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GENETIC AND NUTRITIONAL PROFILING OF COMMON BEAN (*PHASEOLUS VULGARIS* L) GERMPLASM FROM AZAD JAMMU AND KASHMIR AND EXOTIC ACCESSIONS

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an important legume around the globe and major crop in Jammu and Kashmir after maize and wheat. Despite of its consumption and importance, the farmers are deprived of any improved cultivar and very little attention has been given to the crop for genetic improvement in Pakistan. The exploitation, utilization and conservation of indigenous gene pool and its comparison with exotic germplasm have a very significant role for sustainable nutritional requirements of the mountain communities. The most important bean growing areas of Azad Jammu and Kashmir were selected for germplasm collection. Protein fingerprinting through SDS-PAGE along with nutritional and morphological profiling of the collected germplasm and it comparison with the exotic accessions was performed. Among the seed characteristics, seed coat color and seed shape were found the most diverse attributes while all studied morphological characters showed highly significant variability with accession Lipa-1 as most diverse among all. Similarly evidences from biochemical profiling revealed significant variation with highest protein contents in Neelum-1, i.e. 26%. The accession Lipa-4 showed less cooking time i.e. 79 minutes. Significant molecular diversity on the basis of seed storage protein was evaluated using SDS-PAGE. Similarly the accession Rawalakot has been found rich in protein content, promising in yield and maturity time hence is recommended for general cultivation. Genetically diverse pattern in common bean accessions provided a base for comprehensive breeding program in Pakistan and AJK in future and recommended local accession will help in diversification of conventional agriculture system.

Key words: Biochemical Profiling, Exotic, Germplasm, Indigenous, Genetic Diversity, SDS-PAGE, Significant.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the ten most important crops around the globe due to its high nutrient values against alarming trend of malnourishment (Amanullah *et al.,* 2006). It is traditionally a basic food crop consumed dually as pods and grains and is a source of inexpensive protein for rural and urban masses in many developing countries including Pakistan (Amanulla *et al*., 2006; Romero *et al*., 2013). In Azad Kashmir, common bean is considered as staple food other than cereals. It is a major crop of District Neelum, Muzaffarabad, Bagh and Poonch in Azad Kashmir after maize, rice and wheat (Danish *et al*., 2002; Qureshi *et al*., 2002; Khan *et al*., 2012). It is potentially declared as nearly perfect diet as it contains low fat/high protein contents, dietary fibers and complex carbohydrates (Broughton *et al*., 2003; Słupski and Lisiewska, 2013). Beside the caloric source it also provides minerals, fibers, thiamine, folate, and phytochemicals with neuro-protective and analgesic properties while anti nutrients like phytic acid and polyphenols were targeted to improve its nutritional and health related aspects (Jha *et al.,* 2015; Clemen, 2014; Petry *et al.,* 2015). Common bean is not only fulfilling the malnutrition requirements but it is also defensive against various diseases including cardiac disease, cancer, osteoporosis, obesity and diabetes (Bazzano *et al*., 2001; Hangen and Bennick, 2002). Although, Graham and Ranalli (1997) reported significant nutritional and health values but the crop was seriously neglected in some pockets of the world.

Khan *et al*. (2012) declared the mountain areas of Azad Jammu and Kashmir and Northern Areas, rich in incredible natural resources including expedient and unexplored gene pool. Same is the case with common beans (*Phaseolus* specie) within the area having enormous unexploited germplasm. It has not been given attention and is confined only to the mountain regions and subtropical zones of the country (Amanullah *et al.,* 2006). Lower yield problem among the farmers has been reported by Graham and Ranalli (1997) and Beebe *et al.* (2013) due to disease, drought stress, insects/pests, non-availability of improved cultivars, ozone stress and mineral deficiencies. Moreover, due to unavailability of a defined variety of common bean in Azad Kashmir, the farmers rely on the seed conserved by them. The prospects of crop are very bright in Pakistan if comprehensive and integrated efforts to improve the

existing germplasm are made. Its exploitation and cultivation will not only reduce the genetic erosion but will also lead to diversification of the conventional agricultural system that can become a profitable venture for poor farmers (Amanullah *et al.,* 2006). The importance of genepool diversity is very well established fact now for increasing the genetic potential of crops (Lin, 2011)

Genetic diversity on the basis of protein profiling was reportedly characterized in the bean genotypes using SDS-PAGE analysis by Perriera *et al* (2009) and Ismat and Yasar (2011). Characterization on the basis of phaseolin protein along with some molecular markers and microsatellites explained the major basis of diversity evaluation and phylogenetic relationship within the species. While molecular diversity, on the basis of seed size, using SSR markers revealed significant genomic variation among common bean germplasm and was reported by Blair *et al*. (2009) and Roderiguez (2015). Breeding programs and harnessing of natural diversity along with advanced biotechnological innovations added more information about some specific traits, their improvement and adaptations to different environments to meet global food security issues (Norma *et al*., 2016). But neither the germplasm resources of crop nor its diversity and nutritional aspects have been investigated in Azad Kashmir. The main objective of the research was collection of some landraces of common beans from diverse pockets of Azad Kashmir and their diversity evaluation along with some exotic accessions by screening on the basis of seed attributes, morphological performance, biochemical compounds and seed storage protein.

MATERIALS AND METHODS

Plant material: A survey was conducted to collect the representative germplasm comprised of eight landraces of common bean (*Phaseolus vulgaris* L.) from diverse altitudes of Azad Jammu and Kashmir including District Neelum, Lipa valley, Forward Kahota and Rawalakot (Fig. 1). This germplasm was used in the present research for comparison with fourteen accessions from International Centre for Tropical Agriculture (CIAT) obtained from the gene bank of Plant Genetic Resources Institute (PGRI), Islamabad, Pakistan as shown in (Table 1). Total twenty two bean types were sown under the agro climatic conditions of Rawalakot, Azad Jammu and Kashmir in Randomized Complete Block Design (RCBD) with three replications. All the accessions were provided with recommended agronomic practices.

Data Collection and Analysis

Seed attributes: Seeds of twenty two (22) accessions were analyzed by using descriptor of Biodiversity International for Seed Characteristics. The data was collected for seed shape as round, oval, cuboid and kidney; seed coat color; presence and absence of spots, spots color; spots pattern as constant mottled, striped, rhomboid spotted and speckled; seeds quantitative color as white, yellow pale yellow and cream; hilum as prominent and non prominent and brilliance of seeds as matt, medium and shiny.

Morphological attributes: The data for ten quantitative traits was recorded from each plot of RCBD for randomly selected plants of each ecotype. Date of seedling emergence (50% germination), date of flowering (50% flowers bloomed), date of seed maturity (90% maturity) were recorded by single value for each ecotype while pods/plant in cm from ten plants and leaf area in cm^2 were analyzed from leaves of ten randomly selected plants of each type. Chlorophyll contents were recorded in (mg/cm^2) in three replications. Pods length and seeds per pod were recorded on ten pods sampled randomly within each type from each plot. The 100-seed weight was recorded in grams as described earlier using descriptor of Bidiversity International for Common beans.

Chlorophyll contents: Chlorophyll contents were determined by Lichtenthaler and Wellburn (1985) method from green leaves by using ethanol as solvent by photo spectrometer at 645nm and 663nm. Total chlorophyll contents were determined in mg/cm^2 by using the formula:

$$8.02(\text{OD at } 645\text{nm} + \text{OD at } 663\text{nm})$$

Biochemical Attributes

Moisture contents: Dry seeds were ground and moisture contents were determined by taking 2g sample of each ecotype and drying in the hot air oven provided with openings for ventilation. Samples were maintained at 130ºC for 60 minutes. The loss in sample weight was expressed as percentage moisture (AOAC, Method no.925.10).

$$\text{Moisture \%age} = \frac{\text{Wt. of samples and dish after moisture loss - initial wt. of dish}}{\text{Wt. of sample}} \times 100$$

Ash contents: Washed and clean crucibles were dried properly in hot air oven at 105 ºC for 1hour and cooled in desiccator and weighed as W_1. Then 2g of sample in each crucible was weighed as W_2. Samples were subjected to 550 ºC overnight, crucibles were cooled by placing in desicators and weighed as W_3 (AOAC Method no. 923.03).

$$\text{Ash percentage} = \frac{\text{Weight of ash}}{\text{Sample weight}} \times 100 = \frac{(W_3 - W_1)}{W_2} \times 100$$

Protein Assay: Protein was analyzed by using AutoKjeldahl Analyzer and Digestion system with scrubber. The samples 0.5g was digested with 10ml

H_2SO_4 in the presences of catalyst, 2 kjeltabs per tube. All organic nitrogenous compounds were converted into ammonium sulphate. Automatic distillation and titration included addition of strong alkali (NaOH) and boiling which converted ammonium sulphate to ammonium, which was distilled as NH_4OH and this amount was determined by titrating it with known normality of H_2SO_4. The percentage of nitrogen was converted to protein by multiplying it with nitrogenous factor, which was different for various kind of food (Buchi-Autokjeldahl Manual).

Percent protein = %N × Protein factor
Protein factor for wheat flour = 5.7
Others = 6.25

Crude fat: Crude fat present in sample was extracted by Buchi extraction system by n-hexane at its boiling point. Samples flour 3g of each ecotype was weighed in duplicate and put in the thimbles. After turning on the reticulating chiller and completion of the whole cycle the solvent was evaporated and fat content was determined by weighing the beaker (AOAC Method no. 923.03).

$$\text{Crude fat \%age} = \frac{\text{Weight of beaker after fat extraction - Weight of empty beaker}}{\text{Weight of sample}} \times 100$$

Crude fiber: An indigestible organic residue was determined by crude fiber digestion apparatus and Fibertech filtration unit. Seed flour 2-3g as sample of each accession was weighed in a berzilius beaker and 200ml. H_2SO_4 was added and boiled for 30 minutes. After removing from heat 10 ml of NaOH was added and boiled for an additional 30 minutes. Crude fiber was separated by filtering through the filtration unit and residues were washed with hot water to remove excess alkali. Crucible were dried and cooled and weighed as W_1. Residues were ignited at 550°C overnight and cooled and weighed as W_2 (William and Starkey, 1982).

$$\text{Fiber \%age} = \frac{\text{Wt. of crucibles and sample - Wt of crucible and sample after ignition}}{\text{Weight of sample}} \times 100$$

Carbohydrate contents: Carbohydrate contents were measured by difference method:
Carbohydrate % = 100 - % moisture + % ash + % protein + % fats + % fibers

Total energy: Total energy was measured by using following formula.
Total energy = (% proteins × 4) + (% fats × 9) + (% carbohydrates × 4)

Hydration capacity and swelling capacity: Hydration capacity and swelling capacity were analyzed by method described by Sooraj and Khetarpal (1993) using 5g dry seeds in 50ml of distilled water. The seeds of common bean were cleaned and weighed (5g) and counted accurately. The seed were transferred to the cylinder containing 5ml water and kept over night at room temperature. After 24 hours seeds were drained by removing upper water with filter paper and swollen seeds were reweighed to calculate hydration capacity as:
Hydration capacity/seed = wt. of soaked seed – wt. of seeds before soaking / no. of seeds.
Hydration index = hydration capacity per seed / wt. of one seed (g)
Swelling Capacity = volume after soaking - volume before soaking/ no. of seeds
Swelling index =swelling capacity per seed/ volume of one seed (ml)

Cooking time: Approximately 10g of seeds were taken in beakers fitted with condensers to avoid evaporation during boiling. Water was added in the ratio 1:4 (w/v). Samples were stirred at 2 min intervals. After 45 min, one seed was withdrawn without interrupting the boiling. 'Degree of cooking' was tested by pressing the seed between index finger and thumb.

Seed storage protein analysis: Molecular weight of the dissociated polypeptides were determined by using molecular weight protein standards “MW-SDS-70 kit” (Sigma, USA). The SDS-PAGE for total seed protein was carried out in the discontinuous buffer system according to Laemmli (1970). After staining and destaining data was scored as (0) and (1) for absence and presence of bands in white light.

Statistical analysis: Analysis of Variance (ANOVA), mean squares with coefficient of variance and least significant difference (LSD) values for morphological attributes were evaluated using Statistix 8.1. While diversity on the basis of morphological, biochemical and seed storage protein was analyzed through tree diagram using Wards method in window based computer software XLSTAT.

RESULTS AND DISCUSSIONS

Diversity in seeds attributes: The representative gene pool from AJK and gene bank of PGRI of the crop revealed striking diversity on the basis of eight seed attributes and morphological pattern (Table 2). Seeds were categorized in four distinct groups for their shape as cuboid, kidney, round and oval. All bean types were found incredibly diverse in seed coat color as white, maroon red, pale cream, brown, black, yellow and orange colors of seeds coat were found in all accessions. Presence and absence of seed spots were evaluated and only four types were found with spots on their seeds. Rawalakot beans carried cream speckled spots on red coat color while black constant mottled spots were observed on pale cream colored Lipa-4. Similarly Bravo and Ducato were found with speckled dark orange

colored pattern on orange seed coat. Seed coat pattern was absent in all other accessions. Seed brilliance was diverse characteristic with shiny, medium and matt types. Quantitative seeds color also showed variation with white, pale white, yellow, pale yellow and cream colors. Hilum was not found prominent in seven seed type. Among all studied seed attributes seed shape and seed coat color was investigated as more variant. Marillia *et al.* (2011) also reported significant diversity in seed shape and coat color in common bean accessions. Results showed significant variation in other seed characteristics and are in accordance to the diversity reports of Sofi *et al.* (2011) in defined seed attributes.

Morphological diversity: Germplasm for ten agro-morphological attributes was evaluated on the basis of ANOVA and p values. Significant diversity in mean squares with LSD values in morphological attributes of common bean accessions was shown (Table 3). Days to flowering was found non-significant with $p>0.05$ when evaluated for ANOVA. While other morphological attributes like pod length and seeds per pod were found significant for variability with $p<0.05$. Some other attributes like days to germination, days to pods formation, days to maturity, leaf area, chlorophyll contents, pods per plant and 100 seed weight were found highly significant for their mean square values on the basis of p value ($p<0.01$). Similarly early maturity was found in Forward Kahota followed by Rawalakot. Accession Bravo has greater seed weight followed by Lipa-1. Leaf area of Lipa-1 was also found greater among all studied types. Yield parameters like pods per plant, seeds per pod and seed weight were found high in Lipa-1 followed by Rawalakot type. Cluster analysis on the basis of morphological attributes depicted greater diversity among twenty two accessions (Fig. 2). On the basis of dissimilarity for quantitative parameters, all accessions were grouped in two clusters. Cluster I comprised of only three accessions while rest of all were grouped in cluster II. Accession Lipa-1 followed by Forward Kahota and Rawalakot were found diverse among twenty two for their morphological pattern in hierarchical clustering. It was clear from tree diagram that Forward Kahota and French Mild Maxi are at distant pattern on the basis of morphological attributes (Fig 2). Present results revealed that local and exotic accessions were grouped in the same cluster despite of differences in some seed characters.

Current investigations revealed more variations in 100 grains weight whereas non significant results were reported in previous studies, though the gene pool was comparatively very small (Coelho *et al.,* 2009). However these results were found in accordance with previous research of Ammanullah *et al.* (2006); Periera *et al.* (2009) and Sofi *et al.* (2011). Lipa-1 followed by Rawalakot was found with higher seed yield among all the studied members of gene pool.

Biochemical profiling: A range of diversity was observed in different metabolites among twenty two accessions. All accessions exhibited the biochemical constituents within reported range with significantly diverse pattern. The mean values of twelve observed biochemical constituents have been displayed (Table 4). The protein contents were found in range of 18.9-26%. Neelum-1 has highest protein percentage followed by Rawalakot showing the lowest. Beans, being low fat food, contained 1.43-2.08% fats contents, Rawalakot has maximum contents while Lipa-2 exhibited the minimum value for fat contents. The percentage of crude fibers in eight accessions was within the range of 3.38-5.26%. Rawalakot contained maximum fibers in their dry beans as compared to other seven while accession 1a has minimum. Estimated carbohydrates percentage range was from 51.3-58.92% with maximum values displayed by Lipa-2 and minimum values in 2f. Striking diversity was found in cooking time across twenty two accessions with the range of 79-127 minutes. Lipa-3 took maximum time to cook while minimum time was consumed by Lipa-4 during cooking.

Dendogram constructed on the basis of biochemical studies categorized twenty two accessions in two main groups at 500 % dissimilarity index (Fig. 3). Accession Lipa-3 was found more variant while accession 2e and Lipa-2 were found distantly apart and showed greater difference on the basis of their phylogenetic pattern.

Present study showed a comprehensive biochemical report of twenty two common bean accessions. Moisture contents were high in present investigation as compared to earlier studies, Kara *et al*. (2013) reported 3.39-6.40% moisture in seeds of different bean cultivars. This difference may be due to type of cultivar, localization or processing of food type. Srogi (2005); Trinidad *et al*. (2010) and Filipiak-Florkiewicz *et al*. (2012) declared these factors a sound reason of such variations in nutrient composition of common beans. Encouraging range in protein contents was investigated in local and exotic germplasm which was in range of previous studies that is 19-31% (Coelho *et al*., 2009). Broader range in earlier studies might be due to wider genetic base of the studied germplasm. Total energy, ash, fats, fibers and carbohydrates were also comparable to previously studied nutritional profile of legume by Saroj and Khetarpal (1993) and Romero *et al*. (2013). The carbohydrates and fibers percentage in twenty two accessions was found in accordance with prior report of Reddy *et al*. (1984) on red kidney bean. Results related to cooking time including the associated factors like hydration capacity and swelling capacity were of greater importance. High hydration capacity and swelling capacity of beans reduced cooking period. These results were somehow in consistence to those described earlier. In previous report Coelho *et al*. (2009) described

correlation between cooking time and protein contents. They also reported high protein contents in beans associated with shortening of cooking time. In present investigation some accessions with high protein percentage also revealed short time period for cooking. While cluster analysis revealed four major groups of all twenty two accessions, with local and exotic bean accessions in the same clusters. It indicated variable pattern of the metabolites in all bean types despite of their geographical differences. Nutritionally the bean genotypes revealed enriched pattern of the vital and indispensable nutrients which may be useful to fulfill not only the dietary requirements of the mountain community but may be helpful for further nutritional fortification of the crop.

Molecular diversity using SDS-PAGE: Molecular diversity through SDS-PAGE on the basis of seed storage protein was evaluated. Total protein profile in twenty two accessions from different areas of Azad Kashmir showed variation in some of the observed bands (Fig. 5). Total ten (10) protein bands were found after electrophoresis, out of which, five (5) were found monomorphic in their pattern. While rest were polymorphic in nature with significant variability among twenty two accessions. It depicted 50 % polymorphism in observed accessions. It was also observed that some accessions with different phenotype and different ecogeographic zones are grouped in the same cluster. Tree diagram of twenty two accessions divided the germplasm in two main clusters on the basis of differences in the banding pattern (Fig. 4). Cluster I comprised of an outlier, i.e. 2g, at larger linkage distance. This accession showed more diversity in its protein profile among other accessions. Cluster II was grouped in further two sub clusters, cluster IIa and cluster IIb. Accession Forward Kahota and Lipa-2 were clustered in same group of cluster IIa due to some similarities in their storage proteins. While cluster IIa included ten accessions with French mild maxi as an outlier and more diverse for their total protein estimated through SDS-PAGE. While IIb grouped eight accessions with relatively slim variation in their banding pattern. Greater distance was observed in Lipa-3 and Ducato which indicated greater difference in protein pattern among these local and exotic accessions.

SDS-PAGE analysis of the germplasm confirmed the variation, found out, on the basis of phenotype of seeds and plant characteristics. Earlier studies of Ulukan (2011) declared protein analysis technique as reliable technology to study the genetic diversity. Nisar *et al.,* (2008) declared SDS-PAGE as more authentic because somehow seed storage protein was found independent of environmental influences.

Our findings revealed clear discrimination in banding pattern for seed protein in different bean genotypes. Accessions from different geographical zones showed a range of diversity among all morphological and biochemical attributes that was confirmed by their protein banding pattern analysis. Variation and polymorphism in banding pattern was revealed in bean types in different earlier studies of Sarikamis *et al.* (2009) and Ismet and Yasar (2011).

Figure 1. Seeds of common bean (*Phaseolus vulgaris* L.)

Table 1. Common Bean germplasm with collection sites.

Sr. No.	Accessions	Collection Source	Sr. No.	Accessions	Collection Source
1	Lipa- 1	AJK	12	2c GB-4404-3-1999	PGRI, NARC
2	Forward Kahota	AJK	13	2d GB-4404-4-1999	PGRI, NARC
3	Rawalakot	AJK	14	2e-long beans	PGRI, NARC
4	Lipa-2	AJK	15	2f	PGRI, NARC
5	Neelum-1	AJK	16	Local Kashmiri	PGRI, NARC
6	Lipa-3	AJK	17	French mild maxi	PGRI, NARC
7	Lipa-4	AJK	18	French Polisia	PGRI, NARC
8	Neelum-2	AJK	19	Bravo	PGRI, NARC
9	1a GB-4404-1-1999	PGRI, NARC	20	Ducato	PGRI, NARC
10	2a GB-4404-1-1999	PGRI, NARC	21	Local Balakot	KPK, Pakistan
11	2b GB-4404-2-1999	PGRI, NARC	22	2g	PGRI, NARC

Table 2. Seed attributes of twenty two common bean accessions.

Sr. No	Accessions	Seed shape	Seed color	Seed spots	Spot color	Spot color pattern	Quantitative color	Hilum	Seed brilliance
1	Lipa-1	Kidney	White	Absent	Absent	Absent	White	Not prominent	Medium
2	Forward kahota	Oval	Maroon	Absent	Absent	Absent	Pale white	Not prominent	Medium
3	Rawalakot	Cuboid	Red	Present	Creamish	Speckled	Pale yellow	Not prominent	Medium
4	Lipa-2	Kidney	Pale creamish	Absent	Absent	Absent	Yellow	Not prominent	Medium
5	Neelum-1	Oval	Black	Absent	Absent	Absent	White	Not prominent	Shiny
6	Lipa-3	Cuboid	Yellow	Absent	Absent	Absent	White	Not prominent	Medium
7	Lipa-4	Cuboid	Pale creamish	Present	Black	Constant mottled	Creamish	Not prominent	Medium
8	Neelum-2	Kidney	Maroon	Absent	Absent	Absent	White	Not prominent	Shiny
9	1a GB-4404-1-1999	Kidney	Brown	Absent	Absent	Absent	Cream	Not Prominent	Matt
10	2a GB-4404-1-1999	Kidney	White	Absent	Absent	Absent	White	Not prominent	Medium
11	2b	Kidney	Brown	Absent	Absent	Absent	Yellow	Not prominent	Matt
12	2c GB-s4404-3-1999	Kidney	Dark brown	Absent	Absent	Absent	Yellow	Prominent	Matt
13	2d GB-4404-4-1999	Oval	Dull brown	Absent	Absent	Absent	Pale white	Prominent	Matt
14	2e-long beans	Oval	Brown	Absent	Absent	Absent	Yellow	Prominent	Matt
15	2f	Oval	Maroon	Absent	Absent	Absent	Cream	Not Prominant	Matts
16	Local Kashmiri	Round	Pale white	Absent	Absent	Absent	Yellow	Prominent	Matt
17	French mild maxi	Long oval	Pale white	Absent	Absent	Absent	Yellow	Prominent	Matt
18	French polisia	Oval	White	Absent	Absent	Absent	Pale white	Not Prominent	Matt
19	Bravo	Oval	Orange	Present	Dark orange	Speckled	Pale white	Not prominent	Medium
20	Ducato	Oval	Orange	Present	Dark orange	Speckled	Pale white	Not prominent	Medium
21	Local Balakot	Cuboid	Maroon	Absent	Absent	Absent	Pale white	Not Prominent	Shiny
22	2g	Oval	Maroon	Absent	Absent	Absent	Yellow	Prominent	Matt

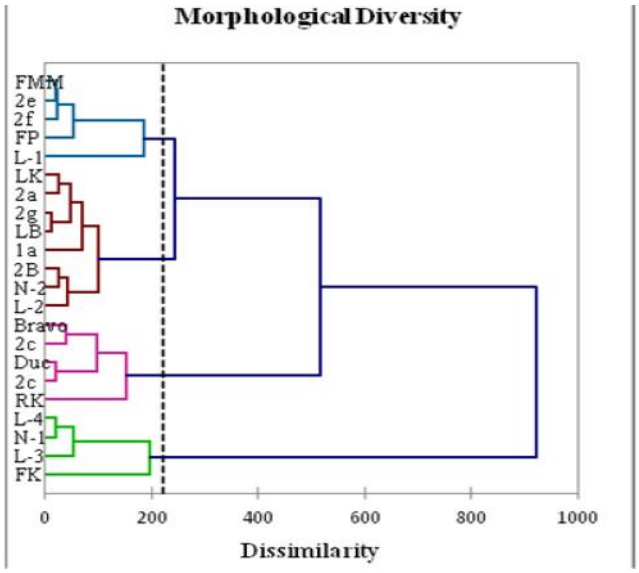


Figure 2. Dendogram showing diversity among 22 common bean accessions on the basis of morphological attributes

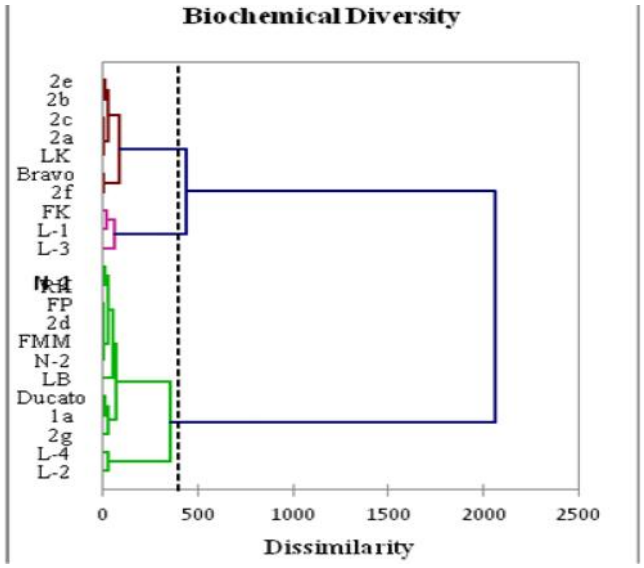


Figure 3. Dendogram showing diversity among 22 common bean accessions the basis of biochemical profile

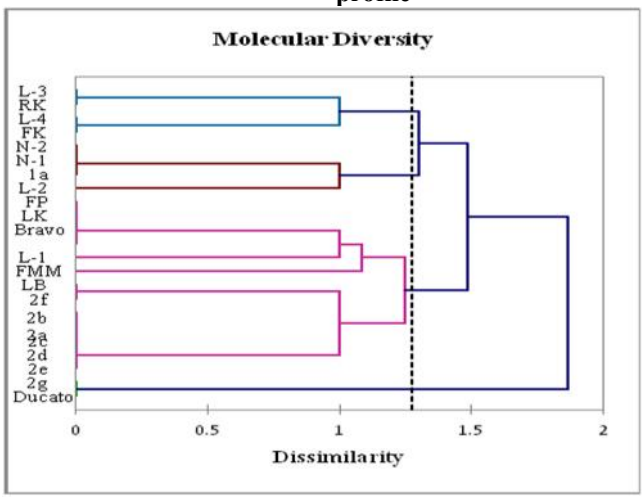


Figure 4. Dendogram showing diversity among 22 common bean accessions on the basis of SDS-PAGE.

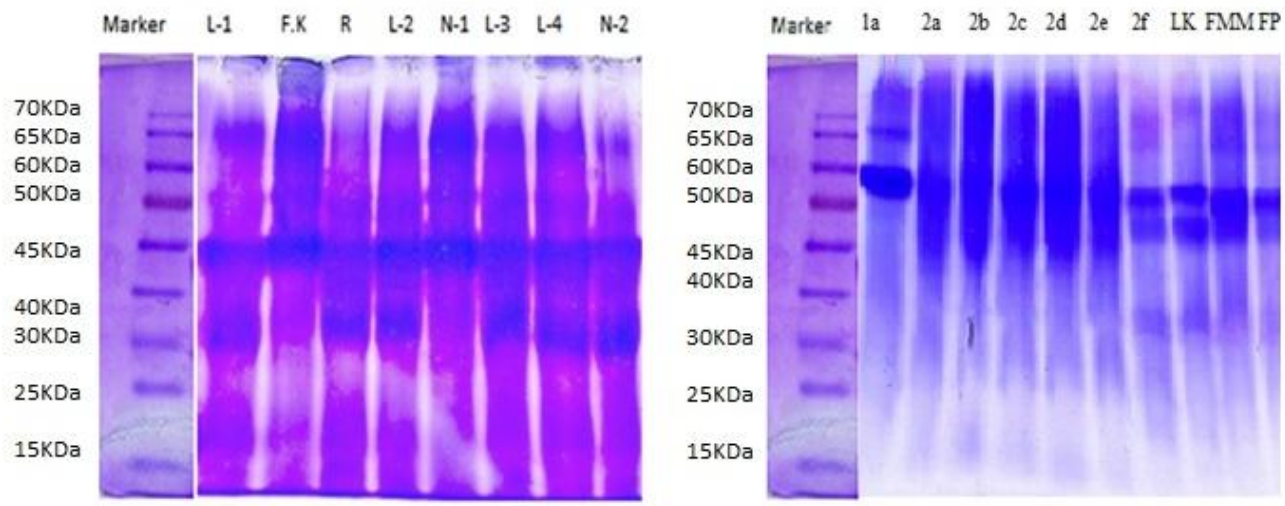
Table 3: ANOVA Mean square values and CV of morphological attributes in common bean accessions

		Man Squares									
SOV	**DF**	**Days to germination**	**Days to flowering**	**Days to pods formation**	**Days to maturity**	**Leaf area**	**Chlorophyll contents**	**Pods per Plant**	**Pods length**	**Seeds per pod**	**100 seed weight**
Rep	2	5.19697	782.742	28.4242	71.0152	17.8909	0.60857	52.788	0.56061	2.22727	21.184
Ecotypes	21	1.63997 **	24.428 ns	24.7395 **	93.225 **	28.6165**	5.78620 **	108.179**	9.12843 *	1.06782 *	168.866 **
Error	42	0.37157	19.203	7.2179	18.1580	4.6176	0.35118	37.439	2.57648	0.38600	4.083
CV		6.92	7.78	4.29	4.45	8.34	6.31	34.11	13.24	9.90	7.97
LSD value		1.0044	7.2206	4.4269	7.0215	3.5408	0.9765	10.082	2.6449	1.0237	3.3297

ns at p> 0.05, *, ** and *** significant at p< 0.05, 0.01, 0.001.

Table 4. Mean and LSD values for biochemical attributes of twenty two common bean accessions.

Sr. No.	Accessions	Moisture (%age)	Ash (%age)	Protein (%age)	Fats (%age)	Fibers (%age)	Carbohydrates (%age)	Energy (Kcal.)	Hydration Capacity g/seeds	Hydration Index	Swelling Capacity ml/seeds	Swelling Index	Cooking Time (min)
1	Lipa-1	11.02	4.36	23.48	1.75	3.42	55.84	332.8	0.2	0.6	0.11	0.12	120
2	Forward Kahota	12.1	4.4	22.75	1.59	4.1	55.43	327.02	0.08	0.5	0.05	0.03	122
3	Rawalakot	12.7	4.47	25.28	2.08	5.26	51.82	327.04	0.3	0.6	0.27	0.03	96
4	Lipa-2	11.02	4.47	22.64	1.43	3.45	58.92	331.34	0.27	0.4	0.35	0.15	85
5	Neelum-1	10.84	4.4	26	1.79	4.49	52.92	330.1	0.17	0.4	0.19	0.04	98
6	Lipa-3	12.19	4.47	22.6	1.72	3.75	52.46	336.92	0.24	0.6	0.13	0.03	127
7	Lipa-4	11.25	4.66	23.26	1.57	3.84	55.17	328.85	0.38	0.6	0.34	0.02	79
8	Neelum-2	11.46	4.24	23.86	1.58	3.75	55.4	330.08	0.03	0.21	0.27	0.05	96.00
9	1a	11.2	4.29	22.56	1.65	3.38	52.8	328.1	0.18	0.5	0.08	0.11	99
10	2a	11.7	4.51	22.12	1.48	3.61	52.3	328.4	0.09	0.4	0.16	0.08	107
11	2b	12.1	4.31	23.47	1.47	3.77	55.7	330.7	0.08	0.4	0.24	0.13	110
12	2c	11.6	4.59	22.15	1.58	3.39	56.4	332.1	0.15	0.4	0.13	0.07	106
13	2d	10.9	4.61	22.45	1.56	4.27	54.1	333.7	0.23	0.6	0.19	0.13	96
14	2e	11.3	4.47	24.7	1.61	3.49	56.7	331.5	0.19	0.5	0.29	0.14	112
15	2f	12.2	4.46	22.11	1.74	5	51.3	328.3	0.31	0.3	0.22	0.03	111
16	Local Kashmiri	11.4	4.35	23.2	1.52	3.54	56.9	335.7	0.27	0.3	0.18	0.04	107
17	French mild maxi	12.1	4.6	22.78	1.86	3.67	53.3	329.4	0.26	0.4	0.3	0.08	96
18	French polisia	11.6	4.56	23.01	1.88	3.8	52.3	331.5	0.23	0.6	0.14	0.11	95
19	Bravo	12.3	4.33	22.66	1.58	4.5	54.7	329.2	0.16	0.5	0.23	0.12	113
20	Ducato	11.01	4.4	23.1	1.71	4.19	53.8	334.2	0.13	0.6	0.15	0.09	101
21	Local Balakot	12	4.54	22.5	1.66	4.63	55.2	331.2	0.06	0.4	0.11	0.1	91
22	2g	11.06	4.41	18.9	1.45	3.89	55.6	330.3	0.09	0.5	0.29	0.12	97
LSD values		0.3711	0.0776	0.1603	0.2456	0.1142	1.0538	4.7237	0.0279	0.2075	0.2693	9.743	7.7237



The molecular marker used in this gel was SDS-70 KIT from SIGMA chemical company.

Figure 5. Electrophoretic banding pattern produced by SDS-PAGE of total seed proteins of Common bean accessions

Conflict of Interest: There is no conflict of interest regarding present research work

Conclusion: Morphological, biochemical and molecular analysis (SDS-PAGE) of the germplasm provided the nutritional and genetic facts as well as helped to scrutinize the requisite accessions from the gene pool for hybridization program for crops improvement. Accession Rawalakot was recommended for general cultivation to the farmers. Screening and biochemical profiling of diversely collected ecotypes and their comparison may lead to meet the malnutrition dilemma in one hand and conservation and improvement of the local germplasm on the other hand. The common bean improvement program shall be cushioned by the base provided by present investigation. This promising variability on the basis of preliminary evaluation provided a base to start a comprehensive and integrated breeding program which can lead to the evolution of common bean variety for Azad Kashmir and Pakistan. It will be helpful for diversification in present agriculture system that may become a profitable venture for poor farmers of country.

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