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ELECTROPHORETIC EVIDENCE FOR SUBGENERIC AND SECTIONAL RELATIONSHIPS OF SOME SPECIES IN *VICIA* L.

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Abstract

Electrophoretic profiles of native seed protein of 26 species of the genus *Vicia* representing sections *Australe*, *Vicilla* and *Cracca* in subgenus *Vicilla* and sections *Hypechusa*, *Peregrinae* and *Faba* in subgenus *Vicia* have been revealed by PAGE. Numerical analysis of the results indicated that the delimitation of the majority of the examined species agree with their previous classification based on morphological characters. However the grouping of some species i.e., *V. dasycarpa*, *V. haeniscyamus*, *V. dichronantha* and *V. peregrina* was found to be anomalous to earlier classifications. *V. michauxii* is also clearly delimited from the other species.

Introduction

The genus *Vicia* belongs to tribe Vicieae (*Leguminosae- Paplionoideae*) which comprises of *Vicia* L., *Lathyrus* L., *Lens* Mill., *Pisum* L., and the closely related *Vavilovia feodorov*. The tribe is characterized by several advanced characters and the lack of distinct relation to other tribes of the family (Kupichia, 1977). It is generally assumed that within the tribe *Vicieae* the two small genera *Lens* and *Pisum* are derived from the larger and more heterogenous genera *Vicia* and *Lathyrus* (Hanelt & Mettin, 1989).

The genus *Vicia* is widely distributed worldwide, but species diversity is clearly found in the Mediterranean region. A number of in frageneric classifications for the genus have been proposed, but due to insufficient coverage of species and the selective weighing of a few morphological characters, these treatments are controversial. Davis & Plitmann (1970) delimited 59 species from Turkey and the east Aegean islands in three groups A,B & C. Similarly Komarov (1972) has grouped the 83 species recorded in the flora of the USSR in three subgenera based on pod characters.

The first most elaborate and comprehensive taxonomic revision of the infrageneric taxonomic structure of the genus as a whole is that of Kupicha (1976). She realized that the dichotomy of character states within *Vicia* can be expressed in the information of just two subgenera; *Vicia* L. and *Vicilla* (Schur) Rouy. Species with nectariferous spot on abaxial surface of the stipules; inflorescence shorter than the subtending leaf and usually one - flowered were placed in section *Vicia*. Species without nectariferous spots, inflorescence equals or subtending leaf and many-flowered were delimited in subgenous *Vicilla*. The 32 species placed in subgenus *Vicia* were divided into 5,

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sections, while subgenus *Vicilla* which comprised of 101 species was delimited in 17 sections, 7 of which are monospecific. According to her concept of primitiveness and specialization in the genus, the species in subgenus *Vicia* were considered more specialized than those of subgenus *Vicilla*. Cvelev (1980) proposed a third subgenus (subgen. *Ervum* (L.) Taub. in which he placed annual species with small simply constructed flowers in loose raceme which may be the result of evolutionary reductions from several ancestral groups.

Hanelt & Mettin (1989) supported the dichotomous arrangement of all sections of *Vicia* in two subgenera, the subgen. *Cracca* (Dumort.) Petern., which largely corresponds to subgenus *Vicilla*, was divided into 20 sections and subgenus *Vicia* was divided into six sections. They estimated the number of species in the former section to be 140-160 of cosompolitan distribution and in the latter to be 40-50 distributed in the Mediterraean region and the Near and the Middle East. The subgenus *Cracca* is heterogenous and comprises of a majority of perennial species with less derived character states e.g., many flowers, long peduncled inflorescences and simple stipules. The species in subgenus *Vicia* are almost exclusively annual and the subgenus was considered as a monophyletic group with some synapomorphic character states e.g., reduced racemes and nectariferous stipules.

There has been a number of attempts to utilize other lines of evidence to address the systematic relationships within *Vicia*. Evidence from cytological studies revealed that the basic chromosome number x = 7, 6 or 5 (Mettin & Hanelt 1968; 1973; Moore, 1977). Polyploid numbers have been recorded in small number of species (Rudyka, 1986; Efimov, 1988, El-Shanshoury 1991). The x = 7 is the most common number in the two subgenera, x= 6 is found in about 37.36% of the species while x=5 has only been recorded in 9 out of the 120 cytologically examined species (Hanelt & Mettin, 1989). However, in *Vicia* variation in other karyotype features are of more important for taxonomic and phylogenetic considerations than chromosome numbers. In connection with the chromosomal variation in the genus, genome size (Chooi, 1971; Raina & Bisht, 1988), repetitive DNA sequences (Bassi *et. al.*, 1982; Yakura *et al.*, 1987), Amino acids and related compounds (Bell, 1965. Perrino *et al.*, 1989); low molecular weight carbohydrates (Yasui *et. al.*, 1987) have been investigated in a limited number of species. The contribution of these evidence to the taxonomy and phylogeny of species in *Vicia* has been of little significance.

The use of seed protein electrophoresis has provided valid evidence for taxonomic and genetic relationships in some genera of the *Fabaceae*. Protein extracts separated by using polyacrylamide gel electrophoresis (PAGE) produced banding patterns that have been important in *Phaseolus* (Adrianse *et al.*, 1969) *Crotolaria* (Boulter *et al.*, 1970), *Lotus* (Heider, 1987), and *Trifolium* (Badr, 1995). This technique has also been used for the study of the relationships of few species in *Vicia* (Sammour, 1989; El-Shanshoury, 1991). The results of these authors have encouraged us to use electrophoretic studies combined with numerical method to evaluate the relationships of species representing the two subgenera of the genus. The resulting relationships are discussed in the light of the morphological and cytological criteria and compared to previous classifications of the studied taxa.

Materials and Methods

The material of the examined species of *Vicia* were obtained from the International Center of Agricultural Research in the Dry Areas (ICARDA). The Egyptian material were collected by the authors. The origin of the examined species is listed in Table 1. Majority of the used species are of Mediterranean or European origin but one species from Canada and one from Japan were also used.

To extract seed proteins, 0.5 g of mature healthy seeds were powdered using electric mill (IKA-Labortechnike A10). The meal was defatted by stirring with pure acetone (30 minutes 3 - times) and filtered. The residue was dried and protein were extracted in 0.2 M Tris-HCl buffer and 10% sucrose at pH 8.0 for 2 h. After centrifugation at 15,000 rpm for 20 minutes, the supernatant (protein extract) was used for electrophoresis. Samples were loaded directly onto 12% acrylamide (as described by Sambrook *et al.*, 1989). pH 8.3 Tris glycine electrode buffer (pH 8) was used. Electrophoresis was made using a vertical slab gel (Hoefer SE 400) at 140 V for the first 15 minutes and 150 V until the bromophenol blue tract dye reached the bottom of the gel. Protein bands were stained using coomassie blue R-250 for one h and destained for two days.

The bands produced by each species were counted and measured by comparison of each species to the others. The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0 respectively) using the program NTSYS written by Rohlf (1988) and analyzed using the UPGM methods to produce the phenetic relationships. The procedure applied is based on the UPGM method and cluster analysis which expresses the relationships between the taxa (OTUS) as an average taxonomic distance illustrated by the results in a dendrogram.

Results

The electropherograms of the examined 28 taxa revealed a total number of 42 bands, a minimum number of 17 bands was recorded in *V. hyaeniscyamus*, while the minimum number of bands was scored in an accession of *V. villosa*. A diagramatic representation of the banding pattern of the studied taxa is illustrated in Figs.1, 2 and 3. The relationships between these species, based on the variation in the banding profile among them using the NTSYS program are shown by the dendrogram illustrated in Fig.4.

The studied 28 taxa have an average taxonomic distance of about 1.55. At this level the two species *V. hyaeniscyamus* and *V. dasycarpa* were delimited from the rest of the species. These two species were separated from each other at a taxonomic distance of about 1.27 which indicates a high level of difference between them. *V. peregrina* and *V. dichronantha* were delimited from the remaining 24 taxa at an average taxonomic distance of about 1.51, but were also separated from each other at a relatively high level (about 1.37) *V. michauxii* which is placed with *V. peregrina* in the same section *Peregrinae* was delimited from the remaining species at an average taxonomic distance of about 1.48.

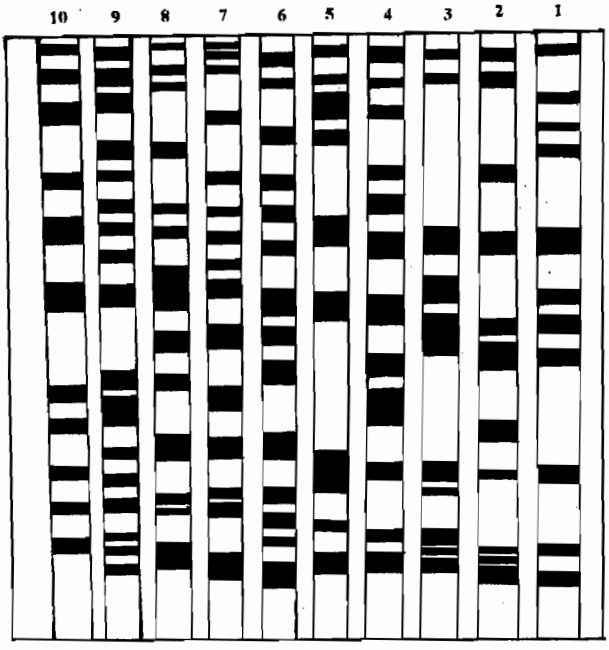

Fig.1. Electropherograms produced by PAGE seed proteins of samples of *Vicia* numbered as in Table 1. under non reducing conditions.

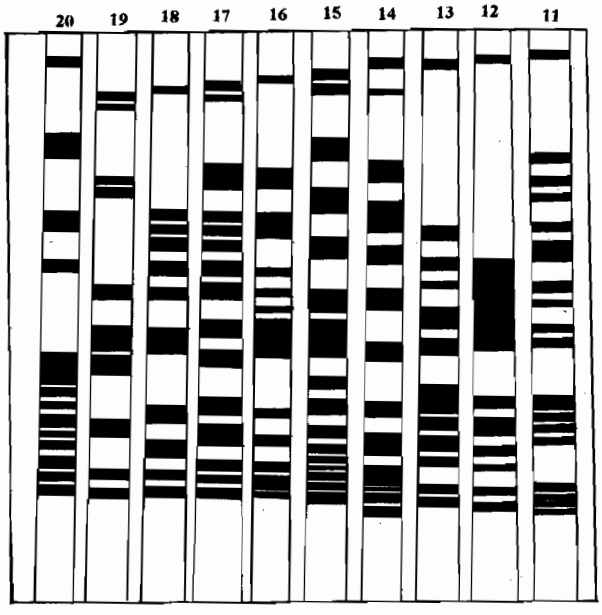

Fig.2. Electropherograms produced by PAGE seed proteins of samples of *Vicia* numbered as in Table 1. under non reducing conditions.

Table 1. Source and origin of the studied *Vicia* species (arranged after Kupicha revision 1976 as modified by Hanelt & Mettin 1989).

No.	Species	Section	Subgenous	Source	Origin
1.	*V melonops* Sibth & Smith	Hypechusa	Vicia	ICARDA	Japan
2.	*V. hybrida* L.	Hypechusa	Vicia	ICARDA	Syria
3.	*V. pannonica* Crantz	Hypechusa	Vicia	ICARDA	Hungary
4.	*V. anatolica* Turrill	Hypechusa	Vicia	ICARDA	UN.K.
5.	*V. lutea* L.	Hypechusa	Vicia	Egypt	Egypt
6.	*V. michauxii* Sprengei	Peregrenae	Vicia	ICARDA	Syria
7.	*V. peregrina* L.	Peregrenae	Vicia	Egypt	Egypt
8.	*V. dichronantha* Diels	Vicilla	Vicilla	ICARDA	Italy
9.	*V. hyaeniscyamus* Mouterde	Faba	Vicia	ICARDA	Syria
10.	*V. dasycarpa* (Ten.) Cav.	Cracca (Davis 1970)	Vicilla	ICARDA	Canada
11.	*V. syeriocarpa* Feuzl	Hypechusa	Vicia	ICARDA	Syria
12.	*V. johannis* Tamamschlan	Vicia	Faba	ICARDA	Syria
13.	*V. eriocarpa* Fenzl	Cracca (Davis 1970)	Vicilla	ICARDA	Syria
14.	*V. noeana* Renter ex Boiss	Hypechusa	Vicia	ICARDA	Syria
15.	*V. serratifolia* Kunth	Austerales	Vicilla	ICARDA	Italy
16.	*V. molis* Boiss	Peregrenae	Vicia	ICARDA	Libanon
17.	*V. aintabensis* Boiss. & Hussain	Peregrenae	Vicia	ICARDA	Syria
18.	*V. mulijuga* (Boiss.) Rech. F.	Cracca	Vicilla	ICARDA	Syria
19.	*V. benthamiana*			ICARDA	Syria
20.	*V. montevidensis* Vogel.	Austerales	Vicilla	ICARDA	Italy
21.	*V. palaestina* Blss.	Cracca	Vicilla	ICARDA	Syria
22.	*V. villosa* Roth agg.	Cracca	Vicilla	ICARDA	Polland
23.	*V. villosa* Roth agg.	Cracca	Vicilla	ICARDA	Sun.
24.	*V. villosa* Roth agg.	Cracca	Vicilla	ICARDA	Sun.
25.	*V. benghalensis* L.	Cracca	Vicilla	ICARDA	UNK
26.	*V. cracca* L. agg.	Cracca	Vicilla	Holland	Holland
27.	*V. monantha* Retz.	Cracca	Vicia	Egypt	Egypt
28.	*V. bithynica* (L.) L.,	Faba	Vicia	Holland	Holland

The remaining 23 taxa were divided into two major groups at a distance level of about 1.45, one small group comprising of 5 species viz., *V. melanops*, *V. hybrida*, *V. panonica*, *V. anatolica* and *V. lutea*. All these species have been placed together in section *Hypechusa* of subgenus *Vicia*, however some other species that have been classified in the same section and included in the present study were not included in this group. Within this group the 3 species *V. melanops*, *V. hybrida* and *V. pannonica* were delimited together from *V. anatolica* and *V. lutea*.

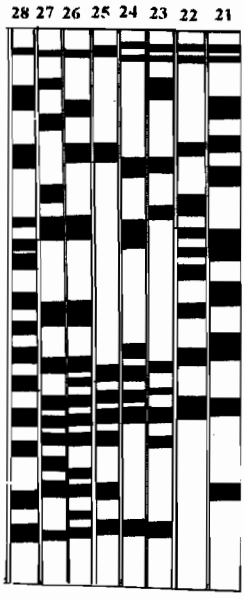

Fig.3. Electropherograms produced by PAGE seed proteins of sample of *Vicia* numbered as in Table 1. under non reducing conditions.

The second major large group was divided into two subgroups, one comprising of 8 species and the other 10 species. In the first subgroup *V. serratifolia* was distinct from the other seven species at a distance level of about 1.37. At a distance level of 1.27, *V. eriocarpa* was also delimited as a single phenetic line . The remaining 6 species were subdivided into two clusters, one including *V. mollis* and *V. benthamiana* and the other comprised of *V. johanis*, *V. noeana*, *V. aintabensis* and *V. multijuga*, the latter two species showed low level of taxonomic distance, indicating close similarity between them.

In the second subgroup *V. sericocarpa* and *V. palastina* were delimited in two distinct phenetic lines at a distance of about 1.3. The remaining 8 species were delimited in two small groups, one comprised of two accession of *V. villosa* and *V. montevidensis* and the other comprising five species, distinguished into two small clusters; one cluster includes an accession of *V. villosa*, *V. benghalensis* and *V. cracca* and the other cluster comprises *V. monantha* and *V. bithynica*. A relatively high degree of resemblance is evident among the species delimited in the latter subgroup.

Discussion

The phenetic relationships between the studied taxa as expressed by the numerical analysis of their seed protien PAGE profiles clearly demonstrated the isolation of *V. dasycarpa* and *V. hyaeniscyamus* from the other taxa. *V. dasycarpa*, has been placed in section *Cracca* by both Komarov (1972) and Davis (1970), however, this species is

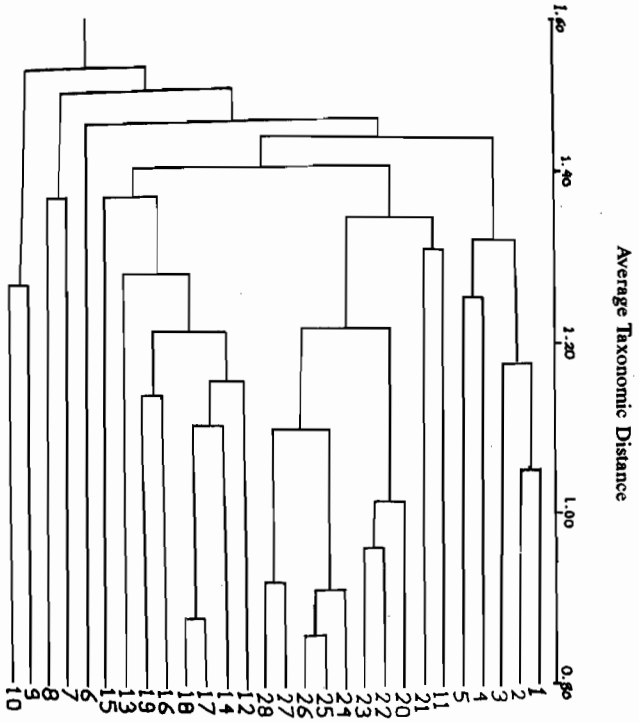

Fig.4. Dendrogram illustrating the relationships among the 28 studied taxa of *Vicia* numbered as in Table 1, produced by PAGE seed protein data under non reducing conditions.

clearly distinguished from the other species of this section by features of stem, calyx margin, limb of standard and seed hilum (Kupicha, 1976). *V. hyaeniscyamus*, on the other hand, has been delimited in section *Faba* of subgen. *Vicia*. This species was considered to closely resemble *V. faba, V. narborensis* and *V. galilaea*, and none of them is included in the present study (Kupicha, 1976). However, its protein banding profile is clearly different from the two species of the same section studied i.e., *V. bithynica* and *V. johannis*.

Other two species which are clearly distinguished from the other species by PAGE profiles are *V. dichronantha* and *V. peregrina*. The former species has been placed by Kupicha (1976) under section *Villosa* and was considered to be the type species for this section. *V. peregrina* was considered by Cvelev (1980) to be the type species of section *Peregrinae*, but was also placed in sect. *Euvicia* (Komarov, 1972), or sect. *Vicia* (Davis, 1970). The morphological resemblance which led Kupicha (1976) to place this species in section *Peregrince* together with *V. michauxii*, *V. aintabensis* and *V. mollis* are the annual growth form inflorescence 2- flowered, not pedunculate but flowers are borne in long pedicels, legume rhomboidal (sutures not parallel), seed with very short hilum, and smooth testa. These morphological similarities are not correlated with the data of PAGE of seed proteins.

The isolation of *V. dichronantha* by the PAGE data as revealed here is consistent with its morphological characters which make it considerably different from the other species studied i.e., hypostomatic paripinnate leaves, dimorphic stipules, oblong vexillum, and dorsally compressed style, both types of characters confirm the view of Kupicha (1976) who delimited *V. dichronantha* in a separate section *Vicilla*.

V. michauxii which has been placed with *V. peregrina* in one section in some treatments of the genus is clearly separated as a single group by the data of seed proteins. This contradiction is correlated with differences between the two species in their morphological criteria (Kupicha, 1976). The systematic treatment of this species has been controversial, and has been placed by different authors in different sections. Its isolation by the analysis of PAGE profiles is consistent with some morphological features e.g. larger seeds, yellowish-pink flowers and calyx teeth shorter than tube. The isolation of this species is in agreement with the opinion of Kupicha (1976) that its presence in the section in unusuall except for its large seeds.

A major group of 8 species was delimited from the remaining 15 species. In this group 5 species were previously delimited in subgen. Vicia and 3 in subgen. *Vicilla*. *V. serratifolia* was placed in section *Euvicia* by Komarov (1970) which comprised species now delimited in subgen. *Vicia* (Kupicha, 1976; Hanelt & Mettin, 1989). Kupicha (1976) delimited *V. serratifolia* in section *Australes* of subgen. Vicilla however, the electrophoretic profile of this species is clearly different from the other species of section *Australes* included in the present study i.e., *V. montevidensis* which showed substantial affinity to the species of subgen. *Vicilla*. *V. eriocapa* of the same group has been placed in section *Vicilla* of subgen. *Vicilla* by Kupicha (1976) and in section *Cracca* by Pietro *et al.*, (1984) and was considered as a subspecies of *V. villosa* by Davis (1970). The results of the present study clearly distinguish this species from the other species of section *Vicilla* i.e., *V. dichronantha* and from the taxa in section *Cracca* which are delimited together as one major group. The data further contradict the inclusion of *V. eriocarpa* as a subspecies of *V. villosa*. Another species of this group that has been placed in section *Cracca* of subgen. *Vicilla* is *V. mulijuga*. This species showed a high level of phenetic similarity to *V. aintabensis* (17) of section *Peregrinae* in subgen. *Vicia*. The other species previously placed in this section i.e., *V. michauxii*, *V. peregrina* and *V. mollis* showed considerable variation in their native seed protein composition. It seems that the evolution of the species in this section has not been associated with changes in storage seed proteins.

The present data may indicate that section *Peregrinae* may be a convenient assemblage of species with considerable heterogeniety. Of this group. *V. johannis* has been delimited in section *Faba* of subgen *Vicia*. Another species that has been placed in the same section i.e. *V. bithynica* by Kupicha (1976) is delimited here in another major group mainly comprised of species of section *Cracca* of subgen. *Vicilla*. This delimation agrees with Komarov (1972) who considered *V. johannis* in section *Cracca*. The results of seed protein electrophoresis may support the inclusion of this species in section *Cracca* as proposed by the latter author. It is to be noted that Kupicha (1976) stated that *V. bithynica* is a distinctive species with unsettled taxonomic history.

The remaining species have been delimited in two major groups. One of these groups includes 5 species that have been delimited together in the same section in 3

treatments of the genus by Komarov (1972) *Euvicia*, Davis (1970) *Vicia* and Kupicha (1976) *Hypechusa*. These are: *V. melanops*, *V. hybrida*, *V. panonica*, *V. analotica* and *V. lutea*. Other species which have been delimited with these five species in the same section *Hypechusa* of subgen. *Vicia* i.e., *V. noeana*, *V. seriocarpa* are delimited here in different groups. The situation of both species has been discussed above. The controversy in the treatment of these species may be considered as support for their delimitation by seed protein PAGE analysis.

The grouping of species 21,22,23,24,25,26 and 27 by protein electrophoresis is also in accordance with their delimitation together in section *Cracca* as in previous classifications as proposed by Komarov (1972), Davis (1970) and Kupicha (1976) on morphological characters. All these species have the same chromosome number 2n = 14. *V. montevidensis* also with 2n = 14 is grouped with these species, however it was placed by Kupicha (1976) in section *Austurales*. *V. seriocarpa* which has been placed by previous classification in section *Hypechusa* was found to be more similar to species of section *Cracca*. The grouping of *V. bithynica* with species of section *Cracca* by PAGE contradict the previous classification by Davis (1970), Kupicha (1976) and Cvelev (1980) but is in agreement with Komarov (1972) and Perrino *et al.*, (1984).

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