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Electrophoretic analysis of seed storage proteins for different *Pisum* and *Lathyrus* species

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Abstract. *Lathyrus* and *Pisum* species are members of the tribe *Vicieae* in the Fabaceae family. These species are of economic importance. *Pisum sativum* L. is a basic model in plant genetics. Seed storage proteins have been used as a biochemical marker to determine the phylogenetic relationship among studied *Lathyrus* and *Pisum* species. The polymorphism of the studied *Lathyrus* species was 86.05%, while that of the studied *Pisum* species was 51.35%. The highest similarity matrix was among *P. sativum* subsp. sativum covariates. The highest value was 0.96 detected between *P. sativum* subsp. *sativum* Convar. *speciosum* & *axiphium*, *speciosum* & *medullare,* and *speciosum* & *sativum*, while the lowest similarity matrix was detected between *Pisum fulvum* and all the studied *P. sativum* subsp. *sativum* covariates. Similarity matrix analysis among the *Lathyrus* species revealed the next genetic relationships: *L. articulates* L. and *L. aureus* are the most closely related to each other (0.76), followed by *L. pratensis* L. which is the most related to *L. pannonicus* subsp. *varius*. All studied species of both genera, *Lathyrus* and *Pisum*, were split into two main clusters. The first cluster contains all the studied *Pisum* species, while the second cluster contains all the studied *Lathyrus* species. However, the genetic relationship or similarity matrix among the studied *Pisum* and *Lathyrus* species was very low.

Keywords: genetic relationship, *Lathyrus*, *Pisum*, Seed storage proteins, SDS-PAGE.

Introduction

Leguminosae (Fabaceae family) is the third largest family of flowering plants. This family contains about 21,000 species **(**Christou 1993). Legumes vary in size and habit, ranging from herbaceous to woody plants. The seeds of legumes have a high amount of protein content, ranging from 20% in peas to 40% in lupin (Cereletti 1979), and they are considered the main sources of proteins in the human diet and are also used as feed for animals. Moreover, species belonging to the family Leguminosae have many secondary metabolites with potential medical use (Andrea 2011).

The genus *Lathyrus* and *Pisum* are members of the tribe *Vicieae* (Fabaceae, Papilionoideae). The genus *Lathyrus* comprises nearly 160 annual and perennial species, many of which are cultivated in temperate zones of the northern hemisphere, South America, and tropical East Africa (Kupicha 1983). This economically important genus is used as forage, human food, or ornamental plant (Chtourou-Ghorbel et al. 2001, Ben Brahim et al. 2002, Lewis et al. 2005). The third most widely grown legume is pea (*Pisum sativum*), cultivated in temperate zones worldwide. The pea genus (*Pisum*) includes a thousand varieties maintained in numerous germplasm banks worldwide. Pea seeds contain numerous proteins that benefit human nutrition (Ambrose 1995, Zohary & Hopf 2000, Smýkal et al. 2008).

The modern technological tools based upon mutant induction are becoming pervasive in *P. sativum* subsp. *sativum*. The induced variants in stem features, leaf, flower, pod, and seed have led to several criteria for assorting infraspecific diversity (Makaševa 1979, Lehmann & Blixt 1984). Several convarieties have been created and defined, for example, the field pea (convar. *speciosum* (Dierb.) Alef) that is known as grain forage, or the convariety *axiphium* Alef., which is recognized as the sugar pea with edible pods.

Genetic diversity is a substantial process for studying the adaptation of species to environmental changes and the natural selection process (Grassi et al. 2006). However, a comparative study on the variation of seed storage proteins is desirable for increasing our knowledge about the genetic resemblance among the most important grain legumes. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a simple and effective biochemical technique for describing plant species' genetic structure. Seed storage proteins are the third-hand copy of genomic DNA and largely independent of environmental changes (Sammour 1987, Javaid et al. 2004, Iqbal et al. 2005), So it is utilized for differentiation of the variation among different species, examining the purity of huge cultivars, detecting the genome relationships, determining the essential characters of landraces and wild relatives to supply increasing yield and crop production (Sammour 1991), and utilizing the information on genetic diversity to make decisions concerning selection of superior genotypes for enhancement plant criteria and yield.

This research was conducted to determine the relationship among different *Lathyrus* species and *Pisum* species and subspecies based on seed storage proteins profile using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique.

Material and methods

Plant material

Seeds of 14 *Lathyrus* species and 9 *Pisum* species and subspecies were obtained from the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany (Table 1).

SDS-PAGE

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), as modified by Studier (1973). Seed storage proteins of studied *Lathyrus* and *Pisum* species were extracted from their seed. Protein fractionations were performed exclusively on vertical slab gel (19.8 cm x 26.8 cm x 0.2 cm) using the vertical electrophoresis apparatus "Claver" manufactured in the UK. Seeds of studied *Lathyrus* and

Pisum species were ground well in Eppendorf; after that, the 2x Lan's buffer [1M Tris-HCl (ph 8.8), SDS (10%), 0.25M EDTA, Glycerol] was added and mixed well then incubated in refrigerator over-night, then vortexed for 15 seconds and centrifuged at 12,000 rpm at 4˚C for 20 min. The supernatant containing the total seed storage proteins was transferred to new Eppendorf tubes and stored at deep freeze until the SDS-PAGE protein electrophoresis technique was used. 50 µl of the extracted protein was mixed with the same volume of 2x Lan's buff [1M Tris-HCl (ph 8.8), SDS (10%), 0.25M EDTA, Glycerol] in an Eppendorf tube. ß-mercaptoethanol (5µl) was added to each tube and boiled in the water bath for 10 min, and then 10 µl bromophenol blue was added to each tube before samples loading. BL Ultra Prestained Protein Ladder (GeneDirex, Cat No. PM001-0500) and protein samples were loaded on a 10% resolving gel. After that, the protein ladder and protein samples were run in running buffer [Tris-base, SDS (10%), Glycine] at 100V until the resolving gel was reached, and then separation was done at 250V. After completing the run, protein gels were stained with 1% Coomassie brilliant blue R250 dye and destained by gentle agitation in a destaining solution (glacial acetic acid and methanol) until the gel background was clear.

Gel analysis

A Sony digital camera (Japan) was used to capture gel images. Then, a software program (Total Lab) was applied to detect the molecular weight of each protein band. Finally, these data were introduced in Past software program in Past software program (Paleontological statistics software package for education and data analysis, Hammer et al. 2001) to detect the similarity matrix (UPGMA, using Jaccard's coefficient), which determines the phylogenetic relationships among the examined species.

Table 1. Sample number, accession number, and the origin of each studied *Lathyrus* and *Pisum* species and subspecies.

Samples	Genus	Scientific name	Accession name	Origin
1	*Lathyrus*	*L. articulates* L.	LAT 117/79	Unknown
2		*L. aureus* (ster) Brandra	LAT 15/79	Soviet Union
3		*L. davidii* Hance	LAT 21/82	Japan
4		*L. digitatus* (M.Bieb) Fiori	LAT 8/84	Ukraine
5		*L. hierosolymitanus* Boiss	LAT 142/84	Unknown
6		*L. laxiflorus* (Desf) O. kuuntze	LAT 20/81	Greece
7		*L. latifolius* L.	LAT 7/82	France
8		*L. neurolobus* Boisset Helder	LAT 25/84	Unknown
9		*L. niger* L. Bernh	LAT 6/78	Hungary
10		*L. pannonicus* (Jacq) Garcke subsp. *varius*	LAT 24/83	Italy
11		*L. pratensis* L.	LAT 22/83	German
12		*L. pseudocicera* pamp	LAT 346/99	Israel
13		*L. sylvestris* L.	LAT 2/79	Canada
14		*L. tuberosus* L.	LAT 23	Soviet Union
15	*Pisum*	*P. fulvum* sibth etsm	PIS 1665/91	-
16		*P. sativum* L.S.I. subsp. *asiaticum* Gov	PIS 1803/86	-
17		*P. sativum* L.S.I. subsp. *abyssinicum* (A. Braun) Berger var. *varilovianum* (Gov.)	PIS 1223/73	-
18		*P. sativum* L.S.I. subsp. *elatius* (M. Bieb.) Schmalh	PIS 1140/75	-
19		*P. sativum* L.S.I. subsp. *sativum* Convar. *axiphium* Alf "Dvargsabel"	PIS 643/73	-
20		*P. sativum* L.S.I. subsp. *sativum* Convar. *medullare*	PIS 1814/80	-
21		*P. sativum* L.S.I. subsp. *sativum* Convar. *medullosaccharatum* (Kȍrn) lehm "Artemis"	PIS 640/74	-
22		*P. sativum* L.S.I. subsp. *sativum* Convar. *sativum*	PIS 1523/75	-
23		*P. sativum* L.S.I. subsp. *sativum* Convar. *speciosum* (Dieb.) Alef	PIS 1667/78	-

Results

Seed storage proteins of *Lathyrus* species

Seed storage protein fractions by SDS-PAGE were considered a biochemical marker for studying the phylogenetic relationship among the Lathyrus species studied. The seed storage proteins profile detected 43 bands with molecular weight (Mw) ranging from 110 to 13 KiloDalton (KDa). Six monomorphic bands were detected at Mw 98, 92, 49, 33, 29, and 25 KDa, and the polymorphism was 86.05%. A maximum of 35 bands were detected in *L. tuberosus* L., whereas the minimum was 20 bands in *L. pannonicus* subsp. *varius*. Two negative unique bands were detected in *L. neurolobus* and *L. pseudocicera* at Mw 36 and 15 KDa, respectively. The band at Mw27 KDa was detected only in *L. latifolius* L. and *L. pseudocicera*. Six bands were absent at different molecular weights in the studied *Lathyrus* species as follows, at 47 KD in *L. niger* L. and *L. pannonicus* subsp. *varius*, at 40 KD in *L. pannonicus* subsp. *varius* and *L. pseudocicera*, at 39 KD in *L. articulates* L. and *L. aureus*, at 38 KD in *L. pseudocicera* and *L. tuberosus* L., at 37 KD in *L. hierosolymitanus* and *L. neurolobus* and at 34 KD in *L. davidii* and *L. niger* (Table 2 and Figure 1).

Table 2. Approximate molecular weight of total seed storage protein bands in different *Lathyrus* species (*1- L. articulates; 2- L. aureus; 3- L. davidii; 4- L. Digitatus; 5- L. hierosolymitanus; 6- L. Laxiflorus; 7- L. latifolius; 8- L. neurolobus; 9- L. niger, 10- L. pannonicus; 11- L. pratensis; 12- L. pseudocicera; 13- L. sylvestris; 14- L. tuberosus).*

Ser. no.	**Mw**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**Polymorphism (%)**
1	110	+	+	+	-	-	+	-	-	-	-	+	+	-	-	P (Polymorhic)
2	105	-	-	-	-	-	-	+	-	+	-	-	-	+	+	P
3	98	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M (Monomorphic)
4	92	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
5	80	+	+	-	+	+	-	-	-	-	-	-	-	-	-	P
6	77	-	-	+	+	+	+	+	+	-	-	-	-	+	+	P
7	73	+	+	-	+	+	+	+	-	-	+	+	+	-	+	P
8	70	-	-	+	-	+	-	-	-	-	-	+	+	-	+	P
9	65	+	+	+	+	-	-	-	+	-	-	-	-	+	+	P
10	62	-	-	-	+	+	+	-	+	+	-	+	-	-	+	P
11	60	-	-	+	-	-	-	+	-	-	-	-	+	+	-	P
12	59	+	+	-	+	-	-	-	+	+	+	+	-	-	+	P
13	56	+	+	+	+	+	-	+	+	-	-	+	-	+	+	P
14	54	-	-	-	-	-	-	+	+	+	-	-	-	+	-	P
15	52	+	+	+	+	+	+	+	+	+	+	+	+	-	+	P
16	51	+	+	-	-	-	-	-	+	-	-	+	+	-	+	P
17	49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
18	47	+	+	+	+	+	+	+	+	-	-	+	+	+	+	P
19	45	+	-	-	+	-	-	-	+	-	-	-	-	+	+	P
20	42	+	+	+	-	-	-	+	+	-	-	+	+	-	-	P
21	40	+	+	+	+	+	+	+	+	+	-	+	-	+	+	P
22	39	-	-	+	+	+	+	+	+	+	+	+	+	+	+	P
23	38	+	+	+	+	+	+	+	+	+	+	+	-	+	-	P
24	37	+	+	+	+	-	+	+	-	+	+	+	+	+	+	P
25	36	+	+	+	+	+	+	+	-	+	+	+	+	+	+	P
26	35	+	-	-	+	+	-	-	-	+	-	-	-	-	+	P
27	34	+	+	-	+	+	-	+	+	+	+	+	+	+	+	P
28	33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
29	32	-	+	-	-	-	+	+	+	+	+	+	-	-	+	P
30	31	-	+	+	-	+	+	+	+	+	-	-	-	+	+	P
31	30	+	-	-	-	-	-	-	-	-	-	-	+	+	+	P
32	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
33	27	-	-	-	-	-	-	+	-	-	-	-	+	-	-	P
34	25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
35	24	-	-	-	+	-	-	+	-	-	-	-	-	-	+	P
36	23	-	+	+	-	+	+	-	+	+	+	+	+	+	+	P
37	22	+	+	+	+	+	+	-	-	-	-	-	+	+	+	P
38	21	+	+	-	-	+	+	+	+	+	+	+	+	+	+	P
39	19	-	-	-	-	-	+	+	+	+	+	+	+	-	+	P
40	16	+	-	-	-	-	-	-	-	-	-	-	+	+	+	P
41	15	+	+	+	+	+	+	+	+	+	+	+	-	+	+	P
42	14	+	+	+	-	+	+	+	+	-	-	-	+	-	-	P
43	13	+	-	+	-	-	-	-	-	-	+	+	-	+	+	P
Total Bands		30	28	26	26	26	25	29	28	24	20	28	26	28	35	86.05

<u>Genetic similarity</u>

The similarity index among *Lathyrus* species was determined for each pair-wise group (Table 3 and Figure 2). Depending on seed storage protein analysis using SDS-PAGE, the similarity ranged from 0.76 to 0.39 (Table 3). The highest similarity index (0.76) was detected between *L. articulates* L.

and *L. aureus*, while the lowest similarity index (0.37) was documented between *L. niger* L. and *L. pseudocicera*. Figure 2 represents the dendrogram from UPGMA cluster analysis among different *Lathyrus* species. It gave two main genetic clusters; the first includes *L. pseudocicera* only, while the second was split into two sub-clusters. The first sub-cluster is divided into two groups: the first group contains *L. latifolius* L. and *L. neurolobus,* and the second group is split into two subgroups: the first subgroup contains *L. pratensis* L. and *L. pannonicus* subsp. *varius*, while the second subgroup contains *L. niger* L. only. The second sub-cluster split into two groups, the first group contains *L. sylvestris* L. and *L. tuberosus* L. and the second group split into two subgroups, the first subgroup split into two clades, the first clade contains *L. articulates* L. and *L. aureus* while the second clade contains *L. digitatus* only. The second subgroup is divided into two clades; the first contains *L. hierosolymitanus* L. and *L. laxiflorus*, while the second contains *L. davidii* only.

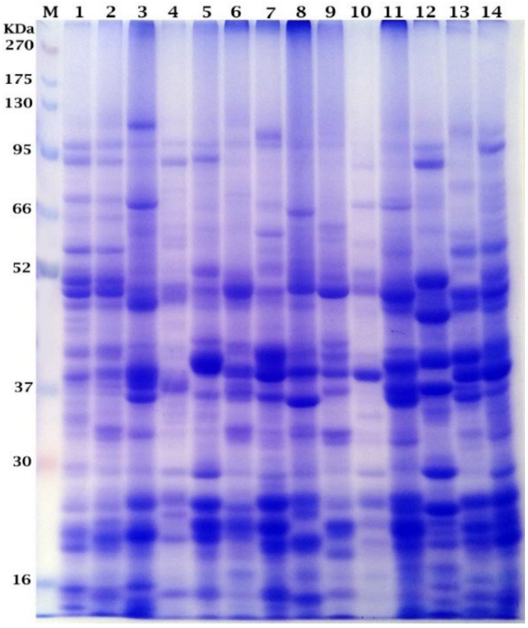

Figure 1. Electrophoretic patterns of different *Lathyrus* species for total seed storage proteins. M: Protein marker (270, 175, 130, 95, 66, 52, 37, 30 and 16 KDa); (*1- L. articulates; 2- L. aureus; 3- L. davidii; 4- L. digitatus; 5- L. hierosolymitanus; 6- L. laxiflorus; 7- L. latifolius; 8- L. neurolobus; 9- L. niger; 10- L. pannonicus;11- L. pratensis; 12- L. pseudocicera; 13- L. sylvestris; 14- L. tuberosus).*

Table 3. Similarity matrix among studied *lathyrus* species as computed according to Jaccard' Coefficient revealed by protein markers (*1- L. articulates; 2- L. aureus; 3- L. davidii; 4- L. digitatus; 5- L. hierosolymitanus; 6- L. laxiflorus; 7- L. latifolius; 8- L. neurolobus; 9- L. niger; 10- L. pannonicus;11- L. pratensis; 12- L. pseudocicera; 13- L. sylvestris; 14- L. tuberosus)*

1	1.00													
2	***0.76***	1.00												
3	0.56	0.64	1.00											
4	0.65	0.59	0.53	1.00										
5	0.56	0.64	0.63	0.68	1.00									
6	0.49	0.66	0.65	0.55	0.70	1.00								
7	0.48	0.58	0.57	0.53	0.57	0.64	1.00							
8	0.53	0.65	0.54	0.54	0.59	0.61	0.63	1.00						
9	0.42	0.53	0.43	0.52	0.56	0.63	0.61	0.63	1.00					
10	0.47	0.55	0.44	0.48	0.48	0.61	0.53	0.50	0.69	1.00				
11	0.61	0.70	0.59	0.54	0.59	0.66	0.58	0.65	0.63	***0.71***	1.00			
12	0.56	0.54	0.53	***0.37***	0.49	0.55	0.53	0.42	0.39	0.48	0.59	1.00		
13	0.57	0.51	0.64	0.54	0.54	0.51	0.58	0.56	0.53	0.45	0.47	0.46	1.00	
14	0.63	0.58	0.53	0.65	0.61	0.58	0.56	0.62	0.59	0.53	0.66	0.53	0.66	1.00
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**

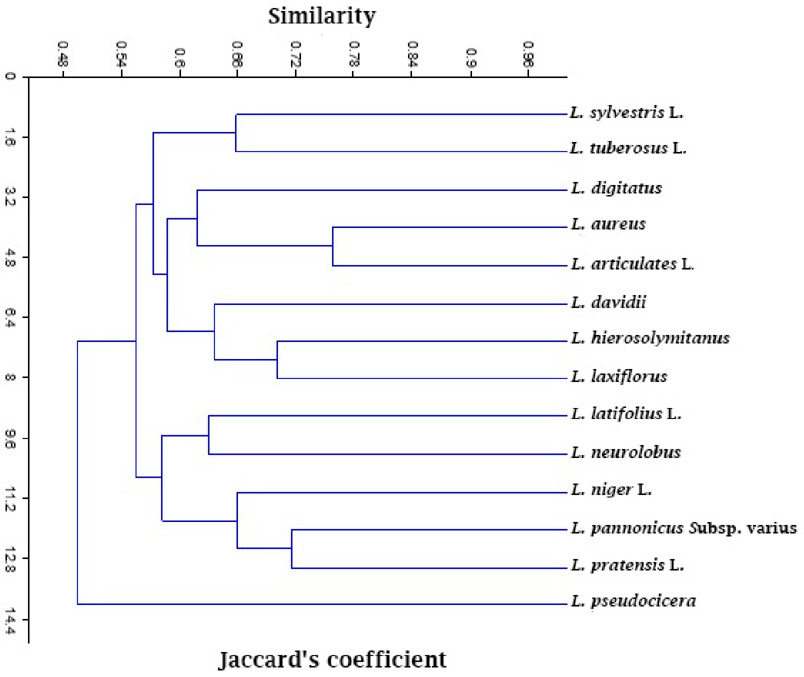

Figure 2. UPGMA dendrogram of studied *Lathyrus* species based on analysis of total seed storage proteins

Seed storage proteins of *Pisum* species

Seed storage proteins profile using SDS-PAGE detected 37 bands with molecular weight (Mw) ranging from 110 to 15 KiloDalton (KDa). There were eighteen monomorphic bands at Mw 100, 64, 50, 43, 38, 37, 36, 34, 33, 32, 29, 25, 23, 22, 20, 19, 17 and 15 KDa, while the polymorphism was 51.35%. A maximum of 28 bands were detected in two *P. sativum,* such as in subspecies *elatius* and *sativum* Convar. *medullosaccharatum*. The minimum number was 24 bands, detected twice in Convar. *axiphium* and *medullare*. Five negative unique bands were detected in subspecies *abyssinicum* var. *varilovianum* at Mw 75 and 71 KDa, in *P. fulvum* at Mw 60 and 55 KDa, and in subspecies *asiaticum* at Mw 48 KDa. Also, eight positive unique bands were detected in *P. fulvum* at Mw 110, 83, and 40 KDa, in subspecies *abyssinicum* var. *varilovianum* at Mw 77 and 45 KDa, in subspecies *elatius* at Mw 88 KDa, and subsp. *sativum* Convar. *medullosaccharatum* at Mw 90 and 39 KDa. The Mw 95 and 47 KDa bands were detected in subspecies *asiaticum* & *sativum* Convar. *medullosaccharatum* and *asiaticum* & *sativum* Convar. *sativum*, respectively. The band at Mw 54 KDa was absent in subspecies *asiaticum* and *sativum* Convar. *axiphium* (Table 4 and Figure 3).

Genetic similarity

The similarity index among *Pisum* species was determined for each pair-wise group (Table 5 and Figure 4). The similarity relationships depending on seed storage protein analysis using SDS-PAGE ranged from 0.96 to 0.68 (Table 5). The highest similarity index (0.96) was documented between Convar. *speciosum* & *axiphium*, *speciosum* & *medullare,* and *medullare* & *sativum*, while the lowest similarity index (0.68) was documented between *P. fulvum & P. sativum* subsp. *asiaticum* and *P. fulvum & P. sativum* subsp. *abyssinicum* var. *varilovianum*. Figure (4) represents the dendrogram from UPGMA cluster analysis among different *Pisum* species. It gave two main genetic clusters; the first cluster includes *P. fulvum* only, while the second cluster consists of all subspecies of *Pisum sativum*. The second cluster was further split into two sub-clusters. The first sub-cluster includes *P. sativum* subsp. *abyssinicum* var. *varilovianum* only. The second sub-cluster is split into two groups; the first group comprises *P. sativum* subsp. *asiaticum* only. However, the second group split into two subgroups; the first subgroup includes *P. sativum* subsp. *sativum* Convar. *medullosaccharatum* only, while the second subgroup split into two clades; the first contains *P. sativum* subsp. *elatius* only, but the second clade split into two sub-clades. The first sub-clade contains *P. sativum* subsp. *sativum* Convar. *medullare* only, but the second sub-clade is split into two sections; the first contains *P. sativum* subsp. *sativum* Convar. *axiphium* only, while the second section contains Convar. *sativum* and *speciosum*.

Genetic similarity based on combined data of *Lathyrus* and *Pisum* proteins profile

The genetic similarity coefficient and dendrogram tree (Rooted phylogenetic tree) were gathered between different *Pisum* and *Lathyrus* species as computed according to Jaccard's Coefficient from_seed storage proteins profile analysis Table (6) and Figure (5). The genetic similarity among the studied *Lathyrus* and *Pisum* species was very low, but both studied genera originated from the same ancestor and formed two main clusters. The first cluster contains all studied *Pisum* species, while the second includes all studied *Lathyrus* species. Table (6) represents the relationship among different studied *Pisum* and *Lathyrus* species. The highest similarity index (0.38) was documented between *P. sativum* subsp. *sativum* Convar. *axiphium* and *L. pannonicus* subsp. *varius,* followed by *P. sativum* subsp. *sativum* Convar. *speciosum* and *L. niger* L. & *L. pannonicus* subsp. *varius,* and *P. sativum* subsp. *abyssinicum* var. *varilovianum* & *L. sylvestris* L. with similarity index (0.36). The lowest similarity index (0.20) was documented between *P. sativum* subsp. *sativum* Convar. *medullare* and *L. articulates* L.

Table 4. Approximate molecular weight of total seed storage protein bands in different *Pisum* species and subspecies (*1- P. fulvum; 2- P. sativum* subsp. *asiaticum; 3- P. sativum* subsp. *abyssinicum* var. *varilovianum; 4- P. sativum* subsp. *elatius; 5- P. sativum* subsp. *sativum* Convar. *axiphium; 6- P. sativum* subsp. *sativum* Convar. *medullare; 7- P. sativum* subsp. *sativum* Convar. *medullosaccharatum; 8- P. sativum* subsp. *sativum* Convar. *sativum; 9- P. sativum* subsp. *sativum* Convar. *speciosum).*

B. no.	**Mw**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Polymorphism (%)**
1	**110**	+	-	-	-	-	-	-	-	-	P
2	**100**	+	+	+	+	+	+	+	+	+	M (Monomorphic)
3	**95**	-	+	-	-	-	-	+	-	-	P (Polymorphic)
4	**90**	-	-	-	-	-	-	+	-	-	P
5	**88**	-	-	-	+	-	-	-	-	-	P
6	**83**	+	-	-	-	-	-	-	-	-	P
7	**77**	-	-	+	-	-	-	-	-	-	P
8	**75**	+	+	-	+	+	+	+	+	+	P
9	**71**	+	+	-	+	+	+	+	+	+	P
10	**67**	+	+	+	+	-	-	-	-	-	P
11	**64**	+	+	+	+	+	+	+	+	+	M
12	**60**	-	+	+	+	+	+	+	+	+	P
13	**56**	+	-	-	+	-	-	+	-	-	P
14	**55**	-	+	+	+	+	+	+	+	+	P
15	**54**	+	-	+	+	-	+	+	+	+	P
16	**52**	-	-	-	+	+	-	-	+	+	P
17	**50**	+	+	+	+	+	+	+	+	+	M
18	**48**	+	-	+	+	+	+	+	+	+	P
19	**47**	-	+	-	-	-	-	-	+	-	P
20	**45**	-	-	+	-	-	-	-	-	-	P
21	**43**	+	+	+	+	+	+	+	+	+	M
22	**40**	+	-	-	-	-	-	-	-	-	P
23	**39**	-	-	-	-	-	-	+	-	-	P
24	**38**	+	+	+	+	+	+	+	+	+	M
25	**37**	+	+	+	+	+	+	+	+	+	M
26	**36**	+	+	+	+	+	+	+	+	+	M
27	**34**	+	+	+	+	+	+	+	+	+	M
28	**33**	+	+	+	+	+	+	+	+	+	M
29	**32**	+	+	+	+	+	+	+	+	+	M
30	**29**	+	+	+	+	+	+	+	+	+	M
31	**25**	+	+	+	+	+	+	+	+	+	M
32	**23**	+	+	+	+	+	+	+	+	+	M
33	**22**	+	+	+	+	+	+	+	+	+	M
34	**20**	+	+	+	+	+	+	+	+	+	M
35	**19**	+	+	+	+	+	+	+	+	+	M
36	**17**	+	+	+	+	+	+	+	+	+	M
37	**15**	+	+	+	+	+	+	+	+	+	M
Total Bands		27	25	25	28	24	24	28	26	25	51.35%

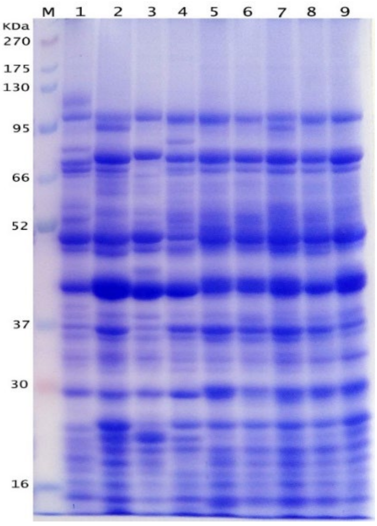

Figure 3. Electrophoretic patterns of different *Pisun* species and subspecies for total seed storage proteins. M: Protein marker (270, 175, 130, 95, 66, 52, 37, 30 and 16 KDa); (*1 - P. fulvum; 2 - P. sativum* subsp. *Asiaticum; 3 - P. sativum* subsp. *abyssinicum* var. *varilovianum; 4 - P. sativum* subsp. *Elatius; 5 - P. sativum* subsp. *sativum* Convar. *axiphium; 6 - P. sativum* subsp. *sativum* Convar. *medullare; 7 - P. sativum* subsp. *sativum* Convar. *medullosaccharatum; 8 - P. sativum* subsp. *sativum* Convar. *sativum; 9 - P. sativum* subsp. *sativum* Convar. *speciosum).*

Table 5. Similarity matrix among studied *Pisum* species and subspecies as computed according to Jaccard' Coefficient revealed by protein markers (*1 - P. fulvum; 2 - P. sativum* subsp. *Asiaticum; 3 - P. sativum* subsp. *abyssinicum* var. *varilovianum; 4 - P. sativum* subsp. *Elatius; 5 - P. sativum* subsp. *sativum* Convar. *axiphium; 6 - P. sativum* subsp. *sativum* Convar. *medullare; 7 - P. sativum* subsp. *sativum* Convar. *medullosaccharatum; 8 - P. sativum* subsp. *sativum* Convar. *sativum; 9 - P. sativum* subsp. *sativum* Convar. *speciosum).*

1	1.00								
2	***0.68***	1.00							
3	***0.68***	0.72	1.00						
4	0.77	0.77	0.77	1.00					
5	0.70	0.81	0.75	0.86	1.00				
6	0.76	0.81	0.81	0.86	0.92	1.00			
7	0.72	0.77	0.71	0.81	0.79	0.86	1.00		
8	0.71	0.82	0.76	0.86	0.92	0.92	0.80	1.00	
9	0.73	0.79	0.79	0.89	***0.96***	***0.96***	0.83	***0.96***	1.00
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

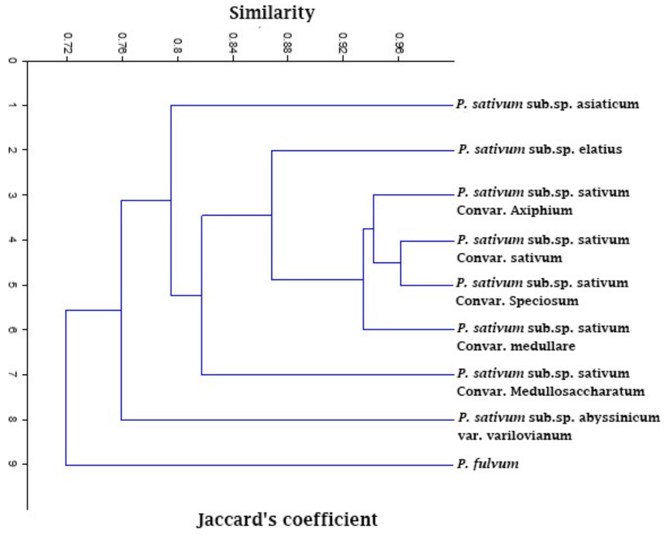

Figure 4. UPGMA dendrogram of studied *Pisum* species and subspecies based on analysis of total seed storage proteins.

Table 6. Similarity matrix among studied *Lathyrus* species and *Pisum* species and subspecies as computed according to Jaccard' Coefficient as revealed by protein markers (for species, see Table 1).

1	1.00																						
2	***0.76***	1.00																					
3	0.56	0.64	1.00																				
4	0.65	0.59	0.53	1.00																			
5	0.56	0.64	0.63	0.68	1.00																		
6	0.49	0.66	0.65	0.55	0.70	1.00																	
7	0.48	0.58	0.57	0.53	0.57	0.64	1.00																
8	0.53	0.65	0.54	0.54	0.59	0.61	0.63	1.00															
9	0.42	0.53	0.43	0.52	0.56	0.63	0.61	0.63	1.00														
10	0.47	0.55	0.44	0.48	0.48	0.61	0.53	0.50	0.69	1.00													
11	0.61	0.70	0.59	0.54	0.59	0.66	0.58	0.65	0.63	0.71	1.00												
12	0.56	0.54	0.53	0.37	0.49	0.55	0.53	0.42	***0.39***	0.48	0.59	1.00											
13	0.57	0.51	0.64	0.54	0.54	0.51	0.58	0.56	0.53	0.45	0.47	0.46	1.00										
14	0.63	0.58	0.53	0.65	0.61	0.58	0.56	0.62	0.59	0.53	0.66	0.53	0.66	1.00									
15	0.27	0.34	0.29	0.26	0.26	0.33	0.30	0.28	0.34	0.31	0.34	0.23	0.31	0.27	1.00								
16	0.22	0.29	0.28	0.24	0.24	0.32	0.29	0.23	0.29	0.32	0.29	0.28	0.29	0.25	***0.68***	1.00							
17	0.22	0.26	0.28	0.28	0.24	0.32	0.32	0.29	0.32	0.32	0.26	0.24	***0.36***	0.28	***0.68***	0.72	1.00						
18	0.23	0.30	0.29	0.26	0.26	0.29	0.33	0.27	0.33	0.33	0.30	0.26	0.30	0.26	0.77	0.77	0.77	1.00					
19	0.23	0.30	0.28	0.25	0.25	0.32	0.29	0.24	0.33	***0.38***	0.30	0.28	0.27	0.26	0.70	0.81	0.75	0.86	1.00				
20	***0.20***	0.27	0.25	0.22	0.22	0.29	0.29	0.24	0.33	0.33	0.27	0.25	0.30	0.23	0.76	0.81	0.81	0.86	0.92	1.00			
21	0.21	0.27	0.29	0.26	0.26	0.29	0.33	0.27	0.33	0.33	0.30	0.26	0.33	0.26	0.72	0.77	0.71	0.81	0.79	0.86	1.00		
22	0.24	0.32	0.30	0.27	0.27	0.34	0.34	0.29	0.35	0.35	0.32	0.30	0.32	0.27	0.71	0.82	0.76	0.86	0.92	0.92	0.80	1.00	
23	0.22	0.29	0.28	0.24	0.24	0.32	0.32	0.26	***0.36***	***0.36***	0.29	0.28	0.29	0.25	0.73	0.79	0.79	0.89	***0.96***	***0.96***	0.83	***0.96***	1.00
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**

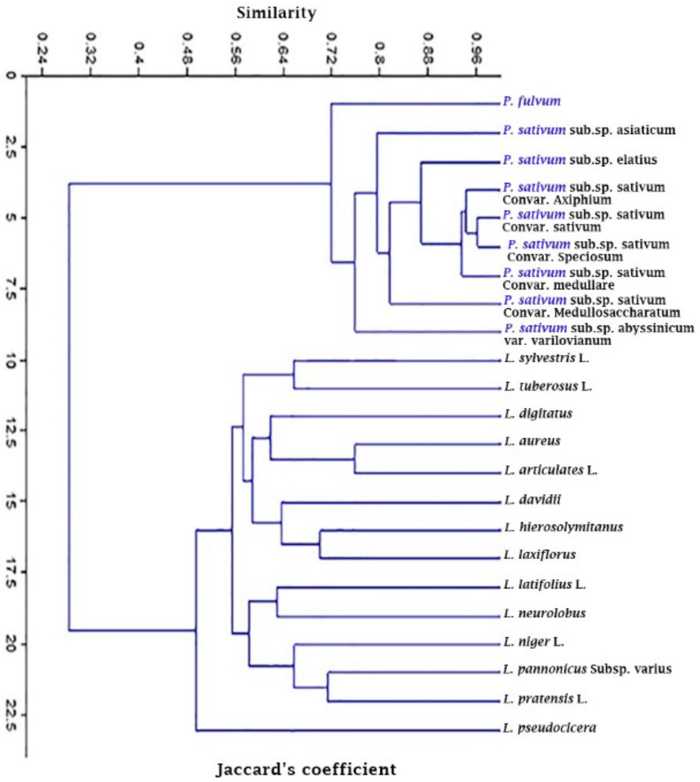

Figure 5. The dendrogram of studied *lathyrus* species and *Pisum* species and subspecies as constructed using protein patterns Unweighted Pair-group Arithmetic (UPGMA) and similarity matrices computed according to Jaccard' Coefficient.

Discussion

Seed storage protein pattern obtained by electrophoresis is an effective method used as a biochemical marker. It reflects the genetic information of DNA and is suitable for studying genetic diversity and classifying many crop species (Isemura et al. 2001, Ghafoor & Ahmad 2005, Nagy et al. 2009). Seed storage protein investigation via the SDS-PAGE technique is considered practical, inexpensive, and reliable. This technique is mostly independent of environmental fluctuations compared to isozyme analysis (Iqbal et al. 2005, Dutta & Mallick 2012). Seed proteins examined by SDS-PAGE have been used for detecting intraspecific variation and interspecific relationships (Çelebi et al. 2009, Hameed et al. 2009). Many researches based on the electrophoretic analysis of seed storage proteins have been used to study the genetic variability in numerous legumes, for instance, the genus *Lupin* (Vaz et al. 2004), *Pisum* (Jha & Ohri 2002), *Lathyrus* (Emre et al. 2007a, Emre, 2009), *Onobrychis* (Emre et al. 2007b), *Phaseolus* (Przybylska & Przybylska 1993), Soyabean (Rayan & Osman 2019), *Vicia* (Mirali et al. 2007, Emre 2007, Osman et al. 2020), *Lupinus* (Ali & Heiba 2021) and *Cicer* (Khalid et al. 2023).

There are numerous studies on various legumes, such as chickpeas (Ghafoor et al. 2003b), lentils (Piergiovanni & Taranto 2003), groundnut (Javaid et al. 2004), pigeon peas (Jha & Ohri 1996) and black gram (Ghafoor et al. 2003a), which reported a low level of intra-specific variation. This agrees with our results, as we observed that the protein profile of *Pisum* species provided low polymorphism (51.35%) but disagrees with Ladizinsky (1979), who observed a high level of polymorphism in peas.

Genetic similarity information could be used to make decisions regarding selecting superior genotypes for improvement or to be used as parents to develop future cultivars through hybridization (Ghafoor & Arshad 2008). Based on combined data, I presented the genetic similarity and a dendrogram, which were gathered by seed storage protein analysis of several species and subspecies of the *Pisum* and *Lathyrus* genera. Both genera originated from the same ancestor into two main clusters. The first cluster contains all studied *Pisum* species (Figure 5). The highest similarity index (0.96) was documented between Convar. *speciosum* & *axiphium*, *speciosum* & *medullare* and *medullare* & *sativum* of *P. sativum* subsp. *sativum*, this indicates that these convars. are closely related, while the lowest similarity index (0.68) was documented between *P. fulvum & P. sativum* subsp. *asiaticum* and *P. fulvum & P. sativum* subsp. *abyssinicum* var. *varilovianum*, this indicates that these species are more distantly related. The second cluster contains all studied *Lathyrus* species. The highest similarity index (0.76) was documented between *L. articulates* L. and *L. aureus;* this indicates that these species are closely related, while the lowest similarity index (0.39) was documented between *L. niger* L. and *L. pseudocicera,* which indicates that, these species are more distantly related. My results agree with the study of Emre et al. (2010), who investigated the variation among eight *Lathyrus* species collected from different regions in Turkey by analyzing their seed protein patterns using the SDS-PAGE technique.

Schaefer et al. (2012) documented that *P. sativum* and *P. fulvum* are closely related, and wild *P. fulvum* is the ancestor of the cultivated *P. sativum.* Also, other studies documented that *P. fulvum* diverged from other species of the *Pisum* genus (Palmer et al. 1985, Polans & Saar 2002, Saar & Polans 2000).

The highest similarity indexes among species of both genera were *P. sativum* subsp. *sativum* Convar. *axiphium* and *L. pannonicus* subsp. *varius* were closely related with a similarity index of 0.38, followed by *L. niger* L. and *L. pannonicus* subsp. *varius* with *P. sativum* subsp. *sativum* Convar. *speciosum,* and then *L. sylvestris* L. with *P. sativum* subsp. *abyssinicum* var. *varilovianum*. The lowest similarity index (0.20) was documented between *P. sativum* subsp. *sativum* Convar. *medullare* and *L. articulates* L., which indicates that these species were less related, as shown in Table 6. This agrees with Omar et al. (2019), who studied the phylogenetic relationship among some species of different genera, such as *Lens, Vicia, Lathyrus,* and *Pisum,* by sequencing the internal transcribed spacer (ITS) region. They found that the Pisum and Lathyrus genera species were placed in the same clade, while the studied species of the other different genera were found in another clade. The clade that contained *Pisum* and *Lathyrus* split into two subclades; the first subclade contained all *Pisum* species, while the second clade contained all *Lathyrus* species. My results are also in agreement with the results of Osman & Ali (2020, 2021), who studied the genetic relationship among five *Lathyrus* species and six *Pisum sativum* subspecies based on collective data of three molecular markers (RAPD, ISSR, and SCoT). They reported the relationship in this dissenting order: *L. tuberosus* is more related to *L. hierosolymitanus* than comes *L. latifolius* and then *L. articulates*, whereas *L. pseudocicera* was at a distance from the other species, while the relationship among subspecies of *Pisum sativum* was as follows: subsp. *sativum* Convar. *speciosum* is more related to Convar. *medullare* then comes Convar. *axiphium* and followed by subsp. *elatus,* which is more related to subsp. *abyssinicum* than to subsp. *asiaticum*.

From my results, it could be concluded that the genetic similarity among studied *Pisum* and *Lathyrus* species represented a very low similarity between both studied genera. The highest similarity matrix was documented between *P. sativum* subsp. *sativum* Convar. *axiphium* and *L. pannonicus* Subsp. varius (0.38), which indicates that these species were relatively related. The lowest similarity index (0.20) was documented between *P. sativum* subsp. *sativum* Convar. *medullare* and *L. articulates* L., which indicates that, these species were less related. The dendrogram represented that both genera originated from the same ancestor into two main clusters. The first cluster contains all studied *Pisum* species, while the second contains all studied *Lathyrus* species. In the first cluster, the highest similarity matrix (0.96) was documented between Convar. *speciosum* & *axiphium*, *speciosum* & *medullare* and *medullare* & *sativum* of *P. sativum* subsp. *sativum*, which indicates that these Convars. of *P. sativum* subsp. *sativum* were closely related. The highest similarity matrix (0.76) was documented in the second cluster between *L. articulates* L. and *L. aureus.*

List of abbreviations

SDS-PAGE - Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

UPGMA - Unweighted Pair-group Arithmetic
M - Monomorphic bands
P - Polymorphic bands
KDa - KiloDalton
DNA - Deoxyribonucleic acid
ITS - Internal transcribed spacer region

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