Seed Storage Protein Profiles of Pea (*Pisum sativum* L.) Genotypes using SDS-PAGE

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Using polyacrylamide slab gel electrophoresis, the water soluble seed proteins could be resolved in 42 bands distributed in six zones. One hundred and thirteen cultivars were categorized into 28 groups; each group had distinct and different electropherotic banding patterns. However, within the group cultivars were also further distinguishable by the intensity of staining of bands. It was possible through electrophoresis to differentiate phenotypically similar cultivars for example UU-12, Multifreezer, Piourette, Starcovert, Bonneville and IP-3. Cultivar Meethi Phali with a specific characteristic of edible pod type formed a separate group with 9 bands. Most of the varieties showed no distinct patterns which would allow their characterization. There were a few specific bands in each variety and most of them; although exclusive, appeared at such a low frequency that varietal characterization was not possible.

Key Words: Electropherogram, Genotypes, Pea, SDS-PAGE, Seed protein

Introduction

The use of the seed protein profile, obtained by electrophoresis, for resolving taxonomic and evolutionary problems has been greatly expanded in the last decade. Stability is one of the main features of the seed protein. For this reason it has been suggested as an additional tool for species identification besides other traditional approaches (Wolf, 1980; Bushuk *et al.*, 1987).

Characterization of germplasm using biochemical fingerprinting has got special attention due to its increased used in crop improvement and the selection of desirable genotypes for breeding crops. Protein profiling, an independent, emerging sub-specialty of proteomics, is poised to provide unprecedented insight into biological events. Unlike DNA sequencing, where capillary electrophoresis changed everything by allowing high-throughput sequencing, no equivalent exists for proteins. The use of genetic markers and protein profiling is also successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky and Hymowitz, 1979). Unfortunately, except for a few genera, extensive screening of germplasms to uncover variability in the seed protein profile is generally lacking. The concept of genetic distance has been widely used as a tool in determining diversity through multivariate analysis to choose genetically diverse parents (Ghosh and Gulati, 2002). Very little information is available on the geneology of pea varieties, the amount of variability still available to breeding populations and the relationship between the pea varieties used for fodder, food or feed. The present study has been undertaken to get better knowledge on the architecture of genetic diversity within *Pisum sativum* L.. In view of above considerations, the electrophoretic characterization of available germplasms based on SDS-PAGE of seed protein had been done in the present investigation.

Materials and Methods

One-dimensional SDS-polyacrylamide vertical slab gel electrophoresis was carried out to determine the protein profiles per banding patterns of buffer extracted (Tris base, SDS and Mercaptoethanol) seed proteins excluding seed coat in 113 vegetable pea genotypes (Garffin 1990). The extraction procedure of Matta and Gatehouse (1982) was followed. The procedure developed by Laemmli (1970) was followed for gel preparation and running. The electropherogram of each gel was prepared diagrammatically locating the bands in lines on the basis of their relative front mobility values (rf value). Relative front mobility was calculated as per following formula:

$$Rf = \frac{\text{Distance between origin and band}}{\text{Distance between origin and tracking dye}}$$

The electropherograms (banding patterns) are shown in Plate 1.

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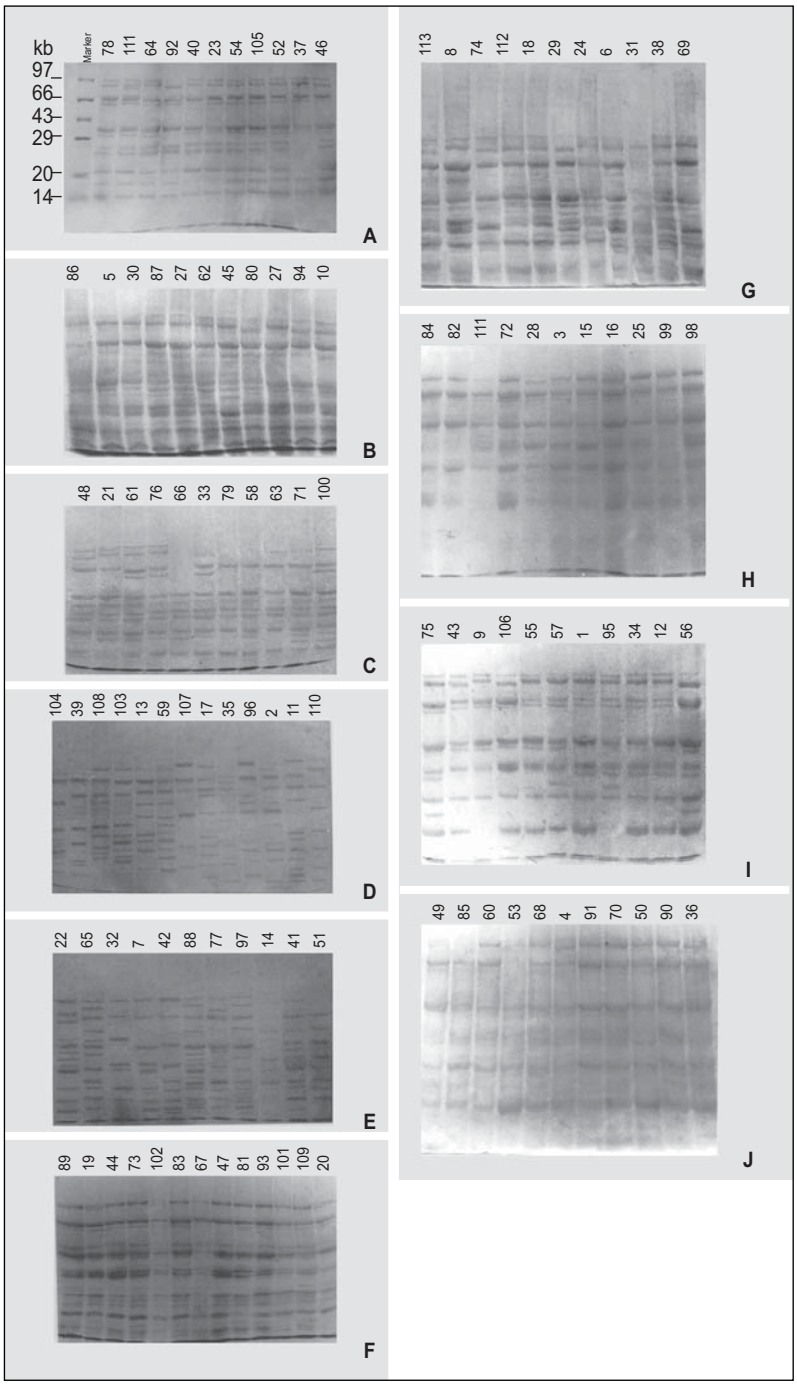

Fig. 1. Electropherogram of 113 genotypes of vegetable Pea. See Table 1 for legends

The banding patterns were characterized by 6 clear distinct zones viz., A, B, C, D, E and F. Zone A (around 97kDa) was nearest and F (around 14kDa) was farthest from the origin *i.e.* the point of protein sample application. The protein migrated from cathode to anode passing through separating gel. The protein bands were stacked according to their molecular weight *i.e.* high molecular weight protein were located in upper region and low molecular weight proteins in the middle to lower regions of the gel, respectively (Table 2).

On the basis of molecular weight markers pattern, method used by Rosa and Jouve (1992) was followed

Table 1. Legend for Fig. 1.

S. No.	Genotype number	Genotype	S. No.	Genotype number	Genotype
Gel A			Gel F		
1.	78	Boach Selection	1.	89	Alderman Dwarf
2.	111	Alaska	2.	19	KS-245
3.	64	Waverplus	3.	44	Ecoli
4.	92	JP-4	4.	73	Early December
5.	40	NDVP-10	5.	102	Profino
6.	23	Cobric	6.	83	ELF
7.	54	Starcovert	7.	67	PMR-8
8.	105	P-388-1	8.	47	Borpeena
9.	52	PMR	9.	81	Bonneville
10.	37	PMR-7	10.	93	VP-8002
11.	46	KS-246	11.	101	Arkel
Gel B			12.	109	King
1.	86	VP-8902	13.	20	Bridger
2.	5	JP-83	Gel G		
3.	30	Dwarf Gray Sugar	1.	113	Frosty
4.	87	VRP-1	2.	8	Midivert
5.	26	PM-1	3.	74	Rover
6.	62	Primette	4.	112	PI-3
7.	45	Tuinya Pratapgarh	5.	18	PMR-3
8.	80	Sutton Early Giant	6.	29	Cobrette
9.	27	PMR-11	7.	24	Selection-23
10.	94	Sel-82	8.	6	GC-141
11.	10	PM-5	9.	31	VL-6
Gel C			10.	38	Alderman
1.	48	Sel-30	11.	69	NDVP-9
2.	21	Deleketesse	Gel H		
3.	61	PC-121	1.	84	Meethi Phali
4.	76	Lincoln	2.	82	PMR-13
5.	66	Stop	3.	26	PM-1
6.	33	VL-7	4.	72	GC-245
7.	79	GC-152	5.	28	PMR-18
8.	58	Sel-14-3-2	6.	3	Manova Sugar
9.	63	UD-3	7.	15	PMR-21
10.	71	Corona Imperette	8.	16	PMR-15
11.	100	P-88	9.	25	6588-1
Gel D			10.	99	KT-15-4-4
1.	104	Azad Pea-3	11.	98	KS-123
2.	39	NDVP-1	Gel I		
3.	108	Recette	1.	75	Multifreezer
4.	103	Progress	2.	43	KS-226
5.	13	DPP-62	3.	9	Thomas Laxton
6.	59	Harabona	4.	106	Lay long Progress
7.	107	Superlaska	5.	55	PRS-18-6
8.	17	VRP-2	6.	57	Little Marvel
9.	35	JP-171	7.	1	UU-12
10.	96	Victory Freezer	8.	95	PMR-12
11.	2	6587-1	9.	34	NDVP-25
12.	11	VP-7802	10.	12	Pioneer
13.	110	Green Gulf	11.	56	P-23
Gel E			Gel J		
1.	22	PMR-9	1.	49	PMR-17
2.	65	Waverex	2.	85	Meethi Phali
3.	32	Polerette	3.	60	NDVP-5
4.	7	PM-2	4.	53	UD-2
5.	42	Piourette	5.	68	E-6
6.	88	Frizette	6.	4	Vitalis
7.	77	Petit Breton	7.	91	VL-3
8.	97	J-506	8.	70	NLP
9.	14	Asauji	9.	50	PH-2
10.	41	Early Queen	10.	90	Mammoth Melting Sugar
11.	51	PMR-16	11.	36	Sel-93

Table 2. SDS-PAGE banding patterns for seed protein in 113 genotypes of vegetable pea classified into 28 groups

Zone Rf	Value	Groups																											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
A_1	1.0	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_2	1.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–
A_3	1.5	–	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–
A_4	1.7	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_5	2.0	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	+	–	–	–
A_6	2.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B_1	2.5	–	+	–	–	–	+	+	–	+	+	–	+	–	+	+	+	–	+	–	–	–	–	–	–	+	–	–	–
B_2	2.7	–	–	–	–	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B_3	2.9	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B_4	3.0	–	–	–	+	+	–	–	+	–	+	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	+	+
B_5	3.2	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	+	–	+	–	–	+	–	–	–	–	–	–
B_6	3.4	–	+	–	–	–	+	–	+	–	–	+	+	+	+	+	+	–	+	–	–	+	–	–	–	+	+	–	+
B_7	3.6	–	–	–	–	–	–	+	+	+	+	–	+	–	+	+	–	+	–	–	–	+	–	–	–	–	–	+	–
B_8	3.8	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–
C_1	4.0	+	–	–	–	–	–	–	–	+	+	–	–	–	–	+	+	+	–	+	–	–	+	+	–	–	–	–	+
C_2	4.2	+	–	–	–	–	–	–	+	+	–	+	–	+	–	–	–	+	–	–	–	–	–	+	–	–	+	–	–
C_3	4.5	–	–	+	+	+	–	–	–	+	+	–	+	–	+	+	–	–	+	–	–	–	+	–	–	+	–	–	–
C_4	4.7	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–	+	–	–
C_5	5.0	–	–	+	+	+	+	–	+	+	+	–	–	+	–	+	–	–	–	–	+	–	–	+	–	+	+	–	–
C_6	5.2	–	–	–	–	–	+	–	–	–	–	–	+	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–
C_7	5.4	+	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	+	–	–	–	+	–
C_8	5.5	–	+	+	–	+	+	+	–	–	+	+	–	+	–	–	+	–	+	+	–	+	–	+	+	+	–	–	–
C_9	5.8	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	+
C_{10}	6.0	–	–	+	+	+	–	–	+	–	+	+	+	–	–	+	+	+	–	–	–	–	–	–	+	–	+	–	+
C_{11}	6.2	–	–	+	–	–	–	–	+	–	–	+	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–
C_{12}	6.5	–	+	–	–	–	+	+	–	+	–	+	+	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+
C_{13}	6.7	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	+	–	–
C_{14}	6.8	+	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D_1	7.0	–	–	–	+	+	–	–	+	–	–	+	+	–	+	+	+	–	–	+	–	+	+	+	+	–	+	–	+
D_2	7.2	–	–	+	–	–	+	–	–	–	+	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–
D_3	7.4	–	–	–	–	–	–	–	+	+	–	–	+	+	+	+	+	+	–	+	–	+	+	–	–	+	+	–	–
D_4	7.6	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–	+	–	–
D_5	7.8	–	+	–	–	–	–	–	–	–	–	+	–	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–
D_6	8.0	–	–	–	–	+	–	+	+	–	+	–	+	+	+	–	–	+	–	–	–	+	–	–	–	–	+	+	+
E_1	8.2	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	–	+	–	+	+	–	+	–	–	–	–	–	–
E_2	8.5	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+	–	+	–	–	–	–	–	+	+	–	+	–	–
E_3	8.8	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
E_4	9.0	–	–	–	–	–	–	+	–	–	+	–	+	+	+	–	+	–	+	+	+	–	+	–	–	–	–	–	–
F_1	9.2	–	–	–	–	–	–	–	+	+	–	–	–	+	+	+	–	+	–	–	+	–	–	–	–	–	+	–	–
F_2	9.5	–	–	–	–	–	–	–	+	–	–	+	+	–	+	+	–	+	–	+	–	+	+	–	+	–	–	–	+
F_3	9.7	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	+
F_4	9.8	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Total		7	9	9	9	10	11	11	13	13	14	15	16	17	18	20	11	19	11	11	8	9	9	10	6	9	14	7	10

for band differentiation, zone A representing the heaviest molecular weight protein was sub divisible into six distinct bands i.e. A_1, A_2, A_3, A_4, A_5 and A_6. Among these A_3 and A_5 were comparatively sharp bands. Similarly, zone B representing mostly thicker and dark bands, was sub divisible into eight bands i.e. B_1, B_2, B_3, B_4, B_5, B_6, B_7 and B_8. However, B_1, B_4 and B_6 were relatively dark bands. The next zone C representing dark to lighter bands with few faint bands was sub divisible into C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, and C_{14}. The next zone D characterized by lighter to faint bands was sub divisible into D_1, D_2, D_3, D_4, D_5 and D_6. Similarly, zone E representing mostly sharp bands were sub divisible into E_1, E_2, E_3 and E_4. Among these, E_2 and E_3 were comparatively thicker and darker bands. The last zone F was characterized by comparatively lighter bands and was sub divisible into F_1, F_2, F_3 and F_4. Thus a total of 42 bands could be resolved in seed protein (Table 2).

The different bands of HMW (97-43 kDa) were used for calculation of similarity indices. Presence of bands was scored as 1 and its absence as 0 for all the genotypes. These data matrix were then entered into NTSYS-PC. The grouping was carried out by unweighted pair group method using arithmetic averages (UPGMA) cluster analysis (Seneath and Sokal, 1973). The calculations were done using computer software package NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System Programme) (Rohlf, 1993).

Results and Discussion

The seed protein of 113 pea lines was subjected to sodium dodecyl sulphate polyacrylamide slab gel electrophoresis. The results obtained in the present study demonstrated the existence of 42 protein bands located in six zones (A, B, C, D, E and F). Different protein electropherotic patterns exhibited by 113 cultivars could be identified solely by the cultivar specific electropherogram(Fig. 1). On the basis of protein profile, 113 genotypes were classified into 28 groups. The genotypes within the group had similar protein banding pattern (Table 3).

The cultivars which were indistinguishable on the basis of simple identifiable morphological traits like growth habit; flower colour etc. could be distinguished on the basis of their electrophoretic patterns, example,

Table 3. Name of cultivars with in groups

Group	Cultivars
1.	6587-1, Asauji, Primette and NDVP-9
2.	Manova Sugar, Cobric, Dwarf Gray Sugar, Early Queen, VL-3, JP-4, Sel-82, Profino, Recette, Green Gulf, IP-3 and Frosty
3.	JP-83, VP-7802, UD-2, and PRS-18-6
4.	GC-141 and NDVP-5
5.	NDVP-1 and Ecoli
6.	P-23, Little Marvel, Progress, E-6, KS-246, Multifreezer, and NLP
7.	PMR-8 and Lincoln
8.	UU-12, VRP-2, PMR-3, PM-1, PMR-21, PMR-18, Sel-93, Alderman, NDVP-10, Tuinya Pratapgarh, Bridger, Borpeena, Piourette, PH-2, Sel-30, Deleketesse, UD-3, Waverex, PMR-11, Rover, PMR-13, Vitalette, VP-8902, P-88 and Azad Pea-3
9.	Cobrette, KS-245, VL-7, Stop, Petit Breton, KT-15-4-4, Sutton Early Giant, Victory Freezer and Waverplus
10.	PM-2, PMR-15, PMR-9, Starcovert, PMR-12 and P-388-1
11.	NDVP-25, Herabona, PC-121, ELF, VRP-1, Frizette, Boach Selection, Alderman Dwarf and Superlaska
12.	PM-5, Sel-23, VL-6, Polerette and J-506
13.	KS-226, PMR-17, VP-8002 and Arkel
14.	PMR, Corona Imperette, GC-245, GC-152, Bonneville, Lay Long Progress and King
15.	DPP-62
16.	Vitalis
17.	Midivert
18.	Thomas Laxton
19.	Pioneer
20.	6588-1
21.	JP-171
22.	PMR-7
23.	PMR-16
24.	Early December
25.	Meethi Phali
26.	Mammoth Melting Sugar
27.	KS-123
28.	Alaska

cultivars UU-12, Multifreezer, Piourette, Starcovert, Bonneville and IP-3 got separated which was not possible by morphological markers. Cultivar Meethi Phali with a specific characteristic of edible pod formed a separate group with 9 bands.

Some of the cultivars placed in the same group on the basis of their similar banding pattern were distinguishable by the intensity of staining of bands, for example, UU-12, VRP-2, PMR-3, PM-1, PMR-21, PMR-18, Sel-93, Tuinya Pratapgarh, PH-2, UD-3, PMR-11, PMR-13, Azad Pea-3 had a very dark and thick B_4 band as compared to KS-245, Alderman, NDVP-10, Bridger, Borpeena, Waverex, Rover, Vitalette and P-88 where this B_4 band was medium dark. Differences between accessions in the darkness and thickness of various bands are the most commonly reported types of variation, suggesting that the formation of many of the bands in the seed protein profile are under control of quantitative gene systems. This theory was supported by the study of Ladizinsky and Hymowitz (1979) and they also suggested that this kind of variation may be due to differential extraction or solubility of seed protein from different accessions.

Variation in band intensity observed within electropherograms of different groups may be attributed to large variation in the amount of various polypeptides present in the protein extract. Similar trends were observed in the protein-banding pattern of other leguminous crops like cowpea (Gomathinayagam and Ramaswary, 1994), fieldpea (Hussain *et al*., 1988) and also pea (Mishra *et al*., 1998).

Although, uniformity and uniqueness of seed protein profile are typical of plants, variation in the number of bands and their position in the profile have been reported, especially where great number of accessions were examined (Salmanowiz and Przybylska, 1992 and Singh *et al*., 1994). There was pattern in grouping of genotypes cultivars UU-12, Multifreezer, Piourette, Starcovert, Bonneville and IP-3.Cultivar Meethi Phali related to agronomic traits and selected genotypes from various clusters are suggested to use in pea improvement program (Ghafoor and Arshad, 2008)

Cluster analysis (Fig. 2; Table 4), after quantifying the protein bands, using UPGMA procedure, indicated that broadly the germplasms were grouped into two clusters, KS-226, Harabona and Multifreezer in cluster I and rest 110 genotypes in cluster II. The cluster II again formed 12 small clusters having 50-100% similarity. Dwarf and early varieties KS-226, Multifreezer and Herabona were included in one cluster which was most distinct from other showing greater divergence from other cultivars. The above varieties showed greater internal homogeneity and were more distinct from the other varieties. Within this clusters genotypes Multifreezer and KS-226 were having 68% similarity and from this Harabona included with a branching having 48% similarity with the above two. This may be due to the fact that Multifreezer and KS-220 are received from Holland and Harabona must be split off from an earlier common ancestor to form the secondary gene pool. This study had been in agreement with the work of Labdi *et al*. (1996).

Similarly relatively higher similarity percentage indicated a closeness among the genotypes as perusal of result presented 100% similarity between Progress and Recette and Alderman and VL-6. The closeness might be due to common parentage as former three cultivars were released from Europe and VL-6 had parentage of European origin or confluence of similar genes from different parents in the development of varieties. Similar pattern had been reported by Mishra *et al*. (1998). The phylogenetic evolutionary tree developed from the analysis indicated that most of the pea varieties did not form distinct clusters; rather, the relatively close and small clusters of varieties were distributed within one broad cluster as can be seen in the phenogram obtained in cluster II with 12 groups of 110 varieties of pea The relatedness of varieties in dendrogram were of mixed pattern as per their geographical distribution (Table 4). This may be due to the fact that till now different selection pressure must have been applied for different yield and yield related characters in different genotypes which caused the diverse expression of genes for those characters in genotypes. This need to the fact that formation of many bands in seed protein profiles are under control of quantitative gene systems and proteins are primary products of structural gene changes in coding base sequence will under many circumstances result in corresponding changes in the primary structure of protein. Even single amino acid substitution, deletion or addition can have marked effects on the migration of proteins under an electric field during electrophoresis (Ladizinsky and Hymowitz, 1979).

The similarity percentage varied between 50–100%. This corresponds with the fact that little genetic diversity found in pea used in study and most cultivars have the

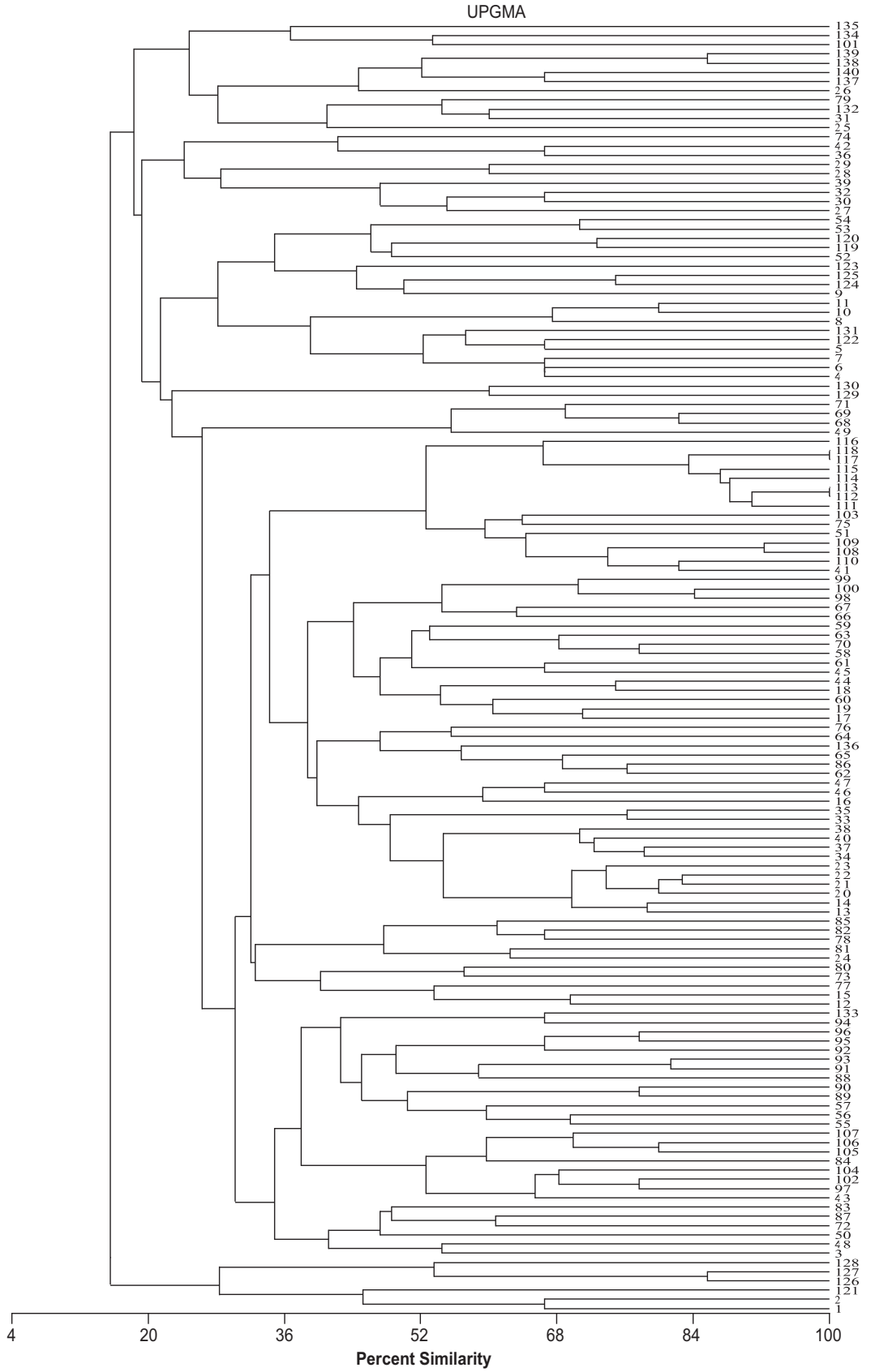

Fig. 2. Dendrogram generated based on seed protein bands of pea genotypes

Table 3. Arrangement of genotype in dendrogram

S. No.	Genotype No.	Genotypes	S. No.	Genotype No.	Genotypes
1.	82	PMR-13	58.	83	ELF
2.	97	J-506	59.	51	PMR-16
3.	15	PMR-21	60.	27	PMR-11
4.	84	Viodetti	61.	80	Sutton Early Giant
5.	86	VP-8902	62.	22	PMR-9
6.	91	VL-3	63.	94	Sel-82
7.	49	PMR-17	64.	78	Boach Selection
8.	28	PMR-18	65.	36	Sel-93
9.	72	GC-245	66.	102	Profino
10.	53	UD-2	67.	98	KS-123
11.	16	PMR-15	68.	25	6588-1
12.	3	Manoa Sugar	69.	66	Stop
13.	110	Green Gulf	70.	68	E-6
14.	54	Starcovert	71.	85	Meethi Phali
15.	23	Cobric	72.	99	KT-15-4-4
16.	13	DPP-62	73.	109	King
17.	40	NDVP-10	74.	101	Arkel
18.	107	Superlaska	75.	93	VP-8002
19.	17	VRP-2	76.	81	Bonneville
20.	34	NDVP-25	77.	44	Ecoli
21.	56	P-23	78.	19	KS-245
22.	12	Pioneer	79.	65	Waverex
23.	95	PMR-12	80.	42	Piourette
24.	55	PRS-18-6	81.	14	Asauji
25.	1	UU-12	82.	88	Frizette
26.	57	Little Marvel	83.	20	Bridger
27.	106	Lay Long Progress	84.	77	Petit Breton
28.	30	Dwarf Gray Sugar	85.	5	JP-83
29.	87	VRP-1	86.	41	Early Queen
30.	26	PM-1	87.	73	Early December
31.	64	Waver Plus	88.	89	Alderman Dwarf
32.	39	NDVP-1	89.	33	VL-7
33.	103	Progress	90.	79	GC-152
34.	108	Recette	91.	60	NDVP-5
35.	104	Azad Pea-3	92.	76	Lincoln
36.	69	NDVP-9	93.	21	Deleketesse
37.	38	Alderman	94.	61	PC-121
38.	31	VL-6	95.	37	PMR-7
39.	6	GC-141	96.	52	PMR
40.	113	Frosty	97.	105	P-388-1
41.	92	JP-4	98.	18	PMR-3
42.	29	Cobrette	99.	112	IP-3
43.	24	Sel-23	100.	74	Rover
44.	4	Vitalis	101.	32	Polerelte
45.	63	UD-3	102.	8	Midivert
46.	71	Corona Imperatte	103.	100	P-88
47.	62	Primette	104.	58	Sel-14-3-2
48.	45	Tuinya Pratapgarh	105.	70	NLP
49.	35	JP-171	106.	7	PM-2
50.	2	6587-1	107.	48	Sel-30
51.	46	KS-246	108.	11	VP-7802
52.	10	PM-5	109.	111	Alaska
53.	90	Mammoth Melting Sugar	110.	9	Thomas Laxton
54.	50	PH-2	111.	59	Harabona
55.	67	PMR-8	112.	43	KS-226
56.	96	Victory Freezer	113.	75	Multifreezer
57.	47	Borpeena			

Table 4. List of genotypes, their source and features

S.No.	Genotype	Source	Plant height	Growth	Flower colour	Foliage colour	Seed colour	Seed type
1.	UU-12	Pantnagar	Medium	Mid	W	YG	Green	Wrinkled
2.	6587-1	Jabalpur	Tall	Late	W	YG	Yellow	Smooth
3.	Manova sugar	Ludhiana	Medium	Mid	W	YG	Yellow	Smooth
4.	Vitalis	Holland	Tall	Late	P	YG	Green	Wrinkled
5.	JP-83	Jabalpur	Medium	Mid	W	YG	Yellow	Smooth
6.	GC-141	Gwaliar	Tall	Early	W	G	Green	Wrinkled
7.	PM-2	Pantnagar	Dwarf	Early	W	G	Green	Wrinkled
8.	Midivert	Holland	Medium	Early	W	YG	Green	Wrinkled
9.	Thoms laxton	Etawah	Dwarf	Mid	W	DG	Yellow	Smooth
10.	PM-5	Pantnagar	Dwarf	Mid	W	DG	Green	Wrinkled
11.	VP-7802	Almora	Medium	Mid	W	G	Green	Wrinkled
12.	Pioneer	Etawah	Medium	Mid	W	DG	Green	Wrinkled
13.	DPP-62	N.Delhi	Dwarf	Mid	W	DG	Green	Wrinkled
14.	Asauji	Kalyanpur	Tall	Mid	W	YG	Green	Wrinkled
15.	PMR-21	Pantnagar	Dwarf	Early	W	DG	Green	Smooth
16.	PMR-15	Pantnagar	Medium	Mid	P	YG	Green	Smooth
17.	VRP-2	Varanasi	Medium	Mid	W	YG	Green	Wrinkled
18.	PMR-3	Pantnagar	Medium	Mid	W	G	Green	Wrinkled
19.	KS-245	Kalyanpur	Dwarf	Mid	P	G	Yellow	Wrinkled
20.	Bridger	Etawah	Medium	Mid	W	DG	Green	Wrinkled
21.	Deleketesse	Holland	Tall	Late	W	G	Green	Wrinkled
22.	PMR-9	Pantnagar	Medium	Mid	W	YG	Yellow	Smooth
23.	Cobric	Holland	Medium	Mid	W	G	Green	Smooth
24.	Selection-23	Pantnagar	Dwarf	Mid	W	G	Green	Wrinkled
25.	6588-1	Jabalpur	Tall	Mid	W	G	Yellow	Wrinkled
26.	PM-1	Pantnagar	Dwarf	Early	W	G	Green	Wrinkled
27.	PMR-11	Pantnagar	Medium	Late	W	G	Green	Wrinkled
28.	PMR-18	Pantnagar	Dwarf	Mid	W	DG	Yellow	Smooth
29.	Cobrette	Holland	Tall	Late	W	YG	Yellow	Smooth
30.	Dwarf gray sugar	Etawah	Tall	Late	P	YG	Green	Wrinkled
31.	VL-6	Almora	Medium	Mid	W	G	Yellow	Wrinkled
32.	Polerette	Holland	Medium	Mid	W	G	Green	Smooth
33.	VL-7	Almora	Dwarf	Early	W	G	Green	Wrinkled
34.	NDVP-25	Faizabad	Dwarf	Mid	W	G	Yellow	Wrinkled
35.	JP-171	Jabalpur	Dwarf	Mid	W	YG	Yellow	Wrinkled
36.	Sel-93	Jabalpur	Tall	Late	W	G	Yellow	Smooth
37.	PMR-7	Pantnagar	Medium	Late	W	YG	Yellow	Smooth
38.	Aldarman	England	Medium	Late	W	DG	Green	Wrinkled
39.	NDVP-1	Faizabad	Dwarf	Early	W	G	Yellow	Wrinkled
40.	NDVP-10	Faizabad	Dwarf	Mid	W	G	Green	Wrinkled
41.	Early queen	Ludhiyana	Dwarf	Mid	W	G	Green	Wrinkled
42.	Piourette	Faizabad	Medium	Mid	W	DG	Green	Wrinkled
43.	KS-226	Holland	Dwarf	Mid	W	G	Yellow	Smooth
44.	Ecoli	Kalyanpur	Tall	Mid	W	G	Yellow	Wrinkled
45.	Tuyina Pratapgarh	Lucknow	Dwarf	Early	W	G	Green	Smooth
46.	KS-246	Kalyanpur	Dwarf	Mid	W	YG	Green	Wrinkled
47.	Borpeena	Etawah	Medium	Mid	W	G	Green	Wrinkled
48.	Sel-30	N.Delhi	Dwarf	Early	W	YG	Yellow	Wrinkled
49.	PMR-17	Pantnagar	Dwarf	Early	W	G	Green	Wrinkled
50.	PH-2	Hissar	Dwarf	Mid	W	DG	Green	Wrinkled
51.	PMR-16	Pantnagar	Medium	Mid	W	G	Green	Wrinkled
52.	PMR	Pantnagar	Medium	Late	W	YG	Green	Wrinkled
53.	UD-2	Udaipur	Tall	Late	W	YG	Yellow	Wrinkled
54.	Starcovert	Holland	Medium	Mid	W	G	Green	Wrinkled
55.	PRS-18-6	Jabalpur	Dwarf	Mid	W	G	Green	Wrinkled
56.	P-23	Ludhiana	Dwarf	Mid	W	G	Green	Smooth
57.	Little Marvel	N.Delhi	Dwarf	Early	W	G	Green	Wrinkled
58.	Sel-14-3-2	Pantnagar	Tall	Late	W	G	Green	Wrinkled
59.	Herabona	Ludhiana	Dwarf	Early	W	G	Green	Wrinkled
60.	NDVP-5	Faizabad	Dwarf	Mid	W	G	Green	Wrinkled
61.	PC-121	Pantnagar	Dwarf	Mid	W	G	Yellow	Wrinkled
62.	Primette	Holland	Dwarf	Early	W	YG	Yellow	Smooth

Contd.

Table 4. Contd...

S.No.	Genotype	Source	Plant height	Growth	Flower colour	Foliage colour	Seed colour	Seed type
63.	UD-3	Udaipur	Dwarf	Early	W	YG	Yellow	Smooth
64.	Waverplus	Holland	Medium	Mid	W	DG	Yellow	Wrinkled
65.	Waverex	England	Dwarf	Mid	W	DG	Green	Wrinkled
66.	Stop	Holland	Dwarf	Mid	W	YG	Yellow	Wrinkled
67.	PMR-8	Pantnagar	Medium	Mid	W	YG	Yellow	Smooth
68.	E-6	Almora	Dwarf	Early	W	G	Green	Wrinkled
69.	NDVP-9	Faizabad	Dwarf	Early	W	G	Green	Wrinkled
70.	NLP	Etawah	Medium	Mid	W	DG	Green	Wrinkled
71.	Corona Imperette	Holland	Dwarf	Mid	W	G	Green	Wrinkled
72.	GC-245	Gwaliar	Dwarf	Mid	W	G	Yellow	Wrinkled
73.	Early December	N. Delhi	Dwarf	Mid	W	YG	Yellow	Wrinkled
74.	Rover	Holland	Dwarf	Mid	W	G	Green	Wrinkled
75.	Multifreezer	Etawah	Dwarf	Mid	W	YG	Green	Wrinkled
76.	Lincoln	Shimla	Medium	Late	W	YG	Green	Wrinkled
77.	Petit Breton	Holland	Dwarf	Mid	W	G	Yellow	Smooth
78.	Boach Selection	Solan	Medium	Mid	W	DG	Green	Wrinkled
79.	GC-152	Gwaliar	Tall	Late	W	YG	Yellow	Wrinkled
80.	Sutton Early Giant	Solan	Tall	Late	W	G	Yellow	Wrinkled
81.	Bonniville	Kalyanpur	Medium	Mid	W	YG	Green	Wrinkled
82.	PMR-13	Pantnagar	Dwarf	Mid	W	YG	Green	Smooth
83.	ELF	Holland	Medium	Mid	W	G	Yellow	Wrinkled
84.	Vitalette	Holland	Dwarf	Mid	W	G	Green	Wrinkled
85.	Meethi Phali	Ludhiana	Dwarf	Mid	W	G	Green	Wrinkled
86.	VP-8902	Almora	Dwarf	Mid	W	G	Yellow	Wrinkled
87.	VRP-1	Varanasi	Dwarf	Mid	W	YG	Yellow	Smooth
88.	Frizette	Holland	Medium	Mid	W	G	Green	Wrinkled
89.	Alderman Dwarf	Etawah	Dwarf	Mid	W	G	Green	Wrinkled
90.	Mammoth Melting Sugar	Etawah	Medium	Mid	P	YG	Green	Wrinkled
91.	VL-3	Almora	Dwarf	Mid	W	YG	Yellow	Wrinkled
92.	JP-4	Jabalpur	Medium	Late	W	YG	Green	Smooth
93.	VP-8002	Almora	Dwarf	Mid	W	G	Green	Wrinkled
94.	Sel-82	Hissar	Dwarf	Late	P	DG	Green	Wrinkled
95.	PMR-12	Pantnagar	Dwarf	Early	W	G	Green	Wrinkled
96.	Victory Freezer	Etawah	Medium	Mid	P	G	Green	Wrinkled
97.	J-506	England	Medium	Mid	W	G	Yellow	Smooth
98.	KS-123	Kalyanpur	Dwarf	Mid	W	YG	Yellow	Wrinkled
99.	KT-15-4-4	Katrain	Medium	Mid	W	G	Green	Wrinkled
100.	P-88	Ludhiana	Dwarf	Mid	W	G	Green	Wrinkled
101.	Arkel	Katrain	Dwarf	Early	W	G	Green	Wrinkled
102.	Profino	Holland	Dwarf	Early	W	G	Green	Smooth
103.	Progress	Etawah	Dwarf	Mid	W	YG	Yellow	Smooth
104.	Azad Pea-3	Kalyanpur	Dwarf	Mid	W	G	Green	Wrinkled
105.	P-388-1	Jabalpur	Medium	Late	W	G	Green	Wrinkled
106.	Lay Long Progress	Solan	Medium	Late	W	G	Green	Wrinkled
107.	Superlaska	Solan	Tall	Late	W	DG	Yellow	Wrinkled
108.	Recette	Holland	Dwarf	Mid	W	G	Yellow	Wrinkled
109.	King	Etawah	Dwarf	Mid	W	DG	Green	Wrinkled
110.	Green Gulf	Holland	Dwarf	Mid	W	YG	Yellow	Smooth
111.	Alaska	Etawah	Dwarf	Early	W	G	Green	Wrinkled
112.	IP-3	Etawah	Medium	Mid	W	DG	Green	Wrinkled
113.	Frosty	Etawah	Medium	Mid	W	DG	Green	Wrinkled

W = white YG = yellow green G = green P = purple DG = dark green

same ancestors. Similar results have been reported by Petr Samee and Vit Nasinec (1996) in 42 genotypes of *Pisum sativum* L. based on Jaccard's similarity coefficient.

Though considerable inter-varietal variability was found in the present study, most of the varieties showed no distinct patterns which would allow their characterization. There were a few specific bands in each variety and most of them; although exclusive, appeared at such a low frequency that varietal characterization was not possible. Since pure seeds maintained by plant breeders were used in this study, the polymorphic protein patterns were considered to be representative of the varieties examined.

References

Bushuk W, WR Johonson and HD Saperstein (1987) Standard method No. 143, International Association for Cereal Science and Technology, Vienna.

Garffin DE (1990) One dimensional gel electrophoresis. *In:* MP Deutscher (ed) *Methods in Enzymology*. Academic Press Inc., Son Siegai, C.A. Vol. 182, pp 425–441.

Garffin DE (1990) One dimensional gel electrophovesis. In: MP Deutscher (ed), Methods in Enzymology, Academic Press Inc, Son Seigai, CA, Vol. 182, pp 425–411.

Ghafoor A and M Arshad (2008) Seed protein profiling of *Pisum sativum* L., germplasms using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for investigation of biodiversity. *Pak. J. Bot*. **40:** 2315–2321,

Ghosh SK and SC Gulati (2002) Selection of practically significant yield component for genetic diversity analysis in Indian mustard (*Brassica juncea* L.). *Crop Res*. **23:** 49–54.

Gomathinayagam P and NM Ramaswary (1994) Cultivar identification in cowpea using SDS-PAGE electrophoresis. *Plant Biochem. Biotechnol.* **18:** 149.

Hussain A, ST Ali-Khan and W Bushuk (1988) Field pea cultivar identification by electrophoretic patterns of cotyledon proteins. *Canadian J. Plant Sci*. **68:** 1143–1147.

Ladizinskhy G and T Hymowitz (1979) Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet.* **54:** 145–151.

Labdi M, LD Robertson, KB Singh and A Charrier (1996) Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphism. *Euphytica* **88:** 181–188.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Matta NK and JA Gatehouse (1982) Inheritance and mapping of storage protein genes in *Pisum sativum* L. *Heredity* **48:** 383–392.

Mishra SK, SK Dasgupta, B Sharma and R Kahlon (1998) Diversity in seed protein pattern in garden pea and its wild relatives. *Am. Agric. Res.* **19:** 311–316.

Petr Samee and Vit Nasinec (1996) The use of RAPD technique for the identification and classification of *Pisum sativum* L. genotypes. *Euphytica* **89:** 229–234.

Rohlf FJ (1993) NTSYS-PC: numerical taxonomy and multivariate analysis system. Exeter Software, Setanket, NY, USA.

Rosa L and N Jouve (1992) Genetic variation for isozyme genes and proteins in Spanish primitive cultivars and wild subspecies of *Lens. Euphytica* **59:** 181–187.

Salmanowiez BP and Przybylaska J (1992) Seed albumins from some species of *Lathyrus lens* and *Cicer* genera: comparative analysis by gel filtration and electrophoresis. *Genetica Polonica* **33:** 107–113.

Sneath PHA and RR Sokal (1973) *Numerical Taxonomy. Freeman*, San Francisco.

Singh MN, B Rai and RM Singh (1994) Potentialities of heterosis breeding in *Pisum sativum* L. *Indian J. Genet. Plant Breed.* **54:** 398-401.

Wolf G (1980) Investigations on the relations within the family Papilionaceae on the basis of the electrophoretic banding patterns. *Theor. Appl. Genet.* **57:** 225–232.