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MICROMORPHOLOGY OF SEED IN SOME *VICIA* TAXA BELONGING TO SECTION *CRACCA* FROM AZERBAIJAN**Kamala Valeh Asadova, Aydin Musa Asgarov**

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For the first time, some taxa of the genus Vetch (*Vicia* L.) belonging to subgenus *Vicilla* and section *Cracca* (according to the system of Kupicha) from the Azerbaijan were subjected to morphological and scanning electron microscopic (SEM) analyses: *V. tenuifolia* Roth subsp. *variabilis* (Freyn & Sint. ex Freyn) Dinsm., *V. villosa* subsp. *villosa* Roth, *V. villosa* Roth subsp. *varia* (Host) Corb. (*V. dasycarpa* auct.), *V. monantha* Retz. (*V. cinerea* M. Bieb.), *V. elegans* Guss.

Seed patterns were collected in 2015-2017 from different region of Azerbaijan (Zagatala, Ordubad and Shamakhi). In all seed samples, the seed's surface was papillate. However, the size of the ribs in the papillas and the density of papillas were different in different taxa. The papillae with medium density were observed in *V. tenuifolia* subsp. *variabilis*. Dense papilla were observed in *V. villosa* subsp. *villosa*, *V. villosa* subsp. *varia*, *V. monantha* and *V. elegans*. *V. villosa* subsp. *varia* had conical, multirib papillas with waxy specks. However, small conical multirib were observed in *V. tenuifolia* subsp. *variabilis* and *V. monantha*. Conical multirib was found in *V. villosa* subsp. *villosa* and broad conical in *V. elegans*. Ovate hilum was observed in *V. villosa* subsp. *varia*. Oblong-elliptic hilum is peculiar to *V. tenuifolia* subsp. *variabilis*, *V. villosa* subsp. *villosa*, *V. monantha*, and *V. elegans*. The largest seeds are found in *V. tenuifolia* subsp. *variabilis*, and the smallest ones in *V. villosa* subsp. *varia*. Detected micromorphological features play an important role in the determination of different taxa of the genus *Vicia* L.

Keywords: seed, SEM, vetch, leguminous, intraspecific, section, species

Introduction

Azerbaijan is the biggest country of Southern Caucasus according to its area and population. The Republic that is situated in the junction of Europe and Asia has got a unique geographical position. Location in the subtropical latitude, richness and diversity of its nature are the factors that constantly influence the progress of Azerbaijan.

Azerbaijan is distinguished not only by climate and diversity of its soil types, but also by the genetic resources of this plants. Even though natural flora contains rich gene fund of important food, technical, medicine, vitamin rich plants, only a part of this wealth is used [2].

Vetch (*Vicia* L.) is one of the most widespread genera of the family *Fabaceae* Lindl. As with other leguminous plants, taxa of *Vicia* are also selected for their nitrogen-fixing properties. In the roots of the plant, there are nitrogen-fixing bacteria that adopt the atmospheric free nitrogen and convert it into a mineral form that plants can use. During the vegetation period, vetches release these mineral substances into the soil through the roots that enriches the microflora of the soil and fertilize it. Due to this, the productivity of plants grown on the soil after the vegetation period and the quality of crop products rises [17]. Representatives of the genus *Vicia* L. are considered to be good predecessors. This genus contains 150-210 species occurring in Europe, Asia, North America, temperate regions of South America, and partly in tropical Africa [15; 25]. It has been established that in the flora of Azerbaijan the genus *Vicia* is represented by 2 subgenera, 11 sections, 39 species, 5 subspecies and 8 varieties ranging in their distribution from the sea level to mid-mountain zones [1; 3; 4].

The monographic study of the genus was carried out by F.K. Kupicha [23; 25; 26]. Using the classic methods, Kupicha, based on selected morphological signs, subdivided

Vicia into two subgenera: *Vicia* and *Vicilla* (*Cracca*). These subgenera differ in the relative length of the inflorescence and the presence or absence of nectar spots on the stipule. In their taxonomic studies of the genus *Vicia*, many scientists used the seed morphology [6; 11; 16; 24; 27; 28; 33], including micromorphology of the seed texture [7; 11; 12; 13; 16; 22; 29].

Signs of the seeds can be used as an important feature in the classification of species and in the determination of the phylogenetic relationships of intraspecific taxa.

In the first description of a number of vetch taxa, as well as in the Floras (Azerbaijan, the Caucasus flora), no signs of the seeds were mentioned. With this in mind, for the first time were studied the morphology and especially micromorphology of seed samples of species and subspecies of *Vicia* L. distributed in the flora of Azerbaijan.

The aim was to clarify the relationship between the intraspecific groups belonging to the genus *Vicia* L.

Material and methods

The material for the study consisted of seeds of Vetch, which we collected during the expeditions to different regions of Azerbaijan under the guidance of A. Asgarov in 2015-2017. Information on the collection area, biotope, geographical coordinates and altitude of each seed sample was indicated in the special descriptor forms (Table 1). We indicated information on the collection area, biotope, geographical coordinates and altitude of each seed sample in the special descriptions. Hipsometric altitude and area coordinates were measured with a Garmin eTex 20 model GPS device according to WGS-84 (G873) (World Geodetic System).

The most commonly accepted classification is the classification of Kupicha, so we accepted that classification in own work [23]. However, it should be noted that new phylogenetic results indicate that the genus *Vicia*, as traditionally understood, is not monophyletic [4; 9; 10; 32]. We used terminology adopted by some authors cited in our article [3; 10; 11; 14; 15; 18; 19; 20; 22; 24, 29].

In the samples, the surface structures, shape, density or waxy layer of papillae (buds with suction surface) were studied by scanning electron microscopy (SEM).

SEM analysis of plant seeds has been done on a microscope JEOL JSM 6610 LV. Modern plant seeds (both living and herbarium specimens) usually do not require special treatment for SEM. They are sufficiently dried in air. Seeds were transferred with a special tool to previously prepared special tables covered with double adhesive carbon tape. To minimize the static effect, the samples were sprayed with golden powder for 1-2 minutes through the JEOL JFC-1100E (JEOL Ltd., Tokyo, Japan) ion sputter coater.

Seeds can also be examined in the low vacuum mode without metal spraying. After that the table was placed in a microscope for SEM analysis. A general view of the seed surface and the observed features of the sculpture at high and low magnifications were photographed with a JEOL JSM6610 LV electron microscope (JEOL Ltd., USA). This microscope photographed nearly 1500 times or more in order to explore the microstructure of the seed's surface. Sometimes it was found useful to tilt the table; for example, to consider a weakly expressed sculpture. Also, on the cracked objects we can see the internal structure of the shells, which is also more convenient to do by tilting the table. The obtained data were stored on removable storage media. Morphological characters, including general shape, color and size of seeds, were studied under the stereomicroscope Leica EZ4D (USA).

Table 1

Deskriptor information about collected seed samples

Seed patterns	Locality code	Collection area	Biotope	Geographical coordinates	Altitude, m
<i>V. monantha</i>	AZE16D6	Shamakhi district, Shamakhi-Agsu highway	roadside	N 40°38'550 E 48°28'450	794
<i>V. tenuifolia</i> subsp. <i>variabilis</i>	AZE17K19	Shamakhi region, Pirgulu village	forest	N 40°46'864 E 48°36'168	1430
<i>V. villosa</i> subsp. <i>villosa</i>	AZE17K20	Shamakhi region, Mirzandiya village	meadow	N 40°34'737 E 48°43'648	584
<i>V. varia</i>	AZE15Z2	Zagatala region, Yukhari Tala village, Parzivan area	roadside	N 46°35'29 E 41°34'227	358
<i>V. elegans</i>	AZE16E4	Ordubad region, Tivi village	foothills	N 39°06'476 E 45°54'702	1920

The most commonly accepted classification is the classification of Kupicha, so we accepted that classification in own work [23]. However, it should be noted that new phylogenetic results indicate that the genus *Vicia*, as traditionally understood, is not monophyletic [8; 9; 10; 31]. We used terminology adopted by some authors cited in our article [7; 10; 13; 16; 19; 20; 21; 28; 30; 32].

Results and discussion

During expeditions were collected seeds of taxa of *Vicia* belonging to subgenus *Vicilla* and section *Cracca* (according to the system of Kupicha): *V. tenuifolia* Roth subsp. *variabilis* (Freyn & Sint. ex Freyn) Dinsm., *V. villosa* subsp. *villosa* Roth, *V. villosa* Roth subsp. *varia* (Host) Corb. (*V. dasycarpa* auct.), *V. monantha* Retz. (*V. cinerea* M. Bieb.), *V. elegans* Guss.

The distribution of *Vicia* taxa collected from different research areas and descriptions of the collected seed samples are provided below:

Subgenus: *Vicilla* (Schur) Rouy

Sect.: *Cracca* S. F. Gray – Annual to perennial, often climbing. Stipules without a dark nectariferous spot. Leaflets few- many- paired, small to large. Peduncle usually much longer than the 1- many flowers. Flowers usually blue- lilac to purplish- red, rarely yellow. Calyx usually irregular and gibbous. Style compressed, equally pubescent all round near the apex. Legumes with coriaceous, glabrous or hairy valves, not torulose; seeds few to many.

Seed patterns: *V. tenuifolia* Roth subsp. *variabilis* (Freyn & Sint. ex Freyn) Dinsm., *V. villosa* Roth subsp. *villosa*, *V. villosa* Roth subsp. *varia* (Host) Corb. (*V. dasycarpa* auct.), *V. monantha* Retz. (*V. cinerea* M. Bieb.), *V. elegans* Guss.

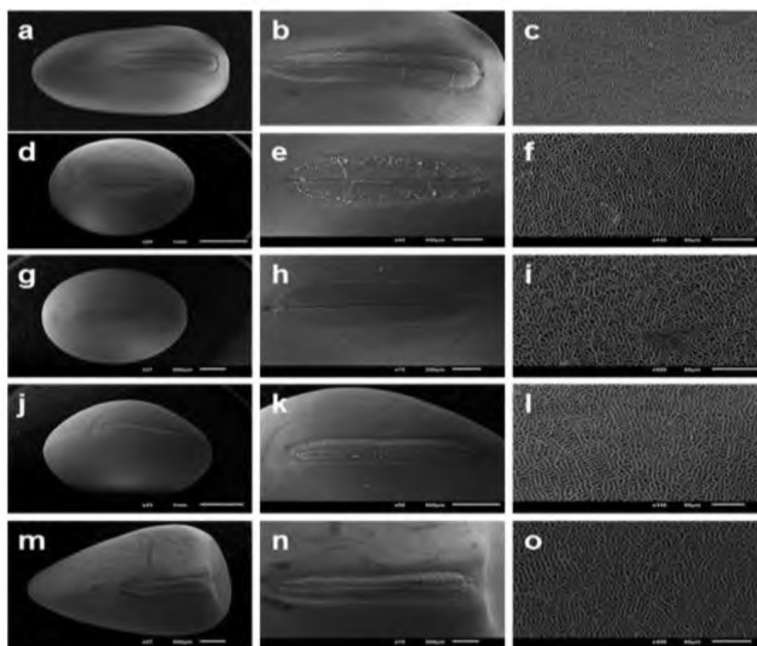


Fig. 1 General shape, hilum shape and seed sculpture some of examined taxa (SEM) a-c: *V. tenuifolia* subsp. *variabilis*; d-f: *V. villosa* subsp. *villosa*; g-i: *V. villosa* subsp. *varia*; j-l: *V. monantha*; m-o: *V. elegans* (scale bar: a-c 1mm, 500, 50 μ m; d-f 1mm, 200, 50 μ m; g-i 500, 200, 10 μ m; j-l 1 mm, 500, 50 μ m; m-o 500, 200, 50 μ m)

Micromorphological structure characteristics of seeds have been analyzed and important diagnostic elements have been identified that may be useful for systematics of controversial taxa. The majority of seed samples are spherical and oblong. The largest seeds are found in *V. tenuifolia* subsp. *variabilis*, and the smallest ones in *V. villosa* subsp. *varia*. Seeds are stained and striped. Hilum can be different of different patterns: long, loose or oval. In all seed samples, the seed coat is papillate. Papillas are large and small. Density, size and shape of papillae differ depending on the type of the papilla. The density of the papillae is estimated to be moderate to high.

Table 2

Testa texture of examined taxa

Signs Seeds	Seed shape	Seed size (mm)	Seed colour	Density of papillas	Shape of papillas	Texture	Shape of hilum	Hilum size (mm)
<i>V. tenuifolia</i> subsp. <i>variabilis</i>	spherical-elliptic	4,168 x 2,760	light brown, with black spot	medium	small conical multirib	papillose	oblong-elliptic	2,115 x 0,424
<i>V. villosa</i> subsp. <i>villosa</i>	spherical	3,240 x 3,048	black, with brown spot	density	conical multirib	papillose	oblong-elliptic	1,297 x 0,448
<i>V. villosa</i> subsp. <i>varia</i>	round	2,821 x 2,771	black	density	conical, multirib, with waxy speckled	papillose	ovate	1,319 x 0,467
<i>V. monantha</i>	spherical	3,903 x 3,711	light brown, with dark spot	density	small conical, multirib	papillose	oblong-elliptic	2,040 x 0,309
<i>V. elegans</i>	ovate	3,947 x 3,083	black, marble	density	broad conical	papillose	oblong-elliptic	1,462 x 0,281

In this study, some characteristics of the seeds were used for the taxonomic delimitation and the identification of them was held according to these characteristics (Table 2). Ovate hilum was observed in *V. villosa* subsp. *varia*. Oblong-elliptic hilum is peculiar to *V. tenuifolia* subsp. *variabilis*, *V. villosa* subsp. *villosa*, *V. monantha*, and *V. elegans*. The papillae with medium density were observed in *V. tenuifolia* subsp. *variabilis* (Fig.1. a-c). Dense papilla were observed in *V. villosa* subsp. *villosa* (Fig.1. d-f), *V. villosa* subsp. *varia* (Fig.1. g-i), *V. monantha* and *V. elegans* (Fig.1. j-l, Fig.1. m-o). The result showed that *V. villosa* subsp. *varia* had conical, multirib papillas with waxy specks. However, small conical multirib were observed in *V. tenuifolia* subsp. *variabilis* and *V. monantha*. Conical multirib was found in *V. villosa* subsp. *villosa* and broad conical in *V. elegans*.

General shape, colour and size of seeds were studied under a stereomicroscope (Fig.2).

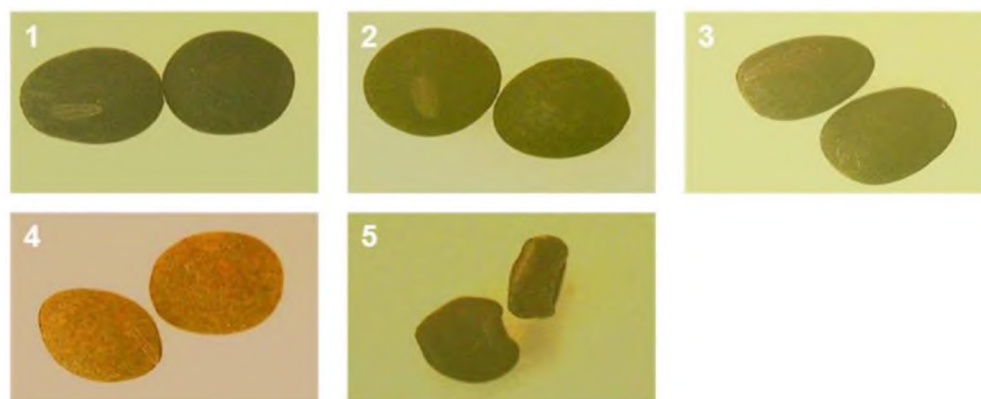


Fig. 2 The general appearance of the seeds under stereomicroscope

1. *V. tenuifolia* subsp. *variabilis*; 2. *V. villosa* subsp. *villosa*; 3. *V. villosa* subsp. *varia*; 4. *V. monantha*; 5. *V. elegans*

Conclusion

Micromorphological analysis of the seeds of important taxa of the genus *Vicia* L. was carried out to eliminate the gaps in seed identification in different Floras.

In result was detected that seeds of taxa of *Vicia* belonging to subgenus *Vicilla* and section *Cracca* had different micromorphological signs: Oblong-elliptic hilum is peculiar to *V. tenuifolia* subsp. *variabilis*, *V. villosa* subsp. *villosa*, *V. monantha*, and *V. elegans* (only ovate hilum in *V. villosa* subsp. *varia*). Dense papilla were observed in *V. villosa* subsp. *villosa*, *V. villosa* subsp. *varia*, *V. monantha* and *V. elegans* (only *V. tenuifolia* subsp. *variabilis* had papillae with medium density). *V. villosa* subsp. *varia* had conical, multirib papillas with waxy specks. Other taxa had simple (without waxy specks) conical papillas.

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Впервые некоторые таксоны рода Вики (*Vicia* L.) из Азербайджана были подвергнуты морфологическому и сканирующему электронно-микроскопическому (SEM) анализу: *V. elegans* Guss., *V. monantha* Retz. (*V. cinerea* M. Bieb.), *V. tenuifolia* Roth subsp. *variabilis* (Freyn et Sint. ex Freyn) Dinsm., *V. villosa* Roth subsp. *varia* (Host) Corb. (*V. dasycarpa* auct.), *V. villosa* subsp. *villosa* Roth.

Образцы семян были собраны в 2015-2017 годах из разных регионов Азербайджана (Закатала, Ордубад и Шамахи). Собранные таксоны были сгруппированы в подвид *Vicilla* и в секцию *Cracca*. В образцах структура поверхности, форма, плотность, поверхность или воскообразный слой папилл (почки с поверхностью всасывания) были изучены методом сканирующей электронной микроскопии (СЭМ).

Морфологические признаки, общая форма, размер, цвет, структура поверхности семян и размеры семян были изучены под стереомикроскопом во время исследования.

Ключевые слова: семена, SEM, вика, бобовые, внутривидовые, сечение, вид

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БИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ РЕДКОГО ВИДА *ALLIUM GRANDE* LIPSKY В БАШКИРСКОМ ПРЕДУРАЛЬЕ

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Приводятся результаты интродукционного изучения редкого вида *Allium grande*: биометрические параметры, сезонный ритм роста и развития, семенная продуктивность и особенности размножения. Луковичный вид, из семейства Alliaceae составляет таксонометрическую секцию *Megaloprason* Wendenbo подрода *Melanocrommyum* (Webb & Berth.) Rouy рода *Allium* L. Вид включен в Красную книгу РФ, статус 2. Эндемик Восточного Кавказа. Феноритмотип – коротковегетирующий,