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Seed proteins and the classification of *Brassicaceae* (*Magnoliopsida*) in Egypt

Abstract

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Seed proteins of eleven species of *Brassicaceae* were investigated by polyacrylamide gel electrophoresis. In total 50 different bands were identified. Some of the bands are characteristic and represent constant markers of each species, which allow the unequivocal identification of their electrophoregram. The obtained data have been treated numerically using the cluster analysis method of unweighted pair group (UPGMA). The electrophoregram gives support to the idea that the tribe *Sisymbrieae* is an unnatural group and suggests its merge with the tribe *Brassiceae*. On the other hand the distinct position of *Zilla spinosa* in the dendrograms supports the traditional treatment of this taxon as a monotypic subtribe *Zillinae*.

Introduction

The family *Brassicaceae (Cruciferae)* is frequently described as a natural family in account of its a remarkable uniformity in the fundamental structure of flowers, fruits and seeds and also in certain anatomical and chemical characters (Schulz 1936, Turrill ·1939, Janchen 1942, Hedge 1976, Bowman & Symth 1998, Goffman & al. 1999). However, it is generally recognized that it is difficult to make a satisfactory classification within the family especially at the tribal and generic levels (Hedge 1976, Heywood 1976, Al Shehbaz 1984). In the last three decades, the employment of chemical characters in plant taxonomy and evolution has become a widely accepted approach (Cronquist 1980, Gershenzen & Mabry 1983, Fairbrathers 1983, Waterman & Gray 1987). Certain chemical constituents have been used in taxonomic and evolutionary studies of *Brassicaceae*. These are fatty acids (Appelqvist 1976); sterols (Knights & Berrie 1971); glucosinolates (Kjaer 1976, Heaney & Fenwick 1980, Gland & al. 1981, Rodman & al. 1981, Horn and Vaughan 1983, Mithen & al. 1987, Waterman & Gray 1987, Lockwood & Belkhiri 1991); storage seed proteins (Vaughan & White 1967, Vaughan & Denford 1968, Vaughan & al. 1966,

Finlayson 1976) and serology (Kolbe 1981). Proteins are usually considered as the immediate products of the genome and are less affected by the environmental conditions. Consequently, the high stability of protein characters particularly those of seeds, makes them a powerful tool in elucidation the origin, evolution and relationship of the taxa (Davis & Heywood 1963, Ladizinsky & Hymowitz 1979). In order to apply a more objective approach in classification of *Brassicaceae* in Egypt, in addition to the morphological characters, seed proteins of eleven wild species in Egypt have been studied using the polyacrylamide gel electrophoresis technique. The obtained data were analyzed by numerical analysis (Cluster analysis) based on Jaccard's coefficient (Sneath & Sokal 1973).

Material and methods

The morphological studies were based mainly on herbarium specimens deposited in CAI, CAIM (abbreviation according to Holmgren and Stafleu, 1983) and AST (Herbarium of the Botany Department, Faculty of Science, University of Assiut, proposed abbreviation). Studies on seed proteins were carried out on mature seeds of eleven species of *Brassicaceae* growing in different localities in Egypt (Table 1). Voucher specimens of studied taxa are deposited in AST and CAI. Seeds of each species were ground separately to a fine flour in a prechilled mortar and pestle. Proteins were extracted (1 g seed flour to 3 ml extract) in a buffer containing 10% glycerol, 5% 2-mercaptoethanol, 2.3% sodium lauryl sulfate and 0.75% Tris. at 0° C with addition of 4 ml of an aqueous solution of polyvinylpyrovolidone. The extract was centrifuged for 20 minutes and the supernatant was decanted. Proteins were precipitated with saturated ammonium sulfate. The pellet was taken up in 1 ml of the extraction buffer and used in 30 µl aliquots for PAGE. Gels were stained in 0.1% comassie blue and destained in 300 ml of destained solution (7% glacial acetic acid, 40% methanol and 53% distilled water). These gels were washed with water, dried and then photographed. The electrophoretic banding patterns and their corresponding R_f value of the studied taxa are shown in fig. 1 and table 2. In total, 50 different bands were identified

For numerical analysis, were studied 46 characters concerned with habit, leaf, stem, flower, fruit and seed (Table 4). The data for numerical analysis thus consisted of the 46 morphological characters and 50 protein characters, scored for each of the 11 OTU's. Each

Table 1. Localities of the studied taxa.

Taxon	Localities
1- *Brasica tournefortii*	Cairo - Alexandria desert Road, 16.4 1986, S.M. El Naggar, s.n. (CAI, AST)
2- *Sinapis arvensis*	Assiut University campus, Assiut, 23.3 1997, S.M. El Naggar, s.n. (CAI, AST)
3- *Diplotaxis harra*	Wadi Al Assiuty, Eastern Desert, 27.3.1997, S.M. El Naggar, s.n. (CAI, AST)
4- *D. acris*	Wadi Al Assiuty, Eastern Desert, 27.3.1997, S.M. El Naggar, s.n. (CAI, AST)
5- *Raphanus raphanistrum*	Banha, 15.1.1985, S.M. El Naggar, s.n. (CAI, AST)
6- *Enarthrocarpus strangulatus*	Burg El Arab, Mariut, 17.4.1986, S.M. El Naggar, s.n. (CAI, AST)
7- *Zilla spinosa*	Wadi Al Assiuty, Eastern Desert, 16.4.1986, S.M. El Naggar, s.n. (CAI, AST)
8- *Schouwia purpurea*	Siwa Oasis, Western Desert, 16.4.1986, S.M. El Naggar, s.n. (CAI, AST)
9- *Lepidium sativum*	Siwa Oasis, Western Desert16.4.1986, S.M. El Naggar, s.n. (CAI, AST)
10- *Capsella bursa-pastoris*	Banha, 15.1.1985, S.M. El Naggar, s.n. (CAI, AST)
11- *Sisymbrium irio*	Assiut, cultivated land, Assiut, 12.3.1998, S.M. El Naggar, s.n. (CAI, AST)

Table 2. Distribution of protein and morphological characters in the studied taxa.
Present = 2, absent = 1.

	Protein characters												Morphological characters										
	1	2	3	4	5	6	7	8	9	10	11		1	2	3	4	5	6	7	8	9	10	11
1-	1	1	1	1	1	1	1	2	1	1	1		2	2	2	2	1	2	2	2	2	2	2
2-	1	1	2	2	1	1	1	1	1	1	1		2	2	2	2	1	2	2	2	2	2	2
3-	1	1	1	1	1	1	1	1	1	1	2		1	1	1	1	2	1	1	1	1	1	1
4-	1	1	1	1	1	2	1	1	1	1	1		1	1	1	1	2	1	1	2	1	1	1
5-	1	1	1	1	1	1	1	1	2	1	1		2	2	2	2	1	2	2	1	2	1	2
6-	2	2	1	1	1	1	1	2	1	1	1		1	1	1	1	1	1	1	1	1	2	1
7-	1	1	1	1	2	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	2	1
8-	2	2	1	1	2	1	1	1	1	1	1		1	1	1	1	2	1	1	2	1	1	1
9-	2	2	1	1	1	1	1	2	1	1	1		2	1	1	1	2	1	1	2	2	2	1
10-	1	1	1	1	1	1	1	1	1	2	1		1	1	1	1	1	1	1	2	1	2	1
11-	1	1	1	1	2	2	1	1	1	1	1		1	1	1	1	1	1	2	1	1	1	1
12-	1	2	1	1	2	1	2	1	1	1	1		1	1	1	1	1	1	1	1	2	2	2
13-	1	2	1	1	1	1	1	1	2	2	1		2	1	1	1	2	2	2	2	1	1	1
14-	2	2	1	1	2	1	1	1	1	1	1		1	2	2	2	1	2	1	2	1	1	1
15-	2	2	1	1	1	2	1	2	2	1	1		2	2	2	1	1	1	2	1	1	1	2
16-	2	2	1	1	2	1	2	2	1	1	1		1	1	1	1	1	1	1	1	2	2	1
17-	1	1	1	1	1	1	1	1	2	2	1		1	1	1	2	2	2	1	2	1	1	1
18-	1	2	1	1	2	2	1	2	2	1	1		2	2	2	2	1	2	1	2	1	1	1
19-	2	2	1	1	2	1	2	1	2	2	1		1	1	1	2	1	2	2	2	1	1	1
20-	1	1	1	1	1	1	1	2	1	1	1		1	2	2	2	1	2	1	2	1	1	1
21-	1	1	1	1	1	1	1	1	2	1	1		2	2	2	2	2	2	2	1	1	1	2
22-	1	1	1	1	1	2	2	1	2	1	1		1	1	1	1	2	1	1	2	1	1	1
23-	1	1	1	1	2	1	1	1	1	1	1		2	2	2	2	1	2	2	1	1	1	2
24-	2	2	1	1	1	1	1	1	1	2	1		1	2	2	2	1	2	2	1	2	2	2
25-	1	1	1	1	1	2	1	2	1	1	1		2	2	2	2	1	2	2	1	1	1	2
26-	1	1	1	1	1	1	1	1	2	2	1		2	2	2	2	1	1	1	2	2	2	2
27-	1	1	2	1	1	1	1	1	1	1	1		1	1	1	1	1	2	2	1	1	1	1
28-	1	2	1	1	1	1	1	1	1	1	1		1	1	1	1	2	1	1	1	1	1	1
29-	1	1	2	1	1	1	1	1	1	1	2		1	1	1	1	1	1	1	2	2	1	1
30-	1	1	1	1	1	2	1	1	1	1	1		1	1	1	1	1	1	1	1	1	2	1
31-	1	2	1	2	1	1	2	2	1	1	1		1	1	1	1	1	1	1	2	1	1	1
32-	1	1	1	1	1	1	1	1	1	2	1		1	1	1	1	2	1	1	2	1	1	1
33-	2	2	2	2	2	2	2	1	1	1	2		1	1	1	1	1	1	1	2	2	2	1
34-	1	2	1	1	1	1	1	1	1	1	2		1	1	2	2	1	1	1	1	1	1	1
35-	2	1	1	1	2	1	1	1	1	2	1		2	2	1	1	1	1	1	2	1	1	1
36-	1	1	2	1	1	1	1	1	1	1	1		2	2	2	2	2	1	1	2	2	2	2
37-	1	1	1	1	1	1	2	1	1	1	1		2	2	2	2	2	2	2	1	2	2	1
38-	1	1	1	2	2	1	1	1	2	2	1		1	1	1	1	1	1	1	2	2	2	2
39-	2	2	2	1	1	1	1	1	2	1	1		1	2	1	1	1	1	1	2	1	2	2
40-	1	1	1	1	1	2	1	1	1	1	1		1	2	1	1	1	1	1	1	2	1	
41-	1	1	1	1	1	1	1	2	1	2	1		1	2	2	1	1	1	1	1	2	1	1
42-	1	1	2	1	1	1	1	1	1	1	2		2	1	1	2	2	2	2	1	1	1	1
43-	2	1	2	2	1	2	1	1	1	1	2		2	1	1	1	2	2	2	1	1	1	2
44-	1	1	1	1	1	1	1	2	2	2	1		1	2	1	1	1	1	1	2	1	2	2
45-	2	1	1	2	2	1	2	1	2	2	2		2	2	2	2	2	2	2	2	1	1	1
46-	1	1	2	1	1	1	2	1	1	1	2		1	1	1	1	1	1	1	2	2	2	2
47-	1	2	1	1	1	1	2	2	2	2	1												
48-	1	1	1	1	1	1	1	1	2	1	1												
49-	2	1	1	1	1	1	1	1	1	1	1												
50-	1	2	1	1	1	1	1	1	1	1	1												

Table 3. Matrix of similarity between all pairs of studied taxa based on protein characters.

11	10	9	8	7	6	5	4	3	2	1	
										1.00	**1**
									1.00	0.52	**2**
								1.00	0.80	0.54	**3**
							1.00	0.70	0.58	0.54	**4**
						1.00	0.23	0.14	0.12	0.33	**5**
					1.00	0.26	0.75	0.52	0.44	0.52	**6**
				1.00	0.70	0.24	0.50	0.43	0.37	0.57	**7**
			1.00	0.17	0.31	0.34	0.36	0.28	0.36	0.32	**8**
		1.00	0.21	0.19	0.17	0.11	0.28	0.33	0.28	0.28	**9**
	1.00	0.54	0.26	0.12	0.12	0.09	0.20	0.20	0.29	0.20	**10**
1.00	0.44	0.39	0.24	0.37	0.29	0.10	0.36	0.41	0.52	0.41	**11**

character was scored for presence (2) and absence (1). The data were analyzed using the Jaccard's coefficient $Sj=$ (where a is the number of characters shared by a pair of samples, b is the number of characters found in one of a pair only, and c is the number of characters found only in the other one of a pair). This formula was used as a measure of similarity of pattern (Sneath and Sokal, 1973). The matrix of Jaccard's coefficient was then used in a pair-wise cluster analysis using the unweighted pair group method using arithmetic average (UPGMA) to produce a phenogram of similarities.

Results and discussion

The analysis of results reveals that some bands are characteristic and constant markers for each species and allow the unequivocal identification of their electrophorgrams. Other bands are common in more than one species. Characteristic (marker) bands of species are No 1, 5, 20 for *Shouwia purpurea*; No. 3 for *Sisymbrium irio*; No. 4, 30, 40 for *Zilla spinosa;* No. 7, 23 for *Raphanus raphanistrum;* No. 10, 32 for *Capsella bursa-pastoris;* No. 21, 48 for *Lepidium sativum*; No. 27, 36 for *Diplotaxis harra*; No. 28, 50 for *Sinapis arvensis*; No. 49 for *Brassica tournefortii* and No. 37 for *Enarthrocarpus strangulatus.*

The seed protein banding patterns in the studied taxa show the close relationships of taxa and distinguish and differentiate them to their distinct status. From the two dendrograms based on protein analysis (Fig. 2) and morphological characters (Fig. 3), the very distinct position of *Zilla spinosa* agrees with the previous treatments of this taxon based on the morphological evidence. Schulz (1936) using the morphological criteria put *Zilla spinosa* in a very distinct position as the monotypic subtribe *Zilliinae,* which is clearly different from all other genera in our area particularly those that are investigated here, and is very unlikely to be confused with them.

The first aggregate of the dendrogram based on protein characters (Fig. 2) consists of five species namely *Brassica tournefortii, Sinapis arvensis, Raphanus raphanistrum, Enarthrocapus strangulatus* and *Schouwia purpurea.* This aggregate agrees with the

Table 4. Morphological characters of the studied taxa used in the numerical study.

Habit characters	
1 - Annual	Perennial
2 - Herb	Shrub
3 - Spiny plants	Not spiny plants
4 - Glabrous	Hairs of any type present on at least one part
5 - Simple hairs present	Simple hairs absent
7 - Furcate hairs present	Furcate hairs absent
7 - Stellate hairs present	Stellate hairs absent
Leaf characters	
8 - Lower leaves simple entire	Lower leaves otherwise
9 - Upper leave simple entire	Upper leaves otherwise
10- Upper leaves lobed or pinnatsect	Upper leaves otherwise
Floral characters	
11- Bract present	Bract absent
12- Sepals equal	Sepals unequal
13- Sepals saccate at the base	Sepals not saccate
14- Sepal lenghth 5 mm or longer	Sepal lenghth less than 5 mm long
15- Petals yellow	Petal not yellow
16- Petals white	Petal not white
17- Petals violet or red	Petal nither violet nor red
18- Petal lenghth 5 mm or longer	Petal lenghth less than 5 mm long
19- Darke veins present	Dark veins absent
20- Filament length 5 mm or longer	Filament lenghth less than 5 mm long
21- Anther linear	Anther not linear
22- Anther sagittate at the base	Anthe not sagittate at the base
23- Stigma bilobed	Stigma not bilobed
24- Stigma capitate	Stigma not capitate
Fruit characters	
25- Fruit siliqua	Fruit cilicula
26- Fruit dehiscent	Fruit indehiscent
27- Fruit 2-joint	Fruit not 2-joint
28- Fruit globose	Fruit not globose
29- Fruit orbicular	Fruit not orbicular
30- Fruit obcordate	Fruit not obcordate
31- Valves winged	Valves wingless
32- Beak spine- shaped	Beak not spine- shaped
33- Septutum perpendiculare with valves	Septum paralell to the valves
Seed characters	
34- Seeds arranged in 2 rows	Seeds not arranged in rows
35- Seed globose	Seed not globose
36- Epidermal cell well developed	Epidermal cell not well developed
37- Anticlinal cell boundaries raised	Anticlinal cell boundaries channeled
38- Periclinal cells wall domate	Periclinal cell walls notdomate
39- Periclinal cells wall with central portion	Periclinal cell walls witout central portion
40- Central portion raised	Central portion not raised
41- Periclinal cell wall flat	Periclinal cell wall otherwise
42- Periclinal cell wall concave	Periclinal cell wall otherwise
43- Periclinal cell wall folded	Periclinal cell wall not folded
44- Periclinal cell wall striated	Periclinal cell wall not striated
45- Embryo conduplicate	Embryo otherwise
46- Embryo incumpent	Embryo otherwise

Table 5. Matrix of similarity between all pairs of studied taxa based on morphological characters.

11	10	9	8	7	6	5	4	3	2	1	
										1.00	**1**
									1.00	0.52	**2**
								1.00	0.80	0.54	**3**
							1.00	0.70	0.58	0.54	**4**
						1.00	0.23	0.14	0.12	0.33	**5**
					1.00	0.26	0.75	0.52	0.44	0.52	**6**
				1.00	0.70	0.24	0.50	0.43	0.37	0.57	**7**
			1.00	0.17	0.31	0.34	0.36	0.28	0.36	0.32	**8**
		1.00	0.21	0.19	0.17	0.11	0.28	0.33	0.28	0.28	**9**
	1.00	0.54	0.26	0.12	0.12	0.09	0.20	0.20	0.29	0.20	**10**
1.00	0.44	0.39	0.24	0.37	0.29	0.10	0.36	0.41	0.52	0.41	**11**

classification of Schulz (1936) in which all these taxa were delimited in one tribe (*Brassiceae*), but in different subtribes. Morphologically *Brassica* is closely similar to *Sinapis* and some taxonomists have disputed the position of species in them. Linnaeus (1753) recognized *Brassica nigra* and *Brassica juncea* under *Sinapis*. Muschler (1912), Ascherson & Schweinfurth (1887) and Ramis (1929) followed Linnaeus in that respect. In the present study analysis of seed protein data indicated that, *Brassica* and *Sinapis* are more closely allied to each other than to any of studied taxa (Fig. 2). This result agrees with those of Vaughan & Denford, (1968). On the other hand from the dendrogram based on the morphological characters shows that *Brassica* could be affiliated to the aggregate of *Diplotaxis acris/ Raphanus raphanistrum/ Enarthrocarpus strangulatus* whereas *Sinapis arvensis* is more related to *Diplotaxis harra* (Fig. 3). This indicates that in some cases protein characters are more reliable as taxonomic characters than morphological ones. *Raphanus raphanistrum* and *Enarthrocarpus strangulatus* are more distinctive than the preceeding two genera, on account of their indehiscent fruits and dark veined petals. Based on protein data *Raphanus raphanistrum* appears more close to the aggregate of *Brassica / Sinapis* than to *Enarthrocarpus strangulatus* (Fig. 2). On the other hand, *Raphanus raphanistrum* is morphologically more related to *Diplotaxis acris* than to *Enarthrocapus* (Fig. 3). It may be concluded that *Raphanus* and *Enarthrocapus* are less allied to each other. Our results thus agree with the early founding of Rytz (1932) who put *Raphanus* and *Enarthrocarpus* in two separate subtribes *Raphaninae* and *Erucarinae* respectively.

Schouwia purpurea, which was recognized by Schulz (1936) in subtribe *Villinae*. is distinguished from the other members of the above aggregate with its orbicular, winged and dehiscent silicula with long and conical beak and violet or pink petals and ebracteate inflorescence. The position of *Schouwia* in the dendrograms based on protein data (Fig. 2) and the morphological characters (Fig. 3) reflects the distinctive characters and classification of this taxon. *Schouwia purpurea* was regarded as a very distinctive taxon based on its morphological and seed coat characteristics (Fayed & El Naggar 1988, El Naggar & Soliman 1999).
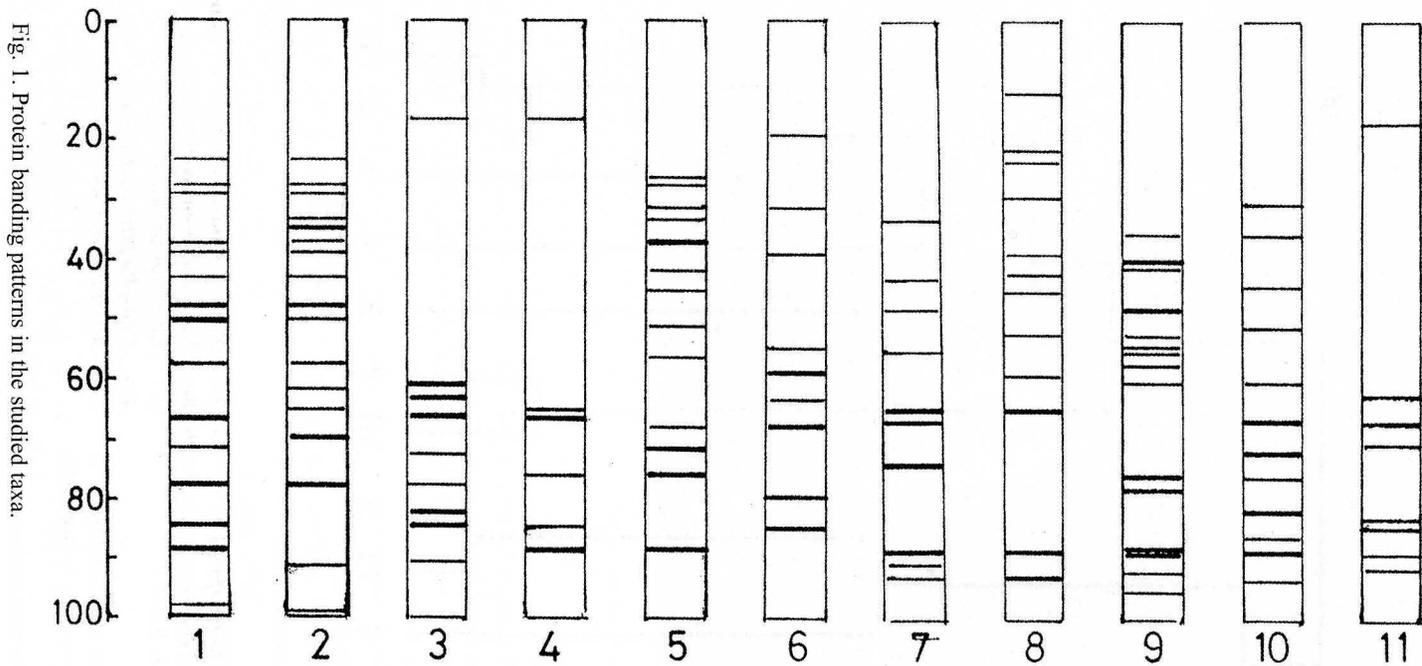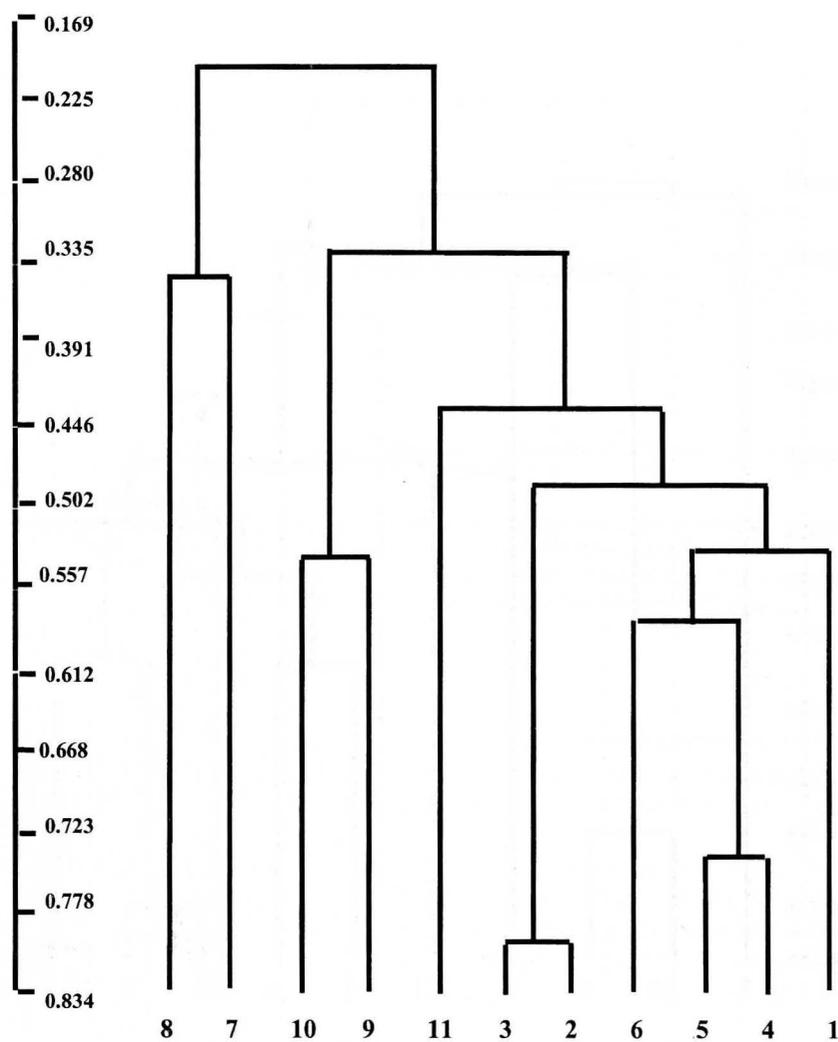
Fig. 1. Protein banding patterns in the studied taxa.

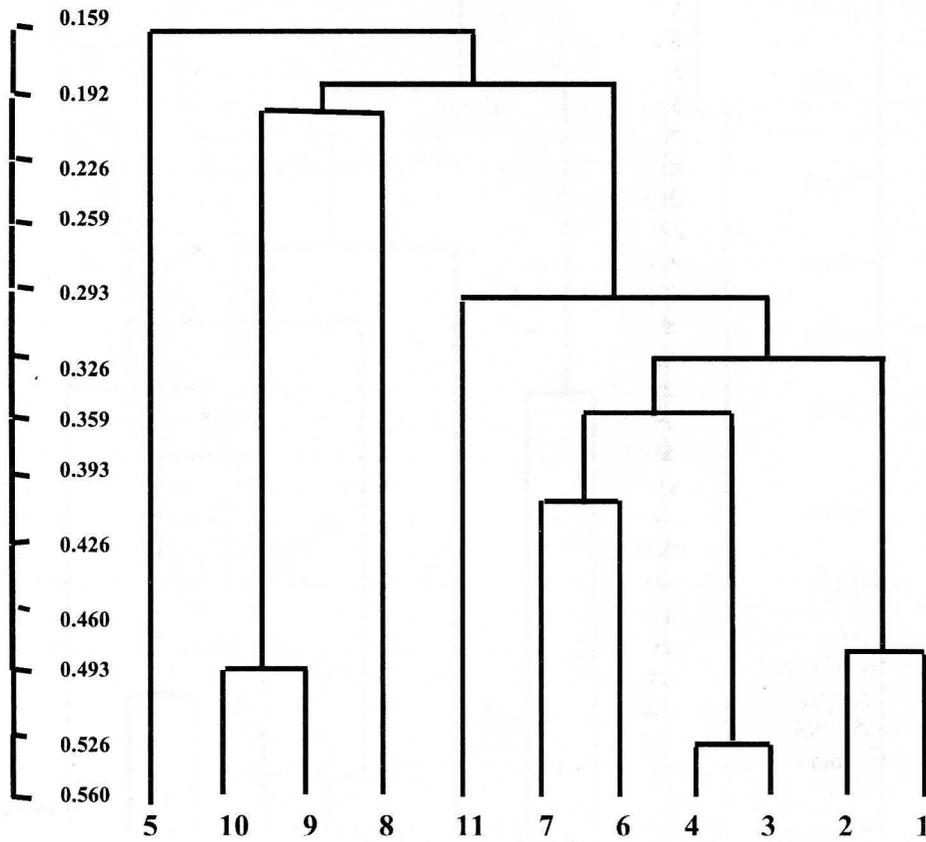| 1-	Brassica tournifortii	7-	Zilla spinosa
2-	Sinapis arvensis	8-	Schouwia purpurea
3-	Diplotaxis harra	9-	Lepidium sativum
4-	D. acris	10-	Capsella pursa-pastoris
5-	Raphanus raphanistrum	11-	Sisymbrium irio
6-	Enarthrocarpus strangulatus		
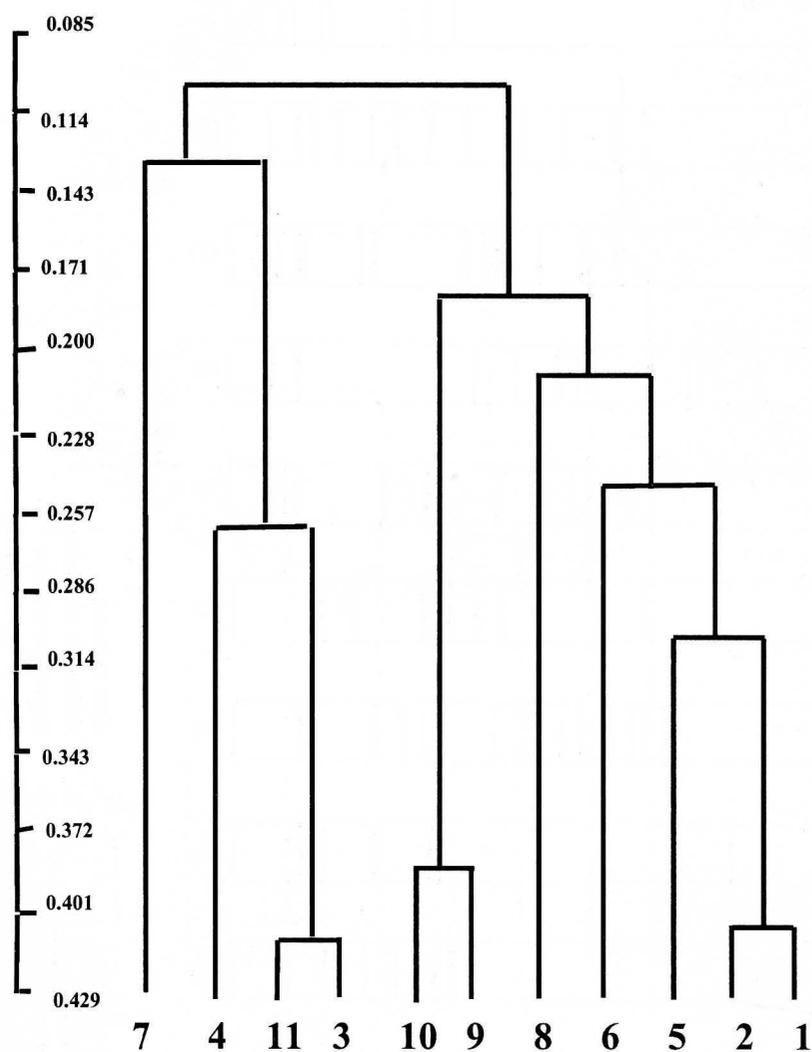
1-   *Brassica tournefortii*   7-   *Zilla spinosa*
2-   *Sinapis arvensis*   8-   *Schouwia purpurea*
3-   *Diplotaxis harra*   9-   *Lepidium sativum*
4-   *D. acris*   10-   *Capsella pursa-pastoris*
5-   *Raphanus raphanistrum*   11-   *Sisymbrium irio*
6-   *Enarthrocarpus strangulatus*

Fig. 2. Dendrogram shows the relationships between the studied taxa based on protein characters.

1-	*Brassica tournefortii*	7-	*Zilla spinosa*
2-	*Sinapis arvensis*	8-	*Schouwia purpurea*
3-	*Diplotaxis harra*	9-	*Lepidium sativum*
4-	*D. acris*	10-	*Capsella pursa-pastoris*
5-	*Raphanus raphanistrum*	11-	*Sisymbrium irio*
6-	*Enarthrocarpus strangulatus*		

Fig. 3. Dendrogram shows the relationships between the studied tax based on the morphological characters.

Fig. 4. Dendrogram shows the relationships between the studied taxa based on the protein and the morphological characters.

The second aggregate is a distinct group comprising two species: *Lepidium sativum* and *Capsella bursa-pastoris* in two different subtribes but both belong to one tribe *Lepidieae. Lepidium* with its orbicular or ellipsoid, dehiscent fruit with one seed in each locule and sessile or petiolated upper cauline leaves and terminal inflorescence was recognized in subtribe *Lipidiniae. Capsella* with its small and white flowers, branched hairs and obcordated or obtriangular, dehiscent silicula, and more than one seed in each locule was placed in the subtribe *Capselliniae* (Schulz 1936). Interestingly the present results, revealed that both taxa are grouped in one aggregate in the two different dendrograms based on seed protein data (Fig. 2) and on morphology (Fig. 3) but at different levels of similarity: 39% and 54% respectively (Tables 3, 5). This proves that *Lepidium* and *Capsella* are closely allied according to their protein and morphological characters.

The last aggregate , *Diplotaxis harra/ Sisymbrium irio/ Diplotaxis acris* may seem to be an unnatural cluster because each of them, based on morphological evidence, belong to different tribes: *Sisymbrium irio* to *Sisymbrieae* and *Diplotaxis* to *Brassiceae. Sisymbrieae* was considered by Hedge (1976), Al Shehbaz (1984) and Heywood (1976) as an unnatural tribe. In *Diplotaxis* there is a beak (in some species) the seeds are in two parallel rows in each locule, and the cotyledons are longitudinally folded around the incumbent radicle; in *Sisymbrium* there is a beakless fruit, usually in uniseriate arrangement of seeds in each locule of the fruit and cotyledons are not folded. Both genera have yellow petals, a siliqua with readily dehiscent valves and glabrous or with simple hairs (Table 4). Morphological similarities between *Sisymbrium* and *Diplotaxis* was noted by the early taxonomists. In this respect it is interesting to note that de Jussieu (in De Candolle 1821) treated *Diplotaxis harra* as *Sisymbrium aegyptium* while Vahl (1791) considered *Diplotaxis harra* as *Sisymbrium hispidum*. The protein patterns found in this investigation could support the idea that *Diplotaxis* and *Sisymbrium* are clearly allied.

It can be generally concluded that in *Brassicaceae*, seed protein characters could not be separately used as a taxonomic evidence but it is reliable to be combined with other evidences as the morphological ones (Fig. 4 and Table 6).

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