

MICROMORPHOLOGY OF SEED IN SOME *VICIA* SPECIES FROM IRAN*

Z. HOSSEINZADEH, M. PAKRAVAN** and
A. TAVASSOLI

Department of Biology, Faculty of Science, Alzahra University

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Abstract

The morphology of 11 species of the genus *Vicia* from Iran was studied using Scanning Electron Microscopy (SEM). The main pattern in seed coat sculpturing in the species was papillose, but the size and the number of side ribs in papillae and the density of papillae, were different in various species. Four morphological characters of seeds: relative hilum length and shape, relative position of the lens to hilum and seed circumference were constant. The results indicate that seed morphology is not useful in circumscribing sections in *Vicia*, but applicable in delimiting species and seed coat microsculpturing and testa patterns and that can provide additional characters for delimiting natural groups. However, the variation of these characters was too little to be applied for separating close species.

Key words: Seed morphology, SEM, *Vicia variegata* group, Iran

* Corresponding author (E-mail: pakravan@alzahra.ac.ir)

Introduction

The genus *Vicia* L. (Fabaceae) comprises 166 species in the world and its diversity centre is the Mediterranean region (MAXTED 1990). CHERTKOVA-ZERTOVA (1971) has mentioned 46 species from Iran. PAKRAVAN (1990) has reported 40 species from Iran. KUPICHA (1976) divided *Vicia* species into two subgenera, *Vicia* and *Viciella* (Schur.) Roug. and has subdivided the latter subgenus into eight sections.

In contribution to the taxonomy of the family Fabaceae and *Vicia* species, many taxonomists have used seed morphology (SWEDERSKI 1924, LEOKENE 1966, ZERTOVA 1962, GUNN 1968, GUNN 1970, UTKIN 1965), as well as micromorphology of seed coat (GUNN 1971, 1981, LERSTEN 1979, LERSTEN & GUNN 1982, PERRINO *et al.* 1984, VORONCHIKHIN 1988, CHERNOFF *et al.* 1992, KIKBRIDE *et al.* 2003). In this study we focus on the species of *V. variegata* group and for comparison, some species from other sections of the subgenus *Viciella* were used. *Vicia variegata* group comprises seven species with distribution from Turkey, Iran and Armenia to Caucasia. *V. persica* Boiss. and *V. aucheri* Jaub. & Spach are endemic to Iran. There are several opinions for classification of these species. CHERTKOVA-ZERTOVA (1979) has distinguished seven species as distinct species in the group. DAVIS (1970) has considered them as subspecies/varieties of *V. canescens* Labill. PAKRAVAN (2000) followed Davis (*l.c.*) and distinguished two subspecies in *V. canescens* as follows:

V. canescens Labill. subsp. *gregaria* (Boiss. & Helder.) Davis

Syn.: *V. gregaria* Boiss. & Helder. in Boiss., Diagn. Pl. Or. Nov. ser. 1(9): 121, 1849. *V. armena* Boiss., *l.c.* ser. 1(2): 102, 1843; *V. persica* Boiss. var. *stenophylla* Boiss., Fl. Or. 2: 583, 1827; *V. akhmaganica* KAZ., Fedd. Repert. 36: 389, 1934. *V. rechingeri* Chertkova-Zertova, Folia Geobot. Phytotax. 12: 101, 1977.

--subsp. *variegata* (Willd.) Davis, Fl. Turkey 3: 287, 1960

Syn.: *V. variegata* Willd., Sp. Pl. 3: 1096, 1802; *V. aucheri* Jaub. & Spach, I11. Pl. Or. 1: 80, 1842; *V. persica* Boiss., Diagn. Pl. Or. Nov. ser. 1(9): 121, 1849

These species were separated by CHERTKOVA-ZERTOVA (1977) based on the hair on the legume surface, flower and stipule size, yet there are several specimens with intermediate characters. *Vicia gregaria* could be distinguished by having large stipules while *V. aucheri* often has villose legume and in *V. akmaganica*, *V. rechingeri*, *V. persica* and *V. armena*, hair of legume, standard size and the leaflet size could separate them. However, there are many polymorphisms in these characters and they share a common habitat.

In this study, seed morphology and micromorphology were studied in order to verify relationships within species groups.

Materials and Methods

Mature seeds of 11 species of the genus *Vicia* were collected from herbarium specimens of Alzahra University ("AZUH") (Table 1).

Five to ten seeds of each species were studied using a JEOL 840 scanning electron microscope. The seed dimensions measured by using an Olympus (model BX15) light microscope. In order to observe the density of papillae on the seed coat, the number of papillae was scored in 9-cm² area of × 1000 magnification prints (representing actual area of 900 μm²) with at least three scores per seed.

Main categories of density were as follows: dense (51-100), dense-moderate (31-51) and medium (11-30). Other potentially useful characters such as papillae height, size, shapes and the number of side ribs were taken into account in the overall assessment of grouping.

The following species were studied: *V. aucheri*, *V. akmaganica*, *V. rechingeri*, *V. persica*, *V. armena* and *V. variegata* (sect. *Variegata* Radzhi), *V. cracca*, *V. monantha*, *V. variabilis* and *V. villosa* (sect. *Cracca* Gray) and *V. tetrasperma* (sect. *Ervum* L.) Taub. The terminology used was adopted from CHERNOFF *et al.* (1993), Gunn (1970), LERSTEN & GUNN (1982) and PERRINO (1982).

Table 1. Species of *Vicia* used in the present study

Species	Section	No.	Locality	Collectors
<i>V. cracca</i> L.	<i>Cracca</i>	1839	Chalus, Kandovan road, Siahbisheh	Amini
<i>V. monantha</i> Retz.	<i>Cracca</i>	481	Zanjan, Fil-e Khameh village	Amini
<i>V. variabilis</i> Freyn & Sint.	<i>Cracca</i>	4804	Charmahal va Bakhtiari Babaheidar	Amini
<i>V. villosa</i> Roth.	<i>Cracca</i>	1843	Tehran, Evin, IRIPP	Hosseinzadeh & Abedinzadeh
<i>V. ervilia</i> (L.) Willd.	<i>Ervilia</i>	TN-44-201	Hamedan, Malayer	Hosseinzadeh & Abedinzadeh
<i>V. ervilia</i> (L.) Willd.	<i>Ervilia</i>	TN-44-104	Lorestan, Koohdasht	Hosseinzadeh & Abedinzadeh
<i>V. ervilia</i> (L.) Willd.	<i>Ervilia</i>	TN-44-90	Hamedan, Tuiserkan	Hosseinzadeh & Abedinzadeh
<i>V. ervilia</i> (L.) Willd.	<i>Ervilia</i>	TN-44-88	Hamedan, Nahavand	Hosseinzadeh & Abedinzadeh
<i>V. tetrasperma</i> (L.) Schreb.	<i>Ervum</i>	1844	Mazandaran, Sisangan	Gharib & Memarian
<i>V. tetrasperma</i> (L.) Schreb.	<i>Ervum</i>	1845	Alborz, Chaboksar	Pakravan
<i>V. akhmaganica</i> Kaz.	<i>Variegatae</i>	1832	Chalus, Kandovan road, Kandovan tunnel	Amini & Abbasi
<i>V. akhmaganica</i> Kaz.	<i>Variegatae</i>	1833	Alborz, Darbandsar	Hosseinzadeh
<i>V. akhmaganica</i> Kaz.	<i>Variegatae</i>	1834	Alborz, Shahrestanak	Pakravan & Hosseinzadeh
<i>V. akhmaganica</i> Kaz.	<i>Variegatae</i>	1835	Alborz, Shemshak	Hosseinzadeh
<i>V. armena</i> Boiss.	<i>Variegatae</i>	1836	Alborz, Darbandsar	Hosseinzadeh
<i>V. armena</i> Boiss.	<i>Variegatae</i>	1837	Chalus, Kandovan road, Pol-e Zanguleh	Amini & Abbasi

Table 1. (contd.)

<i>V. armena</i> Boiss	<i>Variiegatae</i>	1838	Alborz, Shemshak	Hosseinzadeh
<i>V. aucheri</i> Jaub. & Spach	<i>Variiegatae</i>	1505-2	Alborz, 3 Km Dizin to Shemshak	Pakravan
<i>V. persica</i> Boiss.	<i>Variiegatae</i>	1505-1	Alborz, 3 Km Dizin to Shemshak	Pakravan
<i>V. persica</i> Boiss.	<i>Variiegatae</i>	1840	Alborz, Shemshak	Hosseinzadeh
<i>V. variegata</i> Willd.	<i>Variiegatae</i>	1841	Chalus, Kandovan road, Pol-e Zanguleh	Amini
<i>V. variegata</i> Willd.	<i>Variiegatae</i>	1842	Alborz, Shemshak	Hosseinzadeh

The voucher specimens are deposited in the herbarium of Alzahra University ("AZUH").

Results

Morphological characters and SEM patterns of seed coat were analyzed in 11 taxa of *Vicia*. Some of the data obtained are presented in Table 2. It was found that the discriminating seed characters by which taxonomic entities can be defined are: seed size and general shape, location, shape and length of hilum and also lens and microscopic texture of testa.

Seed shapes of majority of species were spherical or oblong, only in *Vicia ervilia* the seed shape was pyramid with compression. *V. variabilis* had the largest seed and *V. tetrasperma* the smallest one. The seeds were mottled and striate. Hilum shape varied from linear, oblong to oval.

The patterns of sculpturing in all of the species were papillose. Conical and broad conical papillae were observed. Density of the papillae, size and the tip-shape of the papillae, number of side ribs of papillae varied in different species. The papillae density was medium to dense. The number of ribs in papillae varied between 7-11 in some taxa, also multirib papillae were found in some other species.

Table 2. Characters of seeds of species of *Vicia* distributed in Iran

Characters	Species					
	<i>V. akhmaganica</i>	<i>V. armena</i>	<i>V. aucheri</i>	<i>V. persica</i>	<i>V. variegata</i>	<i>V. cracca</i>
seed shape	spherical	spherical	oblong	spherical	spherical	spherical-oblong
length (mm)	4.8	5	5	4.4	3.8	3.6-4.9
width (mm)	4.6	4.3	4.5	4	3.7	3.3-3.7
thickness (mm)	3.7	3.3	2.6	3.6	3.3	2.7-3.1
circumference (mm)	13.4	12.4	14.1	12.7	11.2	10.2-13.2
background colour	light brown	light brown	light brown	cream	cream	brown
spot	dark brown	dark brown	dark brown	light brown	dark brown	dark brown
rib density	medium	medium	medium	medium	medium	medium
rib shape	broad conical	broad conical	broad conical	broad conical	broad conical	conical
rib numbers	7-8	8	7-8	8	7-8	>10
texture	papillose	papillose	papillose	papillose	papillose	papillose
hilum colour	cream	cream	cream	cream	cream	green brown
hilum margin	brown	brown	brown	brown	brown	brown
hilum shape	oblong	oblong	oblong	oblong	oval	oblong
hilum length	1.4	1.5	1.5	1.6	1.6	1.8
hilum/per meter	10	12	11	13	14	18
chalaz length	1.3	1.2	1.7	1.2	1	0.9
chalaz colour	brown	brown	brown	brown	black	black
projection	± prominent	± prominent	prominent	prominent	±prominent	prominent

Table 2. (contd.)

Characters	Species					
	<i>V. monantha</i>	<i>V. armena</i>	<i>V. variabilis</i>	<i>V. villosa</i>	<i>V. ervilia</i>	<i>V. tetrasperma</i>
seed shape	spherical	spherical	spherical-elliptic	spherical	conical	spherical
length (mm)	4	5	5.8	4.5	4.3	1.4
width (mm)	3.6	4.3	4.1	4.1	3.9	1.3
thickness (mm)	3.5	3.3	2.9	3.6	3.7	0.7
circumference (mm)	11.6	12.4	14.3	11.6	11.7	4.2
background colour	light brown	light brown	light brown	green	cream	green
spot	black brown	dark brown	black	brown	black	black
rib density	dense	medium	medium	dense	medium	dense
rib shape	conical	broad conical	small conical	conical	conical	truncate
rib numbers	>15	8	>15	>15	9-11	0
texture	papillose	papillose	papillose	papillose	papillose	papillose
hilum colour	white	cream	brown	dark cream	cream	brown
hilum margin	light brown	brown	brown	brown	brown	brown
hilum shape	oblong	oblong	linear	oblong	oval	oval
hilum length	1.5	1.5	2	1.7	0.9	0.8
hilum/per meter	13	12	14	15	7	19
chalaz length	1.1	1.2	1	1.3	1.5	0.4
chalaz colour	brown	brown	brown	dark brown	brown	brown
projection	strongly prominent	± prominent	strongly prominent	strongly prominent	prominent	prominent

Discussion

In the present study, some seed characters were used for the taxonomic delimitation and related them for species identification. Oblong hilum was observed in *Vicia akhmaganica*, *V. monantha*, *V. armena*, *V. aucheri*, *V. cracca*, *V. persica*, *V. villosa* and *V. variegata*. *V. ervilia* and *V. tetrasperma* had oval hilum and linear hilum was observed in *V. variabilis*. PERRINO *et al.* (1984) have distinguished 11 groups in *Vicia* species based on 19 seed characters. Based on their studies our species occur in the following groups: *V. cracca*: group I; *V. ervilia*, *V. akhmaganica*, *V. aucheri* in group IV; *V. monantha*, *V. villosa*, *V. variegata*, *V. variabilis* in group VII; *V. tetrasperma* in group VIII. *V. armena* and *V. persica* are intermediate between the two groups IV and VII.

The results obtained from the present work, agree with those of CHERNOFF *et al.* (1992) and PERRINO (1984). The papillae with medium density were observed in *Vicia akhmaganica*, *V. aucheri*, *V. persica*, *V. variabilis*, *V. variegata* and *V. ervilia* (Fig. 1 A, C, D, E, H, J). Dense papillae were observed in *V. villosa* (Fig. 1-I) and *V. tetrasperma* (Fig. 1 K). Conical papillae were observed in *V. monantha*, *V. variabilis*, *V. villosa*, *V. ervilia* (Fig. 1 G-I), and broad-conical papillae in *V. akhmaganica*, *V. armena*, *V. aucheri*, *V. persica*, *V. variegata* and *V. cracca* (Fig. 1 A-F). Large papillae were found in *V. akhmaganica*, *V. armena*, *V. cracca*, *V. persica* and *V. variegata*. Small papillae were observed in *V. aucheri*, *V. variabilis* (Fig. 1 C, H). Papillae with medium size were observed in *V. aucheri*, *V. variabilis*, *V. variegata* and *V. ervilia* (Fig. 1 C, H, E, J). Papillae with 7-8 ribs were observed in *V. akhmaganica*, *V. armena*, *V. aucheri*, *V. cracca*, *V. variegata* and *V. persica*. Papillae with 9-11 ribs were observed in *V. ervilia*. Side ribs in *V. variabilis*, *V. monantha* and *V. villosa* were very thin and there was not any conspicuous rib in side of papillae in *V. tetrasperma* (Fig. 2 C, D). The pattern of seed coat in *V. tetrasperma* was markedly different from those of other species.

The results showed similar seed patterns in *Vicia variegata* group. They had broad conical and dense papillae with 7-8 ribs. Only in *V. aucheri* papillae were medium size. *V. variabilis* and *V. cracca* are closely related. They differ only in flower size and standard shape and the seed coat sculpturing was somewhat different as *V. variabilis* had typical sculpturing and different pattern. Two other species of

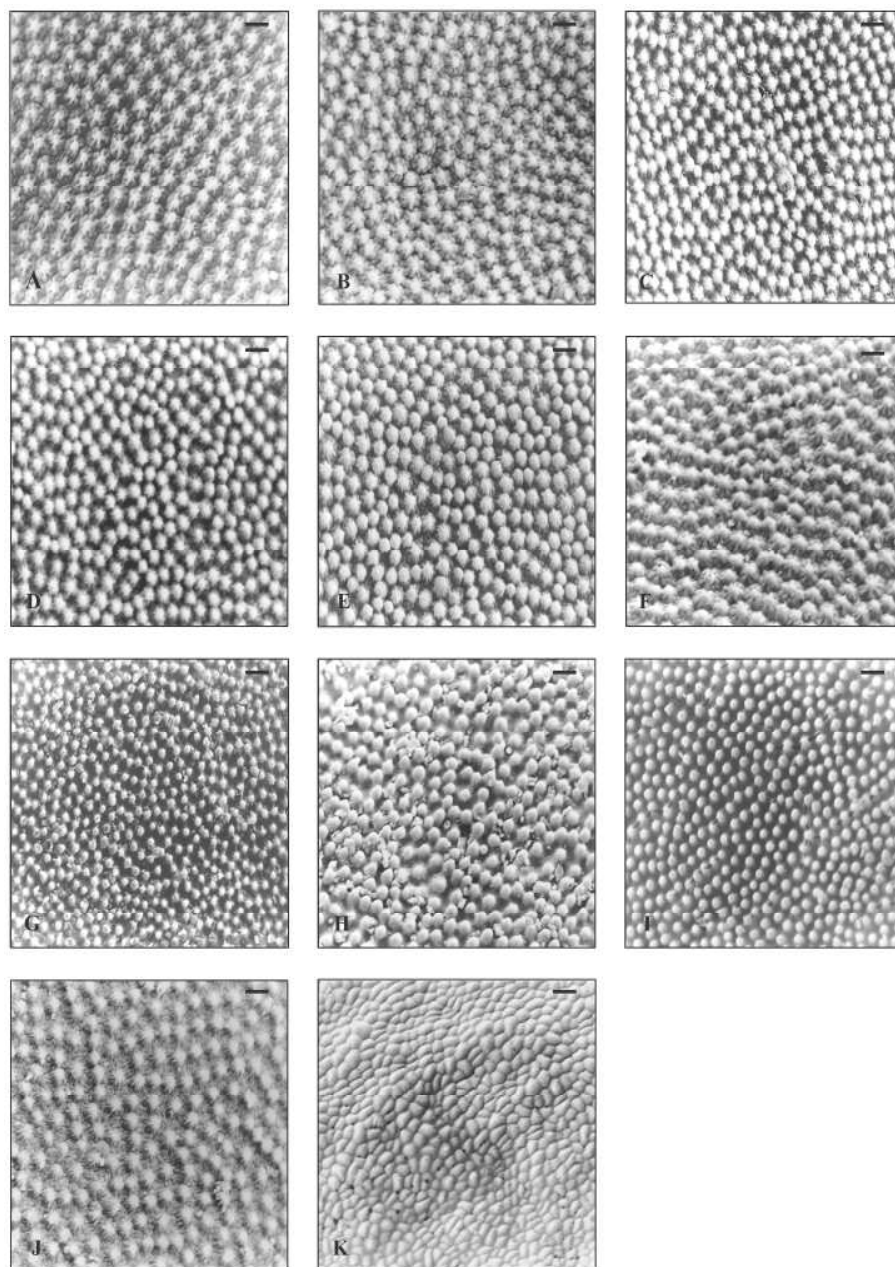


Fig. 1. SEM of seed coat in: A. *V. akhmaganica*, B. *V. armena*, C. *V. aucheri*, D. *V. persica*, E. *V. variegata*, F. *V. cracca*, G. *V. monantha*, H. *V. variabilis*, I. *V. villosa*, J. *V. ervilia*, K. *V. tetrasperma* (Scale bar = 10 μ m).

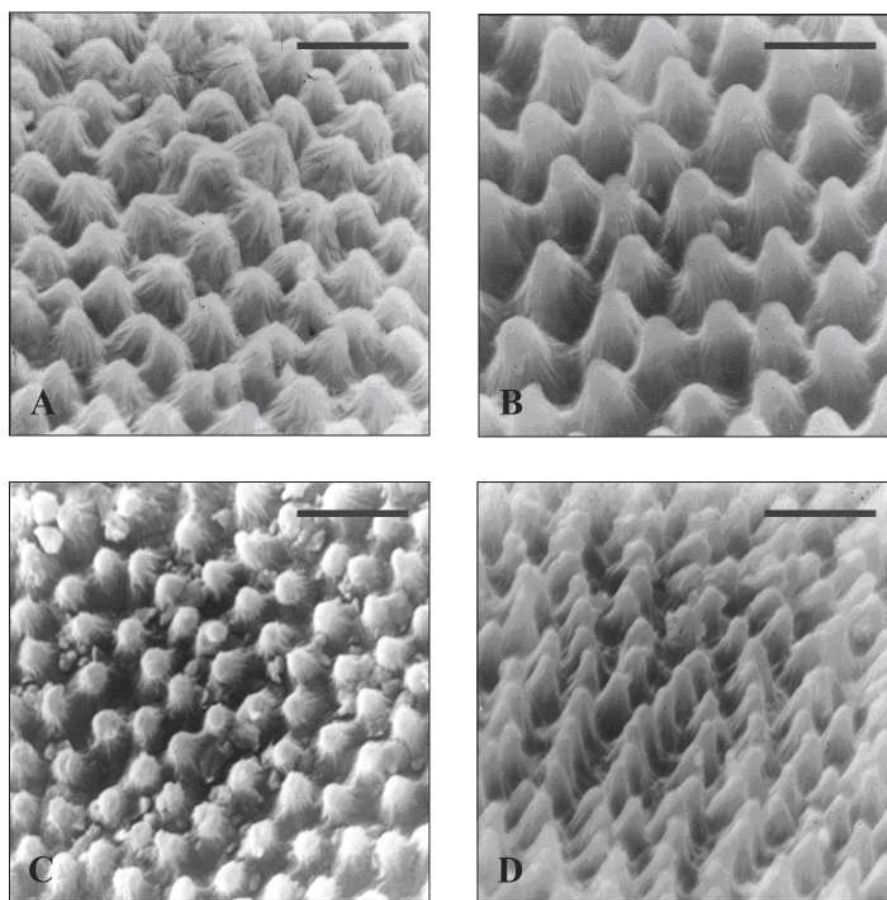


Fig. 2. SEM of seed coat in: A. *V. aucheri*, B. *V. cracca*, C. *V. monantha*, D. *V. villosa* (Scale bar = 10 μ m).

section *Cracca* are *V. monantha* and *V. villosa* with similar morphology. They also had same seed coat characters that are dense, small and conical papillae with narrow ribs.

Vicia tetrasperma which belongs to the section *Ervum*, had special characters of seed morphology and testa characters. *V. ervilia* belonging to the section *Ervilia* also had special characters in seed morphology and testa characters.

Finally, the results of this study indicate that, shape and morphology of seeds are too variable to be applied for delimiting natural species groups. These characters can provide additional characters for delimiting species. Seed sculpturing characters are too few to be applied for separating species, but in closely related species show

common patterns. For example, *Vicia persica* and *V. aucheri* are very closely related and seed sculpturing is similar in these species.

In *Vicia variegata* group, similar characters were observed so these species can remained as a species complex with some polymorphism in morphological characters. The results of this study support those of PAKRAVAN (2000) and DAVIS (1970) on this species complex.

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Address of the authors: Z. HOSSEINZADEH, Dr. M. PAKRAVAN and Dr. A. TAVASSOLI, Faculty of Science, Alzahra University, P.O. Box 19935-567, Iran.