RESEARCH ARTICLE Legume Research- An International Journal, Volume 43 Issue 4: 470-479 (August 2020)

Studies on genetic diversity of French bean (*Phaseolus vulgaris* L.) landraces of Himachal Pradesh based on morphological traits and molecular markers

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10.18805/LR-4089

ABSTRACT

The knowledge of the extent of underlying diversity in the germplasm can be effectively employed to breed desirable cultivars and to challenge the consequences of the unprecedented biological, physical and chemical stresses of the future growing conditions. In the present study, total 26 geographically diverse French bean genotypes collected from different agro-climatic zones of Himachal Pradesh and two check varieties *viz.,* Baspa and Jawala were grown under randomized complete block design with three replications for deciphering the extent of genetic diversity. The extent of variability was assessed using DUS morphological traits and PCR based approach. Analysis of variance for quantitative traits showed significant differences among all the genotypes for all the characters studied. The genotypes RL-19 and RL-20 gave higher seed yield and performed better for many of the horticultural traits over all the checks. Further, 15 RAPD primers were used for scoring polymorphism at the DNA level. The French bean landraces produced 79 PCR fragments, of which 59 were polymorphic with an average of 3.93 polymorphic fragments per primer. The overall percentage of polymorphism was recorded 74.62 per cent. The average Jaccard similarity index among genotypes ranged from 0.48 to 0.98, thereby showing sufficient genetic variability. Geographical affiliations and morphological associations were observed while studying the genotypic patterns within each group i.e. A, B, C and D. Such variability is desirable in breeding programs.

Key words: Dendrogram, Genetic diversity, Morphological traits, *Phaseolus vulgaris*, RAPD markers.

INTRODUCTION

French bean (*Phaseolus vulgaris* L.; Family: Leguminosae; annual diploid species 2n= 22) is also known as 'Rajmash' or haricot bean or kidney bean or common bean. It is one of the most important leguminous pulse crop grown for its tender pods, shelled beans and dry beans. French bean is one of the most important protein sources for human consumption in the developing countries. Besides this, they are also rich in calcium required for bone structure and general health, iron required for blood making and different types of vitamin B which are effective on the nervous system (Karasu and Oz, 2010).

Himachal Pradesh is endowed with varied agro-climatic zones from sub tropical to dry temperate desert and possesses a distinct position among other states with regard to bean germplasm. Anonymous (2016-17) reported nearly 3.69 thousand hectares area under bean cultivation with the production of 44.76 thousand metric tons in Himachal Pradesh. The main hot spot regions of French bean cultivation in Himachal Pradesh are Shimla, Chamba, Sirmour, Kullu, Kinnaur and some areas of Mandi and Solan district. Unlike high yielding varieties, the landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period. Genetic diversity has the evolutionary significance for the survival and adaptation of species in different agro-climatic conditions. It is also one of the prerequisites for crop improvement programmes. Kumar *et al*. (2014) envisaged that if there is not enough genetic diversity among genotypes, it is practically not feasible to bring improvement in any characteristic and further increase in yield, because selection of desirable genes depends upon the extent of genetic variability present among the available gene pool. Hence, it is important to know the proportion of variability in the collected genetic germplasm.

The traditional method of characterization based on morphological descriptors of plant is a common step in plant breeding for establishing the distinctiveness of the varieties. However, morphological characterization has some limitations, as it is descriptive, error-prone, expression of characteristics are affected by the environment and does not expose the genetic diversity between the morphologically overlapping cultivars and identical accessions when

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How to cite this article: Kanwar, R., Mehta, D.K., Sharma, R. and Dogra, R.K. (2020). Studies on genetic diversity of French bean (*Phaseolus vulgaris* L.) landraces of Himachal Pradesh based on morphological traits and molecular markers. Legume Research. 43(4): 470-479.

Submitted: 09-10-2018 **Accepted:** 28-02-2019 **Published:** 14-08-2019

collected from a particular niche. Therefore, efforts are being directed to use molecular markers (RAPD, AFLP, SSR etc.) for revealing polymorphism at the DNA level. The molecular markers, including RFLP (Restriction Fragment Length Polymorphism) (Metais *et al*. 2000), RAPD (Random Amplified Polymorphic DNA) (Chiorato *et al*. 2007) and AFLP (Amplified Fragment Length Polymorphism) (Kumar *et al*. 2008) have been explored in common bean diversity studies. RAPD markers are the most widely used markers for genetic diversity study because no prior DNA sequence information is needed for designing PCR primers as required for other PCR based genetic marker systems. The PCR-based RAPD approach using arbitrary primers requires only nanogram quantities of template DNA, no radioactive probes and is relatively simple compared to other techniques (Biswas *et al*. 2010).

Characterization both at phenotypic level using morphological characters supplemented with molecular characterization at DNA level will serve as a principle tool in establishing the identity and distinctness of the genotypes and a key medium to find out the existing polymorphism in the concerned species for breeding purpose. Such information on characterized genotypes will not only help in building a strong national gene pool but will also assist seed certification programmes via documenting cultivar purity. In view of these facts, the present investigation was designed to document and characterize the existing genetic variability in French bean both at phenotypic and genotypic level.

MATERIALS AND METHODS

Plant genetic material

During the year 2014, survey and exploration trips to 36 sites in districts *viz*., Shimla, Chamba, Sirmour, Kullu, Kinnaur and some areas of Mandi and Solan were conducted and different indigenous genotypes of French bean were collected. The samples collected comprised of seeds (1 kg each or less depending upon the availability). The collection was made from farm store, farmer's field and threshing yards. Each collected sample was brought to the laboratory where it was cleaned, and stored in the air tight plastic containers. Then, these genotypes were assigned the collection number constituting of alpha numeric characters like Rajmash Local 1 (RL-1), Rajmash Local 2 (RL-2) and so on until every collected germplasm line had its own identification number. In total, 26 diverse lines and 2 check varieties recommended from CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur *viz*., Baspa and Jawala were incorporated in the study programme (Table 1).

Field evaluation and data collection

The field experiment was carried out at Experimental Farm of the Department of Seed Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, H.P. during the Kharif season of 2015 and 2016. The experiment was laid-out in Randomized Complete Block Design (RCBD) with three replications. The seeds were sown at a spacing of 45 cm × 15 cm (dwarf types) and 90 cm × 15 cm (pole types). Two seeds per hill were dibbled at a depth of 2.0 to 2.5 cm. Thinning operation was carried out on 21st day after sowing and retained one healthy and vigorous plant per hill. The standard cultural practices recommended for growing French bean in the mid hills as included in the "Package of Practices for *Kharif* crops (in Hindi)" published by the Directorate of Extension Education, CSK HPKV, Palampur, Himachal Pradesh were followed (Anonymous, 2011). Ten plants were randomly selected and tagged for recording observations. Observations were recorded on eleven qualitative traits *viz*., plant growth habit, stem pigmentation, hairiness on the stem, flower colour, hairiness on the flower, leaflet shape, pod colour at immature stage, orientation of pods, pod shape, pod pubescence and pod colour at physiological maturity and sixteen quantitative characteristics *viz*., early plant vigour (cm), leaflet length (cm), leaflet width (cm), plant height at final harvest (cm), number of branches per plant, days to 50 % flowering, inflorescence length (cm), days to first harvest, harvest duration, pod length (cm), pod width (cm), number of pods

Table 1: French bean genotypes alongwith their place of collection used in the present study.

Genotype	Place of Collection
RL- 1	Sangla (Kinnaur)
RL- 2	Poweri (Kinnaur)
RL- 3	Kanam, Poo (Kinnaur)
RL- 4	Sapni (Kinnaur)
RL- 5	Bhavanagar (Kinnaur)
RL- 6	Pangi (Chamba)
RL- 7	Pangi (Chamba)
RL- 8	Tissa (Chamba)
RL- 9	Gharer, Bharmaur (Chamba)
RL-10	Panjsei, Bharmaur (Chamba)
RL- 11	Satnala, Bharmaur (Chamba)
RL-12	Satnala, Bharmaur (Chamba)
RL- 13	Sunarly, Chopal (Shimla)
RL-14	Khashdhar (Chirgaon)
RL- 15	Gassopul, Rampur (Shimla)
RL-16	Khaltudhar, Kumarsain (Shimla)
RL- 17	Jubbal (Shimla)
RL- 18	Dhar Gaura, Rampur (Shimla)
RL- 19	Khanag (Kullu)
RL- 20	Khanag (Kullu)
RL- 21	Bhutti (Kullu)
RL- 22	Bhalyani (Kullu)
RL- 23	Anni, Banjar (Kullu)
RL-24	Ser Jaghas, Rajgarh (Sirmour)
RL- 25	Ratoli, Rajgarh (Sirmour)
RL- 26	Kotla- Nagali (Solan)
Baspa	Mountain Agril. Research & Extension Centre, Sangla (Kinnaur)
Jawala	Regional Horticulture Research and Training Station, Sharboo (Kinnaur)

per plant, number of seeds per pod, 100 seed weight (g), seed yield per plant (g) and seed yield per hectare (q). An analysis of variance was made for each quantitative trait combined over years (Gomez and Gomez, 1984).

DNA extraction

Total genomic DNA was extracted from fresh young leaves, collected from each genotype, following CTAB method (Doyle and Doyle, 1987) with some modifications. Each fresh leaf tissue (200 mg) was ground to a fine powder in liquid nitrogen and transferred to a 2 mL autoclaved microcentrifuge tube, then 1.0 mL prewarmed CTAB buffer (containing 10% CTAB, 0.5M EDTA (pH 8.0), 5M NaCl, 1M Tris-HCl (pH 8.0), 2% β-mercaptoethanol) was added. The mixture was incubated at 60°C for 1 hour and then mixed with equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1 v/v). The suspension was centrifuged at 11000 rpm for 20 minutes at room temperature and the supernatant (800 µL) was transferred to another 2 mL autoclaved microcentrifuge tube and an equal volume of chloroform: isoamyl alcohol (24:1 v/v) was added. The mixture was again centrifuged at 11000 rpm for 15 minutes at 10°C. The supernatant (600 µL) was then precipitated by adding 200 µL of ice-cold isopropanol. Keep the mixture at -20°C for 1 hr, then spinned at 11000 rpm for 20 min at 4°C to take out the DNA pellet. Wash the DNA pellet with 100 µl of 70% ethanol for 20 min and centrifuge at 10000 rpm for 10 min at 4°C. The tubes containing DNA pellet were kept overnight under laminar air flow (LAF) hood for complete drying. The dried pellet was dissolved in 50 µl of TE buffer (pH 8.0).

Purification of DNA

For purification, 1 µl of RNase (10 mg/ml) was added to TE containing DNA, mixed gently and incubated for 1 hr at 37°C. Then equal volume (50 µl) of phenol: chloroform: isoamylalcohol (25: 24: 1 v/v) was added and mixed thoroughly to emulsify. Centrifuged at 11000 rpm for 10 min and carefully took out the aqueous phase to fresh autoclaved microcentrifuge (2.0 ml) tube. Added 1/10th volume of 3M sodium-acetate (pH 4.8) and mixed thoroughly. Added about two volumes of absolute ethanol (95%) and mixed by gentle inversion and kept on ice for 20 min. Spinned at 11000 rpm for 15 min at 4°C to form DNA pellet. The DNA pellet was washed with 70% ethanol, dried and dissolved in 50µl of TE buffer. (pH 8.0). The aliquates of DNA were stored at -20°C for future use.

Polymerase Chain Reaction (PCR) and Gel Electrophoresis

Fifteen RAPD primers obtained from operon technologies were used to generate markers assisted information on genetic diversity. The polymerase chain reaction was performed in the Thermo Cycler. The reaction volume of 20 µl contained 10.2 µl of sterile distilled water, 2 µl Taq DNA polymerase buffer (1X), 1.5 µl $MgCl_2$ (2 mM) , 2 µl dNTPs (1 mM), 0.3 µl Taq DNA polymerase (1U/ µl), 2 µl random primer (10 pmol) and 2 µl genomic DNA (50-100 ng). All the samples were given initial denaturation at 94°C for 5 minutes in a thermal cycler. PCR was carried out with a total of 45 cycles. Each cycle consisted of 30 seconds of denaturation at 94°C, 1 minute of annealing at temperature specific to the melting point of primer, 2 minutes of extension at 72°C and a final extension at 72°C for 10 minutes. PCR products were allowed to stand at 4°C for 5 minutes. The amplified products were separated by electrophoresis on agarose gel (1.2%) under submerged conditions. 1X TAE buffer was used to prepare the agarose gel and tray buffer. To each PCR amplified DNA, 3 µl of 6X loading dye was added and 20 µl of sample was loaded into each well. 100 bp ladder (0.1-1.5Kb), Gene Ruler TM was used as standard and the gel was run at 70 V until the loading dye reached the gel front. The amplified DNA was viewed under the UV trans-illuminator and image was taken through gel documentation system.

Band scoring and RAPD analysis

Each amplified product was scored for their presence or absence. Co-migrating bands were considered to represent the same locus and thus treated as the same band while scoring. Presence of an amplified product was designated as '1' and absence was marked as '0'. Intensity of the bands was not taken into account while scoring. A pair wise similarity index was constructed by using Jaccard coefficients (Jaccard, 1908). This was further, subjected to UPGMA cluster analysis and a dendrogram was constructed. NTSYS-pc, version 2.02h was used to perform cluster analysis of the complete RAPD data.

RESULTS AND DISCUSSION

Qualitative characteristics

The observations obtained on qualitative traits (Table 2) revealed that based on plant growth habit, fourteen genotypes with alphanumeric accession number RL- 2, 5, 6, 7, 8, 10, 12, 14, 15, 16, 18, 21 and two check varieties Baspa and Jawala were categorized as bush type while remaining fourteen genotypes RL- 1, 3, 4, 9, 11, 13, 17, 19, 20, 22, 23, 24, 25 and 26 possessed pole type growth habit. The French bean genotypes varied for stem pigmentation. The genotype RL- 13 had green with red streaks pigmentation on stem, RL- 3, 24 and 26 had green with purple streaks pigmentation on stem whereas, remaining genotypes and check varieties were with green stem pigmentation. Hairiness on stem varied among different genotypes. The five French bean genotypes and two check varieties *viz*., RL- 9, 15, 18, 21, 24, Baspa and Jawala were glabrous (without hairs), eleven genotypes *viz*., RL- 1, 3, 4, 6, 8, 11, 13, 16, 17, 19 and 20 had sparse hairiness and ten genotypes *viz*., RL- 2, 5, 7, 10, 12, 14, 22, 23, 25 and 26 had intermediate hairs on the stem. The flower colour of genotypes RL- 4, 5, 6, 8, 10, 12, 13, 14, 15, 16, 18, 23, 24, 25, Baspa and Jawala were pink, genotypes RL- 1, 2, 7, 17 and 21 had violet flowers and genotypes RL- 3, 9, 11, 19, 20, 22 and 26 had white petal colour. The flowers of all the French bean genotypes including check varieties Baspa and

Jawala were glabrous (without hairs). The leaflet shape of eighteen genotypes *viz*., RL- 1, 3, 4, 8, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25 and 26 was round whereas ten genotypes including check varieties *viz*., RL- 2, 5, 6, 7, 9, 10, 14, 23, Baspa and Jawala had ovate form of leaflet shape. Green with red streaks pods at immature stage were observed in three genotypes (RL- 4, 12 and 23), green with purple streaks pods were recorded in three genotypes (RL-11, 17 and 26) and green coloured pods were obtained in twenty two genotypes including check varieties (RL- 1, 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24, 25, Baspa and Jawala). All the genotypes alongwith check varieties under study had prostrate growing habit of pods. Among twenty eight French bean genotypes studied for pod shape, six genotypes and a check variety *viz*., RL- 1, 3, 13, 17, 19, 20 and Baspa had straight pods, seventeen genotypes and a check variety *viz*., RL- 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 18, 22, 23, 24, 26 and Jawala produced slightly curved pods, and three genotypes *viz*., RL- 16, 21 and 25 produced curved pods. Among twenty eight French bean genotypes studied for hairiness on pod, six genotypes *viz*., RL- 1, 8, 15, 21, 22 and 24 were glabrous (without hairs), twelve genotypes *viz*., RL- 2, 6, 7, 9, 10, 11, 17, 19, 20, 23, 25 and 26 were having sparse hairs and ten genotypes alongwith check varieties *viz*., RL- 3, 4, 5, 12, 13, 14, 16, 18, Baspa and Jawala had intermediate pod pubescence. Copper brown pod colour was observed in all the genotypes including check varieties at maturity. Similar studies on characterization of French bean genotypes were conducted by Prashanth (2003), Neupane *et al*. (2005), Kar *et al.* (2006) and Okii *et al*. (2014) using qualitative morphological characteristics.

Quantitative characteristics

Based on the pooled data of two years (Table 3), analysis of variance revealed significant differences among the genotypes for all the traits. The genotype RL-20 recorded maximum plant height (45.33 cm) after 30 days of sowing indicating vigourous growth and minimum plant height (20.78 cm) was observed in genotype RL-10. The genotype RL-26 produced maximum (10.65 cm) leaflet length while genotype RL- 22 recorded minimum (8.03 cm) leaflet length. Maximum leaflet width (7.79 cm) was recorded in genotype RL-26 and minimum leaflet width (5.58 cm) was recorded in RL-2. Maximum plant height (170.59 cm) was depicted by genotype RL -19, whereas minimum plant height (26.19 cm) was recorded in genotype RL-16. The genotype RL-20 observed maximum number of branches per plant (18.67), whereas minimum number of branches per plant (5.75) was recorded in RL-16. The genotype RL-11 recorded minimum days to 50% flowering (42.17 days), while RL-19 recorded maximum days to 50% flowering (63.00 days). Maximum inflorescence length (4.26 cm) was recorded in RL-3 and minimum inflorescence length (2.38 cm) was observed in RL-16. Minimum days to first harvest (76.00 days) were recorded in check variety Jawala, whereas maximum days

Table 2: Variation of different qualitative characters in French bean.

Name of Descriptor	Descriptor state	Number of accessions	% of accessions
Plant growth habit	Bush	12 + 2 check varieties (Baspa and Jawala)	50
	Pole	14	50
Stem pigmentation	Green with red streaks	1	4
	Green with purple streaks	3	11
	Green	22 + 2 check varieties (Baspa and Jawala)	85
Hairiness on the stem	Glabrous	5 + 2 check varieties (Baspa and Jawala)	25
	Sparse	11	39
	Intermediate	10	36
Flower colour	Pink	14 + 2 check varieties (Baspa and Jawala)	57
	Violet	5	18
	White	7	25
Hairiness on the flower	Glabrous	26 + 2 check varieties (Baspa and Jawala)	100
Leaflet shape	Round	18	64
	Ovate	8 + 2 check varieties (Baspa and Jawala)	36
Pod colour at immature stage	Green with red streaks	3	11
	Green with purple streaks	3	11
	Green	20 + 2 check varieties (Baspa and Jawala)	78
Orientation of pod	Prostrate	26 + 2 check varieties (Baspa and Jawala)	100
Pod shape	Straight	6 + 1 check varieties (Baspa)	25
	Slightly curved	17 + 1 check varieties (Jawala)	64
	Curved	3	11
Pod pubescence	Glabrous	6	21
	Sparse	12	43
	Intermediate	8 + 2 check varieties (Baspa and Jawala)	36
Pod colour at physiological maturity	Copper brown	26 + 2 check varieties (Baspa and Jawala)	100

Table 3: Mean Quantitative characteristics of 28 indigenous French bean genotypes. (as recorded in the year 2015 and 2016)

Genotype	Characters															
	EPV (cm)	LL (cm)	LW (cm)	PHFH (cm)	NB	DT 50%F	IL(cm)	DTFH	HD (days)	PL (cm)	PW (cm)	NPPP	NSPP	100 SW (g)	SYPP (g)	SYPH (q)
RL-1	35.24	9.74	7.73	100.67	12.50	53.34	3.54	83.84	21.00	9.68	1.27	19.17	6.27	24.98	18.05	10.65
RL-2	21.69	8.67	5.58	41.09	8.37	45.50	2.95	78.34	14.00	8.27	1.27	9.19	5.08	41.90	10.40	12.45
RL-3	36.35	10.2	7.43	102.75	14.98	50.67	4.26	82.67	23.50	12.79	1.23	18.83	7.13	27.69	23.34	13.77
RL-4	39.79	9.40	6.78	121.44	12.57	49.34	2.51	79.67	23.83	11.36	1.17	12.75	6.03	40.58	18.00	10.62
RL-5	25.55	9.40	6.40	34.00	7.42	48.83	3.14	78.34	13.83	10.57	1.20	7.87	5.03	49.09	12.33	15.58
RL-6	24.40	9.78	6.62	33.75	7.00	49.00	3.27	76.67	14.83	7.32	1.32	5.95	4.47	27.04	8.41	10.02
RL-7	23.58	9.29	6.22	34.50	6.82	46.67	2.85	77.50	17.00	10.22	1.33	7.97	4.17	31.67	13.44	15.99
RL-8	23.97	8.73	6.23	40.00	6.90	44.84	2.98	81.50	13.50	9.91	1.33	8.84	4.33	45.35	13.25	15.77
RL-9	36.81	9.89	5.86	157.09	15.72	58.00	3.09	86.34	23.33	12.54	1.36	18.96	6.18	55.83	24.94	14.72
RL-10	20.78	8.72	6.40	51.72	7.23	44.17	2.74	80.67	21.50	8.84	1.33	8.01	4.92	34.77	12.45	14.82
RL-11	37.18	9.85	7.44	94.02	14.08	42.17	3.06	85.50	23.00	12.79	1.19	21.34	6.12	25.28	21.65	12.77
RL-12	22.93	9.74	7.73	132.12	9.58	47.50	3.21	77.50	16.83	8.60	1.25	8.43	5.10	42.22	10.25	12.20
RL-13	38.62	9.85	6.86	158.82	13.58	53.67	2.84	87.17	23.67	12.42	1.26	15.80	6.23	32.76	20.09	11.85
RL-14	22.67	9.45	6.17	35.05	6.72	48.50	2.94	79.84	14.00	8.74	1.38	8.28	4.23	33.60	12.06	14.35
RL-15	23.17	8.75	6.44	118.67	10.67	56.17	3.24	97.34	17.00	8.98	1.24	7.21	4.60	26.48	11.16	13.28
RL-16	22.95	9.32	6.59	26.19	5.75	49.17	2.38	83.67	19.67	6.83	1.31	5.04	3.82	26.68	4.91	5.84
RL-17	36.57	9.35	6.69	150.62	13.99	53.84	2.56	82.50	23.17	10.99	1.18	15.92	5.95	47.11	21.26	12.54
RL-18	24.55	9.36	7.23	54.84	6.00	55.84	3.06	77.67	15.67	9.22	1.20	6.76	4.41	22.48	10.27	12.22
RL-19	42.65	9.79	7.64	170.59	17.83	63.00	3.54	83.00	24.67	12.69	1.24	21.17	6.90	32.11	33.38	19.70
RL-20	45.33	9.99	7.37	161.54	18.67	62.84	3.99	82.34	23.83	13.34	1.19	20.09	7.25	39.91	35.04	20.67
RL-21	26.38	9.36	7.12	39.70	8.12	45.50	3.46	78.67	17.33	8.68	1.18	10.07	5.13	36.48	10.66	12.69
RL-22	34.58	8.03	6.45	94.34	12.38	49.17	2.89	79.50	25.00	9.70	1.16	15.40	5.75	39.54	17.21	10.15
RL- 23	35.11	9.70	6.60	124.60	12.37	44.67	3.36	76.17	24.67	9.91	1.18	14.07	6.05	46.05	17.40	10.27
RL-24	36.18	9.36	6.52	147.75	12.95	55.84	3.36	78.00	25.00	10.29	1.21	17.76	5.88	32.18	18.25	10.77
RL-25	24.73	8.26	6.75	83.82	6.87	49.34	3.20	78.67	17.33	7.35	1.09	9.11	5.15	19.19	8.77	10.43
RL-26	38.64	10.65	7.79	121.20	16.09	55.50	3.25	100.00	16.50	12.90	0.79	16.59	6.75	35.28	26.22	15.47
Baspa	21.91	10.38	6.69	47.84	8.15	45.17	3.18	78.50	21.80	9.59	1.26	9.70	4.88	41.45	12.64	15.03
Jawala	24.12	8.92	5.84	27.92	9.00	50.00	2.66	76.00	23.50	10.83	1.32	8.34	5.08	46.05	15.10	17.98
Mean	**30.23**	**9.43**	**6.76**	**88.25**	**11.77**	**50.65**	**3.13**	**81.70**	**19.96**	**10.19**	**1.23**	**12.45**	**5.46**	**35.85**	**16.46**	**13.31**
±SE (m)	**1.28**	**0.39**	**0.31**	**7.07**	**0.58**	**0.64**	**0.16**	**1.22**	**1.17**	**0.29**	**0.06**	**0.82**	**0.24**	**0.98**	**1.67**	**1.43**
CD (5%)	**3.65**	**1.11**	**0.88**	**20.09**	**1.66**	**1.81**	**0.45**	**3.47**	**3.32**	**0.82**	**0.14**	**2.32**	**0.67**	**2.92**	**4.75**	**4.07**

EPV= Early plant vigour, LL= Leaflet length, LW= Leaflet width, PHFH= Plant height at final harvest, NB= Number of branches per plant, DT50% F= Days to 50 per cent flowering, IL= Inflorescence length, DTFH= Days to first harvest, HD= Harvest duration, PL= Pod length, PW= Pod width, NPPP= Number of pods per plant, NSPP= Number of seeds per pod,100 SW= Hundred seed weight, SYPP= Seed yield per plant, SYPH= Seed yield per hectare.

Table 4: Clustering pattern of twenty eight genotypes of French bean.

Cluster	Number of genotypes	Genotypes
I	8	RL- 2, 7, 8, 14, 15, 16, 25 and Jawala
II	13	RL- 1, 3, 4, 9, 11, 13, 17,19, 20, 22, 23, 24 and 26
III	7	RL- 5, 6, 10,12, 18, 21 and Baspa

to first harvest (100.00 days) were observed in RL-26. Longest harvest duration was observed in genotypes *viz*., RL-22 and RL-24 (25.00 days), while shortest harvest duration (13.50 days) was recorded in RL- 8. The genotype RL-20 recorded maximum mean pod length (13.34 cm), while minimum pod length (6.83 cm) was observed in genotype RL-16. Maximum pod width (1.38 cm) was recorded in genotype RL-14 and minimum pod width (0.79 cm) was observed in genotype RL-26. The genotype RL-11 observed maximum number of pods per plant (21.34), whereas minimum number of pods per plant (5.04) was observed in RL-16. Maximum and minimum number of seeds per pod was observed in genotype RL-20 (7.25) and RL-16 (3.82), respectively. The genotype RL-9 recorded maximum 100 seed weight (55.83 g), while genotype RL-25 observed minimum (19.19 g) 100 seed weight. Maximum and minimum seed yield per plant was recorded in genotype RL-20 (35.04 g) and RL-16 (4.91 g), respectively. Similarly, maximum and minimum seed yield per hectare was observed in genotype RL-20 (20.67 q/ha) and RL-16 (5.84 q/ha), respectively. Das *et al*. (2014) also evaluated the performance of fourteen different bush type French bean genotypes and observed that highest seed yield/plant, per plot and per hectare was obtained from Arka Suvidha (39.56 g, 1099.19 g and 2180.92 kg respectively) followed by Falguni (36.86g, 1032.25 g and 2047.97 kg respectively) and lowest result in this respect was shown by Shillong Local-3 (5.56 g, 155.81 g and 312.84 kg respectively). Similar studies on seed yield/plant and seed yield/ha were conducted by Galindo *et al*. (2004), Bhushan *et al*. (2007) and Prakash and Ram (2014) in French bean.

Table 5: Average intra and inter cluster distance (D^2).

Cluster	I	II	III
I	**2.209**		
II	5.152	**3.024**	
III	2.044	4.809	**1.654**

Genetic divergence

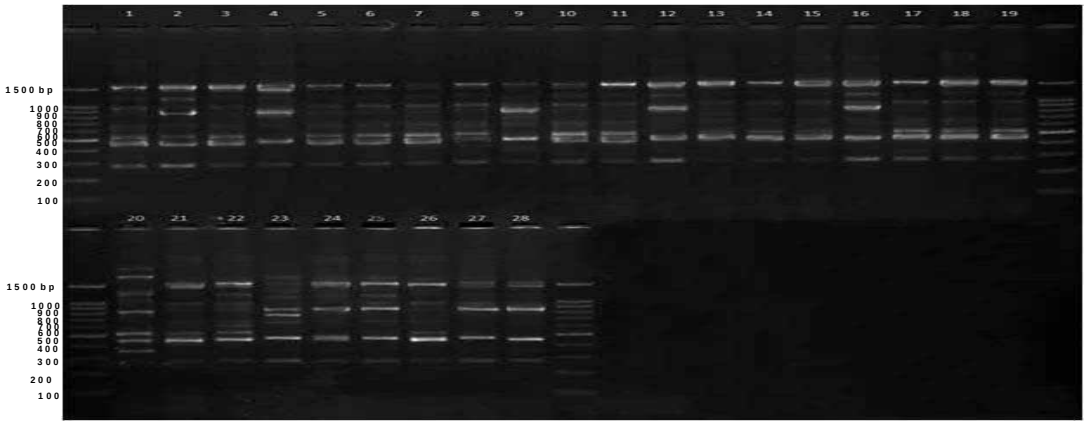
By estimating D^2 values in all possible combinations of the genotypes, the 28 genotypes of French bean were grouped into three clusters revealing moderate genetic diversity (Table 4). Maximum number of genotypes (13) were accommodated in cluster II while minimum (7) in cluster III. Average intra cluster distance was maximum (3.024) in cluster II and minimum (1.654) in cluster III. The inter cluster distance was recorded maximum (5.152) between cluster I and II, indicating that hybridization between the genotypes from cluster I and II can be utilized for getting superior recombinants/transgressive segregants in segregating generations of French bean (Table 5). Furthermore, cluster means of the various characters indicated that among the three clusters, cluster II was found superior for important traits *viz*., early plant vigour, leaflet length, leaflet width, plant height at final harvest, number of branches per plant, days to 50 per cent flowering, inflorescence length, days to first harvest, harvest duration, pod length, number of pods per plant, number of seeds per pod, 100 seed weight and seed yield per plant, whereas cluster I was found superior for the trait *viz*., pod width. Superior candidate genotypes

Table 6: Cluster means for different characters among twenty eight genotypes of French bean.

Characters	Clusters		
	I	II	III
Early plant vigour (cm)	23.36	37.93	23.79
Leaflet length (cm)	8.92	9.80	9.53
Leaflet width (cm)	6.23	7.01	6.88
Plant height at final harvest (cm)	46.45	131.19	56.28
Number of branches per plant	7.53	14.44	7.77
Days to 50% flowering	48.77	53.23	48.00
Inflorescence length (cm)	2.90	3.25	3.15
Days to first harvest	81.60	83.59	78.29
Harvest duration	17.00	23.17	17.40
Pod length (cm)	8.89	11.65	8.97
Pod width (cm)	1.28	1.18	1.25
Number of pods per plant	7.99	17.52	8.11
Number of seeds per pod	4.56	6.34	4.85
Hundred seed weight (g)	33.87	36.87	36.22
Seed yield per plant (g)	11.13	22.68	11.00

Table 7: Total number of bands amplified with 15 random decamer primers by PCR and number of polymorphic bands.

Primer	Sequence(5′ -3′)	Annealing temperature (°C)	No. of amplified bands	No. of polymorphic bands	Polymorphism bands (%)
OPB-07	-GGTGACGCAG-	34	**6**	4	66.67
OPB-17	-AGGGAACGAG-	32	8	6	75.00
OPE-08	-TCACCACGGT-	32	3	3	100.00
OPE-10	-CACCAGGTGA-	32	5	4	80.00
OPE-19	-ACGGCGTATG-	32	8	7	87.50
OPAC-03	-CACTGGCCCA-	34	7	4	57.14
OPAC-11	-CCTGGGTCAG-	34	7	6	85.71
OPAK-12	-AGTGTAGCCC-	32	7	3	42.86
OPL-12	-GGGCGGTACT-	34	2	1	50.00
OPL-13	-ACCGCCTGCT-	34	4	4	100.00
OPL-14	-GTGACAGGCT-	32	1	0	Monomorphic
OPM-03	-GGGGGATGAG-	34	4	4	100.00
OPN-01	-CTCACGTTGG-	32	3	3	100.00
OPN-10	-ACAACTGGGG-	32	6	5	83.33
OPN-18	-GGTGAGGTCA-	32	6	5	83.33
TOTAL			**79**	**59**	**74.62**



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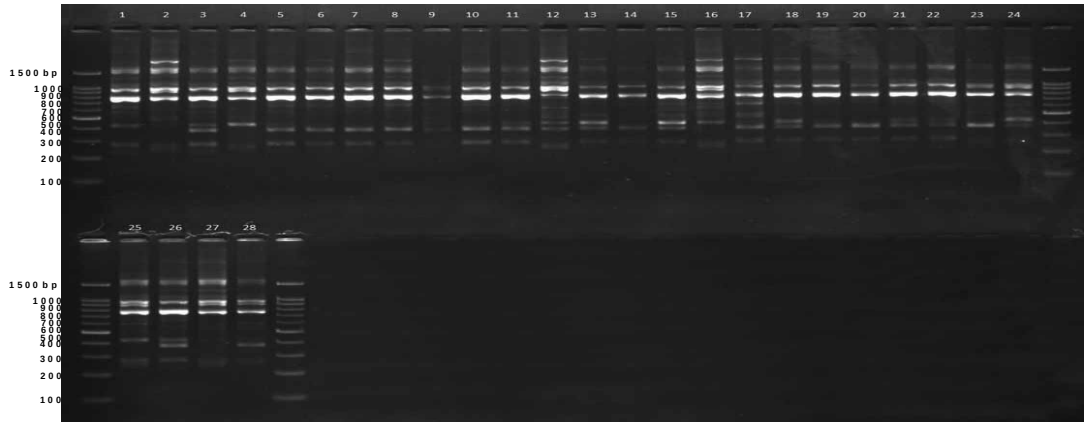


O P B - 1 7

Code given to different genotypes: 1-26 (RL-1 to RL-26): 27 (Baspa); 28 (Jawala)

Fig 1: RAPD profiles of French Bean genotypes use in primer OPB-07 and OPB-17.

from these clusters can be selected for trait specific improvement program (Table 6).

Molecular diversity

In the present study, a set of randomly chosen DNA markers were used to assess the genetic variation among 28 French bean genotypes. In total 33 RAPD primers screened among 28 French bean genotypes, 15 were used for scoring of data to evaluate the genetic diversity and others could not be used because either some primers did not amplify the genomic DNA of the selected 28 French bean genotypes or the bands amplified were not scorable for cluster analysis (Table 7).

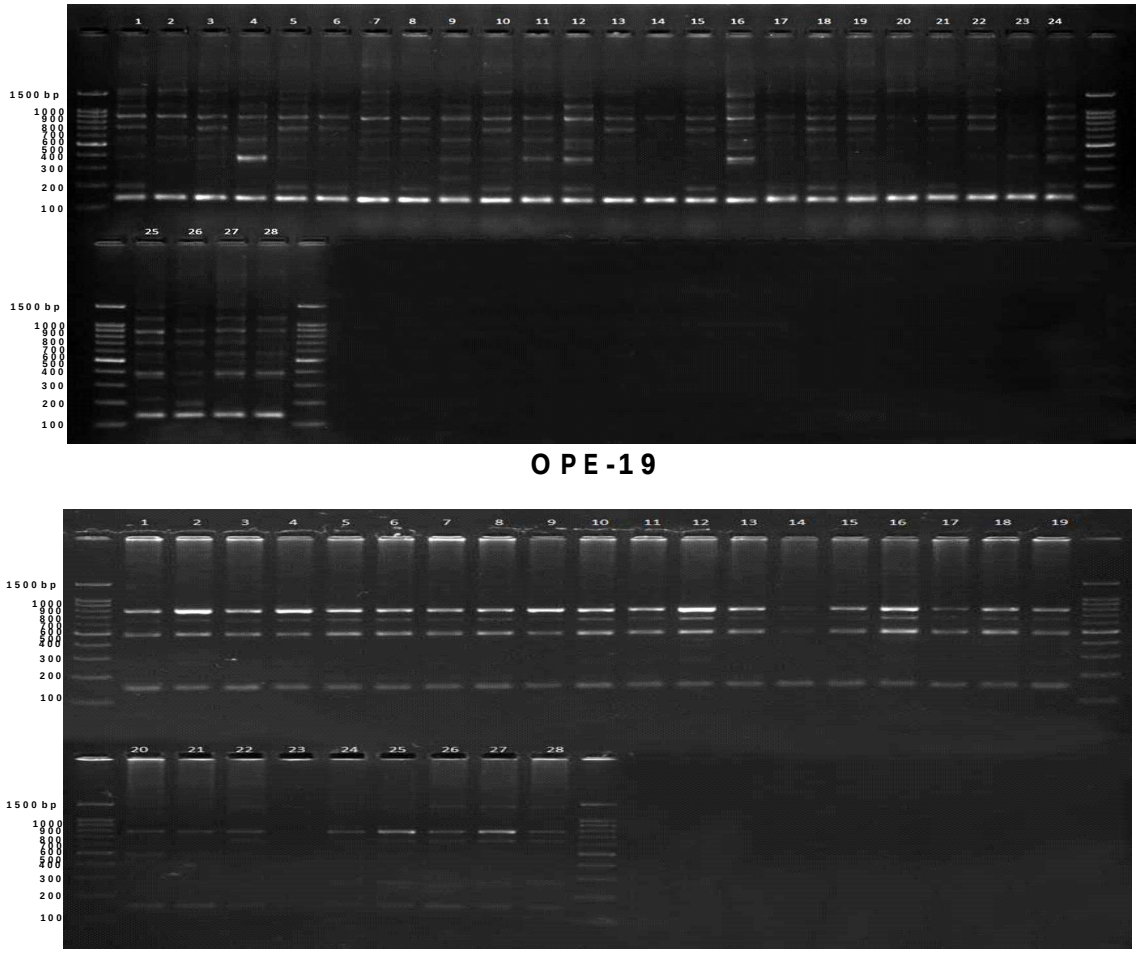
A band locus was considered as polymorphic if the band differentiated at least two of the genotypes. The RAPD profiles of all samples with few RAPD markers are presented in Fig 1 and 2. A total of 79 bands were amplified with 15 primers, of which 59 were polymorphic among 28 French bean genotypes with an average of 3.93 polymorphic bands per primer and overall percentage of polymorphism was 74.62 (Table 8). This value was higher than those reported earlier by Biswas *et al.* (2010) and Kumar *et al.* (2014) in *Phaseolus vulgaris* L. In the present investigation the highest (8) PCR fragments were observed with primers *viz.*, OPB-17 while the lowest (1) was observed with OPL-14.

Cluster analysis based on RAPD profile

The pair wise genetic distances obtained based on Jaccard's coefficients by combined scores of all 15 informative primers were used for clustering bean cultivars using Unweighted Pair Group of Arithmetic Averages (UPGMA) method (SAHN

Table 8: Summary statistics of PCR-RAPD analysis of French bean genotypes.

Particulars	Numbers
Total number of band levels	79
Total number of polymorphic bands	59
Number of primers studied for scoring	15
Maximum number of levels observed	8
Minimum number of levels observed	1
Average number of band levels /primer	5.27
Average number of polymorphic bands /primer	3.93
Overall polymorphism percentage obtained	74.62%



Code given to different genotypes: 1-26 (RL-1 to RL-26): 27 (Baspa); 28 (Jawala)

Fig 2: RAPD profiles of French Bean genotypes use in primer OPE-19 and OPL-13.

module of NTSYS-PC version 2.02), clusters obtained are presented in dendrogram (Fig 3).

The dendrogram separated majority of the French bean genotypes into one main cluster and two sub clusters (Ia and Ib) which further branched into four groups (A,B,C and D). Geographical affiliations in grouping were also observed in some groups. The average Jaccard similarity index among cultivars ranged from 0.48 to 0.98, values which are wider and more diverse than those reported by Mavromatis *et al*. (2010) for RAPDs for common bean and that indicates the high genetic diversity of the material used in this study. Cluster analysis revealed that genotype RL-21 collected from Bhutti (Kullu) was the most diverse and came out as an outliner sharing minimum (48%) similarity with other genotypes. Genotypes RL-18, RL-17 and RL-22 which were collected from Dhar Gaura, Rampur (Shimla), Jubbal (Shimla) and Bhalyani (Kullu) shared 60 to 70% similarity with other genotypes. These genotypes had the distinct seed coat colour compared to other genotypes. The interesting point which can be interpreted is that though the genotypes RL-5 and RL-6 have been collected from different regions but shares maximum similarity. This indicates that these two genotypes could be of same genetic architecture and at the time of evolution might have dispersed from the same geographical region. Further, genotypes RL-9, RL-11, RL-13, RL-15, RL-16 and Jawala have also been organized under the same group and shares the prominent distinguishing characteristic i.e. seed coat colour. The genotypes RL-14 and Baspa also had similar seed coat pattern and seed shape. Similar characterization programmes based on RAPD (PCR based system) has been carried out by various researchers *viz*., Biswas *et al*. (2010), Mavromatis *et al*. (2010), Szilagyi *et al*. (2011) and Kumar *et al*. (2014) in *Phaseolus vulgaris* L.

Genetic variability observed in the French bean genotypes could prove a valuable genetic resource for breeders and a basic tool for developing an efficient conservation strategy. Clustering analysis strongly supports that the genotypes under study are quite distinct from each other and the differences (polymorphism) demonstrates the usefulness of germplasm collection.

Clustering patterns based on morphological (D^2) and molecular (RAPD) marker analysis

The clusters generated by D^2 analysis through phenotyping (Table 4) and RAPD markers analysis through genotyping (Fig 3) depicted that the genotypes *viz*., RL-3, 4, 9, 11, 13; RL-5 and RL-6; RL-7, check variety Jawala and RL-8; RL-10 and RL-12; RL-19 and RL-20 and RL-23 and RL-24 clustered together based on D^2 analysis and molecular analysis. However the genotypes *viz*., RL-1 and RL-2; RL-15 and RL-16; RL-25 and RL-26 and RL-14 and check variety Baspa differed in the scattering pattern. On the basis of quantitative data these genotypes were dropped into different clusters while at the molecular level these were grouped together with each other. The association of these genotypes may be attributed to the fact that these genotypes shared qualitative characteristics with each other and some had geographical linkages. In D^2 analysis, high numerical nature of the quantitative data not only leads to the masking of smaller numerical data but also restricts the use of highly significant qualitative parameters in germplasm characterization, leading to a lesser precision and misclassifications even at the species level. Hence, the molecular markers are relied upon for final conclusions. In general, the landraces seems to have sufficient variability both at morphological and molecular level. Such variability is desirable in breeding programs. (Kumar *et al*. 2014).

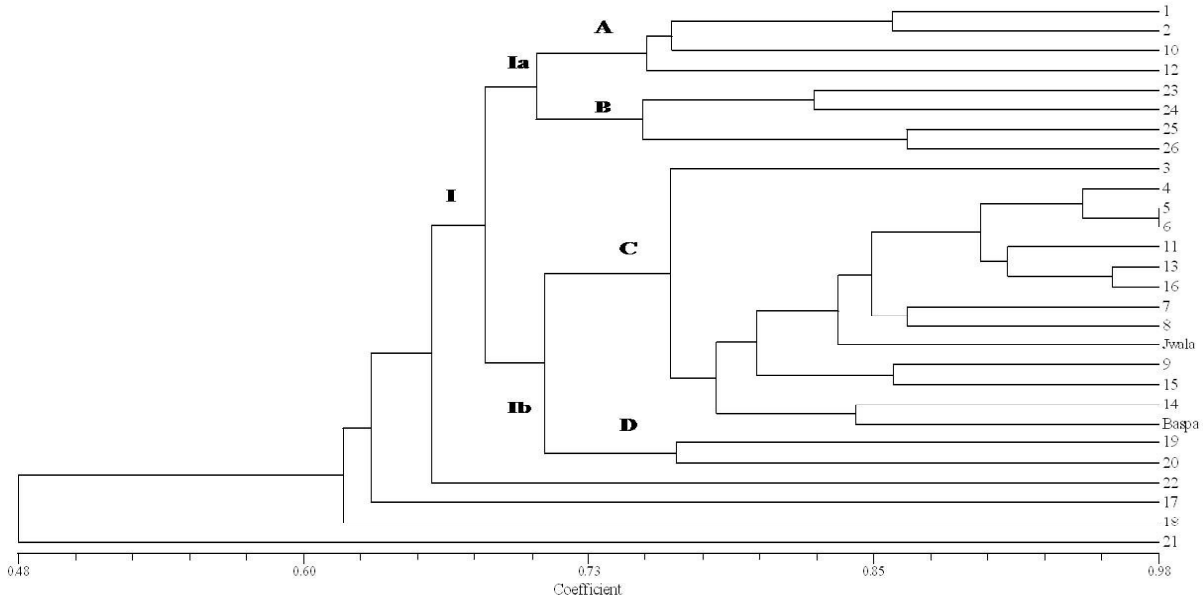


Fig 3: Cluster analysis dendrogram of twenty eight commercial cultivars of common bean (*Phaseolus vulgaris* L.) using RAPD data and UPGMA method.

The collected indigenous genotypes would constitute an essential gene pool for future breeding programme. Characterization of germplasm is an important mechanism for identifying and cataloguing useful agronomic traits of existing genetic resources. Such activities will catalyze the effective involvement of present gene pool in future crop improvement programmes. The genotypes used in the present study showed a wide variation in morphological characters like plant height at final harvest, number of branches per plant, pod length, number of pods per plant, number of seeds per pod, seed yield/plant and seed yield–/ hectare. The genotypes RL-19 and RL-20 gave higher seed yield and performed better for many of the agronomical traits over all the checks. So, these genotypes offer a scope for their adaptability in the state after their multilocation testing. Considerable genetic diversity (Jaccard Similarity Index which ranged between 0.48 to 0.98 was observed among the landraces at molecular level even with the use of limited set of primers. To sum up, the landraces seems to have sufficient variability both at morphological and molecular level. This study would prove a basic primary step in germplasm recognition activity for future breeding programmes and will project the local available genetic footprints to entire agro associated enterprises for necessary consideration.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Science and Technology, Ministry of Science and Technology, Government of India, New Delhi for their financial support.

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