

## ASSESSMENT OF GENETIC DIVERSITY IN PEA (*PISUM SATIVUM* L.) LANDRACES BASED ON PHYSICO-CHEMICAL AND NUTRITIVE QUALITY USING CLUSTER AND PRINCIPAL COMPONENT ANALYSIS

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### Abstract

Field pea (*Pisum sativum* L.) is an important protein-rich pulse crop produced globally. Genetic and environmental factors lead to a variation in protein content and also influence the quality of seed of dry pea (*Pisum sativum* L.). The area and production for dry peas are increasing but knowledge is lacking on how the pea landraces differ in the seed quality. The purpose of the present study was biochemical characterization of 46 pea landraces in order to evaluate the moisture content, fat, fiber, protein, ash, phenolic content, carbohydrates, pH, chlorophyll content and TSS. The data obtained after biochemical analysis were subjected to statistical analysis. The cluster analysis indicated the probable association within 46 pea landraces. Landraces L7, L13, L19, L25, L26, L27, L30 and A43 were showing variation in term of analyzed biochemical traits. Correlation coefficients among all the biochemical traits were computed. Ash content was showing maximum positive and highly significant correlation with moisture content (0.583\*\*), phenolic content (0.76\*\*) and fat (0.371\*\*). Fiber was showing highly significant and positive correlation with three characters which were: protein (0.455\*\*), total soluble solid (0.437\*\*), and fat (0.399\*\*). Positive and highly significant association was found among carbohydrate and pH (0.418\*\*). Fat content was showing positive and significant correlation with moisture content (0.316\*), protein (0.313\*) and total soluble solid (0.312\*), respectively. These findings can be used to guide future breeding studies and germplasm management of these pea landraces. Based on our findings, it is recommended that pea landraces which performed well in the experiment and bear bright future prospects should be considered in designing future hybridization programs to elucidate the correlation among various biochemical aspects at molecular level among different pea genotypes.

**Key words:** Diversity, Principal component analysis, Cluster analysis, Pea landraces.

### Introduction

Pea (*Pisum sativum* L.) is grown as leguminous crop in many regions of the world. Its production ranks fifth in the world after soybean, peanut, drybean, and chickpea. Field pea is mainly used as a protein source, as it has a relatively rich and unique protein profile, different from other natural protein sources (Anon., 2010). Pea proteins are esteemed for its high digestibility (90-95%) and have fewer allergenic-responses and no health controversy also. It is gluten-free, little in the sulfurous amino-acids (cysteine and methionine), more in lysine, an important amino-acid for human health (Pownall *et al.*, 2010). Field peas have 23% protein, on the other hand novel pea genotypes have around 30% protein and advanced pea lines with 30% protein (Bing, 2007, 2010a, 2010b, 2012).

Dried-peas are frequently used within soup and also directly consumed (Nawab *et al.*, 2008). The best pH ranged for pea production is 5.5-7.0. The fat material of peas is normally very low with no cholesterol at all (Anon., 2015). Most important part of beans, peas, and lentils is fiber. Fiber is an ingredient of plant foods that cannot be digested. Legumes contain 7 g of dietary fiber and good sources of various vitamins and minerals, such as folate, Fe, K, and mg (Anon., 2015).

Peas are complete source of nutrition in light of the fact that their grains are full of protein (27.8%), complex-sugars (42.65%), nutrients, minerals, fibers

and antioxidant-compounds (Urbano *et al.*, 2003; 2005). It ranges from 15.5-39.7% (Davies *et al.*, 1985; Bressani & Elias, 1988). Fresh peas have per 100 g: 44 calories, 75.6% water, 6.2 g protein, 0.4 g fat, 16.9 g carbohydrate, 2.4 g crude-fiber and 0.9 g ash, while dried peas contain: 10.9% water, 22.9% protein, 1.4% fat, 60.7% starch, 1.4% crude-fiber, and 2.7% ash (Duke, 1981; Hulse, 1994).

TSS is the most essential parameter that helps to designate sugariness of fresh and processed horticultural food yield. Peas contain large quantities of water in proportion to their weight (Xue & Yang, 2009). They generally contain 90-96% water. Water content has important effect on pea's quality characteristics (Liu *et al.*, 2007). Keeping in view the biochemical importance of peas, the present study was conducted to investigate genetic diversity through physico-chemical and quality related traits in various pea landraces.

### Material and Methods

The present study was conducted during 2015-2016 at University of the Poonch, Rawalakot. The landraces were collected from different locations of district Poonch (Banjosa, Devi Gali, Jandali, Dhoke, and Rawalakot), Bagh (Harigal, Sudhan Gali, Mallot, and Dirkot), Sudhnoti (Trarkhal, Mang, Bloch, and Plandari), Mirpur and NARC (PGRI) Islamabad. Forty six landraces of field pea were used to assess the biochemical diversity in pea germplasm (Table 1).

**Table 1. List of different landraces used for biochemical study.**

Landraces	Location	Landraces	Location
CH	Meteor	L-24	Sudhan Gali
L-2	Banjosa	L-25	Sudhan Gali
L-3	Banjosa	L-26	Mallot
L-4	Devi Gali	L-27	Mallot
L-5	Devi Gali	L-28	Dirkot
L-6	Jandali	L-29	Dirkot
L-7	Jandali	L-30	Mirpur
L-8	Dhoke	L-31	Mirpur
L-9	Dhoke	L-32	Mirpur
L-10	Rawalakot	L-33	Mirpur
L-11	Rawalakot	L-34	Mirpur
L-12	Rawalakot	A-35	NARC
L-13	Rawalakot	A-36	NARC
L-14	Trarkhal	A-37	NARC
L-15	Trarkhal	A-38	NARC
L-16	Mang	A-39	NARC
L-17	Mang	A-40	NARC
L-18	Bloch	A-41	NARC
L-19	Bloch	A-42	NARC
L-20	Plandari	A-43	NARC
L-21	Plandari	A-44	NARC
L-22	Harigal	A-45	NARC
L-23	Harigal	A-46	NARC

**Determination of moisture, fat, fiber, protein, carbohydrate and ash contents:** The total moisture content, fat, fiber, ash content and protein contents were estimated by the technique earlier used by the Anon., (1994). The carbohydrates were estimated by difference method as described by Anon., (1990).

**Leaf chlorophyll content, total soluble solids and total phenolic content:** Leaf chlorophyll content was determined spectrophotometrically (Arnon, 1949). TSS was estimated by means of refractometer (Anon., 1994). The total phenolic content was estimated by a Folin-ciocalteu assay using gallic-acid as usual (Singleton *et al.*, 1999).

**Statistical analysis:** The collected data were analyzed to determine the phenotypic correlation coefficients among

various parameters using SPSS 16.1. Mean values of the bio-chemical character for landraces were standardized and used for computing Euclidean distance among them. Dendrogram was formed by using computer software PAST. PCA and cluster-analyses were used to find Euclidean distance among landraces and to distinguish the relation to the most important character.

## Results and Discussion

Descriptive statistics was used to study different biochemical traits among 46 pea landraces. High coefficient of variance was observed for phenolic (33.2%) followed by total soluble solids (28.65 %), chlorophyll (26.27%), ash (19.00%), fiber (16.12%), protein (8.17%), fat (7.91%), moisture (7.91%), pH (6.96%) and carbohydrates (3.20%) (Table 2).

### Cluster analysis

**Average linkage distance among pea landraces:** The cluster-analysis indicated the probable relationship among 46 pea's landraces. Cluster 1 included 2 sub-clusters 1A and 1B. Cluster 1 comprised of fifteen landraces. In sub-cluster 1A, landraces L16 and L20, L18 and L35, L11 and L12 were significantly correlating each other, while landraces L-13 and L-21 were outliers in this sub-cluster. Sub-cluster 1B was comprised of landraces, L23 and L22, L8 and L33 present at same linkage distance showing maximum similarity in term of traits studied, while L25, L7 and L19 were outliers.

Cluster II comprised of fourteen landraces and was sub-divided into two sub-clusters, IIA and IIB. Sub-cluster IIA comprised of landraces, L15 and L38, Check and L2, L5 and A42, L17 and L28 at same linkage distance, whereas landraces L27 and L31 were outliers for the cluster. While sub-cluster IIB comprised of landraces, L14 and L30, L24 and L34 were correlating each other at same linkage distance, respectively.

Cluster III was further sub-divided into two sub-clusters, IIIA and IIIB. Sub-cluster IIIA consisted of landraces L3 and L4 were at same linkage distance. Sub-cluster IIIB was composed of L9 and L29, L36 and A46, L10 and A40, A41 and A44, A39 and A45 were significantly correlating to each other at same linkage distance. Landraces L37, L32, L26 and A43 were outliers for this cluster, showing variation (Figs. 1 and 2).

**Table 2. Descriptive statistics for different biochemical traits among 46 pea landraces.**

	Moisture (%)	TSS (%)	Phenolic (mg/g)	pH	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)	Chlorophyll (mg/g)
Minimum	7.10	1.10	1.16	5.30	17.67	1.21	0.83	2.10	59.32	11.10
Maximum	9.90	2.90	3.91	6.90	27.01	1.62	1.98	4.40	69.59	33.92
Sum	377.0	89.20	105.9	285.0	1058.2	65.06	59.72	141.4	2959.1	1039.8
Mean	8.20	1.94	2.30	6.20	23.01	1.41	1.30	3.07	64.33	22.61
Std. Error	0.10	0.08	0.11	0.06	0.28	0.02	0.03	0.09	0.30	0.88
Variance	0.42	0.31	0.59	0.19	3.53	0.01	0.04	0.34	4.25	35.26
Std. Dev	0.65	0.56	0.77	0.43	1.88	0.11	0.21	0.58	2.06	5.94
CV (%)	7.91	28.65	33.27	6.96	8.17	7.91	16.12	19.00	3.20	26.27

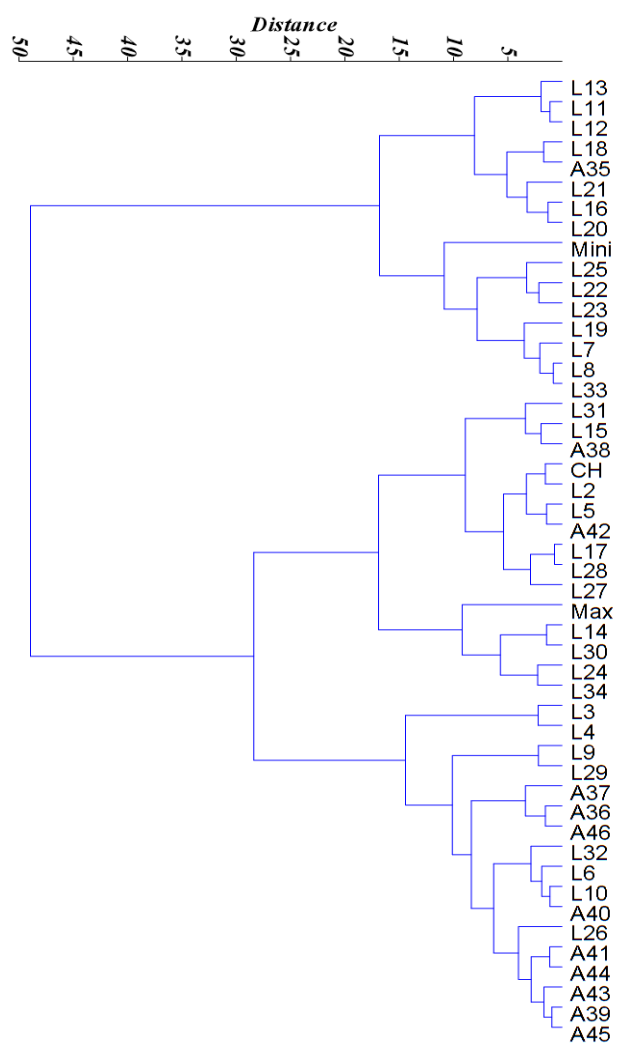


Fig. 1. Dendrogram based on average linkage distance for 46 pea landraces.

**Simple correlation coefficient:** Table 3 represents the correlation coefficients among all the biochemical traits. Ash was showing maximum positive and highly significant correlation with moisture content (0.583\*\*), phenolic content (0.76\*\*) and fat (0.371\*\*). Fiber was showing highly significant and positive correlation with three characters i.e., protein (0.455\*\*), total soluble solid (0.437\*\*) and fat (0.399\*\*). Highly significant correlation was found among carbohydrate and pH (0.418\*\*). Fat showed positive and significant correlation with moisture content (0.316\*), protein (0.313\*) and total soluble solid (0.312\*). Maximum positive and significant correlation was recorded between chlorophyll content and phenolic contents (0.342\*), whereas maximum positive and significant correlation was observed for total soluble solids and moisture content (0.338\*). A strong negative and highly significant correlation was observed between carbohydrate and protein (-0.447\*\*).

As starch accounts for variations in the crude protein of field-peas, stated by Holl & Vose (1980); Reichert & MacKenzie (1982); Bastianelli *et al.*, (1998) and Wang *et al.*, (2008). This varies in the associations among the ingredients for the period of maturation might be possible reason for describes correlations between further nutrients like CP and sugar. Reichert & MacKenzie (1982) and Nikolopoulou *et al.*, (2006) found stronger correlations among CP and ash and further correlations among ash and EE, ash and starch, as well as EE and starch. Hence, correlations are not suitable for predicting the composition of harvested crops due to a high changeability of hereditary and environmental influences on the stage of maturity (Weber *et al.*, 2005; Gutierrez *et al.*, 2007; Gallardo *et al.*, 2008).

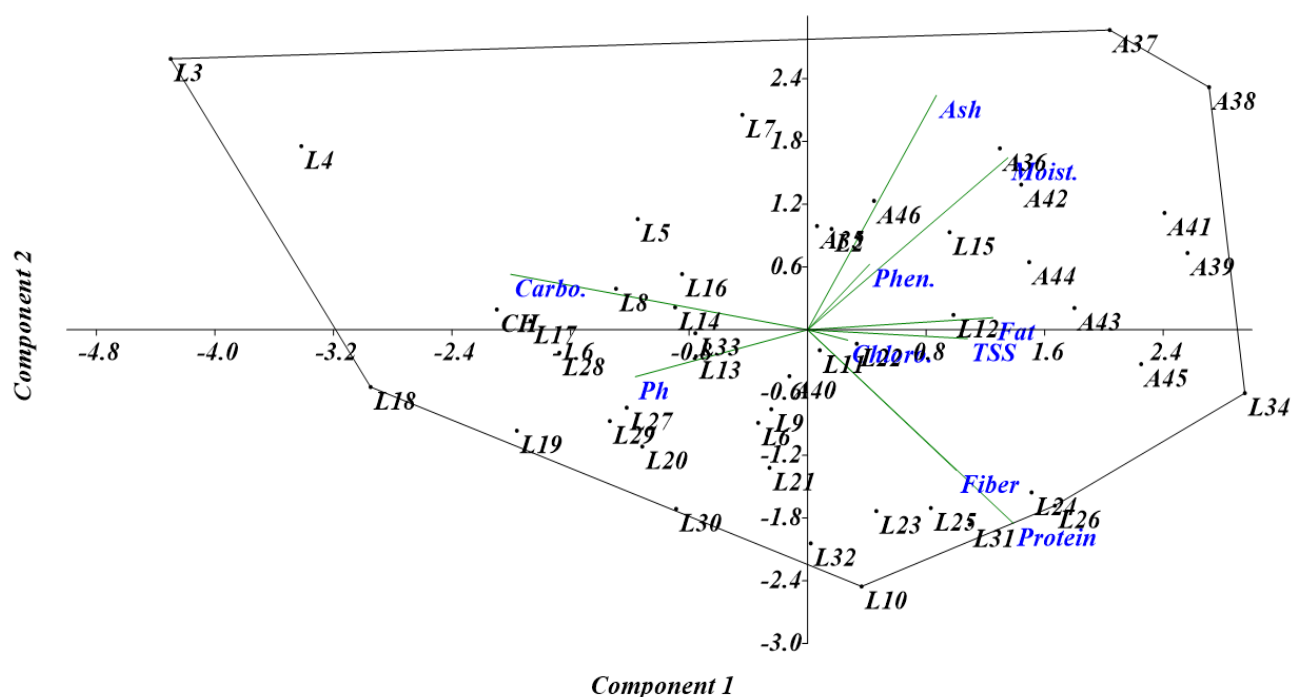


Fig. 2. Scatter Biplot diagram of 46 landraces of peas on the basis of biochemical characterization.

**Table 3. Correlation matrix for ten studied traits.**

Traits	Moisture	TSS	Phenolic	pH	Protein	Fat	Fiber	Ash	Carbohydrate	Chlorophyll
Moisture (%)	1.000									
TSS (%)	0.338*	1.000								
Phenol (mg/g)	0.209	0.076	1.000							
Ph	-0.208	-0.064	0.174	1.000						
Protein (%)	0.141	0.251	0.226	0.050	1.000					
Fat (%)	0.316*	0.312*	0.113	0.017	0.313*	1.000				
Fiber (%)	0.273	0.437**	0.195	0.081	0.455**	0.399**	1.000			
Ash (%)	0.583**	0.220	0.376**	0.039	0.010	0.371**	0.041	1.000		
Carbohydrate (%)	-0.143	-0.041	0.026	0.418**	-0.447**	-0.127	0.037	-0.043	1.000	
Chlorophyll (mg/g)	0.203	-0.019	0.342*	0.154	0.284	0.219	0.143	0.245	0.027	1.000

\*\*Correlation is highly significant at 0.01 level (2-tailed); \*Correlation is significant at 0.05 level (2-tailed)

Where; TSS = Total soluble solid

**Table 4. The Eigen-values for 10 traits of *Pisum sativum* landraces.**

PC	1	2	3	4
Eigenvalue	2.71	1.71	1.47	1.00
% variance	27.06	17.06	14.71	10.03
Cumulative variance (%)	27.06	44.12	58.83	68.86

**Principal component analysis (PCA):** PCA simplify the complex data by breaking it into smaller component called principal components. PCA was performed based on ten characters. The first four principal components (PC) accounted for 68.86% of the variation (27.06, 17.06, 14.71 and 10.03 for PC1, PC2, PC3 and PC4, respectively). The highest Eigen value was 2.71, whereas the least was 1.00. Wang *et al.*, (2015) has demonstrated 41% of differentiation among populations in 266 grass pea accessions (Table 4).

**Factor loadings for various components:** Figure 3 shows the factor loadings for ten biochemical traits in pea genotypes. In factor loading for PC 01 maximum positive load was contributed by protein content (0.3698) followed by moisture content (0.3637), fat (0.3379), total soluble solids (0.2836), fiber (0.2641), ash content (0.2389), phenolic content (0.1212) and chlorophyll content (0.111), while maximum negative load was explained by carbohydrates (-0.5372) followed by pH (-0.3066). So this factor is known as protein factor.

Figure 4 shows the factor loadings for ten biochemical traits in peas genotypes. In factor loading for PC 02 maximum positive load was contributed by ash (0.6027) followed by moisture (0.4054), phenolic content (0.2358), chlorophyll content (0.1458), carbohydrates (0.1179) and fat (0.0151), while maximum negative load was explained by protein (-0.4568) followed by fiber (-0.3961), total soluble solids (-0.0989) and pH (0.0770). So this factor is known as ash factor.

Figure 5 shows the factor loadings for ten biochemical traits in pea genotypes. In factor loading for

PC 03 maximum positive load was contributed by chlorophyll content (0.5436) followed by phenolic content (0.4474), protein content (0.3326), and pH (0.2755), while maximum negative load was explained by total soluble solids (-0.4234), followed by moisture content (-0.2278), carbohydrates (-0.2269), fiber (-0.1686), fat (-0.0816) and ash (-0.006). This factor is known as chlorophyll factor.

Figure 6 shows the factor loadings for ten biochemical traits in peas genotypes. In factor loading for PC 04 maximum positive load was contributed by pH (0.5468) followed by fat (0.4307), fiber (0.3943), phenolic content (0.3358), total soluble solids (0.3158), carbohydrates (0.1888), ash (0.1649), and chlorophyll (0.0107), while maximum negative load was explained by protein (-0.2182) and moisture (-0.1947). So this factor is known as pH factor.

Figure 7 shows the factor loadings for ten biochemical traits in peas genotypes. In factor loading for PC 05 maximum positive load was contributed by phenolic content (0.6306), followed by total soluble solids (0.272), fiber (0.1629), moisture content (0.0700), protein (0.0254) and carbohydrate (0.0101), while maximum negative load was explained by fat (-0.6352) followed by chlorophyll content (-0.2793), pH (-0.1074) and ash (-0.0554). This factor is known as phenolic factor.

Figure 8 shows the factor loadings for six quantitative traits in peas genotypes. In factor loading for PC 06 maximum positive load was contributed by chlorophyll content (0.628) followed by fiber (0.4159), carbohydrates (0.3059), moisture (0.0986), and phenolic content (0.0459), while maximum negative load was explained by pH (-0.4097) followed by ash (-0.2856), protein content (-0.2773), fat (-0.0280) and total soluble solids (-0.0069). This factor is known as chlorophyll factor.

Scree plot diagram constructed for ten traits showed that out of 10, six traits were showing the Eigen value greater than 0.7. Eigen value greater than 0.7 (Jolif cutoff) indicated that six component or traits were showing maximum variance in term of variability (Fig. 9).

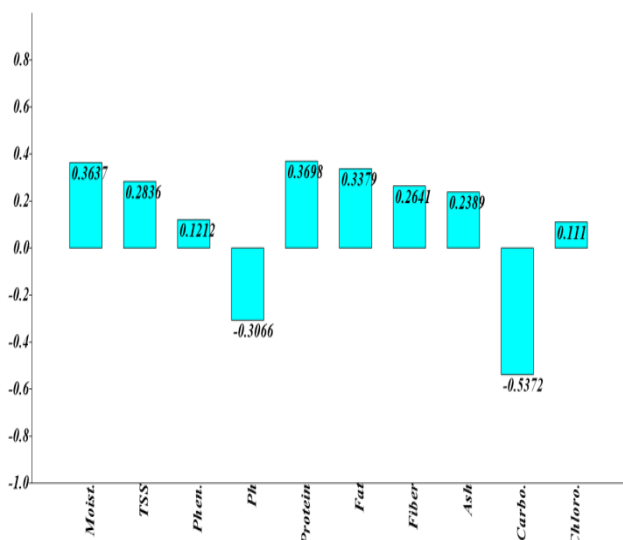


Fig. 3. Factor loadings for PC1 for biochemical traits of 46 pea landraces.

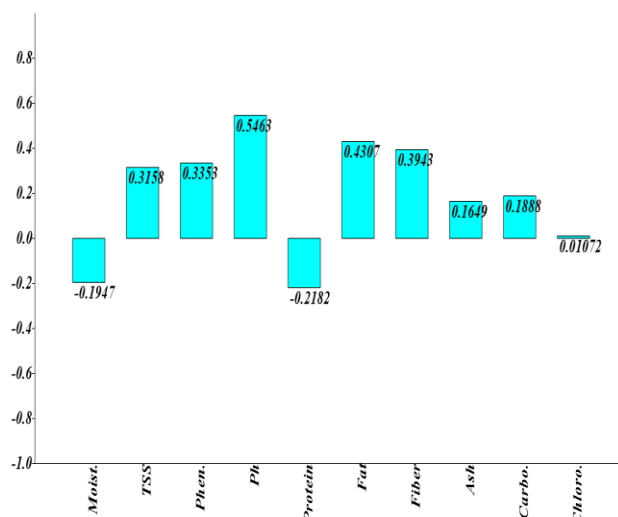


Fig. 6. Factor loadings for PC4 for biochemical traits of 46 pea landraces.

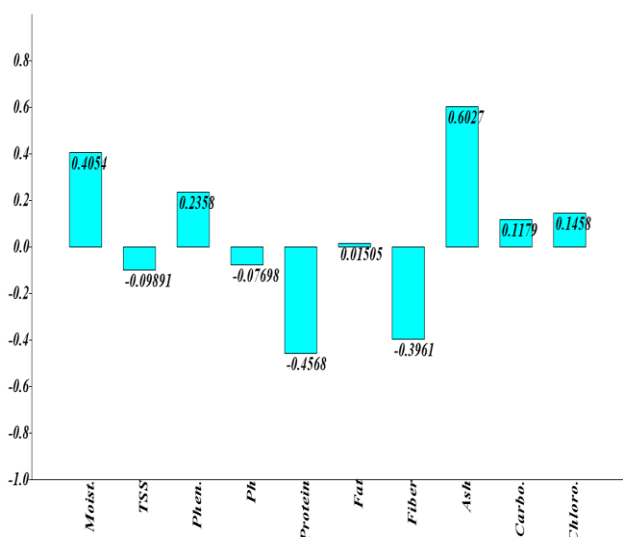


Fig. 4. Factor loadings for PC2 for biochemical traits of 46 pea landraces.

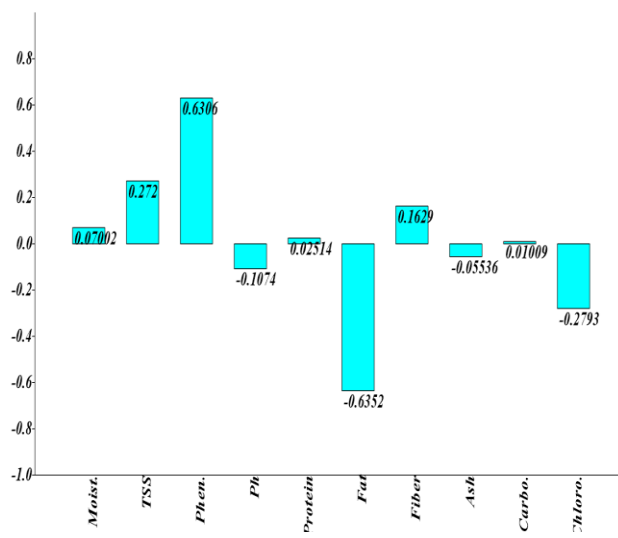


Fig. 7. Factor loadings for PC5 for biochemical traits of 46 pea landraces.

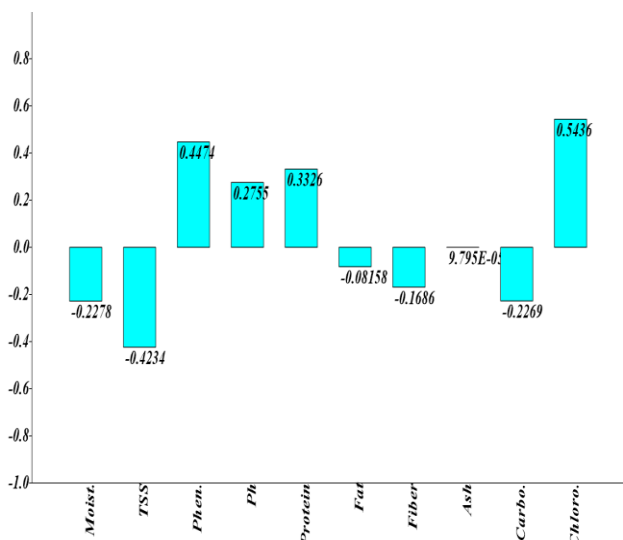


Fig. 5. Factor loading for PC3 for biochemical traits of 46 pea landraces.

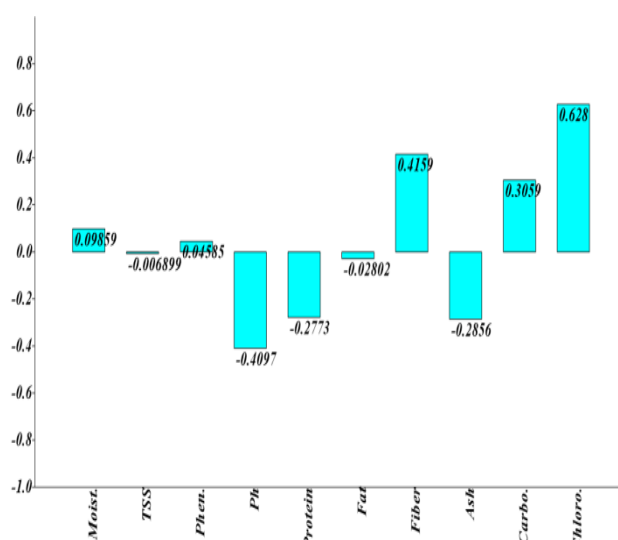


Fig. 8. Factor loadings for PC6 for biochemical traits of 46 pea landraces.

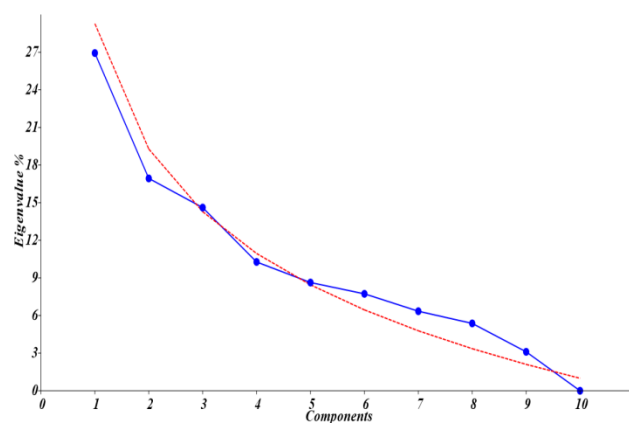


Fig. 9. Scree plot diagram of various factors.

## Conclusions

Results revealed that maximum variability was contributed by carbohydrates and total soluble solids, while landraces L13, L21, L25, L7, L19, L27, L31, L32, L34 and L26 were most diverse contributing maximum variation. In principal component analysis first four components (PCA) accounted for 68.86% of the diversity. Ash showed maximum positive and highly significant correlation with moisture content (0.58\*\*), phenolic content (0.76\*\*) and fat (0.37\*\*). Collectively, landraces (L13, L21, L25, L7, L19, L27, L31, L32, L34 and L26) with better biochemical traits could be used in breeding programs to exploit their maximum potential.

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