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Electrophoretic Characterization and the Relationship Between Some Egyptian Cruciferae

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Abstract: The present study was carried out on 17 Egyptian taxa of the *Cruciferae* representing 10 genera and 13 species collected from different localities. Polyacrylamide gel electrophoresis (SDS-PAGE) was employed to characterize those taxa. The data obtained were analyzed by the NTsys-pc program package using the UPGMA clustering method. The produced dendrogram from SDS-PAGE analysis showed a close affinity and the monophyllay among the species of the genera; *Enarthrocarpus* Labill., *Farsetia* Turra, *Zilla* Forssk. and the paraphyllaly within the species of the genus *Brassica* L.

Key words: *Cruciferae* (*Brassicaceae*), electrophoresis, seed protein analysis, numerical analysis

Introduction

The *Cruciferae* = *Brassicaceae* are the largest family of the *Brassicales*. It is a natural family of major economic importance. Heywood (1993), recorded about 380 genera and 3000 species in this family, whereas, Mabberley (1997) reported the number of genera to be 365 and the number of species to be 3250. On the other hand, Judd *et al.* (1999) recorded 419 genera and 4130 species belonging to this family.

The *Cruciferae* is classified into 13 tribes, *Thelypodieae*, *Pringleae*, *Sisymbrieae*, *Hesperideae*, *Arabideae*, *Alysseae*, *Lepidieae*, *Brassiceae*, *Chamireae*, *Schizopetaleae*, *Stenopetaleae*, *Helphileae* and *Cremolobeae*. Only two of the tribes, the *Brassiceae* and *Lepidieae*, can be regarded as natural, apart from the monotypic ones (*Pringleae* and *Chamireae*) which are confined to South Africa (Heywood, 1993).

The family is considered to be monophyletic on the basis of the elongate gynophore and elongate exserted stamens. Additional synapomorphies include the type of glucosinolates, the structure of the endoplasmic reticulum and *rbcL* sequences (Judd *et al.* 1999).

Cladistic analysis based on sequence *rbcL* data indicates that many genera of the *Brassicoideae* such as *Cleome*, *Brassica*, *Draba* and *Arabidopsis* are nonmonphyltic (Judd *et al.* 1999).

In the flora of Egypt *Cruciferae* is well represented. Täckholm (1974) reported 61 genera and 106 species distributed in the different habitat types of the country. On the other hand, El-Hadidi and Fayed (1995) recorded 55 genera and 108 species for the family.

Most members of ***Cruciferae*** are food or ornamental plants, e.g. ***Raphanus sativus*** L., ***Brassica rapa***, ***Brassica oleracea*** (L.) var. ***capitata***. Very few e.g. mustard and ***Brassica nigra*** L. are of medicinal value. Mustard plants or their oils are included in ointments of rheumatic pains. It is used as emetic in cases of poisoning. It may be used as stimulant for the heart (Naim ***et al.***, 1984).

Seed protein is highly stable, being unaffected by environmental conditions (Harbone and Turner, 1984). Thus electrophoretic banding patterns of total seed protein as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) have provided a valid source of taxonomic evidences and were used to address taxonomic relationships at the generic and specific levels, for example ***Trifolium*** (Badr, 1995), ***Sesbania*** (Badr ***et al.***, 1998), the genus ***Vicia*** (Kamel and Al-Mashad, 1999) the genus ***Lathyrus*** (Badr ***et al.***, 2000), ***Lens esculenta*** (Hassan, 2001), ***Hordeum vulgare*** (El-Rabey ***et al.***, 2002) and ***Psidium*** (Hassan, 2002).

Sanches-Yelamo ***et al.*** (1992) carried out a comparative electrophoretic study of seed proteins in 29 wild taxa of the genera ***Diplotaxais***, ***Erucastrum*** and ***Brassica*** (***Cruciferae***). Their results supported the close affinity among the species of each genus and are largely consistent with evolutionary patterns that had been inferred previously through the study of seed, seedling and fruits morphology.

In the present study, seed protein analysis (SDS-PAGE) was carried out on 17 taxa of the ***Cruciferae*** collected from their natural habitats from the flora of Egypt from different localities. The data obtained were analyzed by the NTsys-pc program package using the UPGMA clustering method to characterize the taxa studied and find out the relationship between them.

Materials and Methods

Materials of the 17 taxa were collected from various habitats in Egypt. Voucher specimens are deposited at the herbarium of Biological Sciences and Geology Department, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt. The studied species, the localities from which they were collected are given in Table 1.

Seed protein electrophoresis

Seed proteins of the studied taxa were extracted using Tris-HCl (pH 8.8). SDS-polyacrylamide gel electrophoresis was performed in 10% acrylamide slab gels following the system of Laemmli (1970). Protein extraction was conducted by mixing 0.02 g of seeds with an equal weight of pure, clean, sterile fine sand. The seeds were then ground to fine powder using a mortar and pestle and homogenized with 1 M Tris-HCl buffer (pH 8.8) in clean eppendorf tube and left in refrigerator over night, then centrifuged at 10.000 rpm for 10 min. The supernatant of each sample was kept in deep-freeze until use for electrophoretic analysis.

A volume of 30 µl protein extract was added to equal volume of treatment buffer and boiled for 10 min. in water bath before loading in the gel. About 30 µl of this mixture was loaded on the gels. Electrophoresis was carried out at 100 V for 5 h using a low molecular weight protein mixture of Sigma as a marker. Gels were stained in Comassie brilliant blue R-250, destained and then photographed. The number of bands revealed in each lane of the gel were counted and analyzed using Doc 2000 Bio-Rad system.

Numerical analysis

The total number of the recorded attributes (47) obtained from seed protein analysis in each taxon were scored and coded for creating the data matrix of computation. The presence or absence of each 47 different characters was treated as a binary character in a data matrix i.e. coded 1 and 0 respectively.

The relationships between the studied taxa, expressed by average taxonomic distance (dissimilarity), have been demonstrated as phenogram, based on the analysis of the recorded characters using the NTsys program package for IBM-pc as described by Rohlf (1993).

Results and Discussion

SDS-PAGE electropherogram of the storage seed proteins

Seed protein analysis was carried out on 17 taxa of 10 genera collected from the flora of Egypt from different localities (Table 1). The seed protein banding profiles of the 17 taxa are illustrated in Fig. 1 and 2. A total number of 47 protein bands were observed within the species studied. ***Brassica tournefortii*** collected from Al-Nobaraia (16) was found to have the highest number of bands (17), while the lowest number of bands (4) was observed in the sample of the same species collected from Rasheed (15). The highest molecular weight protein band (136.00 KDa) among the studied samples was recorded in ***Lobularia arabica*** (6), while the lowest one (10.85 KDa) was detected in the two species of the genus ***Enarthrocarpus*** (3 and 4) and in ***Sisymbrium irio*** (8).

Table 1: Source and origin of the studied taxa

No.	Taxa	Source
1	***Cakile maritima*** Scop.	Cairo-Alex. Des. R.
2	***Coronopus niloticus*** (Del.) Spreng.	Roxy, Cairo
3	***Enarthrocarpus lyratus*** (Forssk.) DC.	Bremly cave, Alex.
4	***Enarthrocarpus strangulatus*** Boiss.	Burg El-Arab
5	***Erucaria hispanica*** (L.) Druce.	Burg El-Arab
6	***Lobularia arabica*** (Boiss.) Muschl.	Rasheed, Alex.
7	***Matthiola longipetala*** (Vent.) DC.	Burg El-Arab
8	***Sisymbrium irio*** L.	Roxy, Cairo
9	***Farsetia oblongata*** Presl.	Cairo-Alex. Des. R.
10	***Farsetia aegyptia*** Turra.	Cairo-Suez Des. R.
11	***Zilla spinosa*** (Turra) Prantl.	Cairo-Alex. Des. R.
12	***Zilla spinosa*** (Turra) Prantl.	Cairo-Suez Des. R.
13	***Zilla biparmata*** O. E. Schulz.	Cairo-Suez Des. R.
14	***Brassica tournefortii*** Gouan.	Burg El-Arab
15	***Brassica tournefortii*** Gouan.	Rasheed, Alex.
16	***Brassica tournefortii*** Gouan.	Al-Nobaria
17	***Brassica tournefortii*** Gouan.	Roxy, Cairo

Table 2: The molecular weights of protein bands extracted in Tris-HCl buffer in the studied taxa

Band No.	Mol. Wt. (Kda)	Taxa 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	136.00	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
2	134.21	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	+	+
3	128.00	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
4	74.28	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	73.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
6	70.00	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
7	66.00	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+
8	62.16	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
9	59.40	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
10	52.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
11	47.65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
12	45.50	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+
13	45.00	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	44.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
15	38.44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
16	36.00	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
17	32.42	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
18	30.23	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
19	29.14	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+
20	28.43	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
21	28.00	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	27.89	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
23	27.50	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	27.13	+	-	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+
25	26.06	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
26	25.90	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
27	25.25	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+
28	24.50	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-
29	24.00	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-
30	23.63	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+
31	22.70	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+
32	22.02	+	-	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-
33	21.25	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+
34	20.60	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
35	19.20	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
36	18.89	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
37	17.67	-	-	-	-	-	-	+	-	+	+	-	-	-	+	+	+	+
38	17.30	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
39	16.95	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
40	16.60	-	-	-	-	-	-	+	-	-	+	+	+	+	-	-	+	+
41	16.31	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-
42	15.90	-		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
43	15.45	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-

Table 2: Continued

Band No.	Mol. Wt. (Kda)	Taxa 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
44	14.24	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
45	13.49	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
46	12.55	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-
47	10.85	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-
Total number of bands		12	8	8	7	5	5	7	14	8	7	6	11	6	5	4	17	16

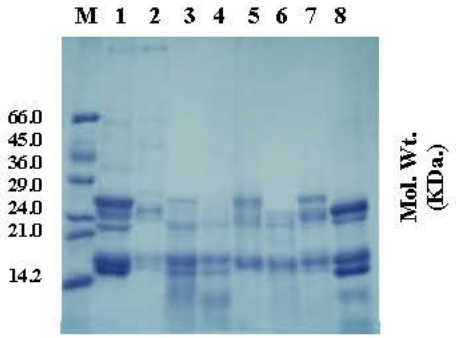

Fig. 1: Electrophoretic banding profiles of seed proteins extracted in Tris-HCl buffer of the studied taxa (1-8)

Fig. 2: Electrophoretic banding profiles of seed proteins extracted in Tris-HCl buffer of the studied taxa (9-17)

The electropherograms produced from seed protein analysis using Tris-HCl buffer revealed great polymorphism among these taxa as illustrated in Table 2. Twelve bands were detected in *Cakile* Miller, which was characterized by the presence of two unique bands at the molecular weights of 74.28 and 28.00 KDa. Also, two unique bands (45.00 and 27.50 KDa) were distinguished *Coronopus* Zinn. from the other species. Eight and seven bands were recognized in the two samples of the *Enarthrocarpus* (3 and 4). The band recorded at the molecular weight of 25.72 Kda

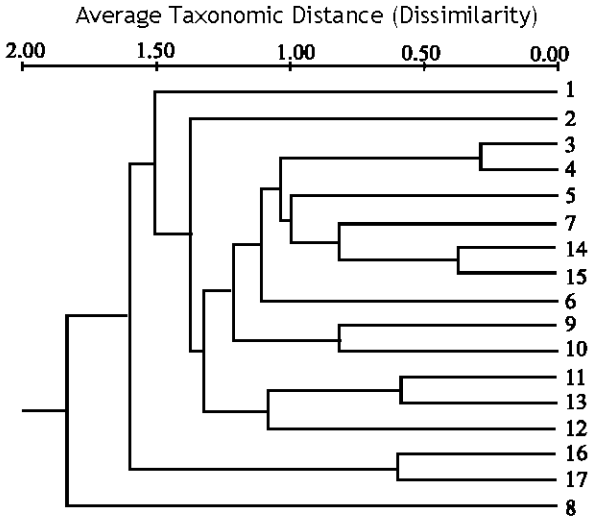

Fig. 3: UPGMA- phenogram based on coding of 47 attributes obtained from SDS-PAGE profiles of seed proteins extracted in Tris-HCl buffer.

could be considered as species specific bands due to its presence in the two species of the genus ***Enarthrocarpus*** (3 and 4). Five bands were recorded in the two species ***Erucaria*** and ***Lobularia*** (5 and 6), also the band recorded at the molecular weight of 15.90 KDa distinguished these later species from the other taxa. Each of these species was characterized by the presence of one unique band at the molecular weights of 26.06 and 136.00 KDa (respectively).

Four unique bands among 14 bands were recorded in ***Sisymbrium irio*** L. (9) at molecular weights of 70.00, 59.40, 20.60 and 19.20 KDa. These unique bands discriminated this species from the other species studied. Within the two species of ***Farsetia*** (9and10), eight and seven bands were recorded respectively. Among them the two bands at 30.23 and 16.95 KDa could be considered as genus specific bands due to their presence in the two species only, whereas ***Farsetia aegyptia*** Turra. (10) was distinguish from the other species by the presence of a unique band at the molecular weight of 28.43 Kda.

Within the three samples belonging to the two species studied of the genus ***Zilla*** Forssk., six and eleven bands were recorded in the protein profiles (Fig. 2). Two unique bands were recorded at the molecular weights of 18.89 and 14.24 KDa. These two bands discriminated the sample of *Z. **spinosa*** collected from Cairo-Sues Road (12) from the other studied samples. Moreover, the band recorded at the molecular weight of 24.50 KDa distinguished the two species of *Z. **spinosa*** (11and12) from the third species *Z. **biparmata*** (13). Obviously, from the studied protein profiles of the wild taxa that the four bands recorded at the molecular weights of 128.00, 24.00, 17.30 and 13.49 KDa could be considered as genus specific bands due to their presence in the three samples belonging to genus ***Zilla*** Forssk. (Table 2).

Four samples belonging to ***Brassica tournefortii*** Gouan. were collected from different localities as shown in Table 1, these samples (14, 15, 16 and16) showed great variations in the

number of bands recorded in their protein profile patterns (Fig. 2). Five and four bands were recorded in the samples 14 and 15, while within the samples 16 and 17 the highest number of bands (17 and 16) were detected. The later two samples of ***Brassica*** Gouan. (16 and 17) could be distinguished from the former ones (14 and 15) by the presence of four bands recorded at the molecular weights of 73.48, 52.05, 47.65 and 44.00 KDa Only one unique band was detected at 38.44 KDa in the sample number (16), this band discriminated this sample from all other studied species.

It is also obvious that the band recorded at the molecular weight of 25.25 KDa could be considered as species specific band due to its presence in all studied samples belonging to the genus ***Brassica tournefortii*** Gouan.

Numerical analysis

The phenogram produced by the analysis of 17 taxa studied based on coding of 47 attributes obtained from Tris-HCl extracted proteins are shown in Fig. 3. This phenogram shows that the examined taxa have a total taxonomic distance of about 1.88. At this level, ***Sisymbrium irio*** L. (8) was split off from the other taxa, then at 1.63 level the two samples of the genus ***Brassica*** L. (16 and 17) were split off from the remaining taxa and are distinguished from each other at the level of 0.58. At the levels 1.50 and 1.38, ***Cakile maritima*** Scop. (1) and ***Coronopus niloticus*** (Del.) Spreng. (2) were also split off from the remaining taxa (respectively).

At the level 1.30, the three species of the genus ***Zilla*** Forssk. were separated together in a small group from the other taxa, within this group, ***Zilla spinosa*** (Turra) Prantl. (12) collected from Cairo-Suez desert road was split off from the other two species (11 and 13), then the latter species were distinguished from each other at the 0.56 level.

At the level 1.22, the two species of the genus ***Farsetia*** Turra (9 and 10) were separated together from the other taxa and then distinguished from each other at the 0.89. At the level 1.18, ***Lobularia arabica*** (Boiss.) Muschl. (6) was split off from the remaining taxa. Then at the level 1.10, the two species of the genus ***Enarthrocarpus*** Labill. (3 and 4) were separated together and distinguished from each other at the 0.25 level.

At the levels 1.00 and 0.89, ***Erucaria hispanica*** (L.) Druce. (5) and ***Matthiola longipetala*** (Vent.) DC. (7) were split off from the other species respectively. Then at 0.89 level, the other two species of the genus ***Brassica*** L. (14 and 15) were separated together and distinguished from each other at the 0.38 level.

The electropherograms produced from seed protein analysis of the 17 Egyptian wild taxa belonging to 10 genera collected from different localities revealed great polymorphism among these different genera. 13 unique bands were detected, while no monomorphic band was recorded. One genus specific band was recorded in the species of the genus ***Zilla*** L. and two species specific bands were detected within the species of ***Brassica*** L. and ***Enarthrocarpus*** Labill. Although, there was resemblance among the species of each genus, there were characteristic variations among the different genera.

The present investigation is in agreement with the study of Sanches-Yelamo ***et al.*** (1992). This study supports the close affinity among the species of each genus.

Seed protein banding patterns as revealed by SDS-PAGE produces reproducible band pattern (profile) when proteins are prepared in a standard method and hence have valid value in taxonomic purposes. Consequently, proteins with identical electrophoretic mobility are deemed to represent the same unit character. Therefore characters derived from seed proteins have been utilized in plant taxonomy at different levels to construct phenetic classifications (Boulter, 1981; Smith, 1984 and Echeverrigaray ***et al.***, 1998). Hence they can be considered as traits to study genetic variation among the plant taxa. However, for the study of the taxonomic relationships among species and higher taxonomic ranks more valid assessment should necessarily be obtained when these data are compiled with other lines of evidence from morphology and cytology.

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