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# Electrophoretic seed albumin patterns in the *Vicia sativa* L. aggregate

Janina PRZYBYLSKA, Zdzisława ZIMNIAK-PRZYBYLSKA, Paweł KRAJEWSKI

Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland

**Abstract.** Electrophoretic analysis of seed albumins covered 201 accessions representing six subspecies of *Vicia sativa* L.: *nigra, amphicarpa*, *incisa*, *sativa*, *macrocarpa* and *cordata*. At least five individuals of each accession were examined separately. All the taxa under study showed variation with respect to albumin banding patterns, which was due to differences between accessions and individual variation within accessions. The number of albumin bands distinguished in particular taxa varied from 13 to 19. The statistical analysis of the electrophoretic data consisted in hierarchical grouping by the UPGMA method based on EUCLIDEAN distances. In the case of subsp. *nigra* and subsp. *sativa*, accessions originating from North Africa tended to form a group showing some distinctness from the remaining accessions. As to relationships among the studied taxa, subsp. *nigra* and subsp. *cordata* were shown to be the most closely related and their affinities to other members of the *V. sativa* aggregate were decreasing in the following order: subsp. *macrocarpa*, subsp. *sativa*, subsp. *amphicarpa*, subsp. *incisa*. The obtained results are discussed with reference to taxonomic relationships among the members of the *V. sativa* aggregate.

**Key words:** electrophoresis, genetic resources, seed albumins, taxonomy, variation, vetches, *Vicia sativa*.

## Introduction

The *Vicia sativa* aggregate, comprising a group of closely related autogamous taxa, is morphologically, karyologically and ecologically variable. As reported by HANELT and METTIN (1989), "... it is very difficult to arrange this variability into a formal taxonomic classification". The main controversies concern the rank of the members of the *V. sativa* aggregate. For example, METTIN and HANELT (1964) consider them as separate species, while MAXTED (1995)

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Correspondence: J. PRZYBYLSKA, Institute of Plant Genetics, Polish Academy of Sciences, ul. Strzeszyńska 34, 60-479 Poznań, Poland, email: jprz@igr.poznan.pl.

and LADIZINSKY (1978) treat them, respectively, as subspecies or varieties of *Vicia sativa* L.

In view of the controversial taxonomy of the *V. sativa* aggregate, some comparative biochemical studies have been performed. LADIZINSKY and WAINES (1982) as well as POTOKINA and EGGI (1991) carried out electrophoretic analysis of seed proteins in the aggregate. Later on, JAASKA (1997) and POTOKINA et al. (1999) included the *V. sativa* aggregate in their studies of isoenzyme variation and DNA polymorphism, respectively, in *Vicia* subgenus *Vicia*. The different lines of investigations provided rather inconsistent results as to interrelationships within the *V. sativa* group. This paper is aimed at contributing to taxonomic classification of the *V. sativa* aggregate by electrophoretic analysis of seed albumins. The studied taxa are treated as subspecies of *V. sativa* L. according to MAXTED (1995). Of six subspecies recognized by MAXTED, only subsp. *devia* was not examined because it was not available. On the other hand, subsp. *cordata* – omitted in MAXTED's classification – was included in this study. To acquire a better knowledge on relationships among the members of the *V. sativa* aggregate, much attention was given to variation within the examined taxa, especially within the widely distributed subsp. *nigra* and subsp. *sativa*.

## Material and methods

### Plant material

The study covered 201 accessions representing six subspecies of *Vicia sativa* L.; at least five individuals of each accession were examined separately. Information on the analysed material is given in Table 1. In the description of the results, some particular accessions are indicated by a catalogue number, donor's designation (A-C) and country of origin, if known. Both in the text and in Table 1 country codes are used (LIPMAN et al. 1996).

### Analytical techniques

All techniques used in the study were described by PRZYBYLSKA et al. (1977). Crude protein extracts containing a large proportion of albumins were obtained using 0.15 M acetate buffer, pH 4.6.

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 methylene-bisacrylamide was 20 : 1. Protein bands were detected with 0.1% Coomassie Brillant Blue G 250 in 12.5% trichloroacetic acid.

Homology of albumin bands within and between taxa was established as reported previously (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995).

**Table 1**. Information on the examined accessions of the *Vicia sativa* aggregate

| Subspecies | Abbr[1] | Accessions | | |
|---|---|---|---|---|
| | | Number[2] | Geographical region and country of origin[3] | Donor[4] |
| *nigra* (L.) Ehrh. | Ni | 61 (312) | **S.W. Europe:** ESP 3, FRA 1, PRT 2; **Central Europe:** DEU 1, HUN 1, SVK 1; **S. Europe:** BGR 1; GRC 2, ROM 1; **N. Africa:** DZA 2, EGY 1, TUN 1; **S. Africa:** ZAF 2; **S.W. Asia:** CYP 1, IRN 5, ISR 5, SYR 3, TUR 7, UZB 1; **S. central Asia:** AFG 2, PAK 6; **E. Asia:** JPN 8, TWN 1; **S. America:** ARG 1, URY 1; **Australia:** AUS 1 | A, B |
| *amphicarpa* (L.) Batt. | Am | 11 (59) | **N. Africa:** DZA 1; **S.W.Asia**: JOR 2, SYR 5, TUR 1; **E.Asia**: JPN 1; unknown 1 | B, C |
| *incisa* (M.Bieb.) Arcang. | In | 3 (16) | **Central Europe**: DEU 1; **S.W.Asia**: TUR 1; unknown 1 | B, C |
| *sativa* | Sa | 86 (508) | **W.& S.W. Europe:** ESP 4, FRA 3, GBR 1, PRT 3; **Central Europe:** BEL 2, CSK 4, DEU 5, HUN 4, LTU 1, NDL 1, POL 4, SVK 2; **S.& S.E. Europe:** ALB 1, BGR 7, GRC 9 , ITA 2, ROM 2, YUG 2; **N. Europe:** FIN 1, SWE 1; **N. Africa:** DZA 1, MAR 2, TUN 1; **S.W. Asia:** ARM 1, CYP 5, GEO 1, IRN 3, IRQ 1, ISR 1, JOR 1, SYR 2, TUR 5; **Central Asia:** MNG 1, CHN 1; **E. Asia:** JPN 1 | A, B, C |
| *macrocarpa* (Moris) Arcang. | Ma | 14 (70) | **W.& S.W. Europe**: FRA 1, PRT 1; **S. Europe**: GRC 1, ITA 5; **N. Africa**: DZA 3; **S.W. Asia**: TUR 2; unknown 1 | B, C |
| *cordata* (Wulfen ex Hoppe) Asch. & Graebner | Co | 26 (132) | **W.& S.W. Europe**: ESP 1, FRA 1, PRT 1; **S. Europe**: GRC 3, ITA 13; **N. Africa**: EGY 1, MAR 1; **S.W. Asia:** TUR 3; unknown 2 | B, C |

[1]Abbreviations of the taxa names.
[2] Numbers of examined individuals are indicated in parentheses.
[3] Numbers of accessions from particular countries are indicated.
[4] Donors: A - USDA, ARS, Washington State University, Regional Plant Introduction Station, Pullman, Washington, USA, B - International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, C - Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany.

### Statistical analysis

Accessions of *V. sativa* L. subsp. *nigra* and subsp. *sativa* were classified into groups called populations on the basis of geographical origin. In the statistical analysis only populations represented by at least three accessions were considered. The coefficients of similarity between populations and between the studied taxa, based on EUCLIDEAN distances calculated from frequencies of albumin bands, were used for UPGMA hierarchical grouping of the populations and of the taxa and for constructing dendrograms. All computations were made by Genstat 5 software (Genstat 5 Committee, 1996).

## Results

In the previous investigations of different leguminous plants it was found that electrophoretic screening for seed albumin patterns may be performed using crude protein extracts obtained with acetate buffer, pH 4.6 (PRZYBYLSKA et al. 1977, 1999, PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1993, 1995). Electrophoretic patterns produced by such extracts and the corresponding patterns of albumins isolated by a standard procedure proved to be almost indistinguishable. Therefore, in the present work crude protein extracts were used, which enabled an analysis of more than one thousand individuals. As in the earlier studies, only well-defined and reproducible bands were recorded.

Figure 1 illustrates variation in electrophoretic seed albumin patterns detected in the studied subspecies of *V. sativa*. Frequency distribution of seed albumin bands recorded in the examined taxa is shown in Table 2.

### Variation within the taxa

***V. sativa* subsp. *nigra***. In total, 19 albumin bands were distinguished in subsp. *nigra*; in particular accessions from 4 (PI 532870/A/PAK) to 11 (IFVI 4402/B/DZA) bands were recorded. Of 61 examined accessions, as many as 25 accessions proved to be heterogeneous with respect to electrophoretic seed albumin patterns. The distinctness of some accessions was evident. For example, the band with $R_f$ value of 0.42 seemed to be characteristic of two accessions, IFVI 4753/B/DZA and PI 294277/A/ISR. On the other hand, the commonly occurring band with $R_f$ of 0.54 was not observed in three accessions, PI 221965/A/AFG, PI 532869/A/PAK and PI 532870/A/PAK.

An attempt was made to relate electrophoretic seed albumin data to geographical origin of the studied accessions. Frequencies of seed albumin bands in populations originating from seven geographical regions were subjected to statistical analysis and the obtained results are illustrated in the form of a dendrogram (Figure 2A). Some populations appeared to be closely related: populations from Southwest Europe and from Southwest Asia grouped at 0.78 similarity, and popu-

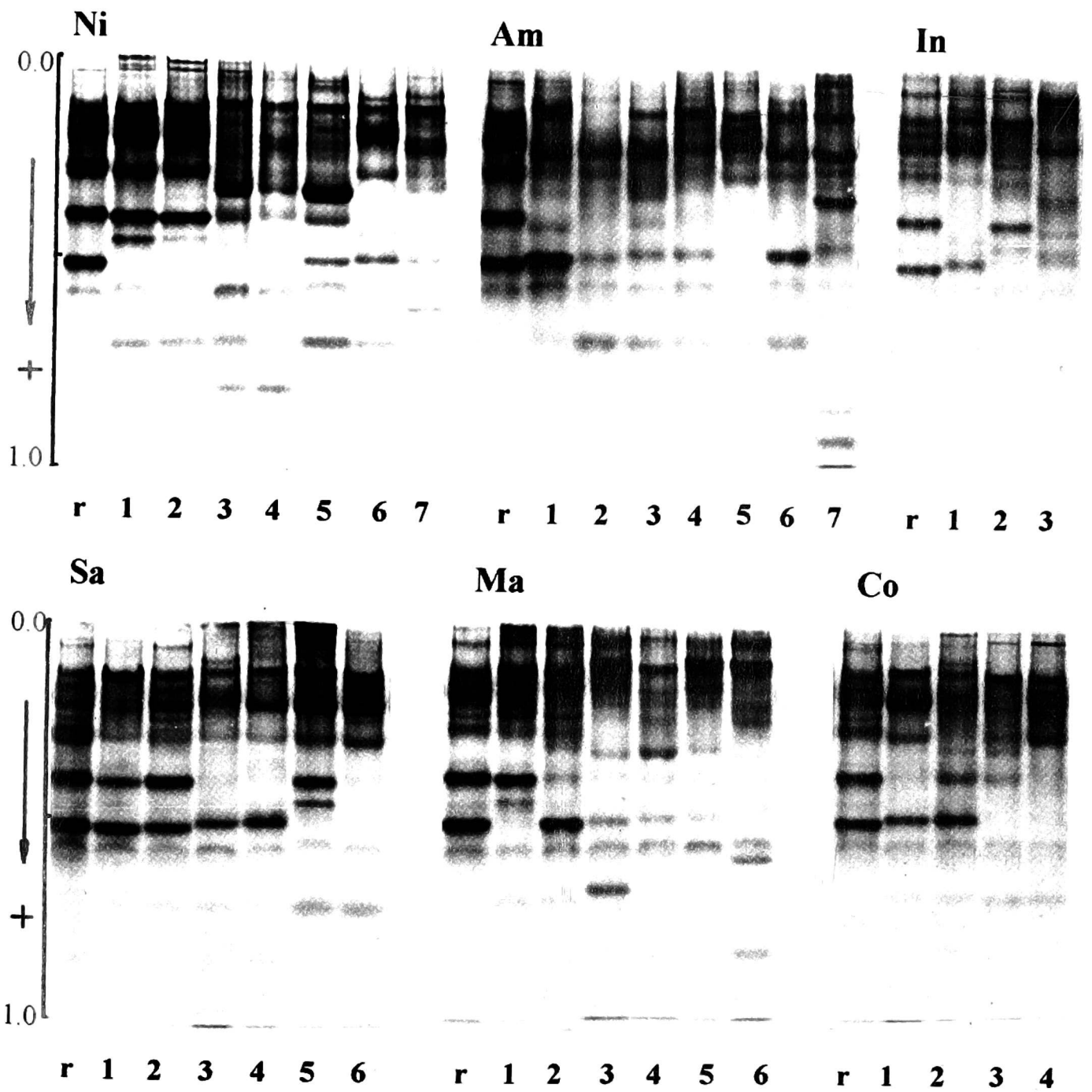


Figure 1. Gels illustrating variation in electrophoretic seed albumin patterns in the studied taxa of the *Vicia sativa* aggregate. Taxa designations as in Table 1. r reference pattern produced by the *V. sativa* subsp. sativa accession, PI 393904/A/BEL.

**Table 2.** Frequency (%) of seed albumin bands in the studied taxa of the *Vicia sativa* aggregate.

| Band | | Taxon[1] | | | | | |
|---|---|---|---|---|---|---|---|
| No. | $R_f$ value | Ni | Am | In | Sa | Ma | Co |
| 1 | 0.09 | 26 | 29 | 0 | 11 | 19 | 20 |
| 2 | 0.10 | 0 | 0 | 0 | 0 | 20 | 0 |
| 3 | 0.12 | 38 | 54 | 0 | 75 | 6 | 18 |
| 4 | 0.13 | 47 | 25 | 31 | 0 | 70 | 63 |
| 5 | 0.14 | 15 | 20 | 38 | 30 | 17 | 20 |
| 6 | 0.16 | 14 | 47 | 13 | 25 | 36 | 0 |
| 7 | 0.18 | 41 | 0 | 31 | 6 | 0 | 49 |
| 8 | 0.19 | 0 | 0 | 31 | 0 | 0 | 30 |
| 9 | 0.20 | 64 | 19 | 69 | 91 | 26 | 86 |
| 10 | 0.21 | 0 | 29 | 0 | 66 | 39 | 0 |
| 11 | 0.22 | 0 | 59 | 0 | 0 | 0 | 0 |
| 12 | 0.23 | 24 | 0 | 38 | 0 | 0 | 0 |
| 13 | 0.27 | 0 | 31 | 0 | 0 | 0 | 0 |
| 14 | 0.28 | 57 | 10 | 0 | 54 | 14 | 98 |
| 15 | 0.33 | 49 | 10 | 100 | 0 | 20 | 0 |
| 16 | 0.38 | 16 | 0 | 0 | 5 | 0 | 0 |
| 17 | 0.39 | 47 | 17 | 25 | 39 | 37 | 11 |
| 18 | 0.40 | 0 | 0 | 31 | 0 | 0 | 0 |
| 19 | 0.42 | 3 | 0 | 31 | 0 | 0 | 0 |
| 20 | 0.44 | 13 | 0 | 25 | 18 | 21 | 0 |
| 21 | 0.47 | 0 | 63 | 0 | 0 | 0 | 0 |
| 22 | 0.48 | 67 | 0 | 63 | 6 | 51 | 92 |
| 23 | 0.49 | 0 | 0 | 0 | 16 | 7 | 2 |
| 24 | 0.51 | 0 | 0 | 0 | 2 | 0 | 0 |
| 25 | 0.54 | 93 | 88 | 100 | 96 | 93 | 74 |
| 26 | 0.56 | 18 | 14 | 0 | 0 | 14 | 0 |
| 27 | 0.65 | 0 | 0 | 0 | 0 | 26 | 0 |
| 28 | 0.68 | 72 | 54 | 69 | 55 | 21 | 30 |
| 29 | 0.82 | 19 | 0 | 0 | 0 | 64 | 0 |
| 30 | 0.83 | 0 | 10 | 0 | 0 | 0 | 0 |
| 31 | 0.92 | 0 | 10 | 0 | 0 | 0 | 0 |

[1] Taxa designations as in Table 1.

lations from South central Asia and from East Asia clustered at 0.74. The population from North Africa was the most distant and joined the remaining populations at 0.42 similarity.

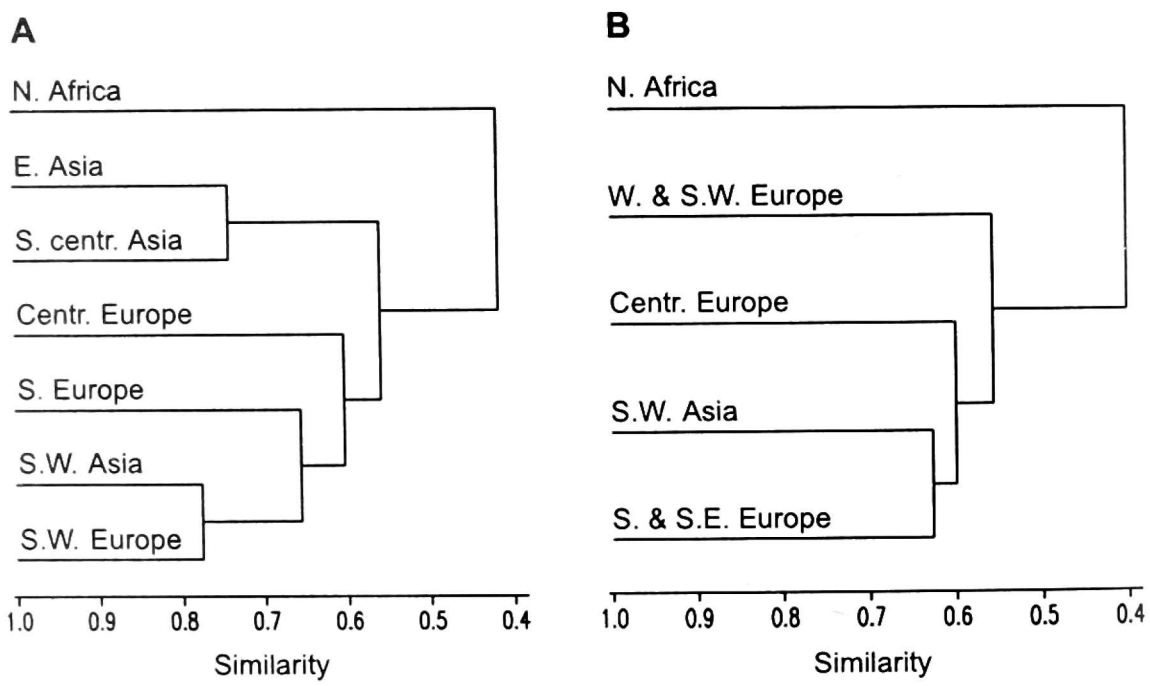


Figure 2. UPGMA dendrograms of *Vicia sativa* subsp. *nigra* (A) and subsp. *sativa* (B) populations from different geographical regions, based on EUCLIDEAN distances calculated from frequencies of electrophoretic seed albumin bands.

***V. sativa* subsp. *amphicarpa***. Eighteen seed albumin bands were recorded in the 11 examined accessions of subsp. *amphicarpa*; particular accessions showed from 5 (IFVI 4322/B/JOR, IFVI 2599/B/JPN) to 9 (IFVI 4539/B/DZA, VIC 724/C) bands. Individual variation was detected in 7 accessions. The distinctness of the accession IFVI 4233/B/JOR was especially marked since this accession showed 3 bands ($R_f$ 0.33, 0.83, 0.92) absent in other forms, and lacked one band ($R_f$ 0.54) which was present in all the remaining accessions.

***V. sativa* subsp. *incisa***. A total of 15 seed albumin bands were recorded in the 3 examined accessions of subsp. *incisa*; particular accessions displayed from 5 (VIC 1001/C) to 9 (IFVI 739/B/TUR) bands. One accession (IFVI 739/B/TUR) proved to be heterogeneous with respect to electrophoretic seed albumin patterns. Each of the studied accessions showed some bands not recorded in other accessions.

***V. sativa* subsp. *sativa***. Sixteen seed albumin bands were distinguished in the 86 studied accessions. Particular accessions displayed from 3 (PI 284080/A/GRC) to 11 (PI 393887/A/DEU) bands. Individual variation was found in 51 accessions. None of the examined accessions could be distinguished by a characteristic band.

Relationships between populations from five geographical regions, based on the frequency of seed albumin bands, are presented in Figure 2B. The populations from Southwest Asia and from South and Southeast Europe are most closely related and group at 0.63 similarity. The population from North Africa is peripheral and joins the remaining populations at 0.40 similarity.

***V. sativa* subsp. *macrocarpa***. Nineteen seed albumin bands were recorded in the 14 studied accessions; particular accessions showed from 2 (IFVI 416/B/FRA) to 13 (IFVI 1942/B/ITA) bands. Individual variation – observed in 7 accessions – was especially marked in the accession IFVI 1942/B/ITA and was responsible for a relatively high number of bands distinguished in this accession. Some differences between accessions concerned the presence/absence of the bands with $R_f$ values of 0.28, 0.54 and 0.56.

***V. sativa* subsp. *cordata***. In the 26 examined accessions 13 seed albumin bands were detected; particular accessions showed from 4 (IFVI906/B/ITA and IFVI 910/B/ITA) to 8 (several accessions) bands. Fifteen accessions proved to be heterogeneous with respect to the presence/absence of some albumin bands.

## Relationships among the taxa

In total, 31 seed albumin bands were recorded in the subspecies of *V. sativa*, from 13 in subsp. *cordata* to 19 in subsp. *nigra* and subsp. *macrocarpa*. None of the examined taxa could be distinguished by a characteristic band(s), though some

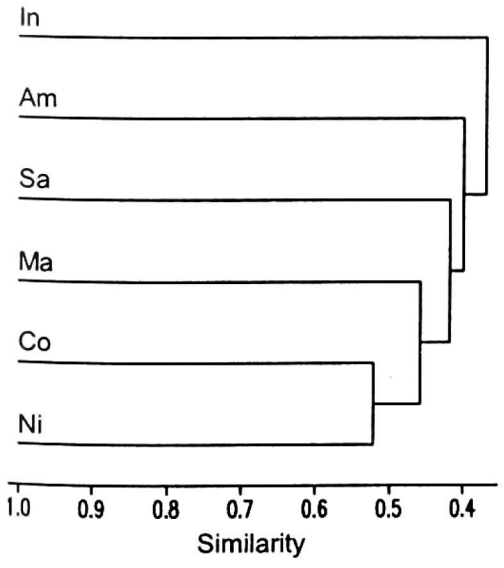


Figure 3. UPGMA dendrogram of the studied taxa of the *Vicia sativa* aggregate based on EUCLIDEAN distances calculated from frequencies of electrophoretic seed albumin bands. Taxa designations as in Table 1.

bands were detected only in some individuals of one taxon. Therefore, different frequencies of particular bands were responsible for differences between the studied taxa. Hierarchical grouping of the taxa based on frequencies of seed albumin bands showed subsp. *nigra* and subsp. *cordata* to be the most closely related: they clustered at 0.52 similarity (Figure 3). Affinities of these two taxa to the remaining members of the *V. sativa* aggregate decrease in the following order: subsp. *macro-*

*carpa,* subsp. *sativa,* subsp. *amphicarpa*, subsp. *incisa.* The peripheral taxon of the aggregate, *V. sativa* subsp. *incisa*, joined the remaining subspecies at 0.37 similarity.

## Discussion

Electrophoretic analysis of seed albumins has proved to be a useful tool in taxonomical studies of various legumes (PRZYBYLSKA 1986, PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1993, 1995, PRZYBYLSKA et al. 1998, 1999, ZIMNIAK-PRZYBYLSKA, PRZYBYLSKA 1994). Therefore, this approach seemed to be promising in clarifying the controversial taxonomy of the *Vicia sativa* aggregate.

In all the studied subspecies of *V. sativa* a marked variation in electrophoretic seed albumin patterns was observed, which was due to differences between accessions and individual variation within accessions. A statistical analysis of the data obtained for *V. sativa* subsp. *nigra (= V. angustifolia* L.) and for *V. sativa* subsp. *sativa* showed in both taxa some genetic distinctness of the accessions originating from North Africa. This finding is worth mentioning but should be treated with a proper reservation and checked by examination of additional accessions. In future studies of the *V. sativa* aggregate it might be interesting to look for possible correlations between biochemical characteristics and ecological niches. LADIZINSKY (1978) reported the relationship between the chromosome number and specific habitats in wild populations of *Vicia sativa* L.

As regards interrelationships among the studied members of the *V. sativa* aggregate, the electrophoretic seed albumin data support their treatment as subspecific taxa. Particular taxa showed no characteristic bands or sets of bands, which may indicate some outcrossing between the taxa distinguished in the essentially inbreeding species *V. sativa.* It should also be added that differences between most distant taxa, concerning band frequency, are of a similar order as differences between North African and other accessions in *V. sativa* subsp. *nigra* and in *V. sativa* subsp. *sativa.* The electrophoretic seed albumin data are generally in agreement with the results of electrophoretic analysis of seed proteins reported by LADIZINSKY and WAINES (1982). Our data are also concordant with the results of the study of isoenzyme variation (JAASKA 1997); four members of the *V. sativa* aggregate were reported to have most allozymes in common and no examined taxon could be distinguished by a specific allozyme(s). In contrast, POTOKINA and EGGI (1991) reported species-specific seed globulin patterns for particular members of the *V. sativa* aggregate. This, however, was not reflected in the analysis of DNA polymorphism, which showed the taxa of the *V. sativa* aggregate to be closely related, with the notable exception of *V. sativa* subsp. *incisa* (POTOKINA et al. 1999). In this connection it should be mentioned that, according to the electro-

phoretic seed albumin data presented in this paper, *V. sativa* subsp. *incisa* is genetically a peripheral member of the *V. sativa* aggregate.

The presented electrophoretic seed albumin data reflect the confused taxonomic status of the *V. sativa* aggregate. There is no clear morphological distinction between some members of this group; diagnostic features used for their identification overlap considerably, which results in the occurrence of intermediate forms (ZOHARY, PLITMANN 1979, GIL, CUBERO 1993, POTOKINA 1997). The characterization of the *V. sativa* aggregate is also problematic cytologically. Three different chromosome numbers, 2n = 10, 2n = 12 and 2n = 14, were reported for the taxa involved and plants at the same chromosome level show a wide range of different karyotypes. There is no general agreement concerning correlation between karyotype and taxon. On the one hand, such a correlation was found by METTIN and HANELT (1964). On the other hand, a marked intrataxon karyotypic variation was described by other authors (HOLLINGS, STACE 1974, LADIZINSKY 1978). As reported by HOLLINGS and STACE (1974) "...there are often much closer resemblances between certain karyotypes of different taxa than between the different karyotypes within each taxon". Reproductive barriers have not yet been fully developed in the *V. sativa* aggregate and spontaneous hybridizations and recombinations between the different karyotypes are responsible for the confusing chromosomal polymorphism in this group (HOLLINGS, STACE 1974, LADIZINSKY, TEMKIN 1978, ZOHARY, PLITMANN 1979, HANELT, METTIN 1989). The *V. sativa* complex represents a unique case of rapid evolution and initial speciation (METTIN, HANELT 1973, LADIZINSKY, TEMKIN 1978). HANELT and METTIN (1989) conclude that the *V. sativa* aggregate "...is a typical example of variable swarm of mostly sympatric, incompletely isolated microspecies which are continuously evolving". According to LADIZINSKY and TEMKIN (1978), for practical and breeding purposes, the *V. sativa* aggregate can be considered as one gene pool. A similar conclusion may be drawn from the electrophoretic seed albumin data presented in this paper.

**Note added in proof**

Recently, POTOKINA et al. (2000) reported interspecific variation between taxa in the *Vicia sativa* aggregate revealed using RAPD and seed protein analyses.

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