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Genetic divergence and population structure analysis of common bean accessions from Garhwal Himalayas

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Abstract

Aim of study: Collection of common bean accessions from different villages of Garhwal Himalayan region and assessment of their genetic diversity.

Area of study: Field trials were conducted at New Tehri, Tehri Garhwal, Uttarakhand. The lab work was performed at Seed Science & Technology departmental laboratory, HNB Garhwal University, Srinagar Garhwal, Uttarakhand, India.

Material and Methods: 176 common bean accessions were collected from different villages of Garhwal Himalayas. Fourteen quantitative and ten qualitative traits were used to assess morphological diversity and for molecular diversity, twenty SSR primers were used.

Main results: Less variation was observed in genotypic and phenotypic variance in the different parameters studied, which indicates that the observed trait variation and expression are primarily due to genetic factors. The principal component analysis revealed that all the 15 quantitatively measured traits have been loaded on five components, however, major portion of variance (45.38 %) in common bean germplasm was explained by first two components. 293 alleles were scored by SSR markers for the accessions studied. The Bayesian clustering model implemented in STRUCTURE software, on the primary level (K = 2) clearly indicate the presence of both Mesoamerican and Andean gene pool. However, the Mesoamerican gene pool was predominant. The average allele/loci were 4.25 and 32.38 for Andean and Mesoamerican genepool respectively.

Research highlights: All the accessions were divided in Mesoamerican and Andean gene pool. Mesoamerican gene pool accounts for the vast majority. The Andean gene pool was only detected at higher altitude while Mesoamerican gene pool was found at both higher and lower altitude. Approximately 21 accessions showed agronomic superiority.

Keywords: common bean; genetic diversity; germplasm; gene pool; principal component analysis; SSR.

Análisis de divergencia genética y estructura poblacional de accesiones de judía del Himalaya Garhwal

Resumen

Objetivo del estudio: Recolección de accesiones de judía de diferentes aldeas de la región del Himalaya de Garhwal y evaluación de su diversidad genética.

Área de estudio: Se realizaron ensayos de campo en New Tehri, Tehri Garhwal, Uttarakhand y el trabajo de laboratorio se realizó en el laboratorio departamental de Ciencia y Tecnología de Semillas, Universidad HNB Garhwal, Srinagar Garhwal, Uttarakhand, India.

Material y métodos: Se recolectaron 176 muestras de judía de diferentes aldeas de Garhwal Himalayas. Se utilizaron catorce rasgos cuantitativos y diez cualitativos para evaluar la diversidad morfológica y, para la diversidad molecular, se utilizaron veinte cebadores SSR.

Principales resultados: Se observó menor variación en la varianza genotípica y fenotípica en los diferentes parámetros estudiados, lo que indica que la variación y expresión de los rasgos observados se deben principalmente a factores genéticos. El análisis de componentes principales reveló que los 15 rasgos medidos cuantitativamente se han cargado en cinco componentes; sin embargo, la mayor parte de la variación (45.38 %) en el germoplasma de judía se explicó por los dos primeros componentes. Se puntuaron 293 alelos mediante marcadores SSR para las accesiones estudiadas. El modelo de agrupamiento bayesiano implementado en el software STRUCTURE, en el nivel primario (K = 2) indica claramente la presencia de un acervo genético tanto mesoamericano como andino.

Sin embargo, el acervo genético mesoamericano fue predominante. El alelo/loci promedio fue 4.25 y 32.38 para el acervo genético andino y mesoamericano, respectivamente.

Conclusiones: Todas las accesiones se dividieron en acervo genético mesoamericano y andino. El acervo genético mesoamericano representa la gran mayoría. El acervo genético andino pertenece a mayor altitud, mientras que el acervo genético mesoamericano pertenece tanto a mayor como a menor altitud. Aproximadamente 21 accesiones mostraron superioridad agronómica.

Palabras clave: diversidad genética; judía; germoplasma; resistencia a enfermedades; SSR.

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Introduction

In India, the common bean (*Phaseolus vulgaris* L.) is commonly known as *Rajmah*. It is a diploid (2n = 2x = 22) and self-pollinated species, however, less than 3% outcrossing was reported, while greater values have been recorded on different times (Ibarra-Perez et al., 1997). It is considered as an economically, nutritionally, and socially important crop because of paramount source of lipids, proteins, carbohydrates, fibers and ashes (Silva et al., 2014). Common bean is the most important source of protein in Indians diet. Common bean, a major food crop in many parts of world (Leitao et al., 2023), is noted for its diversity. India has a significant diversity in the domesticated gene pool, even if it is not the primary center of diversity of common bean. It comprises accessions from the two major gene pools, Mesoamerican and Andean, which can be discriminated by seed size (Andean genotypes being large seeded), biochemical characteristics and by microsatellite markers (Arunga et al., 2020). The genetic diversity observed in India is similar to that of European and Chinese beans (Beebe et al., 2001). Wide geographic distribution among various habitats has increased the spectrum of genetic diversity not only in India but around the world (Meza et al., 2013). In the traditional production system, climbing beans are rotated with vegetables and intercropped with grain amaranth and maize during the rainy season in the mountains, while bush types are grown as the sole crop in the Indian plains during winter.

The most well-known landraces grown in India's northwestern region are Harshil rajmah, Joshimath, Auli, Chamba, Barot, Munsiyari, Kinnauree, Bhaderwah and Kashmiri. These landraces have been named on the basis of geographical areas in which they are raised. The spread of common bean in India, particularly in the Himalayan region especially in Uttarakhand, has high genetic diversity which is still unexplored (Rana et al., 2015; Shama et al., 2022). According to Baseline Data on Horticultural Crops in Uttarakhand (2018), common bean is cultivated on a 5,776-ha area of Uttarakhand with a production of 38,112 Mt. Here, diversity in seed shape, color, plant type, and other important traits can be seen, however, the crop's major weaknesses include non-synchronous maturity, ineffective plant architecture, a higher rate of flower drop, response to diseases and a lower variety profile which are some of the main obstacles to increased production. Traditionally, farmers grow 10 or 12 landraces together, but economic factors and agronomic advancements have changed the focus to single-component varieties rather than multi-component combinations. As a result, the displacement of traditional genetic diversity with improved varieties is now visible not only in the high cropping intensive areas but also in the traditional common bean growing areas (Rana et al., 2000; Sofi et al., 2014). Even so, the common bean cropping system is still diverse in the Garhwal Himalayas. Therefore, germplasm collection and evaluation are necessary to conserve available germplasm. The study of diversity in plant genetic resources provides useful information for plant breeders to develop new and improved varieties with advantageous characteristics, including farmers and consumers preferred traits.

As climate change continues to impact global agriculture, preserving genetic diversity in crops is important. With a diverse gene pool, breeders can develop crops that are more resilient to changing climatic conditions, including drought, heat, and extreme weather events (Rana et al., 2009). Preserving genetic diversity also helps to maintain a healthy agro-ecosystem. A diverse gene pool in common beans means that there are more potential sources of resistance to pests and diseases, reducing the need for pesticides and herbicides. Additionally, genetic diversity can enhance soil health and promote sustainable agriculture practices such as crop rotation (Thomas et al., 2019). Hence, it is necessary to conduct a more comprehensive analysis of the genetic variability and population structure of the common bean accessions collected from the primary bean cultivation areas in the Garhwal region. For this research, 176 local common bean accessions were obtained from six districts in Garhwal, Uttarakhand, India. This investigation offers plant breeders valuable insights into the genetic diversity of common bean germplasm from the Garhwal Himalayas, potentially serving as valuable breeding material for developing new cultivars.

Material and Methods

Collection of accessions and field trials

A total of 176 common bean accessions (175 *Phaseolus vulgaris* L. and one *Phaseolus coccineus* L.*)* were collected from various districts of the Garhwal region *i.e.,* Tehri Garhwal, Pauri Garhwal, Chamoli, Rudraprayag, Dehradun and Uttarkashi (Supplementary Table S1). At each collection sites, collected plants were a mixture of common bean accessions. These accessions were first separated on the basis of physical appearance then purified by two cycles of single seed descent method. Ten seeds per row were sown under field condition and seeds were harvested separately from each plant. Then, for morphological diversity, two field trials were conducted in the years 2019 and 2020 from April to November at a farmer's field situated at New Tehri, Tehri Garhwal, Uttarakhand, India which is located at an elevation of 1,750 masl. The field trials were conducted using Randomized Block Design (RBD) with three replications followed by the spacing 30 x 15cm. The latitude and longitude coordinates are 30.376251° N and 78.435379° E respectively. The temperature in Tehri Garhwal rises up to 30 °C in summers and 15 °C in winters. The average annual rainfall (though fluctuating) remains about 70 cm.

Genetic diversity analysis by morphological markers

Assessed morphological traits included 15 quantitative traits *i.e.,* first flowering, 50 % flowering, days to first pod, days to 50 % pod, first harvesting, 50 % harvesting, number of pods/plant, length of pods, width of pods, number of seeds/pod, weight of five green pods, length of seeds, width of seeds, 100 seed weight and yield per plant; and ten qualitative traits *i.e.,* growth habit, leaf colour, stem colour, flower colour, pod colour, seed coat pattern, seed coat colour, seed size, seed shape and seed coat texture (smooth or rough). All quantitative data were the average of ten plants. Three different growth habits were recorded. Determinate plants essentially halt vegetative growth (100-120 days) once reproductive growth (flowering) begins, while indeterminate plants continue to grow (210-240 days) after flowering has begun. The semi-determinate growth habit (maturing in 140-180 days) lies between the determinate and indeterminate growth habits (Suppl. Table S2).

Genetic diversity analysis by molecular markers

Twenty SSR primers were carefully chosen from earlier studies based on their consistent amplification patterns and high polymorphic information contents (Suppl. Table S3). Ten plants of each accession were grown in pots for DNA isolation. Genomic DNA was isolated from five grams of leaf tissue per accession by Cetyl Trimethyl-Ammonium Bromide (CTAB) method as described by Doyle & Doyle, 1990. For amplification, a reaction mixture (20 µl) containing 50 ng DNA, 2X PCR Buffer, 1µM primer, 100 µM of each dNTP, 0.3 U Taq DNA polymerase was used. The amplification reaction was done in 20µl PCR tube. The primary PCR cycle consisted of 35 cycles of initial denaturation for 30 sec at 94 °C, annealing for 60 sec, extension at 72 ºC for 90 sec and final extension on at 72 ºC for min then hold at 4 ºC. Amplification was done in a BioRadC1000™ Thermal cycler.

Statistical analysis

Frequency distribution graphs were prepared for all traits. Data transformation details of qualitative data are given in Suppl. Table S2. The relative frequencies were calculated by dividing the number of frequencies for one observation by the total number of observations. Histogram for qualitative data were prepared by calculating the frequency of each category observed in the common bean collection. Data on quantitative phenotypic traits were subjected to analysis of variance using OPSTAT (version14.139.232). The quantitative traits were further analyzed for various statistical parameters such as mean, range, variance, coefficient of variance, phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), heritability genetic advance, and genetic advance as a percentage of mean in RStudio version 2021.05.29 and R version 4.1.1. To eliminate the redunancy in quantitative data the PCA was performed by the software XLSTAT.

The genetic polymorphism was recorded by taking the band size of all markers and the presence and absence of the band was recorded in the binary form (0 and 1). AMOVA for sample size, number of alleles, Shannon's information index and heterozygosity were calculated using the GenAIEx 6.502 software. The STRUCTURE analysis v2.3.4 program was used to determine the population structure (Pritchard et al., 2000). The program was run with a burn in period of 5,000 and 50,000 MCMC interactions, in a continuous series of groupings (K) ranging from 1 to 10 in 10 repetitions. The best K (DELTA K) was estimated by the method proposed by Evanno et al. (2005) through the STRUCTURE HARVESTER program (Earl et al., 2012). Genetic distance among accessions were calculated using C.S. Chord distance (Cavalli-Saforza et al., 1967). A neighbor- joining (NJ) tree was conducted with Power marker. Genetic relationship among accessions was analysed by Principal Coordinate Analysis (PCoA) using the GenAIEx 6.502 software.

Polymorphism information content (PIC) for each marker was calculated based on the formula –

$n.PIC = 1\text{-}\Sigma\,(Pij).\ 2\ \mathrm{i} = 1\mathrm{i}.1$

Where Pi is the allele frequency for the j^{th} allele in i^{th} primer and summation extended over 'n' patterns (Nei et al., 1973).

Results

Assessment of genetic diversity by morphological markers

Frequency distribution for qualitative traits

Figure 1. Diversity in seed shape, size, colour, flower and pod colour in common bean accessions collected from Garhwal Himalayas. Flower colour- A- GFB-123, B-GFB-6, C-GFB-102 (Phaseolus coccineus), D- GFB-101, E- GFB-2, F-GFB-45, G-GFB-25, Bean colour- A-GFB-01, B- GFB-02, C- GFB-65, D- GFB-03, E- GFB-17, F-GFB-04, G- GFB-22, H-11, I- GFB-100, J- GFB-51, K- GFB-15.

All the qualitative traits measured in this study showed a wide range of variation among the 176 accessions (Fig. 1 and 2). The frequency for indeterminate growth was 86.36%, followed by semi-determinate (10.79%) and determinate (2.84%) growth habits (Fig. 2).

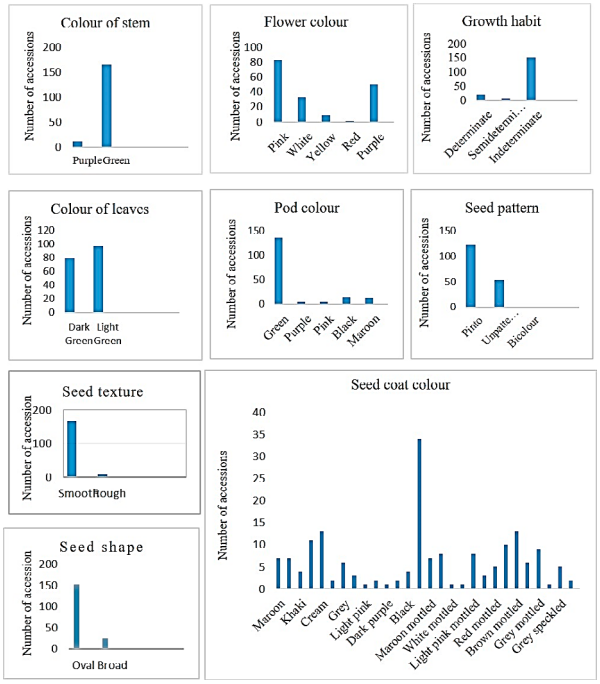

Figure 2. Histogram of nine qualitative traits to assess the morphological diversity of common bean accessions collected from Garhwal Himalayas.

The colour of the leaves was dark green in 45.45% of the accessions and light green in 54.54% of the accessions. The colour of the stem was green in 93.75% and purple in 6.25% of the accessions. Five different flower colours were observed in this study *viz.,*purple, pink, white, yellow, and red; and the frequency was maximum (47.72%) for pink coloured flowers while minimum (0.56%) for red coloured flowers. The red flower colour belongs to the species *P. coccineus*

This species also showed the largest seed size and shape. Maximum frequency (79.54%) in pod colour was recorded for the green colour pods. Most accessions showed pinto (mottled) seed pattern (69.31%), followed by unpattern (30.11%), and bicolored (0.56%). Twenty-eight different seed colours were recorded. The predominant seed colour was cream mottled (19.31%) followed by cream and brown mottled (7.36%). The seed sizes were large (1.6-2.0 cm) for 22.15% accessions, medium (1.0-1.5 cm) for 17.04% accessions, and small (0.5-1.0 cm) for 60.79% accessions. For seed coat texture, most of the accessions (95.45%) were found with smooth seed coat texture. The shape of the seed was oval in the majority of the accessions *i.e.*, 92.04% (Table S2).

Frequency distribution for quantitative traits

All the quantitative traits are described as a frequency distribution graph in Fig. 3. The majority of accessions had medium pod length (10-16 cm). The medium pod length was recorded for 84.09% accessions. The majority of the accessions (84.65%) had pod widths ranging from 1.1 cm to 1.5 cm. The most common number of seeds/pod was 6-8 which was observed in 52.27% of the accessions. The weight of five green pods was categorized into two groups where 60.22% accessions were having less than 20-30 g weight while 39.20% accessions weighed between 30-50 g. The weight of 5 green pods for accession GFB-102 was 53 g. The length of seeds was recorded between 0.5-1.0 cm for the majority of the accessions (67.04%). The width of seeds was found 0.3 cm to 0.5 cm in 98.96 % accessions. The highest 100 seed weight was recorded for the accession GFB-102 (63.28 gm) while for 65.34% accessions 100 seed weight ranged from 20-40 g. The yield/plant was high (150-170 g) for 18.75% accessions and low (75-100 g) for 68.18% accessions. The yield of 13.06 % accessions ranged between 100-150 g.

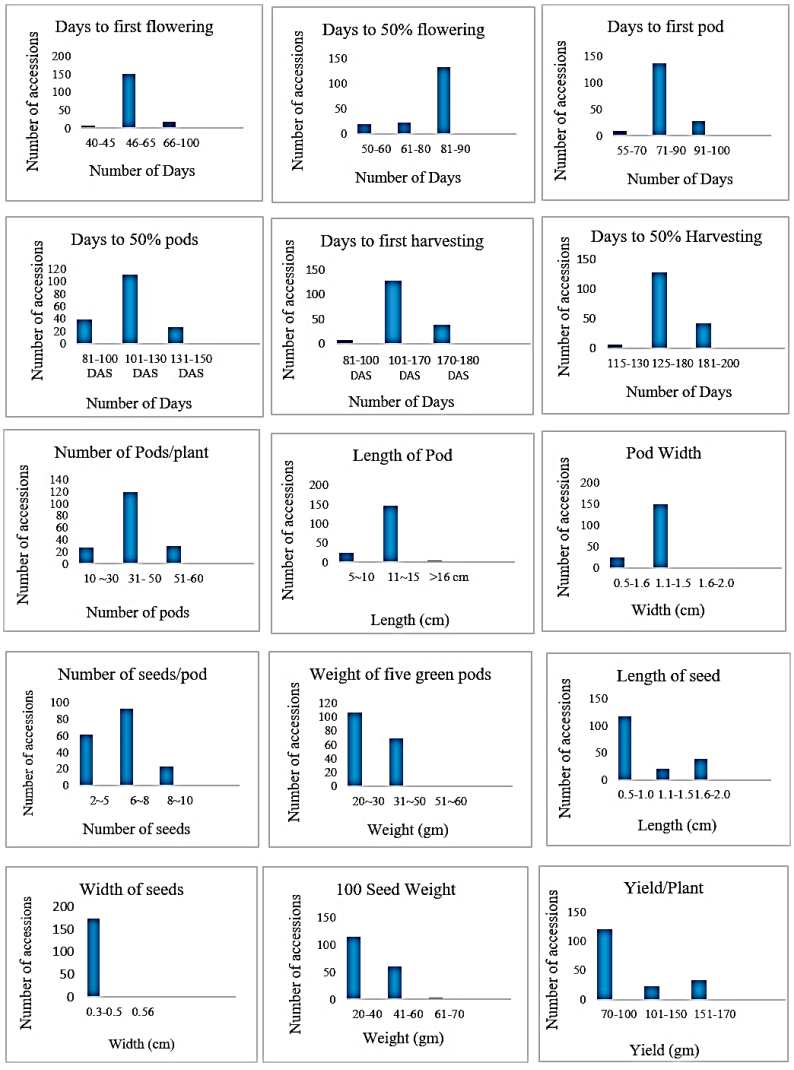

Figure 3. Histogram of fifteen quantitative traits to assess the morphological diversity of common bean accessions collected from Garhwal Himalayas.

Mean, range, variance, coefficient of variance, heritability and genetic advance for qantitative traits

The maximum phenotypic (P) and genotypic (G) variance were recorded for yield/plant which was 2,100.8 and 1,906.2 respectively while the minimum phenotypic (P) and genotypic (G) variance were recorded for the width of seed *i.e.,* 0.01 and 0.009 respectively.

Table 1. Statistical parameters of genetic variability in 176 common bean accessions collected from Garhwal Himalayas.

Traits	Range	Mean ± SE	P	G	PCV (%)	GCV (%)	Heritability (%)	Genetic advance	Genetic advance (% of mean)
DFF	40.8 -71.4	55.7 ± 2.8	47.4	31.4	12.3	10.06	66.2	9.3	16.6
FF	48.40-86.2	67.3 ± 2.8	66.3	50.1	12.0	10.5	75.5	12.6	18.7
FPI	48.6-9	74.9 ± 4.6	74.7	51.0	11.5	9.5	68.2	12.0	16.0
FP	55.2-134.9	101 ± 8.2	159.2	123.1	12.4	10.9	77.3	19.9	19.7
FH	83.2-172.3	126.1 ± 10.5	362.8	325.2	15.1	14.3	89.6	34.7	27.5
FPH	107.1-186.8	157.3 ± 1.8	382.6	289.1	12.4	10.8	75.5	30.0	19.0
PPP	20.4-59.4	30.1 ± 0.8	80	69	29.7	27.59	86.25	3.1	10.2
LP	7.1-23.2	13 ± 0.05	4.7	4.2	16.6	15.7	89.3	3.9	30.0
WP	0.5-1.9	1.2 ± 0.01	0.1	0.07	26.3	22.0	70.0	0.4	33.3
SPP	3.4-90	5.8 ± 0.09	1.1	0.92	18.0	16.53	83.6	1.7	29.3
WFGP	13.5-53.0	29.7 ± 0.1	61.2	53.4	26.3	24.60	87.2	13.9	46.8
LS	0.4-2.5	1 ± 0.06	0.08	0.07	28.2	26.45	87.5	0.5	50.0
WS	0.1-0.9	0.3 ± 0.04	0.011	0.009	33.3	31.6	81.8	0.1	33.3
HSWT	17.1-65.2	37.09 ± 0.43	79.1	69.5	23.9	22.47	87.8	15.8	42.7
YPP	30.12-270.21	99.5708 ± 0.44	2100.8	1906.2	46.06	43.87	90.7	84.5	84.9
CV			2.334	2.437	0.467	0.499	0.103	1.342	0.59

P: phenotypic variance. G: Genotypic variance. GCV: Genotypic Coefficient of Variance. PCV: Phenotypic Coefficient of Variance. DFI: Days to flower initiation. DFF: Days to 50 % flowering. DPI: Days to pod initiation. FH: First harvesting. DFH: Days to 50% harvesting. NOP: Number of pods/plant. PL: Pod length (cm). PW: Pod width (cm). SPP: Seed per plant. WFGP: Weight of five green pods (g). LS: Length of seeds (cm). WS: Width of seed (cm). HSW: 100 seed weight (g). YPP: Yield per plant (g).

The maximum PCV (46.06%) and GCV (43.87%) were recorded for yield/plant while minimum PCV and GCV were recorded for the initiation of the first pod *i.e.,* 11.54 % and 9.5% respectively. The maximum heritability was recorded for yield/plant (90.7) followed by first harvesting (89.6%) while minimum heritability was recorded for the width of the pod (70.0%).

Principal Component Analysis

Genetic diversity and principal component analysis

The PCA used to eliminate the redundancy in data set revealed that all the 15 quantitatively measured traits have been loaded on five components, however, major portion of variance (45.38%) in common bean germplasm is explained by the first two components. The first component (PC1) accounted for 28.647% while the second component (PC2) accounted for 16.737% of variation

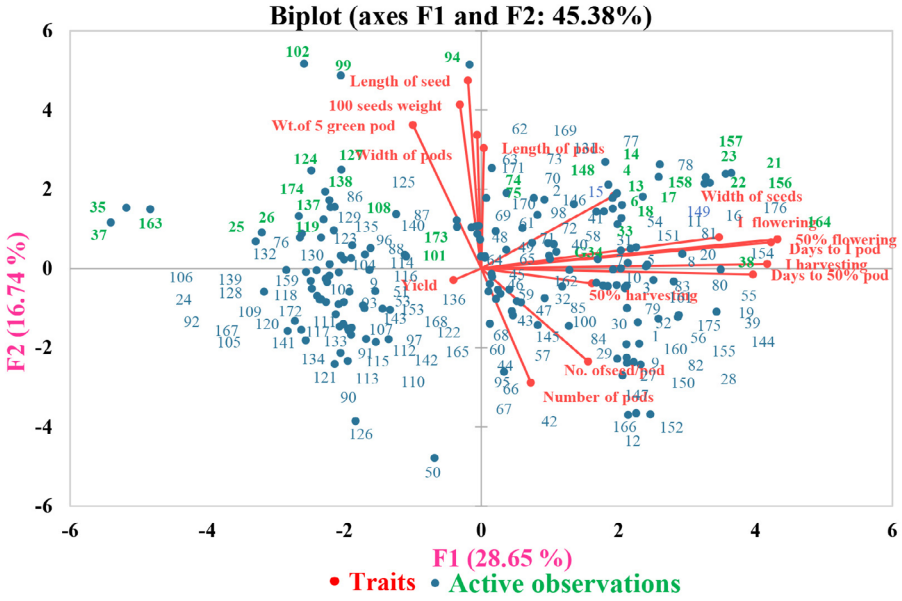

Figure 4. Two-dimensional biplot of 176 accessions of common bean and fifteen quantitative traits loaded on PC1 and PC2. (Accessions in green colour are of large seed size accessions and blue coloured accessions are of medium and small seed size accessions).

The Eigen values were found highest for PC1 which was 4.297 (Suppl. Table S4).The traits fifty percent flowering and days to first pod initiation showing maximum contribution in PC1. In PC2 maximum contributing traits were length of seed and hundred seed weight. Maximum (101) accessions clustered in PC1 followed by PC2 (31), PC3 (25), PC4 (16) and PC5 (3) (Table 2).

Table 2. Contribution of different variables of quantitative traits under five Principal Components and number of bean accessions (Garhwal Himalayas) in each PC.

Variables	PC1	PC2	PC3	PC4	PC5
DFF	16.547	0.617	0.529	0.356	1.471
FF	**18.712**	0.174	0.130	0.010	1.029
FPI	**18.448**	0.088	0.637	1.076	3.569
FP	14.549	0.132	0.000	0.261	5.968
FH	17.700	0.029	0.020	0.443	0.953
FPH	2.565	0.359	13.562	2.623	**53.838**
PPP	2.291	8.316	**17.691**	2.299	0.329
LP	0.021	10.299	5.849	**25.277**	4.897
WP	0.015	7.577	0.007	**24.675**	0.231
SPP	2.609	4.160	**21.547**	0.019	**16.860**
WFGP	0.529	12.115	11.123	5.925	4.202
LS	0.003	**24.585**	0.205	6.930	0.286
WS	5.069	4.337	0.578	9.023	0.644
HSWT	0.017	**20.302**	3.511	9.510	0.442
YPP	0.926	6.909	**24.610**	11.573	5.281
No. of accession	**101**	**31**	**25**	**16**	**3**

DFI: Days of flower initiation. DFF: Days to 50 % flowering. DPI: Days to pod initiation. FH: First harvesting. DFH: Days to 50% harvesting. NOP: Number of pods/plant. PL: Pod length. PW: Pod width. SPP: Seed per plant. WFGP: Weight of five green pods. LS: Length of seeds. WS: Width of seed. HSW: 100 seed weight. YPP: Yield per plant.

Correlation matrix of quantitative traits to determine positive and negative correlation

The correlation analysis was used to evaluate desirable correlated characters since it is a basic technique to describe relationship among independent traits (Suppl. Table S5, Fig. 4). In this matrix, positive and negative correlation among different traits was recorded. Yield is a quantitative character with multiplex nature, which is controlled by many genes (major and minor) and affected by variations in the environmental effect. We recorded that yield/plant was significantly correlated with number

of pod/plant, length of pod, number of seed/pod, weight of five green pods, length of seed, width of seed and 100 seed weight. Similarly, the correlation matrix showed significant positive correlation of hundred seed weight with length of seed, width of seed, length of pod and width of pod while negative with days to 50% pods, number of pods, and number of seed/pod. Number of seed/pod showed positive correlation with all the quantitative traits except width of pods.

Assessment of Genetic Diversity by molecular markers (SSR analysis)

Genetic polymorphism on the basis of SSR primers

Out of the 20 microsatellite markers, eight (BM218, BM278, BM236, GATS91, PVBR20, PVBR25, PVBR5, and PVBR14) were found polymorphic, and they were successful in identifying the genetic diversity of all 176 accessions. These markers were able to differentiate two gene pools, the Andean and the Mesoamerican.

The reliability of microsatellite markers was determined by the presence of peaks of expected size and shape across the studied samples. Analysis of molecular diversity was performed by AMOVA, which was associated with genetic variance (Andean and Mesoamerican) among the population, among accessions, and within accessions. The proportion of molecular variation among the population was 31 % and among individuals was 69 % (Table 3). A total of 293 alleles were scored which included both the Mesoamerican and the Andean gene pools. The average allele/loci were 4.25 for the Andean gene pool ranging from two (BM218) to eight (BM278 and BM236) while the average allele /loci for Mesoamerican gene pool was 32.38 ranging from twenty-one (BM218) to fifty-six (BM278) (Table 4).

Table 3. Analysis of molecular variance (AMOVA) of 176 common bean accessions from Garhwal Himalayas.

Source	df	SS	MS	Est. Var.	Percentage of molecular variance
Among populations	1	268.580	268.580	2.214	31%
Among accessions	174	1695.548	9.745	4.871	69%
Total	175	1964.628		7.088	100%

df- degree of freedom, SS- Sum of square, MS- Mean square, Est.Var.- Estimated variation.

Table 4. Number of accessions (N), Number of Alleles (Na), Shannon's Information Index (I), Heterozygosity (Ho) and Polymorphism Information Content (PIC) analyzed by SSR markers on 176 common bean accessions.

Population	Locus	N	Na	I	Ho	PIC
Andean	BM218	32	2	0.63	0.44	0.34
	BM278	12	8	0.93	0.58	0.48
	BM236	28	8	1.10	0.67	**0.59**
	GATS91	27	2	1.10	0.67	**0.59**
	PVBR20	28	5	1.10	0.67	**0.59**
	PVBR25	38	3	0.76	0.51	0.39
	PVBR5	32	3	0.61	0.42	0.33
	PVBR14	37	3	0.58	0.39	0.32
Mean		**29.25**	**4.25**	**0.85**	**0.54**	**0.46**
SE		**2.85**	**0.88**	**0.08**	**0.04**	**0.04**
Mesoamerican	BM218	144	21	0.62	0.35	0.30
	BM278	164	56	1.05	0.63	**0.56**
	BM236	148	28	0.99	0.59	**0.53**
	GATS91	149	42	0.86	0.53	**0.45**
	PVBR20	148	39	0.84	0.48	0.43
	PVBR25	138	27	0.48	0.27	0.24
	PVBR5	144	23	0.72	0.44	0.37
	PVBR14	139	23	0.69	0.50	0.37
Mean		**146.75**	**32.38**	**0.78**	**0.47**	**0.41**
SE		**2.85**	**4.3**	**0.06**	**0.04**	**0.03**

SE-Standard error

Number of Alleles (Na), Shannon's Information Index (I), Heterozygosity (Ho) and Polymorphism Information Index (PIC) of common bean accessions

In the Andean gene pool, the I was found maximum for markers BM236, GATS91 and PVBR20 *i.e.,* 1.09 followed by BM218 (0.43), PVBR5 (0.41) and PVBR14 0.39. The marker BM278 was found to have 0.57 Index with the mean values of 0.84. The highest Index in the Mesoamerican gene pool was 1.04 for marker BM278 followed by marker BM236 (0.99) and GATS91 (0.86) while the lowest Index was found for marker PVBR5 (0.48 (Table 4).

The observed heterozygosity (Ho) was found maximum (0.66) for the markers BM236, GATS91 and PVBR20 followed by BM278 (0.57) and PVBR25 (0.51) for the Andean gene pool, while minimum Ho was found for PVBR14 (0.39) followed by PVBR5 (0.41) and BM218 (0.43). On the other hand, in Mesoamerican gene pool the Ho was found maximum for the marker BM278 *i.e.,* 0.63 while the minimum was found for PVBR25 which was 0.26 (Table 4). The PIC value was found maximum for the Andean gene pools by markers BM236, GATS91 and PVBR20 *i.e.,* 0.59 and the minimum PIC value was found in PVBR14 (0.31). For the Mesoamerican genepool the maximum PIC was found with marker BM278 (0.55) followed by BM236 (0.52) while the minimum PIC value was found for PVBR25 marker (0.23).

Population structure analysis based on SSR markers

Based on the previous studies of the genetic diversity of common bean with SSR markers, we hypothesized that the collection of common bean accessions from the Garhwal region is composed of accessions of both the Mesoamerican and the Andean gene pools and we found that there is a strong differentiation between these two gene pools (Fig. 5, Suppl. Table S6).

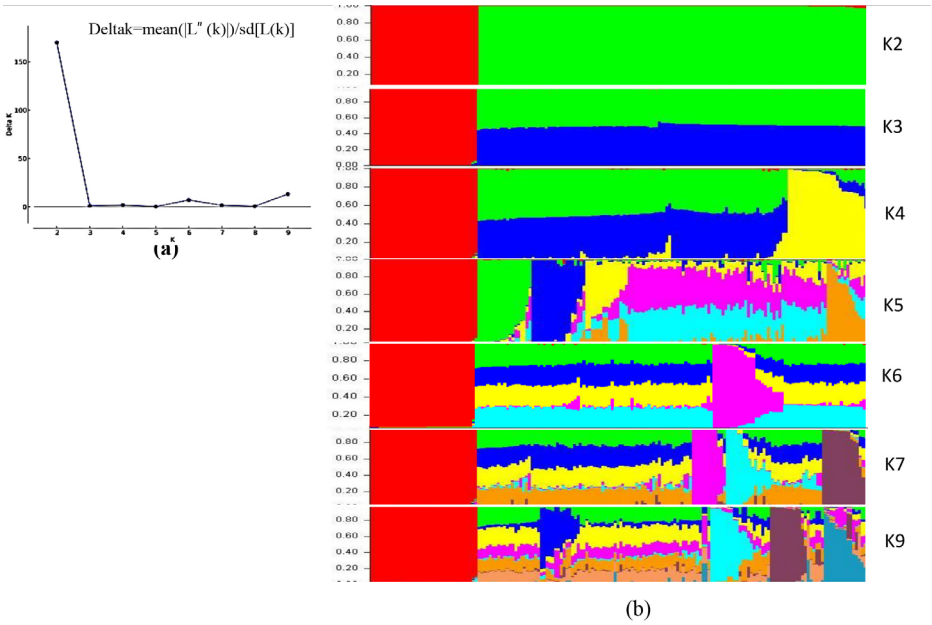

Figure 5. a. Evanno analysis results for determination the best number of clusters (DELTA K). b. Population structure analyzed for 176 bean accessions from Garhwal Himalayas and grouped at k=2, k=3, k=4, k=5, k=6, k=7 and k=9. The red group contains Andean gene pool while other Mesoamerican gene pool.

On structure analysis, at K = 2 the accessions were distributed in two groups, which clearly indicates the presence of these two major gene pools in the studied samples. Thirty nine accessions were grouped in cluster I (red colour) and 138 accessions (green colour) were grouped in cluster II. Cluster I included large-sized seeds (1.5 to 2.0 cm), which is a characteristic feature of the Andean gene pool. The green cluster consisted accessions of small seed size (<1.0 cm) and medium seed size (1.0-1.5 cm). Small seed size is characteristic of the Mesoamerican gene pool. K=3 is presenting three clusters, in which the blue and green colour clusters were classified as mixed groups *i.e.,* all the small and medium-sized seeds were mixed. K=4 is re-presenting adding up of one more colour cluster *i.e.,* yellow. In this yellow cluster, all the small seeds were classified (<1.0 cm). Cluster K=6 represents the most diminutive cluster compared to the others, encompassing small seeds with a similar flower colour. These accessions are sourced from various villages, yet share a comparable altitude ranging from 1,750 to 1,760 amsl. In other groups *viz.,* K=7, K=8, K=9, and K=10 small and medium seeds showed a mixture while the Andean seeds remain in the same cluster (Red).

Further the marker data of the individual primer was analysed for structure and it was found that the two markers BM218 (pv06) and GATS91 (pv02) were able to differentiate the two populations (Suppl. Fig S1). The population division (based on structure), NJ tree based on genetic distance and the PCoA, all revealed the significant divergence in Adean-Mesoamerican gene pool. The NJ tree subdivided the accessions into six major groups and clearly separated Andean gene pool from Mesoamerican gene pool (Fig. 6). Andean gene pool separated in red colour at the branch length of 2.301, which consist of all large seeded accessions. The majority of hybrid accessions were found in Mesoamerican gene pool, which is similar to the results of structure. Mesoamerican gene pool grouped in to five major groups ie. green, brown, blue, pink and purple. Green group separated at 6.309 branch length, blue at 9.647 and purple at 8.993 and so on. This study also helps us to quantify the duplication of accessions. Some of the accessions were found in duplication *i.e.,* GFB-4/ GFB-148, GFB-19/GFB-175, GFB-51/GFB-121, GFB-37/GFB-163, GFB-42/GFB-164, GFB-69/GFB-170, GFB-101/ GFB-173, GFB-108/GFB-174, GFB-109/ GFB-172 (Fig. 6, Suppl. Table S7).

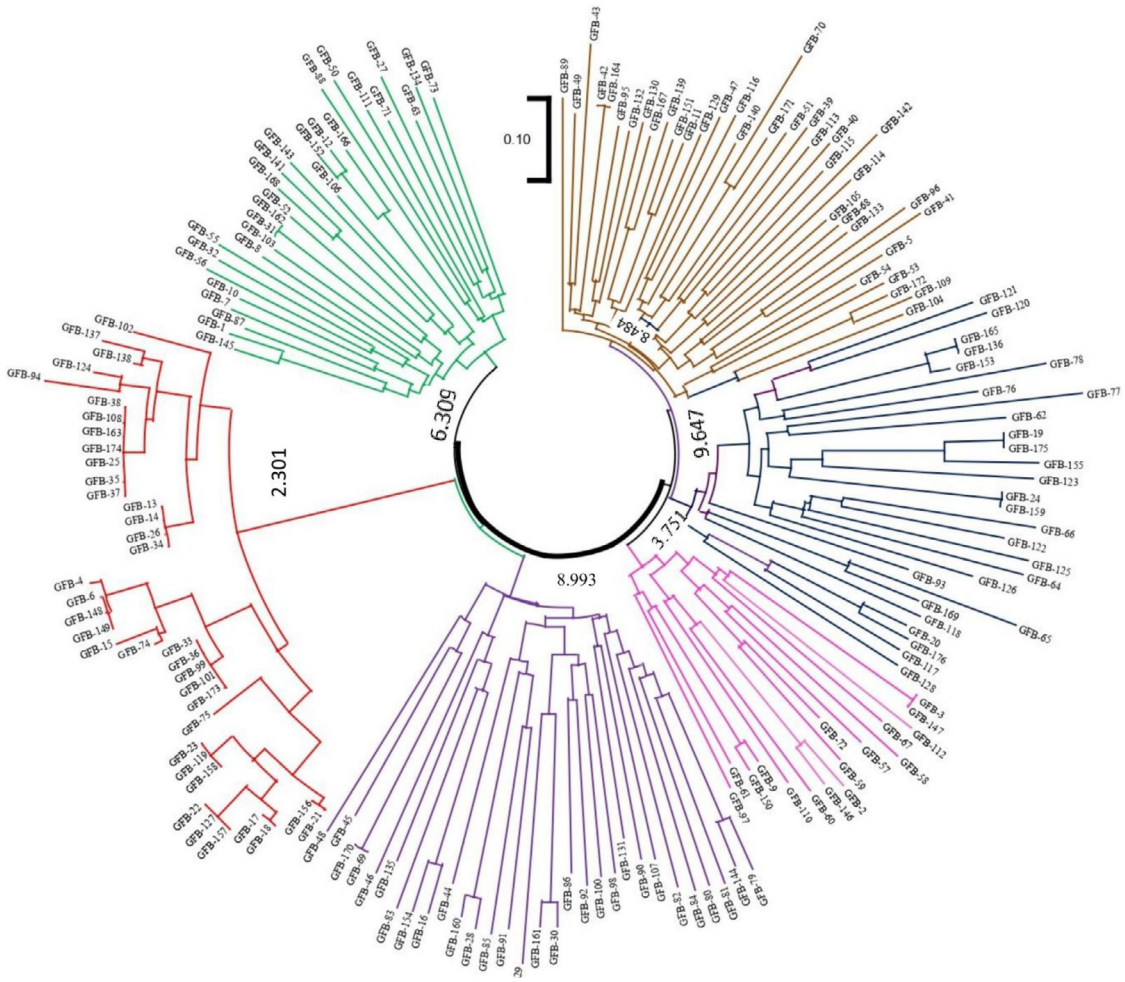

Figure 6. Neighbor-joining tree of microsatellite diversity of bean accessions from Garhwal Himalayas based on the C. S. Chord distance implemented in the Powermarker program. Branch with red color show the common bean accessions of Andean gene pool, other groups belong to the accessions of Mesoamerican gene pool. Numbers next to corresponding nodes indicate the branch lengths in the same units of the genetic distances that are computed based on the marker data for each accession.

Analysis of genetic diversity and geographic distribution (PCoA)

Principal coordinate 1 (16.82%) and 2 (23.20%) clearly separated Andean genepool from Mesoamerican genepool in two different groups (Fig. 7). The Figure 7 is showing the geographic distribution of both the gene pools which is linked with PCoA graph. The present collection represents six districts of the Garhwal region which includes accessions from lower (800 amsl) to higher altitudes (2,745 amsl). In this study we reported that the common bean accessions of Andean gene pool (Bean with red colour) belong to higher altitude while Mesoamerican gene pool (Bean in green colour) in both higher and lower altitude. The altitude range of accessions of Andean gene pool was from 2,000 amsl to 2,745 amsl.

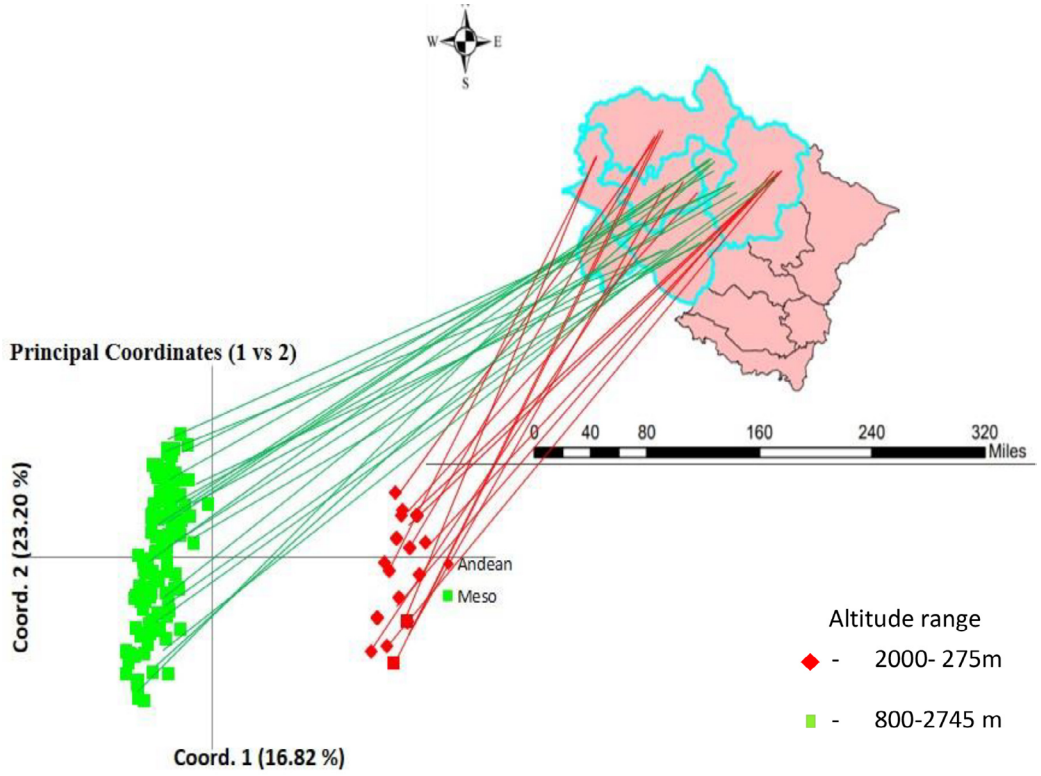

Figure 7. Geographical and genetic distribution of common bean accessions from Garhwal Himalayas. Lower left plot is the result of Principal coordinates analysis (PCoA) of all accessions. PCoA was analysed from microsatellites diversity based on the presence and absence of alleles. Two Gene pool were represented in red and green color.

Discussion

Many characteristics of Uttarakhand common bean accessions were revealed in the present investigation. Our observations confirm the presence of two major gene pools, Mesoamerican and Andean, in the Uttarakhand Garhwal Himalayas. This study however, clearly revealed that the Mesoamerican gene pool accounts for the vast majority of common bean accessions. This predominance of the latter gene pool could be attributed to the multiple introductions of Mesoamerican accessions both before and after the conquest (Gepts et al., 1988). To understand the origin and degree of variability and the genetic basis for sustainable germplasm management and utilization, there is a requirement of assessing genetic diversity in large collections (Blair et al., 2010; Szilgayi et al., 2011). The simplified morphological approach has been reported to be useful for the initial evaluation of accessions and their selection in order to understand the level of genetic diversity (Szilgayi et al., 2011).

Among various traits, seed traits were discovered to be the most important in common bean and a major determinant of commercial adoption of genotypes (Rana et al., 2014). Seed traits are also identified as high heritable qualities, making them crucial in breeding programs (Rana et al., 2010). Significant variance has been discovered in the present findings on seed colour, ranging from a single colour to mottled or speckled seeds with varying tonalities. The common bean with red, maroon, cream and yellow colours are most popular among bean consumers and producers (Rana et al., 2010; Rana et al., 2015). Similarly, Fonseca et al. (2007) also reported the preference for these seed colours from all over the world. Apart from seed colour, the variability in seed shape and size was substantially greater among the accessions (Fig. 1, 2). Three shapes were recorded in common bean germplasm *i.e.,* large (22.15 %), medium (17.04 %), and small (60.79 %). These observations are in line with the findings of other researchers who have documented a broad range in seed shape and size in bean germplasm (Cabral et al., 2010; Mavromatis et al., 2010; Lioi et al., 2012; Chamoli et al., 2021). Other than seed traits ie. seed size, shape, colour, texture etc., the growth habit of common bean ranged from bushy to pole type. Indeterminate growth ((86.36 %) in common bean was prevalent in the accessions from the Garhwal, which take more days to complete its growing cycle because these plants exhibit continuous growth throughout the growing season. They tend to grow as long as the favourable conditions persist. This ongoing vegetative growth extends the overall growth period (210-240 days). The supremacy of one type of growth habit is related to both ecological adaptability and the cropping strategy used. In the Himalayan region, the common bean is usually grown with maize and amaranth, therefore pole type common bean is preferred. It was reported from the present study that pole type beans have a longer life cycle, their yield is greater than the bush ones, and they mature later, while bush beans mature early. These findings are consistent with those of Garcia et al. (1997), who found that increasing climbing ability increases the time

required to reach physiological maturity and that crops with indeterminate growth habits are more productive than plants with determinate growth habits. According to Piergiovanni & Lioi (2010), 90 % of landraces from Italy, planted in Basilicata, had a climbing habit. These findings are consistent with those reported in bean germplasm from the Iberian Peninsula (Rodino et al., 2003), Nicaragua (Gomez et al., 2004) and Portugal (Coelho et al., 2009). Flower colour is also an important trait that separates clusters into different groups. In our study, five types of flower colours were recorded *i.e.,* pink, purple, white, yellow, and red (Fig. 1). Red flower colour belongs to the runner bean. The pink-colour flowers were found in most accessions. A similar colour combination was reported earlier by different researchers in their studies (Caproni et al., 2019; Nkhata et al., 2020).

Estimating GCV in relation to PCV also helped in determining the extent of genetic variation. Pods/plant, the weight of five green pods, LS, SW, and YPP showed high level of variance with high heritability and genetic advance in common bean landraces. Less variation was observed in genotypic and phenotypic variance in different parameters studied, which indicates that observed trait variation and expression is primarily due to genetic factors (Table 1). Accessions such as GFB-01, GFB-03, GFB-09, GFB-12, GFB-20, GFB-22, GFB-23, GFB-25, GFB-30, GFB-35, GFB-37, GFB-74, GFB-75, GFB-94, GFB-101, GFB-102, GFB-111, GFB-145, GFB-147, GFB-153, and GFB-173 showed high values for traits *i.e.,* Pods/plant, the weight of five green pods, LS, SW, YPP and can be subjected for further testing and selection for yield gains through simple selection based on these traits. Accessions GFB-14, GFB-78, GFB-87, GFB-88, GFB-99, GFB-117 and GFB-123 showed low variance, heritability, genetic advance for fifty percent flowering, days to first pod initiation, and fifty percent harvesting. This revealed the presence of non-additive genes for which hybridization would be needed to improve interactions (Rai et al., 2010; Sharma et al., 2012).

Principal component analysis was used in order to analyze the relative contributions of various components to the total divergence. The PCA has shown that the first component (PC1) contributed 28.647 %, while the second component (PC2) accounted for 16.737 % of the variation. Rana et al. (2015) also recorded in their study that the first component (PC1) accounted for 34.5 % variation and PC2 accounted for 27.5 % variation (Table 2). Similar results were reported by Cabral et al. (2010) and Nkhata et al. (2020). With PCA analysis, we have been able to measure the genetic divergence in the bean germplasm and the contribution of different characters in producing diversity in the population. The correlation coefficient among traits is found useful in plant breeding, as it quantifies the degree of genetic and non-genetic relationship between two or more traits (Dewey et al., 1959). If the two traits are highly correlated, the selection for one trait will cause a change in the mean of the other trait of selected individuals because of additive gene effects (Singh et al., 2011). The number of pods per plant was found highly correlated with the number of seeds/pod and yield while the length of the pod was highly correlated with the width of the pod, the weight of five green pods, length of the seed, 100 seed weight and yield which indicates that selection for any of these traits may favour improvement in other traits (Suppl Table S5). The data on the accessions showed that small pod sizes invariably have smaller seeds while large seeded accessions have medium to long and wide pods. In the present study, seed size (width of pod and length of the pod) was positively correlated with the different yield traits like 100 seed weight and yield/plant. Similar positive associations of seed weight with various other yield traits have been reported in bean germplasm (Sofi et al., 2011; Sofi et al., 2014). According to Blair et al. (2009) dry beans varieties that do not meet minimum seed weight criteria can be rejected by the market.

The morphological markers are limited in number and can be easily influenced by environmental factors as well as the growth stage of the plant. Molecular markers show several advantages over morphological markers *i.e.,* abundance, co-dominance, and environment-independent expression. SSR primers are frequently used to estimate the genetic diversity in many crops by scientists because of their reproducibility, multi-allelic inheritance, relatively abundant, and wide distribution across the genome (Powell et al., 1996). Therefore, SSR primers were selected for the present study that provides a complete picture of the diversity and structure in a geographically broad representative collection of common bean landraces from Uttarakhand. In the Garhwal region of Uttarakhand, more diversity in common bean was observed in the Mesoamerican gene pool. The best markers, as determined by the number of alleles, Shannon's information index, heterozygosity, and PIC were BM236, GATS91, PVBR20, and BM278. These four markers successfully separated 176 accessions based on different gene pools and were found to be highly informative in the current study. This suggested that the primers GATS91, PVBR20, BM278 and BM236 could be used to determine genetic divergence among common bean accessions and to study phylogenetic relationships in common bean accessions. Primers BM218 and GATS91were able to differentiate the two populations. This indicate that the two population have diversity on genes located on pv02 and pv06. Further screening using more markers located on these chromosomes are needed to establish this hypothesis. According to Sicard et al. (2005) the high polymorphism of microsatellite markers, as well as their broad cross-species transferability makes them useful for mapping and molecular characterization. The molecular variation among individuals was 69 % which is indicative of the existence of heterogeneity in the accessions (Table 4). The high heterozygosity within landraces is the fact that seeds of individual landraces could be mixtures of different genotypes with similar seed characteristics (Nkhata et al., 2020; Kimaro et al., 2020).

The structural analysis was performed for all 176 accessions on the basis of the banding pattern of SSR markers. The population structure analysis successfully identified two gene pools. Similar observations have been recorded by several other authors. They found that the Mesoamerican and Andean gene pools were separated at k=2 (Tigits et al., 2019; Valdisser et al., 2020). Through NJ tree, the duplication in accessions was reported. Finding two identical samples at two different places indicate that it must have been carried by local farmers or people. It is common for farmers in traditional production systems to grow 10-12

landraces together, which displaced traditional genetic diversity not only in high-cropping-intensive areas but also in traditional kidney bean growing areas (Fig. 6). It was also observed from PCoA and geographic distribution that the accessions of larger seed size or Andean gene pool, belong to higher altitudes but the small and medium seed sized accessions were found in both altitudes. The reason for this assortment can be the natural selection and adaptability of Andean beans for higher altitudes (Adesoye et al., 2012).

The identification of agronomically superior accessions will be helpful for local communities, researchers, and breeders to develop varieties that can not only adapt to environmental changes but also meet the increasing food demands of a continuously growing population. This collection represents a precious genetic heritage of common bean germplasm from the Garhwal Himalayas with great potential for future bean breeding.

Supplementary material (Tables S1, S2, S3, S4, S5, S6 and S7, and Fig. S1) accompanies the paper on SJAR's website".

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Authors' contributions: **Navneeti Chamoli:** Methodology, Experimentation, Formal analysis, Investigation, Resources, Data curation, Writing—original draft preparation. **Deepti Prabha:** Conceptualization, Methodology, Experimentation, Formal analysis, Investigation, Resources, Writing—review and editing. **Jai Singh Chauhan:** Writing—review and editing.

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