Research Article

Primary Evaluation of Seed Characteristics of Common Bean Landraces Collected from Himalaya Region of Pakistan

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Abstract | Germplasm evaluation is of great importance in breeding and crop improvement. Common bean accessions grown by the farming community in Himalaya region are an important source of genetic diversity. A total of 96 indigenous accessions of common beans (*Phaseolus vulgaris* L.) were collected from three different areas of Himalaya region of Pakistan. In all accessions, 78.1% were collected from Khyber Pakhtunkhwa while 11.5% from Kashmir and 10.4% from Gilgit Baltistan. Primary evaluation of seed characteristics was done according to International Board of Plant Genetic Resources (IBPGR) descriptors. Significant variation was observed for seed color, seed shape and seed coat pattern. Twenty-nine different colours of common beans were observed in these 96 accessions with pre-dominance of red colour. Four different seed shapes were observed i.e., Cuboid, oval, truncate fastigiated and kidney shape. Cuboid seed shape was predominant with a frequency of 46.8%. Seed coat pattern was absent in 51% accessions. Five different seed coat patterns i.e., constant mottled, stripped, circular mottling, rhomboid spotted and speckled were recorded in remaining 49% accessions. Cluster analysis was performed and 96 accessions were grouped in four clusters (G1, G2, G3 and G4) based on primary seed characters. The G4 was further divided into two sub groups i.e., G4-A and G4-B. G4-A comprised 25 accessions whereas G4-B contained 30 accessions. The G3, G2 and G1 had 17, 16 and 8 accessions, respectively. Considerable variation was found among all the groups. Conservation and characterization of this diverse germplasm is recommended for future breeding programmes.

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Introduction

Germplasm collection is a global effort to conserve plant biodiversity. The diversity of indigenous genetic resources is invaluable for breeding programmes. These resources can be used for basic research such as resistance to biotic and abiotic stresses, evolution and gene expression for improvement in crop plants (Dudnik et al., 2001; Mario et al., 2010). Common bean *(Phaseolus vulgaris L)* is 3rd crop in the world in terms of grain legumes' production after soybean (*Glycine max* L.) and ground nut (*Arachis hypogea* L.) (Nedumaran et al., 2015). It is an important and easily accessible source of protein in developing countries (Broughton et al., 2003; Mora-Avilés et al., 2007). It also contains starch, dietary fibers, minerals and vitamins other than proteins (Broughton, 2003). Knowledge about the origin and

domestication is a crucial pre requisite in conservation and use of existing germplasm for future breeding programmes. Two main gene pools of common bean are described by Kwak and Gepts (2009), one is Mesoamerican and other is Andes. Mesoamerican beans are small seeded (< 25g/100seeds) while Andean beans have larger seed size (> 40g/100seeds) (Singh et al., 1991). Mesoamerican gene pool includes beans from southern and central Mexico, Central America Colombia and Guatemala while Andes gene pool principally comprised of Peru, Bolivia and Argentina (Bitocchi et al., 2012). These two gene pools are further distinguished in various races such as Mesoamerica, Jalisco, Durango, and Guatemala in Mesoamerican gene pool and Nueve Granda, Chile and Peru in Andean Gene pool (Beebe et al., 2000). Bean seeds have a uniform color which is known as primary color like red, black, beige, white etc. and a secondary color present in the form of stripes, spots and streaks of different shades (Silva and Costa, 2003). The preference of a particular seed color along with the seed coat pattern depends on region, country and consumers' choice (Possobom et al., 2015). A huge phenotypic variability in common beans has usually been expressed in terms of color, size, shape and brightness (Corte et al., 2010).

Common beans are grown by small scale farmers in most of the developing countries. Myanmar, India, Brazil, China and Mexico are top producers of common bean (Nedumaran et al., 2015). In Pakistan, common bean is grown by farmers in Himalaya belt. Himalaya region is rich in legume production, because of favorable climatic conditions for legume production. It includes common bean, peas, cowpea and lentil (Ghafoor and Arshad, 2011).

In Pakistan, the genetic resources of indigenous common bean are unexploited and underutilized with a very inadequate crop improvement work. Mostly local landraces are grown expressing a significant genetic diversity. These land races and primitive types are found in mixed form and not a single registered variety has been reported so far in our country. Due to its broad genetic base, there exists high genetic variability in seed coat color, seed shape, seed size and growth habit. Genetic diversity reduces the susceptibility of food crops to catastrophic losses due to biotic stress (pests or pathogen) as well as abiotic stress (drought, high temperature, chilling etc.) (Ghafoor and Arshad, 2011). According to Nakano et al. (1994) black seeded snap bean varieties have high potential for heat tolerance to be further utilized in breeding programs. No evidence is reported in literature about the characterization of common bean of Pakistan. Therefore, there is need to focus on the characterization and conservation of indigenous common bean of Himalaya region which is exclusively a negligible discipline of legumes. These genetic resources need protection, characterization and conservation. The conservation and sustainable utilization of genetic resources can play an important role in agricultural productivity. It can also significantly contribute to the food security, poverty elimination and national development.

In Pakistan, there is intense need to conserve indigenous germplasm of common bean for its proper utilization at present and in future for crop improvement. The objectives of this study were to collect the indigenous common bean germplasm from Himalaya region of Pakistan and to evaluate for seed characteristics.

Materials and Methods

Common bean has high potential in Himalaya Region of Pakistan which includes northern areas of Khyber Pakhtunkhwa, Gilgit Baltistan and Kashmir. Mostly collection of common bean germplasm was done from the farmers' fields and stores of these specified areas. Some accessions were obtained from the research institutes. Agricultural Research Institute Mingora Swat provided 16 indigenous accessions while two were collected from National Agriculture Research Center Islamabad. Primary evaluation of seed characteristics was done according to the International Board of Plant Genetic Resources (IBPGR) descriptors. Data were recorded on seed color, seed shape and seed coat pattern. Cluster Analysis was done based on basic seed traits and variance dendrogram was generated using R-Statistical Package.

Results and Discussion

Common beans are grown in high altitude areas of Himalaya region. It is grown in kharif season in combination with maize crop as maize plants provide trellis for the climbing types of beans. Local accessions show important genetic resources directly used by the marginal farmers in the remote areas of Himalaya region. Farmers are growing these types for many

years that were selected for their adaptation to local agro climatic conditions. A total of 96 accessions were collected from the Himalaya region of Pakistan. There exists significant diversity in color, shape and seed coat pattern. Seventy-five accessions were collected from Khyber Pakhtunkhwa which comprises 78.1% of total collection while 10 accessions were from Gilgit Baltistan and 11 were from Kashmir showing 10.4% and 11.5% of total respectively (Figure 1A).

Table 1: *Total Number of accessions from Himalaya Region of Pakistan.*

Locations	Sampling Points	Number of Accessions
Khyber Pakhtunkhwa	Siren valley Manda ghuccha	06
	Konsh Valley	05
	Mansehra	07
	Batgram	09
	Kohistan	12
	Chitral	11
	Swat	08
	Shangla	06
	Parachinar	06
	Upper dir	02
	Naran	02
	Battakundi	01
Gilgit	Ghizar	10
Kashmir	Azad Kashmir	11
Total		96

Collection from Khyber Pakhtunkhwa

Common bean is grown in northern areas of Khyber Pakhtunkhwa (KPK) which lies in Himalaya range. Total of 76 accessions were collected from Khyber Pakhtunkhwa. Fifty-eight accessions were obtained from farmers' field of Siran valley, Konsh valley, Naran, Chitral, Batgram, Kohistan, Batakundi, Shangla and Parachinar, while remaining accessions were obtained from Agricultural Research Institute Mingora, Swat. All of these accessions were locally cultivated by the farmers and supplied to the local retail seed shops. They had no accession name. These accessions had been maintained by the farmers for many years by successive cultivation. Seed colour and shape are important indicators of genetic variability and consumer habit. These accessions had different seed colors and shapes (Figure 2A). High variability was observed in seed color. Almost 24 different colors of seed were found in KPK (Table 1). Red color beans are abundant (26.6%) in above mentioned areas of KPK. Similar result was indicated by Sultan et al. (2014). Red colour beans are preferred by the consumers as compared to others (Sexton et al., 1997). Seed coat pattern also varied for each accession (Table 1). Four different shapes of seed were recorded with five different types of seed coat patterns while it is absent in (53.3%) of accessions. Cuboid seed shape was most common (49.4%) followed by oval (32.0%), truncate fastigiated (16.0%) and kidney shapes (2.6%). Okii et al. (2014) observed same trend in seed shape and most common seed shape in his study was Cuboid with frequency of 46%. Similar trends of seed shape were also reported by Sultan et al. (2014). No registered variety of common beans was found or reported in these areas inspite of the most suitable climatic conditions for its production.

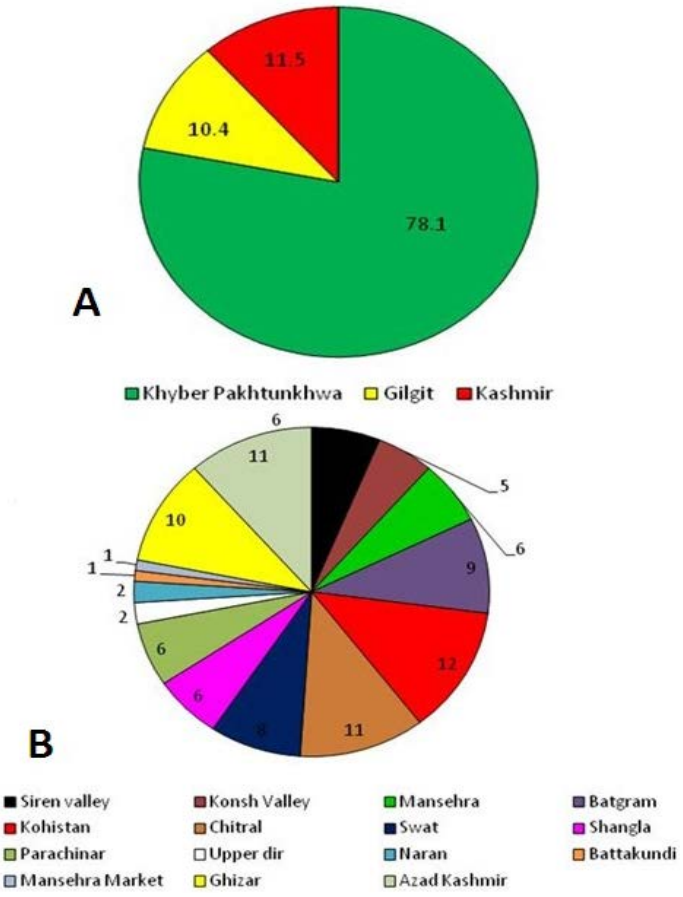

Figure 1: *A, Percentages of collected germplasm from Khyber Pakhtunkhwa, Gilgit Baltistan and Kashmir; B, Number of accessions collected from different areas.*

Collection from Gilgit Baltistan

Gilgit Baltistan has a rich agricultural biodiversity due to its peculiar geographic and climatic conditions. Climatic conditions of Gilgit Baltistan vary widely ranging from monsoon influenced moist temperate zone in western Himalayas to semi-arid and arid cold desert in northern Karakoram and Hindukush.

Table 1: *Seed color, shape and seed coat pattern of accession from Khyber Pakhtunkhwa.*

S.N	Accession code	Location	Seed colour	Seed shape	Seed coat pattern
1	SnMLB	Siren valley	Light Brown	Cuboid	Constant mottled
2	SnKLB	Siren valley	Light Brown	Cuboid	Constant mottled
3	KnCkLB	Konsh valley	Light Brown	Cuboid	Constant mottled
4	BALB	Battgram	Light Brown	Cuboid	Constant mottled
5	MALB	Mansehra	Light brown	Cuboid	Speckled
6	ChMLB	Chitral	Light Brown	Oval	Constant mottled
7	ChPS	Chitral	Skin	Oval	Constant mottled
8	ChBLB	Chitral	Light Brown	Cuboid	Constant mottled
9	ShLS	Shangla	Skin	Cuboid	Constant mottled
10	BAS	Battgram	Skin	Truncate fastigiated	Constant mottled
11	KtLB	Kohistan	Light Brown	Cuboid	Constant mottled
12	StLB	Swat	Light Brown	Cuboid	Constant mottled
13	BADB	Battgram	Dark Brown	Cuboid	Circular mottling
14	PcB	Parachinar	Brown	Oval	Constant mottled
15	KtS	Kohistan	Skin	Oval	Constant mottled
16	SnMBBS	Siren valley	Brown with black strips	Cuboid	Striped
17	KnCkBBS	Konsh valley	Brown with black strips	Cuboid	Striped
18	KnIcBBS	Konsh valley	Brown with black strips	Cuboid	Striped
19	ShLBBS	Shangla	Brown with black strips	Oval	Striped
20	BABBS	Battgram	Brown with black strips	Oval	Striped
21	KtBBS	Kohistan	Brown with black strips	Oval	Striped
22	KnIcBBS	Konsh valley	Brown with black strips	Cuboid	Striped
23	ChPBBS	Chitral	Brown with black strips	Truncate fastigiated	Striped
24	BASBS	Battgram	Skin with black strips	Oval	Striped
25	KtSBS	Kohistan	Skin with black strips	Oval	Striped
26	UdSY	Upper dir	Yellow	Truncate fastigiated	Absent
27	NrR	Naran	Red	Cuboid	Absent
28	ChMR	Chitral	Red	Truncate fastigiated	Absent
29	ChPR	Chitral	Red	Truncate fastigiated	Absent
30	ChBR	Chitral	Red	Cuboid	Absent
31	ShLR	Shangla	Red	Oval	Absent
32	BAR	Battgram	Red	Cuboid	Absent
33	UdSR	Upper dir	Red	Oval	Absent
34	MnR	Mansehra	Red	Oval	Absent
35	MnR	Mansehra	Red	Cuboid	Absent
36	MnR	Mansehra	Red	Truncate fastigiated	Absent
37	StR	Swat	Red	Truncate fastigiated	Absent
38	StR	Swat	Red	Cuboid	Absent
39	StR	Swat	Red	Truncate fastigiated	Absent
40	StR	Swat	Red	Cuboid	Absent
41	StR	Swat	Red	Truncate fastigiated	Absent
42	KtR	Kohistan	Red	Oval	Absent
43	MnR	Mansehra	Red	Truncate fastigiated	Absent
44	PcR	Parachinar	Red	Cuboid	Absent
45	MnBR	Mansehra	Brick Red	Cuboid	Absent
46	KtR	Kohistan	Red	Cuboid	Absent
47	PcDM	Parachinar	Dark maroon	Cuboid	Absent
48	KtM	Kohistan	Maroon	Cuboid	Absent

49	KtRB	Kohistan	Redish Brown	Cuboid	Absent
50	KtPB	Kohistan	Purpish brown	Cuboid	Absent
51	SnMB	Siren Valley	Black	Cuboid	Absent
52	ChMB	Chitral	Black	Cuboid	Absent
53	ChBSB	Chitral	Shiny black	Truncate fastigiated	Absent
54	ShLBsh	Shangla	Blackish	Oval	Absent
55	BAB	Battgram	Black	Truncate fastigiated	Absent
56	PcB	Parachinar	Black	Cuboid	Absent
57	StB	Swat	Black	Cuboid	Absent
58	KtB	Kohistan	Black	Oval	Absent
59	SnKSBS	Siren valley	Skin with brown spots	Oval	Constant mottled
60	SnKLBBS	Siren valley	Light brown with black spots	Oval	Speckled
61	KnChLBRS	Konsh valley	Light Brown with Red spots	Oval	Circular mottling
62	ShLSMS	Shangla	Skin with maroon strips	Oval	Striped
63	NrPSP	Naran	Purplish with skin Spots	Cuboid	Rhomboid spotted
64	BKbPSP	Battgram	Purplish with skin spots	Cuboid	Rhomboid spotted
65	BkPSP	Battakundi	Purplish with skin spots	Cuboid	Rhomboid spotted
66	StPBS	Swat	Purplish with brown spots	Oval	Rhomboid spotted
67	KtPBS	Kohistan	Purplish with Brown spots	Oval	Rhomboid spotted
68	ChMTP	Chitral	Tea pink	Cuboid	Absent
69	ChBLP	Chitral	Light Pink	Cuboid	Absent
70	PcW	Parachinar	White	Oval	Speckled
71	ShLW	Shangla	White	Cuboid	Absent
72	BAW	Battgram	White	Oval	Absent
73	KtW	Kohistan	White	Kidney shaped	Absent
74	PcWBD	Parachinar	White w black dot on one side	Oval	Absent
75	MDR	Mansehra	Dark Red	Kidney shaped	Absent

Table 2: *Seed colors, shapes and seed coat patterns of accession from Gilgit Baltistan.*

S.No	Accession Code	Location	Seed Color	Seed Shape	Seed coat pattern
1	GtGBr	Gilgit	Brown	Cuboid	Constant mottled
2	GtGBBS	Gilgit	Brown with black strips	Oval	Striped
3	GtGR	Gilgit	Red	Oval	Absent
4	GtGB	Gilgit	Black	Cuboid	Absent
5	GtGLBMS	Gilgit	Light brown with maroon strips	Oval	Rhomboid spotted
6	GtGGB	Gilgit	Greenish brown	Cuboid	Absent
7	GtGGB	Gilgit	Greenish brown	Kidney shaped	Absent
8	GtGBGS	Gilgit	Brown with green strips	Kidney shaped	Striped
9	GtGBPS	Gilgit	Brown with purple spots	Kidney shaped	Rhomboid spotted
10	GtGPB	Gilgit	Purplish brown	Cuboid	Constant mottled

Temperatures vary in the valley bottoms from 40°C in summer to -10°C in winter. Commonly grown pluses are beans, peas, black gram, lentil, and chickpea (Khan et al., 2014).

During exploration of indigenous germplasm, ten accessions of common bean were obtained from farmers' field of Gilgit Baltistan. All accessions were of different colours, shapes and seed coat patterns (Figure 2B). Nine different colors, three different shapes and three seed coat patterns were identified according to the descriptor. Seed coat pattern was absent in 40.0% accessions of Gilgit Baltistan (Table 2.2). Similar results were observed by Okii et al. (2014). In his study he found that 45.3% of the total germplasm has no seed coat pattern. Three seed shapes i.e.

oval (30.0%), cuboid (40.0%) and kidney (30.0%) were recorded in collection from Gilgit Baltistan. Mario et al. (2010) presented that he found two seed shapes i.e. ovate and kidney seed shape with a percentage of 50.5% and 21.9% respectively in his study on Chilean common beans germplasm. The other seed shapes presented in their study were round, cylindrical and Rhomboid. As far as seed coat pattern was concerned, three different seed coat patterns were recorded. Different seed coat patterns including constant mottled, striped and rhomboid spotted were represented respectively in 20.0%, 20.0% and 20.0% in collection from Gilgit Baltistan. These are mostly grown in Ghizar district of Gilgit Baltistan.

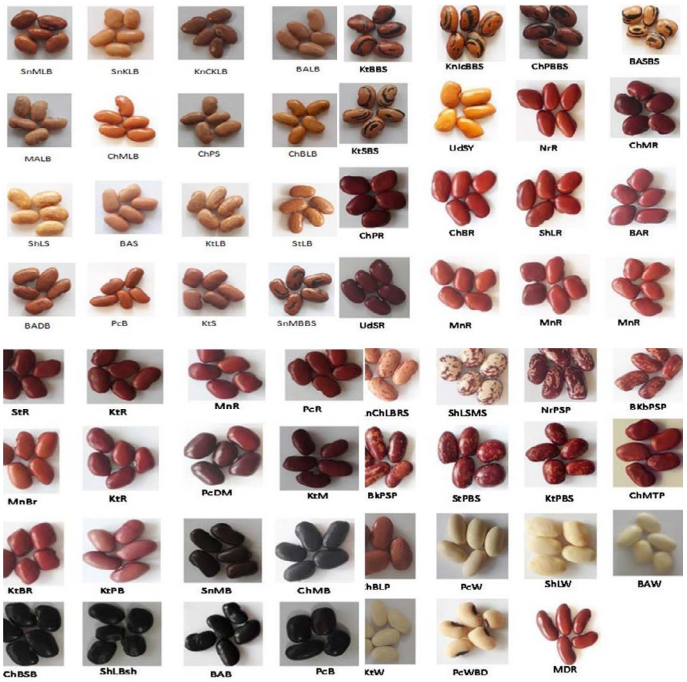

Figure 2A: *Collection from Khyber Pakhtunkhwa.*

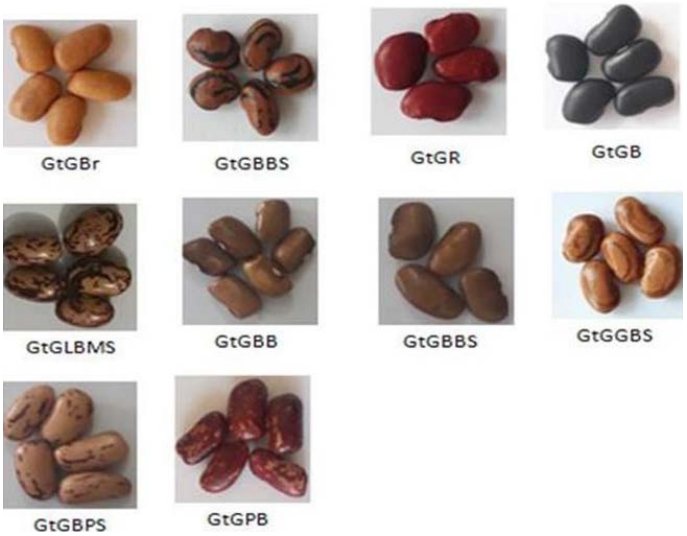

Figure 2B: *Collection from Gilgit Baltistan.*

Collection from Kashmir

The hilly areas of Kashmir are famous for the production of common bean. It is usually grown by the dry land marginal farmers in high altitudes (Sultan et al., 2014). Eight accessions were collected from farmers' field while two accessions were obtained from National Agricultural Research Center Islamabad. Considerable diversity in colour, shape and size was observed in these accessions (Figure 2C). All accessions were of different colors with three variable shapes and three types of seed coat patterns while it was absent in 45.4% accessions (Table 3). Seed coat pattern included constant mottled (9.0%), striped (27.2%) and Rhomboid spotted (18.2%). Three seed shapes i.e. oval (45.45%), cuboid (36.4%) and Truncate fastigiated (18.2%) were recorded in collection from Kashmir. These results are consistent with the findings of different scientists who have reported wide variation in seed shape of common bean germplasm (Rodino et al., 2003; Rai et al., 2006; Rodino et al., 2006; Cabral et al., 2010; Lioi et al., 2012).

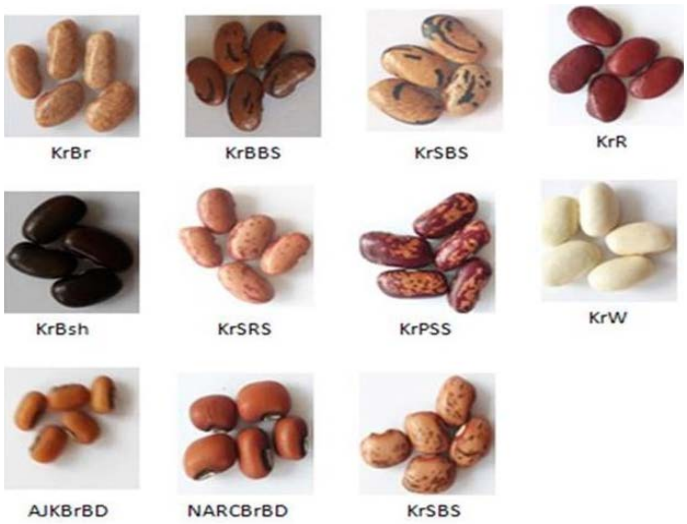

Figure 2C: *Collection from Kashmir.*

Cluster analysis

Cluster analysis was performed on all collection from three different areas of Himalaya Region which produced four clusters (G1, G2, G3 and G4) of 96 genotypes (Figure 3). G4 was further divided into two sub groups i.e. G4-A and G4-B. G4-A contained 25 accessions while G4-B composed of 30 accessions, followed by G3 and G2 containing 17 and 16 accessions respectively. G1 was the smallest group having eight accessions. The 96 accessions occurred in four major clusters (G1, G2, G3, G4). The G1 group contained eight accessions having oval, kidney shaped and cuboid seed shapes. The G2 group was

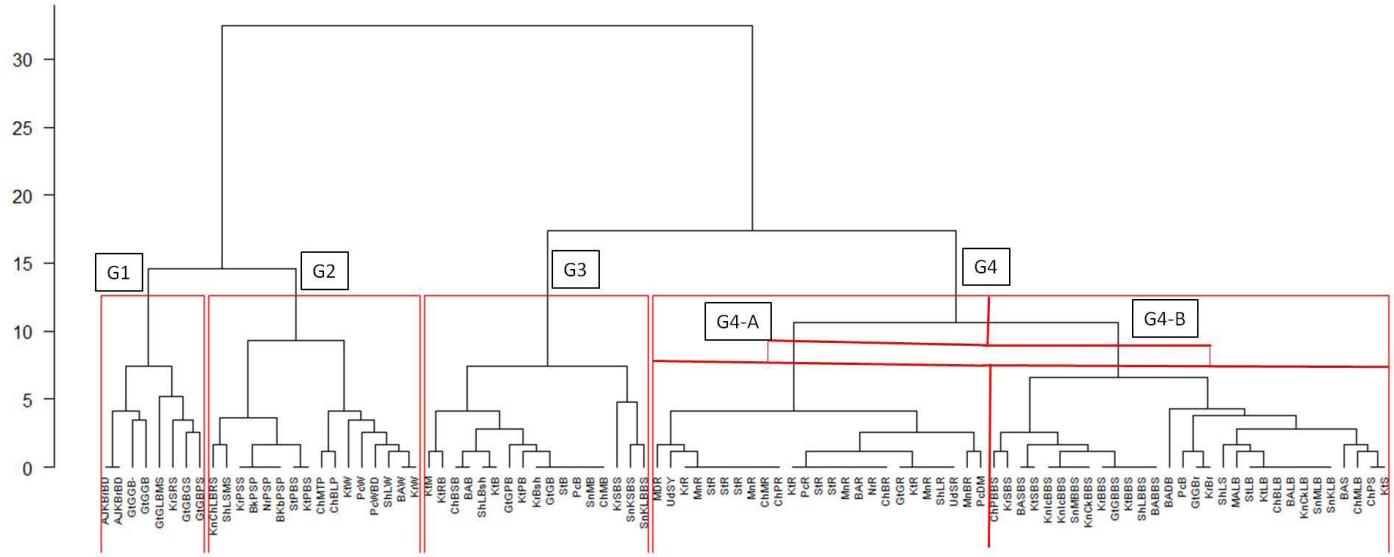

Figure 3: *Dendrogram obtained by cluster analysis showing the similarity rate of 96 common beans accessions.*

Table 3: *Seed colors, shapes and seed coat patterns of accessions from Kashmir.*

S.No	Accession code	Location	Color	Shape	Seed coat pattern
1	KrBr	Kashmir	Brown	Cuboid	Constant mottled
2	KrBBS	Kashmir	Brown with black strips	Oval	Striped
3	KrSBS	Kashmir	Skin with black strips	Truncate fastigiated	Striped
4	KrR	Kashmir	Red	Truncate fastigiated	Absent
5	KrBsh	Kashmir	Blackish	Cuboid	Absent
6	KrSRS	Kashmir	Skin with Red Spots	Oval	Striped
7	KrPSS	Kashmir	Purple with skin spots	Cuboid	Rhomboid spotted
8	KrW	Kashmir	White	Oval	Absent
9	AJKBrBD	Azad Jammu & Kashmir	Brown with black dot on one side	Oval	Absent
10	NARCBrBD	Azad Jammu & Kashmir	Brown with black dot on one side	Oval	Absent
11	KrSBS	Kashmir	Skin with brown spots	Cuboid	Rhomboid spotted

predominated by oval seed shaped followed by cuboid seed shape. Sixteen accessions were included in G2 without a clear distinction in seed color. For example, some accessions had brown primary color with black as secondary color in form of dots while other have skin and brown as primary color with purple, green, red or maroon strips or spots. Seed coat pattern had a mixed trend in both G1 and G2. The G3 was dominated by cuboid seed shape followed by oval and truncate fastigiate, respectively. Most dominant seed colour in this group was black without any seed coat pattern. G4 was the largest group containing 55 accessions. It was further subdivided into two sub groups i.e. G4-A and G4-B. All of 25 accessions in G4-A were of single primary colour i.e. red and maroon. The Seed coat pattern was absent in all accessions of G4-A. In G4-B, 17 accessions had single primary seed colour i.e. skin to brown. The seed coat pattern of these accessions was constant mottled followed by circular mottling and speckled. The remaining 13 accessions of G4-B contained secondary seed colour in form of stripped seed coat pattern i.e. brown primary color with black as secondary color in stripped seed coat pattern. Asfaw et al. (2009) also conducted a study on common beans from Ethiopia and Kenya. They found the similar trend of seed color in their study. Most of accessions in their study were of single primary color and had no secondary seed color, however among those red and cream mottled seed types were also present. Blair et al. (2010) studied land races from Central Africa and observed considerable variations in seed size and color predominated by red which was also frequent in our study. Stoilova et al. (2013) also observed three seed shapes i.e. kidney shaped, cuboid and oval with dominance of white color seeds followed by reddish or mottled seeds in their study. The G4-A and G4-B in our study showed a mixed trend of seed shape that is cuboid, oval, truncate fastigiated and kidney shaped.

This remarkable diversity in seed characters can be useful for proper characterization and conservation of indigenous germplasm. During the course of this study all accessions were collected from local villages which are known for marginal and risk prone farming systems so the diversity found in these accessions can provide valuable alleles for adaptation to stressful environments in future breeding programmes.

Conclusions and Recommendations

The significant diversity in 96 accessions collected during the course of present study from three main areas of Himalaya region may provide valuable alleles for biotic and abiotic stress in future breeding programmes. During primary evaluation of seed characteristics, the existence of different seed colors, shape and seed coat patterns showed remarkable variation which may be helpful in characterization and conservation of germplasm. Morphological, molecular, biochemical characterization and conservation of germplasm is recommended for future propagation and investigation of genetic relationship.

Novelty Statement

Collection of common bean landraces and the primary evaluation of their seed traits are important for the maintenance of diversity and food security in the sce-nario of climate change. This genetic diversity can best be conserved and made available for future breeding programs.

Author's Contribution

Iffat Nawaz and Farhatuullah designed and conducted the study. Iffat Nawaz, Farhatullah and Ghulam Muhammad Ali collected germplasm from different areas of Himalaya region. Iffat Nawaz and Sajid Ali conducted analysis of data. Iffat Nawaz and Farhatullah wrote the manuscript and provided final interpretation. Fida Muhammad and Ghulam Muhammad Ali edited manuscript and provided final interpretation. Iffat Nawaz and Farhatullah provided resources for study.

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