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Taxonomic relationships in some *Vicia* species from Egypt, based on seed morphology and SDS-PAGE of seed proteins

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ABSTRACT. The seed morphology and seed proteins of 11 *Vicia* taxa from Egypt were studied. Macro- and micro-morphological characters, including seed shape, color, size, hilum shape and seed sculpture were described and analyzed. The secondary sculpture of the cell wall varies from papillose in nearly all *Vicia* species to lophate in *V. hirsuta.* Further, the seed proteins of studied taxa were investigated by SDS-PAGE and 39 different bands were scored. The similarity analysis based on the SDS-PAGE profile and seed morphology was found to be a useful characteristic for the discrimination of *Vicia* species both on the subgeneric and the sectional levels. The present study did not acknowledge *V. tetrasperma* and *V. hirsuta* as subgenus *Ervum*. As a result, division of *Vicia* into two subgenera i.e. *Vicia* and *Cracca*, is supported.

Keywords: taxonomy, *Vicia*, seed proteins, seed morphology, sections.

As relações taxonômicas das espécies *Vicia* oriundas do Egito, baseadas na morfologia da semente e na SDS-PAGE das proteínas da semente

RESUMO. Analisam-se a morfologia e as proteínas de sementes de 11 táxons de *Vicia* oriundos do Egito. As características macro- e micro-morfológicas, como forma da semente, cor, tamanho, forma do hilo e a escultura da semente são descritas e analisadas. A escultura secundaria da parede celular varia na papilose em quase todas as espécies de *Vicia* até a lofada em *V. hirsuta*. As proteínas das sementes dos táxons foram analisadas por SDS-PAGE onde 39 bandas diferentes foram detectadas. A análise de similaridade baseada no perfil de SDS-PAGE e na morfologia da semente foi uma característica útil para discriminar as espécies de *Vicia* em níveis subgenérico e seccional. Essa investigação rejeitou *V. tetrasperma* e *V. hirsuta* como subgênero de *Ervum*, mas aceitou a divisão de *Vicia* em dois subgêneros, ou seja, *Vicia* e *Cracca.*

Palavras-chave: taxonomia, *Vicia*, proteínas da semente, morfologia da semente, seções.

Introduction

The genus *Vicia* L. belongs to tribe Vicieae of the Fabaceae family. The number of species in the genus varies significantly, ranging from about 150 accepted species by Kupicha (1976) up to about 210 species by Hanelt and Mettin (1989). The genus is mainly distributed throughout the temperate zone of the northern hemisphere and extends into non-tropical South America. *Vicia* is represented by 14 species in Egypt (BOULOS, 1999).

The infrageneric classification of the genus *Vicia* is debatable. Kupicha (1976) divided the genus into two subgenera (subgenus *Vicia* and subgenus *Vicilla*) and 22 sections, instead of three or four sections as described by earlier authors (BALL, 1968; DAVIS; PLITMANN, 1970; FEDTSCHENKO, 1948; RADZHI, 1970).

Different criteria have been used to determine the interspecific relationships within the genus *Vicia*, namely, cytotaxonomy (MAXTED et al., 1991), morphological and molecular characteristics (VAN DE WOUW et al., 2001, 2003), phylogenetic analysis (CHOI et al., 2006; ENDO et al., 2008; JAASKA, 1997, 2005; LEHT, 2005, 2009; LEHT; JAASKA, 2002; MAXTED, 1993a and b, 1994; MAXTED; DOUGLAS, 1997; STEELE; WOJCIECHOWSKI, 2003), RAPD and restriction fragment characters (POTOKINA et al., 1999) and pollen morphology (ENDO; OHASHI, 1996). Although the seed coat characteristics have been studied by Lersten (1979), Lersten and Gunn (1982) and Marin et al. (1998) in some *Vicia* species, little is known about the ultra-structure of the seed surface in most species of the genus. Furthermore, seed protein electrophoresis has become a useful tool in evolutionary studies to determine species relationships. Potokina et al. (2003) found the phylogenetic outcome of such seed protein analyses consistent with the morphological and DNA data and suggested that that simple comparison of electrophoretic patterns of seed proteins was useful to clarify relationships among species of *Vicia*. Mirali et al. (2007) studied the seed protein of some species of

Vicia by SDS-PAGE and showed that there were major differences between studied taxa. These authors did not prove any relationship between the studied storage proteins and the geographical distribution of the accessions. Seed protein electrophoresis techniques have also been employed for the phylogenetic study of a few species of *Vicia* in Egypt (EL-SHANSHOURY; SOILMAN, 1996; SAMMOUR, 1990).

Current investigation deals with the use of seed protein electrophoresis coupled to seed morphological characteristics to evaluate the inter- and intra-specific variability and relationships among the species of the genus *Vicia* in Egypt.

Material and methods

Seed morphology

Analyzed seeds were either collected from mature plants in Egypt or taken from herbarium specimens. A list of voucher specimens and localities is given in Table 1. Only mature seeds were used in current investigation. The dried seeds were first examined by dissecting microscope (Olympus type BH-2) and 10-15 seeds from each taxon were chosen to cover the variation range. Seeds were mounted on stubs with double adhesive tape. The stubs were sputter-coated with gold for 5 min. in a JEOL, JFC 1100 E ion-sputtering device. After coating, the specimens were examined with a JEOL J-SM-T 200 Scanning Electron Microscope, using accelerating voltages at 15-20 KV, at the Electron Microscopy Unit, Assiut University. The terminology employed follows Marin et al. (1998) and Abdel Khalik and Van Der Maesen (2002).

Seed protein electrophoresis

For the extraction of seed proteins, 0.5 g of mature healthy seeds were ground in liquid nitrogen and seed proteins were extracted using Tris-HCl (pH 8.8). SDS-Polyacrylamide gel electrophoresis was performed in 10% acrylamide slab gels following the system by Laemmli (1970). Gels were photographed, scanned and analyzed using gel Doc 2000 Bio-Rad system.

Data analysis

The total number of recorded attributes (49) obtained from seed protein and morphological analysis in each taxon was scored and coded to establish the data matrix of computation. The presence or absence of each of the 39 different electrophoresis bands was treated as a binary characteristic in a data matrix: i.e. coded 1 and 0 respectively. Three types of analysis were carried out by Hierarchical Cluster Analysis using group average measuring similarity percentage (ROMESBURG, 1984). The relationships among the studied taxa were demonstrated as a dendrogram provided by Statistical program PRIMER Ver. 5.0 (CLARKE; GORLEY, 2001).

Results

Morphological characteristics of the seeds

Table 2 shows the morphological characteristics of the seeds of the genus *Vicia* taxa revealed by light microscopy and SEM. They are also partially shown in Figures 1 and 2.

Table 1. List of the studied species of *Vicia*, information on vouchers and synopsis of the infra generic taxa, according to Kupicha (1976), Hanelt and Mettin (1989) and Leht (2009).

N	Taxon	Voucher	Kupicha, 1976	Hanelt & Mettin, 1989	Leht, 2009
1	*Vicianar bonensis* L.	Israel, Negev, Omer N. of Beersheva, Angelis & Amdursky542 (WAG).	Subgenus *Vicia* L. Sect. *Faba* (Mill.) Ldb.	Subgenus *Vicia* L. Sect. *Faba* (Mill.) Ldb.	Subgenus *Vicia* L. Sect. *Narbonensis* (Radzhi) Maxted
2	*V. lutea* L.	Turkey, Diyarbakir, between Elazig and Diyarbakir, E. Hennipman et al. 1495 (WAG).	Subgenus *Vicia* L. Sect. *Hypechusa* (Alef.) Asch. & Graebn	Subgenus *Vicia* L. Sect. *Hypechusa* (Alef.) Asch. & Graebn	Subgenus *Vicia* L. Sect. *Hypechusa* (Alef.) Asch. & Graebn.
3	*V. sativa* L. subsp. *sativa*	Egypt, Allagi village, Abdel Khalik s.n. (SHG).	Subgenus *Vicia L.* Sect. *Vicia* L.	Subgenus *Vicia L.* Sect. *Vicia* L.	Subgenus *Vicia L.* Sect. *Vicia* L.
4	*V. angustifolia* L.	Netherlands, Prov. Gelderland, Wageningen, A. de Boer-Kool 52 (WAG).	Subgenus *Vicia L.* Sect. *Vicia* L.	Subgenus *Vicia L.* Sect. *Vicia* L.	Subgenus *Vicia L.* Sect. *Vicia* L.
5	*V. peregrina* L.	Israel, Judean mountain, Jerusalem, Leinkram & Grizi 661 (WAG).	Subgenus *Vicia L.* Sect. *Peregrinae* Kupicha	Subgenus *Vicia* L. Sect. *Peregrinae* Kupicha	Subgenus *Vicia* L. Sect. *Peregrinae* Kupicha
6	*V. articulata* Hornem.	Egypt, Faiyum, in the garden of the University, Abdel Khaliks. n. (SHG).	Subgenus *Cracca* Peterm. Sect. *Ervoides* (Godr.) Kupicha	Subgenus *Cracca* Peterm. Sect. *Ervoide s*(Godr.) Kupicha	-
7	*V. tetrasperma* (L.) Schreber	Netherlands, Prov. Gelderland, on the ruins of Castle Batenburg, J. de Brujn 836 (WAG).	Subgenus *Cracca* Peterm. Sect. *Ervum*(L.) Taub.	Subgenus *Cracca*Peterm. Sect. *Ervum*(L.) Taub.	Subgenus *Ervum* Sect. *Ervum* (L.) Taub.
8	*V. hirsuta* (L.) Gray	Turkey, Antalya, Kiremithaneler, 12 km SW of Antalya, E. Hennipman et al. 549. (WAG).	Subgenus *Cracca*Peterm. Sect. *Cracca*Dumort.	Subgenus *Cracca* Peterm. Sect. *Lenticula* (Endl.) Asch. & Graebn	Subgenus *Ervum* Sect. *Lenticula*(Endl.) Asch. & Graebn
9	*V. monantha* Retz.	Egypt, Sohag, between fields, Abdel Khaliks. n. (SHG).	Subgenus *Cracca* Peterm. Sect. *Cracca* Dumort.	Subgenus *Cracca* Peterm. Sect. *Cracca* Dumort.	-
10	*V. villosa* Roth subsp. *varia* (Host) Corb.	France, Soler, Milas, de Wilde, P. W. J. s. n. (WAG).	Subgenus *Cracca* Peterm. Sect. *Cracca* Dumort.	Subgenus *Cracca* Peterm. Sect. *Cracca* Dumort.	Subgenus *Cracca* Peterm. Sect. *Cracca* Dumort.
11	*V. hybrida* L.	Israel, Jerusalem, Mt. Scopus, N. Feinbrun, Grizi & Jacobovitch 346 (WAG).	Subgenus *Vicia* L. Sect. *Hypechusa* (Alef.) Asch. & Graebn.	Subgenus *Vicia* L. Sect. *Hypechusa* (Alef.) Asch. & Graebn	Subgenus *Vicia* L. Sect. *Hypechusa* (Alef.) Asch. & Graebn.

Table 2. Seed morphological characteristics of the analyzed species of the genus *Vicia*.

N	Taxon	Seed shape	Seed size (mm)	Seed color	Luster	Hilum shape	Anticlinal walls of epidermal cells			Periclinal walls of epidermal cells	
							Level	Undulation	Secondary sculpture	Level	Secondary sculpture
1	*Vicia narbonensis* L.	Ellipsoid	4-6	Dark brown	Matt	Oblong	Level to sunken	Slightly undulate	Stellate	Papillose	Striate
2	*V. lutea* L.	Spherical	3-4	Brown	Shiny	Oblong	Irregular	Slightly undulate	Stellate	Papillose	Irregular to ribbed
3	*V. sativa* L. subsp. *sativa*	Spherical	4.5-6	Black to brown	Matt	Oblong	Irregular	Slightly undulate	Stellate	Papillose	Irregular to ribbed
4	*V. angustifolia* L.	Spherical	2.5-4	Black	Matt	Oblong	Irregular	Slightly undulate	Stellate	Papillose	Irregular to ribbed
5	*V. peregrina* L.	Spherical	3-4	Dark brown	Matt	Ovate	Leveled to sunken	Slightly undulate	Stellate	Sharply papillose	Irregular to ribbed
6	*V. articulata* Hornem.	Sub-orbicular	2-4	Black to brown	Shiny	Linear	Irregular	Slightly undulate	Stellate	Papillose	Irregular to ribbed
7	*V. tetrasperma* (L.) Schreber	Spherical	1.5-2	Red-brown	Matt	Ovate	Irregular	Slightly undulate	Stellate	Papillose	Irregular to ribbed
8	*V. hirsuta* (L.) Gray	Spherical	3-4	Yellowish	Shiny	Linear	Irregular	Sinuate	Rod-like	Lophate	Ribbed to faveolariate
9	*V. monantha* Retz.	Spherical	3-4	Dark brown	Shiny	Oblong	Irregular	Slightly undulate	Stellate	Sharply Papillose	Irregular to ribbed
10	*V. villosa* Roth	Spherical	3-4	Dark brown	Matt	Oblong	Irregular	Slightly undulate	Stellate	Papillose	Irregular to ribbed
11	*V. hybrida* L.	Ellipsoid	3-4.5	Red-brown	Shiny	Ovate	Irregular	Sinuate	Stellate	Papillose	Irregular to ribbed

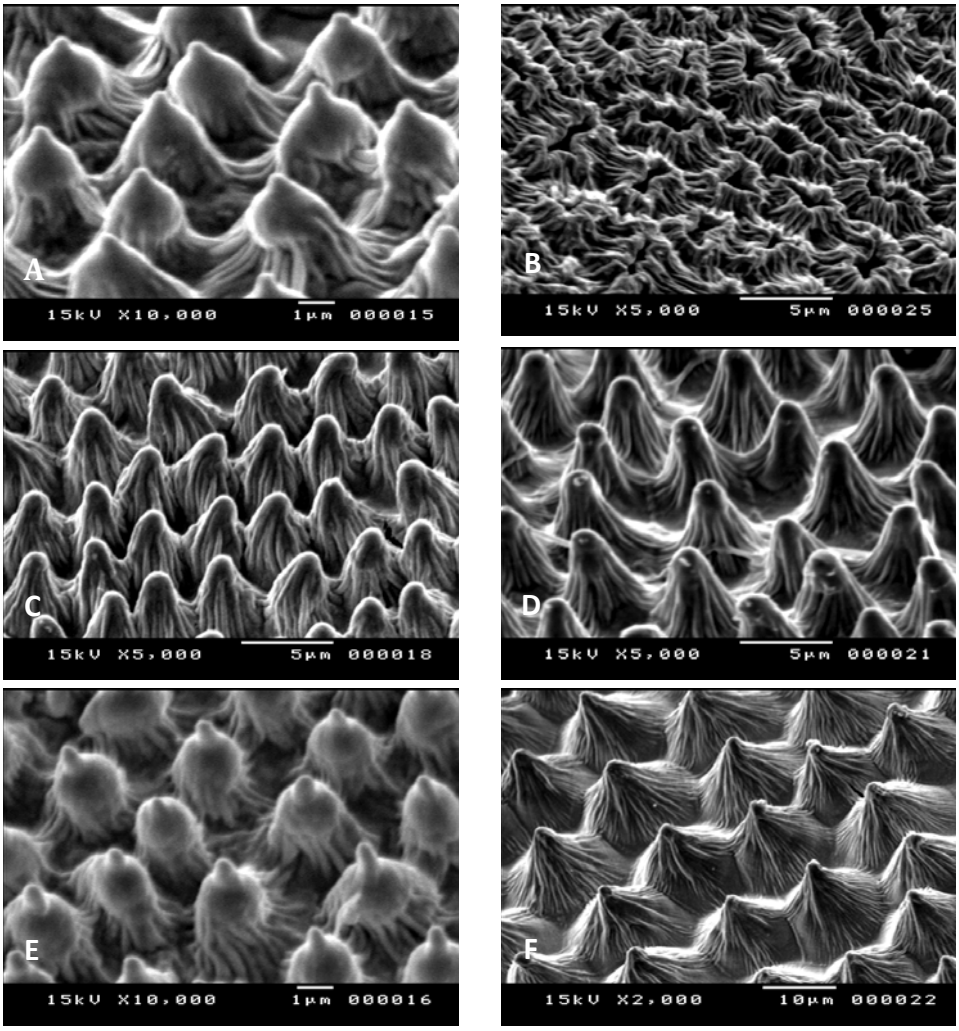
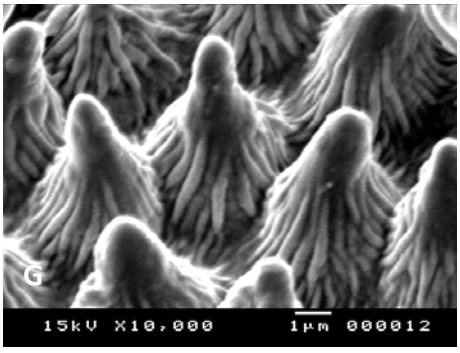


Figure 1. (A-H). SEM photographs of seed surface. A. *Vicia articulata*. B. *V. hirsuta*. C. *V. hybrida*. D. *V. lutea*. E. *V. monantha*. F. *V. narbonensis*. G. *V. peregrina*. H. *V. sativa* subsp. *sativa*. (continue...)



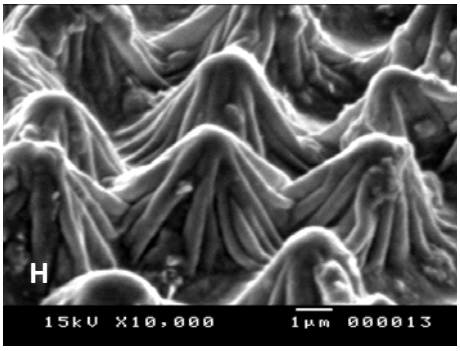
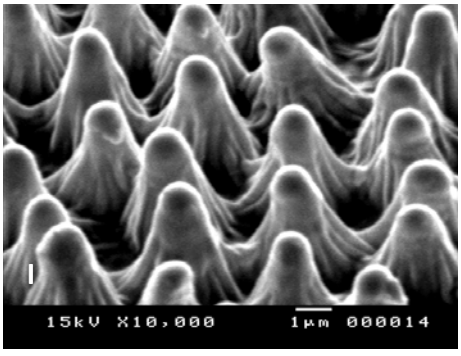
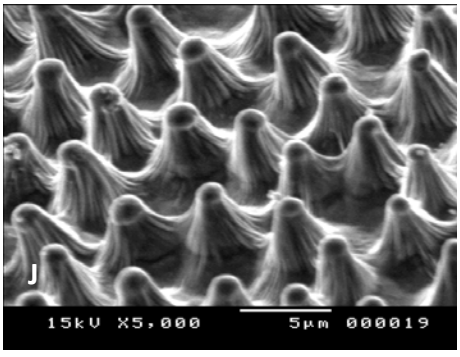


Figure 1. (…continuation)





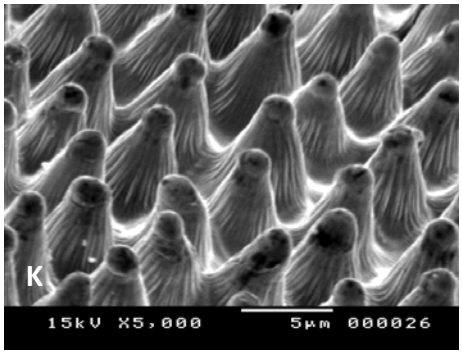


Figure 2. (I-K). SEM photographs of seed surface. I. *V. angustifolia*. J. *V. tetrasperma*. K. *V. villosa* subsp. *varia.*

Seed shape

The seed shape varies from suborbicular in *Vicia articulata*, ellipsoid in *V. narbonensis* and *V. hybrida*, to spherical in the remaining investigated *Vicia* species.

Seed size

Seed sizes vary significantly among the examined taxa. Whereas the largest spherical seeds in *V. sativa* L. subsp. *sativa* are 4.5 - 6 mm in diameter, the smallest ones measure 1.5 - 2 mm in *V. tetrasperma*. The other species have slightly larger seeds, with a diameter measuring 2.5 - 4 mm.

Seed color

Seed colors vary from black in *Vicia angustifolia*, brown-black in *V. sativa* L. subsp. *sativa* and *V. articulata*, brown in *V. lutea*, *V. narbonensis*, *V. peregrina*, *V. monantha* and *V. villosa*, red-brown in *V. tetrasperma* and *V. hybrida* to yellowish in *V. hirsuta*.

Seed surface luster

The luster of seed surface varies from shiny in *Vicia lutea*, *V. articulata, V. hirsuta*, *V. monantha* and *V. hybrida* to matt (dull) in the remaining species.

Hilum shape

Seed hilum shape varies significantly among the examined taxa. It varies from ovate in *Vicia peregrina, V. tetrasperma* and *V. hybrida*, linear in *V. articulata* and *V. hirsuta* to oblong in the other species.

Anticlinal cell wall boundaries

The reliefs of epidermal cells boundaries are generally not well developed. There are three types

of cell wall boundaries: (i) leveled to sunken, slightly undulate, stellate in *Vicia narbonensis* and *V. peregrina* (Figures 1F and 1G); (ii) irregular, sinuate, rod-like in *V. hirsuta* (Figure 1B) and (iii) irregular, slightly undulate, stellate in the remaining taxa.

Periclinal cell wall

Level of periclinal cell walls

The level (curvature) of the outer wall may be a good diagnostic characteristic. There are three different shapes for the level periclinal cell wall: (i) lophate in *Vicia hirsuta* (Figure 1B); (ii) sharply papillose in *V. peregrina* and *V. monantha* (Figure 1E and G); and (iii) papillose in the rest of the taxa.

Secondary sculpture of periclinal cell wall

The surface of the outer cell wall shows great variations among the taxa under analysis: surface is striate in *Vicia narbonensis* and *V. villosa* (Figures 1F and 2K); ribbed to faveolariate in *V. hirsute* (Figure 1B); and irregular to ribbed in the remaining taxa (Figures 1 A, C, D, F, H, 2I, J and K).

SDS-PAGE of storage seed proteins

Electrophoresis analysis of proteins revealed 39 protein bands in the seeds of the 11 species under investigation. Results reveal that some bands are characteristically constant markers for each species and show the unequivocal identification of their electrophoregrams. Other bands are shared by more than one species. The number of bands varies from one species to another, with the largest number (17) in *Vicia narbonensis* and *V. hybrida*, and the lowest number (6) in *V. sativa* L. subsp. *sativa* (Figure 3). For comparison, the 39 protein bands were lumped together with molecular masses and the number of bands from each molecular mass was scored for each species (Table 3).

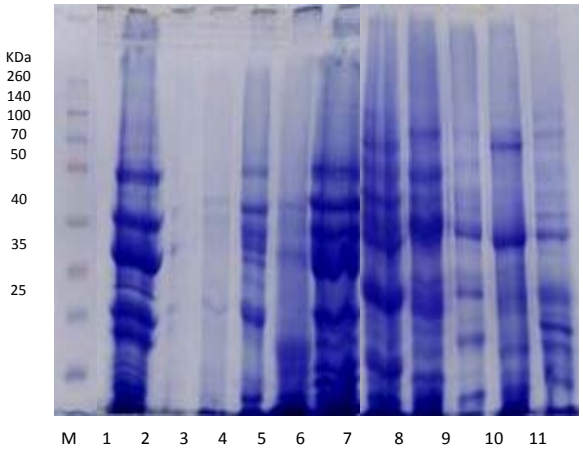


Figure 3. SDS-PAGE analysis of *Vicia* seed proteins numbered as in Table 1.

Table 3. Comparative analysis of molecular weight (Mol.wt), relative front (RF) of SDS-PAGE protein profile of the genus *Vicia*.

Band No	Relative front	Mol.wt. Kda	species										
			1	2	3	4	5	6	7	8	9	10	11
1	0.145	529.85	1	0	0	0	0	0	0	0	0	0	0
2	0.151	500.74	0	0	0	0	0	1	0	0	0	0	0
3	0.188	398.47	0	0	0	1	0	0	0	0	0	0	0
4	0.208	311.60	0	0	0	0	0	1	0	0	0	0	0
5	0.225	283.50	1	0	0	0	0	0	0	0	0	0	0
6	0.231	268.52	0	0	0	0	1	0	0	0	0	0	0
7	0.245	257.10	0	0	0	1	0	0	0	0	0	0	0
8	0.253	236.28	0	0	0	0	0	1	0	0	0	0	0
9	0.281	228.53	0	0	0	1	0	0	0	0	0	0	0
10	0.300	202.66	0	0	0	1	0	0	0	0	0	0	0
11	0.302	167.35	1	0	0	0	0	0	0	0	0	0	0
12	0.310	154.78	1	0	0	0	0	0	0	0	0	0	0
13	0.313	141.33	0	0	0	0	0	1	0	0	0	0	0
14	0.364	110.18	1	0	0	1	0	0	0	0	0	0	0
15	0.275	100.50	0	1	0	0	0	0	1	1	0	1	1
16	0.283	95.51	1	0	1	0	0	0	0	0	0	0	0
17	0.323	87.45	0	1	0	1	1	1	0	0	1	0	0
18	0.346	82.80	1	0	0	0	0	0	1	1	0	1	1
19	0.342	75.02	0	0	0	0	0	0	1	0	0	1	1
20	0.408	67.31	1	0	1	0	0	0	0	0	1	1	1
21	0.414	65.24	0	0	0	0	1	1	1	0	0	0	1
22	0.424	63.76	0	1	1	1	0	0	0	1	0	0	1
23	0.465	58.43	1	0	0	0	1	0	1	1	1	1	1
24	0.493	55.16	0	0	1	1	0	1	0	0	1	1	1
25	0.508	51.12	0	1	0	1	1	0	0	0	0	0	1
26	0.556	44.69	1	0	0	1	0	1	1	1	1	1	1
27	0.596	38.66	0	0	0	0	0	0	1	0	1	0	1
28	0.633	33.14	1	0	1	1	1	0	0	1	1	1	0
29	0.720	28.63	1	1	0	1	1	1	1	1	1	1	1
30	0.756	25.52	0	0	0	0	0	0	1	0	0	1	1
31	0.785	23.73	1	1	0	0	1	1	1	1	1	1	1
32	0.841	19.28	0	0	0	0	0	0	1	1	1	1	1
33	.0.852	17.66	1	0	0	0	1	1	0	0	0	0	0
34	0.879	16.98	0	0	0	0	0	0	1	0	0	0	1
35	0.900	15.79	1	1	1	0	1	1	1	1	1	1	0
36	0.933	14.18	1	0	0	1	0	0	0	0	0	1	1
37	0.937	13.75	0	0	0	0	0	1	1	1	1	0	0
38	0.971	12.30	1	0	0	0	1	1	0	0	0	0	0
39	0.979	12.45	0	0	0	1	0	0	0	0	0	0	0
Total			17	7	6	14	11	14	14	11	12	14	17

Data analysis

A similarity analysis based on the seed proteins and the combination of the SDS-PAGE characters of seed proteins and seed morphology was carried out numerically (Figures 4 and 5). Relationships between the studied taxa are presented in a dendrogram built on the basis of similarity indexes (Table 4). For comparison, the 39 bands were taken together and the 10 number of seed morphological characteristics from each was scored for every species. Three major clusters (A-C) with approximately 67% similarity were obtained (Figure 5). The first cluster (group A) includes *V. sativa* subsp. *sativa* and *V. angustifolia*; the second cluster (group B) includes *V. narbonensis*, and the remaining taxa under analysis comprised group C. Within group C, two subgroups (I and II) were distinguished with 73% similarity. Group I includes three species, with *V. articulata* as the most dissimilar taxon (74%). The other taxa of this group form a well-defined cluster: *V. peregrin*a and *V. lutea* with 78% similarity. Moreover, Group II comprises two well-

defined clusters with 83% similarity: one includes *V. hybrida*, *V. tetrasperma* and *V. villosa*, and the other includes *V. monantha* and *V. hirsuta*.

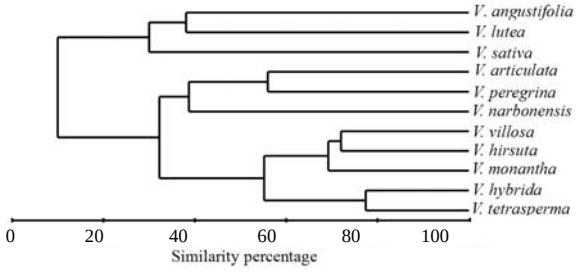


Figure 4. Dendrogram illustrating the relationships of the investigated species of *Vicia* based on seed protein characters.

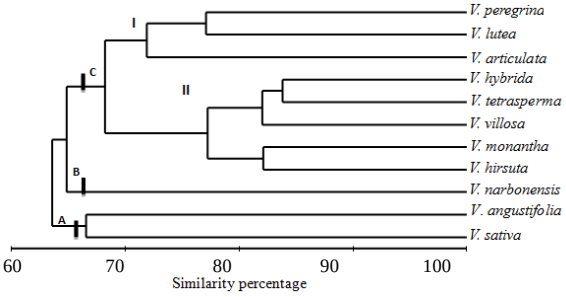


Figure 5. Dendrogram illustrating the relationships of the investigated species of *Vicia* based on the combination of seed proteins and seed morphology by SEM.

Table 4. Similarity matrix between all pairs of studied taxa based on the combination of seed proteins and seed morphology by SEM.

Sp.	1	2	3	4	5	6	7	8	9	10	11
1	100										
2	25	100									
3	34.78	30.76	100								
4	32.25	38.09	30	100							
5	50	55.55	23.52	32	100						
6	38.7	38.09	20	28.57	56	100					
7	38.7	38.09	10	14.28	40	42.85	100				
8	50	55.55	35.29	32	45.45	40	72	100			
9	48.27	42.1	44.44	38.46	52.17	53.84	61.53	69.56	100		
10	58.06	38.09	40	35.71	40	35.71	71.42	72	69.23	100	
11	41.17	41.66	26.08	38.7	35.71	32.25	77.41	57.14	55.17	77.41	100

Discussion

Several authors have tried to provide a natural system to divide the genus *Vicia* into subgenera, sections and subsections (HANELT; METTIN, 1989; KUPICHA, 1976; LEHT, 2009). These studies were largely based on morphological and cytological characters. In the present investigation a large number of seed proteins and seed morphological characteristics were used.

Current results obtained from the mixed analysis generally suggested groups and partially confirmed the subgenera and sectional classification of *Vicia* by Kupicha (1976), Hanelt and Mettin (1989) and Leht (2009).

Subgenus *Vicia*

Section *Vicia*

According to Ball (1968) and Boulos (1999), *Vicia sativa* and *V. angustifolia* belong to *V. sativa* s. l., whereby *V. angustifolia* (subsp. *nigra* (L.) Ehrh.) is treated as subspecies. However, Diklic (1972) and Soó (1996) treated *V. sativa* and *V. angustifolia* as a separate species. Our results indicated that *V. sativa* and *V. angustifolia* formed a group with about 67% similarity. Furthermore, seed morphology confirmed this division since the members of this group had spherical seeds, oblong hilum shape, irregular, slightly undulate, stellate anticlinal boundaries and papillose, irregular to ribbed periclinal cell wall. Above data agreed with the traditional results based on morphology (DIKLIC, 1972; SOÓ, 1996) and with recent results on phylogenetic analyses (POTOKINA et al., 1999; LEHT, 2009) which revealed that section *Vicia* is a well-separated section.

Section Narbonensis

Radzhi (1970), Kupicha (1976), Tzvelev (1980, 1987) and Hanelt and Mettin (1989) classified *V. faba* and *V. narbonensis* in section *Faba* based on morphological characteristics. However, Maxted (1993a) has shown that *V. faba* and *V. narbonenis* are sufficiently distinct to deserve treatment as two sections, *Faba* and *Narbonensis.* Potokina et al. (1999) investigated the molecular data of genomic DNA (RAPDs) and chloroplast DNA (PCR-RFLP) and indicated that *Narbonensis* should be considered a well-separated section which may be related to section *Vicia*. Moreover, Leht (2009) presented cladistic analysis of the morphological data and showed that *V. faba* clustered in the *Narbonensis* group with good support (bootstrap 87) and treated the group as section *Narbonensis.* Furthermore, Maxted et al. (1991) studied the chromosome morphology of the genus *Vicia* and counted $2n = 14$ *in V. narbonensis*.

According to the clustering of the combination of seed proteins and seed morphology analyses, *V. narbonensis* (sect. *Narbonensis)* shows the separation from all other groups (Figure 5), distinct from the others by its elliptical seeds; leveled to sunken, slightly undulate, stellate anticlinal boundaries and papillose with striate periclinal cell wall. As a rule, these results are partially congruent with those of Maxted et al. (1991), Maxted (1993a), Potokina et al. (1999) and Leht (2009).

Sections Peregrinae and Hypechusa

Kupicha (1976) has suggested the existence of a close association between sections *Peregrinae* and

Hypechusa, as shown by the phenetic investigation by Maxted (1994). In their taxonomic treatments, Tzvelev (1987) and Fedoronchuk (1996) retained *V. peregrina* in section *Hypechusa*, accrediting it to subsection *Peregrinae*. Moreover, Leht (2009) has shown the cladistic analysis of the morphological characters of the subgenus *Vicia* and confirmed this agreement. Maxted and Douglas (1997) investigated the phenetic analysis of section *Hypechusa* and split the taxa of *Hypechusa* into two series: *Hyrcanicae* and *Hypechusa*. In current analysis, *V. peregrina* (sect. *Peregrinae*) is linked with *V. lutea* (sect. *Hypechusa*) and *V. articulata* as sister species. Moreover, the seed morphology confirms this division, since the species of sections *Peregrinae* and *Hypechusa* have spherical to elliptic seeds, measuring 3-4 mm; oblong to ovate hilum shape; slightly undulate, stellate anticlinal boundaries and papillose to sharply papillose; irregular to ribbed periclinal cell wall. Furthermore, the two species of section *Hypechusa* (*V. lutea* and *V. hybrida*) occupy two positions (Figures 4 and 5). Therefore, the treatment of *V. lutea* and *V. hybrida* in one section is not supported. These data agree with those of Tzvelev (1987), Maxted (1994), Fedoronchuk (1996), Maxted and Douglas (1997) and Leht (2009).

Subgenus *Cracca*

Sections *Ervoides* and *Ervum*

The Ervoid species of sections *Ervoides* (*V. articulata*) and *Ervum* (*V. tetrasperma*) have been treated either in the separate subgenus *Ervum* (FEDORONCHUK, 1996; LEHT, 2009; RADZHI, 1970; TZVELEV, 1980, 1987) or in the subgenus *Cracca* (HANELT; METTIN, 1989; KUPICHA, 1976; MAXTED, 1993a). Phylogenetic analysis of isozyme variation in subgenus *Cracca* (JAASKA, 2005) did not support the treatment of *Ervoid* species in a separate subgenus *Ervum*, due to their polyphyly. Consequently, the *Ervoid* species were subdivided into two clades. However, Leht (2005, 2009) studied the phylogenetic analysis of the morphological characteristics of the subgenus *Cracca* and indicated that these sections form a separate clade comprising the subgenus *Ervum*; this clade is sister to the clades, consisting of species of subgenus *Vicia* and subgenus *Cracca*.

In the present study, the two species clustered into two different clusters. *V. articulata* form a unique cluster with species from subgenus *Vicia*, whereas *V. tetrasperma* clustered with species from subgenus *Cracca* and *Vicia*. Moreover, seed morphology confirmed this division because both *V. articulata* and *V. tetrasperma* have similar characteristics, such as irregular, slightly undulate, stellate anticlinal boundaries and papillose, irregular to ribbed periclinal cell walls. However, both species also have different characteristics, such as suborbicular seed shape, 2-4 mm size, shiny luster, and linear hilum shape in *V. articulata*, but spherical seed, 1.5-2 mm, matt luster and ovate hilum shape in *V. tetrasperma*. Therefore, treatment of *V. articulata* and *V. tetrasperma* in the subgenus *Ervum* is not supported. These data are congruent with those of Kupicha (1976), Hanelt and Mettin(1989) and Jaaska (2005), but disagree with those of Leht (2005, 2009).

Section *Cracca*

According to Kupicha (1976) and Lersten and Gunn (1982), species of *Vicia villosa, V. monantha* and *V. hirsuta* belong to section *Cracca*. However, Hanelt and Mettin (1989) placed *V. villosa* and *V. monantha* into section *Cracca* and *V. hirsuta* into section *Lenticula*. Moreover, Leht (2009) excluded *V. hirsuta* from subgenus *Cracca,* section *Cracca* and placed it in subgenus *Ervum,* section *Lenticula.* Further, Steele and Wojciechowski (2003) noted that *V. hirsuta* did not support its placement in section *Cracca* and formed a strongly supported group with *V. articulata* (section *Ervoides*). Additionally, Roti-Michelozzi and Serrato-Valenti (1986) studied the seed characteristics of Italian *Vicia*, section *Ervum*, and classified its species into two groups. One group was characterized by a papillose or sharply papillose cell wall pattern and the second one characterized by lophate, regulate or polygonal seed sculpturing, which included *V. hirsuta*, *V. tetrasperma, V. pubescens* and *V. laxiflora*. Marin et al. (1998) studied seed morphology of several species of *Vicia* and they concluded that almost *Vicia* species had a papillose cell wall pattern although *V. hirsuta* possessed a smooth outer cell wall. Current results showed a high degree of similarity among the taxa of section *Cracca* (similarity 75%) based on the analysis of seed proteins and seed characteristics. Additionally, seed morphology confirmed this division because all taxa of this section had spherical seeds, measured 3-4 mm, shiny to matt luster, irregular, slightly undulate, stellate anticlinal wall, and papillose, irregular to ribbed periclinal cell wall. Conversely, *V. hirsuta* had unique characteristics, such as smooth outer cell wall, lophate, ribbed to faveolariate periclinal wall. Consequently, the treatment of *V. villosa*, *V. monantha* and *V. hirsuta* in the section *Cracca* is supported. Above data are congruent with those of Kupicha (1976), Lersten and Gunn (1982), Hanelt and Mettin (1989), Marin et al. (1998) and Jaaska (2005), but disagree with those of Roti-Michelozzi and Serrato-Valenti (1986), and Leht (2005, 2009).

Conclusion

In general, *Vicia* species have a similar seed sculpturing pattern that may in some cases be species-specific. However, seed sculpturing pattern alone could not provide considerable information to distinguish sections of this genus. Seed morphological characteristics of species in the genus *Vicia* could not be used as taxonomic evidence separately but may be reliably combined with seed protein characteristics in this concern. Therefore, determining similarities between species, current results indicated some degree of similarity among the species of sections *Vicia* and *Cracca*. The section *Narbonensis* (*V. narbonensis*) was considered a separate group, while *Hypechusa* was the most heterogeneous section. Moreover, treatment of the Ervoid species of sections *Ervoides* (*V. articulata*) and *Ervum* (*V. tetrasperma*) in a separate subgenus *Ervum* was not recommended. A comprehensive study covering all *Vicia* species would be necessary to make a more thorough classification. The use of molecular data is strongly recommended in further studies.

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References

ABDEL KHALIK, K.; VAN DER MAESEN, L. J. G. Seed morphology of some tribes of Brassicaceae (implications for taxonomy and species identification for the flora of Egypt). **Blumea**, v. 47, n. 2, p. 363-383, 2002.

BALL, P. W. *Vicia* L. In: TUTIN, T. G.; HEYWOOD, V. H.; BURGES, N. A.; MOORE, D. M. (Ed.). **Flora Europaea 2**. Cambridge: Cambridge University Press, 1968. p. 129-136.

BOULOS, L. **Flora of Egypt**. Cairo: Al Hadara Publishing, 1999.

CHOI, B. H.; SEOK, D. I.; ENDO, Y.; OHASHI, H. Phylogenetic significance of stylar features in genus *Vicia* (Leguminosae); an analysis with molecular phylogeny. **Journal of Plant Research**, v. 119, n. 5, p. 513-523, 2006.

CLARKE, K. R.; GORLEY, R. N. **Primer Version 5**. Plymouth: Primer-E, 2001.

DAVIS, P. H.; PLITMAN, U. P. *Vicia* L. In: DAVIS, P. H (Ed.). **Flora of Turkey and East Aegean Islands**. Edinburgh: Edinburgh University Press, 1970. v. 3. p. 274-325.

DIKLIC, N. *Vicia* L. In: JOSIFOVIC, M. (Ed.). **Flora SR Srbije**. Beograd: SANU, 1972. v. 4, p. 315-355.

EL-SHANSHOURY, A. R.; SOLIMAN, S. A. Electrophoretic evidence for subgeneric and sectional relationships of some species in *Vicia* L. **Pakistan Journal of Botany**, v. 28, n. 2, p. 173-182, 1996.

ENDO, Y.; OHASHI, H. The pollen morphology of *Vicia* L. (Leguminosae). **American Journal of Botany**, v. 83, n. 8, p. 955-960, 1996.

ENDO, Y.; CHOI, B. H.; OHASHI, H.; DELGADO, S. A. Phylogenetic relationships of New World *Vicia* L. (Leguminosae) inferred from nrDNA internal transcribed spacer sequences and floral characters. **Systematic Botany**, v. 33, n. 2, p. 56-363, 2008.

FEDORONCHUK, M. M. A revision of species of the genus *Vicia* L. (Fabaceae) in the flora of the Ukraine. **Ukrayins'kyi Botanichnyi Zhurnal**, v. 53, n. 5, p. 587-598, 1996.

FEDTSCHENKO, B. A. *Vicia* L. In: SCHISCHKIN, B.; BOBROV, E. (Ed.). **Flora URSS**. Mosqua-Leningrad: Editio Academiae Scientiarum, 1948. v. 13, p. 406-475.

HANELT, P.; METTIN, D. Biosystematics of the genus *Vicia* L. (Leguminosae). **Annual Review of Ecological Systems**, v. 20, p. 199-223, 1989.

JAASKA, V. Isoenzyme diversity and phylogenetic affinities in *Vicia* subgenus *Vicia* (Fabaceae). **Genetic Resources and Crop Evolution**, v. 44, n. 6, p. 557-574, 1997.

JAASKA, V. Isozyme variation and phylogenetic relationships in *Vicia* subgenus *Cracca* (Fabaceae). **Annals of Botany**, v. 96, n. 6, p. 1085-1096, 2005.

KUPICHA, F. K. The infrageneric structure of *Vicia* L. **Notes from the Royal Botanic Garden Edinburgh**, v. 34, n. 3, p. 287–326, 1976.

LAEMMLI, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature**, v. 227, n. 5259, p. 680-685, 1970.

LEHT, M. Cladistic and phenetic analysis of relationships in *Vicia* subgenus *Cracca* (Fabaceae) based on morphological data. **Taxon**, v. 54, n. 4, p. 1023-1032, 2005.

LEHT, M. Phylogeny of *Vicia* (Fabaceae) based on morphological data. **Feddes Repertorium**, v. 120, n. 7/8, p. 379-393, 2009.

LEHT, M.; JAASKA, V. Cladistic and phenetic analysis of relationships in *Vicia* subgenus *Vicia* (Fabaceae) by morphology and isozymes. **Plant Systematics and Evolution**, v. 232, n. 3-4, p. 237-260, 2002.

LERSTEN, N. R. A distinctive seed coat pattern in the Vicieae (Papilionoideae, Leguminosae). **Proceedings of the Iowa Academy of Science**, v. 86, n. 3, p. 102-104, 1979.

LERSTEN, N. R.; GUNN, C. R. Testa characters in tribe Vicieae, with notes about tribes Abreae, Cicereae, and Trifolieae (Fabaceae). U.S. Department of Agriculture, **Technical Bulletin**, v. 1667, p. 1-40, 1982.

MARIN, P. D.; BOZA, P.; MERKULOV, L. J.; KRSTIC, B.; PETKOVIC, B.; VELJIC, M.; PAJEVIC, S. Seed sculpturing of selected European *Vicia* L. species (Fabaceae) and their taxonomical evaluation. **Seed Science and Technology**, v. 26, n. 1, p. 17-32, 1998.

MAXTED, N. A phenetic investigation of *Vicia* L. subgenus *Vicia* (Leguminosae, Vicieae). **Botanical Journal of the Linnean Society,** v**.** 111, n. 2, p. 155-182, 1993a.

MAXTED, N. A phenetic analysis of *Vicia* section *Atossa* series Truncatulae (Leguminosae:Vicieae). **Kew Bulletin**, v. 48, n. 4, p. 739-753,1993b.

MAXTED, N. A phenetic investigation of *Vicia* L. section *Peregrinae Kupicha* (Leguminosae, Papilionoideae, Vicieae). **Edinburgh Journal of Botany**, v**.** 51, n. 1, p. 75-97, 1994.

MAXTED, N.; DOUGLAS, C. A phenetic investigation of *Vicia* section *Hypechusa* (ALEF). Aschers & Graebner (Leguminosae, Papilionoideae, Vicieae). **Lagascalia**, v. 19, n. 1-2, p. 345-370, 1997.

MAXTED, N.; KHETTAB, M. A.; BISBY, F. A. The newly discovered relatives of *Vicia faba* do little to resolve the enigma of its origin. **Botanika Chronika**, v. 10, p. 435-465, 1991.

MIRALI, N.; EL- KHOURI, S.; RIZQ, F. Genetic diversity and relationships in some *Vicia* species as determined by SDS-PAGE of seed proteins. **Biologia Plantarum**, v. 51, n. 4, p. 660-666, 2007.

POTOKINA, E.; ENDO, Y.; EGGI, E.; OHASHI, H. Electrophoretic patterns of seed proteins in the East Asian *Vicia* species (Leguminosae) and their systematic utility. **Journal of Japanese Botany**, v.78, n. 1, p. 29-37, 2003.

POTOKINA, E.; TOMOOKA, N.; VAUGHAN, D. A.; ALEXANDROVA, T.; RQ, X. U. Phylogeny of *Vicia* L. subgenus *Vicia* (Fabaceae) based on analysis of RAPDs and RFLP of PCR-amplified chloroplast genes. **Genetic Resources and Crop Evolution**, v. 46, n. 2, p. 149-161, 1999.

RADZHI, A. D. Conspectus systematis specierum Caucasicarum Generis *Vicia* L. In: TZVELEV, N. N (Ed.). **Novitates systematicae plantarum vascularium**. Leningrad: Nauka, 1970, v. 6. p. 228-240.

ROMESBURG, H. C. **Cluster analysis for researchers**. **Belmont**: Life-time. Canada: Learning Publications, 1984.

ROTI-MICHELOTTI, G.; SERRATO-VALENTINI, G. Seed characteristics in Italian species of genus *Vicia* section Ervumand their diagnostic value. **Seed Science and Technology**, v. 14, n. 2, p. 391-402, 1986.

SAMMOUR, R. H. Electrophoresis of the seed protein of *Vicia faba* L., and its immediate progenitors. **Plant Breeding**, v. 104, n. 3, p. 196-201, 1990.

SOÓ, R. A. **Magyar flora es vegetacio rendszertani novenyfoldrajzikezi konyve**. Budapest: Akademiai Kiado, 1996. v. 2.

STEELE, K. P.; WOJCIECHOWSKI, M. F. Phylogenetic analyses of tribes Trifolieae and Vicieae, based on sequences of the plastid gene, *mat*K (Papilionoideae: Leguminosae). In: KLITGAARD, B. B.; BRUNAEU, A. (Ed.). **Advances in legume systematics**. London: Royal Botanical Gardens, 2003. p. 355-370.

TZVELEV, N. N. Systema specierum generis *Vicia* L. in parte Europaea URSS. **Novitates Systematicae Plantarum Vascularium**, v. 17, p. 200-208, 1980.

TZVELEV, N. N. *Vicia* L. In: FEDOROV, A. A. (Ed.). **Flora Partis Europaeae URSS**. Leningrad: Nauka, 1987. v. 6, p. 127-147.

VAN DE WOUW, M.; MAXTED, N.; FORD-LLOYD, B. V. A multivariate and cladistic study of *Vicia* ser. *Vicia* (Fabaceae) based on analysis of morphological characters. **Plant Systematics and Evolution**, v. 237, n. 1-2, p. 19-39, 2003.

VAN DE WOUW, M.; MAXTED, N.; CHABANE, K.; FORD-LLOYD, B. V. Molecular taxonomy of *Vicia* ser. *Vicia* L. based on Amplified Fragment Length Polymorphisms. **Plant Systematics and Evolution**, v. 229, n. 1-2, p. 95-105, 2001.

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