



STUDY OF MORPHOLOGICAL CHARACTERISTICS OF POLLEN GRAINS OF *ARONIA MITSCHURINII* A.K. SKVORTSOV & MAITUL.

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Morphology of pollen grain was described for nine *Aronia mitschurinii* A.K. Skvortsov & Maitul. phenotypes (AM-01 – AM-09) at the laboratory of Department of Tropical and Subtropical plants of M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv) and Institute of Biodiversity Conservation and Biosafety at Slovak University of Agriculture in Nitra using an electron microscope Carl Zeiss LS 15. The measurement of morphometric parameters was carried out on 50 pollen grains from each phenotype using the AxioVision Rel. 4.8.2.0 program. The measurements were made in micrometer (μm). The length of polar axis (P) and the equatorial diameter (E) of grain, P/E ratio were measured and their variation was compared among studied genotypes. SEM investigations showed that the pollen grains are radial-symmetrical, isopolar, oblong-ellipsoid and 3- and 4-colporate. Texture is sinuous-tuberculate in equatorial zone and finely bumpy in polar zone. The polar axis and equatorial diameter of pollen grains values were varied from 34.16 to 50.14 μm and from 16.10 μm to 25.71 μm , respectively. This study showed that there were differences among the phenotypes in all measured factors. It is known that phenotypic variability is an evolutionarily fixed response of any group of organisms with a constant genotype to changes in environmental conditions and it is adaptive. Therefore, our research suggests that all individuals forming the introduction population of *Aronia mitschurinii* are sufficiently adapted to the conditions of M.M. Gryshko National Botanical Garden of NAS of Ukraine.

Keywords: *Aronia mitschurinii*, phenotype, pollen, SEM, morphology

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Introduction

Research into the morphological characteristics of pollen grains by scanning electron microscopy (SEM) from specific genotypes and cultivars are important and useful for taxonomy, phylogeny, palaeobotany, breeding programmes, e.g., *Pyrus* spp. (Westwood and Challice, 1978; Motyleva et al., 2017), *Vitis vinifera* L. (Ahmedullah, 1983), *Prunus cerasus* L. (Miaja et al., 2000), *Olea europaea* L. (Javady and Arzani, 2001), *Prunus armeniaca* L. (Arzani et al., 2005), *Cornus mas* L. (Mert, C. 2009), *Diospyros* spp. (Grygorieva et al., 2010, 2013, 2017), *Corylus avellana* L. (Nikolaieva et al., 2014), *Ziziphus jujuba* Mill. (Rouhakhsh et al., 2014), *Castanea sativa* Mill. (Grygorieva et al., 2015), *Cydonia oblonga* Mill. (Radović et al., 2016), and *Cichorium intybus* L. (Adamchuk et al., 2017). Size, shape, surface morphology, and ultrastructure of pollen grains are of great importance in the characterization of the pollen grains (Erdtman, 1966; Fogle, 1977; Martens and Fretz, 1980; Brindza and Brovarskyi, 2013; Dyakova, 2014; Brovarskyi et al., 2017; Chlebo and Adamchuk, 2017).

The characteristics of pollen grains are often additional diagnostic features for taxa of various ranks, as shown for *Caragana arborescence* Lam. (Kuklina et al., 2015), *Lupinus polyphyllus* Lindl. (Vinogradova et al., 2012), *Robinia* spp. (Vinogradova et al., 2013), *Solidago* L. complex (Vinogradova, 2012).

The properties of the pollen grains of different phenotypes of *Aronia mitschurinii* have not been evaluated in detail yet.

The knowledge of pollen morphological characteristics can be an adequate method for identification phenotypes of *Aronia mitschurinii*.

Material and methodology

Locating trees and data collection

The pollen of 9 *Aronia mitschurinii* phenotypes (AM-01 – AM-09) from the collection of M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG) was investigated.

Pollen grains collection

Freshly flowers (not opened) were collected randomly from the different genotypes at the balloon stage (May 2018). Pollen samples released from dry flowers were further dried under laboratory conditions. The dry pollen was used for a microscopic study of morphological characteristics. The samples of pollen grains were applied to double-tape, fastened to metal object tables with 10 mm diameter.

Scanning electron microscopy (SEM)

The pollen grains were studied at the laboratory of Department of Tropical and Subtropical plants of NBG and Institute of Biodiversity Conservation and Biosafety of Slovak University of Agriculture in Nitra (IBS) using an electron microscope Carl Zeiss LS 15, and the microphotographs were taken. 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 ($P = 60$ 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. Typical exine patterns, shape, size and the dimensions of pollen grains for each *Aronia mitschurinii* genotypes were determined by using a scanning electron micrograph (SEM).

Morphometric characteristics

The measurement of morphometric parameters was carried out on 50 pollen grains from each genotype using the AxioVision Rel. 4.8.2.0 program. The measurements were made in micrometers (μm). The characterization of pollen grains was calculated by taking the following parameters: the polar axis (P – line connecting the proximal and distal pole), the equatorial axis (E – a line perpendicular to the polar axis and located in the equatorial plane).

Statistical analysis

Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between genotypes were computed on the basis of the Bray-Curtis similarity index; multi-dimensional scaling (MDS) analyses were performed in PRIMER (Clarke and Gorley, 2006). Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by Stehlíková (1998).

Results and discussion

This study of pollen morphology of tested *Aronia mitschurinii* showed that pollen grains are radially symmetrical, isopolar and according to the localization of apertures are zono-tricolpate. Three compound apertures are according to distribution equidistant. The size, shape of pollen grains and number of apertures are documented on Figure 1.

Pollen grains oblong-ellipsoid, the apertures are long. In the received pictures it is clearly seen that the pollen grains of the presented blackberry breeds are 3 – and 4 – colporate. In polar view pollen grains are circular and triangular with straight or convex sides, in equatorial view – elliptical. Colpi, with uneven edges and pointed or blunted ends, almost converge at the poles. Colpi have granular membrane. Colpi are elliptic, longitudinal and elongated with straight or orbicular-dentate edges. Texture is sinuous-tuberculate in equatorial zone and finely bumpy in polar zone.

The polar axis (P), equatorial diameter (E) and polar axis to equatorial diameter (P/E) ratio of pollen grains of nine *Aronia mitschurinii* phenotypes were measured using scanning electron microscopy (SEM), and the results are displayed in Table 1. An important morphological characteristic is the size of pollen grains. The length of polar axis (P) varied from 34.16 to 50.14 μm and the width of the equatorial axis (E) was in the range of 16.10–25.24 μm . The values of variation coefficient were in the range of 4.20–5.84% for polar axes and in the range of 6.31–9.28% for equatorial axes.

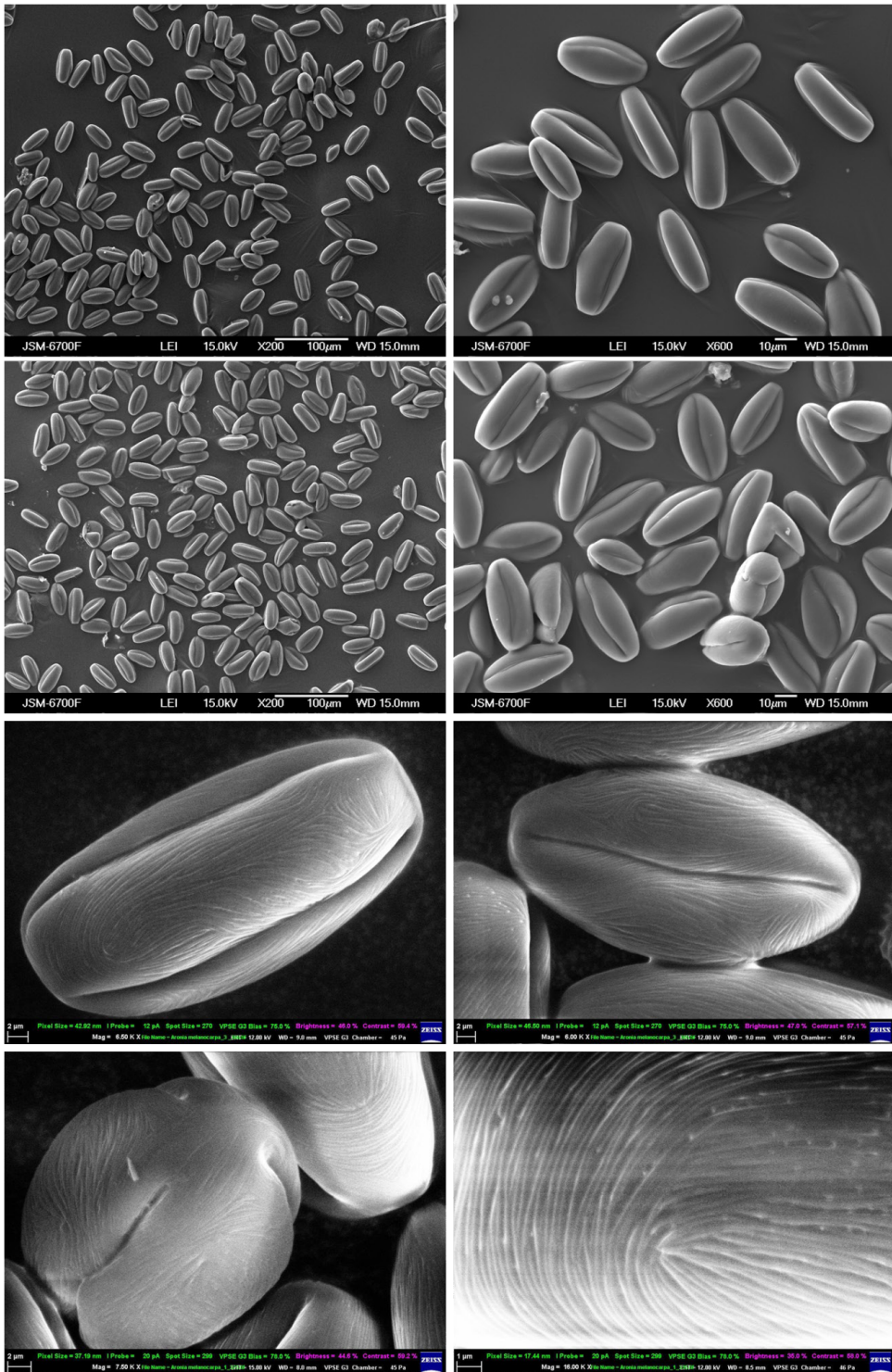


Figure 1 Pollen grains of *Aronia mitschurinii* A.K. Skvortsov & Maitul. species in different positions (Photo: Gurnenko, 2018; Motyleva, 2018)

Table 1 The measured pollen morphological traits of selected phenotypes of *Aronia mitschurinii* A.K.Skvortsov & Maitul.

Phenotypes	min	max	CV %	min	max	CV %	min	max	CV %
	P – Polar axis (µm)			E – Equatorial axis (µm)			SI – shape index (P/E)		
AM-01	39.78	47.39	5.10	17.47	23.58	8.02	1.68	2.53	10.41
AM-02	40.14	50.10	5.84	18.99	25.24	6.55	1.70	2.45	7.97
AM-03	39.38	49.12	4.65	19.35	23.96	6.71	1.80	2.43	8.82
AM-04	36.53	47.12	5.48	18.49	24.29	6.67	1.63	2.30	8.64
AM-05	38.95	50.14	5.56	19.10	25.00	6.31	1.74	2.32	7.83
AM-06	41.26	49.38	4.55	16.51	22.09	7.40	1.90	2.91	8.54
AM-07	40.69	49.50	4.20	16.94	22.35	7.34	1.93	2.59	7.50
AM-08	34.16	41.77	5.47	16.10	20.29	6.38	1.80	2.48	7.78
AM-09	39.04	47.92	5.15	16.23	24.28	9.28	1.83	2.85	10.99

Note: min – minimum value; max – maximum value; CV – variation coefficient (%).

Shape index (SI) of pollen grain depends on parameters of polar (*P*) and equatorial (*E*) axis. Shape index (the *P/E* ratio) of tested species varied from 1.63 to 2.91. Mean values of morphological parameters of pollen demonstrated on Figure 2. According to the average values, the phenotype AM-08 has the smallest pollen grains $38.00 \times 18.22 \mu\text{m}$ (Figure 2A). On the dendrogram (Figure 2B), you can see that the phenotype AM-08 is really separated from the other samples. According to literary data, *Aronia mitschurinii* is a facultative apomict, and all her cultivars have only one genotype (Persson-Hovmalm et al., 2004; Vinogradova and Kuklina, 2014).

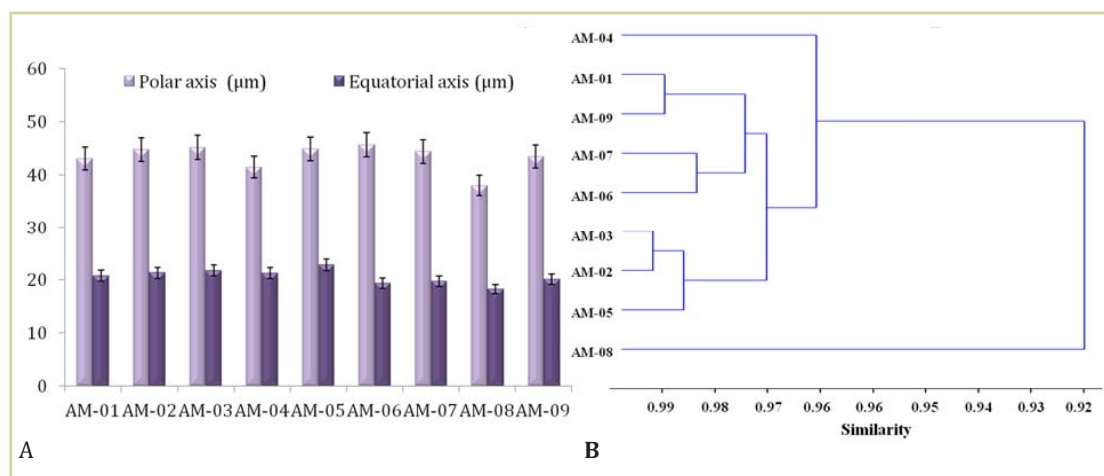


Figure 2A–B The average values and dendrogram of *Aronia mitschurinii* A.K. Skvortsov & Maitul. 9 phenotypes based on morphometric characteristics of pollen Conclusions

However, such a significant difference in the AM-08 phenotype compare with other samples still suggests the possibility of generation some genetic changes in the introduction population. Perhaps they are associated with hybridization or with accumulation of phenetic micromutations, which are not uncommon in botanical gardens, where samples of different geographical origin are cultivated close to each other. In the future, we propose to study the nature of these differences by molecular genetic methods.

Conclusions

Morphology characteristics of pollen grains of any genotypes and cultivars are important for breeding programmes and the studing of germplasm. Thus, the detailed pollen morphological and micro-sculptural characteristics of 9 phenotypes was investigated by using scanning electron microscopy. The analysis of morphological characteristics of pollen showed significant differences among *Aronia mitschurinii* phenotypes concerning the dimensions of pollen grain (length, width, and their ratio). Some of the pollen morphological parameters analysed be used for identification of *Aronia mitschurinii* phenotypes.

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