

## Comparative study of seed protein profiles in the genus *Pisum*

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### Abstract

Seed protein profiles of 24 wild and cultivated taxa of *Pisum* have been compared by sodium dodecyl sulphate polyacrylamide gel electrophoresis. No consistent differences were detected either among wild taxa or between wild and cultivated taxa. This shows that *Pisum* forms a single-species complex on the basis of seed protein profiles.

*Additional key words:* electrophoresis, genetic diversity, pea, seed-storage proteins.

### Introduction

There is still no consensus about the relationships of various species in the genus *Pisum* (*Fabaceae*). It is traditionally classified into four or five species *P. sativum*, *P. elatius*, (*P. sativum* subsp. *elatius*), *P. abyssinicum*, *P. humile* and *P. fulvum* (Lehman and Blixt 1984). Studies based on morphology, ecology, cytogenetics and hybrid performance show *P. fulvum* to be a fully divergent species and other species form a single species complex (Ben Ze'ev and Zohary 1973), while the other researchers consider that *Pisum* is monospecific and that differences observed for *P. fulvum* are more a matter of degree than the basis of

a distinct species (Lemprecht 1966, Blixt 1974, Waines 1975, Marx 1977, Wolff 1980, Palmer *et al.* 1985, Hoey *et al.* 1996, Lu *et al.* 1996). Seed protein electrophoresis has proved to be a powerful tool in solving taxonomic problems and elucidating origin and evolution of a number of cultivated plants (Ladizinsky and Hymowitz 1979, Jha and Ohri 1996, Nath *et al.* 1997, Haider and El-Shanshoury 2000, Vladova *et al.* 2000, El Naggar 2001). In the present study an attempt has been made to follow differentiation patterns in seed protein profiles in 24 wild and cultivated *Pisum* species.

### Materials and methods

All the *Pisum* L. species (Table 1) were obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany. Protein extraction was carried out by homogenising cotyledons in 0.1 M Tris-HCl buffer (pH 7.5). Samples of supernatant obtained after centrifugation at 17 600 g at 4 °C for 20 min were diluted with sample buffer containing 0.0625 M Tris-HCl, pH 6.8, 2.5 % sodium dodecyl sulfate (SDS), 5 % 2-mercaptoethanol and 10 % glycerol and heated in boiling water for 5 min prior to being loaded on gel. Content of total proteins in the samples were estimated following the method of Lowry *et al.* (1951). Electrophoresis was carried out in the modified discontinuous SDS-PAGE

system of Laemmli (1970) using 10 % acrylamide resolving gel (0.375 M Tris-HCl, pH 8.9) and 4 % stack gel (0.125 M Tris-HCl, pH 6.8). The running buffer was Tris-glycine (0.3 % Tris base, 1.44 % glycine and 0.1 % SDS, pH 8.3). Staining of the gels was done in 0.02 % Coomassie Brilliant Blue R-250 containing 55 % methanol and 7 % acetic acid while destaining was done in the same solution but without the dye. The R<sub>f</sub> value for each band was computed from the mean of 4 different runs and 2 extractions. The values were used to prepare a data matrix, with rows corresponding to the characters (unique bands; variables) and column to the species in question (operational taxonomic units, *i.e.* OTUs). The

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scores were 1 for the presence and 0 for the absence of a band. The data file thus written was used as the input for *NTSYS-PC* (Numerical Taxonomy and Multivariate Analysis System), through *SIMQUAL* module (Programme to compute various association coefficients for qualitative data) software to compute Jaccard's

similarity index for all possible pairs of taxa (OTUs) in question. The similarity matrix thus generated was used to construct a dendrogram by the unweighted pair-group method with arithmetic means (UPGMA) using *SAHN*, another module in *NTSYS-PC* package (Sneath and Sokal 1973).

Table 1. List of *Pisum* accessions obtained from the Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben.

Accession number	Species, cultivar
1	PIS 1665/91 <i>P. fulvum</i> Sibth & Sm.
2	PIS 132/84 <i>P. sativum</i> subsp. <i>transcaucasicum</i> Gov.
3	PIS 1141/92 <i>P. sativum</i> L. subsp. <i>elatius</i> (Stev.) Schmalh
4	PIS 150/73 <i>P. sativum</i> L. <i>abyssinicum</i> (A.Br.) Berger var. <i>vavilovianum</i> Gov.
5	PIS 1804/86 <i>P. sativum</i> L. subsp. <i>asiaticum</i> Gov.
6	PIS 1318/91 <i>P. sativum</i> L. subsp. <i>syriacum</i> Berger
7	PIS 975/78 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>sativum</i> var. <i>episcopi</i> Alef.s.l.
8	PIS 505/75 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>speciosum</i> (Dierb.) Alef. var. <i>angulare</i> Gov.
9	PIS 334/75 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>sativum</i> var. <i>superfluens</i> Alef. s.l.
10	PIS 2018/83 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>sativum</i> var. <i>nanoanglicum</i> Korn.
11	PIS 525/74 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>jessenii</i> Alef. s.l.
12	PIS 1545/77 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>medullare</i> Alef. var. <i>balticum</i> Alef. s.l.
13	PIS 968/79 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>medullulosaccharatum</i> (Korn.) Lehm. var. <i>prasinum</i> Lehm.
14	PIS 1530/77 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>medullare</i> Alef. var. <i>pervicax</i> Alef. s.l.
15	PIS 491/89 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>speciosum</i> (Dierb.) Alef. var. <i>zeylanicum</i> Alef.
16	PIS 433/77 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>speciosum</i> (Dierb.) Alef. var. <i>thebaicum</i> (Willd) Alef. s.l.
17	PIS 1521/80 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>speciosum</i> (Dierb.) Alef. var. <i>capucinorum</i> Alef.
18	PIS 496/73 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>speciosum</i> (Dierb.) Alef. var. <i>arvense</i> (L.) Alef.
19	PIS 2116/84 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>axiphium</i> Alef. var. <i>minus</i> Gov.
20	PIS 1527/89 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>axiphium</i> Alef. var. <i>compocarpum</i> Alef.
21	PIS 1686/93 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>medullare</i> Alef. cv. Kelvil
22	PIS 1720/93 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>sativum</i> cv. Sovereign
23	PIS 1651/93 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>medullare</i> Alef. cv. Perk
24	M 1677/93 <i>P. sativum</i> L. s.l. subsp. <i>sativum</i> convar. <i>sativum</i> cv. Primvers

## Results and discussion

In total 68 unique polypeptide bands were noted. The maximum number of bands in any one taxon was 40 recorded in *P. sativum* subsp. *syriacum* and the minimum number was 22 recorded in *P. sativum* subsp. *sativum* var. *pervicax* (Figs. 1, 2). *P. fulvum* shows maximum genetic homology (44 %) with *P. sativum* subsp. *transcaucasicum* while with other subspecies the homology ranges from 18.2 % with *P. sativum* subsp. *abyssinicum* to 30 % with *P. sativum* subsp. *elatius* (Table 2, Figs. 1,3). However, the homology of *P. fulvum* with the cultivated varieties of *P. sativum* ranges from 5.3 % (*P. sativum* subsp. *sativum* convar. *medullare* var. *balticum*) to 35.7 % (*P. sativum* subsp. *sativum* convar. *sativum* var. *nanoanglicum*) (Table 2, Figs. 1,2,3). *P. sativum* subsp. *elatius* shows maximum homology (35.5 %) with *P. sativum* subsp. *abyssinicum* which agrees with the AFLP studies made by Lu *et al.* (1996).

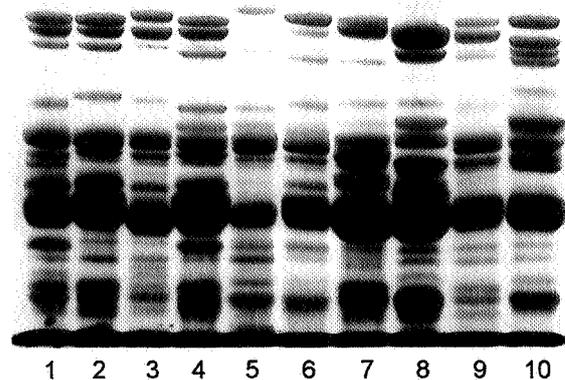


Fig. 1. Electrophoregrams showing seed storage protein banding pattern in *Pisum* taxa (serial number of taxa as in Table 1).

*P. sativum* subsp. *asiaticum* forms a sister group with four varieties of *P. sativum* subsp. *sativum* (taxa 7, 8, 9, 10 in Table 1, Figs. 1,3). *P. sativum* subsp. *syriacum* forms a

sister group to all these taxa and shows maximum homology with *P. sativum* subsp. *sativum* convar. *speciosum* var. *ungulare* (Table 2, Figs. 1,3).

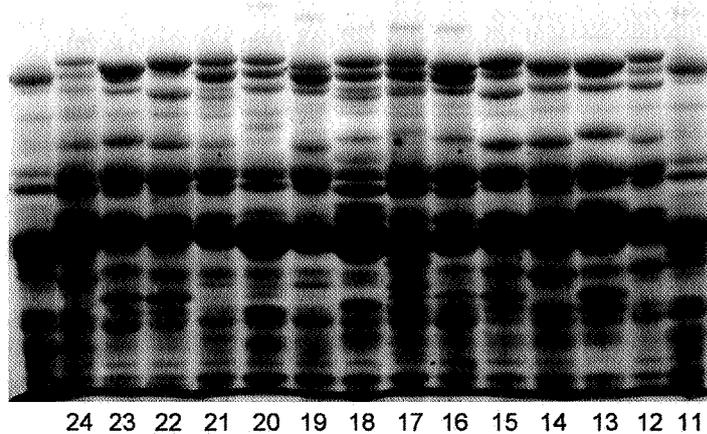


Fig. 2. Electrophoregrams showing seed storage protein banding pattern in *Pisum* taxa (serial number of taxa as in Table 1).

Table 2. Similarity matrix based on the observed seed protein patterns between all possible pairs of 24 species of *Pisum* (serial numbers as in Table 1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
1	1.000																									
2	0.440	1.000																								
3	0.300	0.258	1.000																							
4	0.182	0.219	0.355	1.000																						
5	0.219	0.300	0.273	0.355	1.000																					
6	0.194	0.156	0.250	0.250	0.290	1.000																				
7	0.250	0.212	0.344	0.265	0.303	0.242	1.000																			
8	0.323	0.171	0.222	0.333	0.333	0.313	0.364	1.000																		
9	0.258	0.219	0.313	0.313	0.448	0.212	0.593	0.419	1.000																	
10	0.357	0.310	0.242	0.323	0.367	0.219	0.400	0.483	0.519	1.000																
11	0.083	0.182	0.167	0.167	0.200	0.143	0.194	0.257	0.200	0.242	1.000															
12	0.053	0.081	0.194	0.194	0.194	0.323	0.158	0.286	0.265	0.135	0.344	1.000														
13	0.073	0.048	0.119	0.237	0.237	0.154	0.200	0.256	0.237	0.150	0.270	0.333	1.000													
14	0.147	0.114	0.050	0.167	0.200	0.111	0.132	0.257	0.235	0.171	0.200	0.303	0.469	1.000												
15	0.077	0.077	0.071	0.154	0.184	0.103	0.150	0.175	0.184	0.158	0.406	0.314	0.515	0.552	1.000											
16	0.132	0.075	0.179	0.278	0.150	0.128	0.146	0.231	0.179	0.184	0.394	0.343	0.342	0.394	0.531	1.000										
17	0.135	0.077	0.154	0.184	0.154	0.194	0.179	0.270	0.216	0.128	0.286	0.394	0.389	0.324	0.455	0.485	1.000									
18	0.122	0.070	0.225	0.225	0.140	0.175	0.250	0.275	0.195	0.143	0.324	0.389	0.385	0.256	0.486	0.472	0.486	1.000								
19	0.175	0.146	0.250	0.250	0.190	0.200	0.186	0.268	0.250	0.195	0.316	0.342	0.410	0.351	0.472	0.459	0.514	0.500	1.000							
20	0.194	0.162	0.243	0.278	0.150	0.100	0.237	0.263	0.243	0.250	0.278	0.237	0.378	0.394	0.485	0.471	0.441	0.514	0.636	1.000						
21	0.147	0.054	0.235	0.200	0.135	0.176	0.229	0.222	0.235	0.139	0.273	0.265	0.343	0.313	0.364	0.353	0.324	0.441	0.515	0.484	1.000					
22	0.176	0.111	0.265	0.265	0.162	0.206	0.189	0.286	0.229	0.235	0.265	0.257	0.371	0.344	0.314	0.382	0.353	0.316	0.417	0.469	0.593	1.000				
23	0.111	0.081	0.229	0.303	0.132	0.171	0.257	0.250	0.229	0.167	0.344	0.294	0.412	0.303	0.394	0.469	0.314	0.471	0.457	0.469	0.654	0.571	1.000			
24	0.083	0.054	0.167	0.200	0.167	0.212	0.194	0.222	0.273	0.139	0.331	0.303	0.306	0.313	0.364	0.353	0.452	0.400	0.515	0.438	0.615	0.536	0.483	1.000		

The various varieties and cultivars of *P. sativum* subsp. *sativum* (Table 1, taxa 7 - 24) express similarity in banding pattern ranging from 12.8 to 65.4 % among themselves (Table 2, Figs. 2,3). These results clearly show that differences observed among wild taxa, between wild and cultivated taxa and among cultivated taxa are of the same degree. All these *Pisum* taxa are not much differentiated from each other and are thus closely related. The present results agree with earlier studies

where also no consistent differences were found between seed protein patterns of cultivated and wild taxa (Waines 1975, Wolff 1980). Similarly a cladistic analysis based on morphological, allozyme and RAPD markers confirmed a close relationship among 17 cultivated and wild *Pisum* taxa. All these taxa including *P. fulvum* belong to one species complex (Hoey *et al.* 1996). Furthermore, relationships based on AFLP also clearly show that genetic diversity is evenly distributed within the genus

*Pisum* and its various species and subspecies cannot be markedly distinguished (Lu *et al.* 1996). However, hybridization studies suggest that *P. fulvum* is reproductively isolated and is therefore a fully divergent

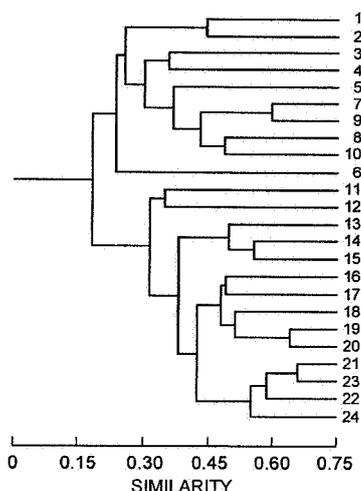


Fig. 3. Dendrogram (UPGMA) of 24 wild and cultivated taxa of *Pisum* based on similarity matrix of seed storage proteins (serial number of taxa as in Table 1).

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