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Analysis of the ultrastructure of pollen grains and seeds to identify plant materials

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Abstract
We studied the ultrasculpture of pollen grains and the surface, the macro- and microstructure of the seeds of broad beans (*Faba bean* L.), peas (*Pisum sativum* L.) and common beans (*Phaseolus vulgaris* L.). It was revealed that pollen grains are single, medium in size, radially symmetric, three-aperture, but the exine surface sculpture and ornamentation of the pollen grain surface are of decisive importance. The main role in determining pollen grains is played by shape and ornamentation. It was also determined that the seeds of beans differ in the maximum sperm thickness, and the thickness of the sclerotest makes the main contribution to the formation of this trait. The bean epidermis of the seed peel is two-row and has the greatest thickness. When seeds germinate, the matrix protein and aleuron grains of the storage parenchyma are first consumed. Its cells and the size of starch grains are larger in broad beans. And the sign "the ratio of the size of starch grains to the size of the cells of the storage parenchyma,%" allows you to identify fragments of the seeds of broad beans, peas and beans without seed peels.

Keywords: sporderm, palynomorphology, pollen grains, spermoderma, histology of seeds, *Vicia faba*, *Pisum sativum*, *Phaseolus vulgaris*

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INTRODUCTION

Without exaggeration, broad beans, peas and common beans can be called the most common of leguminous crops. They have long been used in food, as fodder plants and as a valuable precursor. Currently, the possibility of using in medicine as plants with antitumor activity and hypoglycemic effect has been proven (Sharaf et al., 1972; Wall & Wall, 1975; Key & Marble, 1999; Sukharev, 2005; Saliu, 2016).

Studies on the viability and storage of pollen are widely presented in the literature in connection with breeding issues (Izakovic, 1989; Murin, 1995; Tsvetova & Ishin, 1995; Mundargi et al., 2016; Kurkina et al., 2018), as well as on the influence of environmental factors on pollen (Sénéchal et al., 2015; Minnaar & Anderson, 2019), much less work is done on the fine organization, ultrastructure of pollen grains in the field of plant systematics and medicine (Tokarev, 2002; Zavialova et al., 2016). In connection with the introduction of electron microscopy methods into palynological practice, the possibilities for studying pollen morphology have significantly expanded.

The growing interest in these cultures of biomedical specialists requires the identification of diagnostic signs of plant materials. It is known that the characteristics of pollen grains and the structure of seeds are constant, therefore they are of great value as determinants. So far, there is no comprehensive information on the histology of seeds of the species *Vicia faba* L., *Pisum sativum* L., *Phaseolus vulgaris* L. (Pavlova, 2009). In this regard, the aim of our research was to identify diagnostic signs of pollen grains and seeds of fodder beans, sowing peas and common beans.

MATERIAL AND METHODS

We studied varieties of broad beans and common beans with small seeds comparable to pea seeds. The material was anthers with pollen grains *Vicia faba* (cultivar Severinovsky), *Pisum sativum* (cultivar Madonna), *Phaseolus vulgaris* (cultivar Nerin). Pollen from dry anthers was placed on the metal tables of the microscope without preliminary treatment. In describing the morphology of pollen grains, the terminology proposed by Hesse et al. (2009). Size groups were established by G. Erdtman on the basis of measurements of the length of the major axis of pollen

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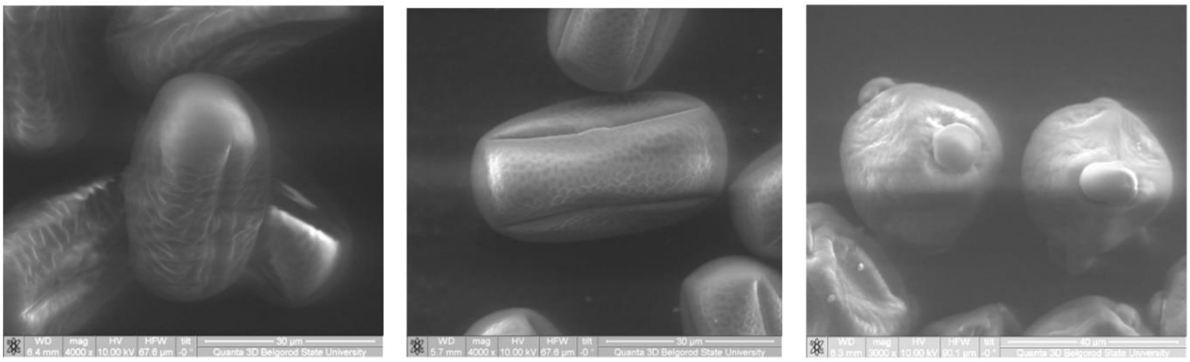

Fig. 1. Pollen grains of *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* (from left to right) with an increase of 4000

Table 1. Morphological characteristics of legume pollen grains

Species of plants	Value (at P> 0.05)			The shape
	P, µm	*E*, µm	*P/E*	
Vicia faba	48.9 – 1.2	23.5 – 24.6	2.08	elongated ellipsoidal
Pisum sativum	44.7 – 45.3	24.5 – 24.3	1.82	ellipsoidal
Phaseolus vulgaris	39.2 – 41.2	40,1 – 40,8	1,00	spheroidal

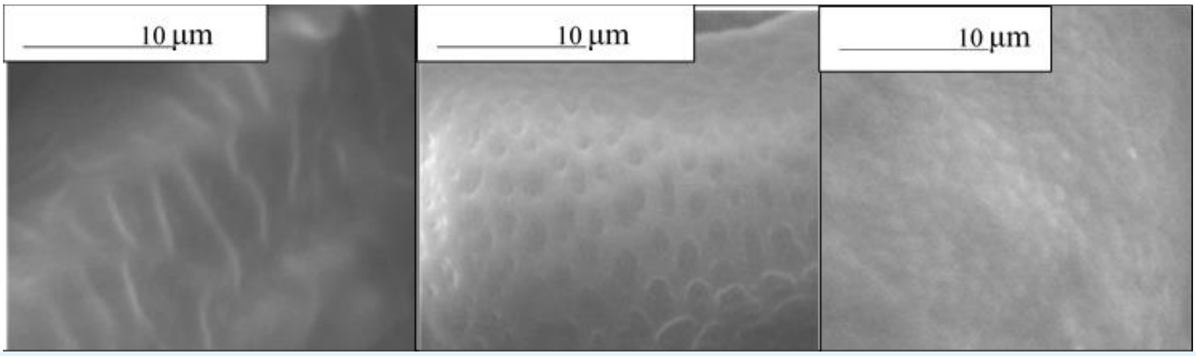

Fig. 2. Sculpture of the surface of legume pollen grains (× 4000): *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* (from left to right) with a magnification of 4000 times

grain. Dry seeds of fodder beans, peas, and common beans were thoroughly cleaned and destroyed their integrity in the mortar. Weighing was carried out in 30-fold repetition on an AR 5120 balance (Ohaus).

The ultrasculpture of pollen grains, surfaces, the microstructure of the sperm (on the lateral side of the seed) and cotyledons (on the adaxial side) were studied on chips using a Quanta 200 3D scanning electron microscope at the Center for Collective Use of Technologies and Materials of Belgorod National Research University. Statistical data processing was carried out on a personal computer using the Microsoft Excel data analysis package. The average error was calculated at a reliability level of 99%.

RESULTS AND DISCUSSION

The analysis of microphotographs of pollen revealed that the pollen grains of the studied plants are single (monads), medium in size (large pollen grains are also found in broad beans), radially symmetric, three-aperture, apertures are symmetrically located (in 3 symmetry planes) (**Fig. 1**).

The polar axis (*P*) in all cases is larger than the equatorial diameter (*E*). The average sizes of *P* and *E* differ at the generic level (**Table 1**).

The shape of the pollen grains in the equatorial projection is rounded for common beans, oblong for broad beans and peas. The pollen grains are spheroidal (common beans), ellipsoidal (peas), and elongated ellipsoidal (broad beans).

By the nature of the apertures, the pollen grains of broad beans and peas are equatorially 3-complexed, while common beans are equatorial-3-pore. Furrows are narrow, long, with straight edges. The pores are round or oval. All pollen grains are of the zonocolporate type.

Of decisive importance in the diagnosis of pollen grains of the studied legume genera is exine surface sculpture (**Fig. 2**), which is consistent with the opinion of L.A. Kupriyanova (1978). So, the sculptural elements of the sporotherm of broad beans and peas are meshes (brochi), and common beans - grains (granula). In broad beans and peas, ornamentation is more or less equally homogeneous (homobrochate).

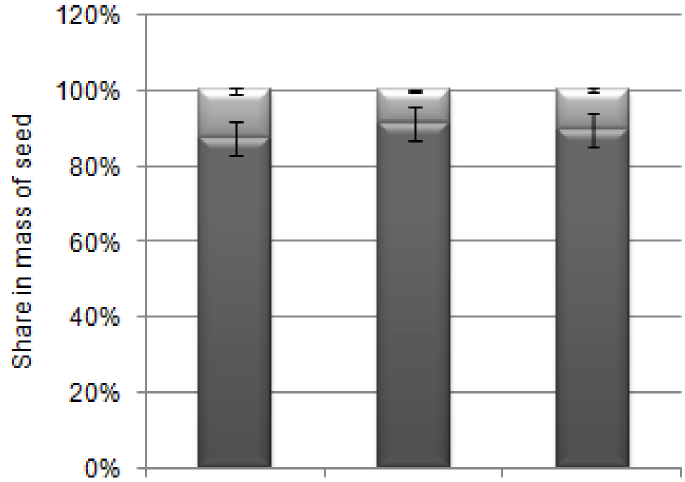

Fig. 3. The proportion of the embryo and seminal peel in the mass of seed: seed peel (top) and seed germ (bottom) of *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* (from left to right)

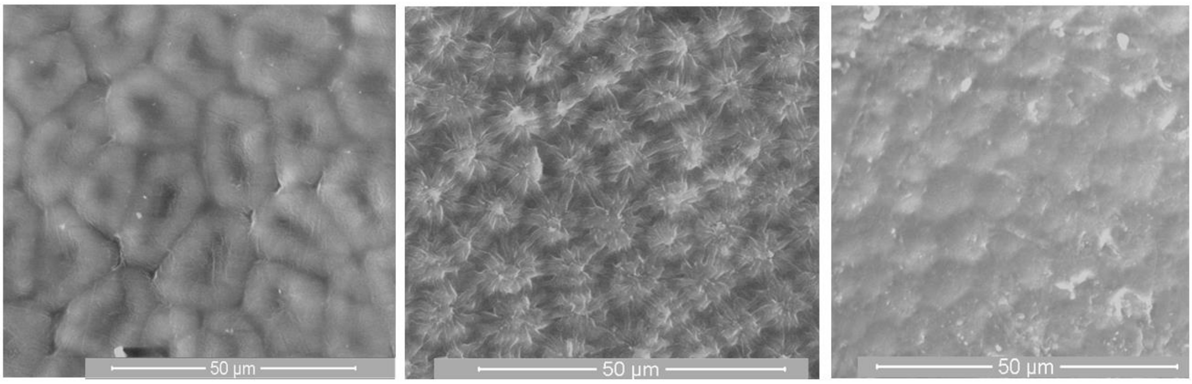

Fig. 4. The surface of the seeds of *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* (from left to right) with an increase of 2000 times

Thus, the shape and ornamentation of pollen grains play a major role in determining the pollen grains of broad beans, sowing peas, and common beans.

Seed analysis showed the following. As can be seen from the data in **Fig. 3**, the largest share of the seed peel in the seed mass was observed in broad beans (12.5%), in common beans and peas, the share of the seed peel in the seed mass was 10.3 and 8.7%, respectively (with 1000 seeds 290, 200, and 220 g, respectively).

There are no stomata on the surface of the seeds. Xylem elements are spiral and ring-shaped. The outer layer - solid, or palisade, consists of thick-walled, densely-closed cells elongated perpendicular to the surface of the seed and forming exesta. The cells of this layer parallel to the surface of the seed are crossed by a light line.

t can be seen from the surface of the seed that the broad bean epidermis consists of multifaceted cells that are almost 2 and 3 times larger than the pea and common bean epidermal cells in size. The surface of the cells has central folds - hollows, which makes the seeds dull. Epidermal cells of star-shaped pea seeds, strongly folded in the radial direction, explaining the “roughness” of the seeds to the touch. The common bean epidermis is represented by cells of almost rounded shape, without folds and indentations, which determines the glossy surface of the seeds (**Fig. 4**).

Thus, the epidermal cells of broad beans and peas are so densely arranged that they do not pass water and folds form on their surface, which gradually disappear when the seeds swell.

The palisade cells of the epidermis form the exesta of the seed coat. The epidermis of common bean sperm is two-row. The light line here passes between two rows of epidermal cells of the same height. The smallest epidermis thickness was observed in peas (**Table 2**). Since there is no significant difference in the thickness of exesta from broad beans and beans, the number of rows of the epidermis is a diagnostic sign. The broad bean expressed and mesotest thickness of 25.8 ± 4.3 µm, consisting of multifaceted densely closed cells.

The hypodermis is represented by coil-shaped, or columnar, cells - osteoscleroids, which form the endotest of the sperm. There were no significant

Table 2. Morphometric characteristics of seeds

Signs, µm	*Vicia faba*	*Pisum sativum*	*Phaseolus vulgaris*
Size of the sperm epidermis cells	21.5 ± 2.7	12.1 ± 0.9	7.5 ± 1.1
Sperm epidermis thickness	67.6 ± 4.5	39.8 ± 1.2	72.1 ± 2.3
Hypodermis thickness	10.1 ± 2.7	8.5 ± 0.8	7.2 ± 0.9
Sclerotest thickness	103.3 ± 8.1	48.3 ± 1.5	79.2 ± 2.5
Parenhotest thickness	31.8 ± 2.8	25.6 ± 2.3	25.2 ± 3.3
Size of cotyledons epidermis cells	74.8 ± 8.1	32.1 ± 4.6	42.9 ± 3.9
Size of storage parenchyma cells	81.1 ± 0.9	68.9 ± 3.7	97.1 + 12.8
Size of starch grains	19.2 ± 3.2	27.6 ± 6.9	31.1 ± 2.2

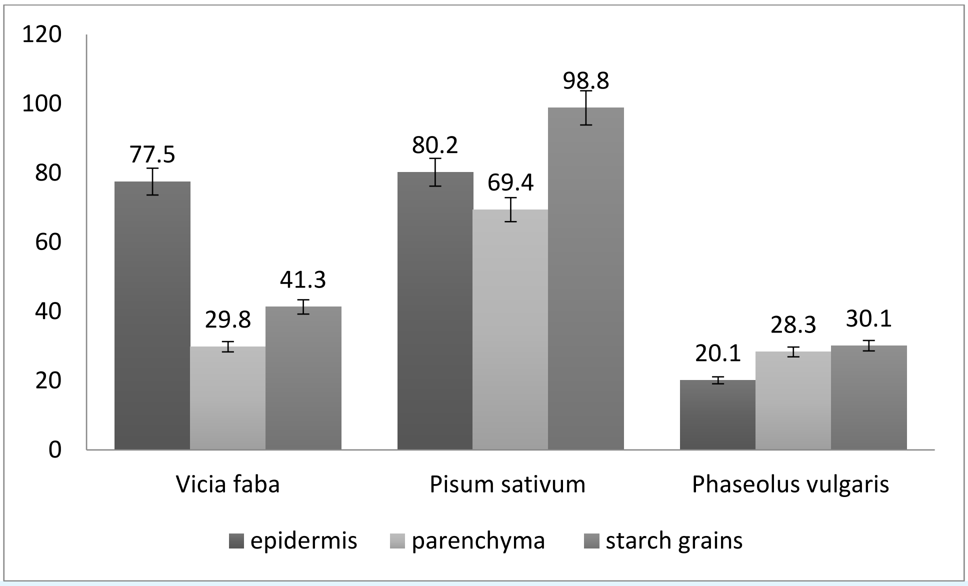

Fig. 5. The cell size (µm) of the cotyledons of the epidermis, parenchyma and starch grains of *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* (from left to right)

differences in the thickness of the hypodermis (see **Table 1**). This layer of sperm is called swelling due to the ability of parenchymal cells to increase in size when water enters them and to provide “swelling” of seeds during germination

The broad bean sclerotest is formed by the exo-, meso- and endotest, while in peas and common beans, the sclerotest is represented by a combination of exo and endotest. The average thickness of the sclerotest of bbroad beans exceeds that of peas by 2 times. Below is the parenhotest. This is a parenchyma, the cells of which gradually decrease towards the embryo. The thickness of the parenhotest is comparable in the seeds of the studied cultures (see **Table 2**). Thus, the average thickness of broad bean sperm is 135.1 µm, common beans - 104.4, and peas - 73.9 µm. From the above data it is seen that the thickness of the sclerotest makes the main contribution to the thickness of the sperm.

The epidermis of the cotyledons of the studied cultures without stomatal apparatus. The cells of the epidermis of the broad bean cotyledon are prosenchymal with slightly pointed, and the beans with rounded ends. The shape of the pea epidermal cells is almost rectangular. From the data of **Table 2** it can be seen that the length of the epidermal cells of the broad bean cotyledons is 1.7 times greater than that of common beans and 2.3 times larger than peas (**Fig. 5**).

The cells of the storage parenchyma are almost isodiametric, thin-walled, the shortest in peas and the longest in common beans. The cotyledon parenchyma cells are filled with starch grains, which are connected by a continuous protein matrix. Common beans and peas have larger starch grains (see **Table 2**) than broad beans and 62 and 44%, respectively. It is known that the seeds of broad beans are characterized by a high protein content, while the selection of common beans and peas (as food crops) was carried out, for the most part, on taste, depending on the starch content

Fig. 6 shows the recesses in the matrix from precipitated starch grains in the seed parenchyma (for example, beans). Protein granules formed during precipitation of a protein that was in vacuoles are located mainly along the cell wall of parenchymal cells (for example, common beans).

In **Fig. 7** (using the example of broad beans), it is seen that during seed germination, matrix protein and protein granules are consumed first. At the same time, the cotyledons become loose, since only strands remain from the protein matrix.

Offering seed attributes, M.E. Pavlova (2009) considers it appropriate to reflect not the absolute sizes of seeds and their parts, but their ratios, expressed as a percentage. So, traditionally used, for example, the ratio of the width or thickness of the seed to the length; for the

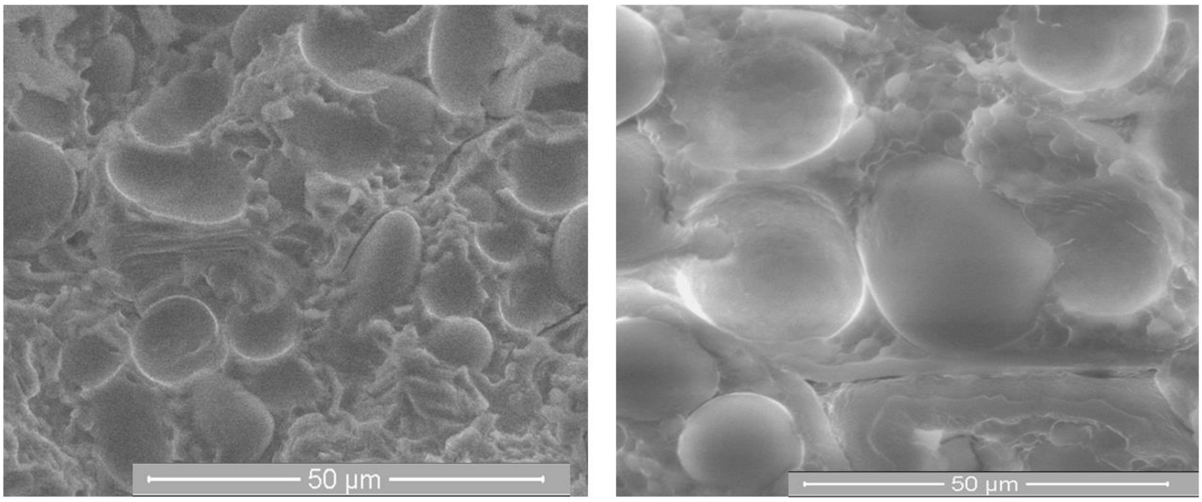

Fig. 6. Micrographs of the cells of the storage parenchyma of the cotyledon broad beans (left) and common beans (right) with an increase of 2000 times

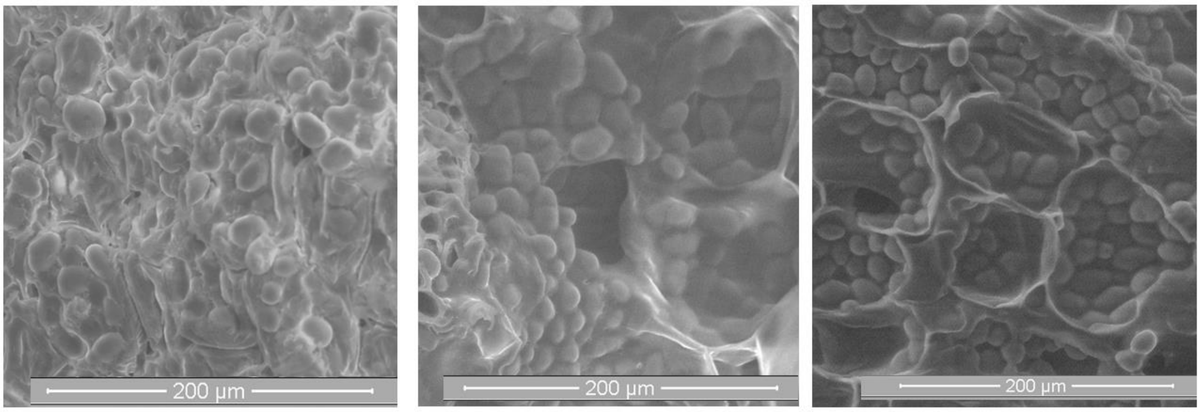

Fig. 7. Micrographs of broad bean cotyledon parenchyma cells taken before germination, on the 9th and 13th day from the start of germination (from left to right)

Table 3. Diagnostic signs of seeds

№	Sign	*Vicia faba*	*Pisum sativum*	*Phaseolus vulgaris*
1	The ratio of sperm thickness to seed thickness, %	2	1.5	2.5
2	The ratio of the size of the cells of the epidermis of sperm (from the surface of the seed) to the thickness of the sperm, %	14 – 15	16	6 – 7
3	The ratio of the size of the cells of the epidermis of the cotyledon (from the surface of the seed) to the size of the cells of the storage parenchyma, %	72 – 105	36 – 54	42 – 53
4	The number of rows of the epidermis	1	1	2
5	The ratio of the size of starch grains to the size of the cells of the storage parenchyma, %	20 – 30	40 – 50	30 – 40
6	The ratio of epidermal thickness to sperm thickness, %	47 – 52	50 – 57	66 – 74
7	The size of the epidermal cells (from the surface of the seed), microns	17 – 25	11 – 13	5 – 8
8	Sperm cell epidermal cell shape from the surface of the seed	polygonal with a central hollow	star	rounded

genus Bauhinia, the ratio of the thickness of the sperm epidermis to the thickness of the sperm was proposed (Kurkina et al., 2018).

Signs of seeds that can be considered diagnostic for broad beans, sowing peas and common beans are shown in **Table 3**.

Thus, depending on the presence of certain fragments of seeds when determining plant materials, the signs given in the table can be used. It can be seen that the first two signs are the most laborious. Sign No. 3 can be used if it is necessary to identify crushed broad bean seeds from common beans and peas; its combination with sign No. 4 becomes informative already for three crops. Sign No. 5 will become the only possible application if there are no fragments of the seed peel in the analyzed mass. Sign No. 6 can be used when comparing fragments of common bean seeds with seeds of broad beans and peas. The simplest of the signs of the seed microstructure is trait No. 8, which can be combined with No. 7 or used independently.

CONCLUSIONS

1. The shape and ornamentation of pollen grains play a major role in determining the pollen grains of *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris*.

2. The maximum thickness of sperm is also characterized by *Vicia faba* seeds. The main contribution to the formation of this trait is made by the thickness of the sclerotest.

3. The epidermis of sperm and cotyledons of the studied cultures does not contain stomatal apparatus. The *Phaseolus vulgaris* epidermis of the seed peel has a double row and a light line passes between two rows of cells.

4. Ultrasculpture of the surface of the seed can serve as a simple diagnostic sign.

5. When seeds germinate, the matrix protein and aleuron grains of the storage parenchyma are first consumed. Its cells and the size of starch grains are larger in *Phaseolus vulgaris*. And the sign "the ratio of the size of starch grains to the size of the cells of the storage parenchyma,%" allows you to identify fragments of the seeds of *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* without seed peels.

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