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I.Yu. Maltsov, P.Ye. Bulakh

The Comparative Morphological Analysis of Pollen Grains of Different Genotypes of the Sweet Cherry (*Cerasus Avium* (L.) Moench) and Apple Tree (*Malus Domestica* Borkh.) Growing in Slovakia

M.M. Gryshko National Botanic Garden NASU,
1, Timiryazevska Str., Kyiv, 01014, Ukraine

The comparative morphological analysis of pollen grains of different genotypes of a sweet cherry (Cerasus avium (L.) Moench.) and apple tree (Malus domestica Borkh.), grown in Slovakia is carried out. The technique of allocation of morphological criteria of their difference is work out. A qualitative criteria (presence or absence perforation on a surface of pollen grains, the shape of striated elements of a sculpture, presence or absence on them coalescence and overstressing), and quantitative criteria (average length of a polar axis and equatorial diameter) are separated. The statistical analysis of values of the separated features for the investigated genotypes of a sweet cherry has allowed to allocate the certain genotypes, or groups of genotypes that shows identification of pollen grains' belonging.

Key words: *Cerasus avium, Malus domestica,* pollen grains, morphometry, a sculpture of exine, statistical methods, beekeeping.

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Introduction

A sweet cherry (*Cerasus avium* (L.) Moench.) and its grades one of the basic entomophilous melliferous fruit plants, playing the important role in maintenance of beekeeping with a forage reserve, which manufacture constantly increases, is (Gonzalez-Gomeza et al., 2009; Lyle, 2006; World Sweet Cherry Review, 2006). An apple tree (*Malus domestica* Borkh.) is the major fruit culture, giving valuable spring bribes to bees (Словарь-справочник пчеловода, 1955). However, both the sweet cherry, and many grades of an apple tree cannot be pollinated by pollen of the grade, and yield a normal crop only at pollination by their other grades (Gluhov, 1960). Therefore, gardens are pollinated by bees, firstly provide beekeeping with a forage reserve, secondly – increase productivity up to 35-50 % (Нуждин, Виноградов, 1971).

Sweet cherry is a large fruiter which is winning first place among stone fruits tree as a meliferous plant, being a valuable April-May meliferous plant, giving to bees nectar, pollen-load and glue-propolis. Blossoms at the end of spring: a tree during 10 days, tree plantations - 15-20 days. Each flower lives about 5 days, allocating 1.5-2 mg of sugar in nectar. Due to at favorable conditions: cross-pollination, weather and correct agricultural technician sweet cherry can give 36-40 kg of honey from 1 hectare. However, almost all honey from a sweet cherry usually early blossoming goes on growing brood. Sweet cherry – typical cross-pollination plant, and within the limits of a grade it is sterile of itself. Therefore gardens of a sweet cherry should consist of 3-4 mutually pollinated grades. The best carriers of pollen are bees during flowering on 1 hectare sweet cherries 2-3 bee families are required (Словарь-справочник пчеловода, 1955).

Apple tree is a tree in height up to 15 m, also there are and procumbent varietis. The apple tree plentifully blossoms and is willingly visited by bees – the basic pollinator of its flowers (Глухов, 1960). Depending on a grade to blossom starts with 3-8-th year of a life, at the end of spring. The flower lives 3-4 days, the tree blossoms about 10 days, tree plantation - about 15-18 days. Nectariferous tissues in the flower of an apple tree is near staminal rings on walls of a receptacle. The flower allocates in nectar on the average about 0,025 mg of sugar. Nectar of an apple tree is light yellow, rather viscous and, very sweet on taste. At plentiful allocation it is possible to notice as light drops around ovary of a fruit. Concentration of sugar in nectar is abaut 20-40 % (Глухов, 1960). In industrial gardens during their flowering a bees collect about 5-8 kg of honey. This honey is efficiency of 25-30-years plantations makes 20-30 kg/hectare (Бурмистров, Никитина, 1990). Honey more liquid, than received from summer plants, but has pleasant taste, fragrance, color of honey is yellow, sometimes a little bit darkish. (Словарь-справочник пчеловода, 1955).

Pollen and honey find is used in the medical purposes at various diseases. There are proteins, amino acids, carbohydrates, vitamins, enzymes, hormones, antibiotics, microcells and other substances are found out in pollen. It is established, that flower pollen renders beneficial influence on an human organism and can be used as a dietary food stuff (Бурмистров, Никитина, 1990; Нуждин, Виноградов, 1982).

Pollen possesses high nutritional value, in it. Undoubtedly, any other product of a natural origin cannot compete to it. While fruit and vegetables consists of water (90-92 %) and, have few valuable components. Pollen, in that kind as it is received to the consumer, practically does not contain some water. At processing in a dryer it loses about 10 % of the weight, fruit and vegetables would lose at the same operation approximately 90 % (Нуждин, Виноградов, 1982). Thus, pollen appears extraordinary rich food stuff, equal to which we cunt find in the nature, and besides it is a high-grade product.

It is necessary to note, that allergic diseases are always caused by pollen, spread by a wind. The pollen collected by bees never the reason of them happens because at the moment of formation pollen loads forager add to it of a few nectar and a saliva which destroy the allergens contained in pollen (Бурмистров, Никитина, 1990). However it is important to remember, that from some kinds of plants of a bee collect the pollen which has poisonous properties. It is also established, that sometimes high toxicity possesses pollen of some agricultural widespread and important plants from families of *Poaceae* and *Compositae* though in they are not poisonous by themselves (Нуждин, Виноградов, 1982). Therefore by manufacture of medical products it is necessary to establish more precisely a specific origin used for this honey, pollen and bee-bread. Besides detection in honey and bee-bread pollen of poisonous plants will allow to diagnose sharply proceeding illnesses of bees, such, as nectareous and pollen toxicoses (Бурмистров, Никитина, 1990).

Researches of morphology of pollen grains of the pollen load, the honey saving up in beehives and bee breads was carried out by A Sala-Llinares, *et al.* (A Sala-Llinares *et al.*, 1992). It is established, that the steadiest is the structure of an exine which were not changed during storage. They weren't practically damaged, were grains in pollen loads and a bee bread. The comparative analysis of morphometric parameters of grains in various products of beekeeping, unfortunately, was not carried out. By other researches (Бурмистров, Никитина, 1990) it is established, that the pollen grains of the same kinds received from a bee bread, honey, and also from flowers of plants and their herbarium samples, on features of a morphological structure and the size do not differ essentially.

For today there are no standards for certification of products of beekeeping, in particular at definition of uniformity and a botanical origin of honey, a pollen load and a bee bread, that, certainly, constrains development of beekeeping. For such introduction of standards it is necessary to develop a technique of definition of uniformity and a botanical origin of products of beekeeping. The most authentic and accessible, in our opinion is such criterion as morphological features of pollen grains present in them. The importance of morphological features in systematization of plants at classification of taxons of a different rank, down to a specific level is confirmed with many researches (Косенко, 1992; Соколова, 2002).

A lot of researches show importance of morphological and morphometric features of pollen grains for identification of a species of fruiters of family *Rosaceae* is carried out (Shim *et al.*, 1988; Hebda, Chinnappa, 1990; Xiang and Sheng, 1991; Joneghani, 2008). In particular, Thakur and Thakur (Thakur, Thakur, 1970) asserted, that figure of an exine of pollen is so genetically steady, that for a various species stone fruits plant it can be used for identification of a species. Hebda, *et al.* (Hebda *et al.*, 1988) has shown, that the sculpture of an exine, the aperture and structure of a zone of the aperture, the form and the size of a pollen grain (a line of the attributes) allowing to identify genera and even a species *Rosaceae*. Fogle (Fogle, 1977-1) used length and width of a pollen grain, height of crests of an exine and a ledge of pores for definition of a species of fruiters. It also marked that structure of an exine of pollen grains between various genotypes of *Malus domestica* diferes (Fogle, 1977-2).

Distinction in morphology pollen grains of a different species of a sweet cherry is established by S.A Gilani, *et al.* (Gilani *et al.*, 2010). Such as differences in characteristics of pollen grains between various genotypes of one species such researches devoted to a sweet cherry practically are absent. It is possible to allocate S.M. Motyleva *et al.* works (Мотылева, *et al.*, 2011) and Ostrovsky *et al.*, (Ostrovsky *et al.,* 2011) in which morphometric features of various grades and genotypes were studied, the sufficient comparative analysis of the received results however is not noticed. The opportunity can be useful for definition of an accessory of pollen grains to this or that genotype can be useful for definition of a high-quality belonging of pollen loads or bee breads. There for such is obligat multi variety forage reserves in beekeeping of a sweet cherry gardens. Besides such opportunity is necessary for specification of strategy of gathering by bees of pollen and nectar from fodder trees.

In the present work results of research which purpose is allocation of morphological criteria of difference of pollen grains of different genotypes of two representatives of family *Rosaceae* Juss. - sweet cherries (*Cerasus avium* (L.) Moench.) and apple trees (*Malus domestica* Borkh.) (growing in Slovakia) are reflected. The basic morphometric parameters of pollen grains and features of an ornamentation of an exine have been investigated and the comparative statistical and morphological analysis is carried out. For identification of genotypes as qualitative criteria (presence or absence of perforation on a surface of pollen grains, accretions and overstressings at striated elements of a sculpture, the form of striated elements of a sculpture), and quantitative (the average sizes of attributes: lengths of a polar axis and equatorial diameter). Such approach has allowed to allocate the majority of the investigated genotypes.

I. Materials and Methods

Material for research selected during full flowering from eight genotypes *Cerasus avium* (PA138, PA141, PA174, PA481, PA560, PA916, PA945, PA969) and seven genotypes *Malus domestica* (Md-1/4, Md-1/8, Md-2/10, Md-5/12, Md-9/5, Md-11/2, Md-33/9).

For studying preliminary dried up pollen was used. An anther placed on subject glass, then a preparation needle destroyed an environment of an anther, pollen grains wich a brush transferred on an objective little table of a microscope with bilateral adhesive tape preliminary pasted on it. A material sputter-coated with carbon, and then with gold (Карупу, 1964; Уикли, 1975). For research of morphological parameters used scanning electronic microscopes ZEISS EVO LS 15 (Carl Zeiss) and JSM-6700F (JEOL) was used.

Morphological parameters of pollen grains were measured. For measurements license program AxioVs40 V 4.8.2.0 (Carl Zeiss) was used. such features as a polar axis (P – a direct line between distal and proximal poles of a pollen grain), equatorial diameter (E – a direct line in an equatorial part of a pollen grain perpendicular to a polar axis) were measured. The morphological description of pollen grains was carried out at increase in 2500-3000 times, the description of a sculpture of a surface at increase in 10000 times was carried out.

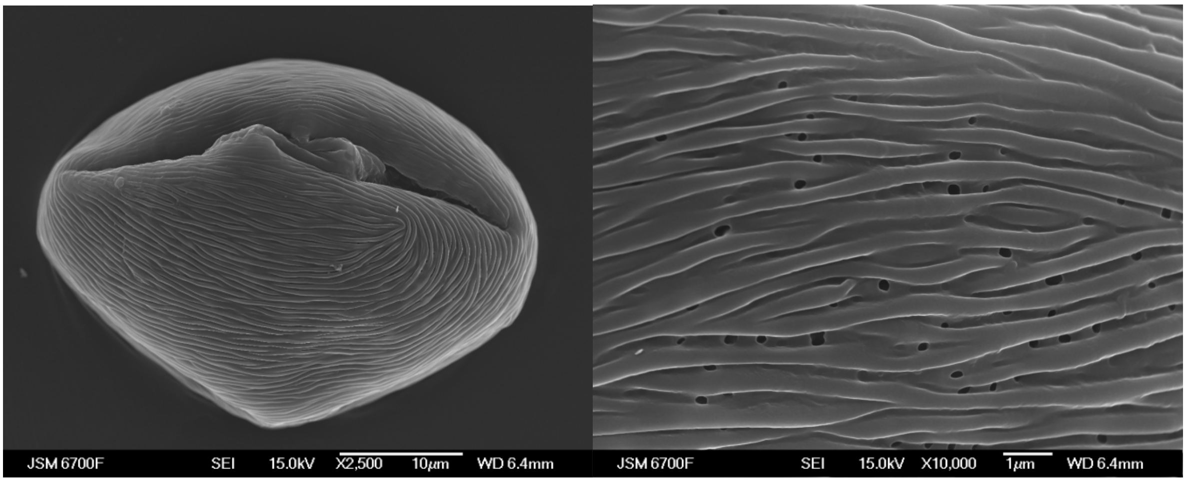
The amount of sampling at measurement of pollen grains for each species has been accepted equal 30, paid off on sample with the greatest factor of a variation at confidential probability q = 0.99 and relative discrepancy ε no more than 5 % (Урбах, 1963).

Estimation of reliability of distinctions between average values of sample carried out{spent} using methods of the dispersive analysis (ANOVA). For the analysis of the received data used also main component method and the clustered analysis. Results were processed in system STATISTICA 6.1.

At the description of pollen grains used the terminological dictionary «Glossary of Pollen and Spore Terminology» (Punt et al., 2007) and also other works (Erdtman, 1943; Куприянова, Алешина, 1972; Токарев, 2002) were used.

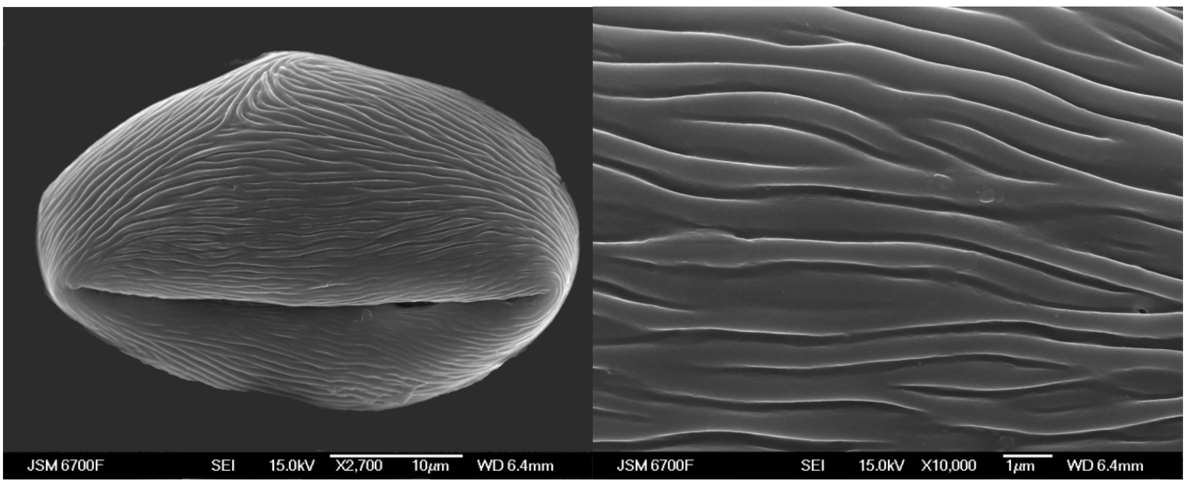
II. Results and Discussion

General view from equator and details of a sculpture of a mesocolpium of pollen grains of investigated genotypes *Cerasus avium* are submitted in figures 1-8.



A B

Figure 1. Pollen grains of *Cerasus avium* PA138. A – equatorial view (X2500);
B – sculpture of the mesocolpium surface (X10000).



A B

Figure 2. Pollen grains of *Cerasus avium* PA141. A – equatorial view (X2700);
B – sculpture of the mesocolpium surface (X10000).

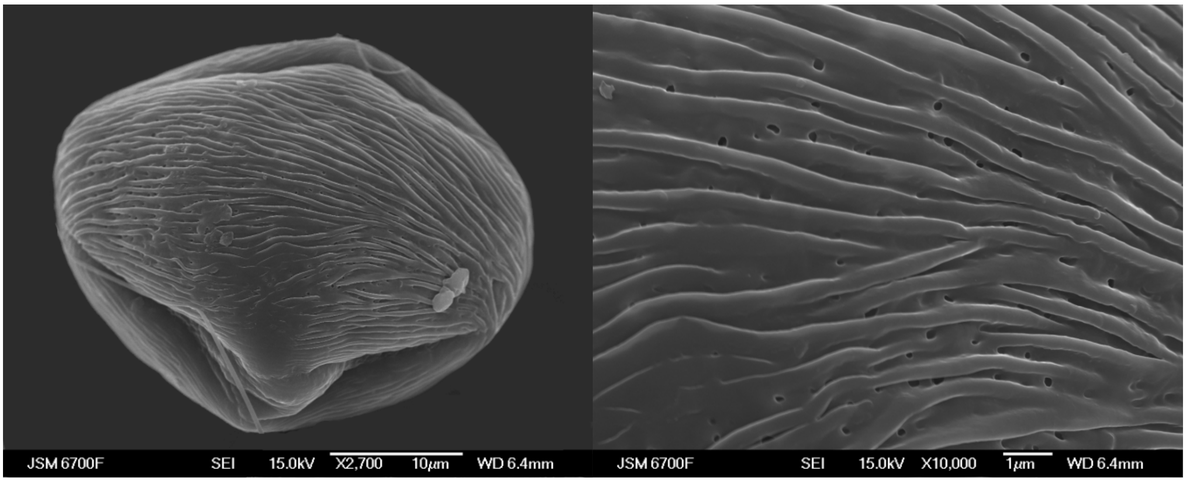


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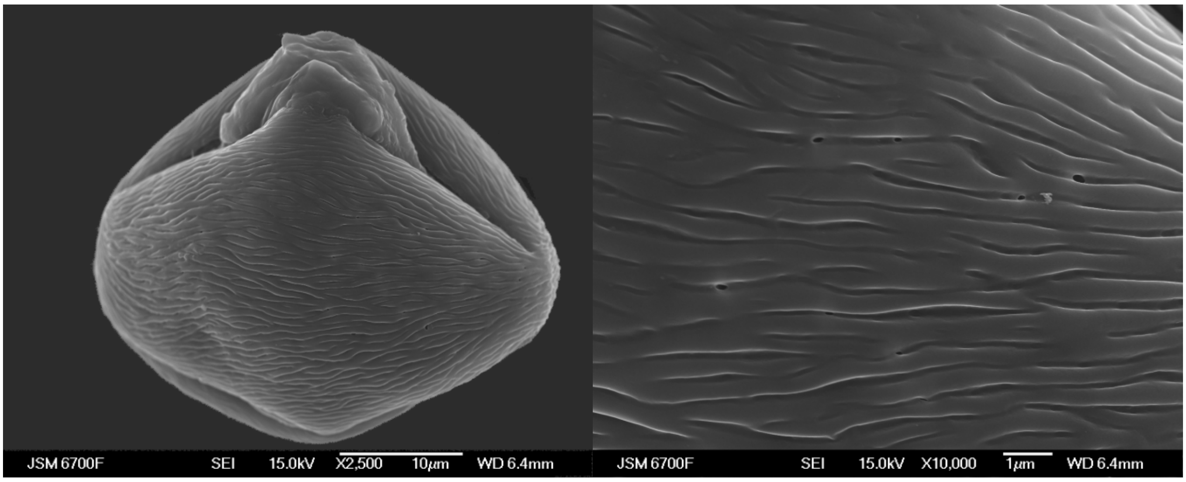
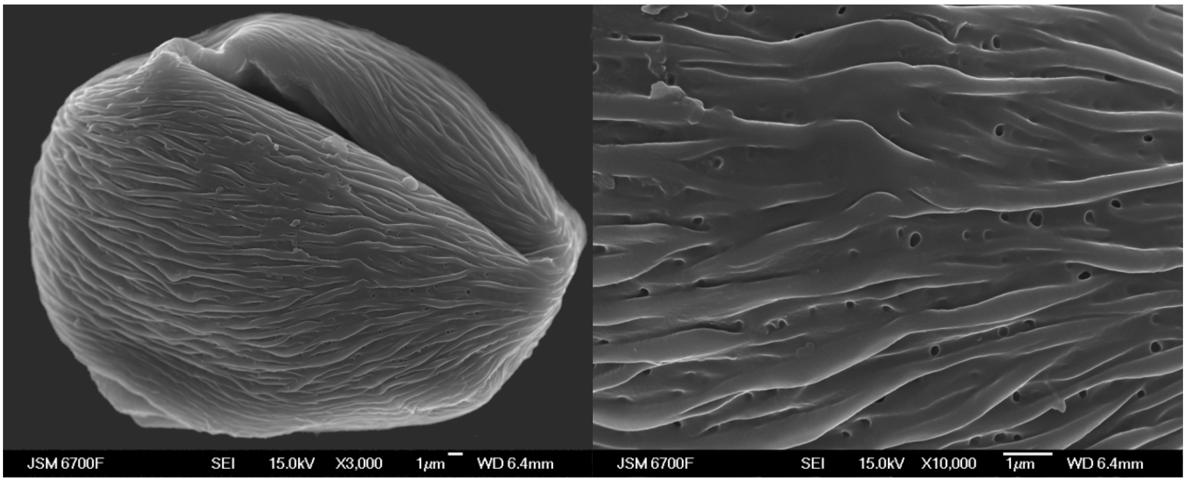
Figure 3. Pollen grains of *Cerasus avium* PA174. A – equatorial view (X2500);
B – sculpture of the mesocolpium surface (X10000).



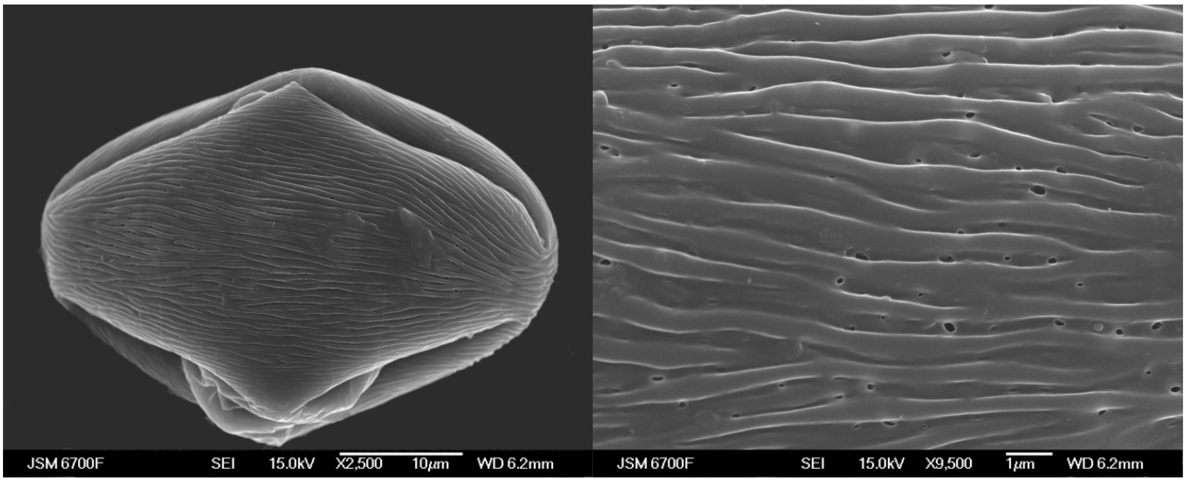
A B

Figure 4. Pollen grains of *Cerasus avium* PA481. A – equatorial view (X3000);
B – sculpture of the mesocolpium surface (X10000).



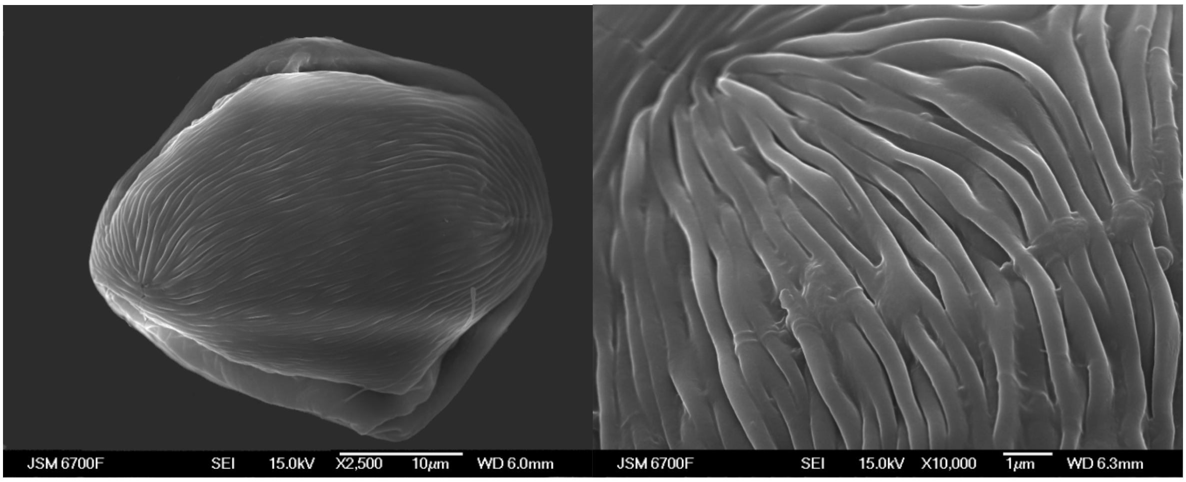
A B

Figure 5. Pollen grains of *Cerasus avium* PA560. A – equatorial view (X2700);
B – sculpture of the mesocolpium surface (X10000).



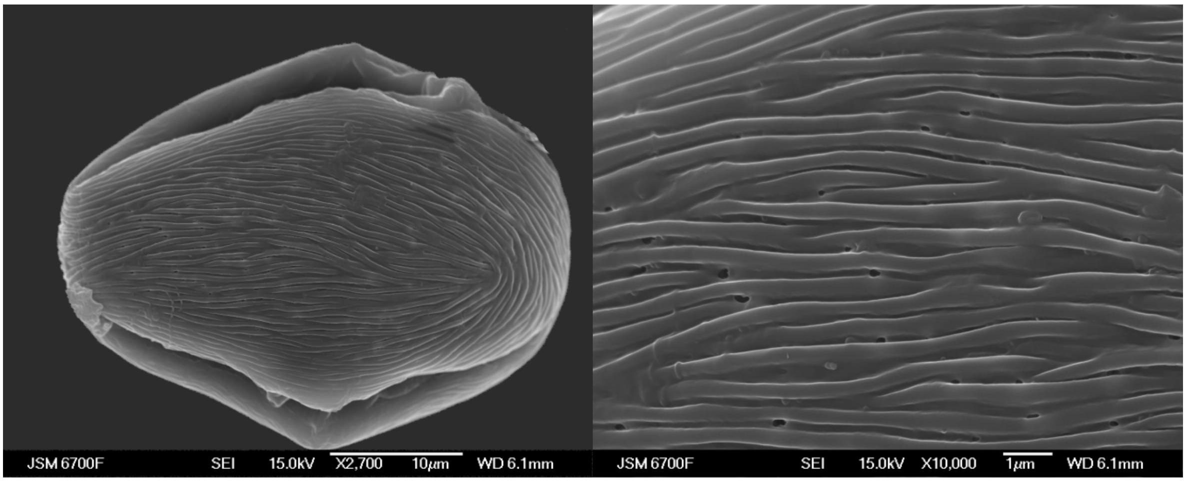
A B

Figure 6. Pollen grains of *Cerasus avium* PA916. A – equatorial view (X2500);
B – sculpture of the mesocolpium surface (X10000).



A B

Figure 7. Pollen grains of *Cerasus avium* PA945. A – equatorial view (X2500);
B – sculpture of the mesocolpium surface (X10000).



A B

Figure 8. Pollen grains of *Cerasus avium* PA969. A – equatorial view (X2700);
B – sculpture of the mesocolpium surface (X10000).

Pollen grains colpate, in an outline from a pole circularly-trilobate or circular- triangular, from equator ellipsoidally-circular (an average index of the form in a range from 1.28 till), isopolar; the average sizes, average length of a polar axis in a range from 36.26 µm till 43.94 µm, equatorial diameter from 25.19 µm till 29.08 µm. Colpi long, with parallel edges, the ends of colpi slightly circular or poorly pointed. A sculpture of an exine striated less often striato-adnate (PE945), sinuous shape (PE560), frequently, perforated (PE138, PE174, PE481, PE560, PE916, PE969).

As criteria of morphometric differences of pollen grains of objects of research we had been chose length of a polar axis and equatorial diameter. Results of measurements are resulted in table 1.

We had been analysing a belonging of sample of values of length of a polar axis (P) and equatorial diameter (E), different genotypes to one general set. The dispersive analysis (ANOVA) has shown (with a significance value 0.01), that samples for different genotypes as for P (p = 0.000023), and for E (p = 0.000039), are not samples of one statistical population. For an estimation of the importance of distinctions between average of concrete genotypes, we had been applied criterion of the least significant difference. Results are submitted in table 2. The analysis of results shows, that significantly different (with a significance value 0.05) on value of average of length of a polar axis ($\overline{x}$ = 36.26 µm) from the others, is a genotype PA560, and on value of average equatorial diameter ($\overline{x}$ = 25.19 µm) – is a genotype PA174. These relations between the investigated genotypes are well illustrated on dendrogram of a clusterization based on morphometric features which is shown in figure 9. More evidently relations between morphometric parameters of various genotypes shows the method of principal component analysis. Results of the analysis are submitted in figure 10. We observe, that the most different on the morphometric criteria (wich we have chosen) are a genotypes PA560 and PA174. To them on properties genotypes PA138 and PA945 are opposite accordingly. Other genotypes are located compactly enough, wich means that they are a little differ from each other on length of a polar axis and equatorial to diameter.

Table 1

Comparison of pollen grains polar axes length and equatorial diameter of tested sweet cherry genotypes (*Cerasus avium* (L.) Moench.)

Genotypes	**P – polar axes (µm)**				**E – equatorial diameter (µm)**				**SI**
	min	**max**	$\overline{x}$	**V%**	**min**	**max**	$\overline{x}$	**V%**	
PA138	39.14	47.93	43.94	8.61	24.43	30.61	28.31	8.28	1.56
PA141	37.19	49.33	41.14	9.77	25.78	29.74	27.18	4.89	1.52
PA174	31.82	44.22	39.52	10.48	22.19	27.32	25.19	6.08	1.57
PA481	36.45	49.76	40.87	8.92	18.84	31.07	26.92	9.93	1.54
PA560	31.21	42.09	36.26	8.68	25.26	31.56	28.45	7.77	1.28
PA916	34.02	45.64	40.83	8.01	24.36	29.57	27.30	6.63	1.50
PA945	37.48	45.54	41.03	5.60	24.45	33.78	29.08	6.89	1.42
PA969	37.86	47.93	41.80	7.24	24.02	31.63	27.73	6.97	1.52

min – minimal value; max – maximal value; $\overline{x}$ – average value; V – variation coefficient (%); SI – shape index (P/E ratio).

Table 2

The significance of distinctions between average values of length of a polar axis (the top value) and equatorial diameter (the bottom value) various genotypes of sweet cherry (*Cerasus avium* (L.) Moench.)

	PA138	**PA141**	**PA174**	**PA481**	**PA560**	**PA916**	**PA945**	**PA969**
PA138		0.127455	0.011689*	0.063888	0.000010**	0.078048	0.061877	0.169053
		0.333659	0.005467**	0.188000	0.895443	0.369876	0.428806	0.497124
PA141	0.127455		0.277833	0.847765	0.000793**	0.835753	0.928999	0.604379
	0.333659		0.038314*	0.773238	0.163116	0.898916	0.020213*	0.573853
PA174	0.011689*	0.277833		0.282035	0.012643*	0.352283	0.183997	0.045287*
	0.005467**	0.038314*		0.032317*	0.000130**	0.019612*	0.000000**	0.000928**
PA481	0.063888	0.847765	0.282035		0.000137**	0.969879	0.877609	0.350749
	0.188000	0.773238	0.032317*		0.043465	0.646346	0.000835**	0.261264
PA560	0.000010**	0.000793**	0.012643*	0.000137**		0.000781**	0.000010**	0.000000**
	0.895443	0.163116	0.000130**	0.043465		0.177546	0.332580	0.221716
PA916	0.078048	0.835753	0.352283	0.969879	0.000781**		0.863247	0.404827
	0.369876	0.898916	0.019612*	0.646346	0.177546		0.017954*	0.654119
PA945	0.061877	0.928999	0.183997	0.877609	0.000010**	0.863247		0.350054
	0.428806	0.020213*	0.000000**	0.000835**	0.332580	0.017954*		0.006723
PA969	0.169053	0.604379	0.045287*	0.350749	0.000000**	0.404827	0.350054	
	0.497124	0.573853	0.000928**	0.261264	0.221716	0.654119	0.006723	

* – $P < 0.05$; ** – $P < 0.01$.

The comparative analysis of a sculpture surface of a pollen grains' mesocolpium of of researched objects allows to allocate the following criteria of difference. The first criterion is presence or absence of perforation on a surface of pollen grains (fig. 1B, 3B, 4B, 5B, 6B, 8B); subcriterion of this criterion is the quantity of perforation on unit of the area : <400000/mm (fig. 2B),> 400000/mm (fig. 1B, 3B, 4B, 5B). The second – character of sculptural elements, namely presence (fig. 7B) or absence (fig. 1B, 2B, 3B, 4B, 5B, 6B, 8B) overstressings at striated elements of a sculpture. The third criterion - the form of sculptural elements: sinuous (fig. 5B), either more or less a straight line (fig. 1B, 2B, 3B, 4B, 6B, 7B, 8B). The data on presence or absence of the separated features at investigated genotypes are shown in table 3. Having accepted these criteria as qualitative, alongside with morphometric parameters, we had been applied the clustered analysis of hierarchical connections between researched genotypes (a rule of association – a method of single connection, a measure of affinity – Chebyshev distance). Results are submitted as dendrogram in figure 11.

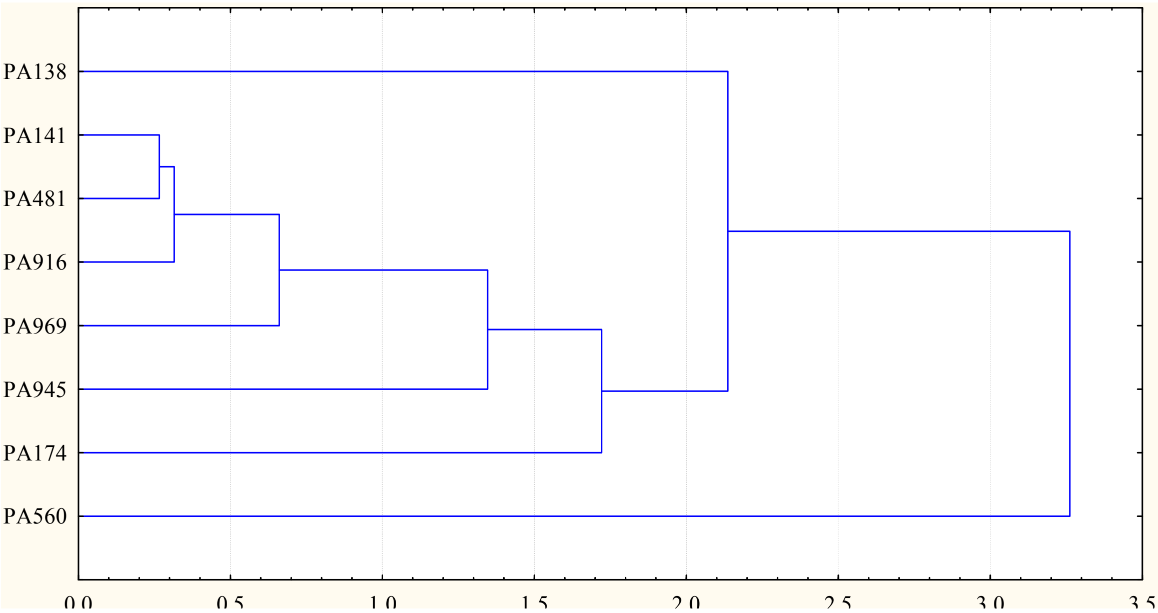


Figure 9. Clustering dendrogram seven genotypes of sweet cherry (*Cerasus avium* (L.) Moench.), based on morphometric feature.

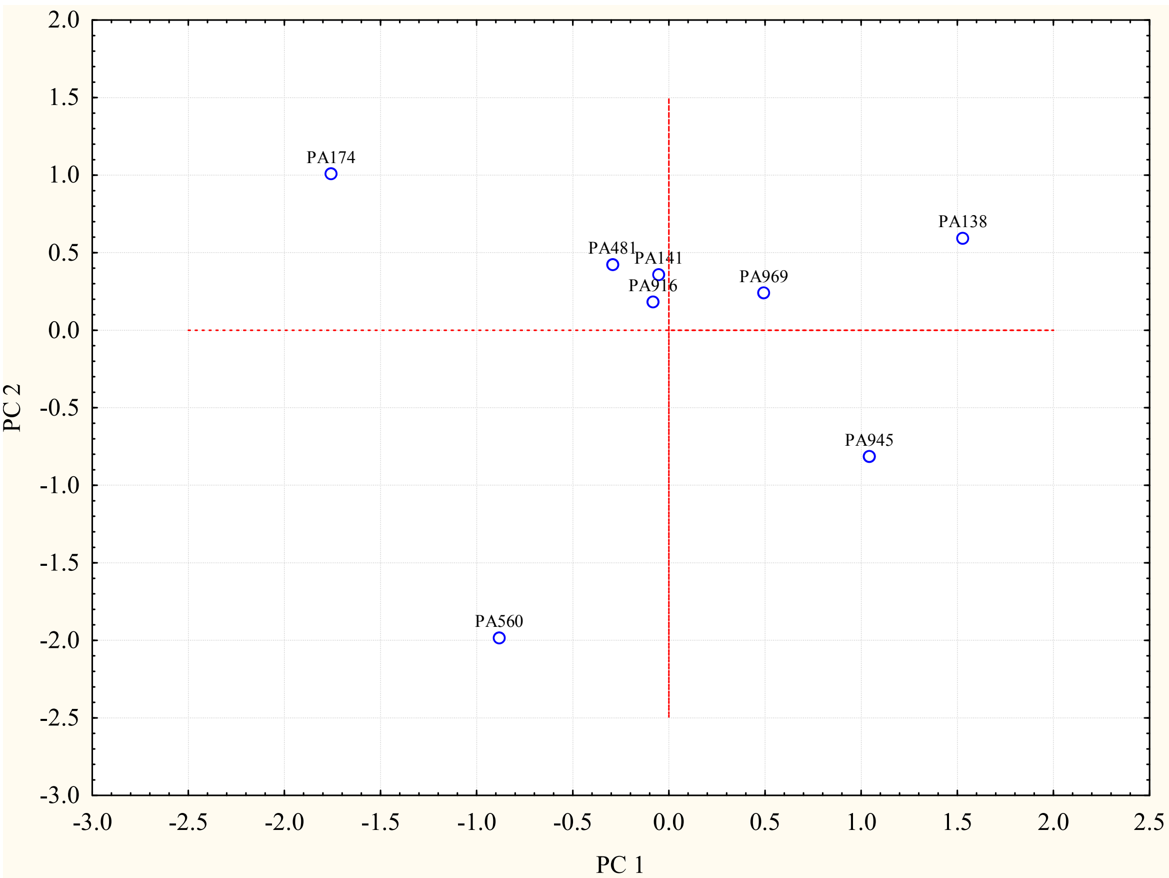


Figure 10. Projection seven genotypes of sweet cherry (*Cerasus avium* (L.) Moench.) on a factorial plane.

Table 3
Comparison characteristic feature sculpture of the mesocolpium surface pollen grains of tested sweet cherry genotypes (*Cerasus avium* (L.) Moench.)

Feature	PE138	PE141	PE174	PE481	PE560	PE916	PE945	PE969
Perforation	X		X	X	X	X		X
> 400000/mm^2	X		X		X	X		
Overstressing							X	
Sinuous shape					X			

X – presence of a feature.

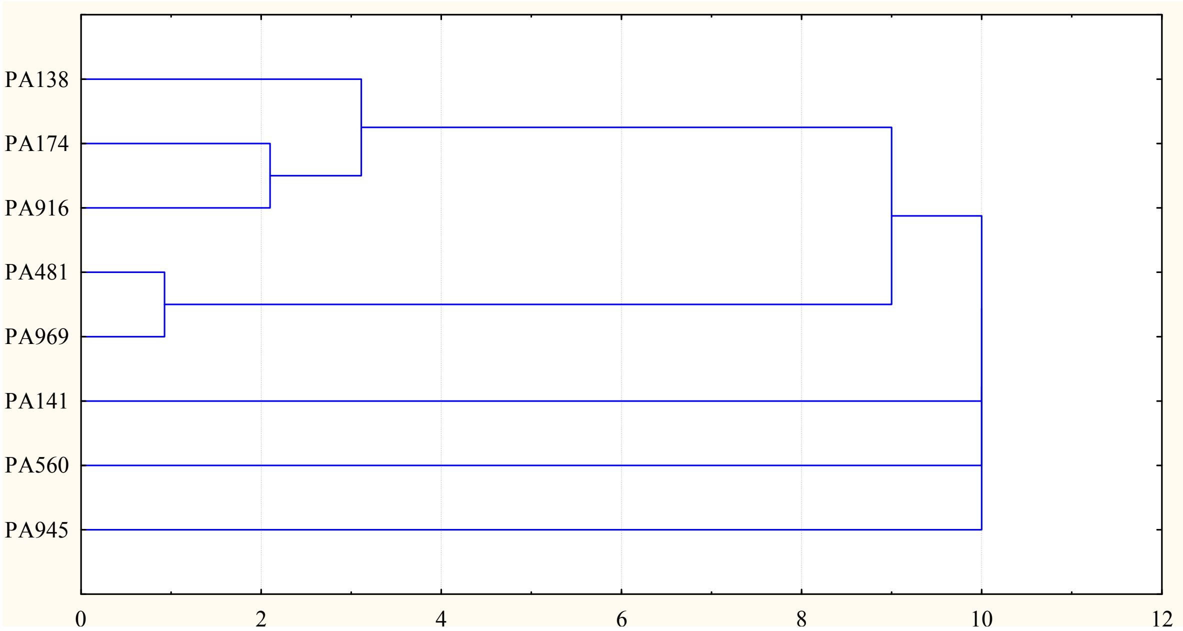


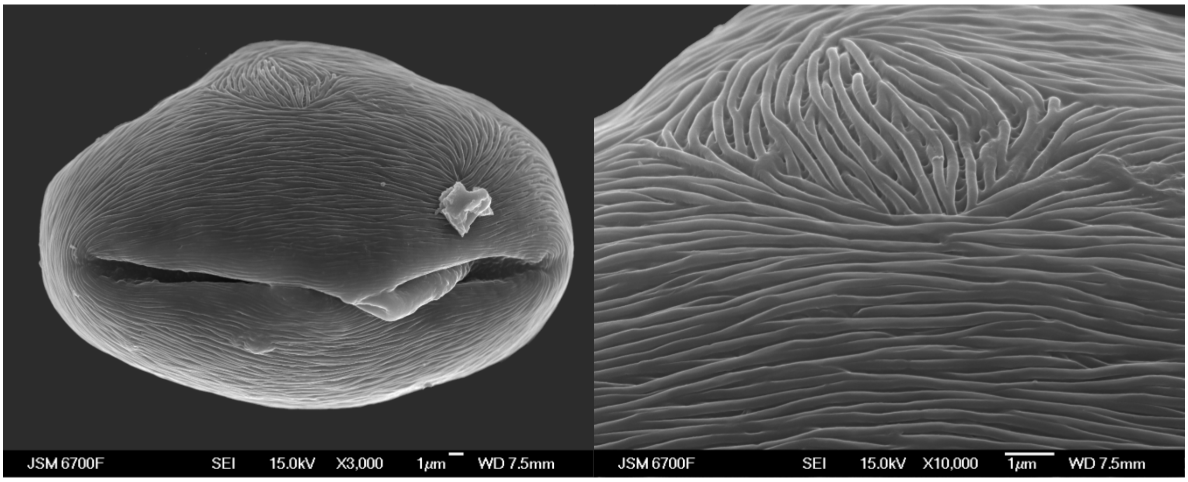
Figure 11. Clustering dendrogram seven genotypes of sweet cherry (*Cerasus avium* (L.) Moench.), based, both on quantitative, and on qualitative feature.

On a dendrogram four groups of one rank are isolated. The analysis of formation of these groups has shown, that criterion of allocation is the combination of qualitative attributes: presence or absence perforation, presence or absence of overstressings at striated elements of a sculpture and the form of striated elements of a sculpture. Group I (PA945) - absence perforation, presence of overstressings, the direct form of striated elements of a sculpture; group II (PA560) - presence perforation, absence of overstressings, the sinuous form of striated elements of a sculpture; group III (PA141) - absence perforation and overstressings, the direct form of striated elements of a sculpture; group IV (PA138, PA174, PA481, PA916, PA969) - presence perforation, absence of overstressings, the direct form of striated elements of a sculpture. Within the limits of group IV distinctive attribute between genotypes between genotypes is the quantity perforation on unit of the area: <400000/mm2 (PA481, PA969) or> 400000/mm2 (PA138, PA174, PA916). Within the limits of these groups the distance between genotypes is determined by morphometric feature. The significant difference is absent between genotypes PA481 and PA969, and also PA138 and PA916 (tab. 2).

In figures 12-18 are submitted a general view from equator of pollen grains and details of a sculpture of a mesocolpium of investigated genotypes *Malus domestica***.** Pollen grains colpate; in an outline from a pole circular-trilobate or circular-triangular, from equator ellipsoidal-circular (an average index of the form in a range from 1.44 till), isopolar; the average sizes, average length of a polar axis in a range from 31.85 µm till 42.85 µm, equatorial diameter from 21.23 µm till 23.93 µm.

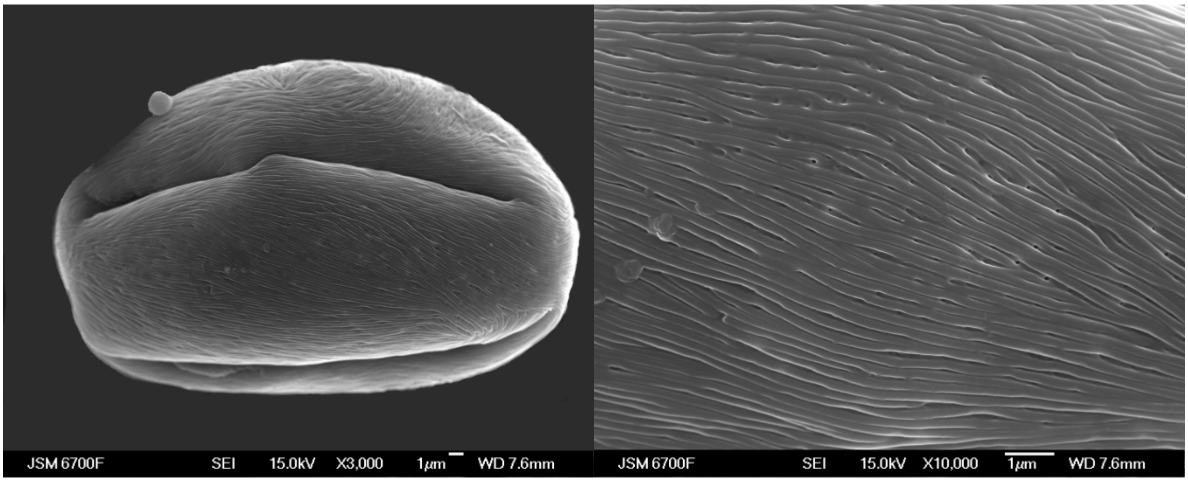
Colpi long, with parallel edges, or pulled together at poles and moved apart in an equatorial zone, with thin edges, in an equatorial zone multigear. Time large, more or less circular, near 10.0 µm in diameter, are covered with edges of colpuses. Diameter of an apocolpium. 7.2-8.0 µm, width of a mesocolpium 15.0-25.2 µm. An exine 1.7 µm, two-layer, integumentary, in regular intervals thicker; a structure spotted, edge of an exine on an optical section a crenulate, spreading layer and a cover of equal thickness. Color of a grain yellowish brown (Куприянова, Алешина, 1978).

Sculpture of an exine striated, less often sinuous - striated (Md-11/20) or striato-adnate (Md-2/10, Md-9/5, Md-33/9), frequently, perforated (Md-1/8, Md-9/5, Md-11/2). Results of measurements of length of a polar axis and equatorial diameter are resulted in table 4.



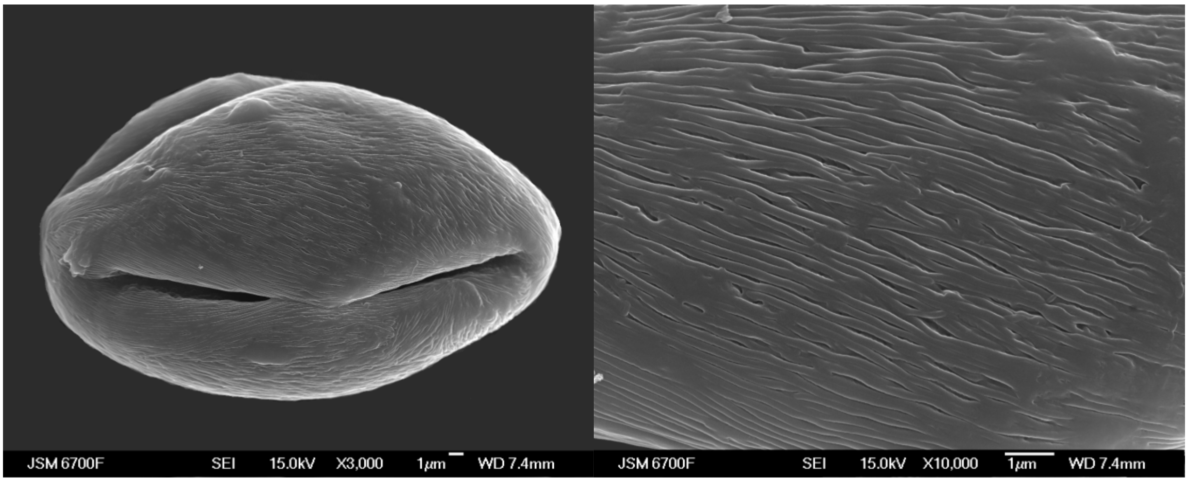
A B

Figure 12. Pollen grains of *Malus domestica* Md-1/4. A – equatorial view (X3000); B – sculpture of the mesocolpium surface (X10000).



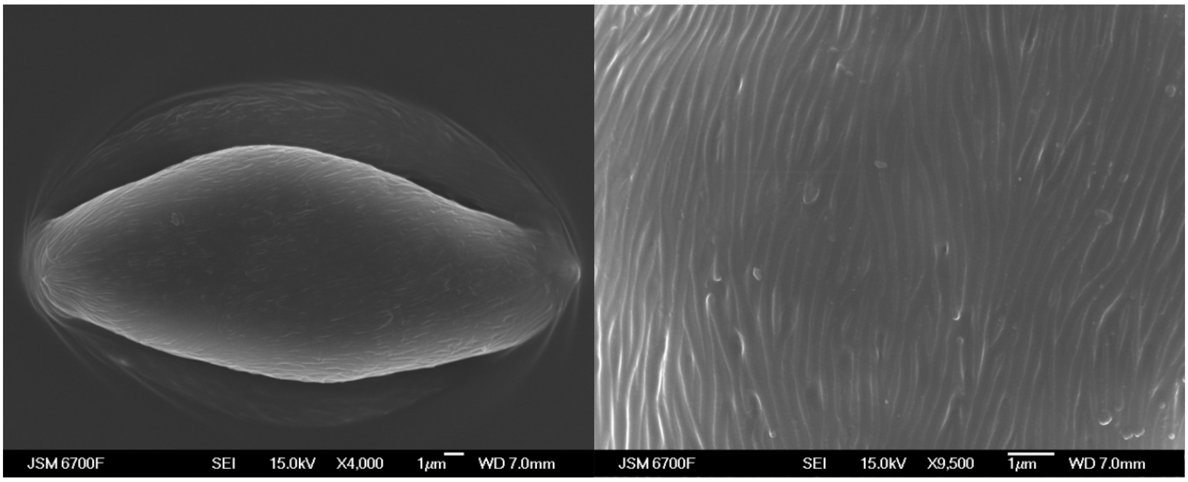
A B

Figure 13. Pollen grains of *Malus domestica* Md-1/8. A – equatorial view (X3000); B – sculpture of the mesocolpium surface (X10000).



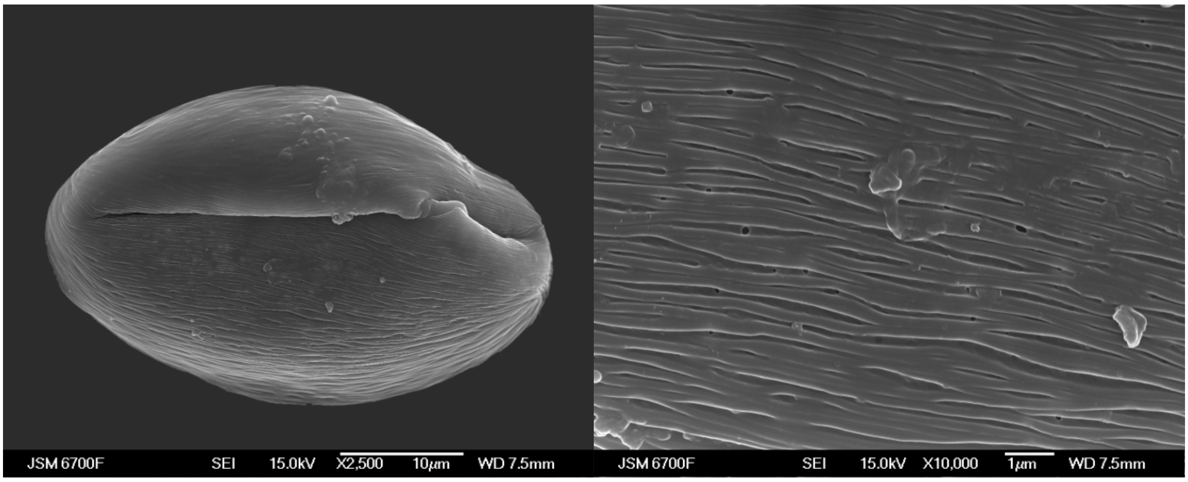
A B

Figure 14. Pollen grains of *Malus domestica* Md-2/10. A – equatorial view (X3000); B – sculpture of the mesocolpium surface (X10000).



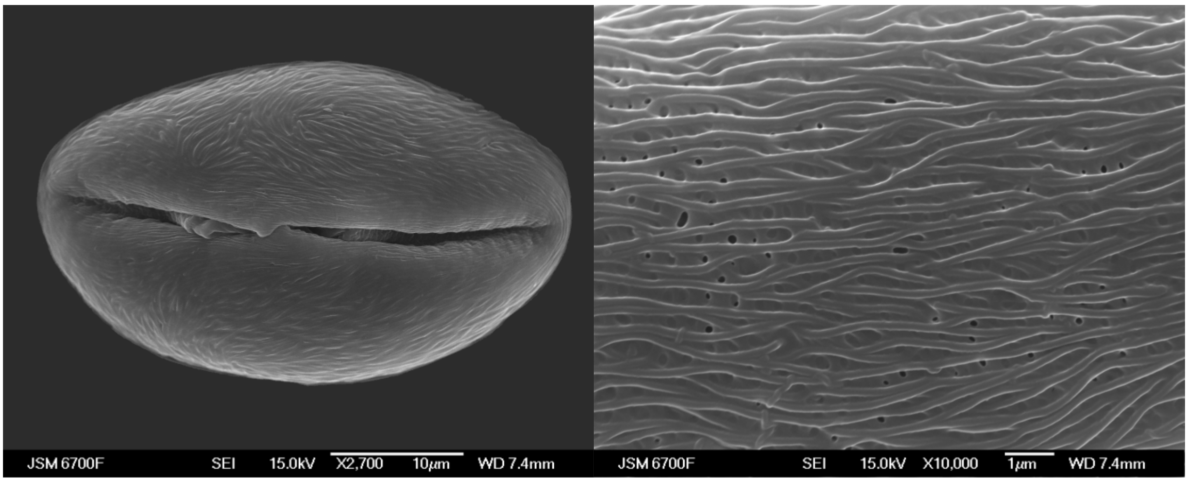
A B

Figure 15. Pollen grains of *Malus domestica* Md-5/12. A – equatorial view (X4000); B – sculpture of the mesocolpium surface (X10000).



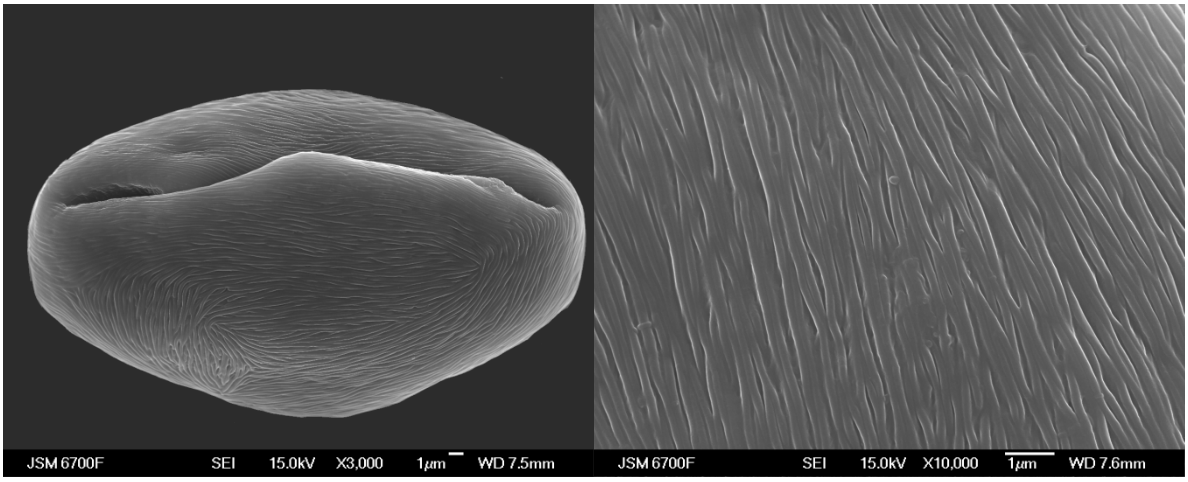
A B

Figure 16. Pollen grains of *Malus domestica* Md-9/5. A – equatorial view (X2500); B – sculpture of the mesocolpium surface (X10000).



A B

Figure 17. Pollen grains of *Malus domestica* Md-11/2. A – equatorial view (X2700); B – sculpture of the mesocolpium surface (X10000).



A B

Figure 18. Pollen grains of *Malus domestica* Md-33/9. A – equatorial view (X3000);
B – sculpture of the mesocolpium surface (X10000).

Table 4

Comparison of pollen grains polar axes length and equatorial diameter of tested apple tree genotypes (*Malus domestica* Borkh.)

Genotypes	**P – polar axes (μm)**				**E – equatorial diameter (μm)**				**SI**
	min	**max**	**$\overline{x}$**	**V%**	**min**	**max**	**$\overline{x}$**	**V%**	
Md-1/4	26.57	37.96	31.85	10.68	16.89	23.82	21.23	10.18	1.51
Md-1/8	29.69	42.82	33.64	8.30	19.35	27.91	23.54	9.65	1.44
Md-2/10	34.47	43.75	38.54	7.07	17.94	26.69	22.96	9.17	1.69
Md-5/12	32.78	40.01	36.14	6.05	22.90	26.02	23.87	4.05	1.52
Md-9/5	34.21	52.11	40.99	12.12	20.45	27.09	23.93	8.10	1.72
Md-11/2	36.13	46.86	42.85	6.30	19.69	29.93	23.09	9.08	1.87
Md-33/9	31.03	42.12	36.07	8.59	20.19	25.75	23.37	6.23	1.55

min – minimal value; max – maximal value; $\overline{x}$ – average value; V – variation coefficient (%); SI – shape index (P/E ratio).

Table 5

The significance of distinctions between average values of length of a polar axis (the top value) and equatorial diameter (the bottom value) various genotypes of apple tree (*Malus domestica* Borkh.)

	Md-1/4	**Md-1/8**	**Md-2/10**	**Md-5/12**	**Md-9/5**	**Md-11/2**	**Md-33/9**
Md-1/4		0.081853	0.000000**	0.001273**	0.000000**	0.000000**	0.000354**
		0.000487**	0.008295**	0.001701**	0.000256**	0.004508**	0.003994**
Md-1/8	0.081853		0.000000**	0.032621*	0.000000**	0.000000**	0.014640*
	0.000487**		0.257990	0.658454	0.531474	0.382591	0.769847
Md-2/10	0.000000**	0.000000**		0.038098*	0.011432*	0.000000**	0.012199*
	0.008295**	0.257990		0.211606	0.110639	0.793605	0.509985
Md-5/12	0.001273**	0.032621*	0.038098*		0.000166**	0.000000**	0.957263
	0.001701**	0.658454	0.211606		0.941762	0.286661	0.530073
Md-9/5	0.000000**	0.000000**	0.011432*	0.000166**		0.053226	0.000015**
	0.000256**	0.531474	0.110639	0.941762		0.169002	0.418042
Md-11/2	0.000000**	0.000000**	0.000000**	0.000000**	0.053226		0.000000**
	0.169002**	0.418042	0.169002	0.418042	0.169002		0.659228
Md-33/9	0.000354**	0.014640*	0.012199*	0.957263	0.000015*	0.000000*	
	0.003994**	0.769847	0.509985	0.530073	0.418042	0.659228	

* – $P < 0.05$; ** – $P < 0.01$.

Table 6

Comparison characteristic feature sculpture of the mesocolpium surface pollen grains of tested apple tree genotypes (*Malus domestica* Borkh.). X – Presence of a feature

Feature	Md-1/4	Md-1/8	Md-2/10	Md-5/12	Md-9/5	Md-11/2	Md-33/9
Perforation		X			X	X	
> 400000/mm^2						X	
Coalescence			X		X		X
Overstressing			X				
Sinuous shape						X	

X – presence of a feature

For the analysis of a belonging samples values of length of a polar axis (P) and equatorial diameter (E), different genotypes to one general set the dispersive analysis (ANOVA) which has shown (with a significance value 0.01) has been used, that samples for different genotypes as for P (p = 0.000001), and for E (p = 0.007170), are not samples of one statistical population.

Results of an estimation of the importance of distinctions between average of concrete genotypes on criterion of the least significant difference are submitted in table 5. The analysis of results shows, that significantly different (with a significance value 0.05) on value of average of length of a polar axis ($\overline{x}$ = 38.54 µm) from the others, is genotype Md-2/10.

Also it is possible to notice, that relatives (the zero hypothesis is not rejected at a significance value 0.05) among themselves are the following pairs genotypes: Md-1/4 and Md-1/8, Md-9/5 and Md-11/2, Md-33/9 and Md-5/12.

On value of average equatorial diameter (with a significance value 0.05) ($\overline{x}$ = 21.23 µm) from the others, genotype Md-1/4 is significantly different. Other genotypes significantly do not differ from each other (the zero hypothesis is not rejected at a significance value 0.05). These relations between the investigated genotypes are well illustrated on dendrogram of a clusterization based on morphometric features which is shown in figure 19.

For visualization of relations between morphometric parameters of various genotypes we shall take advantage of a method of principal component analysis. Results of the analysis are submitted in figure 20 which shows, that researched genotypes represent diverse enough group. Most marginal by the morphometric criteria chosen us, are a genotypes Md-1/4 and Md-11/2.

Generalizing, it is possible to ascertain, that received at the morphometric analysis not it is enough quantitative features for identification of investigated genotypes *Malus domestica.* In connection with what we had been investigated a variety of a sculpture of a surface of a mesocolpium of pollen grains of objects of research which comparative analysis allows to allocate the following criteria of difference. The first criterion is presence (fig. 2B, 5B, 6B) or absence (fig. 1B, 3B, 4B, 7B) perforation on a surface of pollen grains; subcriterion of this criterion is the quantity perforation on unit of the area: <400000/mm (fig. 2B, 5B),> 400000/mm (fig. 6B). The second criterion - character of sculptural elements, namely presence (fig. 3B, 5B, 7B) or absence (fig. 1B, 2B, 4B, 6B) coalescences of striated elements of structure; subcriterion this criterion is presence of overstressings at striated elements of a sculpture (fig. 3B). The third criterion - the form of sculptural elements: sinuous (fig. 6B), either more or less a straight line (fig. 1B, 2B, 3B, 4B, 5B, 7B). The data on presence or absence of the separated features at investigated genotypes are shown in table 6.

Having accepted these criteria as qualitative, alongside with morphometric parameters, we had been applied the clustered analysis of hierarchical connections between researched genotypes (a rule of association - a method of single connection, a measure of affinity - Chebyshev distance). Results are submitted as a dendrogram in figure 21. We see, that five groups of one rank which criterion of allocation is the combination of qualitative features are well isolated: presence or absence perforation, coalescences and the form of striated elements of a sculpture. Group I (Md-11/2) - presence of the sinuous form of striated elements of a sculpture, absence of coalescences, presence perforation; group II (Md-9/5) - the direct form of striated elements of a sculpture, presence perforation and coalescences; group III (Md-2/10, Md-33/9) - the direct form of striated elements of a sculpture, absence perforation and presence of coalescences; group IV (Md-1/8) - the direct form of striated elements of a sculpture, presence perforation and absence of coalescences; group V (Md-1/4, Md-5/12) - the direct form of striated elements of a sculpture, absence perforation and coalescences.

Within the limits of group III distinctive attribute between genotypes between genotypes is presence (Md-2/10) or absence (Md-33/9) overstressings of striated elements of a sculpture. Within the limits of group V the distance between genotypes is determined by morphometric attributes. Thus as table 2 shows, at genotypes Md-1/4 and Md-5/12 (with a significance value 0.01) values of average as lengths of a polar axis, so equatorial diameter are significantly different.

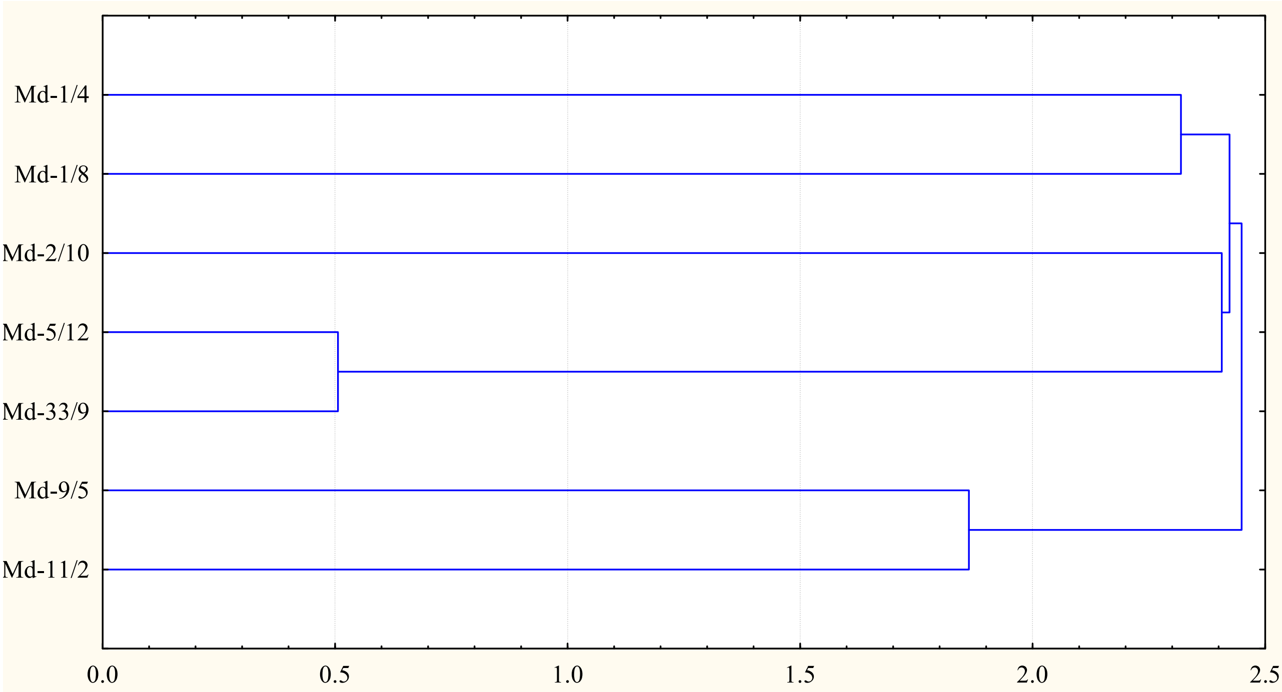


Figure 19. Clustering dendrogram seven genotypes of apple tree (*Malus domestica* Borkh.), based on morphometric feature.

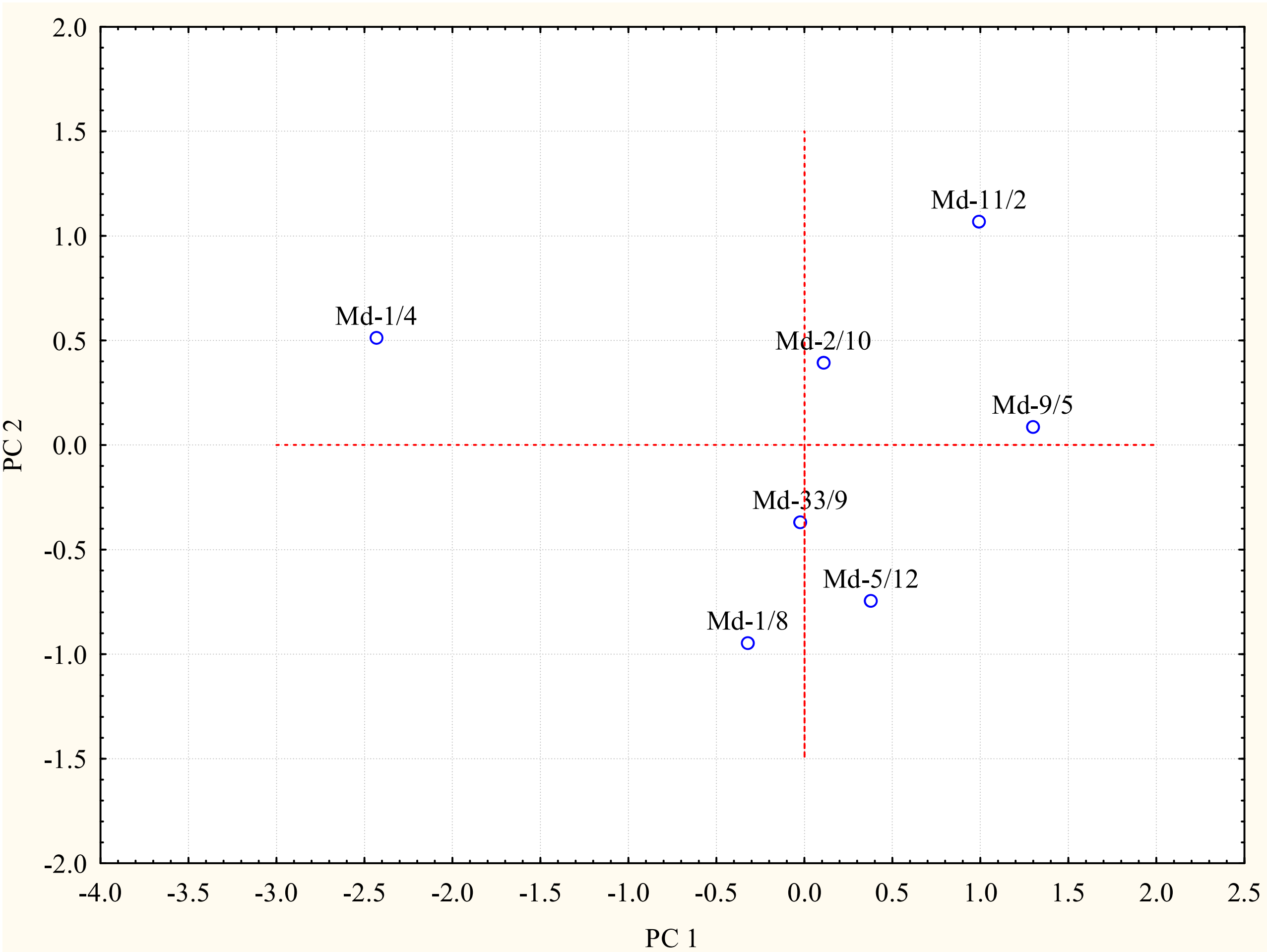


Figure 20. Projection seven genotypes of apple tree (*Malus domestica* Borkh.) on a factorial plane.

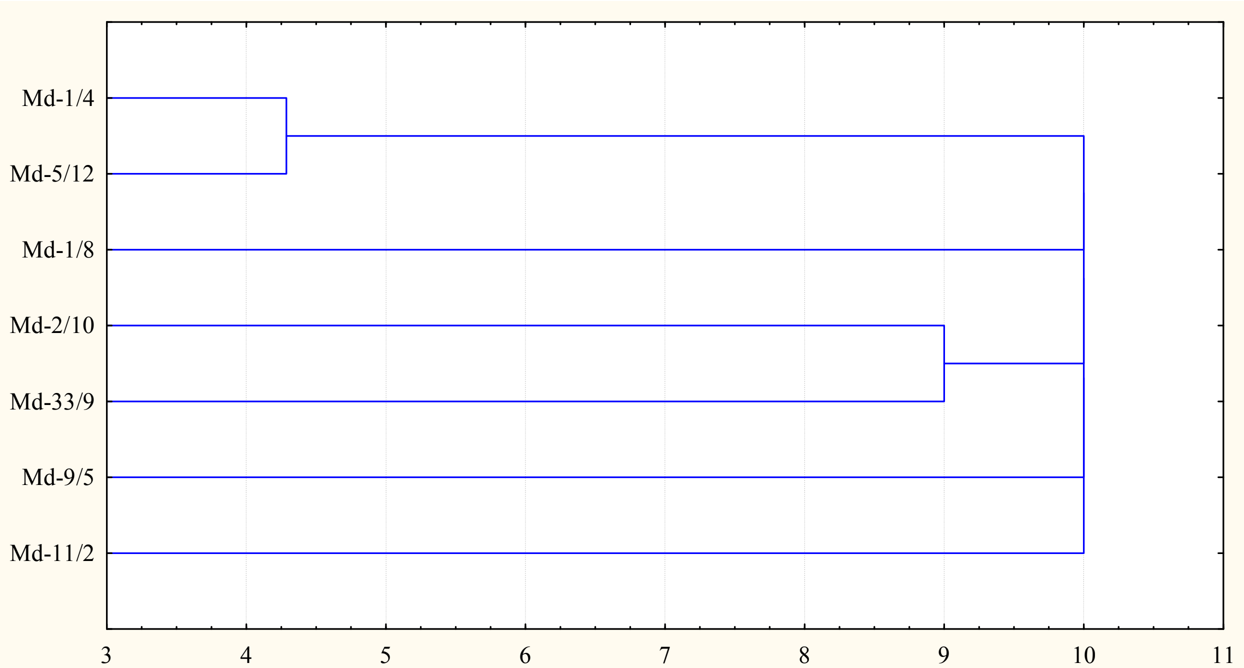


Figure 21. Clustering dendrogram seven genotypes of apple tree (*Malus domestica* Borkh.), based, both on quantitative, and on qualitative feature.

Conclusions

Thus, we carry out the comparative morphological analysis of pollen grains of trees of family *Rosaceae* Juss. By the example of eight genotypes of a sweet cherry (*Cerasus avium* (L.) Moench.**)**, and seven genotypes of an apple tree (*Malus domestica***)**, growing in Slovakia, also the technique of separated of morphological criteria of their difference is fulfilled.

Have been separated as qualitative criteria (presence or absence perforation on surfaces of pollen grains, presence or absence of coalescences of striated elements of structure, and the form of sculptural elements: sinuous either more or less a straight line, and also two criteria: quantity perforation on unit of the area and presence of overstressings at striated elements of a sculpture), and quantitative (average length of a polar axis (P) and equatorial diameter (E)). The separated features can be easily enough determined by express trains - methods that is convenient at identification of investigated pollen grains. The value of coefficient of a variation (up to 10 %) allows to be limited to 30 measurements at definition of the specified morphometric parameters. The statistical analysis of values of the separated features for the investigated genotypes of a sweet cherry has allowed to separate the certain genotypes, or groups of genotypes that facilitates identification of a pollen grains belonging.

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Literature

1. D. Gonzalez-Gomez, M. Lozano, Fernandez-Leon VI. et al., European Food Resources Technology, 229, 223 (2009).
2. G. Erdtman, An introduction to pollen analysis. Waltham, Mass (Chronica Botanica Company, U.S.A, 1943).
3. W.H. Fogle, J. Amer. Soc. Hort. Sci., 102 (55), 548 (1977-1)
4. W.H. Fogle, J. Amer. Soc. Hort. Sci., 102 (5), 552 (1977-2).
5. W. Punt, P.P. Hoen, S. Blackmore, S. Nilsson, A. Le Thomas, Review of Palaeobotany and Palynology, 143, 1 (2007).
6. R.J. Hebda, C.C. Chinnappa, B.M. Smith, Canadian Journal of Botany, 68, 595 (1988).
7. R.J. Hebda, C.C. Chinnappa, Canadian Journal of Botany, 68, 1369 (1990).
8. Joneghani V. Nazeri, Iranian Journal of Science & Technology, 32 (A2), 89 (2008).
9. S. Lyle, Fruit & Nuts (Timber Press Inc., Portland).

10. S.A. Gilani, R.A. Qureshi, A.M. Khan, P. Potter, African Journal of Biotechnology, 9 (20), 2872 (2010).
11. R. Ostrovsky, J. Brindza, O. Vergun, O. Crygorieva, D. Toth, Достижения и перспективы развития селекции, возделывания и использования плодовых культур (Ялта, 2011).
12. K.K. Shim, B.K. Shu, S.H.A. Park, Journal of the Korean Horticultural Society, 29 (2), 95 (1988).
13. A. Sala-Llinares, M. Suarez-Cervera, J. Seoane-Camba, J. Marquez, Journal of Apicultural Research, 31 (2), 53 (1992).
14. World sweet cherry review (Edition a Publication of Belrose, U.S.A., 2006).
15. H.C. Xiang, H.P. Sheng, Acta Phyotaxonomica Sinca, 29 (5), 445 (1991).
16. А.Н. Бурмистров, В.А. Никитина, Медоносные растения и их пыльца (Росагропромиздат, Москва, 1990).
17. М.М. Глухов, Альбом медоносов (Киев, 1960).
18. В.Я. Карупу, Электронная микроскопия (Киев, 1984).
19. В.Н. Косенко, Бот. журн., 77 (3), 23 (1992).
20. Л.А. Куприянова, Л.А. Алешина, Пыльца и споры растений Европейской части СССР. Т.1. (Наука, Ленинград, 1972).
21. Куприянова Л. А., Алешина Л. А. Пыльца двудольных растений Европейской части СССР. – Л.: Наука. – 1978. – 184 с.
22. А.С. Нуждин, В.П. Виноградов, Основы пчеловодства (Колос, Москва, 1982).
23. С.М. Мотылева, Е.Н. Джигадло, М.Н. Кузнецов, М.Е. Мертвищева, Достижения и перспективы развития селекции, возделывания и использования плодовых культур (Ялта, 2011).
24. Ю.А. Бранзбурга, Н.И. Филатовой, Н.И. Емельяновой, Словарь-справочник пчеловода (Государственное издательство сельскохозяйственной литературы, Москва, 1955).
25. Е.А. Соколова, Значение анатомических признаков для систематики представителей подсемейства *Primoideae (Rusaceae),* Диссертация на соискание ученой степени доктора биологических наук (Санкт-Петербург, 2000).
26. П.И. Токарев, Морфология и ультраструктура пыльцевых зерен (Т-во научн. изд. КМК., Москва, 2002).
27. Б. Уикли, Электронная микроскопия для начинающих (Москва, 1975).
28. В.Ю. Урбах, Математическая статистика для биологов и медиков (Москва, 1963).

Мальцов Ігор Юрійович– кандидат біологічних наук, науковий співробітник відділу закритого ґрунту.

Булах Петро Євгенович – доктор біологічних наук, старший науковий співробітник, завідувач лабораторії екології та захисту рослин відділу ландшафтного будівництва.