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Trypsin inhibitor electrophoretic patterns in *Vicia faba* L. and related species

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Abstract. The studied material covered 58 accessions representing *Vicia faba* of section *Faba*, *V. bithynica* of section *Bithynicae* and seven species of section *Narbonensis*. Proteins of individual seeds were separated by polyacrylamide gel electrophoresis and the gels were stained for inhibitory activity against trypsin. The number of trypsin inhibitor (TI) bands recorded in particular species varied from three in *V. eristalioides* and *V. galilaea* to 15 in *V. narbonensis*; in total, 30 bands were distinguished in the examined material. Except for *V. eristalioides*, the studied species showed intraspecific variation with respect to electrophoretic TI patterns. A statistical analysis of the data, using hierarchical UPGMA grouping, resolved the studied taxa into three main clusters. *V. faba* subspecies/varieties formed one cluster. The second cluster consisted of *V. narbonensis* varieties and the rather distantly related *V. eristalioides*. The third cluster comprised *V. bithynica* as a peripheral species and the more closely associated *V. galilaea*, *V. hyaeniscyamus*, *V. johannis*, *V. kalakhensis* and *V. serratifolia*. The obtained results are discussed with reference to taxonomic relationships of the species under study.

Key words: electrophoresis, section *Bithynicae*, section *Faba*, section *Narbonensis*, taxonomy, trypsin inhibitor patterns, *Vicia*.

Introduction

The faba bean, *Vicia faba* L., is an Old-World pulse cultivated since prehistoric times from India to the Western Mediterranean countries (CUBERO 1973). However, there is no general agreement concerning its domestication centre, and its wild ancestor is unknown.

Vicia faba and eight related wild species – the most plausible ancestors of the faba bean – were formerly placed in section *Faba* sensu Kupicha (KUPICHA

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1976, MAXTED et al. 1991). Recently MAXTED (1995) recognized *V. faba* as the only species of section *Faba*, and included wild species of the former section *Faba* in two sections: *V. bithynica* in section *Bithynicae*, and the remaining species in section *Narbonensis*. The latter section comprises the following species of the so-called *V. narbonensis* complex: *V. eristalioides, V. kalakhensis, V. johannis, V. galilaea, V. serratifolia, V. narbonensis* and *V. hyaeniscyamus*.

Several research groups carried out biochemical comparative studies to get a better knowledge of relationships between *V. faba* and its allies. Our group performed electrophoretic analysis of isoenzyme variation in these taxa (PRZYBYLSKA et al. 1992, 1998) as well as a comparative electrophoretic analysis of seed albumins (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995) and globulins (ZIMNIAK-PRZYBYLSKA, PRZYBYLSKA 1995). This paper presents a further step of the comparative protein studies, namely the results of an electrophoretic analysis of trypsin inhibitors (TIs) in the species considered.

Materials and methods

Plant material

The study covered 58 *Vicia* accessions representing *Vicia faba* of section *Faba* and eight species of the related sections *Bithynicae* and *Narbonensis* (MAXTED 1995). The accessions were obtained from the following sources: A – Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany (28 accessions); B – The Vicieae Germplasm Collection, Department of Biology, University of Southampton, Southampton, U.K. (12 accessions); C – Dr. G. Ramsay, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland (9 accessions); D – International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (6 accessions); E – Dr. J.I. Cubero, Departamento de Genetica, Universidad de Cordoba, Cordoba, Spain (1 accession); F – Germplasm Institute, C.N.R., Bari, Italy (1 accession); G – National Department of Plant Genetic Resources, Plant Breeding and Acclimatization Institute, Radzików n/Warsaw, Poland (1 accession). A general characterization of the studied material is given in Table 1.

Original seed samples were analysed. At least four individuals of each accession were examined separately.

In the description of the results, some particular accessions are indicated by a catalogue number, donor's designation (A-G) and geographical origin, if known. Both in the text and in Table 1 country codes are used (LIPMAN et al. 1996).

Analytical techniques

Seed protein extracts were obtained using 0.15 M acetate buffer, pH 4.6, as described earlier (PRZYBYLSKA et al. 1977).

Table 1. Information on the examined *Vicia* accessions. The taxa are listed according to MAXTED (1995)

Taxon	Taxa symbols	Accessions		
		Number[1]	Geographical origin[2]	Source[3]
Section *Bithynicae* (B. FEDTSCH. ex RADZHI) MAXTED				
V. bithynica (L.) L.	B	5 (42)	FRA 1, GRC 1, ITA 2, SYR 1	A, B
Section *Narbonensis* (B. FEDTSCH. ex RADZHI) MAXTED				
A. Series *Rhombocarpae* MAXTED				
V. eristalioides MAXTED	E	1 (20)	TUR 1	B
B. Series *Narbonensis* (B. FEDTSCH. ex RADZHI) MAXTED				
V. kalakhensis KHATTAB, MAXTED & BISBY	K	4 (21)	SYR 4	B, C
V. johannis TAMAMSCHJAN in KARYAGIN	J	7 (28)		
var. *procumbens* H. SCHÄFER	J1	3 (12)	GEO 1, TUR 2	A, C
var. *johannis*	J2	3 (12)	SYR 1, TUR 1, UZB 1	A, B
unknown		1 (4)	TUR 1	A
V. galilaea PLITM. & ZOH. in PLITM.	G	5 (33)		
var. *galilaea*	G1	2 (15)	ISR 2	A, C
var. *faboidea* (PLITM. & ZOH. in PLITM.) H. SCHÄFER	G2	3 (18)	ISR 1, UZB 1, unknown 1	A, C
V. serratifolia JACQ.	S	5 (20)	ESP 1, FRA 1, MLT 1, TUR 1, unknown 1	A, B, C
V. narbonensis L.	N	15 (80)		
var. *salmonea* (MOUT.) H. SCHÄFER	N1	3 (20)	ISR 2, SYR 1	A, B
var. *jordanica* H. SCHÄFER	N2	3 (15)	ISR 1, SYR 1, TUR 1	A, B
var. *affinis* KORNHUBER ex ASCH. & SCHWEINF.	N3	3 (15)	ESP 1, TUN 1, TUR 1	A
var. *aegyptiaca* KORNHUBER ex ASCH. & SCHWEINF.	N4	3 (14)	ESP 1, TUR 2	A, B
var. *narbonensis*	N5	3 (16)	ITA 1, PRT 1, ROM 1	A
V. hyaeniscyamus MOUT.	H	5 (33)	SYR 5	B, C
Section *Faba* (MILLER) LEDEB.				
V. faba L.	F	11 (66)		
subsp. *paucijuga* MURAT.	F1	2 (14)	PAK 1, unknown 1	E, F
subsp. *faba*		9 (52)		
var. minor BECK	F2	3 (20)	ESP 1, IND 1, SDN 1	A, D
var. *equina* PERS.	F3	3 (18)	JOR 1, MAR 1, SDN 1	D
var. *faba*	F4	3 (14)	ITA 1, SYR 1, TUR 1	D, G

[1]Numbers of examined individuals are indicated in parentheses.
[2]Numbers of accessions of a given geographical origin.
[3]For key to the seed sources see: Plant material.

Polyacrylamide gel electrophoresis was conducted in slab gels in a discontinuous buffer system according to DAVIS (1964). The acrylamide concentration in the separation gel was 10% and the ratio of acrylamide to methylenebisacrylamide was 20 : 1. Samples corresponding to 4-8 mg of seed meal were applied to the gel. The gels were stained for TI activity using the method of URIEL and BERGES (1968).

Homology of TI bands within and between species was established as reported previously (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995). Only reproducible bands were recorded.

Statistical analysis

Similarity coefficients among the studied taxa, based on EUCLIDEAN distances calculated from frequencies of TI bands, were used for UPGMA hierarchical grouping of the taxa and for constructing the dendrogram.

Results

The detected variation in TI banding patterns in the studied species is shown in Figure 1. The patterns were produced by protein extracts corresponding to 4 mg of seed meal. However, no qualitative changes in the patterns were observed when 2-fold increased amounts of samples were applied to gels. It should be noted that some bands with low intensities could only be observed in the gels and are not visible in the photograph. The recorded TI bands are listed in Table 2.

Intraspecific variation

Variation in TI patterns was observed within all the studied *Vicia* species except for *V. eristalioides* represented by only one accession. Individual variation partly blurred the differences among the examined accessions. Nevertheless, some data concerning intraspecific variation should be mentioned.

In *V. bithynica,* the accession VIC 793/A/ITA could be distinguished by a TI pattern with the characteristic band with R_f of 0.34 (Figure 1B – pattern 4).

Two botanical varieties of *V. johannis*, var. *procumbens* and var. *johannis*, displayed similar TI patterns, which is reflected in the cluster analysis (Figure 2). In both varieties patterns 1 and 3 were observed (Figure 1J). Of the seven accessions examined, only the accession 867491/B/SYR, labelled var. *johannis,* showed pattern 2.

In *V. galilaea* two TI patterns were recorded (Figure 1G). Pattern 1 was produced by one accession of var. *galilaea*, NAR 44/A/ISR, and pattern 2 was found both in var. *galilaea* and in var. *faboidea*. In the cluster analysis of the examined taxa, var. *galilaea* and var. *faboidea* group with different species (Figure 2).

Of the five studied *V. serratifolia* accessions as many as three showed characteristic TI patterns. Patterns 1, 4 and 5 (Figure 1S) were characteristic of the accessions NAR 123/A/FRA, NAR 120/A/TUR and 810194/C, respectively.

In most of the examined *V. narbonensis* accessions a marked individual variation was observed. No particular accession showed a distinctive TI pattern. According to the electrophoretic TI data, accessions representing different botanical varieties grouped together in the cluster analysis (Figure 2).

V. faba accessions showed a marked heterogeneity except for two accessions of subsp. *paucijuga*. Different subspecies/varieties displayed similar TI patterns and formed one cluster (Figure 2).

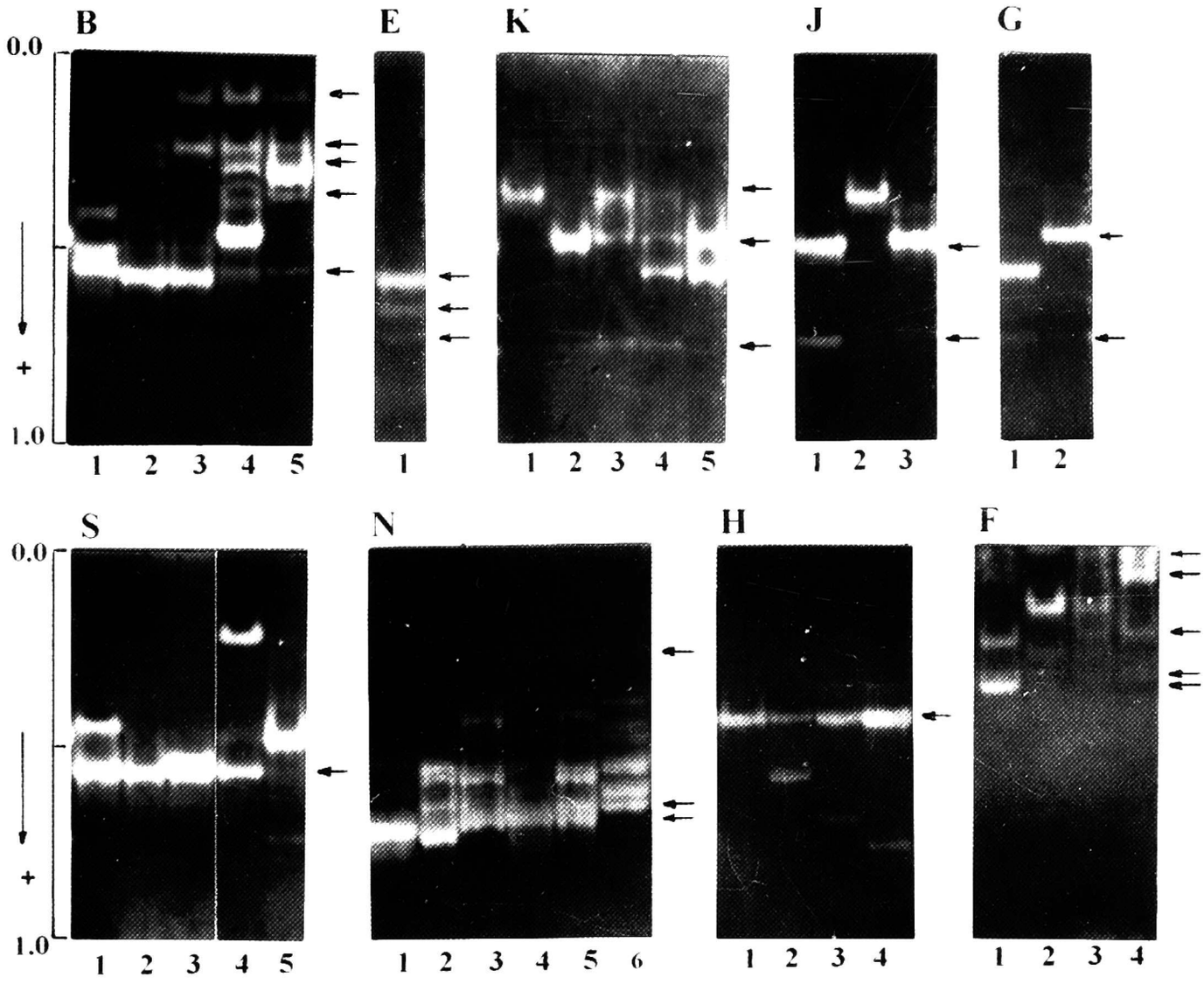

Figure 1. Gels illustrating variation in electrophoretic patterns of trypsin inhibitors in the studied *Vicia* species. For species symbols see Table 1. Bands recorded in at least 50% of the examined individuals of a given species are indicated with arrows.

Table 2. Frequency of the trypsin inhibitor (TI) bands recorded in the studied *Vicia* taxa. The values present percentages of individuals showing particular bands

TI bands		Taxa[1]																
No.	R_f value	B	E	K	J1	J2	G1	G2	S	N1	N2	N3	N4	N5	H	F1	F2	F3	F4
1	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100
2	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	57	60	33	50
3	0.14	81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	67	21
5	0.22	0	0	0	0	0	0	0	20	0	0	0	0	0	0	100	45	56	86
6	0.25	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45	44	0
8	0.29	71	0	0	0	0	0	0	0	100	100	100	36	100	0	0	0	0	0
9	0.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	60	11	86
10	0.32	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	40	67	21
12	0.34	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0.38	57	0	81	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0.39	19	0	0	0	0	0	0	0	25	33	7	36	56	0	0	0	0	0
15	0.43	17	0	0	0	0	0	0	0	0	53	67	36	19	0	0	0	0	0
16	0.45	0	0	0	0	0	0	0	20	40	13	73	64	0	0	0	0	0	0
17	0.46	38	0	67	33	33	47	100	0	0	0	0	0	0	100	0	0	0	0
18	0.47	0	0	0	67	33	0	0	20	0	0	0	0	0	0	0	0	0	0
19	0.50	33	0	0	0	0	0	0	0	30	67	0	36	0	0	0	0	0	0
20	0.53	0	0	0	0	0	0	0	35	0	0	0	0	0	0	0	0	0	0
21	0.55	62	0	43	0	0	53	0	50	15	0	0	0	0	0	0	0	0	0
22	0.58	38	100	0	0	0	0	0	30	75	67	33	93	75	0	0	0	0	0
23	0.59	0	0	0	0	0	0	0	0	0	0	47	0	0	0	0	0	0	0
24	0.60	0	0	0	0	0	0	0	0	30	73	20	36	69	0	0	0	0	0
25	0.62	0	0	0	0	0	0	0	0	0	33	67	0	0	6	0	0	0	0
26	0.65	0	100	0	0	0	0	0	0	30	80	67	36	69	0	0	0	0	0
27	0.68	0	0	0	0	0	0	0	0	55	40	67	29	81	0	0	0	0	0
28	0.70	0	100	0	0	0	0	0	0	35	47	33	64	0	0	0	0	0	0
29	0.73	0	0	71	100	100	100	100	20	30	13	0	0	0	33	0	0	0	0
30	0.75	0	0	0	0	0	0	0	0	15	0	0	0	0	12	0	0	0	0

[1] For taxa symbols see Table 1.

Interspecific relationships

Frequency of TI bands recorded in the studied taxa is shown in Table 2. The data presented reveal characteristic features of some of the examined species. A marked distinctness of *V. faba* is due to a group of slow-moving bands with low

intensities, with R_f values in the range of 0.02-0.33. Of seven bands observed in this species only one band (R_f 0.22) had its counterpart in another species, namely *V. serratifolia*. *V. bithynica* showed frequent occurrence of two bands (R_f 0.14, 0.25), not observed in the remaining species under study. A set of three bands with R_f values above 0.55 was characteristic of *V. eristalioides*. A group of fast-moving bands, with R_f values in the range of 0.58-0.70, may be regarded as a species-specific feature of *V. narbonensis*.

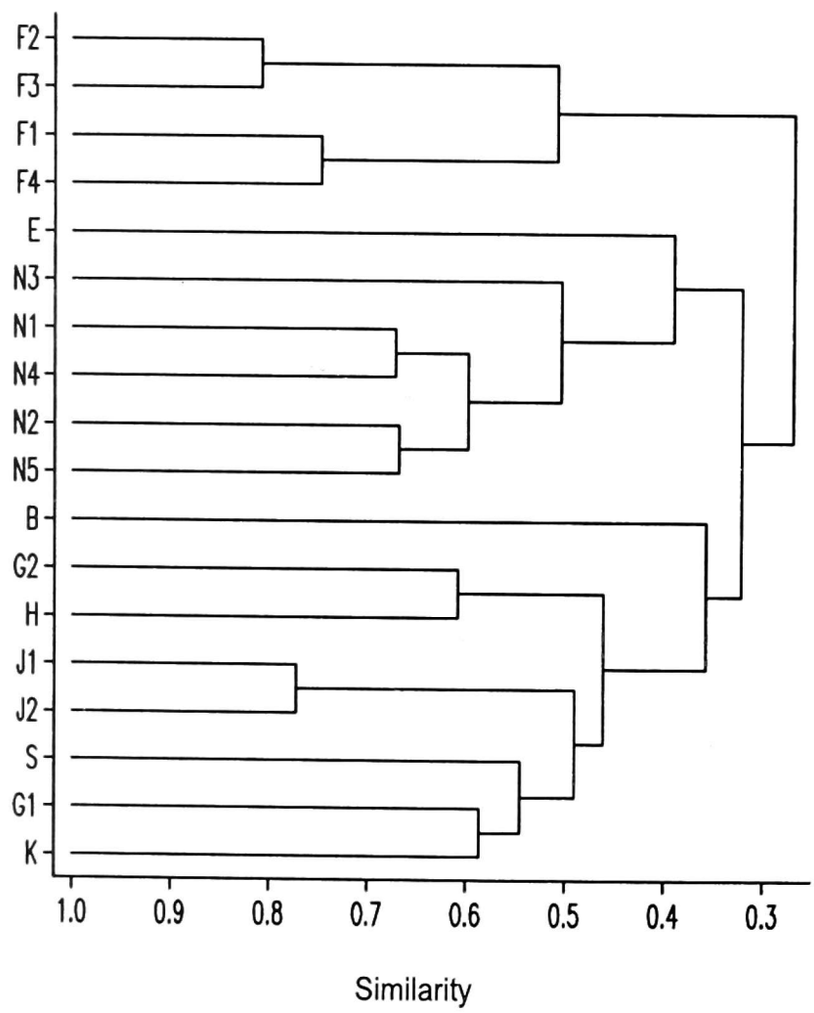

Figure 2. Hierarchical grouping of the studied *Vicia* taxa based on the EUCLIDEAN distances calculated from electrophoretic trypsin inhibitor data. For taxa symbols see Table 1

V. galilaea, *V. hyaeniscyamus*, *V. johannis*, *V. kalakhensis* and *V. serratifolia* exhibited a relatively large proportion of shared TI bands; of 11 bands detected in these species as many as five bands were observed in at least two species. Moreover, some accessions/individuals of different species of this group showed same TI patterns. For example, the *V. serratifolia* accession 810194/C produced a pattern (Figure 1S – pattern 5) indistinguishable from that displayed by four accessions of *V. johannis* (Figure 1J – pattern 1).

Hierarchical grouping of the investigated taxa, based on EUCLIDEAN distances calculated from the TI electrophoretic data, is presented in Figure 2. The studied taxa form three main clusters. *V. faba* subspecies/varieties form one cluster. The second cluster consists of *V. narbonensis* varieties and rather distantly related *V. eristalioides*. The third cluster comprises *V. bithynica* as a peripheral species and more closely associated *V. galilaea, V. hyaeniscyamus, V. johannis, V. kalakhensis* and *V. serratifolia.*

Discussion

Electrophoretic analysis of proteinase inhibitors is rarely used in legume chemotaxonomic studies but has been appeared to disclose their genetic diversity in soybean, pigeonpea and their wild relatives (KOLLIPARA, HYMOWITZ 1992, KOLLIPARA et al. 1994). In this study an attempt was made to check the usefulness of this approach in studying taxonomic relationships among *Vicia faba* and its relatives. Electrophoretic variants with TI activity were used as biochemical markers and the obtained results were compared with the corresponding data coming from the electrophoretic comparative analysis of seed albumins (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995), seed globulins (ZIMNIAK-PRZYBYLSKA, PRZYBYLSKA 1995) and isoenzyme variation (PRZYBYLSKA et al. 1998).

Electrophoretic analysis of both TIs and other proteins revealed intraspecific variation in all the examined species except for *V. eristalioides* represented by only one accession. Distinctness of some particular accessions was revealed in the analysis of different proteins, e.g., the *V. serratifolia* accession 810194 appeared similar to some other species of section *Narbonensis* in the analysis of seed globulins, isoenzymes and TIs, which is consistent with the results of the study of DNA polymorphism (Van de VEN et al. 1993). On the other hand, the analysis of different proteins provided complementary data as to differences among accessions representing particular species; for instance, *V. galilaea* NAR 44 showed distinctive patterns in the analyses of seed albumins and TIs but was not distinguishable in the analyses of seed globulins and isoenzymes.

In the case of species divided into units of a lower taxonomic rank, the results obtained in the analyses of different proteins are essentially consistent. Two botanical varieties of *V. johannis,* var. *procumbens* and var. *johannis*, appeared to be closely associated irrespective of the type of the analysed proteins. Similar associations were observed in the case of botanical varieties of *V. narbonensis* and of subspecies/varieties of *V. faba*. By contrast, distinctness of two botanical varieties of *V. galilaea,* var. *galilaea* and var. *faboidea*, was shown in the analysis of seed albumins, isoenzymes and TIs.

As regards interrelationships among the studied species, electrophoretic analysis of TIs revealed a marked distinctness of *V. faba* and thus confirmed the results of a comparative analysis of seed albumins (PRZYBYLSKA, ZIMNIAK-PRZY-

BYLSKA 1995, SALMANOWICZ, PRZYBYLSKA 1997), seed globulins (ZIMNIAK-PRZYBYLSKA, PRZYBYLSKA 1995), isoenzymes (JAASKA 1997, PRZYBYLSKA et al. 1998) and DNA (Van de VEN et al. 1993, POTOKINA et al. 1999). All the biochemical data support placing *V. faba* in a separate, monospecific section *Faba* (MAXTED 1995).

Distinctness of *V. bithynica*, the only member of section *Bithynicae*, was more pronounced in the analysis of seed globulins (ZIMNIAK-PRZYBYLSKA, PRZYBYLSKA 1995) and isoenzymes (JAASKA 1997, PRZYBYLSKA et al. 1998) than in the comparative electrophoretic study of seed albumins (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995) and TIs. Nevertheless, both the protein and DNA (POTOKINA et al. 1999) data justify recognizing *V. bithynica* as a sufficiently distinct species to form a separate section *Bithynicae* (MAXTED 1995).

Section *Narbonensis* was divided by MAXTED (1995) into two series: series *Rhombocarpae* with only one species – *V. eristalioides* – and series *Narbonensis* comprising the remaining members of the *V. narbonensis* complex. This division was not reflected in electrophoretic patterns of TIs and other proteins; in the analysis of different proteins *V. eristalioides* grouped with different species of series *Narbonensis*. Here it should be added that the DNA data reported by Van de VEN et al. (1993) provided no basis for discriminating between the two series of section *Narbonensis*.

The electrophoretic analysis of TIs showed *V. narbonensis* to be rather remotely related to other members of series *Narbonensis*, even to the morphologically similar *V. serratifolia*. Distinctness of these two species is consistent with the results of electrophoretic analysis of other proteins performed in our laboratory, the isoenzyme data of JAASKA (1997) and the DNA data of Van de VEN et al. (1993). However, the genetic relationship between *V. narbonensis* and *V. serratifolia* remains controversial. According to our protein data and the DNA data of Van de VEN et al. (1993), the two species do not group together. On the other hand, isoenzyme data of JAASKA (1997) and DNA data of POTOKINA et al. (1999) indicate their close association. The discrepancy in the biochemical data concerning the relationship between *V. narbonensis* and *V. serratifolia* may be due to a marked intraspecific variation within *V. serratifolia* and examination of different accessions of this species in different laboratories. Pronounced differences among the *V. serratifolia* accessions were revealed in the comparative protein studies performed in our laboratory and in the analysis of DNA polymorphism reported by Van de VEN et al. (1993).

Except for *V. narbonensis*, the species of series *Narbonensis* form one cluster in the hierarchical grouping of the investigated taxa based on the TI electrophoretic data. This association is generally in agreement with the results of electrophoretic analysis of other proteins and with the data coming from the analysis of DNA polymorphism (Van de VEN et al. 1993, PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995, ZIMNIAK-PRZYBYLSKA, PRZYBYLSKA, 1995, JAASKA 1997, PRZYBYLSKA et al. 1998, POTOKINA et al. 1999).

Data presented in this paper show that electrophoretic TI variants are useful markers in taxonomic investigations. In the light of the TI and other biochemical data, none of the species considered relatives of *V. faba* may be indicated as its close ally and possible progenitor. HANELT and METTIN (1989) conclude that *V. faba* „...is a cultigen isolated from all related wild taxa by strong reproductive barriers...".

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REFERENCES

CUBERO J.I. (1973). Evolutionary trends in *Vicia faba* L. Theor. Appl. Genet. 43: 59-65.

DAVIS B.J. (1964). Disc electrophoresis - II. Method and application to human serum proteins. Ann. New York Acad. Sci. 121: 404-427.

HANELT P., METTIN D. (1989). Biosystematics of the genus *Vicia* L. (Leguminosae). Ann. Rev. Ecol. Syst. 20: 199-223.

JAASKA V. (1997). Isoenzyme diversity and phylogenetic affinities in *Vicia* subgenus *Vicia* (Fabaceae). Genet. Res. Crop Evol. 44: 557-574.

KOLLIPARA K.P., HYMOWITZ T. (1992). Characterization of trypsin and chymotrypsin inhibitors in the wild perennial *Glycine* species. J. Agric. Food Chem. 40: 2356-2363.

KOLLIPARA K.P., SINGH L., HYMOWITZ T. (1994). Genetic variation of trypsin and chymotrypsin inhibitors in pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives. Theor. Appl. Genet. 88: 986-993.

KUPICHA F.K. (1976). The infrageneric structure of *Vicia*. Notes Roy. Bot. Gard. Edinburg 34: 287-326.

LIPMAN E., JONGEN M.W.M., Van HINTUM Th.J.L., GASS T., MAGGIONI L. (1996). Appendix III. Standard country codes (extended list). Central Crop Databases: Tools for Plant Genetic Resources Management. Report of a Workshop, 13-16 October 1996, Budapest, Hungary. International Plant Genetic Resources Institute: 79-83.

MAXTED N. (1995). An ecogeographical study of *Vicia* subgenus *Vicia*. Systematic and ecogeographic studies on crop genepools. 8. International Plant Genetic Resources Institute, Rome, Italy.

MAXTED N., KHATTAB A.M.A., BISBY F.A. (1991). The newly discovered relatives of *Vicia faba* L. do little to resolve the enigma of its origin. Bot. Chron. 10: 435-465.

POTOKINA E., TOMOOKA N., VAUGHAN D.A., ALEXANDROVA T., XU R.-Q. (1999). Phylogeny of *Vicia* subgenus *Vicia* (Fabaceae) based on analysis of RAPDs and RFLP of PCR-amplified chloroplast genes. Genet. Res. Crop Evol. 46: 149-161.

PRZYBYLSKA J., BLIXT S., HURICH J., ZIMNIAK-PRZYBYLSKA Z. (1977). Comparative study of seed proteins in the genus *Pisum*. I. Electrophoretic patterns of different protein fractions. Genet. Pol. 18: 27-38.

PRZYBYLSKA J., ZIMNIAK-PRZYBYLSKA Z. (1995). Electrophoretic seed albumin patterns and species relationships in *Vicia* sect. *Faba* (Fabaceae). Pl. Syst. Evol. 198: 179-194.

PRZYBYLSKA J., ZIMNIAK-PRZYBYLSKA Z., KRAJEWSKI P. (1992). Isoenzyme variation in the genetic resources of *Vicia faba* L. Genet. Pol. 33: 17-25.

PRZYBYLSKA J., ZIMNIAK-PRZYBYLSKA Z., KRAJEWSKI P. (1998). Isoenzyme variation in the wild relatives of *Vicia faba* (Fabaceae). Pl. Syst. Evol. 213: 173-186.

SALMANOWICZ B.P., PRZYBYLSKA J. (1997). Comparative HPLC analysis of seed albumins from *Vicia faba* and *V. kalakhensis* (Fabaceae). Pl. Syst. Evol. 208: 1-9.

URIEL J., BERGES J. (1968). Characterization of natural inhibitors of trypsin and chymotrypsin by electrophoresis in acrylamide-agarose gels. Nature 218: 578-580.

Van de VEN W.T.G., DUNCAN N., RAMSAY G., PHILLIPS M., POWELL W., WAUGH R. (1993). Taxonomic relationships between *V. faba* and its relatives based on nuclear and mitochondrial RFLPs and PCR analysis. Theor. Appl. Genet. 86: 71-80.

ZIMNIAK-PRZYBYLSKA Z., PRZYBYLSKA J. (1995). Electrophoretic seed globulin patterns and species relationships in *Vicia* section *Faba* (Fabaceae). J. Appl. Genet. 36: 299-312.