

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) LANDRACES REVEALED GENETIC SOURCES FOR FUTURE BREEDING AND RESEARCH

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Abstract

Common bean is one of the most significant crops among the main legumes. Landraces of the common bean are grown all over the world. These landraces show remarkable resistance to pests and diseases in addition to their exceptional climate adaptation. Landraces continue to perform well despite low farming inputs. The aim of this work was to identify important germplasm by morphological and biochemical investigation using 96 landraces collected from Azad Kashmir and northern Pakistan. Ninety-six landraces were collected from Azad Kashmir, Gilgit-Baltistan and Sawat district of KPK. The landraces were grown in a field using augmented design for two years. The data were collected on morphological and biochemical traits. Correlation coefficient was performed to assess the association among the traits. Cluster analysis and principal component analysis were performed to assess the diversity among the common bean landraces. A high correlation between seed production and seed weight was observed. Seed weight, seed yield per plant, and the ratio of seeds per pod: pod length, all exhibited positive and statistically significant associations. The results showed a highly significant correlation between pod width, seed weight, and yield. Among biochemical traits including antioxidant activity have shown maximum positive and highly significant correlations with moisture content, ascorbic acid, protein content and crude fiber. Nineteen landraces demonstrated maximal morphological variability based on the average linkage distance. The cluster analysis based on biochemical traits indicated that 27 landraces exhibited the highest level of diversity. These results suggest that common bean landraces are highly valuable genetic resources for future breeding for the common bean improvement.

Key words: *Phaseolus Vulgaris*, Future Breeding; Cluster Analysis; Principal Component Analysis

Introduction

Common beans or kidney beans (*Phaseolus vulgaris* L.) are herbaceous annual plants of the Fabaceae family. Common beans are self-pollinated crops (Ali *et al.*, 2020; García-Fernández *et al.*, 2024). Due to their rich nutritional composition, the common beans hold a position among the top 10 vital crops globally (Hussain *et al.*, 2005; Pereira *et al.*, 2013). Varieties of common beans exhibit diverse shapes, colors, and sizes worldwide, adding complexity to breeding efforts (Welsh *et al.*, 2015; Miklas *et al.*, 2016). Among the primary legumes, the common beans rank as the world's third most significant crop, following soybeans (*Glycine max*) and peanuts (*Arachis hypogaea*) (Gatti *et al.*, 2011; Rambabu *et al.*, 2016; Canci *et al.*, 2019; Nsiri & Krouma, 2023).

Based on morphological and biochemical evidence, two distinct centers of origin for common beans have been identified: one in Mesoamerica and another along the eastern axis of the Andes in South America (Ali *et al.*, 2016). Common beans were domesticated in the highland regions of Latin America over 7000 years ago (Kaplan, 1965). Typically, the color of their seeds is genetically independent of the color of its flowers, which can range from pink and white to purple (Debouck, 2014; Stoilova *et al.*, 2014). Seed shapes vary from round to elliptical, flattened, or elongated and rounded (Nawaz *et al.*, 2019). Common beans exhibit tolerance to temperatures ranging from 27°C to 29.5°C and can thrive across diverse soil depths, with an optimal pH range for fertile soil between

5.5 and 6.5. Flowering times vary among cultivars depending on climatic condition and change in weather pattern (Fahad *et al.*, 2010; Dubey *et al.*, 2023).

Common beans represent a crucial protein source, playing a primary role as a food legume (Assady *et al.*, 2005; Josephine *et al.*, 2018; Kotue *et al.*, 2018). With protein content ranging from 20-25%, predominantly in the form of phaseolin (Tugce *et al.*, 2018), they are extensively cultivated worldwide, holding a significant importance in diverse regions (Anon., 2005; Lin *et al.*, 2008). Serving as a principal protein, dietary fiber, and mineral source in diets (Yeken *et al.*, 2019), common beans contribute significantly to global nutrition and human health (Alonso *et al.*, 2001; Irina *et al.*, 2012). Carbohydrates in beans primarily consist of starch, followed by dietary fiber and sucrose-galactosyl derivatives (Nasar *et al.*, 2022). The major proteins in beans are globulins (54-79%) and albumins (12-30%), accompanied by lectins, lipoxygenase, and various protease inhibitors such as α -amylase, chymotrypsin, and trypsin. However, their nutritional value can be hindered by anti-nutritional factors like phytate, tannins, and oxalate (Celmeli *et al.*, 2018; Mansoor *et al.*, 2023).

The legumes have an essential function within the development of diets (Wani *et al.*, 2017; Long *et al.*, 2020), as they provide a very wealthy supply of dietary constituents and boom soil fertility including nitrogen fixation through symbiosis with rhizobia (Murube *et al.*, 2021). Green beans, contain high vitamin C and nutritional fiber; they are served from time to time in salads but are more regularly organized as a cooked vegetable. Beans are regularly offered as

preserved or frozen (Tomlekova *et al.*, 2024). Beans are harvested while the pods are absolutely developed and desiccated. Beans are wealthy in protein, nutrients B and C, and numerous minerals containing iron, magnesium, phosphorus, and potassium (Rocha *et al.*, 2012).

The genetic evaluation of native common bean is underutilized in Pakistan, with an entirely inadequate crop development program. Nearby genotypes are developed which show significant heritable capability. Such genotypes and primitive types are grown locally but not well defined variety has been established up till now all around the world (Dura *et al.*, 2010). Because of its enormous genetic variability, there is a vast variation in its seed shape, size and growth. Genetic range diminishes the vulnerability of catastrophic losses because of biotic strains in addition to abiotic stress (Jannat *et al.*, 2022).

The genetic variability and characterization of the germplasm of common bean landraces growing in AJK and northern areas are yet unknown. Additionally, Azad Kashmir does not have any registered or well-known varieties of this crucial crop, and none have ever been cultivated. The diverse geographic and climatic conditions in AJK and other areas may lead to significant variability in morphological and biochemical traits among landraces of common bean (Zaffar *et al.*, 2022). It was hypothesized that through comprehensive characterization and analysis, distinct genotypes of common bean exhibiting desirable traits can be identified, offering a potential for improved crop varieties suitable for different environmental conditions and agricultural practices in the region. This study aims (1) to collect landraces of common bean cultivated, (2) to conduct morphological and biochemical

studies for the characterization of different landraces of the common beans, and (3) to identify suitable genotypes of common beans based on the collected data.

Material and Methods

The current research was conducted at the University of Poonch Rawalakot, Azad Kashmir.

Collection of germplasm: For the collection of common bean germplasm, various locations were selected based on genetic and ecological diversity, geographical distribution, pest resistance and cultural significance of the crop. Sites were selected for germplasm collection that capture the diversity and resilience of the crop species, ensuring their long-term conservation and utilization for future generations. In district Poonch, five locations (Devi Gali, Dhoke, Jandali, Banjosa, and Mera) were chosen, along with four locations each in districts Bagh (Sudhan Gali, Mallot, Batharan, and Sanghar), Haveli (Hillan, Bhaidi, Jabbi Saidan, and Kalamola), Muzaffarabad (Charakpura, Raj Puthi, Ghari Dopatta, and Neelum), ten locations in Gilgit (Hopar, Doreal, Aysree, Gulmatee, Mushkoo, Bethrate, Gohrabad, Hayam, Galodass, and Damas), and four locations in Sawat (Dera Alladand, Tamaragar, Malakand, and Ghat Puchar). Germplasm from these selected locations was then planted for two consecutive years, i.e., 2018 and 2019. The common beans exhibit a climbing growth habit, necessitating support structures. Bamboo sticks were employed as temporary supports for the vines, while soft flexible strings were used to provide additional support. Landraces selected were presented in Table 1.

Table 1. Landraces selected for the morphological and biochemical characterization of common beans.

Landraces	Location	Landraces	Location	Landraces	Location	Landraces	Location
L ₁	D Gali ₁	L ₂₅	Batharan ₂	L ₄₉	C Pura ₄	L ₇₃	Gohrabad ₁
L ₂	D Gali ₂	L ₂₆	Batharan ₃	L ₅₀	C Pura ₅	L ₇₄	Gohrabad ₂
L ₃	D Gali ₃	L ₂₇	Batharan ₄	L ₅₁	R Puthi ₁	L ₇₅	Gohrabad ₃
L ₄	D Gali ₄	L ₂₈	Batharan ₅	L ₅₂	R Puthi ₂	L ₇₆	Hayam ₁
L ₅	D Gali ₅	L ₂₉	Batharan ₆	L ₅₃	R Puthi ₃	L ₇₇	Hayam ₂
L ₆	Jandali ₁	L ₃₀	Sanghar ₁	L ₅₄	Neelum ₁	L ₇₈	Hayam ₃
L ₇	Banjosa ₁	L ₃₁	Sanghar ₂	L ₅₅	Neelum ₂	L ₇₉	Galodass ₁
L ₈	Banjosa ₂	L ₃₂	Sanghar ₃	L ₅₆	Neelum ₃	L ₈₀	Galodass ₂
L ₉	Banjosa ₃	L ₃₃	Sanghar ₄	L ₅₇	Neelum ₄	L ₈₁	Galodass ₃
L ₁₀	Banjosa ₄	L ₃₄	Sanghar ₅	L ₅₈	Neelum ₅	L ₈₂	Damas ₁
L ₁₁	Banjosa ₅	L ₃₅	Sanghar ₆	L ₅₉	Hopar ₁	L ₈₃	Damas ₂
L ₁₂	Banjosa ₆	L ₃₆	Hillan ₁	L ₆₀	Hopar ₂	L ₈₄	Alladand ₁
L ₁₃	Dhok ₁	L ₃₇	Hillan ₂	L ₆₁	Hopar ₃	L ₈₅	Alladand ₂
L ₁₄	Dhok ₂	L ₃₈	Hillan ₃	L ₆₂	Doreal ₁	L ₈₆	Tamargar ₁
L ₁₅	Mera ₁	L ₃₉	Hillan ₄	L ₆₃	Doreal ₂	L ₈₇	Tamargar ₂
L ₁₆	Mera ₂	L ₄₀	Baidi ₁	L ₆₄	Gulmatee ₁	L ₈₈	Tamargar ₃
L ₁₇	Mera ₃	L ₄₁	Baidi ₂	L ₆₅	Gulmatee ₂	L ₈₉	Malakand ₁
L ₁₈	S Galli ₁	L ₄₂	Fateh pur	L ₆₆	Aysree ₁	L ₉₀	Malakand ₂
L ₁₉	S Galli ₂	L ₄₃	J. Sidan ₁	L ₆₇	Aysree ₂	L ₉₁	Malakand ₃
L ₂₀	S Galli ₃	L ₄₄	J. Sidan ₂	L ₆₈	Mushkoo ₁	L ₉₂	Malakand ₄
L ₂₁	S. Galli ₄	L ₄₅	Kalamola	L ₆₂	Mushkoo ₂	L ₉₃	G Puchar ₁
L ₂₂	Mallot ₁	L ₄₆	C. Pura ₁	L ₇₀	Bethrate ₁	L ₉₄	G Puchar ₂
L ₂₃	Mallot ₂	L ₄₇	C Pura ₂	L ₇₁	Bethrate ₂	L ₉₅	G Puchar ₃
L ₂₄	Batharan ₁	L ₄₈	C Pura ₃	L ₇₂	Bethrate ₄	L ₉₆	G Puchar ₄

Experimental design used: An augmented design was used for the experiment. The variety Sawat 1 was chosen as the reference for contrasting landraces and was planted five times in blocks after 20 landraces. The conventional procedure was used for planting a single row of the new selection alongside a row of the designated check variety at regular intervals. Subsequently, the yield of the new selection was compared with the nearest check variety following the methodology outlined by Federer & Ragavarao (1975).

Data recording of morphological traits: The data on morphological traits of common beans was recorded on traits such as germination percentage (%), number of leaves per plant, average plant height (cm), distance from cotyledon to primary leaves (cm), leaf area (cm²) per plant, number of branches per plant, days to pod formation, days to flowering initiation, days to flowering completion, number of pods per plant, pod width (cm), pod length (cm), 100 seed weight (g), number of seeds per pod, and seed yield per plant (g).

Data recording of biochemical traits: Total amounts of moisture, fat, fiber, ash, iron, and crude protein in seed were estimated using a method employed by Anon (1994). Carbohydrates were estimated using the method described by Anon (1990). The chlorophyll content of the leaf was determined by spectrophotometrically (Arnon, 1949). Total soluble solids were calculated as Anon (1994). A Folin Ciocalteu assay was used with gallic acid as standard to access total phenolic content (Singleton *et al.*, 1999). Antioxidant activity was accessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reagent following the method of Yang *et al.*, (2013).

Statistical analysis

The two-year data were pooled and analyzed to assess association among the morphological and biochemical traits and to estimate the diversity among common bean landraces (Anony., 1994). The software SPSS Version 20 was used to analyze data to examine the phenotypic correlation coefficients between morphological and biochemical traits separately. Landraces' average morphological and biochemical parameter values were standardized and utilized to calculate Euclidean distance between them. Using the computer program PAST, a dendrogram was constructed. Euclidean distance between landraces and the most significant character were determined using the cluster analysis.

Results

Simple correlation coefficient of morphological traits: Seed yield per plant showed a strong association with seed weight (1.000**). Leaf length showed association with leaf width (0.983) and leaf area (0.977), with leaf length and leaf width (0.975) coming in second and third, respectively (Table 2). The number of seeds per pod showed correlation with pod length (0.950**), seed weight (0.925**), and seed yield per plant (0.925**). Pod length had a significant

association (0.902**) with seed weight and seed yield per plant. A strong correlation (0.611**) was observed between pod width and seed weight and seed yield per plant. The number of pods per plant was significantly correlated with leaf length ($r = 0.215$), leaf breadth ($r = 0.239$), and plant leaf count ($r = 0.219$). Leaf length and width showed correlation with seed yield per plant (0.227* and 0.252*, respectively).

Cluster analysis of morphological traits: Figure 1 showed two-main clusters at a distance of 900. Cluster I includes two sub clusters, I-A and I-B. Cluster I-A further includes IA-1 and IA-2. IA-1 includes 13 landraces L8 (Banjosa₂ Poonch) and L82 (Damas₁ Gilgit), L53 (Raj Puthi₃ Muzaffarabad) and L1 (Devi Gali₁ Poonch), L91 (Malakand₃ Sawat) and L54 (Neelum₁), L11 (Banjosa₅ Poonch) and L18 (Sudhan Galli₁ Bagh), L59 (Hopar₁ Gilgit), L5 (Devi Gali₅ Poonch) and L2 (Devi Gali₂ Poonch) which were at the same distance. Landraces L14 (Dreak Dhok₂ Poonch) and L92 (Malakand₄ Sawat) were the outlier in this sub-cluster (IA-1) showing variability. Sub-cluster IA-2 consisted of 11 landraces L93 (Ghatt Puchar₁ Sawat) and L39 (Hillan₄ Haveli), L67 (Aysree₂ Gilgit) and L52 (Raj Puthi₂ Muzaffarabad), L57 (Neelum₄) and L10 (Banjosa₄ Poonch), L84 (Alladand₁ Sawat), L21 (Sudhan Galli₄ Bagh) and L87 (Tamargar₂ Sawat) that were morphologically similar, while L3 (Devi Gali₃ Poonch) and L47 (Charak Pura₂ Muzaffarabad) were outlier in the subgroup IA-2. Cluster I-B was further divided in two subgroups IB-1 and IB-2. IB-1 cluster includes 6 landraces L64 (Gulmatee₁ Gilgit) and L77 (Hayam₂ Gilgit), L24 (Batharan₁ Bagh) and L9 (Banjosa₃ Poonch), while L29 (Batharan₆ Bagh) and L 83 (Damas₂ Gilgit) were the outlier in this cluster. The cluster IB-2 includes 6 landraces L13 (Dreak Dhok₁ Poonch) and L17 (Hussain kot Mera₃ Poonch), L15 (Hussain kot Mera₁ Poonch) and L96 (Ghatt Puchar₄ Sawat) while L12 (Banjosa₆ Poonch) and L19 (Sudhan Galli₂ Bagh) were the outlier in this cluster.

Cluster II also comprised two sub-clusters II-A and II-B. The sub-cluster II-A is further divided into IIA-1 and IIA-2 sub-clusters. IIA-1 cluster includes three landraces L85 (Alladand₂ Sawat) and L20 (Sudhan Galli₃ Bagh) and L86 (Tamargar₁ Sawat), while sub-cluster IIA-2 includes 20 landraces L27 (Batharan₄ Bagh) and L38 (Hillan₃ Haveli), L33 (Sanghar₄ Bagh) and L36, (Hillan₁ Haveli) L76 (Hayam₁ Gilgit) and L37 (Hillan₂ Haveli), L26 (Batharan₃ Bagh) and L49 (Charak Pura₄ Muzaffarabad), L51 (Raj Puthi₁ Muzaffarabad) and L34 (Sanghar₅ Bagh), L75 (Gohrabad₃ Gilgit) and L46 (Charak Pura₁ Muzaffarabad), L80 (Galodass₁ Gilgit) and L61 (Hopar₃ Gilgit), L32 (Sanghar₃ Bagh) and L70 (Bethrate₁ Gilgit), while L42 (Fateh pur Haveli) and L48 (Charak Pura₃ Muzaffarabad), L62 (Dareal₁ Gilgit) and L74 (Gohrabad₂ Gilgit) were the outlier in this cluster. II-B subcluster is further divided into two subclusters IIB-1 and IIB-2. IIB-1 including 16 landraces L40 (Baidi₁ Haveli) and L41 (Baidi₂ Haveli), L28 (Batharan₅ Bagh) and L71 (Bethrate₂ Gilgit), L69 (Mushkoo₂ Gilgit) and L90 (Malakand₂ Sawat), L68 (Mushkoo₁ Gilgit) and L89 (Malakand₁ Sawat), L65 (Gulmatee₂ Gilgit) and L73 (Gohrabad₁ Gilgit), L72 (Bethrate₄ Gilgit) and L43 (Jabbi Sidan₁ Haveli) that were

related to each other, while L63 (Dareal₂ Gilgit) and L66 (Aysree₁ Gilgit), L60 (Hopar₂ Gilgit) and L95 (Ghatt Puchar₃ Sawat) showed high genetic variability. IIB-2 includes 21 landraces L23 (Mallot₂ Bagh) and Check (Sawat-1), L35 (Sanghar₆ Bagh) and L58 (Neelum₅), L45 (Kalamola Haveli) and L25 (Batharan₂ Bagh), L56 (Neelum₃) and L7 (Banjosa₁ Poonch), L81 (Galodass₃ Gilgit) and L50 (Charak Pura₅ Muzaffarabad), L55 (Neelum₂) and L22 (Mallot₁ Bagh), L88 (Tamargar₃ Sawat) and L44 (Jabbi Sidan₂ Haveli), L78 (Hayam₃ Gilgit) and L31 (Sanghar₂ Bagh) that were related to each other, while L16 (Hussain kot Mera₂ Poonch), L4 (Devi Gali₄ Poonch) and L94 (Ghatt Puchar₂ Sawat), L6 (Jandali₁ Poonch) and L30 (Sanghar₁ Bagh) showed diversity.

Principal component analysis: Principal component analysis (Table 2) showed 5 PCs with Eigen values more than 1. First PC accounted for 29.73% variability, second 16.70%, third 11.60%, fourth 7.62%, and fifth 6.90%. All the significant PCs showed a cumulative variance of 72.55%.

Correlation coefficients of biochemical traits:

Antioxidant activity showed a maximum correlation with moisture content (0.733**), ascorbic acid (0.425**), protein content (0.878**) and crude fiber (0.191**). Crude fiber displayed association with moisture percentage (0.667**), TSS (0.748**), protein content (0.830**) and crude fat (0.181**). Crude fat showed positive and highly significant correlation with TSS (0.568) and protein content (0.165*). The moisture percentage and TSS have shown a positive and significant relationship (0.212*). The strong positive association was observed between ash and crude fiber (0.338**) and phenolics (0.831**). Phenolic content and iron content were highly and positively correlated (0.101*). The ash content and crude fat (-0.113**) showed a strong negative correlation (Table 3).

Cluster analysis of biochemical traits: At the genetic distance of 900, the dendrogram prepared for the assessment of biochemical diversity among landraces depicted two main clusters (I and II) (Fig. 2). Cluster I included two subclusters I-A and I-B. Cluster I-A further included IA-1 and IA-2. The subcluster IA-1 included 8 landraces L49 (Charak Pura₄ Muzaffarabad) and L45 (Kalamola Haveli), L38 (Hillan₃ Haveli) and L18 (Sudhan Galli₁ Bagh), L75 (Gohrabad₃ Gilgit) and L35 (Sanghar₆ Bagh). Landrace L16 (Hussain kot Mera₂ Poonch) and L63 (Dareal₂ Gilgit) were the outliers in this subcluster (IA-1) showing variations. Subcluster IA-2 consisted of 15 landraces L56 (Neelum₃) and L62 (Dareal₁ Gilgit), L41 (Baidi₂ Haveli) and L72 (Bethrate₄ Gilgit), L10 (Banjosa₄ Poonch) and L22 (Mallot₁ Bagh), L8 (Banjosa₂ Poonch) and L82 (Damas₁ Gilgit), L4 (Devi Gali₄ Poonch) and L11

(Banjosa₅ Poonch), L6 (Jandali₁ Poonch), L9 (Banjosa₃ Poonch) and L13 (Dreak Dhok₁ Poonch) that were similar to each other in terms of their biochemical traits, while L96 (Ghatt Puchar₄ Sawat) and L83 (Damas₂ Gilgit) were outliers for this subgroup IA-2. Cluster I-B was further divided into two subgroups IB-1 and IB-2. IB-1 cluster includes 13 landraces L65 (Gulmatee₂ Gilgit) and L66 (Aysree₁ Gilgit), L24 (Batharan₁ Bagh) and L27 (Batharan₄ Bagh), L28 (Batharan₅ Bagh) and L48 (Charak Pura₃ Muzaffarabad), L57 (Neelum₄) and L71 (Bethrate₂ Gilgit), L5 (Devi Gali₅ Poonch), L23 (Mallot₂ Bagh) and L21 (Sudhan Galli₄ Bagh), while L26 (Batharan₃ Bagh) and L25 (Batharan₂ Bagh) were outliers for this cluster. IB-2 cluster includes 14 landraces L94 (Ghatt Puchar₂ Sawat) and L88 (Tamargar₃ Sawat), L67 (Aysree₂ Gilgit) and L81 (Galodass₃ Gilgit), L58 (Neelum₅) and L64 (Gulmatee₁ Gilgit), L69 (Mushkoo₂ Gilgit) and L90 (Malakand₂ Sawat), L42 (Fateh pur Haveli) and L68 (Mushkoo₁ Gilgit), L89 (Malakand₁ Sawat) and L50 (Charak Pura₅ Muzaffarabad), while L7 (Banjosa₁ Poonch) and L87 (Tamargar₂ Sawat) were outliers.

Cluster II was further divided into subcluster II-A and II-B. Similarly, II-A subcluster was further divided into II A-1 and II A-2 subclusters. II A-1 cluster includes 12 landraces L14 (Dreak Dhok₂ Poonch) and L59 (Hopar₁ Gilgit), L73 (Gohrabad₁ Gilgit) and L33 (Sanghar₄ Bagh), L91 (Malakand₃ Sawat) and L1 (Devi Gali₁ Poonch), Check (Sawat-1) and L2 (Devi Gali₂ Poonch), L92 (Malakand₄ Sawat) and L34 (Sanghar₅ Bagh), while L15 (Hussain kot Mera₁ Poonch) and L77 (Hayam₂ Gilgit) were outliers within this subgroup. Subcluster IIA-2 includes 14 landraces L37 (Hillan₂ Haveli) and L53 (Raj Puthi₃ Muzaffarabad), L76 (Hayam₁ Gilgit) and L44 (Jabbi Sidan₂ Haveli), L28 (Batharan₆ Bagh) and L40 (Baidi₁ Haveli), L70 (Bethrate₁ Gilgit) and L36 (Hillan₁ Haveli), L39 (Hillan₄ Haveli) and L74 (Gohrabad₂ Gilgit), L84 (Alladand₁ Sawat) and L65 (Neelum₂), while L11 and L79 (Galodass₁ Gilgit) were outliers. II-B subcluster is further divided into two subcluster IIB-1 and IIB-2. IIB-1 includes 15 landraces, L43 (Jabbi Sidan₁ Haveli) and L19 (Sudhan Galli₂ Bagh), L47 (Charak Pura₂ Muzaffarabad) and L52 (Raj Puthi₂ Muzaffarabad), L51 (Raj Puthi₁ Muzaffarabad) and L31 (Sanghar₂ Bagh), L30 (Sanghar₁ Bagh) and L17 (Hussain kot Mera₃ Poonch), L46 (Charak Pura₁ Muzaffarabad) and L54 (Neelum₁), L3 (Devi Gali₃ Poonch), L93 (Ghatt Puchar₁ Sawat) and L95 (Ghatt Puchar₃ Sawat) that were related to each other, while L12 (Banjosa₆ Poonch) and L61 (Hopar₃ Gilgit) displayed variation in this cluster. IIB-2 subcluster included 7 landraces. L32 (Sanghar₃ Bagh) and L60 (Hopar₂ Gilgit), L80 (Galodass₂ Gilgit), L86 (Tamargar₁ Sawat) and L20 (Sudhan Galli₃ Bagh) while L78 (Hayam₃ Gilgit) and L85 (Alladand₂ Sawat) were the outliers in this subcluster.

Table 2. Eigen values for 15 morphological traits of *Phaseolus vulgaris* L. landraces collected from Azad Kashmir, Northern Areas and Sawat.

PC	1	2	3	4	5
Eigen value	4.46	2.50	1.73	1.14	1.03
% Variance	29.73	16.70	11.60	7.62	6.90
Cumulative eigen value	29.73	46.43	58.03	65.65	72.55

Table 3. Correlation coefficients estimated from eleven biochemical traits of common beans landraces collected from Azad Kashmir, Northern Areas and Sawat.

	Moist	TSS	Pheno	VIT	Protein	Fat	Fiber	Ash	Antioxi	Chloro	Iron
Moist	1										
TSS	0.212*	1									
Pheno	0.949	0.991	1								
VIT	-0.605	0.724	-0.245	1							
Protein	0.638	0.236	0.618	0.723	1						
Fat	0.256	0.568*	0.852	-0.177	0.165*	1					
Fiber	0.667**	0.748**	0.370	0.689	0.830**	0.181**	1				
Ash	0.136	0.499	0.831**	0.083	0.426	-0.113**	0.338**	1			
Antioxi	0.734**	0.208	0.760	0.425**	0.878**	0.216	0.191**	0.235	1		
Chloro	-0.618	-0.527	0.538	0.982	0.826	0.227	-0.910	0.297	0.827	1	
Iron	0.565	-0.234	0.101*	0.032	-0.021	-0.112	0.203	0.050	-0.116	0.000	1

** Significant at the 0.01 level (2-tailed); * Significant at the 0.05 level (2-tailed)

Moist= Moisture percentage, TSS= Total soluble solids, Pheno= Phenolic content, VIT= Ascorbic acid, Protein= Protein content, FAT = Crude fat, Fiber = Crude fiber, Ash= Total ash content, Anti = Antioxidant activity, Chloro= Chlorophyll content, Iron = Total iron content

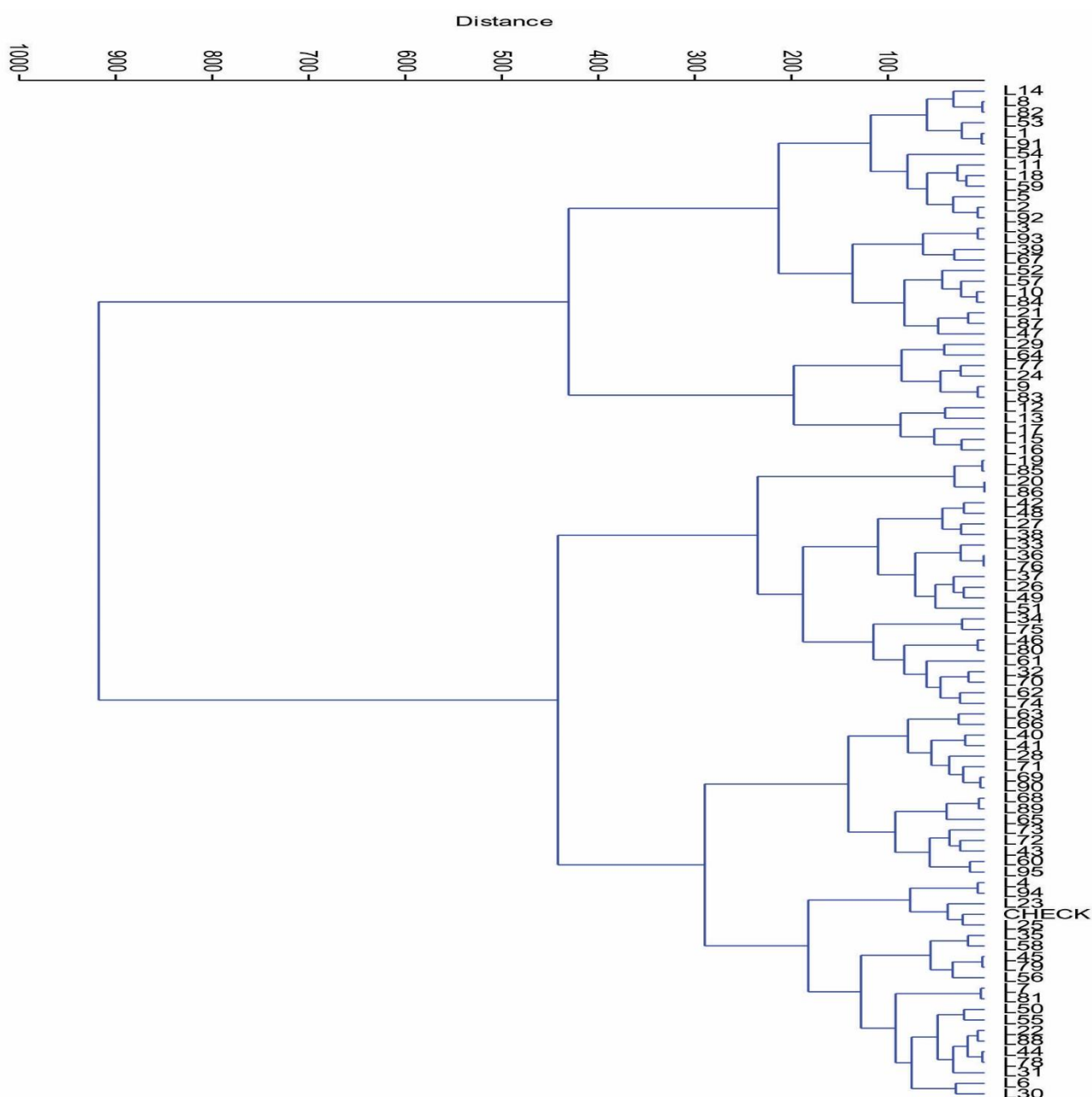


Fig. 1. A dendrogram showing grouping in 96 landraces of common beans collected from Azad Kashmir, Northern Areas and Sawat.

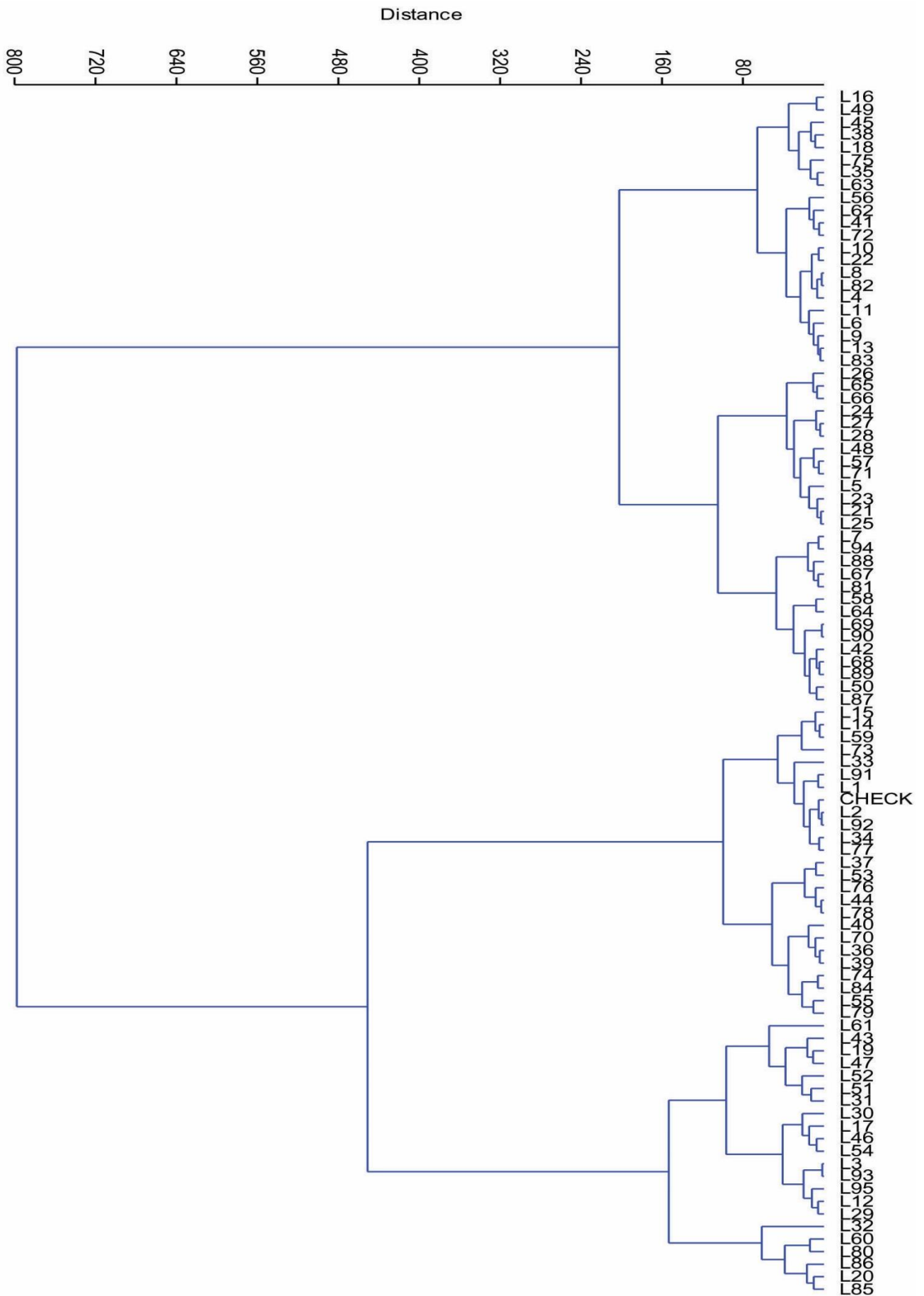


Fig. 2. A dendrogram based on biochemical parameters for the assessment of diversity in *Phaseolus vulgaris* L. landraces collected from Azad Kashmir, Northern Areas and Swat.

Principal component analysis: Principal component analysis (Table 4) showed 4 PCs with Eigen values higher than 1. The first PC accounted for 31.80%, second 13.10%, third 12.60% and fourth 9.80%. All the significant principal components accounted for 67.30% genotypic variability. The Eigen values ranged from 1.1-3.5, respectively.

The highest positive factor loading for PC 01 was explained by antioxidant activity (0.9427), followed by TSS (0.9312), phenolics (0.8983), moisture percentage (0.8085), chlorophyll content (0.373), ascorbic acid (0.29), crude fiber

(0.192), crude fat (0.012), and total ash content (0.012), while the highest negative factor loading was due to iron (-0.09571) and protein (-0.05059), respectively (Fig. 3).

Iron (0.7124) contributed the most positive load for PC 02, followed by chlorophyll content (0.4645), protein (0.3633), crude fat (0.2836), TSS (0.1265), phenolics (0.0125), and total ash content (0.0162), while crude fiber (-0.5512) explained the most negative factor loading, followed by ascorbic acid (-0.2215), moisture percentage (-0.1278), and antioxidant activity (-0.0566) respectively (Fig. 4).

Table 4. Eigen values of 11 biochemical traits of *Phaseolus vulgaris* L. landraces collected from Azad Kashmir, Northern Areas and Sawat.

PC	1	2	3	4
Eigen value	3.50	1.43	1.40	1.10
% Variance	31.80	13.10	12.60	9.80
Cumulative eigen value	31.80	44.90	57.50	67.30

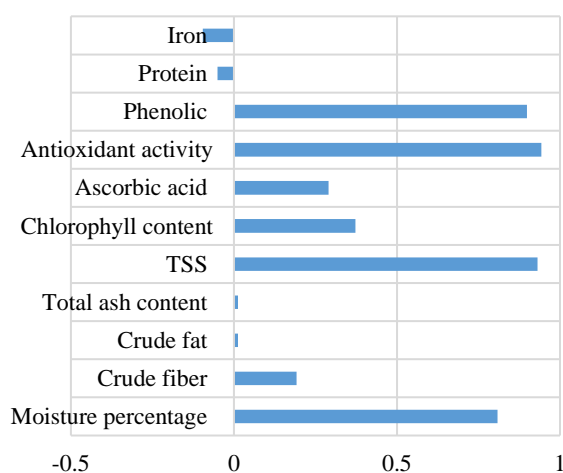


Fig. 3. Factor loadings for PC1 for biochemical traits for the characterization of common bean land races.

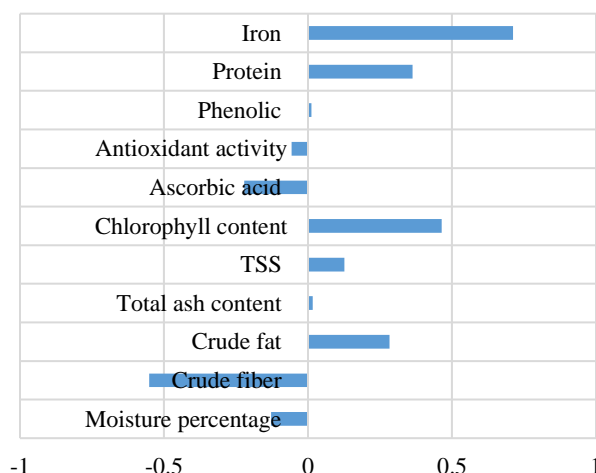


Fig. 4. Factor loadings for PC2 for biochemical traits for the characterization of common bean land races.

Discussion

The correlation coefficient analysis for morphological traits indicates a significant association between grain yield per plant and leaf width as well as leaf length. This relationship is substantiated by the fact that increased leaf width and length lead to a greater leaf area, fostering heightened biochemical processes such as photosynthesis, consequently resulting in higher yields per plant. Basaran *et al.*, (2013) depicted a strong positive correlation of leaf length and leaf width in legume crops. Seed per pod of the plant was negatively correlated by 100 seed weight. Ghatti *et al.*, (2011) reported a negative relationship between these two factors. Similarly, Tekeli *et al.*, (2007) observed a positive correlation of number of seeds per pod with pod length.

Leaf length was strongly associated with leaf width (correlation coefficient of 0.983) and leaf area (correlation coefficient of 0.977). Additionally, there had been a slightly weaker association between leaf length and leaf width (0.975). These correlations suggest that plants with longer leaves tend to have wider leaves and larger leaf areas. This could be due to genetic factors or environmental conditions influencing leaf growth. This trait shows correlations with

pod length (0.950), seed weight (0.925), and seed yield per plant (0.925). These correlations indicate that pods with greater lengths tend to contain more seeds, which in turn contributes to higher seed weights and overall seed yield per plant. These findings could be valuable in understanding factors influencing seed production efficiency (Nogueira *et al.*, 2021). The longer pods tend to contain heavier seeds and contribute more to the overall yield per plant. Understanding this relationship could aid in selecting plants with desirable pod characteristics for maximizing yield (Sheibanirad *et al.*, 2022).

The number of pods per plant showed weak correlations with leaf length (0.215), leaf width (0.239), and plant leaf count (0.219). These correlations suggest that plants with certain leaf characteristics or higher leaf counts may produce more pods. However, these correlations are relatively weak compared to others mentioned, indicating that factors other than leaf traits likely play a more significant role in determining pod production (Ali *et al.*, 2020). The number of pods per plant showed weak correlations with leaf length (0.215), leaf width (0.239), and plant leaf count (0.219). These correlations suggest that plants with certain leaf characteristics or higher leaf counts may produce more

Pods. However, these correlations are relatively weak compared to others mentioned, indicating that factors other than leaf traits likely play a more significant role in determining pod production (Ligarreto & Martínez, 2014).

The correlations identified significant relationships between various components and their impact on antioxidant activity. For instance, the strong positive correlation between antioxidant activity and moisture content, ascorbic acid, protein content, and crude fiber suggests that these factors may play crucial roles in enhancing antioxidant properties (de Andrade *et al.*, 2022). Similarly, the associations observed between crude fiber and other components like moisture percentage, TSS, and protein content indicate the interdependence of these factors in determining the overall quality of the sample (Basaran *et al.*, 2013; Arruda *et al.*, 2018). Furthermore, the negative correlation between ash content and crude fat suggests a potential antagonistic relationship between these two components. Such insights can be valuable in understanding how different factors interact within a sample and how they collectively contribute to its antioxidant activity (Sharifi *et al.*, 2011). These results are consistent with the previous findings, indicating consistency across different research efforts (López-Alcocer *et al.*, 2017). This suggests that the observed correlations may have broader applicability and could potentially be generalized to similar contexts or samples (Nogueira *et al.*, 2021).

Overall, these results provide valuable insights into the factors influencing antioxidant activity in the samples under investigation. Understanding these relationships can aid in optimizing processes or formulations to enhance the antioxidant properties of products, thus potentially offering health benefits to consumers.

Conclusion

The study indicates that several landraces of common bean exhibit maximum diversity in morphological, biochemical, and molecular traits, suggesting their potential for further breeding and high-yield production. Notably, landraces such as L3, L12, L16, L19, L25, L26, L41, L42, L47, L56, L62, L63, L66, and L93 demonstrate superior performance in both morphological and biochemical traits under natural field conditions. The findings serve as a benchmark for future studies, while also highlighting the need to explore additional diversity. The landraces so identified in this investigation warrant further studies for their potential in enhancing common bean breeding and production.

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