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RESEARCH ARTICLE

Estimation of phylogenetic relationships of *Phlomis* species based on seed protein polymorphism

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Keywords: *Phlomis*, *Phlomoides*, *Phlomis* taxa, SDS-PAGE, seed proteins.

Abbreviations: DTT: dithiothreitol
RAPDs: randomly amplified polymorphic DNA
SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis
TES: TE-saline
UPGMA: unweighted pair-group method with arithmetic averages

In this study, phylogenetic relationships among 39 *Phlomis* taxa were investigated based on seed protein profiles produced by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A total of 21 polypeptide bands were scored, of which, 19 were polymorphic among the taxa of the genus *Phlomis*. A distance matrix was generated from the similarity matrix which was computed by using Jaccard's similarity coefficients, based on polymorphic bands and then an UPGMA tree was established through cluster analysis performed on the distance matrix. Genetic distances ranged from 0.00 to 0.50 within subsection *Dendrophlomis*; from 0.00 to 0.625 within subsection *Gymnophlomis* and from 0.00 to 0.769 within subsection *Oxyphlomis*. The UPGMA tree formed four groups. The topology of the tree is in agreement with the taxonomic view regarding the section *Phlomis* as it is divided into three subsections as *Dendrophlomis*, *Gymnophlomis* and *Oxyphlomis* based on morphological characters. The grouping pattern of the tree also indicated that subsection *Dendrophlomis* is more closely related to subsection *Gymnophlomis* than subsection *Oxyphlomis*.

The genus *Phlomis* L. contains over 100 species that have been divided into two main sections: *Phlomoides* and *Phlomis* (Moench, 1794; Albaladejo et al. 2005). Section *Phlomis* was further subdivided into three subsections, *Dendrophlomis*, *Gymnophlomis* and *Oxyphlomis* (Bentham, 1834). The diagnostic character for separating sections is corolla shape. Species of section *Phlomis*, which have corolla with curved upper lip and trifid lower lip with large median and smaller lateral lobes, differs from species of section *Phlomoides* that have corolla with straight upper lip and trifid lower lip with sub equal lobes (Azizian and Moore, 1982). Section *Phlomis* occurs mostly in the Mediterranean region, where Turkey is regarded as one of the primary center of diversification for species of the section (Hedge, 1986). For instance, in the Flora of Turkey, the genus *Phlomis* consists of 34 species and six varieties, of which 22 are endemic to that flora (Huber-Morath, 1982).

Taxonomic relationships among species of the genus *Phlomis* have been assessed by morphological characters (Azizian and Moore, 1982; Huber-Morath, 1982; Taylor,

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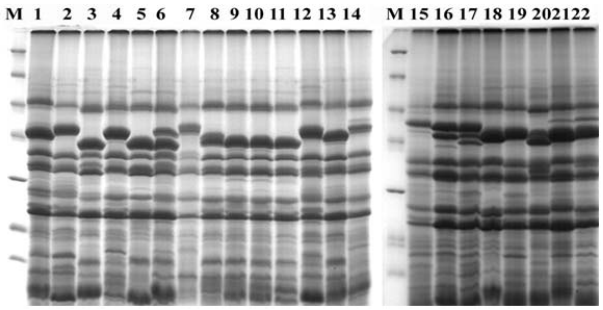

Figure 1. Seed protein profiles of 39 *Phlomis* taxa (M = size marker; numbers 1-22 as in Table 1).

1998). Nevertheless, based on morphological characters alone, it is difficult to distinguish *P. armeniaca*, *P. sieheana*, *P. sintenisii*, *P. lanceolata* and *P. carica* from each other within subsection *Gymnophlomis* because they have overlapping variations in terms of the major delimiting morphological characters such as plant height, basal leaves sizes and shapes, bracteol length, calyx teeth shape and length, indumentum of calyx and number of flowers per verticillaster (Huber-Morath, 1982). Morever, differentiation of *P. carica* from *P. armeniaca*, and of *P. nissoli* from *P. syriaca* within this complex is based on indimentum of calyces and leaves; hovewer, this character shows high variation among individuals of a population and among populations of these species (Huber-Morath, 1982). Also, hybridization is a common incident in this genus, leading to the formation of many interspecific hybrids that increase the taxonomic complexity (Albaladejo et al. 2004; Albaladejo et al. 2005; Albaladejo and Aparicio, 2007; Yuzbasioglu et al. 2008b). Seed storage protein markers have been sucessfully used to resolve taxonomic relationships and characterize cultivated varieties in a number of crop plant species (Igrejas et al. 1999; Vladova et al. 2000; Jha and Ohri, 2002; Karihaloo et al. 2002; Syros et al. 2003; Bhargava et al. 2005; Cherdouh et al. 2005; Alvarez et al. 2006; Stoilova et al. 2006; Mirali et al. 2007; Rout and Chrungoo, 2007; Yuzbasioglu et al. 2008a) because they are stable, uniform, reliable, reproducible and largely independent of environmental fluctuations (Ghafoor et al. 2002; Panigrahi et al. 2007). The taxonomic relationships among species of *Phlomis* based on seed protein electrophoresis have not been studied so far. The objective of the present study was to assess taxonomical relationships among 39 *Phlomis* taxa based on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins.

MATERIALS AND METHODS

Seeds from 39 *Phlomis* taxa examined in the present study were collected from different parts of Turkey (Table 1). Seeds from each taxon were collected from at least 10 single plants within a population of that taxon and then the seeds of each taxon were bulked. The bulked seeds of each taxon were ground into a fine powder with liquid nitrogen. 25 mg of flour samples were transferred into 200 µL of TE-Saline (TES) buffer within 1.5 ml eppendorf tubes containing 100 mM Tris HCl, 50 mM NaCl, 1 mM EDTA, 2% SDS, 2% PVP, 100 µM Nα-P-Tosyl-L-Lysine Chloromethyl Ketone (TLCK), 0.3 µM Phenylmethanesulphonyl Fluoride (PMSF) and 100 mM Phenylalanine Ketone (TPCK). The flour samples were mixed with the TES buffer by vortexing for 10 min at room temperature. Then the mixture was heated for 3 min at between 80 and 90ºC. After cooling at room temperature, 4 µL of DTT (dithiothreitol) solution (3.25 M DTT in dH_2O) was added to the mixture. Having centrifugated the tubes at 15000 x g for 15 min at 4ºC by using a 3K30 cooling centrifuge, the spernatant was transferred into new tubes. Then protein content of each sample was determined according to the modified Bradford assays described by Ramagli and Rodriguez (1985) using Bovine Serum Albumin (BSA) faction as the standard. The protein concentration of each sample was equalized by TES buffer and the equalized protein concentration was either mixed in half with loading buffer (65 mM Tris HCl, pH 6.8, 1% SDS, 2% 2-mercaptoethanol, 20% Glycerol) or kept on ice and stored at -20ºC for further use.

Protein patterns were analyzed by 14% SDS-PAGE (18 x 20 x 0.1 cm; vertical slab gels; Protean Xii, BioRad, CA, USA) according to Laemmli (1970). The molecular weights of the dissociated polypeptides were determined by using molecular size markers in the range between 14.4 and 116 kD (Fermentas SMO431). Gels were stained by a sensitive colloidal Coomassie Brillant Blue G-250 as described by Olivieri et al. (2001) and photographed using a digital image analysis system (Vilber-Lourmat, Bio-Gene V.99). A similarity matrix based on Jaccard's similarity coefficient was produced with NTSYS-pc (Rohlf, 1992) from protein bands scored as 0 (absent) and 1 (present) and then, a distance matrix was constructed by subtracting the similarity coefficients from 1. A dendrogram based on the distance matrix was produced using the unweighted pair-

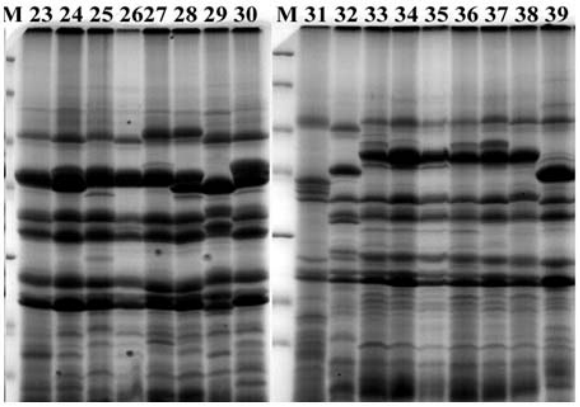

Figure 2. Seed protein profiles of 39 *Phlomis* taxa (M = size marker; numbers 23-39 as in Table 1).

Table 1. *Phlomis* material and its source.

Numbers	Collector Number	*Phlomis* taxa	Collection site	Altitude (m)	Date
1	MYD 1718	*P. russeliana* (Sims) Bentham	Bolu; Around Abant Lake.	1220	12 viii 1999
2	MYD 1451	*P. fruticosa* L.	Izmir; Cesme, Ciftlikkoy.	60-70	27 iv 2000
3	MYD 1102	*P. lunariifolia* Sm.	Antalya; Alanya, East slopes of Kaledran stream.	50	25 vi 1998
4	MYD 1166	*P. grandiflora* var. *grandiflora* H.S.Thompson	Antalya; Kas-Elmali road. Oluk Yaylasi, between Gomuce and Kuruova villages.	1700	28 vi 1998
5	MYD 1103	*P. grandiflora var. fimbrilligera* Hub.-Mor.	Antalya; Alanya, West slopes of Kaledran stream.	60	25 vi 1998
6	MYD 1190	*P. viscosa* Poiret	Osmaniye; Osmaniye-Yarpuz Yaylasi road.	550	6 viii 1998
7	MYD 1678	*P. bourgaei* Boiss.	Antalya; Kemer, around Agva stream.	60	4 vii 2003
8	MYD 1100	*P. leucophracta* P.H. Davis & Hub.-Mor.	Antalya; Between Anamur-Alanya, around Tenzile village.	150	25 vi 1998
9	MYD 1065	*P.longifolia* var. *longifolia* Boiss. & Bl.	Hatay; Between Gedik and Atik.	800	13 vi 1998
10	MYD 1075	*P.longifolia* var. *bailanica* (Vierh.) Hub. -Mor.	Hatay; Above Guzelyayla (Sogukoluk).	980	15 vi 1998
11	MYD 1158	*P. amanica* Vierh.	Hatay; Arsuz (Ulucinar), Haymaseki village, around Aktepe.	250-300	9 viii 1998
12	HD 6253	*P. lycia* D. Don	Antalya; Termessos National Park.	300-400	22 vii 1996
13	MYD 1096	*P. monocephala* P.H. Davis	İçel; Between Kazanci-Anamur, around Abanoz Yaylasi.	1400	25 vi 1998
14	HD 6643	*P. chimerae* Boissieu	Antalya; Kemer, around Cirali.	20-50	15 v 1998
15	MYD 1029	*P. oppositiflora* Boiss. & Haussk.	Sivas; Between Darende-Gurun.	1390	8 viii 1997
16	MYD 1287	*P. bruguieri* Desf.	Gaziantep; Soft Dagi.	1350-1460	16 vi 1999
17	MYD 1349	*P. armeniaca* Willd.	Tokat; Turhal, East slopes of Hastahane hill.	650	3 vii 1999
18	MYD 1384	*P. physocalyx*Hub.-Mor.	Sivas; Gurun-Kayseri 23 km.	1600	11 vii 1999
19	MYD 1251	*P. angustissima* Hub.-Mor.	Afyon; Dirmil; Dirmil pass.	1580	9 vi 1999
20	MYD 1716	*P. capitata* Boiss.	Malatya; Venk village.	1195	12 vii 2003
21	MYD 1178	*P. kotschyana* Hub.-Mor.	Hatay; Arsuz, Haymaseki village, around Aktepe.	250-300	19 v 1999
22	MYD 1463	*P. sieheana* Rech.	Kayseri; Gesi, around Ildem Koop.	c. 1150	28 vi 2000
23	MYD 1405	*P. sintenisii* Rech.	Elazig; Around Harput Castle.	1500	12 vii 1999
24	MYD 1436	*P. lanceolata* Boiss. & Hohen.	Van; Van-Gurpinar 11 km, Karabas	2225	20 vii 1999

25	MYD 1352	*P. linearis* Boiss. & Bal.	Kayseri; Develi-Kayseri 12 km (Erciyes road).	1930	9 vii 1999
26	MYD 1296	*P. brunneogaleata* Hub.-Mor.	Gaziantep; Soft Dagi.	1350-1460	16 vi 1999
27	MYD 1086	*P. nissolii* L.	Nigde; Bor-Aksaray road, between Yesilyurt- Altunhisar villages.	1100	24 vi 1998
28	MYD 1063	*P. syriaca* Boiss.	Adana; Ceyhan, Yilankale.	100-300	12 vi 1998
29	MYD 1036	*P. kurdica* Rech.	Kahramanmaras; Elbistan-Goksun 13 km (old road).	1140	7 viii 1997
30	MYD 1125	*P. carica* Rech.	Denizli; Acipayam, Alaattin town, Degirmendere site.	1100	28 vi 1998
31	MYD 1055	*P. tuberosa* L.	Sivas; Imranlı-Zara 10 km.	1350	16 viii 1997
32	MYD 1163	*P. samia* L.	Adana; Gulek village-Adana 1 km.	1100	5 vii 1998
33	MYD 1321	*P. punges* var. *pungens* Willd.	Kayseri; Yahyali-Maden road 14 km.	1490	27 vi 1999
34	MYD 1057	*P. punges* var. *laxiflora* Velen.	B4 Ankara; Hacettepe university Beytepe campus.	1050-1200	19 viii 1997
35	MYD 1034	*P. punges* var. *hirta* Velen.	Kahramanmaras; Cardak-Elbistan 5 km.	1240	7 viii 1997
36	MYD 1320	*P. punges* var. *hispida* Hub.-Mor.	Kayseri; Yahyali-Maden road 14 km.	1490	27 vi 1999
37	MYD 1429	*P. punges* var. *seticalycina* Nab.	Van; Gurpınar-Catak 25 km.	2090	20 vii 1999
38	MYD 1025	*P. integrifolia* Hub.-Mor.	Malatya; Malatya-Arapkir 30 km, around Kuruçay.	770	7 viii 1997
39	MYD 1038	*P. rigida* Labill.	Malatya; Elbistan-Malatya 53 km.	1600	7 viii 1997

group method with arithmetic averages (UPGMA) under the NJ subprogram in the PHYLIP software package version 3.6a3 (Felsenstein, 2002).

RESULTS AND DISCUSSION

Seed storage proteins of the 39 *Phlomis* taxa examined by SDS-PAGE in the present study produced reproducible and stable protein bands that were used for the classification of the taxa studied in the genus (Figure 1 and Figure 2). A total of 21 polypeptide bands were scored, of which, 2 were universally present and 19 were polymorphic. The cluster analysis of the taxa based on distance matrix produced from Jaccard's coefficient was represented by an UPGMA dendrogram in Figure 3. The dendrogram formed four groups. The first group consisted of taxa from section *Phlomis* subsection *Dendrophlomis*: *P. russeliana*, *P. lycia*, *P. fruticosa*, *P. grandiflora* var. *grandiflora*, *P. bourgaei*, *P. chimerae*, *P. lunariifolia*, *P. viscosa*, *P. grandiflora* var. *fimbrilligera*, *P. longifolia* var. *longifolia*, *P. longifolia* var. *bailanica*, *P. amanica*, *P. monocephala* and *P. leucophracta*. Except for *P. russeliana*, a perennial herb, all other taxa within this subsection are shrubs. In species of this subsection, verticillasters generally have 12-20 flowers and the number of verticillasters range between 1 and 3. In species of this subsection, bracteoles are linear-subulate to lanceolate and ovate in shape, generally 10-18 mm in length and the number of bracteoles is generally between 15 and 30. Relationships among *P. viscosa*, *P. lunariifolia*, *P. grandiflora* var. *fimbrilligera*, *P. longifolia* var. *longifolia*, *P. amanica* and *P. monocephala* obtained by SDS-PAGE analysis in the present study were generally in agreement with the previous study of relationships among species of *Dendrophlomis*, based on randomly amplified polymorphic DNA (RAPDs) markers (Yuzbasioglu and Dadandi, 2008). Nevertheless, different groupings were observed among *P. bourgaei*, *P. leucophracta*, *P. chimerae* and *P. lycia* when compared with the findings of the previous study, which excluded samples of *P. russeliana*, *P. fruticosa* and *P. longifolia* var. *bailanica*. Based on previous RAPD analysis, the grouping of these four taxa agrees better with the traditional taxonomic classification than grouping them based on SDS-PAGE analysis. SDS-PAGE clustering of the species of subsection *Dendrophlomis* into two groups is consistent with the groupings of the previous RAPD study. However, *P. leucophracta* and *P. chimerae* were not clustered in the same group. In contrast, the finding from previous RAPD

Table 2. Genetic distances (as percentages) among 39 *Phlomis* taxa in the genus *Phlomis* (The numbers 1-39 refer to 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	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	-																																						
2	22	-																																					
3	40	40	-																																				
4	13	13	33	-																																			
5	33	33	13	25	-																																		
6	22	22	22	13	13	-																																	
7	33	13	50	25	44	33	-																																
8	38	38	38	29	29	38	29	-																															
9	33	33	13	25	25	33	44	29	-																														
10	33	33	13	25	25	33	44	29	0	-																													
11	33	33	13	25	25	33	44	29	0	0	-																												
12	11	11	45	22	40	30	22	44	40	40	40	-																											
13	25	25	25	14	14	25	38	17	14	14	14	33	-																										
14	40	22	40	33	33	40	33	38	33	33	33	30	25	-																									
15	50	50	64	60	60	64	60	67	60	60	60	40	56	50	-																								
16	55	55	55	64	50	55	64	70	64	64	64	45	60	55	13	-																							
17	50	50	64	60	60	64	60	67	60	60	60	40	56	50	0	13	-																						
18	56	56	70	67	67	70	50	57	67	67	67	44	63	56	29	38	29	-																					
19	56	38	56	50	50	56	50	57	50	50	50	44	43	38	29	38	29	33	-																				
20	73	60	60	70	70	73	56	63	56	56	56	64	67	60	56	60	56	43	43	-																			
21	50	50	64	60	60	64	60	67	60	60	60	40	56	33	25	33	25	29	29	56	-																		
22	50	50	64	60	60	64	60	67	60	60	60	40	56	33	25	33	25	29	29	56	0	-																	
23	44	44	60	56	56	60	56	63	56	56	56	33	50	44	14	25	14	17	17	50	14	14	-																
24	44	44	60	56	56	60	56	63	56	56	56	33	50	44	14	25	14	17	17	50	14	14	0	-															
25	50	50	50	60	44	50	60	67	60	60	60	40	56	50	25	13	25	29	29	56	25	25	14	14	-														
26	64	50	50	60	60	64	60	67	44	44	44	55	56	50	44	50	44	50	29	14	44	44	38	38	44	-													
27	44	44	60	56	56	60	56	63	56	56	56	33	50	44	14	25	14	17	17	50	14	14	0	0	14	38	-												
28	50	50	50	60	44	50	60	67	60	60	60	40	56	50	25	13	25	29	29	56	25	25	14	14	0	44	14	-											
29	60	60	25	56	38	44	70	63	38	38	38	64	50	60	56	44	56	63	43	50	56	56	50	50	38	38	50	38	-										
30	64	50	50	60	60	64	60	67	44	44	44	55	56	50	25	33	25	50	29	38	44	44	38	38	44	25	38	44	38	-									
31	70	82	70	80	67	70	80	75	80	80	80	73	78	82	67	56	67	57	75	78	67	67	63	63	50	80	63	50	63	80	-								
32	82	82	56	80	67	70	80	75	67	67	67	83	78	82	80	70	80	75	75	63	80	80	78	78	67	67	78	67	43	67	57	-							
33	73	73	81	80	80	81	71	77	80	80	80	67	79	73	50	54	50	55	67	69	62	62	58	58	62	71	58	62	79	62	67	77	-						
34	73	73	81	80	80	81	71	77	80	80	80	67	79	73	50	54	50	55	67	69	62	62	58	58	62	71	58	62	79	62	67	77	0	-					
35	71	71	80	79	79	80	69	75	79	79	79	64	77	71	45	50	45	50	64	67	58	58	55	55	58	69	55	58	77	58	64	75	9	9	-				
36	71	80	80	79	79	80	79	75	79	79	79	73	77	80	58	62	58	64	75	77	69	69	67	67	69	79	67	69	77	69	64	75	9	9	18	-			
37	71	80	80	79	79	80	79	75	79	79	79	73	77	80	58	62	58	64	75	77	69	69	67	67	69	79	67	69	77	69	64	75	9	9	18	0	-		
38	75	85	85	83	83	85	83	80	83	83	83	77	82	85	60	64	60	67	80	82	73	73	70	70	73	83	70	73	82	73	50	67	36	36	30	30	30	-	
39	67	67	67	73	64	67	73	79	73	73	73	60	71	67	42	33	42	58	58	71	54	54	50	50	42	64	50	42	62	54	58	69	23	23	17	31	31	42	-

study placed both *P. leucophracta* and *P. chimerae* in one group and the rest of the species of *Dendrophlomis* into another group. *P. longifolia* var. *longifolia*, *P. longifolia* var. *bailanica* and *P. amanica* displayed the same protein pattern based on genetic distances obtained from SDS-PAGE analysis. Morphologically, they are similar to each other in terms of the absence of glandular hairs. The highest genetic distance within subsection *Dendrophlomis* was observed between *P. bourgaei* and *P. lunariifolia* with a genetic distance value of 0.50 in the present analysis (Table 2). *P. bourgaei* has glandular hairs especially on the floral region (calyx and bracteoles) while *P. lunariifolia* is eglandular. Bracteoles are 10-24 mm in length and 0.75-1.5 mm in width in *P. bourgaei* and 7-15 mm in length and 2-3.5 mm in width in *P. lunariifolia*. Calyx teeth lengths are between 3-9 mm in *P. bourgaei* and 2-5 mm in *P. lunariifolia*. The range of genetic distances computed from the present study is concordant with the range (0.145-0.495) obtained from our previous RAPD analysis, however, the lowest genetic distances found between *P. amanica* and *P. monocephala* and the highest between *P. chimerae* and *P. lunariifolia* in the previous study. Differences observed in the groupings of the taxa of subsection *Dendrophlomis* in the dendrograms obtained from SDS-PAGE analysis and the previous RAPD study could be caused by several reasons. For instance, seed proteins are coded by several homologous multigene families (Panigrahi et al. 2007), but RAPD bands may

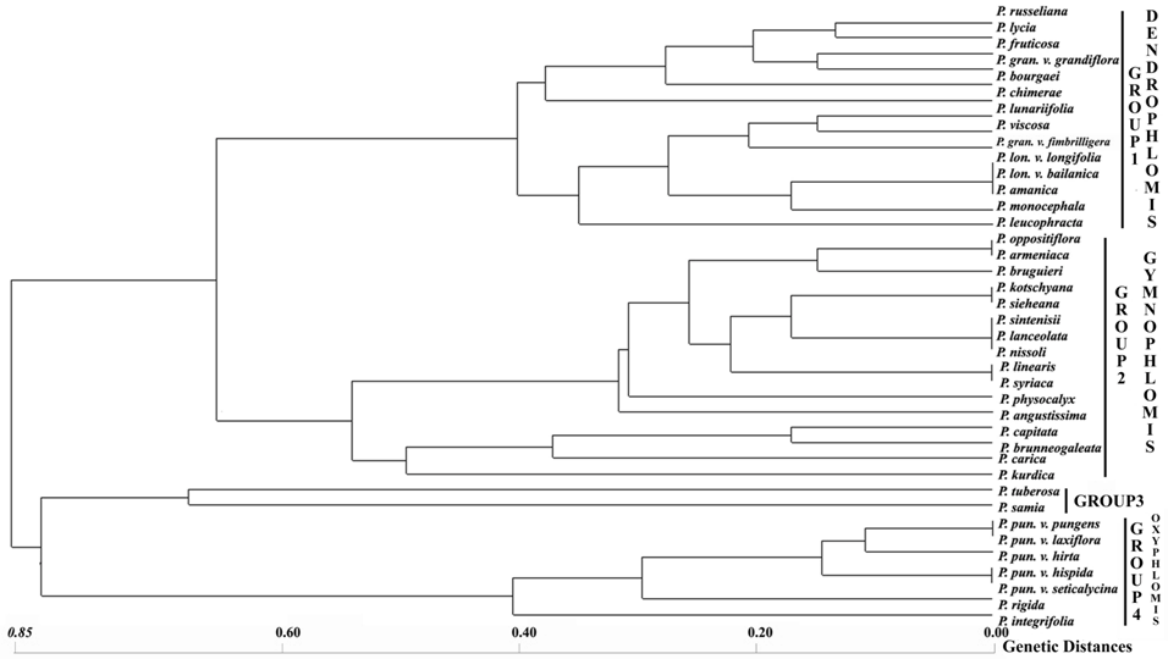

Figure 3. UPGMA dendrogram of 39 *Phlomis* taxa based on distances produced from Jaccard' similarity coefficients of seed storage proteins.

cover coding and non coding regions of DNA (Kirsten et al. 1998; Bussell et al. 2005). In addition to this, the different accessions sampled in the two studies, the different methods used for estimating genetic distances and the number of loci sampled in the two studies could be some other reasons of these differences.

The second group in the dendrogram comprised taxa from section *Phlomis* subsection *Gymnophlomis*: *P. oppositiflora*, *P. armeniaca*, *P. bruguieri*, *P. kotschyana*, *P. sieheana*, *P. sintenisii*, *P. lanceolata*, *P. nissoli*, *P. linearis*, *P. syriaca*, *P. physocalyx*, *P. angustissima*, *P. capitata*, *P. brunneogaleata*, *P. carica* and *P. kurdica*. Species in section *Phlomis* subsection *Gymnophlomis* are perennial herbs. In species of this subsection, bracteoles are weak and subulate to linear-lanceolate in shape, 6-12 in number and 0.5-10 mm in length, but they may be absent, as in *P. oppositiflora* or may reach up to 25 mm, as in *P. bruguieri* (Azizian and Moore, 1982; Huber-Morath, 1982). Verticillasters are usually 4-8 flowered in these species and the number of verticillasters per stem is between 3 and 6 (Huber-Morath, 1982).

In the present study, no variability was observed between *P. kotschyana* and *P. sieheana*, between *P. linearis* and *P. syriaca* and among *P. sintenisii*, *P. lanceolata* and *P. nissoli* within subsection *Gymnophlomis*. In *P. kotchyana* and *P. sieheana*, bracteoles are 2-3 mm in length and leaves length is three times longer than their width in both species. On the other hand, the base of the basal and cauline leaves is cordate in *P. kotschyana* but cuneate or truncate in *P. sieheana*. The shapes of basal and cauline leaves are cordate to broadly triangular cordate in *P. kotschyana* but oblong to oblong-lanceolate in *P. sieheana*. In *P. sintenisii*, *P. lanceolata* and *P. nissoli* bracteoles are stellate-tomentose and these species are also eglandular. Basal leaves are oblong to oblong-lanceolate in *P. sintenisii* and *P. lanceolata* but oblong to ovate in *P. nissoli*. Calyx teeth are lanceolate-acuminate in *P. sintenisii* and *P. lanceolata* but it is ovate-triangular in *P. nissoli*. *P. linearis* and *P. syriaca* are eglandular; their bracteoles are stellate-tomentose and their calyx is slightly campanulate. The highest genetic distance was found between *P. kurdica* and *P. physocalyx*, with a genetic distance value of 0.625 within subsection *Gymnophlomis* in the present study (Table 2). While *P. kurdica* has either glandular or eglandular specimens, *P. physocalyx* has only eglandular specimens. The shape of the base of basal and cauline leaves is cordate in *P. kurdica* and cuneate in *P. physocalyx*. Number of flowers per verticillaster varies from 6 to 10 in *P. kurdica* and from 4 to 6 in *P. physocalyx*.

The third group included *P. tuberosa* from section *Phlomoides* and *P. samia* from section *Phlomis* subsection *Oxyphlomis*. *P. tuberosa* and *P. samia* have pink or purple corolla color in common. The fourth group contained taxa from section *Phlomis* subsection *Oxyphlomis*: *P. pungens* var. *pungens*, *P. pungens* var. *laxiflora*, *P. pungens* var. *hirta*, *P. pungens* var. *hispida*, *P. pungens* var. *seticalycina*, *P. rigida* and *P. integrifolia*. The shape of the base of *P. tuberosa* and *P. samia* is cordate-sagittate, but cuneate, truncate or obtuse in the species of the fourth group. Both

P. tuberosa and *P. samia* have a branched single stem; however, species in the fourth group have many stem above the soil except for *P. rigida*, which has a single stem in generally, but a branched stem occasionally. Also, *P. tuberose* and *P. samia* have bigger cauline leaves than the species in the fourth group. In species of section *Phlomis* subsection *Oxyphlomis* bracteoles are rigid and subulate in shape; 9-40 in number and 10-26 mm in length (Huber-Morath, 1982). Number of verticillasters in these species varies 2 to 6 in general (Huber-Morath, 1982). Within the fourth group, no protein variability was observed between *P. pungens* var. *pungens* and *P. pungens* var. *laxiflora*, and between *P. pungens* var. *hispida* and *P. pungens* var. *seticalycina*. Calyx teeth length is the only morphological diagnostic which discriminates *P. pungens* var. *pungens* from *P. pungens* var. *laxiflora*. Calyx teeth are 1/2 as long as calyx tube or longer in var. *pungens*, but they are 1/3-1/5 as long as calyx tube in var. *laxiflora* (Huber-Morath, 1982). Morphological characters which differentiate *P. pungens* var. *hispida* from *P. pungens* var. *seticalycina* are adaxial suface of leaves and hairy structure on the stem. Adaxial surface of leaves is subglabrose in var. *seticalycina* but stellate-tomentose in var. *hispida*, and stem is covered with sparsely undivided hairs in var. *seticalycina*; however, in var. *hispida* it is covered with dense indumentum of undivided hairs, especially near nodes (Huber-Morath, 1982). *P. samia* was found to be the most distant species to both *P. pungens* var. *pungens* and *P. pungens* var. *laxiflora*, with a genetic distance value of 0.769 within the subsection *Oxyphlomis* in the present analysis (Table 2). While *P. samia* has glandular hairs on stem, leaves and the floral regions such as bracteoles and calyx, *P. pungens* is eglandular. The shape of the base of the cauline leaves is cordate in *P. samia* and cuneate in *P. pungens*. Flowers are shortly pedicellated in *P. samia* and sessile in *P. pungens*. Number of flowers per verticillaster varies from 10 to 18 in *P. samia* and from 6 to 10 in *P. pungens*. Calyx length varies from 8 to 18 mm in *P. pungens* and from 18 to 23 mm in *P. samia*.

In the present study, *P. tuberosa*, the only member of section *Phlomoides* in the Flora of Turkey, was expected to out group with the species of section *Phlomis*, but it clustered with subsection *Oxyphlomis*. This position of *P. tuberosa* could be understandable when considering that this species and species of subsection *Oxyphlomis* have pink or purple corolla color in common. Nevertheless, the inefficiency of section discrimination in the genus might be due to insufficient number of species sampled from section *Phlomoides* and thus, the number of species sampled from section *Phlomoides* need to be increased to examine whether the sectional division of the genus is supported by the SDS-PAGE analysis of seed proteins in future studies. On the other hand, the grouping pattern of the taxa in the present study supports the view of Huber-Morath (1982) in which section *Phlomis* was delimited in three subsections as *Dendrophlomis*, *Gymnophlomis* and *Oxyphlomis*. However, the taxa of subsection *Gymnophlomis* are grouped with the taxa of subsection *Dendrophlomis* in the dendrogram. This topology indicates that subsection *Dendrophlomis* is more closely related to subsection *Gymnophlomis* than subsection *Oxyphlomis*. This result is also in agreement with the morphological data that both subsections have yellow flower color in comparison to subsection *Oxyphlomis* that have purple or pink flower color. Overall, the results of the present study indicate that seed proteins in the genus *Phlomis* are useful characters to differentiate between species and to construct phylogenies for the assessment of evolutionary species relationships.

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