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Implications of seed proteins in *Brassicaceae* systematics

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Abstract

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 unweighed 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*.

Additional key words: cluster analysis, dendrograms, *Sisymbrieae*, *Zilla spinosa*.

Introduction

The family *Brassicaceae (Cruciferae)* is described as a natural family in account of its a remarkable uniformity in the fundamental structure of flowers, fruits, and seeds. Also certain anatomical and chemical characters are considered (Schulz 1936, Turrill 1939, Janchen 1942, Hedge 1976, Bowman and Smyth 1998, Goffman *et al.* 1999). However, it is generally recognized that it is difficult to make a satisfactory classification within the family (Hedge 1976, Heywood 1976, Al Shehbaz 1984). In the last three decades, the employment of certain chemical constituents have been used in taxonomic and evolutionary studies of *Brassicaceae*. These are fatty acids (Appelqvist 1976), sterols (Knights and Berrie 1971), glucosinolates (Kjaer 1976, Heaney and Fenwick 1980, Gland *et al.* 1981, Rodman *et al.* 1981, Horn and Vaughan 1983, Mithen *et al.* 1987, Waterman and Gray 1987, Lockwood and Belkhiri 1991), seed storage proteins (Vaughan and White 1967, Vaughan and Denford 1968, Vaughan *et al.* 1966, Finlayson 1976). The high stability of seed proteins makes them a powerful tool in elucidation the origin, evolution and relationship of the taxa (Davis and Heywood 1963, Ladizinsky and Hymowitz 1979, Haider and El-Shanshoury 2000, Vladova *et al.* 2000). In order to apply a more objective approach in classification of *Brassicaceae* in Egypt, seed proteins of eleven wild species have been studied using the polyacrylamide gel electrophoresis technique in addition to the morphological characters. The obtained data were analyzed by numerical analysis (cluster analysis) based on Jaccard's coefficient (Sneath and Sokal 1973).

Materials and methods

The morphological studies were based mainly on herbarium specimens deposited in CAI, Cairo University Herbarium and CAIM, Agricultural Research Centre Herbarium (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 cm^3 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 cm^3 aqueous solution of polyvinylpyrrolidone. The extract was centrifuged at 4 000 *g* for 20 min and the supernatant was

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decanted. Proteins were precipitated with saturated ammonium sulfate. The pellet was dissolved in 1 cm^3 of the extraction buffer and 0.03 cm^3 aliquots used in PAGE. Gels were stained by 0.1 % Coomassie Brilliant Blue R-250 and destained in 300 cm^3 of destaining 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. In total, 50 different bands were identified.

The data for numerical analysis consisted of the 46 morphological characters (Table 2) and 50 protein characters, scored for each of the 11 Operational Taxonomic Units (OTU's). Each character was classified as presence (1) and absence (0). The Jaccard's coefficient

Table 1. Localities of the studied taxa.

Taxon	Locality
1 - *Brassica tournefortii*	Cairo - Alexandria Desert Road, 16.4. 1986.
2 - *Sinapis arvensis*	Assiut University campus, Assiut, 23.3. 1997.
3 - *Diplotaxis harra*	Wadi Al Assiuty, Eastern Desert, 27.3.1997.
4 - *Diplotaxis acris*	Wadi Al Assiuty, Eastern Desert, 27.3.1997.
5 - *Raphanus raphanistrum*	Banha, 15.1.1985.
6 - *Enarthrocarpus strangulatus*	Burg El Arab, Mariut, 17.4.1986.
7 - *Zilla spinosa*	Wadi Al Assiuty, Eastern Desert,16.4.1986.
8 - *Schouwia purpurea*	Siwa Oasis, Western Desert, 16.4.1986.
9 - *Lepidium sativum*	Siwa Oasis, Western Desert, 16.4.1986.
10 - *Capsella bursa-pastoris*	Banha, 15.1.1985.
11 - *Sisymbrium irio*	Assiut, cultivated land, Assiut, 12.3.1998.

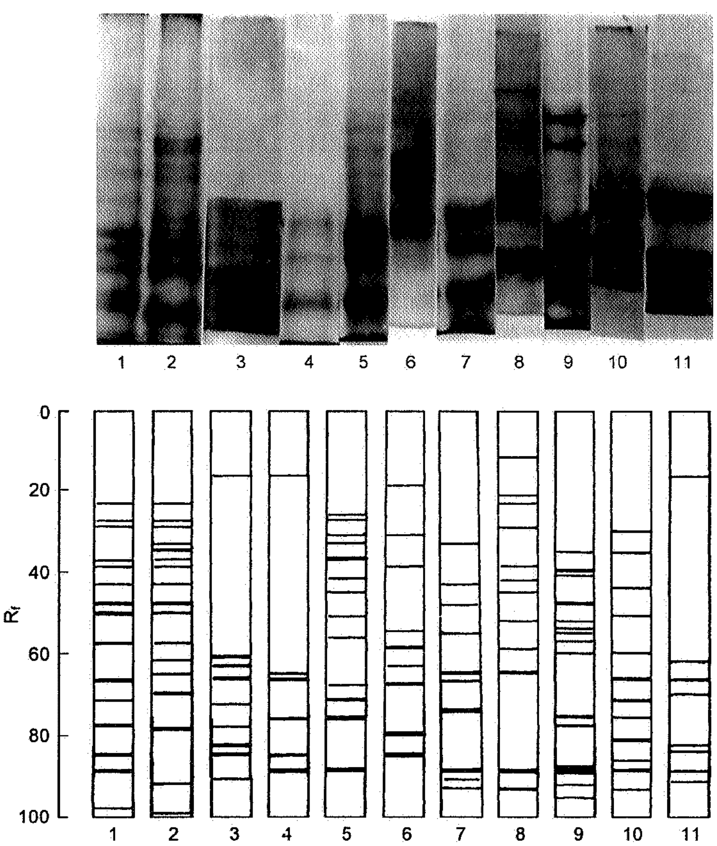

Fig. 1. Protein banding patterns in the studied taxa. The spesies are numbered as in Table 1.

Table 2. Morphological characters of the studied taxa used in the numerical study.

Habitus 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
6 - 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 pinnatisect	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 length 5 mm or longer	sepal length less than 5 mm long
15 - petals yellow	petals not yellow
16 - petals white	petals not white
17 - petals violet or red	petals neither violet nor red
18 - petal length 5 mm or longer	petal length less than 5 mm long
19 - dark veins present	dark veins absent
20 - filament length 5 mm or longer	filament length less than 5 mm long
21 - anther linear	anther not linear
22 - anther sagittate at the base	anther 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 - septum 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 not domate
39 - periclinal cells wall with central portion	periclinal cell walls without 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

$Sj = a / (a + b + c)$, 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) was used as a measure of similarity of pattern (Sneath and Sokal 1973). The matrix of Jaccard's coefficients was used in a pair-wise cluster analysis using the unweighted pair group method (UPGMA) using arithmetic average 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 electrophoregrams. Other bands are shared by more than one species. Characteristic (marker) bands of species are those of R_f values 12, 21, 53 for *Shouwia purpurea*; 17 for *Sisymbrium irio*; 18, 64, 80 for *Zilla spinosa;* 26, 56 for *Raphanus raphanistrum*; 30, 66 for *Capsella bursa-pastoris*; 54, 95 for *Lepidium sativum*; 61, 73 for *Diplotaxis harra*; 62, 98 for *Sinapis arvensis*; 97 for *Brassica tournefortii*, and 74 for *Enarthrocarpus strangulatus.*

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) put *Zilla spinosa* by using the morphological criteria in a very distinct taxonomic position as the monotypic subtribe *Zilliinae.* This subtribe is clearly different from all other genera in our area and is very unlikely to be confused with them.

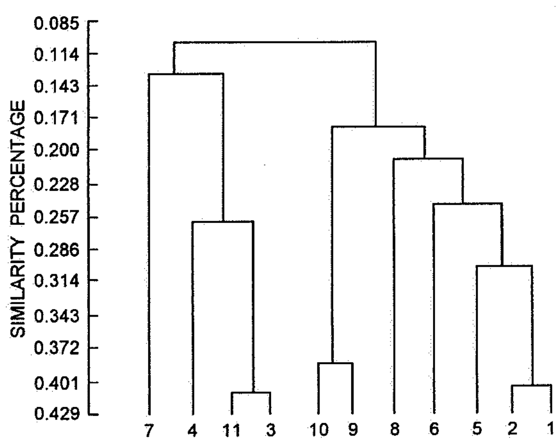

Fig. 2. Dendrogram shows the relationships between the taxa studied based on protein characters. The spesies are numbered as in Table 1.

The first cluster 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 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 and 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 they are to any of studied taxa (Fig. 2). This result agrees with those of Vaughan and Denford (1968). On the other hand, the dendrogram based on the morphological characters (Fig. 3) shows that *Brassica* could be affiliated to the aggregate of *Diplotaxis acris*, *Raphanus raphanistrum*, and *Enarthrocarpus strangulatus* whereas *Sinapis arvensis* is more related to *Diplotaxis harra.* 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* and *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.

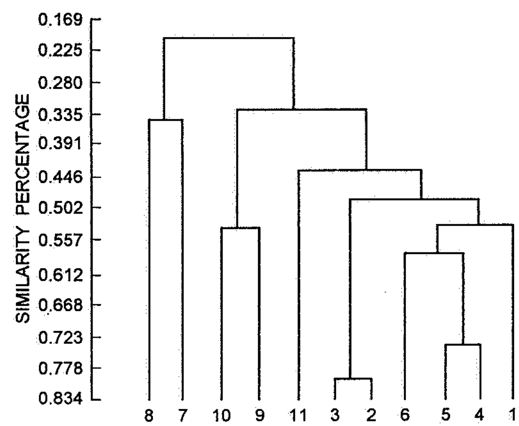

Fig. 3. Dendrogram shows the relationships between the taxa studied based on morphological characters. The spesies are numbered as in Table 1.

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 silicle with long and conical beak and violet or pink petals and bractless inflorescence. The position of *Schouwia* in the dendrograms based on protein data (Fig. 2) and on 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 characters (Fayed and El Naggar 1988; El Naggar and Soliman 1999).

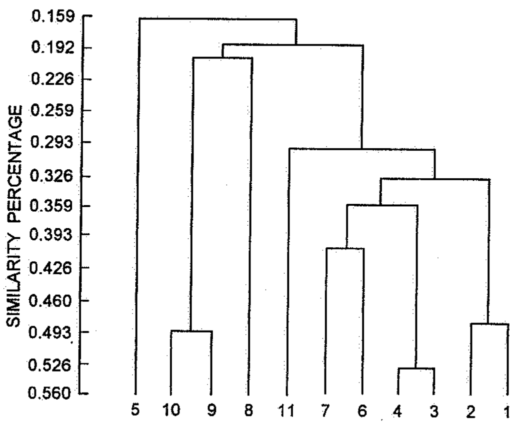

Fig. 4. Dendrogram shows the relationships between the taxa studied based on protein and morphological characters. The species are numbered as in Table 1.

The second aggregate is a distinct group comprising two species: *Lepidium sativum* and *Capsella bursa-pastoris* from 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) even if at different levels of similarity: 39 % and 54 %, respectively (Tables 3, 4). This proves that *Lepidium* and *Capsella* are closely allied according to their protein and morphological characters.

The last aggregate *Diplotaxis harra*, *Sisymbrium irio*, and *Diplotaxis acris* may seem to be an unnatural cluster because each of them based on morphological evidence belong to different tribe: *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 (Table 2). Morphological similarities between *Sisymbrium* and *Diplotaxis* were 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 allied.

It can be generally concluded that in *Brassicaceae* seed proteins characters should not be used as a taxonomic evidence separately but they may be reliable combined with other characters (Fig. 4, Table 5).

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

	1	2	3	4	5	6	7	8	9	10	11
1	1.00										
2	0.45	1.00									
3	0.15	0.08	1.00								
4	0.17	0.09	0.25	1.00							
5	0.35	0.29	0.04	0.18	1.00						
6	0.14	0.12	0.11	0.14	0.15	1.00					
7	0.20	0.27	0.11	0.23	0.27	0.11	1.00				
8	0.17	0.29	0.00	0.05	0.08	0.15	0.15	1.00			
9	0.16	0.23	0.04	0.11	0.17	0.14	0.20	0.17	1.00		
10	0.17	0.14	0.00	0.11	0.18	0.00	0.15	0.13	0.42	1.00	
11	0.15	0.08	0.41	0.27	0.10	0.12	0.20	0.00	0.04	0.05	1.00

Table 4. Matrix of similarity between all pairs of studied taxa based on morphological characters.

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

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

	1	2	3	4	5	6	7	8	9	10	11
1	1.00										
2	0.50	1.00									
3	0.35	0.39	1.00								
4	0.37	0.34	0.56	1.00							
5	0.34	0.20	0.10	0.20	1.00						
6	0.34	0.29	0.35	0.48	0.21	1.00					
7	0.39	0.33	0.30	0.38	0.25	0.42	1.00				
8	0.25	0.33	0.16	0.24	0.22	0.24	0.16	1.00			
9	0.22	0.26	0.19	0.20	0.14	0.16	0.19	0.21	1.00		
10	0.18	0.22	0.11	0.16	0.13	0.07	0.13	0.22	0.48	1.00	
11	0.30	0.29	0.42	0.33	0.10	0.23	0.31	0.14	0.23	0.24	1.00

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