



## THE MORPHOLOGY OF POLLEN GRAINS OF SOME CULTIVARS *RUBUS FRUTICOSUS* L.

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The features of pollen grains sculpture of 8 cultivars *Rubus fruticosus* L. have been studied in details via the scanning electron microscope method. The pollen material picked up in the Central region of Russia and the Republic of Adygeya has been used in the research. The comparative data of morphometric factors are presented, the polymorphism of pollen grains – size and surface is determined. The complex sculptural types are formed by the different combination of 2 or 3 simple sculptural types. The complex structural type of exine is characteristic of the studied cultivars *Rubus fruticosus*. The average size of pollen grains polar axis is within 32.43–37.46 μm; the equatorial diameter is within 16.91–18.88 μm. The ratio of polar and equatorial axis ( $P/E$ ) marks the degree of the pollen grains elongation (roundness). The research showed that the cultivation region gives a certain influence on the change of the pollen average dimensions, but it does not influence  $P/E$  ratio. The pollen grains form is oblong-ellipsoid. In the polar outline the grains are round. The pollen grains of the presented blackberry cultivars are 3- or 4-colporate, the apertures are long. The sculpture of the pollen grain exine is complex, it belongs to the two component sculptural type – rugulose, the picture has the species features. The morphometric and sculptural identical characteristics are separated: the form (the pollen grains elongation degree), the dimensions (the ratio of the polar axis to the equatorial axis ( $P/E$ ), the number of apertures and the surface picture (microstructure).

**Keywords:** blackberry, cultivar, pollen morphology, scanning electron microscope

### Introduction

Blackberry belongs to the absolutely polymorphic plant (Gruner, 2014). Lots of cultivars are complex hybrids and there are many cross-species hybrids among wild-growing forms that make difficult classification. That’s why the possibility to identify the both forms, to determine the phylogenic connections between them using not only external characteristics, but also for

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microstructures applying modern equipment is great interest. Many authors are described pollen of different plant species by scanning electron microscope (Grygorieva et al., 2010, 2017; Brindza and Brovarskyi, 2013; Nikolaeva et al., 2014; Motyleva et al., 2015; Motyleva and Brindza, 2017).

Thereby the study of the pollen grains morphology – the dimensions, the form, the exine structure, the apertures number and place is more actual. The complex of morphological characteristics allows to determine the differences (or similarities) between the close species or cultivars, morphologic inhomogeneity, especially variability of its dimensions because of hybridization and polymorphism (Kupriyanova and Aleshina, 1978; Tzarenko, 2012). The comparative electron-microscopy researches of the blackberry different cultivars pollen growing in the Republic of Adygeya and the Central region of Russia have not been performed before; this fact has determined the purpose of our researches.

## Material and methodology

### Objects of research

The pollen material of *Rubus fruticosus* L. (subgenus *Eubatus* Focke) was picked up in the experimental plantations of Maikop OS VIR experimental station and in VNIISPK (Orel) in 2009–2011. Pollen was studied in 8 cultivars of *Rubus fruticosus* L. The pollen cultivars of Agavam and Thornfree were from different ecological – geographical zones – Russia and the Republic of Adygeya. Pollen cultivars of Darrow, Erie, Flint, Maxwell Early, Mc. Donald, Smoothstem are from Russia.

### Scanning electron microscope (SEM)

Micropictures are taken on the scanning electron microscope JEOL JSM – 6390 in the laboratory of agroecology VNIISPK. Preliminary dried at  $T = 40\text{--}50\text{ }^{\circ}\text{C}$  pollen was accurately pounded with a pestle to annual adhesion and was put on the special carbonic scotch placed on the object table of the scanning electron microscope with a thin metal spreading rod. The comparative morphological studying of the pollen grains was performed according to the working rules on the SEM JEOL JSM-6390 in the conditions of low vacuum ( $P = 60\text{ Pa}$ ) with the following zooming: 500 times – during the measurements; 1000–10000 times – while taking the pictures of the exine sculpture features. Using the regime of low vacuum allows to perform the pollen studying without its preliminary chemical treatment and to receive undistorted data about the research object that makes the process of the probe preparation easier.

### Morphometric characteristics

The measurements of the pollen grains linear dimensions were performed via 200–300 times repeatability in about 30 field microscopy with the average value calculation. All the mentioned in the text dimensions are given in  $\mu\text{m}$ .

### Statistical analysis

For statistical evaluation were used standard methods using statistical software Statgraphics Centurion XVII (StatPoint Inc.USA).

## Results and discussion

The pollen grains morphometric values are given in Table 1. The average size of the polar axis for the *Rubus fruticosus* studied cultivars pollen grains is within 32.43–37.46  $\mu\text{m}$ ; the equatorial diameter is within 16.91–18.88  $\mu\text{m}$ . The ratio of the polar axis to the equatorial one ( $P/E$ ) marks the degree of the pollen grains elongation (roundness). The pollen of Agavam, Flint, Mc. Donald, Smoothstem and Thornfree cultivars is more elongate in comparison with other studying cultivars. Among these cultivars Smoothstem and Thornfree are closely related. It is possible that other 3 cultivars are phylogenically connected with one another. The comparison of the photometric parameters of Agavam and Thornfree cultivars pollen cultivated in different climatic zones (in the Caucasus and in the Central region) showed that the cultivation region gives a certain influence on the change of the pollen average dimensions, but it does not influence  $P/E$  ratio. The received results coincide with the pollen grains description and with the data received while studying *R. caesius* L. pollen from Latvia (Kupriyanova and Aleshina, 1978; Meng and Finn, 2002).

**Table 1** The morphological characteristic of pollen grains of *Rubus fruticosus* L. representatives

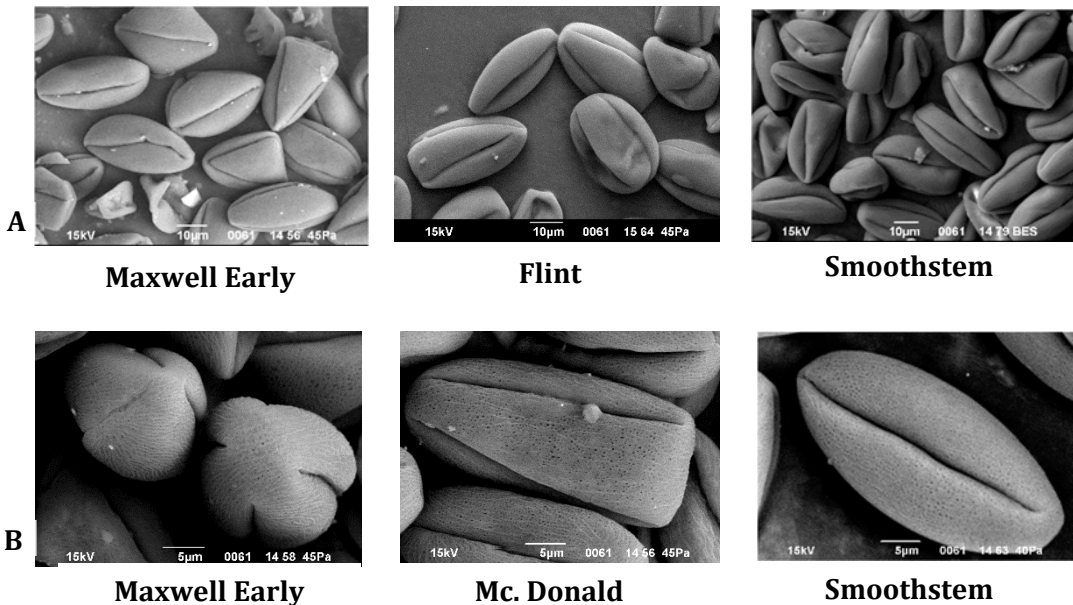
Measurements	<i>n</i>	Min ( $\mu\text{m}$ )	Max ( $\mu\text{m}$ )	Average ( $\mu\text{m}$ )	<i>V</i> (%)	$P/E$
<b>Agavam (Republic of Adygeya)</b>						
<b>P</b>	250	36.49	39.78	37.17	2.76	2.08
<b>E</b>	250	14.35	19.31	17.85	6.48	
<b>Agavam (Russia)</b>						
<b>P</b>	300	27.91	39.62	36.82	6.83	2.06
<b>E</b>	300	15.28	22.01	18.55	5.62	
<b>Darrow (Russia)</b>						
<b>P</b>	245	30.59	39.61	34.83	6.71	1.76
<b>E</b>	245	12.91	19.81			
<b>Erie (Russia)</b>						
<b>P</b>	240	17.61	37.94	32.43	6.71	1.88
<b>E</b>	240	13.59	21.11	17.29	10.31	
<b>Flint (Russia)</b>						
<b>P</b>	250	33.45	39.77	37.46	3.17	2.09
<b>E</b>	250	15.67	19.17	17.85	5.63	
<b>Maxwell Early (Russia)</b>						
<b>P</b>	250	29.31	42.19	35.87	8.64	1.89
<b>E</b>	250	13.31	22.32	18.88	9.59	
<b>Mc. Donald (Russia)</b>						
<b>P</b>	250	22.52	38.97	33.05	8.24	2.02

Continue the Table 1

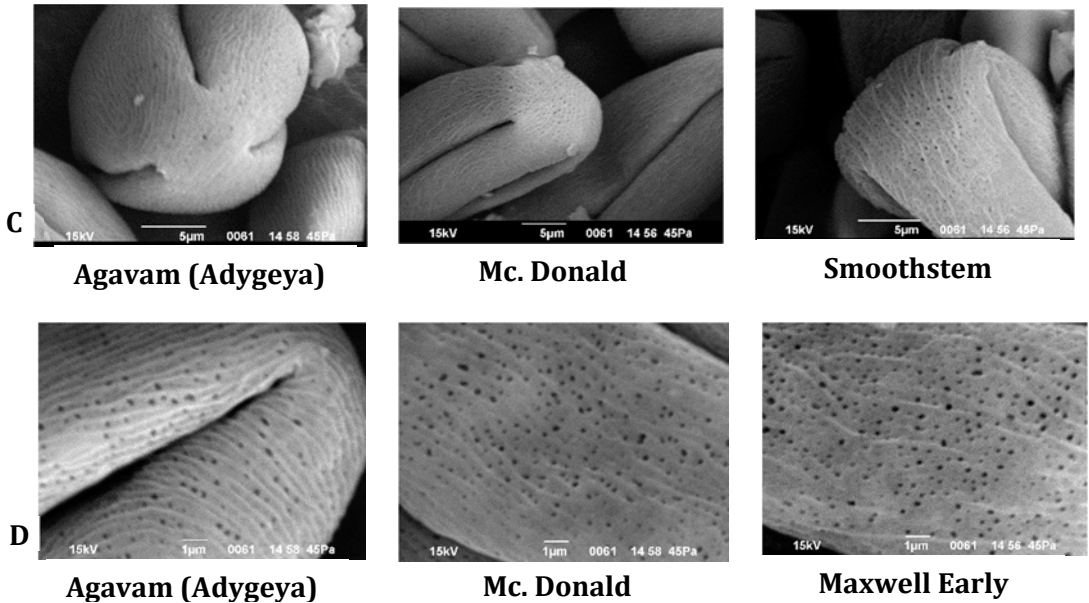
Measurements	<i>n</i>	Min (μm)	Max (μm)	Average (μm)	<i>V</i> (%)	<i>P/E</i>
<b>Smoothstem (Russia)</b>						
<b>P</b>	300	32.76	40.23	35.66	5.72	2.01
<b>E</b>	300	15.23	21.11	17.71	7.87	
<b>Thornfree (The Republic of Adygeya)</b>						
<b>P</b>	300	33.84	39.45	36.33	4.76	2.00
<b>E</b>	300	15.56	21.17	18.14	8.79	
<b>Thornfree (Russia)</b>						
<b>P</b>	240	30.52	39.34	34.92	6.83	1.99
<b>E</b>	240	14.64	23.11	17.58	9.14	

Notes: Min – minimum value; Max – maximum value; *V* – variation coefficient (%); *P* – polar axis; *E* – equatorial one; *P/E* – the ratio of the polar axis to the equatorial axis

The pollen grains form is oblong-ellipsoid, the apertures are long (Figure 1). In the pole outline the grains are round. In the received pictures it is clearly seen that the pollen grains of the presented blackberry cultivars are 3- or 4-colporate (Figure 1B, C), whereas according to the data given by Kupriyanova and Aleshina (1978) that *Rubus fruticosus* pollen is only 3-colporate. Possibly it is connected with the fact that the authors used optical microscope: in the given pictures there is no position “polar view”, but from the equatorial view it is difficult to count the number of apertures.



**Figure 1A–B** The pollen grains of some *Rubus fruticosus* L. cultivars. A – zoom.1000; B–zoom.3300; C–zoom. 5000; D–zoom. 10000



**Figure 1A–B** The pollen grains of some *Rubus fruticosus* L. cultivars. A – zoom.1000; B–zoom.3300; C–zoom. 5000; D–zoom. 10000

The exine pattern is tender-undulate, fine-mesh, the picture has the cultivars features (Figure 1D). The number of the misshapen pollen grains is up to 20–35%. This fact, in some ways, can be connected with the greenness of some pollen, but also with polyploid plants.

## Conclusions

The studying of the blackberry pollen via scanning electron microscope allowed to separate the most important parameters which can be used to identify the representatives of *Rubus fruticosus*. They are the form (the pollen grains elongation, the length and the width ratio). Parameters such as the number of the apertures and the surface picture (microsculpture) are more specific for different cultivars. The certain influence of the blackberry cultivated region climatic conditions on the pollen dimensions was marked.

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