

Using electrophoretic techniques in variation between 35 taxa of *Brassicaceae* Burnett

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Abstract

Seed protein diversity as revealed by variation in SDS-PAGE has been used to reassess the taxonomic relationships between 35 species related to 24 genera of the Brassicaceae. A total of number of 82 protein bands with molecular weight ranging between 0.064 and 0.94 were recorded in the electropherograms. These bands were used as binary characters and analyzed by the STATISCA program package using the UPGMA clustering method. The produced dendrogram from SDS-PAGE analysis showed separation of the two species of genus *Raphanus* at taxonomic level of 62% whereas the remainder are separated into two groups. Also, the dendrogram showed a close affinity between genus *Lepidium* and genus *Capsella*. The phenogram shows that eight species of genus *Brassica* from different geographical locations are not grouped together in one cluster.

Keywords: Brassicaceae (Cruciferae); Seed protein electrophoresis; Numerical analysis

1. Introduction

The *Brassicaceae* Burnett (= *Cruciferae* de Jussieu) is a large natural family of the *Brassicales*. The family is considered to be monophyletic on the basis of the elongate gynophore and elongate, exerted stamens (Rodman, 1991 and Judd *et al.*, 1994) [17, 12]. The family is considered to be monophytic on the basis of the elongate gynotype and elongate exerted stamens Judd *et al.*, 1999) [12]. Electrophoretic separation of seed proteins is a useful technique in plant taxonomy. Polyacrylamide gel electrophoresis for protein and isozyme was used for identification of species, subspecies and variety level (Adrianse *et al.*, 1969, Boulter *et al.*, 1970, Evans and Abernethy, 1983) [1, 6, 9]. Comparing the major storage seed protein in different taxa have provided valid source of evidence for addressing taxonomic relationships at the different taxonomic levels (Nath *et al.*, 1997; Kamel & El-Mashad, 1999; Badr *et al.*, 2000; Hassan, 2001; El-Rabey *et al.*, 2002; and Kamel, 2005) [16, 14, 4, 8, 13]. In Egypt, Tächholm

(1974) reported 61 genera and 106 species and El-Hadidi & Fayed (1995) [7] reported 55 genera and 108 species. Kamel *et al.* (2003) studied characterization and the relationship between 17 Egyptian cruciferous taxa. Also, Amaal Hasan (2009) [3] studied the molecular systematic of 9 tribes of Brassicaceae in Egypt based on different electrophoretic techniques. Aim of the present work is to differentiate between different species gathered from different locations belonging to 24 genera by using seed protein profiles.

2. Materials and Methods

The seeds of 35 taxa representing 24 genera of Brassicaceae were obtained from the Royal Botanic Gardens at Kew, London, UK,), The Botanic Garden Berlin-Dahlem, Germany, and from different localities in Egypt. Studied local taxa were identified according to Tächholm (1974) and Boulos (1999). The studied taxa are given in Table 1.

Table 1: Localities of the studied taxa of the family

No.	Taxa	Serial number	origin	Collection Date
1	<i>Brassica oleracea</i> S.I.L	0070498	England	1988
2	<i>B. oler.</i> var. <i>capitata</i> L.	---	Egypt	2009
3	<i>B. oler.</i> var. <i>botrytis</i> L.	---	Egypt	2009
4	<i>B. rapa</i> L	0020747	Switzerland	1974
5	<i>B.nigra</i> (L.) koch	0070395	England	1988
6	<i>B. tournefortii</i> Gouan	0184696	Egypt	2002
7	<i>Cardamine flexuosa</i>	1071	England	1988
8	<i>Cardamine hirsuta</i>	387109	Brukina-Faso	2007
9	<i>Capsella bursa-pastoris</i> DC.	---	Egypt	2009
10	<i>Coronopus didymus</i> L	0076065	England	1989
11	<i>Descurainia sophia</i> (L.) Webb	0497413	Jordan	2008
12	<i>Diplotaxis rucooides</i> (L.)DC	0113946	Jordan	1996
13	<i>D.harra</i> (forssk) Boiss.	---	Egypt	2009
14	<i>D. tenuifolia</i> (L.)DC	064824	England	1986
15	<i>Eruca Sativa</i> Miller	---	Egypt	2010

16	<i>Erysimum cheiri</i> (L.) Crantz	0026716	France	----
17	<i>Farsetia aegyptia</i> Turra	---	Egypt	2010
18	<i>Hirschfeldia incana</i> (L.) Lagr. Fossat	0115249	Lebanon	1996
19	<i>Lepidium sativum</i> L.	0071831	Oman	1987
20	<i>Raphanus sativus</i> L.	---	Egypt	2010
21	<i>R. raphanistrum</i> L.	0071853	Oman	1989
22	<i>Moricandia sinaica</i> (Boiss.) Boiss.	---	Egypt	2010
23	<i>Matthiola longipetala</i> (Vent) DC.	---	Egypt	2010
24	<i>Sinapis alba</i> L.	200095	Jordan	2003
25	<i>S. arvensis</i> L.	0115272	Lebanon	1996
26	<i>S. allionii</i> Jacq.	---	Egypt	2010
27	<i>Sisymbrium orientale</i> L.	0158808	Jordan	2001
28	<i>S. altissimum</i> L.	0175935	Belgium	1989
29	<i>S. irio</i> L.	---	Egypt	2009
30	<i>S. officinale</i> (L.) Scop	0053257	England	1984
31	<i>Schouwia thebaica</i> Webb.	---	Egypt	2010
32	<i>Thlaspi perfoliatum</i> L	0113876	Jordan	1996
33	<i>Thlaspi caerulescens</i> Presl	1322497	Germany	2004
34	<i>Thlaspi montanum</i> L.	1760604	Germany	2004
35	<i>Thlaspi montanum</i> Vahl	123456	Jordan	2002

3. Extraction and analysis of seed protein

Characterization of seed protein fractions are carried out by using one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Preparation and running of gel were carried out according to Laemmli (1970) [15] and Stegeman *et al.* (1981). The gel was stained with coomassie brilliant blue stain R- 250. Bands were determined and scanned by using Hoefer Scanning densitometr GS 300. Protein gel bands scanned and photographed in Figure 1, 2 & 3.

4. Numerical Analysis

This study is dependent upon the application of a total of 87 comparative anatomical characters and their states as a binary character (0 &1), on each of the taxa studied. The characters and states have been subjected to numerical analysis under a program using similarity and dissimilarity assessment percentage method (Rohlf, 1989) [18]. The method applied is based on cluster analysis by using an UPGMA (unweighted pair-group method with arithmetic means) dendrogram illustrating the interspecific relationships of the studied species as percent similarity.

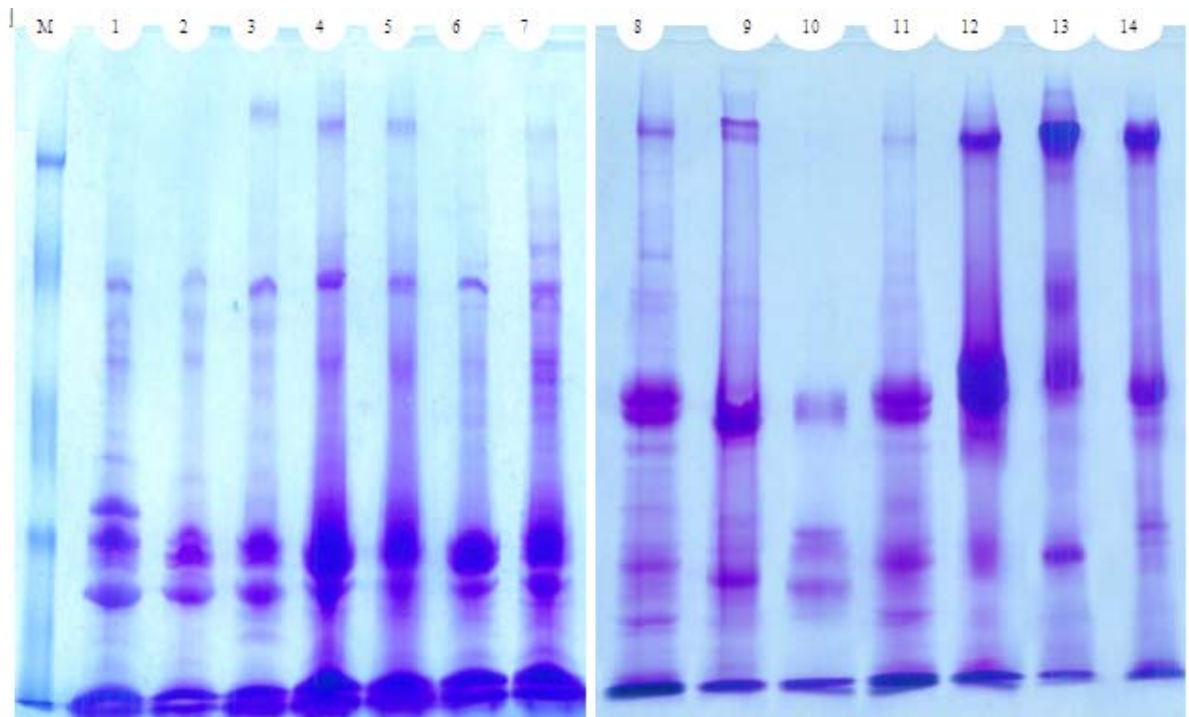


Fig 1: SDS-polyacrylamide gel electrophoresis illustrating storage seed protein of the studied species (1-14 as numbered and listed in Table 1)

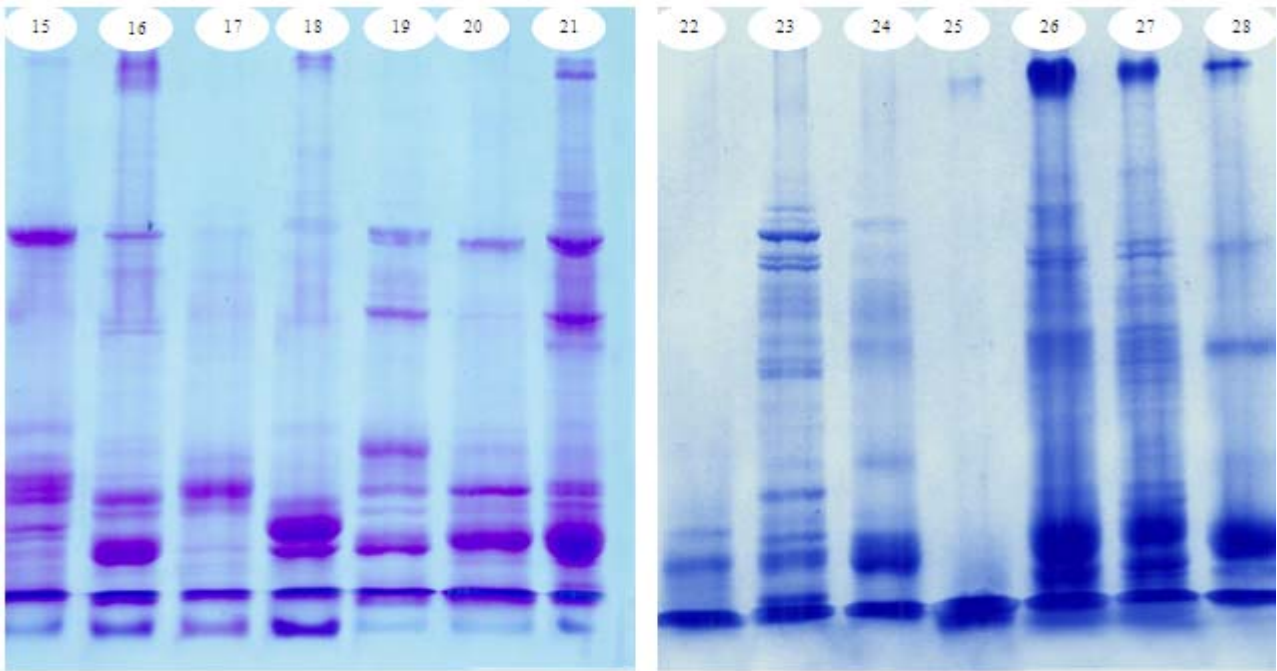


Fig. 2: SDS-polyacrylamide gel electrophoresis illustrating storage seed Protein of the studied species (15-28 as numbered and listed in Table 1)

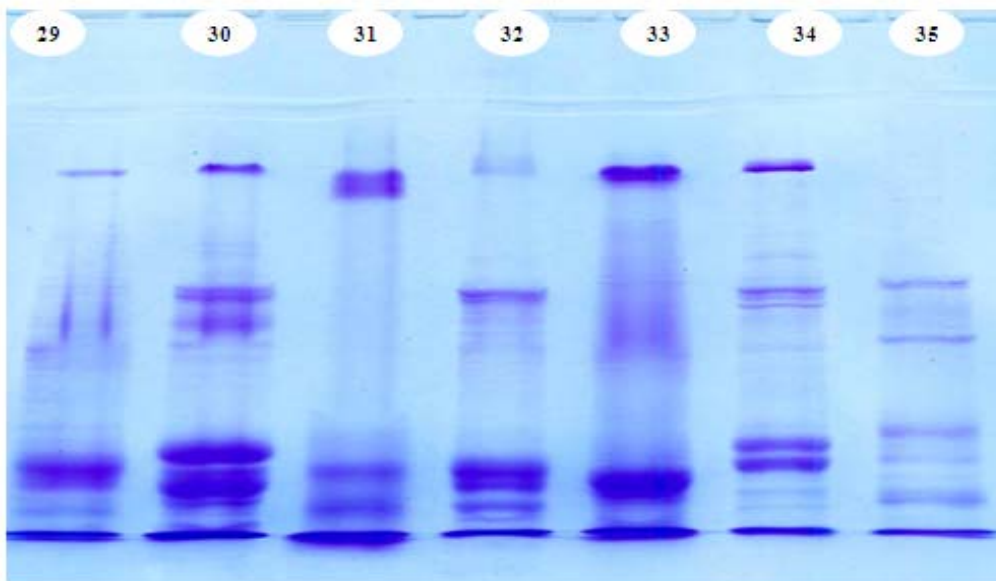


Fig 3: SDS-polyacrylamide gel electrophoresis illustrating storage seed Protein of the studied species (29-35 as numbered and listed in Table 1)

5. Results and Discussion

In the present study, SDS-PAGE seed storage proteins analysis was conducted to elucidate the taxonomic relationship between 35 taxa of the family. 82 different bands ranged between 0.06-0.97 (Table 2, charac., no 1-82). A maximum number of 26 bands, which were not necessarily present in all the studied taxa was detected in *Raphanus sativus* (Egypt), while the lowest one was recorded in samples of *Moricandia sinaica* (Egypt). On the other hand, the 11 bands have been recorded in *Lepidium campestre* (Germany), *Mathiola tricuspidata* (Greece) and *Thlaspi montanum* (Germany). 14

bands have been recorded in *Brassica fruticulosa subsp. fruticulosa* (Spanien), *Erysimum crepidifolium* (Czech Republic) and *S. arvensis* (Lebanon). 20 bands have been recorded in *Lepidium sativum* (Oman) and *Raphanus raphanistrum* (Oman). 23 bands have been recorded in *B. rapa* (Switzerland) and *B. tournefortii* (Egypt). 13 bands have been recorded in *Brassica nigra* (England), *Erysimum cheiri* (France), *Erysimum heritieri var. hierrense* (Spain) and *Sisymbrium irio* (Egypt). 9 bands have been recorded in *Brassica geniculata* (Greece), *Erysimum bicolor* (Portugal) and *Farsetia aegyptia* (Egypt).

Table 2: The relative percentages and position of protein bands of the total protein in the studied taxa (1-17 as numbered and listed in Table 1)

No.	Taxa RF	Taxa																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0.060	---	--	--	--	--	--	--	--	--	--	--	--	1.0	--	--	--	--
2	0.090	---	--	--	--	--	--	--	--	1.0	--	--	1.0	1.0	--	--	--	--
3	0.10	---	--	--	--	--	--	--	1.58	4.29	--	--	3.54	--	--	--	--	--
4	0.11	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
5	0.12	---	--	--	--	--	--	--	6.89	2.58	--	--	--	15.62	--	--	8.28	--
6	0.13	---	--	7.95	--	--	--	--	--	--	--	2.53	10.82	--	19.26	--	--	--
7	0.14	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	6.83	--
8	0.15	---	--	--	6.38	6.26	2.58	--	--	--	--	--	--	--	--	--	--	--
9	0.16	---	--	--	--	--	--	2.61	--	--	--	--	--	--	--	--	--	--
10	0.17	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
11	0.18	---	--	--	--	--	--	--	1.45	--	--	--	--	--	--	--	--	--
12	0.20	---	--	--	--	--	--	--	--	--	--	--	--	6.72	--	--	--	--
13	0.21	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
14	0.24	---	--	--	--	--	--	--	1.29	--	--	--	--	--	--	--	--	--
15	0.25	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
16	0.27	---	--	--	--	3.97	--	2.38	1.1	--	--	--	--	--	--	--	2.51	--
17	0.28	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
18	0.30	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2.49	--
19	0.31	---	--	--	2.55	--	--	--	2.81	--	--	--	--	--	--	--	--	--
20	0.32	---	--	--	--	--	--	2.95	--	--	--	2.77	--	6.46	--	--	--	--
21	0.33	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
22	0.34	2.77	--	--	--	--	2.06	--	--	--	--	--	--	--	--	--	--	--
23	0.35	---	--	--	--	--	--	--	--	--	--	1.98	--	--	5.38	--	--	--
24	0.36	---	--	--	2.67	--	--	--	--	--	--	1.39	--	--	--	--	--	--
25	0.37	6.57	7.89	--	5.62	8.52	5.73	--	1.61	--	--	--	--	13.99	--	--	--	--
26	0.38	---	--	10.33	--	--	--	4.69	--	3.47	--	--	--	--	--	--	--	--
27	0.39	---	--	--	--	--	--	--	2.81	--	--	2.16	--	--	--	--	--	7.55
28	0.40	---	--	--	3.8	--	2.43	--	--	--	--	--	--	--	--	18.69	6.29	--
29	0.41	---	--	--	--	--	--	2.49	--	--	--	--	--	--	--	--	--	--
30	0.42	---	14.32	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
31	0.43	7.32	--	6.56	--	--	3.25	1.44	--	--	--	--	--	--	--	--	2.56	--
32	0.44	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
33	0.45	---	--	--	--	--	--	1.73	2.42	--	--	--	--	--	--	--	--	--
34	0.46	5.05	--	--	--	--	--	--	--	5.11	--	2.18	--	--	5.05	4.12	6.57	8.6
35	0.47	---	--	6.23	--	--	--	2.76	--	--	--	--	--	--	--	--	--	--
36	0.48	---	8.99	--	--	--	8.17	--	--	--	--	--	--	--	--	--	--	--
37	0.49	4.36	--	--	6.54	10.85	--	2.86	--	--	--	--	20.92	--	--	--	--	--
38	0.50	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
39	0.51	---	--	--	--	--	--	--	9.9	--	--	--	--	--	--	--	--	--
40	0.52	4.9	--	--	--	--	--	1.77	--	--	--	--	--	--	--	7.03	--	8.6
41	0.53	---	--	4.12	--	--	--	--	16.84	--	--	15.86	18.15	--	18.45	--	--	--
42	0.54	--	--	--	5.05	--	--	3.81	--	8.14	--	--	--	--	--	--	3.67	--
43	0.55	--	--	--	--	--	4.74	--	10.84	--	26.35	--	--	--	--	--	3.67	--
44	0.56	--	--	--	2.82	--	--	--	--	--	--	10.27	--	--	--	--	--	--
45	0.57	--	--	--	--	9	6.06	2	--	10.3	--	--	--	--	--	--	--	--
46	0.59	3.95	--	2.81	--	--	--	--	--	--	--	--	16.12	--	--	--	--	--
47	0.60	--	--	--	--	--	--	2.03	4.87	7.44	--	--	--	--	6.7	--	--	---
48	0.61	--	--	--	--	--	7.57	--	--	--	--	3.7	--	--	--	--	--	--
49	0.62	4.43	--	--	--	--	--	1.69	--	3.66	--	--	--	--	--	--	--	--
50	0.64	--	--	5.83	--	--	--	3.2	2.72	--	--	2.74	--	--	--	--	--	--
51	0.65	--	5.95	--	--	--	--	--	--	--	--	--	--	--	2.21	--	--	--
52	0.66	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
53	0.67	--	--	--	--	--	--	4.22	--	--	--	--	--	--	--	--	--	--
54	0.68	--	--	8.18	--	--	--	--	--	--	--	--	--	--	--	--	--	--

55	0.70	10.22	--	--	--	10.13	--	--	4.13	--	--	11.53	--	--	--	--	--	--
56	0.71	--	--	--	--	--	--	--	2.4	--	--	--	--	--	--	7.82	--	--
57	0.72	--	--	--	--	--	--	--	0	5.43	--	--	--	--	4.15	--	--	--
58	0.73	--	13.54	--	--	--	--	--	2.58	--	--	--	--	--	--	--	--	--
59	0.74	16.01	--	16.11	--	--	--	--	--	--	16.5	--	--	--	--	--	--	--
60	0.75	--	--	--	36.99	29.72	16.36	13.2	--	--	--	--	--	--	6.69	--	4.14	--
61	0.76	--	9.74	7	--	--	--	--	4.8	5.8	17.9	4.93	--	--	--	4.39	--	8.14
62	0.77	7.68	--	--	--	--	14.83	--	--	--	--	--	16.8	--	4.44	--	--	--
63	0.78	--	--	--	--	--	--	4.65	9.77	--	--	--	--	10.22	--	--	--	--
64	0.79	--	5.85	--	--	--	--	--	--	--	--	14.07	--	--	--	9.87	3.41	--
65	0.80	--	--	--	6.9	9.69	--	--	--	--	--	--	--	--	3.8	--	--	--
66	0.81	--	--	17.72	--	--	9.3	--	2.02	12.78	--	3.65	--	--	2.48	9.88	--	35.73
67	0.82	6.6	17.35	--	--	--	--	8.65	--	--	29.42	--	--	--	--	1.0	1.0	--
68	0.83	--	--	--	7.64	--	--	--	6.92	--	--	4.83	--	--	--	--	--	--
69	0.84	--	--	1.77	--	5.83	5.36	--	--	6.25	--	--	--	--	--	--	1.0	1.0
70	0.85	--	--	--	--	--	4.87	4.36	--	--	--	--	--	--	--	--	--	--
71	0.86	--	--	--	--	--	--	--	--	--	9.83	--	--	--	--	--	--	--
72	0.87	--	--	5.39	--	--	--	3.22	3.68	--	--	5.36	--	--	--	8.61	--	--
73	0.88	--	--	--	--	--	--	--	--	4.46	--	2.83	6.06	4.64	4.36	--	--	--
74	0.89	3.9	--	--	--	--	--	--	4.53	--	--	--	--	--	--	5.32	--	--
75	0.90	--	--	--	4.16	6.05	--	--	--	--	--	--	--	--	--	--	--	3.97
76	0.91	--	8.38	--	--	--	5.06	3.67	--	3.47	--	--	--	--	--	6.83	23.1	--
77	0.92	6.25	--	--	--	--	--	--	2.15	--	--	2.36	--	--	--	--	--	--
78	0.93	--	--	--	--	--	--	--	--	--	--	--	3.32	--	--	5.01	--	4.83
79	0.94	--	--	--	--	--	--	--	--	4.66	--	--	4.28	2.52	--	--	3.01	4.73
80	0.95	--	--	--	--	--	--	--	--	--	--	4.86	--	--	--	--	--	--
81	0.96	--	--	--	--	--	--	19.66	--	--	--	--	--	--	6.7	--	--	--
82	0.97	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Total		14	9	13	12	10	15	23	23	16	5	18	9	13	14	13	16	9

Table 3: The relative percentages and position of protein bands of the total protein in the studied taxa (18-35 as numbered and listed in Table 1)

No	Taxa		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
	RF																				
1	0.060		--	--	--	--	--	1.0	--	--	--	--	--	--	--	--	--	--	--	--	--
2	0.090		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
3	0.10		--	--	--	--	--	2.91	--	--	--	--	--	--	--	--	--	--	--	--	--
4	0.11	3.53	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
5	0.12	2.53	--	--	--	1.88	--	--	--	11.6	7.11	8.36	--	--	--	--	--	--	--	--	--
6	0.13	--	--	--	--	4.26	--	--	--	39.4	--	--	--	--	--	--	--	--	--	--	--
7	0.14	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.94	--	3.08	--	--
8	0.15	--	--	--	--	--	--	--	--	--	--	--	--	--	3.76	--	--	--	--	--	--
9	0.16	--	--	--	--	--	--	--	--	--	--	--	2.53	--	--	3.44	--	7.31	--	--	--
10	0.17	--	--	--	--	--	2.03	--	--	--	--	--	--	--	12.45	--	9.88	--	--	--	--
11	0.18	--	--	--	--	--	--	--	--	--	1.20	--	--	--	--	--	8.74	--	--	--	--
12	0.20	--	--	--	--	--	--	--	--	--	--	--	--	--	14.24	--	--	--	--	--	--
13	0.21	--	--	--	--	--	--	--	--	1.26	1.38	--	--	--	--	--	--	--	--	--	--
14	0.24	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2.4
15	0.25	--	--	--	--	1.34	--	--	--	--	2.68	--	--	--	0.79	--	--	--	--	--	--
16	0.27	3.7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
17	0.28	--	--	--	--	--	1.51	--	--	--	1.93	1.87	--	--	--	--	2.76	--	--	--	--
18	0.30	--	--	--	--	--	--	--	--	2.5	--	--	--	--	--	--	1.47	--	--	--	--
19	0.31	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.42	--	--
20	0.32	--	--	--	--	--	--	--	--	--	--	--	--	1.53	--	--	--	--	--	--	--
21	0.33	1.7	--	--	--	3.4	--	2.21	--	--	--	--	--	--	--	--	--	--	--	--	--
22	0.34	--	2.01	--	--	--	--	--	--	2.39	1.68	--	--	2.5	--	--	--	--	--	--	--
23	0.35	--	--	--	--	--	1.95	3.09	--	--	1.84	--	--	--	--	--	--	--	--	--	--
24	0.36	--	--	--	--	2.31	--	--	1.64	--	2.96	--	--	--	--	--	1.81	--	4.51	--	--
25	0.37	--	--	--	--	--	6.95	--	--	--	--	--	--	--	--	--	1.98	--	--	--	--

26	0.38	6.86	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
27	0.39	--	6.8	--	--	--	--	--	--	--	--	1.34	3.19	--	--	--	--	2.58	--
28	0.40	--	--	--	--	--	3.36	4.52	--	1.82	2.32	8.29	--	--	--	--	3.37	--	--
29	0.41	--	3.19	11.81	9.46	--	--	--	--	--	--	--	1.38	--	--	--	2.6	2.41	--
30	0.42	--	--	--	--	--	3.72	--	--	2.71	1.77	--	--	4.21	--	--	--	--	8.5
31	0.43	--	--	--	2.51	--	--	--	--	1.06	--	--	1.99	--	--	6.7	--	7.49	--
32	0.44	3.46	--	--	--	--	--	--	--	--	--	--	--	6.18	--	--	--	--	--
33	0.45	--	--	--	--	--	3.44	5.37	--	--	2.55	--	1.87	--	--	8.02	--	4.04	--
34	0.46	--	8.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
35	0.47	--	--	--	4.04	--	4.02	--	--	--	2.66	--	--	--	--	--	--	3.58	--
36	0.48	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	8.9
37	0.49	3.64	4.88	--	--	--	5.44	8.79	--	3.89	3.12	--	--	--	--	5.04	--	--	--
38	0.50	--	--	--	--	--	--	--	--	--	--	--	4.9	11.27	--	--	--	2.55	--
39	0.51	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
40	0.52	--	12.7	--	--	--	--	--	--	--	--	--	--	--	--	3.57	--	--	--
41	0.53	5.45	--	--	1.0	1.0	--	--	--	--	--	--	--	--	--	--	--	--	--
42	0.54	--	--	--	--	--	1.0	--	--	--	1.0	--	--	--	--	--	--	--	9.8
43	0.55	--	--	--	4.8	--	--	9.19	--	5.91	4.57	--	7.78	2.24	--	--	--	1.63	--
44	0.56	--	--	--	--	--	--	--	--	--	--	--	--	--	--	6.41	--	2.01	--
45	0.57	--	--	--	3.59	--	3.16	--	--	--	--	13.86	3.74	3.17	--	--	4.58	--	--
46	0.59	--	--	--	--	--	4.66	--	--	4.75	2.63	--	4.72	--	--	5.04	--	--	--
47	0.60	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
48	0.61	--	--	--	--	--	--	--	--	--	--	--	--	1.78	--	--	--	3.24	--
49	0.62	--	--	--	0.91	--	--	--	--	2.9	--	--	--	--	--	--	--	--	--
50	0.64	--	--	--	--	--	4.14	4.74	--	--	--	--	--	--	--	--	--	--	--
51	0.65	--	--	--	0.99	--	--	--	--	--	1.73	--	--	--	--	--	--	--	--
52	0.66	--	--	--	--	--	--	--	--	--	--	--	2.94	--	--	3.42	--	--	--
53	0.67	--	--	--	--	--	--	--	--	--	2.82	--	--	--	--	--	--	--	--
54	0.68	--	--	--	--	--	--	--	--	4.0	--	--	--	--	--	--	--	--	--
55	0.70	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
56	0.71	6.56	--	--	2.75	--	--	--	--	--	--	--	--	--	--	3.42	--	--	--
57	0.72	--	--	4.03	--	--	--	--	--	--	3.48	--	4.81	--	--	--	--	--	--
58	0.73	--	--	--	--	--	3.57	--	--	5.91	--	--	--	--	--	--	--	3.64	--
59	0.74	--	--	--	--	--	--	8.98	--	--	2.67	--	--	--	--	--	--	--	--
60	0.75	--	15.6	6.56	6.04	--	--	--	--	--	--	7.39	6.84	--	--	--	--	--	1.7.
61	0.76	--	--	--	--	--	--	--	--	--	1.59	--	--	--	13.88	--	--	--	--
62	0.77	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
63	0.78	--	--	--	--	--	6.89	--	--	--	3.74	--	--	--	--	--	--	18.4	6.9
64	0.79	--	--	--	--	--	--	--	--	5.01	--	--	--	--	--	--	--	--	--
65	0.80	--	--	--	3.47	--	--	--	--	--	--	--	--	20.55	--	--	--	--	--
66	0.81	--	11.87	18.07	--	--	--	--	--	--	4.89	--	--	--	--	--	--	--	9.7
67	0.82	--	--	--	5.16	--	--	6.27	--	--	--	--	30.83	--	--	--	--	20.2	--
68	0.83	10.27	--	--	--	--	2.77	--	--	--	--	--	--	7.42	29.89	24.33	--	--	--
69	0.84	--	1.0	6.34	4.39	29.5	--	--	--	--	--	--	--	--	--	--	--	--	--
70	0.85	--	--	--	--	--	6.84	14.3	--	9.34	--	--	--	--	--	--	--	--	7.8
71	0.86	--	--	--	--	--	--	--	--	--	20.35	--	18.46	--	--	13.37	34.08	--	--
72	0.87	34.49	--	--	--	--	--	--	--	--	--	--	--	16.1	--	--	--	--	--
73	0.88	--	--	--	--	--	14.5	--	--	16.3	--	44.37	--	--	--	--	--	7.13	6.5
74	0.89	--	11.58	--	26.88	55.1	--	--	--	--	--	--	--	--	--	--	--	--	--
75	0.90	--	--	42	0	--	--	33.0	--	--	--	--	--	6.71	--	--	9.14	--	17.6
76	0.91	17.96	17.26	--	--	--	--	--	--	--	10.55	--	7.89	--	29.54	12.95	--	4.6	--
77	0.92	--	--	7.77	--	--	--	--	--	9.22	--	15.86	--	2.3	--	--	--	--	--
78	0.93	--	--	--	--	15.3	--	--	60.5	--	3.76	--	--	--	--	--	--	--	--
79	0.94	--	--	--	--	--	--	--	--	--	2.2	--	--	--	--	3.95	7.98	--	--
80	0.95	--	--	--	--	--	8.63	--	--	--	--	--	--	5.58	--	--	--	--	--
81	0.96	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0
82	0.97	--	--	--	3.07	--	--	--	--	--	--	--	--	--	--	--	--	--	0
Band Total		12	11	8	20	3	22	11	2	20	26	7	15	17	5	14	13	18	11

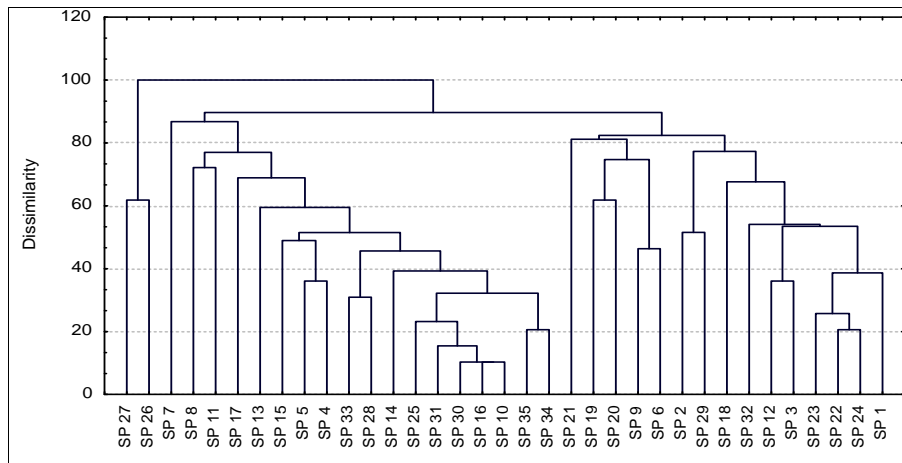


Fig 4: UPGMA-dendrogram based on 82 protein characters illustrating similarity and dissimilarity distances Between the studied taxa as numbered and listed in Table 1.

The phenogram of Fig. 4 produced from the numerical analysis for 82 seed protein characters between 35 taxa of Brassicaceae showed that, all the studied taxa are separated at taxonomic level of 100 %. Two species of genus *Raphanus* (*R. raphanistrum* and *R. sativus*) are separated in single cluster of 62%. Rest of the taxa are separated into two groups the first separated at 85 % dissimilarity level. This group included the two species of genus *Sinapis* (*S. alba* and *S. allionii*), *B. Tournefortii* (Egypt) and *Diplotraxis* (Egypt) are grouped at taxonomic level of 72%, three species of *Erysimum* (*E. Cheiri*, *E. crepidifolium* and *E. heritieri* var. *Hierrense*) and the two species of *Thlaspi caerulescens* and *Thlaspi montanum* which are collected from Germany. On the other hand, *B. Rapa* has separated in single dissimilarity level of 85%. There are a great affinity between *B. oleracea* var. *capitata* and *B. oler.* var. *Botrytis* which are separated at taxonomic levels of 30%. The second group delimited at 82%; at this group the remainder of the studied taxa are recognized; in which the highest similarity was between three species of genus *Mathiola* (*M. fruticulosa*, *M. incana* and *M. tricuspidata*). Also, *Capsella bursa-pastoris* (Egypt) is corellate with *Brassica oleracea* (England) which in turn shared with the three species of *Lepidium* (*L. campestre*, *L. densiflorum* and *L. sativum*) such group is separated at dissimilarity level of 80%.

It is obvious that, the four species belong to genus *Erysimum* were collected from different localities as shown in Table 1 showed a great variations in the number of bands in their protein profile patterns (Figs. 1, 2 &3). From the results, *B. oler.* var. *Capitata* (Egypt) and *B. oler.* var. *Botrytis* (Egypt) are shared in 6 bands (Table 2, bands, no.8,25,37,60,65 &75) in turn shared with *Brassica oleracea* (England) in three bands (Table 2, bands, no. 8, 25 & 60). The most eight species of genus *Brassica* shared in band no.25 with migration distance of 0.37. The low level of protein polymorphism could be attributed to the conservative nature of the seed protein. This conclusion was in accordance with Bonfitto *et al.* (1999) [5] who used SDS-protein profiles to identify nine cultivars of melons. Although the electrophoretic method shows good reproducibility, it failed to identify all of the examined taxa. The detected of the intraspecific polymorphism in the resulted protein profiles could be attributed to some environmental stress (Sammour *et al.*, 1993) [19]. *Brassica* genotypes of each species from different geographical locations in most cases are

not grouped together in one cluster (Fig. 4), such result was in accordance with Kamel (2005) [13]. In addition *Capsella bursa-pastoris* (Egypt) grouped with three species of *Lepidium* at taxonomic level of 80%. On the other hand the two species of genus *Raphanus* are separated in single cluster of 62%. Such cluster grouped with most studied taxa of Tribe *Brassicaceae* such as genus *Brassica*, genus *Diplotraxis*, genus *Sinapis* and genus *Raphanus*. This conclusion in accordance with Al-Shehbaz, *et al.* (2006) [2].

6. References

1. Adrianse A, Kolp W, Robbers JE. Characterization of *Phaseolus vulgaris* cultivators by their electrophoretic patterns. *J Sci. food Agric.* 1969; 20:647-650.
2. Al-Shehbaz A, Beilstein MA, Kellogg EA. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. 2006; 259(2-4):89-120. DOI: 10.1007/s00606-006-0415.
3. Amaal Hasan M. Molecular Systematic of Some Brassicaceae Taxa in Egypt Based on Electrophoretic Isoenzymes Pattern and RAPD Markers. *Australian Journal of Basic and Applied Sciences.* 2009; 3(3):1499-1511,
4. Badr A, El-Shazly HH, Abou El-Enain MM. Seed protein diversity and its implications on the relationships in the genus *Lathyrus* L. (Fabaceae). *Proc. 1st Conf. Biol. (ICBS) Fac. Sci., Tanta Univ.* 2000; 1:333-346.
5. Bonfitto R, Galleschi L, Macchia M, Saviozzi F, Navari-Izzo F. Identification of melon cultivars by gel and capillary electrophoresis. *Seed Science and Technology,* 1999; 27:779-783.
6. Boulter DE, Derbyshire E, Fraham J, Polhill M, Observations on the cytology and seed proteins in various African species of *Crotalaria* L. *Leguminosae, New Phytol.* 1970; 69:117-131.
7. El-Hadidi MN, Fayed AA. Materials for Excursion Flora of Egypt, *Taekholmia* 1995; 15:40-53.
8. El-Rabey H, Ibrahim AM, Badr A, El-Hallafawy K, Salamini F. DNA and seed protein fingerprinting of some Egyptian crop plants I. The relationship of 15 barley cultivars (*Hordeum vulgare* L.). The second International Conference on Biological Science 27-28 April, Fac. Sci., Tanta Univ., Egypt. Abstract 2002, 48.

9. Evaans RK, Abernethy RH, Identification of Cicer millcveth cultivars using SDS-Polyacrylamide gel electrophoresis. *Canadian Journal of Plant Science* 1983; 63; 1087-1090.
10. Harborne JB, Turner BL. *Plant chemo-systematics*, Academic press, New York, 1984.
11. Hassan HZ, Nadia Mansour M, Elham A, Abd El-Hady. Molecular genetic identification of some selected local peach (*Prunus persica* L.) cultivars at Dakhalia. *Egypt. J. Biotechnol.* 2002; 11:282-304.
12. Judd WS, Campbell CS, Kellog EA, Stevens PF. *Plant Systematics. A Phylogenetic Approach* Sinauer Associates, Inc. U.S.A, 1999.
13. Kamel EA. Biochemical and molecular variations in of the genus *Raphanus* L. based on SDS-PAGE seed proteins and isozymes patterns. *Bull. Fac. Sci. Assut Univ*, 2005.
14. Kamel EA, Al-Mashad AAA. Electrophoretic studies of seed proteins and the relationships of some species of the genus *Vicia* L. *FABIS Newsletter.* 1999; 42:5-11.
15. Laemmli U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680- 685.
16. Nath P, Ohri D, Jha SS, Pal M. Seed protein electrophoresis of wild and cultivated species of *Celosia* (Amaranthaceae). *Gen. Res. Crop Evol.* 1997; 44:241-245.
17. Rodman JE. A taxonomic analysis of glucosinolate-producing plants. II. Cladistics. *Syst. Bot.* 1991; 16:619-629.
18. Rohlf FJ. *NTsys-pc, Numerical taxonomy and multivariate analysis system.* Exeter Software Pub. (Ltd.), New York, 1993.
19. Sammour RH, Hamoud MA, Haidar AS, Badr A. Electrophoretic analysis of the seed proteins of some species in the genus *Lotus*. *Feddes Repertorium* 1993; 104(3-4):251-257.