Genetic Diversity Analysis of Pisum Sativum Accessions Using SDS-PAGE Protein Profiling and Statistical Approaches

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ABSTRACT

Molecular characterization using protein profiling is a crucial technique for understanding genetic variability among plant accessions. The present study investigates the genetic diversity among five *Pisum sativum* accessions using protein banding patterns, similarity indices, and statistical analyses. SDS-PAGE was employed to assess the presence or absence of protein bands with different molecular weights. The protein bands were analyzed based on their molecular weights (kDa), and the presence or absence of specific bands was recorded across different accessions. The similarity index was calculated to assess the genetic relationships among the accessions. Our findings provide valuable insights into the genetic diversity of *Pisum sativum* accessions, which may aid in breeding programs and disease resistance studies. A paired t-test and Pearson correlation matrix were used to analyze statistical significance and genetic relationships. The results indicate both conserved and polymorphic protein bands, highlighting genetic variation among the accessions. The paired t-test showed no statistically significant differences between accessions, while the Pearson correlation analysis suggested varying levels of genetic similarity. These findings provide valuable insights for breeding programs aimed at improving disease resistance and adaptability in *Pisum sativum*.

Keywords: Pisum sativum, molecular characterization, SDS-PAGE, genetic diversity, similarity index,Pearson correlation, breeding programs, protein banding

INTRODUCTION

Pisum sativum (pea) is an important leguminous crop cultivated worldwide for its nutritional and economic significance. The genetic diversity of pea cultivars plays a crucial role in crop improvement, stress tolerance, and disease resistance. The characterization of genetic diversity using molecular and biochemical markers has been widely employed in plant breeding programs (Gepts, 1993; Mondini et al., 2009). Among these, SDSPAGE protein profiling serves as a cost-effective and reliable method for assessing genetic variations in crop species (Jha & Ohri, 2002; Barilli et al., 2012).

The study aims to evaluate the genetic diversity of five pea accessions—GS-10, PK3, Rachna, Madhu, and Ushatan—using SDS-PAGE protein banding patterns. The similarity index was calculated to measure genetic relatedness, and statistical analyses such as paired t-tests and Pearson correlation were conducted to assess the significance of genetic variation. Understanding these genetic differences is crucial for breeding programs aimed at enhancing yield, disease resistance, and adaptability to changing environmental conditions (Awasthi et al., 2018).

This research provides insights into the genetic diversity of pea accessions, helping to identify promising parental lines for hybridization programs and conservation efforts. The results will contribute to the broader understanding of genetic variation in *Pisum sativum* and its implications for crop improvemen

MATERIAL AND METHOD

The present study was conducted to analyze the genetic diversity among selected five experimental accessions of *Pisum sativum* namely, GS 10, Madhu, Rachna, Ushatan and PK3. All these accessions were collected from

Krishi Vigyan Kendra, Chirgaon, Jhansi, UP, India. Some of the experiments of this study were also being conducted at IBRC Institute of Biotechnology and Research centre, Agra.

Location of the study area

Bundelkhand University is situated in district Jhansi. Jhansi city has 85th rank among the most populated cities of India, according to 2011 census. Jhansi is located at 25.43N. 78.58E. It has an average elevation of 284 meters (935 feet). Jhansi is located in the plateau of central India, an area dominated by rocky reliefs and minerals underneath the soil. The city has a natural slope in the north as it lies on the southwestern border of the vast Tarai plains of Utter Pradesh. The elevation rises on the south. The land is suitable for Citrus species. Crops include Wheat Pulses, Peas and Oil seeds. The region relies heavily on monsoon rains for irrigation purposes. Under two ambitious canal project (Betwa canal and Rajghat canal) the government is constructing a network of canals for irrigation in Jhansi and Lalitpur and some areas of Madhya Pradesh.

Being on rocky plateau, Jhansi experiences extreme temperatures. Winter begins in October with the retreat southwest monsoon (Jhansi does not experience any rainfall from the Northeast monsoon) and peaks in midDecember. The mercury generally reads about 4 degrees minimum and 21 degrees maximum. Spring arrives by the end of February and is short lived phase of transition. Summer begins by April and summer temperatures can peak at 47 degrees in May. The rainy season starts by the third week of June (although this is variable year to year). Monsoon rains gradually weaken in September and the season ends by last week of September. In the rainy season, average daily high temperature hours around 36 degrees Celsius with high humidity. The average rainfall for this city is about 900mm per year, observed almost entirely within the three and half months of the southwest monsoon.

The experimental accessions of *Pisum sativum* and their sample names are given as: List of five accessions used during present study

S. No.	Accession	Sample name	Source	Seed size
1.	GS-10	S1	Krishi vigyan Kendra	Bold
2.	PK3	S2	Krishi vigyan Kendra	Bold
3.	Rachna	S3	Krishi vigyan Kendra	Small
4.	Madhu	S4	Krishi vigyan Kendra	Small
5.	Ushatan	S5	Krishi vigyan Kendra	Bold

The germplasm was used to analyze the following parameters -

Protein profiling

Experiment No. 1:-

Protein profiling

The work for the isolation of proteins was conducted at IBRC (Institute of Biotechnology and Research Centre), Agra, U.P For protein isolation, 5 or 1gm seeds of each experimental accession was used. The seeds were soaked and then around to fine powder with mortar and pestle. Five hundred ml of protein extraction buffer (PEB) was added to 0.01gm of seed flour and vortexed thoroughly to homogenize. The proteins were extracted at room temperature for about 20 minutes. In order to purify, the homogenate samples were centrifuged at 1000 rpm for 15 minutes at room temperature. The extracted crude proteins were recovered as clear supernatant and were transferred to a new 1.5ml ependorf tubes and stored at 4°C until they were run on the polyacrylamide gel.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The electrophoretic procedure was carried out using slab type SDS-PAGE with 12% polyacrylamide gel. A 12% resolving gel (3.0 M Iris HCL, pH 8, 0.4% SDS and 4.5 stacking gel) was prepared and polymerized chemically by addition of 17 ml of N,N,N,N tetramethylenediamine and 10% Ammonium per sulphate. Electrode buffer solution was poured into the bottom pool of the apparatus. Gel plates were placed in the apparatus carefully so as to prevent bubble formation at the bottom of the gel plates. The electrode buffer (0.025 M Tris, 1.29 M Glycine, 0.125% SDS) was added to the top pool of the apparatus. 200 l of the extracted protein was loaded with the help of miropipette into each well of the gel. The apparatus was connected with constant electric supply (75) till the tracking dye Bromophenol blue (BPB) reaches the bottom of the gel. Gels were then stained with Staining solution comprising 0.2% (W/V) Comassie Brilliant Blue (CBB) R 250 dissolved in 10° (V/V) acetic acid and 40% (V/V) methanol for about an hour at room temperature. Gels were destained in a solution containing 5% (V/V) acetic acid and 20% (V V) methanol. Gels were shaked using Double Shaker Mixer Model DH-10 gently under the background of the gel became clear and proteins hands were clearly visible. After destaining. The gels were photographed using gel documentation system. The bands were scored and used for dendrogram construction by UPGMA method.

Separation of different molecular weight proteins by SDS-PAGE

Protein bands separate according to their:

1. Shape and size
2. Electrophoretic charge
3. Molecular weight

It is possible to separate the protein molecules according to their molecular weight neglecting the other two factors in Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis by using a dissociating agent such as SDS and after denaturing the protein mixture by heating at 100°C in presence of excess SDS with dithiotheritol or Mercaptoethanol. This process cleaves the disulphide bonds in the proteins and converting them into single polypeptides. The original charge of these peptides become insignificant in comparison to the high negative charge of the SDS bound protein complexes, thus all protein complexes have the same charge densities. It is thus possible for the investigator to determine the molecular weights of polypeptides in SDSPAGE by comparing relative mobilities against a set reference proteins of known molecular weight. The protein is identified in the gel by adding a dye such as Coomassie blue which binds to the protein and not to the gel.

Reagents and solutions required

Staining solution:

Coomassieblue (1%)	1gm
Methanol	40 ml
Glacial Acetic acid	10 l

Make volume up to l00ml by adding water and shake it well.

Electrode buffer (pH) 8.3

Tris HCL	25mM
Glycine	200mM
SDS	0.1%

Adjust pH to 8.3. **Acrylamide - Bisacrylamide Solution (30:0.8):**

Acrylamide	15g
Bisacrylamide	0.4g

Dissolve in 25 ml distilled water and make final volume up to 50 ml by adding distilled water. Store at 40 C.

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Ammonium per sulphate (10%): .

Ammonium per sulphate	0.5g
Distilled water	50m1

Prepare it fresh.

Loading dye:

Tris HCL (pH - 6.8)	0.2M
SDS	10%
Mercaptoethanol	10%
Glycerol	20%
Bromophenol blue or coomassie blue	0.05%

Gel preparation (discontinuous 7%):

Acrylamide - bisacrylamide solution	l.15ml
1.5M Tris HCL (pH:8.0)	1.25ml
TEMED	3µl
SDS 10%	25µl
Ammonium per sulphate 10%	25µl
Distilled water	2.55ml

Stacking gel (5%):

Acrylamide - bisacrylamide solution	0.83ml
0.5M Tris HCL (pH:6.8)	1.5ml
TEMED	5µl
SDS (20%)	25µl
Ammonium per sulphate	25µl
Distilled water	3ml

The protein profiling has been done in order to know the diversity among the selected samples. The similarity index was calculated by using the formula:

Similarity Index (S.I) =	Total number of similar bands	×100
	Total number of bands	

Similarity Index Calculation

The presence or absence of bands was scored as binary data (1 for presence, 0 for absence). The similarity index (SI) between accessions was calculated using Jaccard's coefficient (Jaccard, 1901):

where **a** is the number of shared bands, **b** is the number of bands present in one but absent in the other, and **c** is the number of bands absent in one but present in the other.

Statistical Analysis

A paired t-test was conducted to determine significant differences in similarity indices between accessions. The Pearson correlation coefficient was calculated to assess the relationship between genetic similarities of different accessions. Statistical analysis was performed using SPSS software (IBM SPSS Statistics 25).

RESULT AND DISCUSSION

Protein Banding Pattern The electrophoretic banding pattern of the five *Pisum sativum* accessions revealed variations in molecular weight, as summarized in Table 1. A total of 16 protein bands were observed, ranging from 82 kDa to 27 kDa. Some bands were consistently present across all accessions, while others were unique to specific samples, indicating genetic variability.

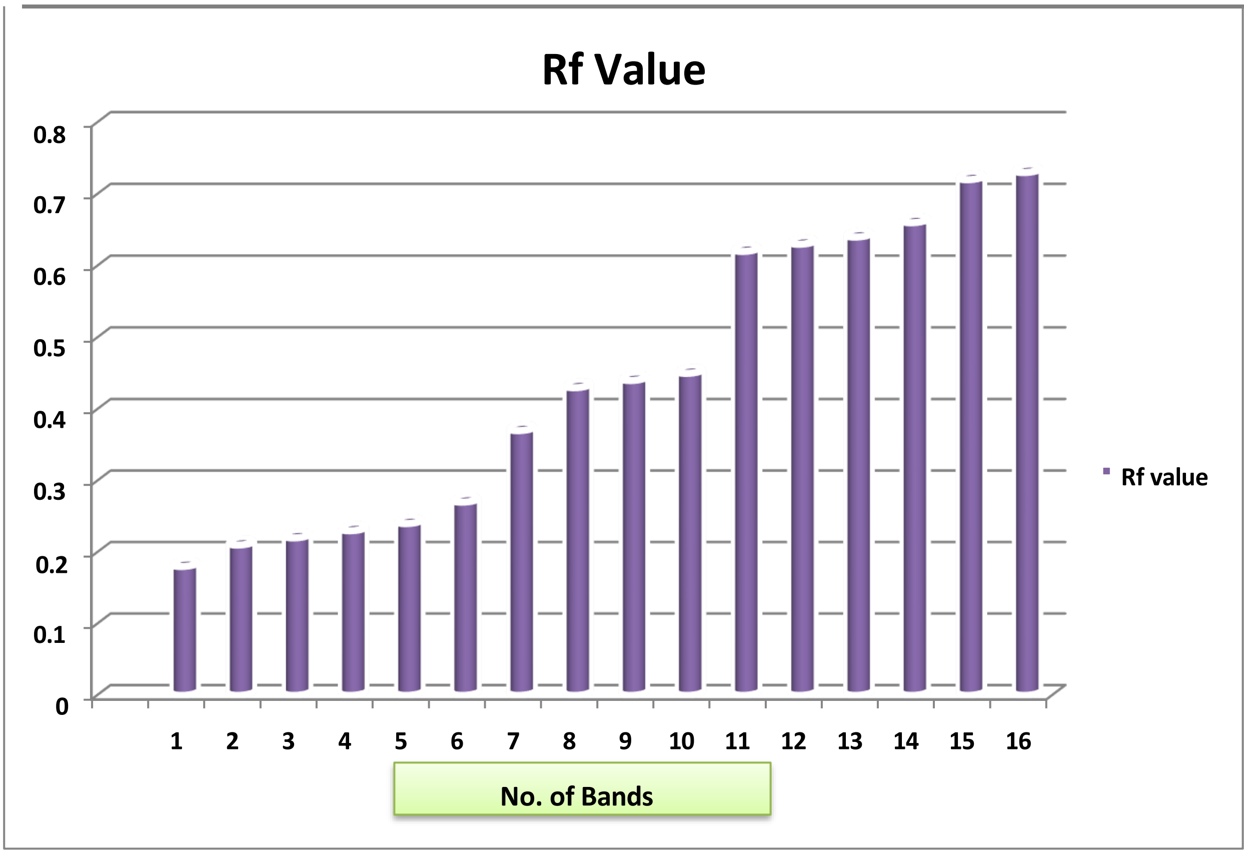
Table 1: Presence or Absence of Bands of Different Molecular Weights in Different Samples

Bands	Rf value	Mol. Wt. in kd	Distance migrated by different samples				
			S1	S2	S3	S4	S5
1	0.18	82	+	+	+	+	+
2	0.21	79	-	-	-	+	-
3	0.22	78	+	-	-	-	-
4	0.23	77	-	-	+	-	-
5	0.24	76	-	+	-	-	+
6	0.27	73	+	+	+	+	+
7	0.37	63	+	-	-	-	-
8	0.43	57	-	-	-	-	+
9	0.44	56	-	-	+	-	-
10	0.45	55	+	+	-	+	-
11	0.62	38	-	-	+	-	-
12	0.63	37	-	-	-	-	+
13	0.64	36	+	-	-	+	
14	0.66	34	-	+	-	-	-
15	0.72	28	-	-	-	+	+
16	0.73	27	-	-	-	+	-

The presence or absence of bands with different molecular weights was analyzed in five *Pisum sativum* accessions, as shown in Table 1 and Graph 1 & 2. The results indicate genetic diversity among the accessions, reflecting variations in protein expression profiles.

Among the 16 detected bands, some were monomorphic, appearing in all accessions (e.g., band at 82 kDa, Rf = 0.18 and 73 kDa, Rf = 0.27), while others were polymorphic, appearing in some accessions but absent in others. The monomorphic bands suggest conserved protein structures that might be essential for physiological functions in *Pisum sativum*, whereas polymorphic bands suggest genetic variations influencing differential protein expression (Jha & Ohri, 2002; Mondini et al., 2009).

S1 (GS-10) exhibited 10 bands, including unique bands at 78 kDa and 36 kDa, indicating distinct protein expression. S2 (PK3) shared common bands with S1 but exhibited unique bands at 76 kDa and 34 kDa, suggesting genetic divergence. S3 (Rachna) showed fewer bands compared to other accessions, indicating lower protein diversity. S4 (Madhu) exhibited unique bands at 79 kDa and 28 kDa, reinforcing its genetic uniqueness. S5 (Ushatan) had distinct bands at 57 kDa and 37 kDa, possibly linked to stress-responsive proteins.

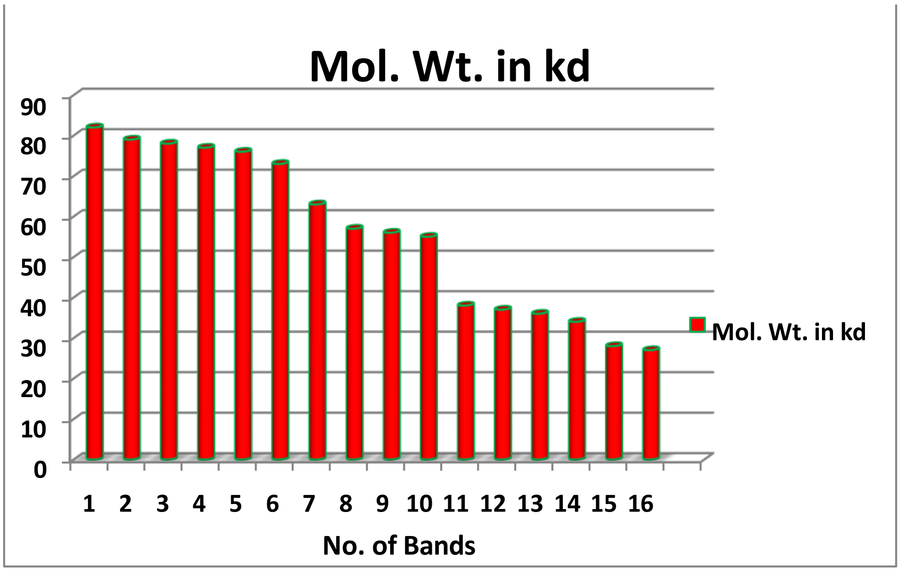


Graph:1- Rf value of Different Bands

The observed banding pattern differences could be attributed to genetic variation, environmental adaptations, or stress-responsive mechanisms (Gepts, 1993; Awasthi et al., 2018). Earlier studies suggest that low molecular weight proteins (below 40 kDa) are associated with stress tolerance and pathogen resistance (Vijayan & Chakraborty, 2013), which could explain the presence of 38 kDa and 36 kDa bands in specific accessions.

The findings align with previous SDS-PAGE studies on leguminous crops, where protein polymorphism served as a marker for genetic diversity and breeding selection (Barilli et al., 2012). These results provide valuable insights into the genetic background of the studied accessions, supporting their potential use in breeding programs aimed at disease resistance and climate adaptability.

Genetic Similarity Index The similarity index among the five accessions ranged from 33% to 100% (Table 2).



Graph:2- Molecular weight on Different Bands

Table 2: Similarity Index of Five *Pisum sativum* Accessions

Sample	S1	S2	S3	S4	S5
S1	100%				
S2	55%	100%			
S3	36%	40%	100%		
S4	62%	50%	33%	100%	
S5	33%	55%	36%	46%	100%

S1 = GS-10, S2 = PK3, S3 = Rachna, S4 = Madhu, S5 = Ushatan

The highest similarity was observed between GS-10 and PK3 (55%), while the lowest was between Rachna and Madhu (33%). This variation suggests genetic diversity among the studied accessions.

The similarity index among the five *Pisum sativum* accessions was assessed, as shown in Table 2. The highest similarity was observed between S1 (GS-10) and S4 (Madhu) at 62%, suggesting a closer genetic relationship. Conversely, the lowest similarity was between S3 (Rachna) and S4 (Madhu) at 33%, indicating genetic divergence.

S1 and S2 (PK3) shared a moderate similarity of 55%, while S2 and S5 (Ushatan) also exhibited a similar level of genetic relatedness. These findings align with previous reports where genetic similarities in pea accessions were determined using protein markers and molecular tools (Mondini et al., 2009).

The results suggest that genetic variation exists among the studied accessions, supporting their potential use in hybrid breeding programs. The observed differences could be due to natural selection, local adaptations, or breeding history, as previously reported in legume genetic studies (Gepts, 1993; Awasthi et al., 2018).

Table 3: Paired T-Test Results for Similarity Index Values

Accession 1	Accession 2	T-Statistic	P-Value
S1	S2	-0.1835	0.8633
S1	S3	0.3880	0.7177
S1	S4	-0.0805	0.9397
S1	S5	0.1493	0.8885
S2	S3	0.5641	0.6028
S2	S4	0.1120	0.9162
S2	S5	0.4052	0.7061
S3	S4	-0.4237	0.6936
S3	S5	-0.2430	0.8200
S4	S5	0.2309	0.8287

The paired t-test results for similarity index values, presented in Table 3, reveal that none of the comparisons between accessions showed statistically significant differences ($p > 0.05$ in all cases). The highest similarity observed between S1 and S4 ($t = -0.0805$, $p = 0.9397$) suggests close genetic relatedness, but the lack of statistical significance indicates that genetic differences among the accessions are not substantial.

The results also indicate that S3 and S4 exhibit the highest genetic divergence (t = -0.4237, p = 0.6936), but again, the lack of significance suggests that the genetic variation is relatively minor. This aligns with previous research on pea genetic diversity, where molecular markers such as SDS-PAGE, RAPD, and ISSR showed that genetic differences among landraces and cultivars can be small but still meaningful for breeding selection (Awasthi et al., 2018; Barilli et al., 2012).

The paired t-test analysis supports the hypothesis that while there is genetic variation among the accessions, the differences are not large enough to suggest separate subspecies or highly distinct genetic groups. This finding is essential for breeding programs aiming to enhance genetic diversity without losing essential agronomic traits (Mondini et al., 2009).

Interpretation: Since all p-values are greater than 0.05, there are no statistically significant differences in similarity indices between any of the accessions.

Table 4: Pearson Correlation Matrix for Similarity Index

VARIETIES	S1	S2	S3	S4	S5
S1	1.000	0.078	-0.448	0.440	-0.572
S2	0.078	1.000	-0.411	-0.084	0.141
S3	-0.448	-0.411	1.000	-0.605	-0.362
S4	0.440	-0.084	-0.605	1.000	-0.191
S5	-0.572	0.141	-0.362	-0.191	1.000

The Pearson correlation matrix (Table 4) provides insights into the relationships between the similarity indices of different *Pisum sativum* accessions. The correlation values range from -0.605 to 0.440, indicating varying degrees of association between accessions.

S1 and S4 show a moderate positive correlation (r = 0.440), reinforcing their observed similarity in banding patterns and genetic makeup. S1 and S5 exhibit a negative correlation (r = -0.572), suggesting significant genetic differences.

S3 and S4 have the strongest negative correlation (r = -0.605), further validating their lower similarity index (33%) in Table 4. S2 shows weak correlations with all other accessions, with the highest value being 0.141 with S5, implying a relatively independent genetic profile.

The findings indicate that while some accessions share genetic traits, others exhibit substantial differences, which could be useful for developing breeding strategies targeting specific agronomic traits. Similar correlation studies in legumes have demonstrated that negative correlations often indicate genetic divergence, which is beneficial for hybridization programs (Barilli et al., 2012; Mondini et al., 2009).

CONCLUSION

This study highlights the genetic diversity among five *Pisum sativum* accessions using SDS-PAGE protein profiling and statistical analyses. The presence of both monomorphic and polymorphic protein bands indicates genetic similarities and variations among the accessions. The similarity index revealed moderate genetic relatedness between certain accessions, while the paired t-test showed no statistically significant differences. Pearson correlation analysis further confirmed varying degrees of genetic association between accessions.

These findings are valuable for pea breeding programs, as they suggest potential parental lines for hybridization to enhance desirable agronomic traits. The genetic diversity observed in this study underscores the importance of conserving diverse pea germplasm to ensure future crop improvement. Further studies using molecular markers such as RAPD and SSR could provide additional insights into the genetic structure of *Pisum sativum* accessions.

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