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Electrophoretic seed globulin patterns and species relationships in *Vicia* section *Faba* (*Fabaceae*)

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Abstract. This work presents electrophoretic seed globulin data obtained for 173 accessions representing nine *Vicia* species of the section *Faba*, which were already investigated for electrophoretic seed albumin patterns (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA, in press). Electrophoretic analysis of seed globulins was performed using sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE). In the examined material totally 38 well-defined polypeptide bands, in the relative molecular mass range of 19-61 kDa, were distinguished. The presence/absence of particular bands was recorded for each analysed individual. The electrophoretic data were used for hierarchical grouping of the examined taxa, based on calculated Bhattacharyya distances. *V. bithynica* proved to be the most distinctive member of *Vicia* section *Faba*. In turn, *V. faba* was clearly different from species of the *V. narbonensis* complex. Taxa of this complex formed two clusters: one consisting of *V. narbonensis* varieties and another – of the remaining species. In the latter group, *V. serratifolia* appeared to be rather distantly related to *V. eristalioides*, *V. galilaea*, *V. hyaeniscyamus*, *V. johannis* and *V. kalakhensis*. The obtained results are compared with the corresponding seed albumin data and discussed with reference to taxonomic relationships in *Vicia* section *Faba*.

Key words: *Vicia* sect. *Faba*, seed globulins, taxonomy, electrophoresis.

The faba bean, *Vicia faba* L., is a member of the subgenus *Vicia* of the section *Faba* sensu KUPICHA (1976). Other members of this section are 8 wild species, i.e. 7 species of the *V. narbonensis* complex and one more distinct species – *V. bithynica*. The *V. narbonensis* complex comprises *V. galilaea*, *V. hyaeniscyamus*, *V. johannis*, *V. narbonensis* and *V. serratifolia* as well as

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two recently discovered taxa – *V. eristalioides* and *V. kalakhensis* (KHATTAB et al. 1988, MAXTED 1988, MAXTED et al. 1991).

In search for a wild ancestor of the faba bean, relationships between *V. faba* and wild species of the section *Faba* were investigated using different approaches (CUBERO 1984, SMARTT 1990, MAXTED et al. 1991), but despite that no closest ally of *V. faba* has been indicated and taxonomic relationships between wild species of the section *Faba* are still a matter of debates.

Our previous paper reported on the electrophoretic analysis of seed albumins in 173 accessions representing nine *Vicia* species of the section *Faba* (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995). This analysis showed a marked intraspecific variation in most of the investigated species as well as some characteristic interspecific relationships. This paper presents results obtained in the electrophoretic analysis of seed globulins in all *Vicia* accessions examined for seed albumin patterns. It seemed interesting to confront data from the analysis of different protein types.

Material and methods

Plant material

An object of the studies were 173 accessions representing nine *Vicia* species of the section *Faba*. In the case of *V. faba, V. narbonensis, V. johannis* and *V. galilaea* an intraspecific classification was taken into account. Therefore, 18 taxa were considered.

The examined accessions were obtained from the following sources: a – Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany (70 accessions), b – The Vicieae Germplasm Collection, Department of Biology, University of Southampton, Southampton, U.K. (24 accessions), c – Western Regional Plant Introduction Station, USDA-ARC, Pullman, Washington, USA (15 accessions), d – Dr. G. Ramsay, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland (12 accessions), e – Institut für Pflanzenbau und Pflanzenzüchtung der FAL, Brunswick, Germany (7 accessions), f – National Department of Plant Genetic Resources, Plant Breeding and Acclimatization Institute, Radzików n/Warsaw, Poland (4 accessions), g – Dr. J. I. Cubero, Departamento de Genetica, Universidad de Cordoba, Cordoba, Spain (4 accessions), h – Germplasm Institute, C.N.R., Bari, Italy (3 accessions), i – International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (2 accessions), j – Agricultural Research Institute, Ministry of Agriculture and Natural Resources, Nicosia, Cyprus (2 accessions).

Table 1. Information on the investigated accessions of the *Vicia* species of the section *Faba*. The taxa are listed as in MAXTED et al. (1991)

Taxon	Accessions		
	Number	Geographical origin*	Source**
V. bithynica (L.) L.	9	France (1), Italy (3), Greece (2), Syria (3)	a, b
V. eristalioides MAXTED	1	Turkey (1)	b
V. kalakhensis KHATTAB, MAXTED & BISBY	3	Syria (3)	b, d
V. johannis TAMAMSCHJAN in KARYAGIN	20		
var. *procumbens* H. SCHÄFER	13	Turkey (9), Syria (2), Georgia (2)	a,b, d
var. *johannis*	7	Turkey (2), Syria (2), Ashkhabad (2), Tashkent (1)	a, b
V. galilaea PLITM. & ZOH. in PLITM.	7		
var. *galilaea*	4	Israel (2), Tashkent (1), unknown (1)	a, b, d
var. *faboidea* (PLITM. & ZOH. in PLITM.) H. SCHÄFER	3	Turkey (1), Israel (1), unknown (1)	a, d
V. serratifolia JACQ.	9	Spain (1), France (3), Hungary (1), Malta (1), Turkey (1), Krasnodar (1), unknown (1)	a, b, d
V. narbonensis L.	46		
var. *salmonea* (MOUT.) H.SCHÄFER	5	Israel (2), Syria (2), unknown (1)	a, b, d
var. *jordanica* H.SCHÄFER	9	France (1), Israel (4), Turkey (1), Syria (3)	a, b
var. *affinis* KORNHUBER ex ASCH. & SCHWEINF.	12	Spain (1), France (1), Tunisia (3), Lebanon (1), Turkey (4), Syria (2)	a, b, d
var. *aegyptiaca* KORNHUBER ex ASCH. & SCHWEINF.	9	Spain (1), Turkey (8)	a, b
var. *narbonensis*	11	Portugal (5), Italy (3), Romania (1) Crete (1), Syria (1)	a, b
V. hyaeniscyamus MOUT.	5	Syria (5)	b, d
V. faba L.	73		
subsp. *paucijuga* MURAT	3	Pakistan (2), unknown (1)	g, h
subsp. *faba*	70		
var. *minor* BECK	25	Spain (2), Italy (1), Greece (2), Sudan (3), Syria (1), Israel (1) Yemen (3), Pakistan (1), Afghanistan (8), India (3)	a,c,e, f, g, i
var. *equina* PERS.c***	33	Morocco (2), Algeria (2), Tunisia (1), Egypt (5), Ethiopia (5), Sudan (3), Turkey (3), Syria (1), Lebanon (1), Israel (1), Jordan (2), Yemen (2), India (1), China (4)	c, f, i
var. *major*	12	Italy (1), Greece (1), Tunisia (1), Cypr (2), Turkey (2), Syria (1), Jordan (2), Lebanon (2),	f, h, i, j

*Numbers of accessions of a given geographical origin are indicated in parentheses

**For key to seed sources see: Plant material

***Accessions of the type *minor/equina* and *equina/major* are included.

Routinely, three individuals of each accession were examined separately. However, in the case of *V. faba* subsp. *paucijuga* 10 individuals were analysed for each of three studied accessions. In *V. eristalioides*, represented only by a single accession, the analysis covered 20 individuals.

Table 1 presents a general characterization of the investigated material. In the text, some particular accessions are indicated by catalogue numbers and donor's designations.

Analytical techniques

Globulins were extracted from cotyledons of single seeds. Essentialy, the extraction was performed according to PASQUALINI et al. (1991). First, albumins were extracted – with 0.15 M acetate buffer, pH 4.6 – and discarded. Second, globulins were extracted with 0.5 M KCl in 0.1 M tris-maleate buffer, pH 6.9, containing 1 mM dithiothreitol and 1 mM ethylenediaminetetracetic acid (EDTA).

Protein samples were dissociated at 100°C for 5 min with 2% SDS in the presence of 5% 2-mercaptoethanol. SDS-PAGE was performed in 12% polyacrylamide slab gels in a discontinuous buffer system according to LAEMMLI (1970); the ratio of acrylamide to methylenebisacrylamide was 30:1. Protein bands were stained with 0.1% Coomassie Brillant Blue G250 in 12.5% trichloroacetic acid. Relative molecular masses (M_rs) of polypeptide bands were estimated by SDS-PAGE using the following standard proteins: bovine serum albumin (M_r 67 kDa), egg albumin (M_r 45 kDa), myoglobin (M_r 17.8 kDa) and cytochrome C (M_r 12.3 kDa) from "SERVA" as well as carbonic anhydrase (M_r 29 kDa) and glyceraldehyde-3-phosphate dehydrogenase (M_r 36 kDa) from "SIGMA".

Registration of the recorded variation: Band homology within and between species was established as described in the previous paper (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995). The detected 38 well-defined and reproducible bands were numbered according to decreasing M_r values (Table 2). Because of errors in M_r estimations by SDS-PAGE (AIKEN, GARDINER 1991), the presented values should be regarded as approximate.

Statistical analysis: A statistical analysis of the obtained data was performed using the method described in MARDIA et al. (1979).

For 18 considered taxa and all 38 bands distinguished in the investigated material the frequencies of individuals falling into two classes (band present, band absent) were calculated. For all pairs of taxa, distances between taxa were calculated using the formula

$$d_{ij} = \sum d_{ij}^{(k)}$$

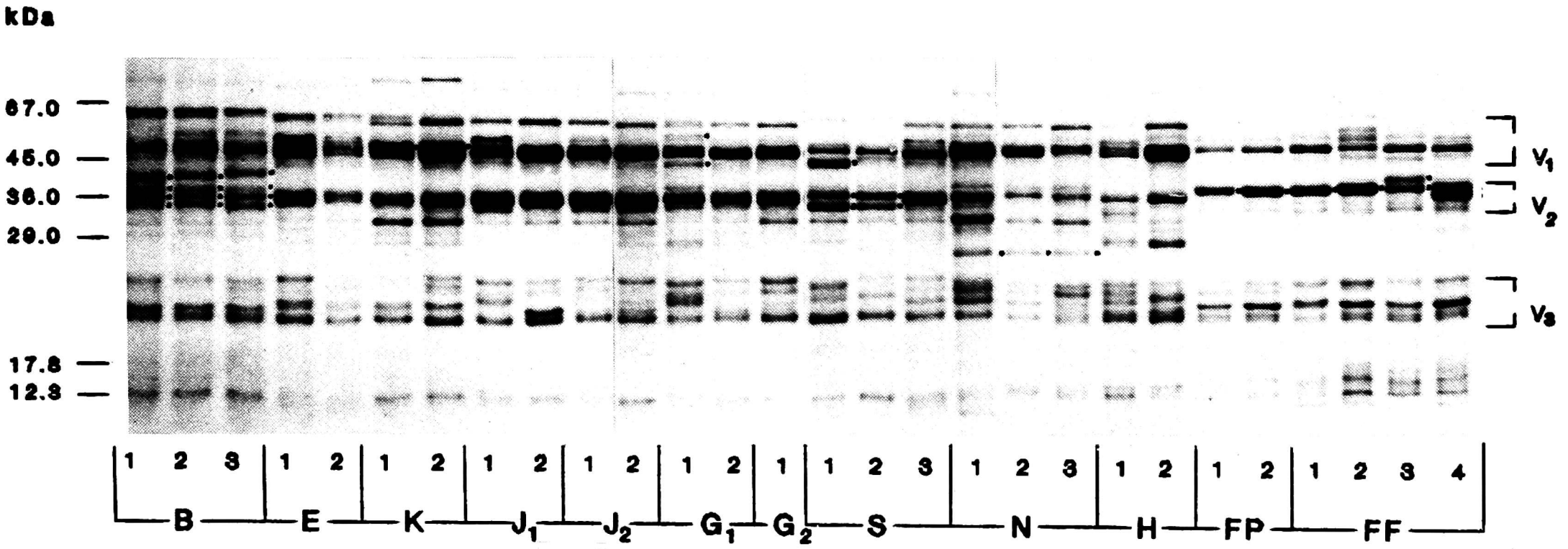

Fig. 1. SDS-PAGE patterns of seed globulins illustrating an overall variation detected in the investigated taxa of the *Vicia* section *Faba*. V1, V2, V3 – variant zones. Bands characteristic of particular species or groups of accessions are marked with dots. For designation of the taxa see Table 2. The presented patterns were produced by individuals of the indicated accessions. B: 1 – VIC 793/a, 2 – VIC 306/a, 3 – VIC 855/a; E: 1-2 – 877321/b; K: 1 – 867166/d, 2 – 867095/d; J1: 1 – NAR 53/a, 2 – 877200/b; J2: 1 – NAR 45/a, 2 – 867491/b; G1: 1 – L2/d, 2 – NAR 137/a; G2: 1 – NAR 132/a; S: 1 – NAR 141/a, 2 – NAR 123/a, 3 – 810194/d; N: 1 – 7/d, 2 – NAR 126/a, 3 – NAR 9/a; H: 1 – 867093/d, 2 – 867152/b; FP: 1-2 – 172/g; FF: 1 – BR 25533/e, 2 – ILB 2930/i, 3 – BR 4444/e, 4 – 244345/f

where $d_{ij}^{(k)}$ denotes Bhattacharyya distance between taxa i and j, calculated for k-th band. For k-th band, the Bhattacharyya distance $d_{ij}^{(k)}$ between taxa was calculated from the frequencies $x_{i1}^{(k)}$, $x_{i2}^{(k)}$ and $x_{j1}^{(k)}$, $x_{j2}^{(k)}$ according to the formula

$$d_{ij} = \sum_{r=1}^{2} [(x_{ir}^{(k)})^{1/2} - (x_{jr}^{(k)})^{1/2}]^2.$$

Thus, the distance used in the analysis measures differences in the frequencies of individuals characterized by the presence/absence of particular bands. The final measure of distance between taxa was calculated as a sum of distances resulting from all bands. On the basis of these distances, a hierarchical clustering was performed using the group average method, and a dendrogram was drawn to illustrate relationships between the examined taxa.

Results

Figure 1 illustrates an overall variation of SDS-PAGE seed globulin patterns detected in the investigated *Vicia* species. It may be seen that well-defined polypeptide bands tend to form three variant zones, V_1, V_2 and V_3, in approximate M_r ranges of 45-61 kDa (V_1), 36-41 kDa (V_2) and 19-25 kDa (V_3). According to other authors' data reported for *Vicia faba,* major polypeptides in the above variant zones apparently correspond to vicilin (V_1), α-legumin (V_2) and β-legumin (V_3) subunits (MATTA et al. 1981, MÜNTZ 1986, PASQUALINI et al. 1991, TUCCI et al. 1991). In some of the examined taxa, well-defined characteristic bands were recorded outside the indicated variant zones. It should be added that differences between the investigated species were detected mainly in the zones V_1 and V_2. As regards zone V_3, individual variation within the accessions often blurred interspecific differences. Totally, 38 polypeptide bands could be distinguished in the investigated material. Their distribution in the considered taxa is shown in Table 2.

SDS-PAGE seed globulin patterns in the investigated *Vicia species*

Of the investigated species, *V. bithynica* could easily be distinguished due to a species-specific triplet of polypeptide bands numbered 20, 23 and 24, in the approx. M_r range of 36-40 kDa. Differences between the examined accessions concerned the occurrence of bands 15, 16 and 17 with approx. M_r values ranging from 41 to 43 kDa; in each accession only one of these bands was recorded. It should be added that bands 15-17 were not observed in other

Table 2. Distribution of globulin polypeptide bands (GPB) in the investigated taxa of *Vicia* section *Faba*: *V. bithynica* (B); *V. eristalioides* (E); *V. kalakhensis* (K); *V. johannis* (J) var. *procumbens* (J1), var. *johannis* (J2); *V. galilaea* (G) var. *galilaea* (G1), var. *faboidea* (G2); *V. serratifolia* (S); *V. narbonensis* (N) var. *salmonea* (N1), var. *jordanica* (N2), var. *aegyptiaca* (N3), var. *affinis* (N4) and var. *narbonensis* (N5); *V. hyaeniscyamus* (H); *V. faba* subsp. *paucijuga* (FP); *V. faba* subsp. *faba* (FF) var. *minor* (FF1), var. *equina* (FF2) and var. *major* (FF3).

GPB		Taxa																	
Band No.	M_r (kDa)	B	E	K	J1	J2	G1	G2	S	N1	N2	N3	N4	N5	H	FP	FF1	FF2	FF3
1	60.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
2	59.6	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
3	59.1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	57.0	–	+	+	–	+	+	+	–	+	+	–	–	–	–	–	–	–	–
5	56.6	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–
6	55.5	–	–	+	–	–	+	–	–	–	+	+	+	+	+	–	–	–	–
7	54.3	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	–	–
8	53.0	–	–	–	–	–	+	+	+	–	–	–	–	–	+	+	+	+	+
9	52.6	–	+	–	+	+	+	+	–	–	–	–	–	–	+	+	+	+	+
10	50.5	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	48.8	+	+	–	–	+	–	–	+	+	+	+	+	+	+	+	+	+	+
12	48.0	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
13	47.3	+	–	–	+	–	+	–	+	–	–	–	–	–	–	+	+	+	+
14	45.4	–	+	+	–	–	+	–	+	–	–	–	–	–	+	–	–	–	–
15	42.6	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	42.0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	41.4	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Band No.	M_r (kDa)	B	E	K	J1	J2	G1	G2	S	N1	N2	N3	N4	N5	H	FP	FF1	FF2	FF3
18	41.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
19	40.5	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
20	39.9	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–
21	39.5	–	–	–	–	–	–	–	–	+	+	+	–	+	–	+	+	+	+
22	39.0	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
23	38.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–
24	36.3	+	–	–	+	+	–	–	+	–	–	–	–	–	+	+	+	+	+
25	34.1	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	30.2	–	+	+	–	–	+	+	+	–	–	–	–	–	+	–	–	–	–
27	27.6	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
28	24.5	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–
29	24.0	+	–	–	+	+	+	–	+	–	+	–	–	–	+	+	+	+	+
30	23.4	–	–	+	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+
31	23.1	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
32	22.9	–	–	–	–	+	–	–	+	+	+	+	+	+	+	–	–	–	–
33	22.6	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
34	21.8	+	–	–	+	+	–	–	+	–	+	–	–	–	+	–	+	+	–
35	21.1	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
36	20.7	+	–	–	+	+	+	–	–	+	+	+	+	+	–	–	+	+	+
37	19.5	–	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–
38	19.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ denotes presence, – denotes absence.

species under study. It also seems worth noticing that none of the nine examined accessions showed individual variation in SDS-PAGE seed globulin pattern.

As regards the *V. narbonensis* complex, species included in this group displayed relatively uniform SDS-PAGE seed globulin patterns, showing prominent band No. 23 (M_r ca 38 kDa) as well as band(s) No. 11 and/or No. 12 with M_r ca 48 kDa. Nevertheless, some characteristic differences could be noted.

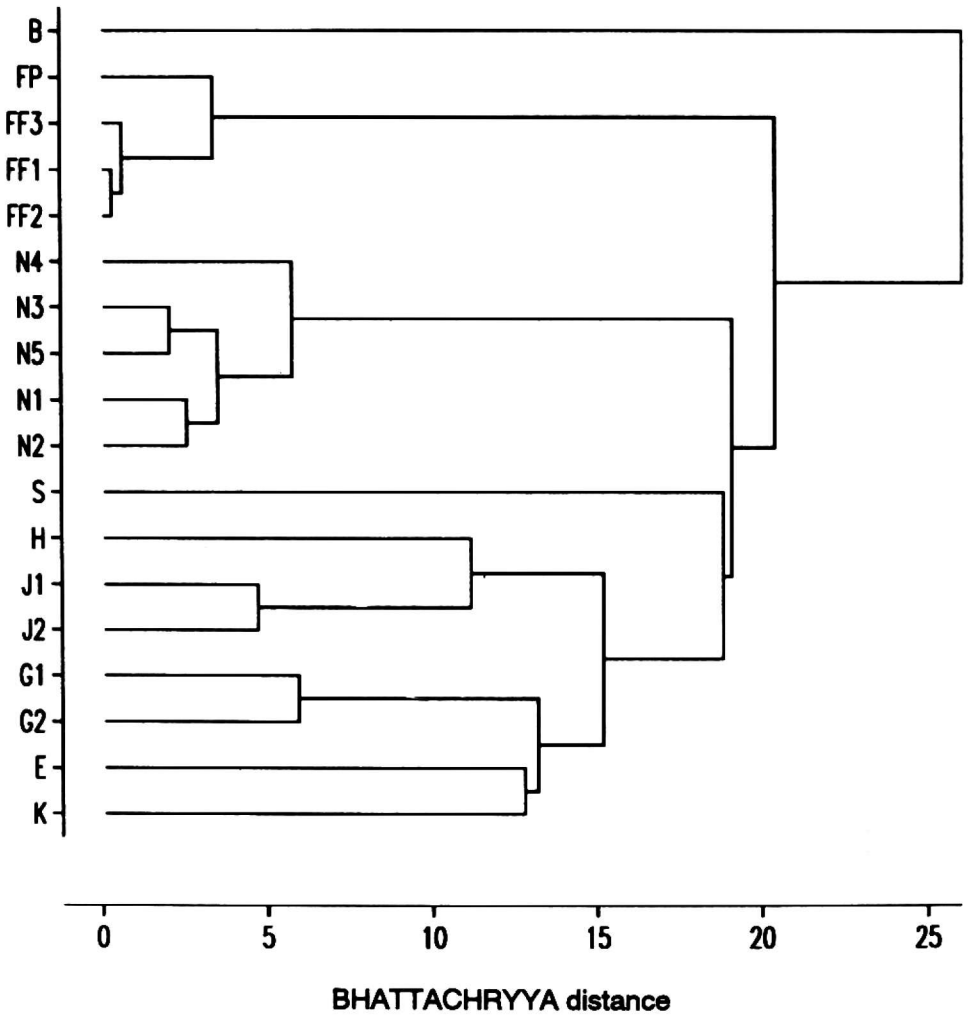

Fig. 2. Hierarchical grouping of the investigated taxa of *Vicia* section *Faba* based on the Bhattacharyya distances calculated from SDS-PAGE seed globulin patterns. Designations of the taxa as in Table 2.

V. narbonensis showed to be the most distinctive species in the *V. narbonensis* complex: all examined individuals of this species displayed a characteristic polypeptide band numbered 27 (M_r ca 28 kDa). A species-specific pattern with characteristic polypeptide band No. 10 (M_r ca 50 kDa) was also found in all analysed individuals of *V. kalakhensis*.

In *V. serratifolia* a marked intraspecific variation was observed. Of nine examined accessions of this species, only the accession 810194/d showed major polypeptide band No. 23 with M_r ca 38 kDa (Fig. 1: S – lane 3), observed as a more or less pronounced band in all species of the *V. narbonensis* complex. The remaining eight accessions under study, instead of band No. 23, displayed polypeptide bands numbered 22 and 24 with approx. M_r 39 and 36 kDa, respectively. Of these eight accessions as many as six forms showed polypeptide band No. 14 with M_r ca 45 kDa (Fig. 1: S – lane 1), not recorded in the remaining three accessions of *V. serratifolia*, but observed as a minor band in *V. eristalioides, V. galilaea, V. kalakhensis* and *V. hyaeniscyamus*. No individual variation was observed in the examined accessions of *V. serratifolia*.

In *V. galilaea*, a slight difference between two botanical varieties could be noticed. In var. *galilaea*, two polypeptide bands not observed in var. *faboidea*, No. 6 (M_r ca 55 kDa) and No. 13 (M_r ca 47 kDa), were recorded. However, one accession classified as var. *galilaea* (NAR 137/a) produced SDS-PAGE seed globulin pattern lacking the above bands.

The cultivated species *V. faba* displayed characteristic prominent band No. 21 with approx. M_r 39.5 kDa. The above band was not recorded only in 3 of 241 examined individuals. These 3 individuals – from different accessions – showed a distinctive pattern with two major bands: No. 20 with M_r ca 40 kDa and No. 23 with M_r ca 38 kDa (Fig. 1: FF – lane 4). About 30% of individuals representing subsp. *faba* showed band No. 18 with M_r ca 41 kDa (Fig. 1: FF – lane 3) not detected in other investigated taxa. SDS-PAGE seed globulin patterns did not discriminate between two subspecies of *V. faba*. In the subsp. *faba*, no variation related either to subdivision of this taxon or to geographic origin of the investigated accessions was observed.

Interspecific relationships revealed by SDS-PAGE seed globulin patterns

The results of hierarchical grouping of the investigated taxa, based on Bhattacharyya distances calculated from electrophoretic data as described in "Material and methods", are presented in Fig. 2.

The dendrogram clearly shows that *V. bithynica* is different from other members of *Vicia* section *Faba*. Next, *V. faba* – represented by subsp. *paucijuga* and 3 varieties of subsp. *faba* – appears to be distinct from members of the *V. narbonensis* complex, which form two groups. Five closely associated varieties of *V. narbonensis* constitute one group. Another group contains taxa whose relationships vary in a relatively wide range. *V. serratifolia* is a peripheral member of this group, remotely related to *V. eristalioides, V. galilaea, V. hyaeniscyamus, V. johannis* and *V. kalakhensis*. Two varieties of *V. johannis*

cluster with *V. hyaeniscyamus*, while two varieties of *V. galilaea* form a cluster with two rather distantly related species – *V. eristalioides* and *V. kalakhensis*.

Discussion

In spite of extensive systematic investigations of *V. faba* and its relatives, an electrophoretic comparative analysis of seed globulins has not been used to study taxonomic relationships in *Vicia* section *Faba* sensu KUPICHA (1976). Variation in the globulin subunit composition was investigated mainly in *V. faba* (MÜNTZ et al.1986, POLIGNANO et al. 1986, PASQUALINI et al. 1991, TUCCI et al. 1993). Like in the present paper, the detected polymorphism could not be related either to taxonomic units at the subspecies/variety level or to geographic origin of the investigated *V. faba* accessions. ABDALLA and GÜNZEL (1979) reported that urea-PAGE seed protein pattern of one *V. narbonensis* accession was different from patterns produced by *V. faba* accessions. SAMMOUR (1989) performed SDS-PAGE analysis of total seed proteins of various *Vicia* species, including a few forms representing 4 species of the section *Faba*, namely *V. faba, V. bithynica, V. narbonensis* and *V. serratifolia*; the obtained results showed similarity of *V. narbonensis* to *V. faba* and a remarkable resemblance between *V. narbonensis* and *V. serratifolia*.

This work presents electrophoretic seed globulin data obtained for all nine species of the section *Faba*, represented by 173 accessions which have already been examined for seed albumin patterns (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995). Results obtained in this work as well as the corresponding seed albumin data are discussed with special reference to the classification of *Vicia* section *Faba* recently proposed by MAXTED et al. (1991). In this classification – based on a phenetic analysis of several hundred specimens and on a literature review of various systematic investigations – three distinct units are distinguished: A. *V. bithynica* (L.) L., B. seven species of the *V. narbonensis* complex and C. *V. faba* L.

Bhattacharyya distances calculated from both electrophoretic seed albumin and seed globulin data show a marked distinctness of *V. bithynica* and *V. faba* which is consistent with division of the section *Faba* into three groups. However, some comments should be made.

According to MAXTED et al. (1991), *V. faba* is the most distinct unit of the three groups in *Vicia* section *Faba*. This view is not supported by seed globulin data showing *V. bithynica* to be a peripheral member of *Vicia* section *Faba*. The above discrepancy is not surprising. Relationships between *V. bithynica*

and *V. faba*, on the one hand, and the remaining members of *Vicia* section *Faba*, on the other, still seem to be debatable.

Electrophoretic seed albumin patterns showed *V. faba* to be quite distinct from other species of *Vicia* section *Faba*. However, a statistical analysis of the albumin data, which took into account not only the presence but also the relative staining intensity of particular bands, showed *V. faba* to be associated with *V. kalakhensis*. This association, though rather remote, is not consistent with other taxonomic evidence including seed globulin data presented in this paper. As mentioned before, it remains to be checked whether the major albumin band apparently responsible for grouping *V. faba* with *V. kalakhensis* represents the same protein in the two species (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995).

As regards interspecific relationships within the *V. narbonensis* complex, data from the electrophoretic analysis of seed albumins and from that of seed globulins are generally consistent or complementary.

Distinctness of *V. eristalioides*, a peripheral member of the *V. narbonensis* complex according to MAXTED et al. (1991), was definitely more evident in the analysis of seed albumins than in the analysis of seed globulins. On the other hand, SDS-PAGE seed globulin data place *V. kalakhensis* in the *V. narbonensis* complex, in agreement with the classification proposed by MAXTED et al. (1991).

Seed albumin patterns of *V. johannis* and *V. galilaea* showed a marked intraspecific variation, not so evident in the SDS-PAGE seed globulin patterns. Nevertheless, the distinctness of one *V. galilaea* accession, namely NAR 137/a labelled var. *galilaea*, revealed in the analysis of seed albumins was confirmed by the globulin data. According to the electrophoretic seed protein patterns, the mentioned accession represents rather var. *faboidea* than var. *galilaea*. A specific overlap between *V. johannis* and *V. galilaea*, shown in a phenetic analysis reported by MAXTED et al. (1991), was found only in the analysis of seed albumins. It should be added here that no other specific overlap between different species of the section *Faba* was recorded either in the analysis of albumins or globulins.

Our data do not confirm the resemblance between seed protein patterns of *V. narbonensis* and *V. serratifolia* reported by SAMMOUR (1989). To the contrary, results of the electrophoretic analysis of both seed albumins and seed globulins clearly indicate distinctness of the two species. This observation is worth emphasizing since *V. narbonensis* and *V. serratifolia* are closely related and some authors consider the latter taxon a subspecies or a variety of *V. narbonesis* (see the reviews: MAXTED et al. 1991, SCHÄFER 1973). Some specific

overlap between *V. narbonensis* and *V. serratifolia* in a phenetic analysis reported by MAXTED et al. (1991) confirms their close relationship. Nevertheless, in the proposed classification of *Vicia* section *Faba, V. serratifolia* is considered a distinct species and our electrophoretic seed protein data confirm this status.

A marked intraspecific variation in *V. serratifolia* was recorded in the analysis of both seed albumins and seed globulins. However, distinctness of *V. serratifolia* accessions as a whole was more evident in the latter analysis; SDS-PAGE seed globulin patterns of most of the examined accessions deviated from the corresponding patterns produced by other examined taxa. In this connection it should be mentioned that the RFLP and PCR data reported by VAN DE VEN et al. (1993) showed an "extreme divergence" of two examined *V. serratifolia* accessions and located one of the accessions outside *Vicia* section *Faba*. In view of the above data, further thorough examination of intraspecific variation in *V. serratifolia* seems to be indispensable.

Distinctiveness of *V. hyaeniscyamus* in the *V. narbonensis* complex was rather due to seed albumin than to seed globulin patterns. As regards relationships of this species with other members of *Vicia* section *Faba*, neither albumin or globulin data are consistent with results of a phenetic analysis reported by MAXTED et al. (1991). According to a statistical analysis of the albumin data, *V. hyaeniscyamus* appeared to be more closely related to *V. narbonensis* than to any other member of the *V. narbonensis* complex. A statistical analysis of the globulin data associates the species with *V. johannis* and more remotely with *V. galilaea, V. kalakhensis* and *V. eristalioides*. A phenetic analysis reported by MAXTED et al. (1991) shows *V. hyaeniscyamus* to be most closely related to *V. kalakhensis*.

Data coming from the electrophoretic analysis of seed proteins reported in this and in the previous paper (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995) contribute to a better knowledge of intraspecific variation within the members of *Vicia* section *Faba*. Moreover, they provide some additional data concerning interspecific relationships in this section. Here, clear distinction between *V. narbonensis* and *V. serratifolia* should be mentioned for the above given reasons.

Unfortunately, neither seed albumin nor seed globulin data helped in revealing a wild progenitor of the cultivated species *V. faba*. Obviously, the investigated material did not include such a progenitor. According to MAXTED (1992), a faba bean progenitor has not been identified yet and future collection missions should concentrate on collecting *Vicia* material in the Central Asian republics of the former Soviet Union. Such collections are suggested by the

discovery of two aberrant *V. narbonensis* specimens with introgressive characteristics of the faba bean (MAXTED 1992). Moreover, some attention should be given to a thorough characterization of the *Vicia* species included in the sections *Hypechusa* and *Peregrinae*. According to molecular DNA data reported by VAN DE VEN et al. (1993), *V. faba* is more closely related to species from the sections *Hypechusa* and *Peregrinae* than to those of the *V. narbonensis* complex.

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