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Research Paper

Evaluation of secondary metabolite profiles in common bean (*Phaseolus vulgaris* L.) genotypes from the Western Himalayan Kashmir

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is a critical staple crop, providing essential nutrients such as proteins, vitamins, minerals, and secondary metabolites. Due to its high protein content (21-25%) and antioxidant properties, especially in beans with pigmented coats, common beans are vital in addressing nutritional deficiencies, particularly in low-income regions. However, deficiencies in these bioactive compounds can lead to metabolic and physiological issues in both humans and plants. This study aimed to enhance the nutritional and nutraceutical quality of common bean germplasm through association mapping, focusing on secondary metabolites and key nutritional traits. A diverse collection of 150 common bean genotypes from the North-Western Himalayas was genotyped using 100 polymorphic SSR markers. The association mapping identified seven SSR markers linked to antioxidants and phytic acid content, explaining 12–34% of the phenotypic variation. These markers were mapped to chromosomes 2, 4, 7 (antioxidants) and 3, 5, 6, 7 (phytic acid). The biochemical analysis showed significant variation among genotypes for traits like protein content (3.27–24.51%), antioxidants (24.19–49.88 µg/g), total phenols (0.30–8.43 mg/g), and flavonoids (2.46–16.98 mg/g). Additionally, significant correlations were found between certain biochemical traits, such as positive associations between lysine and methionine and negative correlations between phytate and antioxidants. Population structure analysis revealed three distinct subpopulations in the germplasm. The study highlights the potential of molecular breeding to improve the nutritional and nutraceutical quality of common beans, providing valuable genetic resources for crop improvement in developing regions.

Key words: Antioxidant, chromosome, common bean, molecular breeding, nutraceutical, secondary metabolite

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Common bean (*Phaseolus vulgaris* L.) is one of the important staple food legumes, and is a potential source of minerals with high content of nutrients. The significant leguminous crop for human food system is the common bean (*Phaseolus vulgaris* L.*)* (Singh *et al*., 2023). Its nutritional value which includes a high concentration of secondary metabolites, protein and minerals are the factors contributing to its success (Hayat *et al*., 2014) and it is special among plant diets from a nutraceutical perspective because they are an inexpensive source of high-quality protein (21–25%) and it is reported that beans with more intensely coloured coats (red, cream, black, pink, and brown) demonstrated the highest antioxidant capabilities. Being a great source of dietary proteins and metabolites (phenol, flavonoid, antioxidant, metionine, lysine and phytic acid) common beans have often been considered a "poor man's meat" (Nazir *et al*., 2022 ; Hayat *et al*., 2014; Yeken *et al*., 2019). Common bean is special among plant diets from a nutraceutical perspective because they are an inexpensive source of essential vitamins, minerals, soluble fiber, starch, phytochemicals along with having a low fat content (Zargar *et al*., 2017). Phenolic chemicals in beans with coloured coats were found to be more effective antioxidants than those with entirely white coats. These secondary metabolites play a pivotal role in the proper growth and development of plants and animals. It is an essential nutrient source having 65% of total protein consumed and 32% of energy for more than 300 million people of the developing countries (Blair *et al*., 2010). It is a nearly perfect food and contains protein as well as antioxidants, due to presence of these attributes with comparatively affordable prices, common bean is the food of choice for developing countries with low GDP (Zargar *et al*., 2017). The deficiency of secondary metabolites hinders the proper functioning of biological processes leading to several metabolic and physiological implications. In many low- and lower-middle income countries, especially those in Asia, Africa, and Latin America, such metabolites are the main components of hidden hunger (Katuuramu & D. N 2017) and it also cause severe yield loss in crops and metabolic disturbances in humans (Gupta *et al*., 2020). To address the secondary metabolites deficiencies, it is important to enhance these secondary metabolites in various staple food items (Wu *et al*., 2012). Enhancement of secondary metabolites in staple food crops through breeding is one of the most promising approaches. Association mapping is one of the modern breeding approaches used to identify genetic loci that determine desired traits (Kojima & Lewontin, 1970). In order to increase the nutritional and nutraceutical qualities of common bean germplasm, a method was created that makes use of association mapping analysis to find QTLs. The number of QTLs that explain variance in the phenotypic trait provides information about genetic make-up. By using association mapping, the QTLs for common bean agronomic features are found (Kamfwa *et al*., 2015).

In the present investigation, we used a diverse core set of 150 common bean genotypes representing a collected from North Himilayan region of India for mapping of QTLs contributing for the enhancement of the nutritional and nutraceutical properties. A set of 100 polymorphic Simple Sequence Repeats markers covering all the chromosomes were used for genotyping and association analysis. By using both GLM and MLM approach in biochemical parameters 7 SSR markers were found associated with Antioxidant and phytic acid and contributing 12-34 % of the phenotypic variation. The markers associated with antioxidants were found to be located on chromosome no. 2,4 and 7 where as markers associated with no. of phytic acid were found to be located on chromosome no. 7,5,6 and 3.

MATERIALS AND METHODS

Germplasm Collection: The germplasm taken under consideration for the present study comprised of one hundred and fifty genotypes (Supplementary table 1) collected mainly from North-Western Himalayas. The collected genotypes were grown at two different locations viz; SKUAST-Kashmir, Shuhama (latitude 35º 30'N, longitude 75º 15'E, altitude of 1,700 meters) and SKUAST-Kashmir, Wadura, Sopore, Baramulla (34.34º N latitude, 74.40º E, altitude of 1590 meters) during year 2022 and 2023 respectively. All the accessions were grown in RCBD with three replications and five row plots of 4 m length, with spacing of 15 cm × 30 cm to provide adequate space for each plant to grow while ensuring proper sunlight exposure and minimizing competition for nutrients. Common beans are moderately drought-tolerant but still require sufficient water for optimal growth. Irrigation was

provided based on the crop's water requirements, field were irrigated prior to sowing to ensure adequate moisture for seed germination.Genotypes were irrigated regularly and provided every 7–10 days, or more frequently if the weather was unusually dry.

Fertilization practices followed standard recommendations for common bean cultivation,well-decomposed farmyard manure (FYM) at a rate of 0.5-0.75 t/kanal was applied before planting to improve soil organic matter and enhance nutrient availability. A balanced application of fertilizers was made using Nitrogen at a rate of 5 kg/kanal, Urea at a rate of 10 kg/kanal, and Phosphorus at a rate of 3kg/kanal. This was to support vegetative growth and boost protein content.

Table 1: List of Common bean genotypes used in the study

K	Reps	Mean Ln P (K)	St. Dev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	3	-1080.13	0.15	-	-	-
2	3	-1093.80	22.42	-13.66	33.70	1.50
3	3	-1073.76	1.84	20.03	60.86	32.99
4	3	-1114.60	13.78	-40.83	58.76	4.26
5	3	-1214.20	126.92	-99.60	38.23	0.30
6	3	-1275.56	105.31	-61.36	142.73	1.35
7	3	-1194.20	93.75	81.36	172.50	1.83
8	3	-1285.33	98.42	-91.13	49.60	0.50
9	3	-1326.86	134.47	-41.53	146.90	1.09
10	3	-1221.50	110.19	105.36	-	-

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Extract Preparation : 0.5 g sample of common bean seed was obtained using a mortar and pestle. 5 ml of 80% methanol was used to homogenise the finely powdered seeds, which were then incubated at 37 °C for 72 hours (Souza *et al*., 2020). After being filtered, the extracts were kept for later study. Following protocols, measurements of phenol, flavonoids, and antioxidative activity were made.

Total phenols, flavonoids and antioxidant: The total phenolic content of common bean seed was carried out by Folin–Ciocalteu's reagent method (Baba & Malik, 2015). In brief, 50 µl of the extract was added to 2.5 ml of ddH2O followed by addition of 125 µl of Folin–Ciocalteu's Phenol Reagent followed by 25% Na2CO3. The mixture was further allowed to stand for 60 min in the dark before absorbance was measured at 700 nm. Total flavonoids content was determined by Aluminum chloride (AlCl3) method (Singleton *et al*., 1999). The antioxidant profiling of the common bean germplasm was done by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams *et al*., 1995).

Estimations of Phytate, Lysine and Methionine : The fine powder of sample has to be extracted with 2.4% HCl and estimated the lysine content by using Reichwaldv & Hatzack, 2008. method method where Sodium phytate (32 mg/ml) was used as a standard. For the estimation of lysine, 100 mg of finely ground sample, 5ml of papain was added and incubated overnight at 65°C and followed by the protocol used by Kalidass & Mohan, 2012 method. Futhermore, Horn et al., 1946 method were used in the estimation of methionine with little modification.

Protein content : The total nitrogen is estimated by micro-Kjeldhal Method as per procedure by Barbano *et al*., 1990. Protein content was calculated and expressed in percentage by applying the formula:

$$\text{Total Protein Content (\%)} = \text{Nitrogen content (\%)} \times 6.25$$

$$\text{Nitrogen content in plant (\%)} = \frac{\text{R(S-B)} \times \text{Normality} \times \text{Atomic wt. of N} \times 100}{\text{Weight of the sample} \times 1000}$$

All observations were taken in three replicates and One-way ANOVA was applied to evaluate the variance of total phenol, flavonoids, Phytate, lysine methionine, and protein among the genotype. The

Pearson's correlation coefficient between these parameters were determined by Pearson's correlation analysis using R software.

DNA isolation and SSR analysis: Genomic DNA was extracted using CTAB method by Doyle and Doyle (1990) with little modification (Doyle, 1990). About 6-8 cm young and actively growing fresh leaves of common bean (Phaseolus vulgaris) genotypes were harvested for genomic DNA extraction. The quality and quantity of genomic DNA was evaluated by Nanodrop and agarose gel electrophoresis. DNA was diluted to a concentration of 25ng/µl for use in the PCR experiment after being quantified. PCR was carried out in 96 well Universal Gradient Thermal Cycler (PEQLAB, Deutschland and Osterrtich, United Kingdom) in a 5 µl reaction mixture. 0.5 µl of template DNA (25ng/µl), 5 µM of forward and reverse primers, 1 U of Taq polymerase (D180-Sigma Aldrich, USA), 1µ l of 10X PCR buffer, 1µl of 25 M MgCl2, and 2.5 M of each dNTP (dTTPs, dGTPs, dCTPs, and dATPs) were included in the reaction mixture. The following programme was used to execute the amplification: 94°C for 4 minutes, followed by 30 cycles of 94°C for 30 seconds, 50°–55°C for 30 seconds, and 72°C for 30 seconds, with a final extension of 72°C for 12 minutes. The PCR product was resolved on PAGE.

Scoring of Bands: For the purpose of scoring alleles for each SSR locus, PCR products produced within the anticipated size range of SSR were employed. The relative mobility of each allele in a gel was used to determine their differences, and the size of each allele was measured using a 100 bp DNA ladder. Data for the appropriate genotype was considered as null allele when a PCR result was not amplified. Using Power marker software, version 3.25 (Liu & Muse, 2005), a variety of metrics were determined, including heterozygosity, major allele frequency, and allele number.

Population structure and diversity analysis: Perrier and Jacquemoud-Collet (2013) method was used to construct a phylogenetic tree using a pairwise distance matrix generated by constructing a dissimilarity matrix using a common allele index with DARwin software (Perrier & Jacquemoud, 2013). In order to create an unweighted neighbour joining tree, the estimated dissimilarity index was used. The NEI coefficient and bootstrap process of re-sampling (1000) across markers and individuals from allele frequencies were used to determine the genetic distances across accessions (Nei, 1972). Model-based methodology and the STRUCTURE version 2.3.4 software were used to evaluate population structure (Pritchard *et al*., 2000). The actual number of subpopulations denoted by K was found using Markov Chain Monte Carlo (MCMC) replications and after 150,000 burn-ins. In order to account for the possibilities of admixture and correlated allele frequency correlation, the data was run ten times for each K value between 2 and 10. Plotting the mean estimate of the log posterior probability of the data L (K) against the specified K value allowed us to identify the ideal K value. The maximum value of L (K) was used to determine the true number of subpopulations. The best ΔK value has also been clearly identified using an ad hoc quantity, K, provided by Evanno *et al*. in 2005 is based on the second order rate of change of the probability function with regard to K (Evanno *et al*., 2005). This estimate was made using Structure Harvester (Earl *et al*., 2012). When the proportion of a genotype's genome in a specific cluster (qK) was higher than a typical cutoff value of 50%, the genotypes were assigned to that cluster. According to Wright *et al*., 2011 the STRUCTURE programme was also used to estimate the degree of genetic divergence, or the wright fixation index of F statistics (Fst), simultaneously between the several bean subpopulations (Wright *et al*., 2011).

Association analysis: In the present study, TASSEL 3.0 was used to identify markers associated with biochemical traits (Total Phenol & Flavonoids, Lysine, Methionine, Phytic Acid, Antioxidants, Protein Estimation). Genotypic microsatellite data analysis and mean content data were considered to be associated with the traits if the markers are significant ($P<0.05$).

RESULTS AND DISCUSSION

Biochemical analysis: The data pertaining to biochemical parameters is presented in the Supplementary table 2 of one year and depicts statistically significant differences among genotypes for total protein content (%), antioxidant, total phenol, flavonoid, phytic acid, methionine, Lysine

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from common bean. Total protein content ranged from 3.27 to 24.51 %, with highest being in genotype WB1664 and the lowest in genotype WB102 with mean value of 13.89%. Antioxidant property in common bean ranged from 24.19 to 49.88 µg/g where in highest content was found in genotype CBZ60 and the lowest in genotype WB634 having an average value of 37.03µg/g. The CBZ19 genotype had the lowest content of total phenols and CBZ60 had the highest which lying in the range of 0.30 to 8.43 mg/g with mean of 4.17mg/g. Additionally, genotypes CBZ14 and WB1282 displayed the lowest flavonoid content reporting 2.46mg/g, while genotype CBZ60 displayed the higest content i.e, 16.98 mg/g and the final average value is 9.29mg/g. The range of phytic acid was 0.59 to 4.84 µg/g, with CBZ24 having the lowest and WB1006 the most being reported with an average of 2.71µg/g. Genotype CBZ2 recorded the poor content of lysine having 4.93 µg/g, while as CBZ64 recorded the higest content of lysine having 17.89 µg/g and their average value were recorded as 11.41µg/g. The methionine content were recorded with lowest content in the genotype CBZ93 having 1.06 µg/g and higest in CBZ95 having 2.70 µg/g with an average value of 2.41µg/g however, in our study, we did not find similar accumulation for all genotypes which were depicted in supplementary table 1.

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ANNOVA for the analysed elements: R software was used to calculate ANNOVA for analysed elements in 150 genotypes of common bean. There were significant differences among the genotypes for phenol, flavonoids, antioxidants phytic acid, Lysine, Methionine and protein ($p<0.01$) content of the seeds (Table 1).

Table 1: Analysis of Variance (ANOVA) table for analysed elements of common bean

Source of Variation	Total Phenols (mg GAE/g)			total Flavonoids (mg QE/g)			Phytic Acids (µg PphyE/ml)			Antioxidants (µg/g)		
	df	ms	F.Ratio	df	ms	F.Ratio	Df	ms	F.Ratio	df	ms	F.Ratio
Replication	2	0.04	8.67**	2	0.13	1.98**	2	0.04	6.27**	2	1.82	5.57***
Treatment	149	6.97	318.63***	149	23.19	333.01***	149	1.68	263.43***	149	87.57	267.69***
Error	297	0.00		298	0.06		298	0.06		298	0.32	
Source of Variation	**Lysine (µg LE/g)**			**Methionine (µg M/g)**			**Protein (%age)**		
	df	ms	F. Ratio	df	ms	F. Ratio	Df	ms	F.Ratio
Replication	2	0.27	0.66	2	0.14	8.42***	2	0.97	19.87***
Treatment	149	12.43	29.7046***	149	0.31	18.05***	149	38.66	791.95***
Error	297	0.41		298	0.179		298	0.04	

Df-degree of freedom; SS-Sum of square; MS-Mean squares; F.Ratio-MS/MSError; Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation analysis for nutritional and nutraceutical traits: The Pearson correlation analysis (using R software version 4.3.1) on the mean of 150 diverse genotypes which indicated that Significant positive correlation was found between (Lysine and Methionine), (Phytate and Protein %age), (Phytate and Flavonoids), (Phenol and Flavonoid) and significant negative correlation was found between (Phytate and Methionine), (Phytate and Antioxidant), (Flavonoids and Methionine) (Fig.1).

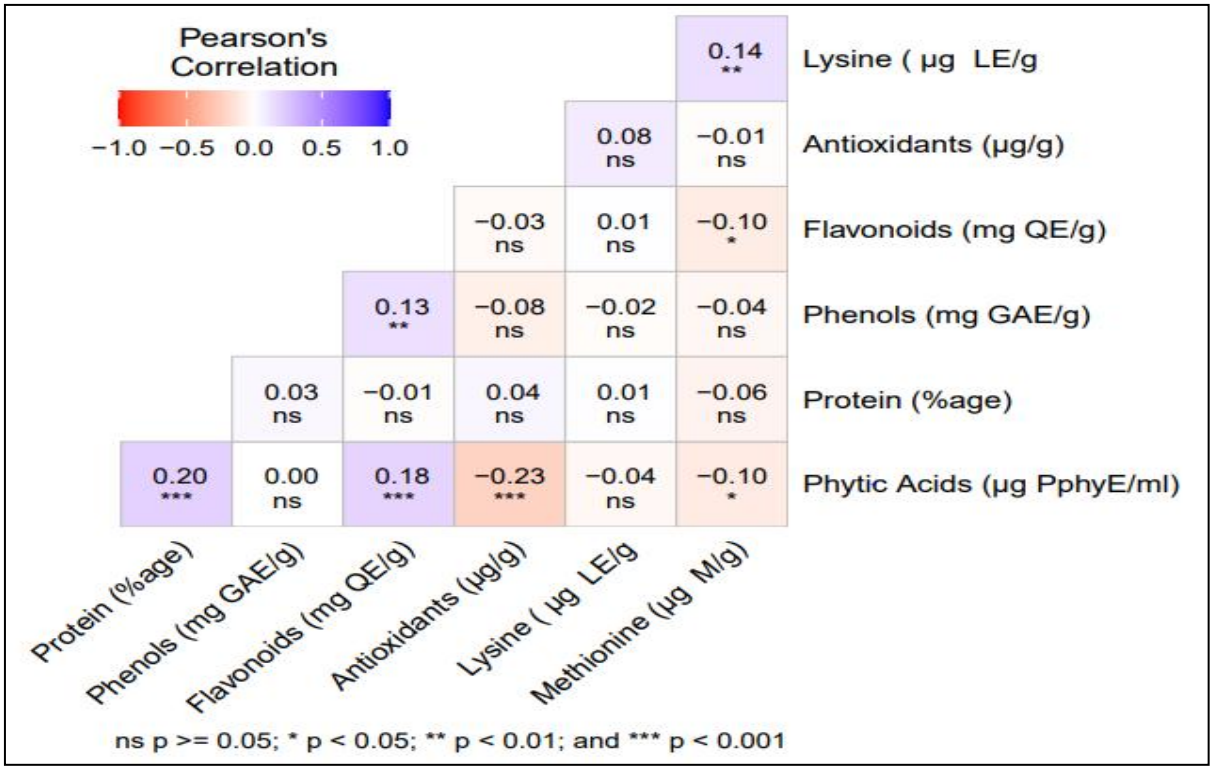


Fig. 1: Correlation between various nutritional elements of common bean

Population Diversity and Structure Analysis: Levels of polymorphism and discriminating power of SSR markers was determined using power marker (Liu and Muse, 2005) and SSR marker system proved to be highly effective in discriminating the 150 genotypes of common bean. A total of 534 alleles ranging from 4 (79722) and (Bmd8) to 19 (BM159) were amplified with an average of 11 alleles per SSR (Table 2).

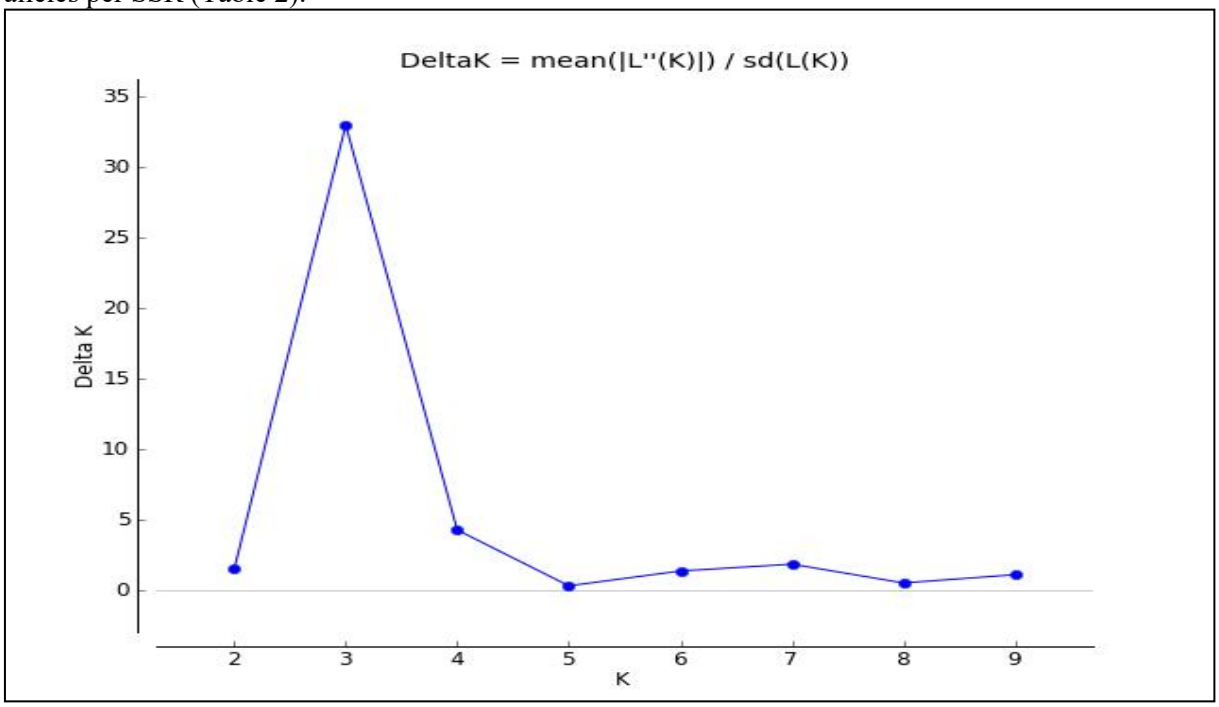


Fig. 2: Graphical representation of optimal number of groups in the program STRUCTURE Harvester inferred using the criterion of Evanno *et al*. (2005). The analysis was based on data obtained from 50 SSR markers loci in common bean collection

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Table 2. Various discriminatory parameters of 50 highly polymorphic (out of 100) SSR markers

S. No.	Marker	Major Allele Frequency	Genotype No	Allele No	Gene Diversity	PIC
01	BM159	0.20	17.00	19.00	0.90	0.89
02	BM151	0.20	13.00	13.00	0.86	0.85
03	PVBR182	0.43	10.00	10.00	0.75	0.73
04	BM185	0.30	10.00	10.00	0.78	0.75
05	PVBR113	0.36	13.00	13.00	0.78	0.75
06	BM156	0.39	12.00	12.00	0.77	0.74
07	PVBR93	0.17	18.00	18.00	0.89	0.88
08	U77935	0.29	16.00	16.00	0.83	0.81
09	BM153	0.33	12.00	12.00	0.80	0.78
10	PVBR213	0.34	8.00	8.00	0.76	0.73
11	BM157	0.19	16.00	16.00	0.86	0.84
12	X57022	0.32	11.00	11.00	0.76	0.72
13	BM154	0.31	13.00	13.00	0.82	0.80
14	BM150	0.29	15.00	15.00	0.84	0.82
15	J04555	0.35	7.000	7.00	0.78	0.75
16	BM149	0.31	11.00	11.00	0.80	0.77
17	BMb654	0.37	11.00	11.00	0.78	0.75
18	PVBR185	0.48	7.00	7.00	0.68	0.63
19	X96999	0.35	8.00	8.00	0.78	0.75
20	X04660	0.39	13.00	13.00	0.78	0.75
21	Bmb152	0.35	10.00	10.00	0.79	0.76
22	Bmb742	0.37	12.00	12.00	0.77	0.74
23	BMC292	0.32	12.00	12.00	0.80	0.77
24	BM200	0.28	15.00	15.00	0.85	0.83
25	X59469	0.32	14.00	14.00	0.79	0.76
26	X74919	0.26	13.00	13.00	0.83	0.81
27	P1v111	0.25	11.00	11.00	0.85	0.84
28	Pvbr87	0.28	9.00	9.00	0.79	0.76
29	X60000	0.18	17.00	17.00	0.87	0.86
30	PVBR5	0.29	13.00	13.00	0.84	0.82
31	BM152	0.38	8.00	9.00	0.72	0.67
32	BMc234	0.24	9.00	9.00	0.82	0.80
33	K03289	0.38	7.00	7.00	0.72	0.68
34	BMC121	0.42	8.00	8.00	0.73	0.70
35	PVBR20	0.36	7.00	7.00	0.73	0.68
36	PVBR69	0.56	9.00	9.00	0.59	0.53
37	BMb793	0.31	10.00	10.00	0.77	0.73
38	BMb115	0.24	12.00	12.00	0.84	0.82
39	BMb152	0.50	5.00	5.00	0.62	0.56
40	X79722	0.42	4.00	4.00	0.70	0.65
41	PVM097	0.24	11.00	11.00	0.83	0.81
42	BM158	0.42	6.00	6.00	0.73	0.70
43	M99497	0.38	12.00	12.00	0.71	0.66
44	Bmd45	0.24	9.00	9.00	0.81	0.78
45	BM172	0.36	6.00	6.00	0.74	0.70
46	BM164	0.30	13.00	13.00	0.82	0.80
47	BM210	0.36	5.00	5.00	0.73	0.68
48	BM161	0.31	10.00	10.00	0.81	0.79
49	Bmd12	0.34	9.00	9.00	0.76	0.72
50	Bmd8	0.40	4.00	4.00	0.70	0.65
Mean		**0.33**	**10.62**	**10.68**	**0.78**	**0.75**

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PIC (Polymorphism Information Content) value varied from 0.5325 (PVBR69) to 0.8926 (BM159) with an average of 0.7553. Gene diversity predicts the likelihood as it is the probability that two randomly chosen alleles from the population are different. For this population, the value varied from 0.628 (BMB152) to 0.900 (BM159) with an average of 0.784. The major allele frequency ranged from 0.173 (PVBR93) to 0.560 (PVBR69) with an average of 0.331 for all SSR markers. Only 4% of SSR markers were found to have value of more than 0.50 which means that the major allele in most of the markers is not shared by most of the common bean germplasm used in our study. Structure analysis was carried out to observe the number of populations in the common bean germplasm. The Evanno test found a maximum of delta K at 3 in the plots of L (K) versus delta (Fig. 2).

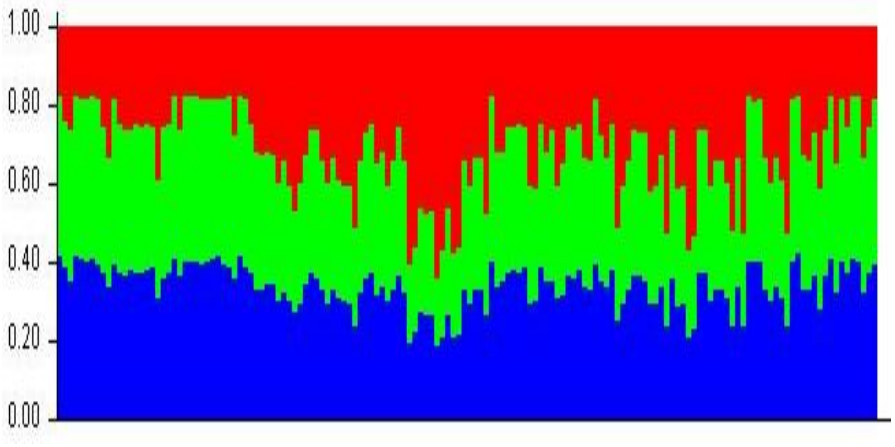
Plots of rate of change of ln(K) against the likely subpopulation (K) generated according to Evanno *et al*., (2005) with subpopulation in common bean germplasm with three subpopulation (K=3). It is generated by STRUCTURE) confirming a likely assignment of the bean germplasm to three sub-groups. The individual membership coefficient at K = 3 from the STRUCTURE run had maximum mean probability of likelihood value of L (K) = -1073.7667 (Supplementary table 3) which led to assignment of common bean germplasm to three (K=3) sub population. The analysis was based on data obtained from 50 highly polymorphic SSR markers loci in common bean collection. Bar plot of individual ancestry proportion for genetic clusters inferred using K=3 and data set of 150 individuals and 50 highly polymorphic SSR markers. Vertical bars represent the individuals of population, Red, Green and blue indicates sub population I, II and III respectively (Fig. 3).

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Supplementary Table 3: Mean LnP (K) and Delta K values of assumed sub-population (K=2 to 10) during STRUCTURE analysis in 150 common bean genotypes

K	Reps	Mean LnP(K)	St. Dev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	3	-1080.13	0.15	-	-	-
2	3	-1093.80	22.42	-13.66	33.70	1.50
3	3	-1073.76	1.84	20.03	60.86	32.99
4	3	-1114.60	13.78	-40.83	58.76	4.26
5	3	-1214.20	126.92	-99.60	38.23	0.30
6	3	-1275.56	105.31	-61.36	142.73	1.35
7	3	-1194.20	93.75	81.36	172.50	1.83
8	3	-1285.33	98.42	-91.13	49.60	0.50
9	3	-1326.86	134.47	-41.53	146.90	1.09
10	3	-1221.50	110.19	105.36	-	-

Fig. 3: Structure of common bean germplasm using STRUCTURE software: Bar plot of individual ancestry proportion for genetic clusters inferred using K=3 and data set of 150 individuals and 50 SSR markers. Vertical bars represents the individuals of population, Red, Green and blue indicates sub population I,II and III respectively.



Association Mapping Analysis: In this present study, TASSEL 3.0 was used to identify markers associated with biochemical traits in common bean (Total Phenol & Flavonoids, Lysine, Methionine, Phytic Acid, Antioxidants, Protein Estimation) in 150

genotypes of common bean. Using GLM and MLM approach seven SSR markers were found associated with Antioxidant and phytic acid parameters and contributing 12-34 % of the variation (Table 3). Out of seven SSR markers, three marker (PVBR93,BM156 and X6000) were associated with antioxidant with R^2 values of 0.34,0.30 and 0.23, four markers (PVBR93, X74919, PVBR20, BM172) were associated with phytic acids with R^2 value of 0.32,0.22,0.28 and 0.12. The markers associated with antioxidants were found to be located on chromosome number 2,4 and 7 where as markers associated with number of phytic acid were found to be located on chromosome number 7,5,6 and 3.

Table 3. Marker Trait association with p value and marker trait regression

Trait	Trait ID	Marker	Chromosome No.	P Value	Marker R_2
Antioxidant	A1	BM156	2	0.001	0.34
Antioxidant	A2	PVBR93	7	0.003	0.30
Antioxidant	A3	X60000	4	0.005	0.23
PhyticAcid	PA1	PVBR93	7	0.047	0.32
PhyticAcid	PA2	X74919	5	0.016	0.22
PhyticAcid	PA3	PVBR20	6	0.003	0.28
PhyticAcid	PA4	BM172	3	0.047	0.12

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In the present study, the total phenolic contents of seeds were range in 0.30 to 8.43 mg GAE/g (supplementary table 2) whereas according to Carbas *et al*., 2020 reported that common bean contained total phenols content ranged from 0.11 to 4.59 mg GAE/g and 0.43 to 3.84 mg GAE/g, besides, according to Rodríguez *et al*., 2021 reported that total phenolic content of the common bean in the range of 350 (line SDP221; colour: white) and 3894 µg GAE/g (SDP061; cream). The total flavonoid content of seeds bean was 16.98 mg QE/g (supplementary table 2) and was higher than the previously reported by Ha *et al*., 2020 for selected common bean 9.29 mg RE/g. The difference in the total contents of phenolic acid and flavonoid may be due to differences in the geographical region, environmental, climatic condition, and storage, and processing methods. Earlier studies on polyphenols and antioxidants in common bean revealed that the samples with high flavonoids and high phenol content show highest antioxidant capacity (Carbas *et al*., 2020). Same effect was reported in our study, genotypes with high phenol and flavonoids content showed highest antioxidant effect by DPPH test. However, the difference in present and earlier studies might be due to the multitude of test systems, different standards used for studying antioxidant activities, different sample preparation and solvent used for conducting the studies. Further, the seeds were analyzed for their protein content and a wide variation was observed ranging from 3.27 to 24.51% with an average of 13.89%. Highest protein content was observed in WB1664 (24.51 %) whereas genotype WB102 had the least protein content of 3.27%. Supplementary table 2 represents the mean values of protein content observed in the seeds of 150 diverse common bean genotypes. Similar results have been reported in previous studies, although variation may be influenced by environmental factors, geographical location and growing season. In earlier reports, protein content in the range of 7.2 to 31.6% with an average of 18.83% (Mahajan *et al*., 2017) 17.4–29% (Durigan *et al*., 1987) has been recorded. Protein content of 36 North American bean cultivars was evaluated and content ranged from 19.6 to 32.2% (Koehler *et al*., 1987). The range of phytic acid was 0.59 to 4.84 µg/g, with CBZ24 having the lowest and WB1006 the most with an average of 2.71µg/g which is in accordance with the reports of Akond *et al*., 2011. Genotype CBZ2 recorded the poor content of lysine having 4.93 µg/g, while as CBZ64 recorded the highest content of lysine having 17.89 µg/g and their average value were recorded as 11.41µg/g. The methionine content was recorded with lowest content in the genotype CBZ93 having 1.06 µg/g and highest in CBZ95 having 2.70 µg/g with an average value of 2.41µg/g and the results were accordance with earlier studies (Flores *et al*., 2020 Marquez *et al*., 1996).

Molecular markers help us to understand the level of genetic diversity that exists among traditional races, varieties and exotic accession that has been well exploited in different crops. The genetic architecture of diverse germplasm lines can be precisely estimated by assessing the structure of the population using SSRs as molecular markers (Varshney *et al*., 2007). In the present investigation, the genetic diversity among the common bean accessions was carried out by model-based clustering approach using SSR genotypic data. Population structure constructed on 150 genotypes divides the germplasm into three sub population (K=3). Association mapping is an efficient tool for identifying genes regulating complex traits. Although mapping using genomic SSR markers has been successfully demonstrated in many agronomically important crops, very few reports are available on marker trait association analysis in common bean. The common bean germplasm collected from north-western Himalayan regions were analyzed for genomic regions associated with nutritional and nutraceutical traits using 50 highly polymorphic SSR (out of 100) markers and TASSEL 3.0. Using both GLM and MLM approach, seven SSR marker loci were identified associated with antioxidant and phytic acids. In another similar study conducted by Mahajan *et al*. (2017) association mapping was done to identify markers associated with minerals and protein contents. Using general Linear Model (GLM), thirteen SSR markers were identified associated with Fe, Zn and protein content at the $p<0.05$ probability level, and contributing 12-33% of the phenotypic variation. Both studies were conducted on Indian common bean germplasm and would therefore act as an important genetic resource for improving nutritional and nutraceutical quality of common bean varieties through molecular breeding and provide an opportunity to the breeders in selecting markers for downstream experimentation towards crop improvement in common bean and related legumes.

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CONCLUSION

In the present investigation, we used a diverse core set of 150 common bean genotypes representing a collection from North Himilayan region of India to identify the markers associated with the traits like phenol, flavonoid antioxidant, protein phytic acid and methionine. In the experimental field, the varied core set of common bean genotypes from Jammu & Kashmir was grown at two separate locations. For the purposes of genotyping and association analysis, a set of 100 SSRs encompassing every chromosome was employed. With an average of 5.34 alleles per SSR, a total of 534 alleles were amplified. With an average of 0.7553, the PIC (Polymorphism Information Content) value ranged from 0.5325 to 0.8926. Seven SSR marker loci were identified associated with antioxidant and phytic acids using the MLM and GLM approaches. These markers account for 14–48% of the diversity in phenotypic traits. It was discovered that BM156, PVBR93 and X60000 were associated with antioxidant and PVBR20, X74919, PVBR93 and BM172 were associated with phytic acid shared by the two methods (MLM and GLM).

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