

First Proteomic Assay of Pakistani *Pisum sativum* L. Germplasm Relation to Geographic Pattern

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Abstract—Proteomic assay was carried out to assess genetic diversity in relation to geographic pattern in 97 genotypes of *Pisum sativum* L., collected from all over Pakistan. In total 34 bands were observed and among these, 26.7% bands were monomorphic, while 73.5% bands showed polymorphism. Based on both Province Wise Analysis (PWA) and Agro-ecological Zones (AEZ) the genotypes collected from Punjab, North West Frontier Province (NWFP) exhibited 70.6%, and 64.7% variation respectively. The germplasm collected from Azad Kashmir showed the lowest level of genetic diversity. Cluster analysis exhibited, moderate level of association, between genetic diversity and geographic pattern of the genotypes.

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INTRIDUCTION

Characterization of genetic diversity in crop species has long been based on morphological traits, however, morphological variation is often found to be of limited because expression of morphological traits may be affected by environmental conditions, thereby constraining the analysis of genetic variation. During recent year, biochemical and molecular genetic techniques have emerged as a complementary strategy in conjunction with traditional approaches in the management of plant genetic resources [1, 2].

For studies on population genetic structure; protein assays are considered as useful tool for most taxa [2–5]. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is widely used due to its reliability and simplicity in describing the genetic structure of crop germplasm [6]. A considerable variation in protein banding pattern has been reported, which was localized to various geographical regions [7].

The present study is the first report on Pakistani pea's germplasm seed storage for investigation of diversity in relation to geographical pattern.

MATERIALS AND METHODS

Seeds of ninety seven genotypes were collected from all over Pakistan (33.40°N and 73.07°E, 540 m). For SDS-PAGE analysis, single seed from each genotype was ground to a fine powder with mortar and pes-

tle. Four hundred µl protein extraction buffers (0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol, was added to 0.01 g of seed flour and mixed well with Automatic Lab-Mixer (DH-10). Bromophenol blue (BPB) was added to the protein extraction buffer as tracking dye to monitor the movement of protein in the gel. The SDS-PAGE of total protein was carried out in the discontinuous buffer system as describe by [8]. It was observed that 15% acrylamide gel concentration with 6 µl of sample gave the best resolution. After staining and destaining, the gel was dried using gel drier (Atto, Rapidery-Mini Japan). In order to check the reproducibility of the method two separate gels for all samples were run under similar electrophoretic condition. The molecular weight of the dissociation polypeptides was determined using molecular weight protein sander's MW-SDS-70 Kit (Sigma, United States). For scoring, electrophoregram was divided into three regions and data was scored for the presence or absence of protein bands.

The SDS-PAGE data were analysed for cluster using computer packages STATISTICA 6.0 in Windows XP-2005. The data were analysed on the basis of Province Wise (PWA) and Agro-Ecological Zones (AEZ) analysis [11]. A similarity index was used to construct a dendrogram by the UPGMA method [9].

RESULTS

The electrophoregram was divided into three regions i.e. region-I (bands # 1–18 ranging in size from 45 – >66.0 kDa),

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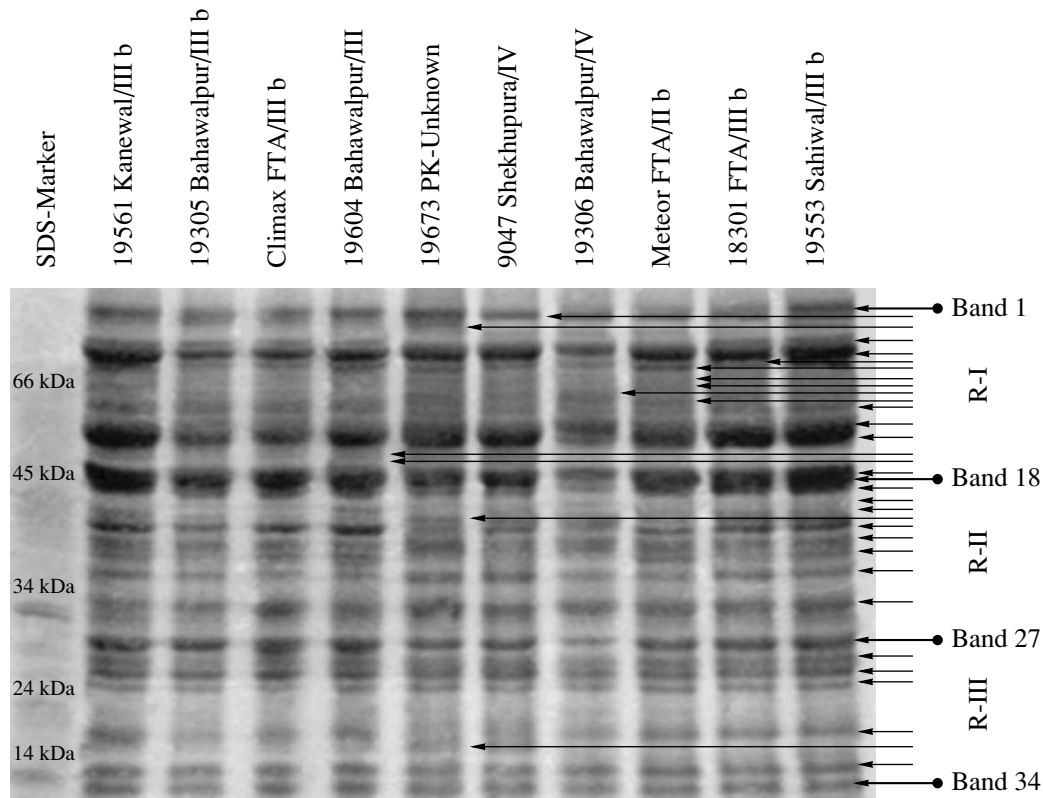


Fig. 1. Proteomic assay of ten Pakistani *Pisum sativum* L. genotypes. Arrow indicate the presence of the number of bands, R-I, R-II and R-III indicates the region of electrophoregram.

region-II (bands # 19–27; 24 – <45.0 kDa) and region-III (bands # 28–34; <14 – 44 kDa). Out of total 34 bands, nine bands # –5, –11, –14, –18, –23, –27, –29, –3, and –34 were monomorphic (Fig. 1). The germplasm collected from Punjab and NWFP showed 70.6% and 64.7%, whereas the germplasm with unknown origin showed 61.8% variation. Among the 34 bands #, three bands viz; # –16, –28 and –31 showed variation at all loci.

Data was analysed on the bases of Agro-ecological zones. Among these germplasm from AEZ-III b (sandy desert), AEZ-IV (north irrigated plan) and Unknown showed the highest levels of variation in the study. While the germplasm of AEZ-V (rain fed lands) showed lowest level of variation (Table 1). Bands # –1, –9, –16, –20, –21 and –31 showed variation in all loci.

Dendrogram at linkage distance 2.7 was divided into eight lineages (major groups L, Fig. 2). At 50% linkage distance 8 lineages were further divided into 66 clusters (C). Lineages I (L-I) comprised of single cluster having 17% of germplasm collected from Balochistan (BL). Lineage II (L-II) comprises of six clusters (C 2 to C 7) encircles mainly the germplasm collected from northern areas, while L-III composed of 3 clusters. L-IV

comprises of 2 clusters whereas L-V comprised 18 clusters consisting of 33% of the total germplasm where as grouped L-V comprised mainly the genotypes collected from Federal Territorial Area (71%), Punjab (48%) and Unknown (30%). Lineages, L-VI and L-VII consisted 12 clusters each. In most of group L-VI, 33% of North West Frontier Province collections were clustered while in L-VIII all the germplasm for Azad Kashmir (100%), 60% for Balochistan and 40% for North West Frontier Province were grouped (Table 2). On the basis of Agro-ecological zone, the germplasm of AEZ-III (sandy desert), AEZ-III b (sandy desert) and AEZ-IV (northern irrigated plain) were mainly distributed in lineage V (L-V). While the germplasm of Western dry mountain AEZ-VIII (67%) and Wet Mountain exhibited maximum representation in L-VIII (Table 2). L-V and L-VIII showed moderate pattern of geographic association for both PWA and AEZ analysis.

DISCUSSION

This is first documented report of estimation of genetic diversity in *Pisum sativum* L., in Pakistan based on proteomic data. In the present study both Province wise and Agro-ecological zone wise analyses showed

Table 1. Proteomic assay of 73.5% polymorphic bands for Province Wise and Agro-ecological zone wise analyses in *Pisum sativum* L. germplasm collected from all over Pakistan

Number of bands	G.f.	1	2	3	4	6	7	8	9	10	12	13	15	16	17	19	20	21	22	24	25	26	28	30	31	32	σ
		Region I (69.5% variation)													Region II (62.5% variation)						Region III (65.71% variation)						
AJK	2	-	+	+	+	±	+	+	-	+	-	-	+	±	-	+	+	-	+	-	+	+	±	+	±	+	11.8
Baluchestan	5	±	+	±	±	+	±	+	±	+	±	±	±	±	±	±	±	±	±	±	+	+	±	±	±	±	55.9
FTA	7	±	±	±	±	+	±	±	±	+	±	±	+	±	±	+	±	±	±	±	±	+	±	+	±	±	55.9
Northern Area	6	±	±	±	±	+	±	+	±	+	±	±	±	±	±	±	±	±	±	±	+	+	±	+	±	±	52.2
NWFP	16	±	±	±	±	±	±	±	±	±	±	±	+	±	±	±	±	±	±	±	±	+	±	+	±	±	64.7
Punjab	41	±	±	±	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	70.6
Unknown	20	±	±	+	±	+	±	±	±	+	±	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±	61.6
Total Bands Var.	97	6	5	5	6	2	6	4	6	6	6	6	3	7	6	5	6	6	6	6	4	2	7	3	7	6	
Agro-ecological zone		(65.93% variation)													(65.27% variation)						(60.0% variation)						
III	5	±	+	+	±	+	±	±	±	±	±	±	+	±	±	+	±	±	±	+	+	±	±	±	±	+	50
IIIB	22	±	±	±	±	+	±	±	±	+	±	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±	64.7
IV	25	±	+	±	±	+	±	±	±	±	±	±	+	±	±	+	±	±	±	±	±	±	±	±	±	±	61.8
IVB	5	±	±	±	±	+	+	±	±	+	±	±	+	±	±	±	±	±	±	±	±	+	±	+	±	±	55.9
V	2	±	±	+	+	+	±	±	±	+	-	-	+	±	+	+	±	±	+	+	+	+	+	+	±	+	26.5
VI	6	±	+	±	±	±	±	+	±	+	±	±	+	±	±	+	±	±	±	±	±	+	±	+	±	+	50
VII	8	±	±	±	+	±	±	+	±	+	±	±	±	±	±	±	±	±	±	±	+	+	±	+	±	±	55.9
VIII	5	±	+	±	±	+	±	+	±	+	±	±	±	±	±	±	±	±	±	±	+	+	±	±	±	±	55.9
O-Unknown zone	20	±	±	+	±	+	±	±	±	+	±	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±	61.8

Notes: G.f., Genotypes frequency, AJK, Azad Jammu-Kashmir, FTA, Federal Territorial Area, Var, variation, Bands, # -5, -11, -14, -18, -23, -27, -29, -33 and -34 are monomorphic bands, III, sandy disert, IIIB, sandy disert, IV, norther irrigated plan, IVB, norther irrigated plan, V, Barani (rainfed) area, VI, wet mountain, VII, northern dry mountain, VIII, western dry mountain.

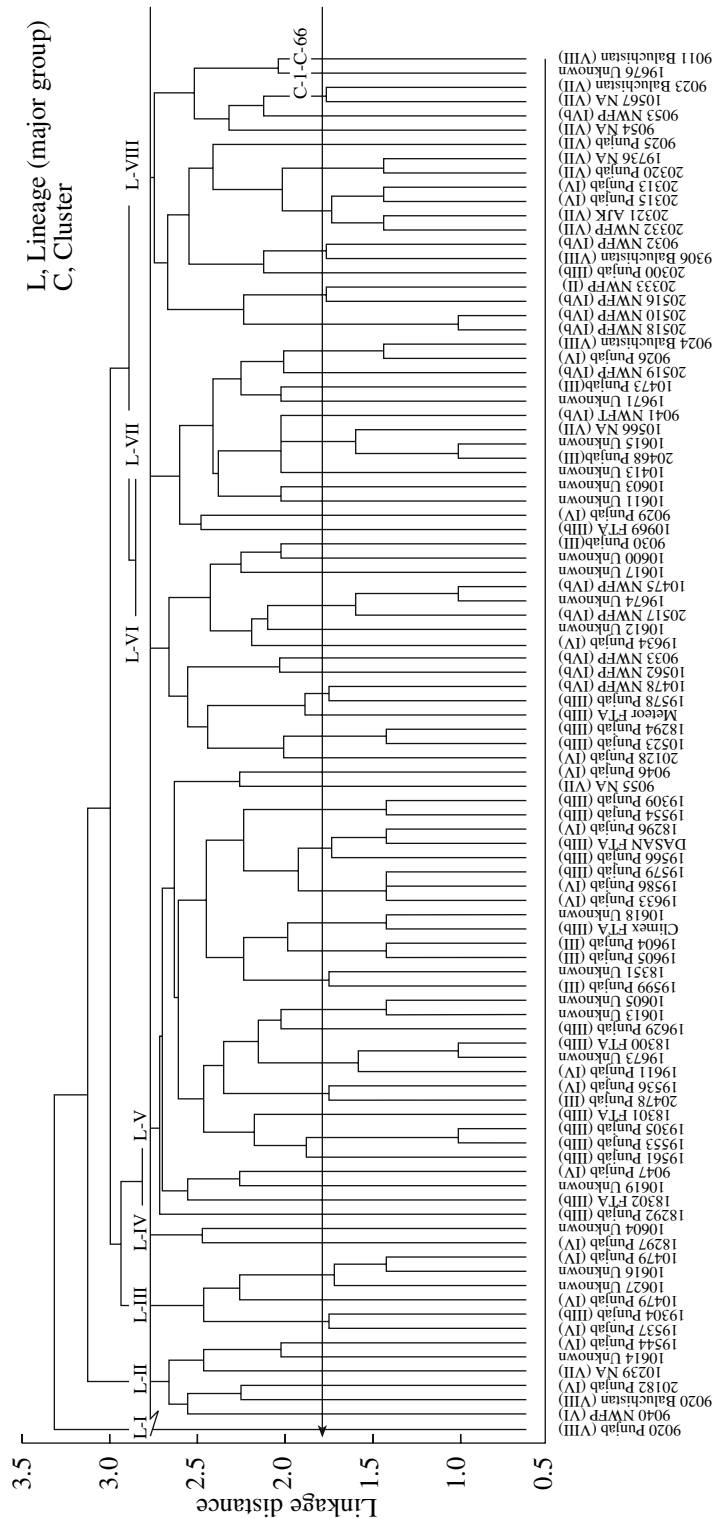


Fig. 2. Cluster diagram of 97 genotypes of *Pisum sativum* L. based on proteomic assay. Roman words showing Aro-ecological zone; NWFP, North West Frontier Province; NA, Nothern Area; L, Lineages and C, clusters.

high level of variation (73%) in contrast to low level of variation reported earlier in various legume species [10] indicating broader genetic base of the germplasm col-

lected during present study. Another possibility behind high variation observed during present study might be the purity of lines. In Pakistan, *Pisum sativum* is used

Table 2. Cluster analysis on the basis of Agro-ecological zone developed from cluster diagram of 97 genotypes of *Pisum sativum* germplasm collected from all over Pakistan

Group 1 (C 1. Genotypes 1)	E Zone	f	Province	f	Group 6 (C 32–C 43. Genotypes 16)	E Zone	f	Province	f
Western dry mountain	VIII	1	Baluchistan	1	Sandy desert	III b	5	FTA	1
Group 2 (C 2–C 7. Genotypes 6)					N Irrigated plain	IV	3	NWFP	5
N Irrigated plain	IV	1	Northern Area	2	N Irrigated plain	IV b	4	Punjab	7
N Irrigated plain	IV b	2	NWFP	2	Barani (rainfed) lands	V	1	Unknown	3
N Dry mountain	VII	2	Punjab	1	Unknown	O	3		
Unknown	O	1	Unknown	1	Group 7 (C 44–C 54. Genotypes 14)				
Group 3 (C 8–C 10. Genotypes 6)					Sandy desert	III	1	Baluchistan	1
Sandy desert	III b	1	Punjab	4	Sandy desert	III b	2	FTA	1
N Irrigated plain	IV	3	Unknown	2	N Irrigated plain	IV	2	NWFP	2
Unknown	O	2			N Irrigated plain	IV b	2	Punjab	4
					N Dry mountain	VII	1	Unknown	5
Group 4 (C 11–C. Genotypes 2)					Western dry mountain	VIII	1		
N Irrigated plain	IV	1	Punjab	1	Unknown	O	5		
Unknown	O	1	Unknown	1	Group 8 (C 55–C 66. Genotypes 20)				
					Sandy desert	III b	1	AJK	2
Group 5 (C 13–C 31. Genotypes 32)					N Irrigated plain	IV	2	Baluchistan	3
Sandy desert	III	4	FTA	5	N Irrigated plain	IV b	4	Northern Area	3
Sandy desert	III b	11	Northern Area	1	Barani (rainfed) lands	V	1	NWFP	6
N Irrigated plain	IV	7	Punjab	20	N Dry mountain	VII	7	Punjab	5
N Dry mountain	VII	4	Unknown	7	Western dry mountain	VIII	4	Unknown	1
Unknown	O	6			Unknown	O	1		

Notes: C, cluster; E Zone, Agro-ecological zone; f, frequency.

as a marginal crop, the stakeholder sowing original stock restricts the contamination of different genotypes. Similarity of banding patterns between genotypes may be due to duplications in the germplasm, however, it is suggested that this should be confirmed with two-dimensional electrophoresis [11].

Cluster analysis of both Province wise and Agro-ecological evaluation during present study revealed proteomic diversity and its association with geographic distribution of the germplasm. In major lineages group L-V and L-VIII more the 80% genotypes collected from sandy desert/FTA and Punjab were grouped, while VIII accommodated more than 60% genotypes of mountainous area. Agro-ecological zone computation

showed clearer association than Province wise computation. During present proteomic assays high level of diversity was observed for each locus in local pea germplasm.

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