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Identification of some wild *Vicia* species using electrophoretic analysis of seed proteins and amino acids composition

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Abstract

We studied protein variation among the Egyptian species of wild *Vicia* that may be of taxonomical importance. Seeds of five wild *Vicia* species (*Vicia sativa* subsp. *amphicarpa, V. articulata, V. ervilia, V. narbonensis* and *V. villosa*) were collected from their natural habitats in Egypt. Protein, albumin and globulin electrophoresis was carried out on the seeds. Moreover, protein content and its amino acids, albumin and globulin were determined. Total bands and their distribution and similarity percentage differed according to the studied *Vicia* species and protein type. Generally, the highest values of total bands for total protein, albumin and globulin were observed in *V. sativa* subsp. *amphicarpa.* Amount of albumin, globulin, total protein, albumin/total protein, globulin/ total protein and amino acids varied according to the studied *Vicia* species. In general, the highest values of determined amino acids, albumin/total protein and globulin/ total protein ratio were recorded in *V. articulata.* Furthermore, numerous taxonomical markers were observed for each studied *Vicia* species can be used as a tool for identifying the genetic diversity in *Vicia* species. These results support the validity of seed protein as a powerful tool for *Vicia* species identification and clarifying taxonomic relationships

Keywords: Vicia, electrophoresis, seed proteins, dendogram, amino acids, legume, gentic diversity, taxonomy

Introduction

Täckholm (1974) recognized twelve species of Vicia in Egypt; where as Boulos (1999) enumerated fourteen species. The genus Vicia belongs to the tribe Vicieae, Papilionoideae and Fabaceae. The legumes are a diverse and important family of angiosperms, with more than 650 genera and 18000 species, legumes are the third largest family of higher plants and are second only to grasses in agricultural importance (Young et al., 2003). Seed storage protein is important for taxonomical studies (Ladizinsky & Waines 1982; Miege, 1982; Vaughan 1983). Moreover, they used protein for identification the geographical origin of different taxa, determination of parents in hybrid species, characterization of polyploids and amphiploids and establishment of similarities between species and the formulation of hypotheses on their phylogeny. They suggested that variation in the seed proteins of Vicia sativa might be an important agent for acquiring ecological flexibility. Daussant et al. (1983) found that the protein content of the legume seeds ranged from 19-30%, rarely less than 20% and often more. Rao (1984) found that Vicia faba albumins composed of approximately about 21% from total seed proteins; but globulins represented about 70% of total seed proteins. On the other hand, Muntz et al. (1986) reported that Vicia faba globulins composed of approximately 30% vicilin and 70% legumin. Przybylska et al. (1992) indicated that 2s albumin is characteristic for tribe Vicieae and important for its systematic. Potokina et

al. (2000) used both seed protein and RAPDs to study intraspecific variation between taxa in V. sativa aggregate and found considerable genetic divergence between and within the studied taxa. They added that seed protein patterns are an effective tool for identifying accessions that can not be identified clearly by morphological criteria alone. Mirali et al. (2007) found major differences in the frequencies of the electrophoretic profiles in the different Vicia species and showed that the abundance of polymorphisms in cross-fertilised species is much higher than those of self-fertilized ones. Moreover, Mustafa (2007) analyzed seed albumins of nine Egyptian cultivars of Vicia faba and identified the electrophoregram profile for each cultivar and assessing genetic variation. Also, he found negative correlation between seed globulins and albumins.

Jackson *et al.* (1969) isolated the amino acids; aspartic, glutamic, threonine, serine, glycine, valine, l/2 cystine, methionine, isoleucine, tyrosine, phenylalanine, lysine, histidine and arginin from *Vicia faba* seeds. Glutamic and aspartic acid residues were predominate in protein seeds, whereas; cystine and methionine residues were inconspicuous. Also, Bailey and Boulter (1970) found that the protein of *Vicia faba* consisted of aspartic, threonine, serine, glutamic, proline, glycine, alanine, methionine, isoleucine, tyrosine, phenylalanine, lysine, histidine and arginine acids. On the other hand, Hassan (1997) determined amino acids (μ mol/g dry wt.) existing in the seeds of seven *Vicia* species; *V. cinerea, V.*



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cordata, V. faba cv. Giza2, *V. monantha, V. nigra, V. peregrina* and *V. sativa* and found the highest values for most determined amino acids; arginine (0.42), glutamine (0.005), serine (0.37), aspartic (0.69), glutamic (0.75), theronine (0.24), glycine (0.42), alanine (0.37), methionine (0.04), valine (0.24), leucine (0.34), lysine (0.63) and total determined amino acids (4.94) (μ mol/g dry wt.) were recorded in *V. sativa* in comparison with the other species.

The objective of this paper is studying the variations in protein, albumin and globulin electrophoresis, in addition, protein, albumin, globulin and amino acids content among studied *Vicia* species to their identification.

Materials and methods

Seeds of five *Vicia* species, *V. sativa* subsp. *amphicarpa*, *V. articulata*, *V. ervilia*, *V. narbonensis* and *V. villosa* were collected from their natural habitats in Egypt. Protein, albumin and globulin electrophoresis was carried out on the seeds of five *Vicia* species. In addition, protein content and its amino acids were determined as follows:

Protein electrophoresis

Seed cotyledons of five studied *Vicia* species were separated and ground to powder in a mortar after removing the testa, 0.5 g of the powder was defatted twice with 25 ml 70% ethanol for 10 min each. All the following steps were carried out at 4°C. Total seed proteins were extracted with extraction buffer (0.1g/ml) which composed of 20 mM Na-borate buffer, 0.5 M NaCl, 1 mM ethylenediaminetetra-acetic acid (EDTA), pH 8.9. After 12 h of stirring, the extract was centrifuged at 10,000 g for 20 min. The supernatant was the total protein; 10 μ l of it was taken for electrophoresis or stored at -20°C according to Juo and Stotzky (1970) and Tucci *et al.* (1991). Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 10% acrylamide slab gels according to Laemmli (1970).

Albumin & globulin electrophoresis

The above supernatant (total protein) was filtered into dialysis bags by running distilled water for 48 h. This homogenate was centrifuged at 10,000 g for 20 min. The supernatant was albumin and the residue was saltsoluble protein (globulin), the residue was washed and suspended in 2 ml from above mentioned extraction buffer. 20 µL of albumin and 10 µL of globulin were taken for electrophoresis according to Juo and Stotzky (1970). Gel electrophoresis was carried out in 10% acrylamide slab gels, with a current of 25 mA and 130 volts per gel until the bromophenol blue marker reached to the bottom of the gel. After electrophoresis, the gels were placed in the solution of coloration which was composed of 0.1% Coomassie blue G solution made of 1 gm Coomassie blue G, 23.5 ml phosphoric acid 85% and 10 g ammonium sulphate and were dissolved in 1 I distilled

water at room temperature for 3 h. Moreover, similarity matrix based on Jaccard's coefficient were estimated by cluster analysis using UPGMA method (Unweighted pair group method with Arithmetic mean) depending on the similarity polymorphism of total protein, albumin and globulin patterns based in protein marker. All computations were performed with NTSYS-PC version 2.1 (Rohlf, 1993).

Determination of protein content

Albumin, globulin and total protein were determined according to Bradford (1976).

Determination of amino acids

Amino acids (arginine, aspargine, glutamine, serine, asparatic, glutamic, threonine, glycine, alanine, methionine, valine, phenylalanine, isoleucine, leucine, histidine, lysine and tyrosine) were determined using computerized High Performance Liquid Chromatography (HPLC) apparatus. Preparation, purification and determination of such samples were described by Moore and Stein (1963). One gram of Vicia species cotyledons was ground in a mortar to powder. The powder was defatted with acetone (3 times) and filtered with filter paper using water pump to obtain the acetone powder. Five mg of each acetone powder was placed into a 16 x 125 mm heavy-walled Pyrex test tube, 1 ml 6 N HCl was added to test tube, and then closed tightly by flame of oxygen. All samples were placed in oven at 110°C for 24 h for hydrolysis. After hydrolysis, the tube was opened carefully and the samples were dried under vacuum in a water bath at 40 to 45°C using water pump. The residues were dissolved in 0.5 ml of distilled water, transferred to 15 ml conical flask and 0.5 ml of 0.2 M sodium phosphate buffer were added. These extracts were, then, evaporated under vacuum to complete dryness. The dried residues were dissolved in 5 ml of sodium phosphate buffer, shacked and then filtered through 0.2 μ membranes. After filtration, 20-75 µl of such cotyledons extracts were taken and injected in HPLC apparatus for estimating the different types of amino acids.

Results

Generally, the protein electrophoresis was appropriated to determine the differences and genetic diversity among the five studied *Vicia* species by cluster analysis using UPGMA depending on the similarity polymorphism of total protein, albumin and globulin patterns. Similar trend was found in this concern in relation to amino acids profile.

Total protein electrophoresis

The electrophoretic data of albumin, globulin and total protein fractions existing in the studied five *Vicia* species seed, which have been investigated using SDS-PAGE, are presented in Table 1-6 and Fig. 1- 6. It is clearly shown from these Tables and Figures that albumin,



Table 1. Seed total protein banding patterns of studied Vicia species obtained by SDS-PAGE.

Band No.	MW (KDa)	Marker	V. sativa subsp. amphicarpa	V. articulata	V. ervilia	V. narbonensis	V. villosa
1	97	6.64	0.00	0.00	3.55	0.00	0.00
2	92	0.00	0.00	0.00	0.00	5.40	0.00
3	89	0.00	0.00	2.99	0.00	0.00	0.00
4	87	0.00	4.35	0.00	0.00	0.00	0.00
5	86	0.00	0.00	0.00	0.00	0.00	1.17
6	83	0.00	0.00	0.00	0.00	0.00	1.13
7	82	0.00	0.00	5.76	0.00	0.00	0.00
8	73	0.00	0.00	0.00	7.12	0.00	0.00
9	68	0.00	0.00	0.00	2.82	0.00	0.00
10	66	12,99	0.00	0.00	0.00	0.00	0.00
11	64	0.00	3.54	0.00	0.00	0.00	0.00
12	62	0.00	2.33	0.00	1.07	0.00	8.32
13	61	0.00	0.00	9.80	0.00	6.27	0.00
14	59	0.00	3.12	2.56	2.59	0.00	0.00
15	58	0.00	0.00	0.00	0.00	20.62	0.00
16	57	0.00	1.56	0.00	11.86	0.00	0.00
17	56	0.00	1.57	0.00	1.57	0.00	3.91
18	55	0.00	4.31	0.00	0.00	0.00	0.00
19	54	0.00	0.00	1.84	2.73	0.00	0.00
20	53	0.00	1.58	0.00	0.00	3.11	0.00
21	52	0.00	3.15	1.81	0.00	0.00	13.66
22	50	0.00	1.58	3.62	1.35	0.00	3.12
23	49	0.00	3.16	0.00	0.00	5.15	0.00
24	48	0.00	0.00	2.18	0.00	0.00	0.00
25	47	0.00	0.00	0.00	0.00	4.99	4.42
26	46	0.00	22.92	0.00	10.54	4.29	0.00
27	45	26.32	3.00	14.86	0.00	0.00	0.00
28	44	0.00	0.00	2.10	1.99	0.00	0.00
29	42	0.00	3.12	0.00	0.00	0.00	0.00
30	41	0.00	0.00	1.30	0.00	0.00	0.00
31	39	0.00	0.00	0.00	15.02	0.00	4.82
32	38	0.00	3.45	3.43	1.98	8.68	0.00
33	37	0.00	10.74	0.00	0.00	0.00	4.87
34	36	0.00	7.54	4.25	3.55	8.07	2.08
35	35	0.00	5.94	14.49	3.64	0.00	17.89
36	32	0.00	0.00	0.00	0.00	6.06	10.83
37	31	0.00	0.00	2.43	0.00	0.00	0.00
38	30	0.00	0.00	1.48	11.33	0.00	0.00
39	29	25.96	0.00	5.88	4.80	4.68	0.00
40	28	0.00	3.93	0.00	0.00	0.00	0.00
41	23	0.00	1.18	0.00	0.00	6.15	0.00
42	22	0.00	0.00	2.11	0.00	0.00	4.47
43	19	0.00	3.29	0.00	12.50.	7.02	0.00
44	18	0.00	0.00	3.97	0.00	0.00	19.32
45	17	0.00	0.00	0.00	0.00	9.52	0.00
46	16	0.00	4.65	13.14	0.00	0.00	0.00
47	14	28.09	0.00	0.00	0.00	0.00	0.00

globulin and total protein fractions varied among the studied *Vicia* species and analyzed into 47, 19 and 38 bands respectively. Electrophoretic data for seed total proteins fractions represented in Table 1 & 2 and Fig.1 show notable variations in total protein banding patterns of the studied *Vicia* species. Data from (Table 1) indicate

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that the highest values of total amount percentage of bands were 22.92, 14.86, 15.02, 20.62 and 19.32 in V. sativa subsp. amphicarpa, V. articulata, V. ervilia, V. narbonensis and V. villosa, respectively, at band no. 26 (MW 46 KDa), band no. 27 (45 KDa), band no. 31 (39 KDa), band no. 15 (58 KDa) and band no. 44 (18 KDa), respectively. In the same time, Table 2 shows that the highest value for seed total protein bands was recorded in V. sativa subsp. amphicarpa (22); whereas, the lowest one (14) was observed in both V. narbonensis and V. villosa. In addition, band no. 4 (MW 87 KDa), band no.11 (64 KDa), band no. 18 (55 KDa), band no. 29 (42 KDa) and band no. 40 (28 KDa) are taxonomical markers for V. sativa subsp. amphicarpa. Band no. 3 (89 KDa), band no. 7 (82 KDa), band no. 24 (48 KDa), band no. 30 (41 KDa) and band no. 37 (31 KDa) are taxonomical markers for V. articulata; band no. 1 (97 KDa), band no. 8 (73 KDa) and band no. 9 (68 KDa) are taxonomical markers for V. ervilia; band no. 2 (92 KDa), band no. 14 (59 KDa) and band no. 45 (17 KDa) are taxonomical markers for V. narbonensis and band no. 5 (86 KDa) and band no.6 (83) are taxonomical markers for V. villosai. Concerning clustering analysis among the five studied Vicia species. The obtained data classified the studied Vicia species into four groups according to similarity percentage. The highest value of similarity percentage (82.35) was observed between both V. articulata and V. narbonensis.

Albumin electrophoresis

Data presented in (Table 3 & 4) and (Fig. 3 & 4) indicate that the highest values of total amount percentage of bands of albumin were 40.80, 38.16, 48.94, 35.91 and 40.89 in V. sativa subsp. amphicarpa, V. articulata, V. ervilia, V. narbonensis and V. villosa, respectively, at band no. 19 (MW 12 KDa), band no. 18 (14 KDa), band no. 16 (21 KDa), band no. 15 (22 KDa) and band no. 18 (14 KDa), respectively. Table 4 shows that, the highest value for seed albumin bands (5) was recorded in V. sativa subsp. amphicarpa, V. ervilia and V. villosa; whereas, the lowest one (4) was observed in both V. articulata and V. narbonensis. On the other hand, three polymorphic bands (band no. 6 (MW 37 KDa), band no. 11 (30 KDa) and band no. 19 (12 KDa) are characteristic for V. sativa subsp. amphicarpa. Band no. 5 (39 KDa) is taxonomical marker for V. articulata; band no. 10 (31 KDa) is taxonomical marker for V. ervilia; bands no. 13, 15 and 17 (28, 22 & 15 KDa) are taxonomical markers for V. narbonensis and band no. 2 (81 KDa) is taxonomical marker for V. Villosa.

Results obtained from UPGMA clustering analysis for albumin fractions basis on the similarity polymorphism in (Fig. 4) separate the five studied *Vicia* species into four groups. The first group consists of *V. ervilia* and *V. villosa* with similarity 90.91%, the second group *V. sativa* subsp. *amphicarpa* and *V. narbonensis* with similarity 88.89%. Moreover, the third group *V. sativa* subsp. *amphicarpa*,

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V. articulata and V. narbonensis with similarity 70.83%. Finally, the fourth group V. sativa subsp. amphicarpa, V. articulata, V. ervilia, V. narbonensis and V. villosa with similarity 67.68%.

Table 3. Seed albumin banding patterns of s	tudied Vicia sativa
sub species obtained by SDS-F	PAGE

Band No.	MW (KDa)	Marker	V. sativa amphicarpa	V. articulata	V. ervilia	V. narbonensis	V. villosa
1	97	14.05	0.00	0.00	0.00	0.00	0.00
2	81	0.00	0.00	0.00	0.00	0.00	3.87
3	66	12.17	0.00	0.00	0.00	0.00	0.00
4	45	33.70	0.00	0.00	0.00	0.00	0.00
5	39	0.00	0.00	32.18	0.00	0.00	0.00
6	37	0.00	37.28	0.00	0.00	0.00	0.00
7	35	0.00	0.00	0.00	25.53	0.00	10.95
8	33	0.00	6.35	0.00	0.00	23.14	12.64
9	32	0.00	0.00	17.37	5.12	0.00	0.00
10	31	0.00	0.00	0.00	3.10	0.00	0.00
11	30	0.00	8.00	0.00	0.00	0.00	0.00
12	29	18.70	0.00	0.00	0.00	0.00	0.00
13	28	0.00	0.00	0.00	0.00	29.45	0.00
14	23	0.00	7.57	0.00	0.00	0.00	31.65
15	22	0.00	0.00	0.00	0.00	35.91	0.00
16	21	0.00	0.00	12.29	48.94	0.00	0.00
17	15	0.00	0.00	0.00	0.00	11.49	0.00
18	14	21.37	0.00	38.16	17.32	0.00	40.89
19	12	0.00	40.80	0.00	0.00	0.00	0.00

Globulin electrophoresis

Globulin banding patterns results are given in (Table 5 & 6) and (Fig. 5 & 6). The highest values of total amount percentage of bands were 17.04, 18.58, 18.25, 20.33 and 26.84 at bands no. 26 (35 KDa), 18 (47 KDa), 14 (54 KDa), 19 (46 KDa) and 20 (45 KDa), respectively, for V. sativa subsp. amphicarpa, V. articulata, V. ervilia, V. narbonensis and V. villosa, respectively. Table 6 shows that the highest value for seed globulin bands (12) was recorded in V. sativa subsp. amphicarpa and V. narbonensis, whereas, the lowest one (8) was observed in V. villosa. Six polymorphic bands (band no. 7 (MW 73 KDa), band no. 8 (66 KDa), band no. 10 (62 KDa), band no. 16 (50 KDa), band no. 26 (35 KDa) and band no. 35 (17 KDa) are characteristic for V. sativa subsp. amphicarpa. Also, six bands (band no. 4 (MW 84 KDa), band no. 5 (77 KDa), band no. 18 (47 KDa), band no. 24 (37 KDa), band no. 30 (28 KDa) and band no. 33 (19 KDa) are characteristic for V. articulata .Band no. 2 (92 KDa), band no. 9 (MW 65 KDa), band no. 12 (57 KDa), band no. 14 (54 KDa), band no. 23 (42 KDa), band no. 25 (36 KDa) and band no. 31 (26 KDa) are taxonomical markers for V. ervilia; bands no. 6, 15, 22 and 32 (75, 53, 43 & 25 KDa) are taxonomical markers for V. narbonensis. As the aforementioned trends which were found for total protein and albumin patterns the cluster

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25	47	-	-	-	-	+	+	Globulin elec
26	46	-	+	-	+	+	-	Globulin
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28	44	-	-	+	+	-	-	percentage of
29	42	-	+	-	-	-	-	26.84 at ba
30	41	-	-	+	-	-	-	KDa) 19 (4)
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35	35	-	+	+	+	-	+	
36	32	-	-	-	-	+	+	narbonensis
37	31	-	-	+	-	-	-	in V. villosa.
38	30	-	-	+	+	-	-	KDa), band
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40	28	-	+	-	-	-	-	(17 KDa)
41	23	-	+	-	-	+	-	amphicarpa.
42	22	-	-	+	-	-	+	band no. 5 (
43	19	-	+	-	+	+	-	(37 KDa), b
44	18	-	-	+	-	-	+	KDa) are ch
45	17	-	-	-	-	+	-	KDa). band
46	16	-	+	+	-	-	-	band no. 14
47	14	+	-	-	-	-	-	25 (36 KDa)
No. of	poly-	-	5	5	3	3	2	markers for
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Table 2. Distribution of seed total protein bands in the studied Vicia species obtained by SDS-PAGE

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analysis differentiate the globulin fractions into four groups. V. articulata and V. villosa located in the first cluster with highest similarity (94.74%) while the lowest similarity (66.66%) between V. ervilia and the others Vicia species under investigation.

Table 4. Distribution of seed albumin bands in the studied
Vicia sativa sub species obtained by SDS-PAGE

Band No.	MW KDa	Marker	V. sativa amphicarpa	V. articulata	V. ervilia	V. narbonensis	V. villosa
1	97	+	-	-	-	-	-
2	81	-	-	-	-	-	+
3	66	+	-	-	-	-	-
4	45	+	-	-	-	-	-
5	39	-	-	+	-	-	-
6	37	-	+	-	-	-	-
7	35	-	-	-	+	-	+
8	33	-	+	-	-	+	+
9	32	-	-	+	+	-	-
10	31	-	-	-	+	-	-
11	30	-	+	-	-	-	-
12	29	+	-	-	-	-	-
13	28	-	-	-	-	+	-
14	23	-	+	-	-	-	+
15	22	-	-	-	-	+	-
16	21	-	-	+	+	-	-
17	15	-	-	-	-	+	-
18	14	+	-	+	+	-	+
19	12	-	+	-	-	-	-
polymo bands	orphic (No.)	-	3	1	1	3	1
Total ba	anding	5	5	4	5	4	5
+ = present - = absent							

Protein content

The amount of albumin, globulin and total protein determined in the seeds of Vicia species under investigation, as well as, the percentage of both albumin and globulin in seed protein are illustrated in Table 7. It is evident that the highest values of albumin, globulin and total protein amounts (4.7, 40.3 & 59.1 mg/g dry wt. respectively) were observed in V ervilia, V. villosa and V. narbonensis, respectively. On the other hand, the minimal value of albumin (2.6 mg/g dry wt.) was recorded in V. articulata, while, globulin and total protein (21.6 & 24 mg/g dry wt.) were recorded in V. sativa subsp. amphicarpa. In the meantime, the percentages of albumins to total protein and globulins to total protein were varied according to the studied Vicia species. The highest percentages of albumins to total protein (15.2%) and globulins to total (89.9%) were recorded in V articulata. Whereas, the lowest one was 5.7% for albumins to total protein in V. narbonensis and 52.8% for globulin to total protein in V. ervilia.

Tab	Table 5. Seed globulin banding patterns of studied Vicia sativa sub species obtained by SDS-PAGE							
Band No.	MW (KDa)	Marker	V. sativa amphicarpa	V. articulata	V. ervilia	V. narbonensis	V. villosa	
1	97	18.60	0.00	0.00	0.00	0.00	0.00	
2	92	0.00	0.00	0.00	9.32	0.00	0.00	
3	85	0.00	13.83	0.00	0.00	11.91	11.17	

No.	(KDa)	Ма	V. s ampl	V. an	V. 6	narbo	Ν. Ν
1	97	18.60	0.00	0.00	0.00	0.00	0.00
2	92	0.00	0.00	0.00	9.32	0.00	0.00
3	85	0.00	13.83	0.00	0.00	11.91	11.17
4	84	0.00	0.00	14.11	0.00	0.00	0.00
5	77	0.00	0.00	4.35	0.00	0.00	0.00
6	75	0.00	0.00	0.00	0.00	1.52	0.00
7	73	0.00	3.45	0.00	0.00	0.00	0.00
8	66	19.65	0.87	0.00	0.00	0.00	0.00
9	65	0.00	0.00	0.00	11.80	0.00	0.00
10	62	0.00	9.37	0.00	0.00	0.00	0.00
11	59	0.00	0.00	10.14	0.00	0.00	7.69
12	57	0.00	0.00	0.00	3.53	0.00	0.00
13	55	0.00	16.67	0.00	0.00	7.52	0.00
14	54	0.00	0.00	0.00	18.25	0.00	0.00
15	53	0.00	0.00	0.00	0.00	16.76	0.00
16	50	0.00	11.58	0.00	0.00	0.00	0.00
17	48	0.00	3.36	0.00	0.00	0.00	10.35
18	47	0.00	0.00	18.58	0.00	0.00	0.00
19	46	0.00	1.71	0.00	0.00	20.33	0.00
20	45	22.97	0.00	0.00	15.57	0.00	26.84
21	44	0.00	1.52	14.92	0.00	5.57	0.00
22	43	0.00	0.00	0.00	0.00	2.35	0.00
23	42	0.00	0.00	0.00	1.96	0.00	0.00
24	37	0.00	0.00	3.30	0.00	0.00	0.00
25	36	0.00	0.00	0.00	13.46	0.00	0.00
26	35	0.00	17.04	0.00	0.00	0.00	0.00
27	34	0.00	0.00	9.54	1.89	0.00	0.00
28	33	0.00	0.00	0.00	0.00	11.22	13.94
29	29	21.09	0.00	0.00	0.00	6.73	7.67
30	28	0.00	0.00	5.36	0.00	0.00	0.00
31	26	0.00	0.00	0.00	10.97	0.00	0.00
32	25	0.00	0.00	0.00	0.00	2.32	0.00
33	19	0.00	0.00	3.40	0.00	0.00	0.00
34	18	0.00	0.00	0.00	4.18	5.58	4.35
35	17	0.00	3.89	0.00	0.00	0.00	0.00
36	16	0.00	0.00	0.00	0.00	8.18	17.98
37	15	0.00	16.72	16.29	9.07	0.00	0.00
38	14	17.70	0.00	0.00	0.00	0.00	0.00

Amino acids

The amounts of different amino acids (µ mol/g dry wt.) in the seeds of various five Vicia species were presented in Table 8. Results in these Tables show obvious increments in the amount of the amino acids; lysine (0.74), valine (0.28), threonine (0.23), isoleucine (0.019), methionine (0.034), glycine (0.38), alanine (0.32), serinine (0.32), phenylalanine (0.19) and histidine (0.11) µ mol/g dry wt. were recorded in V. articulata in comparison with the other amino acids and Vicia species. Whereas, the lowest values for lysine (0.10), Lucien



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Species

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narbonensis

Ζ.

Table 6. Distribution of seed globulin bands in the studied Vicia sativasub species obtained by SDS-PAGE

Band No.	MW (KDa)	Marker	V. sativa amphicarpa	V. articulata	V. ervilia	V. narbonensis	V. villosa
1	97	+	-	-	-	-	-
2	92	-	-	-	+	-	-
3	85	-	+	-	-	+	+
4	84	-	-	+	-	-	-
5	77	-	-	+	-	-	-
6	75	-	-	-	-	+	-
7	73	-	+	-	-	-	-
8	66	+	+	-	-	-	-
9	65	-	-	-	+	-	-
10	62	-	+	-	-	-	-
11	59	-	-	+	-	-	+
12	57	-	-	-	+	-	-
13	55	-	+	-	-	+	-
14	54	-	-	-	+	-	-
15	53	-	-	-	-	+	-
16	50	-	+	-	-	-	-
17	48	-	+	-	-	-	+
18	47	-	-	+	-	-	-
19	46	-	+	-	-	+	-
20	45	+	-	-	+	-	+
21	44	-	+	+	-	+	-
22	43	-	-	-	-	+	-
23	42	-	-	-	+	-	-
24	37	-	-	+	-	-	-
25	36	-	-	-	+	-	-
26	35	-	+	-	-	-	-
27	34	-	-	+	+	-	-
28	33	-	-	-	-	+	+
29	29	+	-	-	-	+	+
30	28	-	-	+	-	-	-
31	26	-	-	-	+	-	-
32	25	-	-	-	-	+	-
33	19	-	-	+	-	-	-
34	18	-	-	-	+	+	+
35	17	-	+	-	-	-	-
36	16	-	-	-	-	+	+
37	15	-	+	+	+	-	-
38	14	+	-	-	-	-	-
Polyn	norphic		c	c	7	4	0
band	s (No.)	-	б	б	/	4	U
Total b	panding	5	12	10	11	12	8
	+	= prese	ent		- = abs	sent	

(0.24), isolucine (0.12), glutamic (0.44), aspartic (0.36), glycine (0.26), serine (0.21), phenylalanine (0.13), histidine (0.07) and asparagines (0.006). Glutamine was absent in *V. ervilia*. The highest value for total amino acids (4.70) was recorded in *V. articulata*, while the lowest one (2.77) was found in *V. narbonensis.*

Discussion

The present investigation reveals that the wild *Vicia* species varied with seed protein and amino acid content as observed from total protein, albumin, globulin, and

+ Albumin (mg/g 2 c 2 c

Recorded data

oť	Albumin (mg/g dry wt.)	3.6	2.6	4.7	4.2	3.6
nount	Globulin (mg/g dry wt.)	21.6	26.4	39.4	31.2	40.3
An	Total protein (mg/g dry wt.)	24.0	45.6	57.6	59.1	45.8
%/	Albumin to total protein	8.0	15.2	7.1	5.7	7.9
% (Globulin to total protein	68.3	89.9	52.8	57.9	88.1

Table 7. Protein content and its percentage in the five

studied Vicia species seeds

articulata

Σ.

ervilia

Ζ.

sativa subsp *amphicarpa*

Ζ.

Table 8. The amino acids composition of the studied Vicia species seeds (calculated as μ mol/g dry wt.)

Species Rec. data	/. <i>sativa</i> subsp. <i>amphicarpa</i>	V. articulata	V. ervilia	/. narbonensis	V. villosa
	1	0 7 4 0	0.040	0.100	0.440
Lysine	0.280	0.740	0.240	0.100	0.410
Leucine	0.320	0.310	0.250	0.240	0.300
Valine	0.230	0.280	0.180	0.170	0.240
Threonine	0.190	0.230	0.150	0.140	0.210
Isoleucine	0.150	0.190	0.130	0.120	0.170
Tyrosine	0.020	0.110	0.170	0.040	0.002
Methionine	0.022	0.034	0.011	0.017	0.021
Glutamic acid	0.620	0.590	0.570	0.440	0.540
Asparatic acid	0.630	0.540	0.440	0.360	0.490
Glycine	0.370	0.380	0.270	0.260	0.340
Alanine	0.260	0.320	0.160	0.250	0.290
Serinine	0.300	0.320	0.220	0.210	0.280
Arginine	0.340	0.350	0.200	0.220	0.290
Phenylalanine	0.150	0.190	0.140	0.130	0.170
Histidine	0.100	0.110	0.080	0.070	0.090
Asparagine	0.016	0.008	0.014	0.006	0.013
Glutamine	0.008	0.004	0.000	0.002	0.003
Total determined amino acids	4.006	4.706	3.225	2.775	3.859

amino acid estimations. The variation also reflected in albumin/total protein and globulin/total protein ratio of the seed powder. This protein variation might have resulted in the polymorphism bands, which gave each species its specific electrophoregram for total protein, albumin and globulin. Such electrophoregram of the studied species can be used as a tool for identifying the genetic diversity in *Vicia* species. These results support the validity of seed protein as a powerful tool for *Vicia* species identification and clarifying taxonomic relationships

villosa

Ζ.

b=V. articulate;

d=V.narbonensis; e=V. villosa

c= V. ervilia;





M= Protein marker; a=V. subsp. amphicarpa b=V. articulate; c= V. ervilia; d=V.narbonensis; e=V. villosa

Fig. 2. Dendogram resulted from UPGMA clustering analysis based on the similarity polymorphism of total Protein patterns of the five studied Vicia spp. using SDS-PAGE





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Fig. 4. Dendogram resulted from UPGMA clustering analysis based on the similarity polymorphism of albumin patterns of the five studied Vicia spp. using SDS-PAGE



Lane[~]1 = protein marker Lane[~]2 = V. sativa subsp. Amphicarpa Lane[~]4 = V. ervilia Lane[~]5 = V. narbonensis

Lane~3 = V. articulata Lane~6 = V. villosa





M= Protein marker; a=V. subsp. amphicarpa b=V. articulate; c= V. ervilia; d=V.narbonensis; e=V. villosa





Lane~1 = protein marker Lane~2 = V. sativa subsp. Amphicarpa Lane~3 = articulata

(Singor *et al.*, 2005; Mustafa *et al.*, 2006; Thanh *et al.*, 2006; Sammour *et al.*, 2007). Also the wide variation observed for seed albumins, globulins and amino acids, which is a genetic trait (Sakr, 1995; Hassan, 1997; Sammour *et al.*, 2007). In addition, Vargas *et al.* (2001) and Mirali *et al.* (2007) attributed the differences between *Vicia* and *Phaseolus* species polymorphism, result from DNA sequences that code for peptides involved in post-transcription changes.

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