



## STUDY OF MORPHOLOGICAL CHARACTERISTICS OF POLLEN GRAINS OF *SAMBUCUS NIGRA* L.

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The aim of the work was to study the general characteristics and significant morphological traits of pollen grains, as the size, shape of pollen grains in *Sambucus nigra* L. The studies were performed on pollen obtained from genotypes cultivated in Ukraine (Kyiv). Morphology of pollen grain was described for thirteen *Sambucus nigra* genotypes (SN-1 – SN-13) at the laboratory of Department of Tropical and Subtropical plants of M.M. Gryshko National Botanical Garden. Pollen grains morphological traits were evaluated using the scanning and transmission electron microscopy. The measurement of morphometric parameters was carried out on 70 pollen grains from each genotype using the AxioVision Rel. 4.8.2.0 program. The measurements were made in micrometer ( $\mu\text{m}$ ). 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 of *Sambucus* species are small to medium-sized, oblat-sphaeroidal-prolat shape, three-colporate and the exine adornments are of reticulate type without perpendicular thickness. This study showed that there were significant differences the genotypes in all measured factors. The polar axis and equatorial diameter of pollen grains values were varied from 22.11 to 29.07  $\mu\text{m}$  and from 11.98  $\mu\text{m}$  to 17.29  $\mu\text{m}$ , respectively. This study confirmed small differences among the genotypes in all measured factors with variation coefficient in the range 2.87–6.02%. It was noted that diversity of surface sculpturing of pollen grains in combination with shape and sizes of them enables to use complex of thin morphologic signs for *Sambucus nigra* pollen identifications.

**Keywords:** *Sambucus nigra*, genotype, pollen, SEM, morphology, Ukraine

### Introduction

The Caprifoliaceae family, which also includes *Sambucus*, *Viburnum*, *Lonicera* and other species has been an object of palynological interest for at least 60 years (Erdtman, 1952; Straka, 1952; Stachurska et al., 1963, 1970, 1971; Weberling, 1966; Kuprianova and Alyoshina, 1972). New information about Caprifoliaceae pollen morphology were obtained due to use light

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microscope (LM) and scanning electron microscope (SEM) by authors Böhnke-Gütlein and Weberling (1981) in Germany, Tank and Donoghue (1985, 2010) in USA, Accorsi et al. (1987) in Italy, Hu and He (1988) and Di Wei Zhong et al. (1993) in China, Maciejewska (1997) in Poland, Tarnavski (1981) and Tamas et al. (1999, 2009) in Romania, Muccifora et al. (2003) in Italy, Tsymbalyuk and Bezusko (2017) in Ukraine.

The pollen grains have a definite shape, size, colour, structure for each species, genus and family and these characters are useful for systematic botany (Erdtman, 1952; Ciobanu, 1971; Brindza and Brovarskyi, 2013; Chlebo and Adamchuk, 2017; Grygorieva et al., 2015, 2017).

The complex of these morphological characteristics and ultrastructure allows determining the differences (or similarities) between the *Sambucus* species (Maciejewska, 2003; Brindza and Brovarskyi, 2013; Tsymbalyuk and Bezusko, 2017).

Using optical microscopy the pollen grain of Caprifoliaceae family, which includes *Sambucus* species is 3-colporate, porate, subsphaeroidal or oblate-sphaeroidal, isopolar, tricellular (Charzyńska and Lewandowska, 1989; Muccifora et al., 2003; Brindza and Brovarskyi, 2013), 3-tetrasymmetric, of small-middle or medium-sized and the exine without perpendicular thickness (Tamas et al., 2009). Authors Tarnavski (1981), Maciejewska (1997), Tsymbalyuk and Bezusko (2017) studied pollen morphology of three species of the genus *Sambucus* (*S. nigra*, *S. ebulus* and *S. racemosa*) represented in the flora of Romania, Poland and Ukraine using light and scanning electron microscopy (SEM). Pollen grains of the studied species are 3-colporate; prolate, spheroidal or oblate-spheroidal in shape; small to medium-sized. Their outline in equatorial view is elliptical or circular, in polar view 3-lobed or slightly 3-lobed. Colpi are long, occasionally of medium length, with pointed and sometimes rounded ends. Pores are indistinct, covered by margins of colpi, or sometimes distinct. Sculpture exine macroreticulate (*S. ebulus*) and microreticulate (*S. racemosa* and *S. nigra*). Polar view is subcircular and equatorial view is suboblate – subcircular (PalDat).

The aim of this study was to obtain the images. The knowledge of pollen morphological characteristics can be an adequate method for identification genotypes of *Sambucus nigra*.

## Materials and methodology

### Locating trees and data collection

The pollen of 13 *Sambucus nigra* genotypes (SN-01 – SN-13) 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 (June 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 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 performing 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 *Sambucus nigra* genotypes were determined by using a scanning electron micrograph (SEM).

## Morphometric characteristics

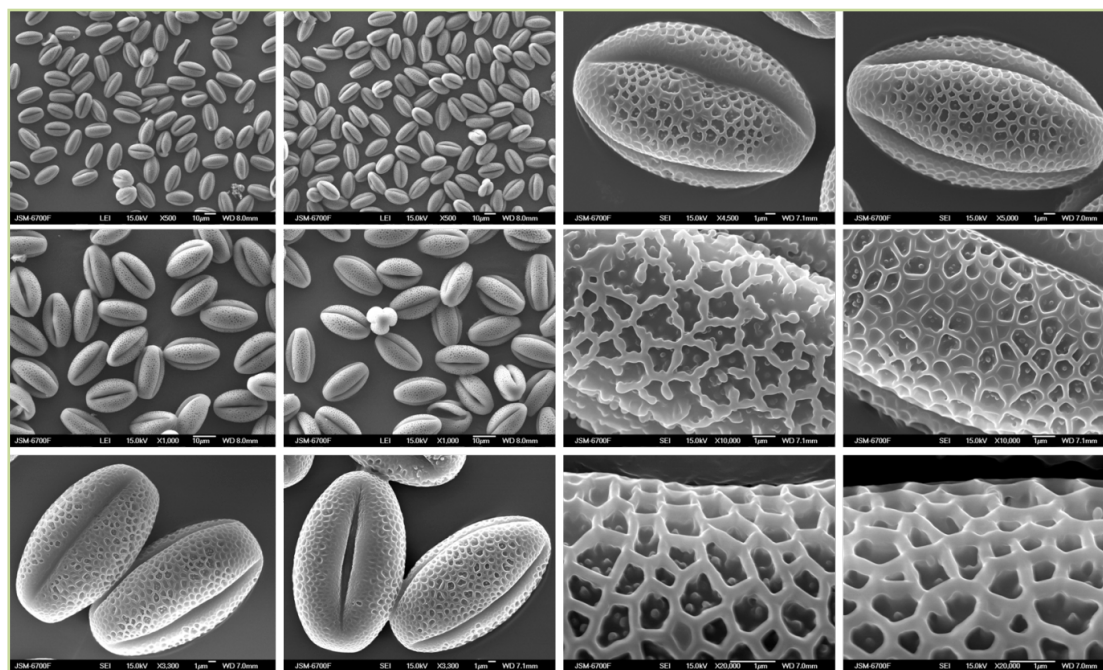
The measurement of morphometric parameters was carried out on 70 pollen grains from each genotype using the AxioVision Rel. 4.8.2.0 program. The measurements were made in micrometers ( $\mu\text{m}$ ). The characterization of pollen grains was calculated by taking the following parameters: the polar axis ( $P$  – the line connecting the proximal and distal pole), the equatorial axis ( $E$  – the 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

Study of 13 tested genotypes of *Sambucus nigra* pollen morphology showed that pollen grains are from small to medium-sized. In accordance with the ratio  $P/E$  (Tab. 1) the pollen tricolporate, oblate or spherical, sometimes oblate-spheroidal by the shape, in polar view pollen grain was 3-lobate and in equatorial view – elliptical or circular (Figure 1). Colpi were long, with more or less equal and clear edges with slightly pointed ends. Membranes of colpi were smooth. Mainly pores were blurred, covered with margins of colpi. But in very rare cases pores were distinct. Pollen wall was with tectum. Ectexine consisted of obvious, short and thin rod-shaped reinforcing elements. They were rarely located. The exsine surface had verrucate sculpturing with rounded cells by the shape. Sculpture of exine was microreticulate. Cells were small or medium size, circular, angled or circular-angled by the shape. Sometimes at the bottom of cells columns are observed. Knowledge from authors Tirnavschi (1981), Donoghue (1985), Maciejewska (1997), Tsymbalyuk and Bezusko (2017) confirmed our results.



**Figure 1** Pollen grains of *Sambucus nigra* L. species in different positions (Photo: Gurnenko, 2018)

**Table 1** The measured pollen morphological traits of selected genotypes of *Sambucus nigra* L.

Genotypes	min	max	<i>x</i>	<i>V</i> (%)	min	max	<i>x</i>	<i>V</i> (%)	min	max	<i>x</i>	<i>V</i> (%)
	<i>P</i> – Polar axis (µm)				<i>E</i> – Equatorial axis (µm)				SI – shape index ( <i>P</i> / <i>E</i> )			
SN-01	22.51	27.95	24.47	3.72	12.93	16.19	14.24	4.73	1.74	1.72	1.72	1.27
SN-02	22.99	26.00	24.51	2.87	12.58	16.21	14.39	5.29	1.83	1.60	1.70	1.84
SN-03	22.58	26.82	24.76	3.50	12.69	16.46	14.43	5.23	1.78	1.63	1.72	1.49
SN-04	22.30	26.15	24.36	3.51	12.61	16.09	14.11	6.02	1.77	1.63	1.73	1.72
SN-05	22.96	26.52	24.69	2.95	12.80	16.18	14.28	5.71	1.79	1.64	1.73	1.94
SN-06	24.07	29.07	26.10	5.49	13.22	17.29	15.19	6.55	1.82	1.68	1.72	1.19
SN-07	22.86	26.43	24.46	3.54	12.48	16.32	14.11	5.97	1.83	1.62	1.73	1.69
SN-08	22.74	26.02	24.43	3.15	12.60	16.52	14.12	4.92	1.80	1.58	1.73	1.56
SN-09	22.83	26.58	24.69	2.98	12.38	15.89	14.09	5.08	1.84	1.67	1.75	1.70
SN-10	22.86	26.45	24.58	2.95	12.31	15.43	13.95	5.13	1.86	1.71	1.76	1.74
SN-11	22.99	26.16	24.34	2.97	12.38	15.70	13.88	5.82	1.86	1.67	1.75	1.96
SN-12	22.11	27.07	24.41	4.33	11.98	16.01	14.12	5.18	1.85	1.69	1.73	1.20
SN-13	22.24	26.36	24.59	3.77	12.32	15.96	14.04	4.65	1.81	1.65	1.75	1.23

Note: min – minimum value; max – maximum value; *V* – variation coefficient (%)

The polar axis ( $P$ ), equatorial diameter ( $E$ ) and polar axis to equatorial diameter ( $P/E$ ) ratio of pollen grains of thirteen *Sambucus nigra* phenotypes were measured using scanning electron microscopy (SEM), and the results are displayed in Table 1.

An important morphological trait is the size of pollen grains. The length of polar axis ( $P$ ) varied from 24.34 (SN-11) to 26.10 (SN-06)  $\mu\text{m}$  and the width of the equatorial axis ( $E$ ) was in the range from 13.88 (SN-11) to 15.19 (SN-06)  $\mu\text{m}$ . According to the average values, the genotype SN-06 has the largest pollen grains  $26.00 \times 15.19 \mu\text{m}$ . The values of variation coefficient were in the range from 2.87 (SN-02) to 5.49 (SN-06) % for polar axes and in the range from 4.65 (SN-13) to 6.55 (SN-06) % for equatorial axes.

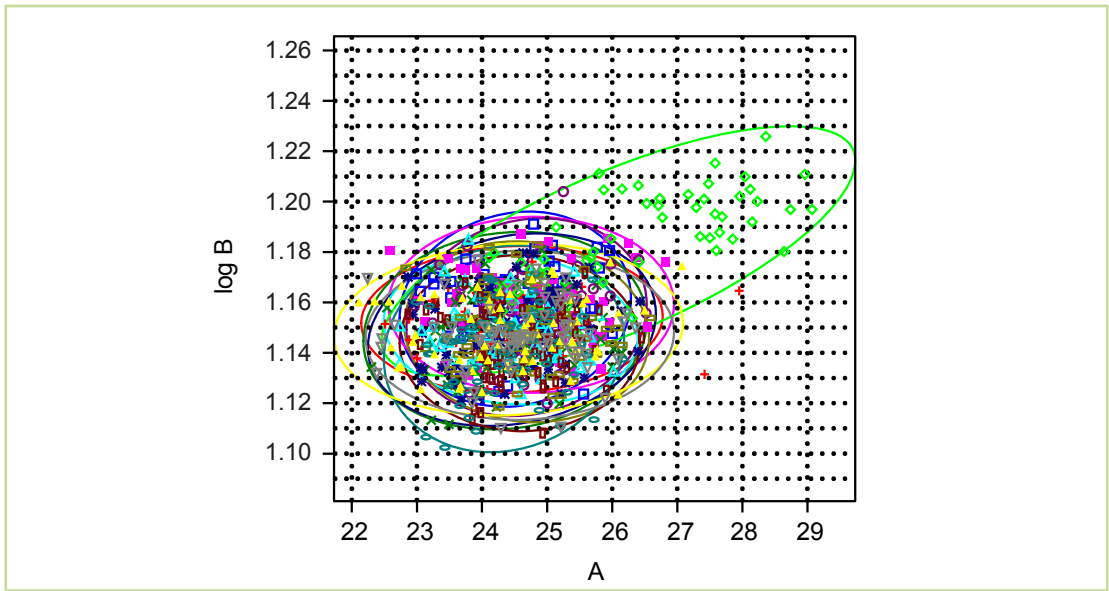
Shape index (SI) of pollen grain depends on parameters of polar ( $P$ ) and equatorial ( $E$ ) axis. Shape index (the  $P/E$  ratio) of tested species varied from 1.70 (SN-02) to 1.76 (SN-10). The sizes of pollen *Sambucus nigra* are very similar, the same for  $P/E$  ratio (1.70–1.76), whereas in comparison with authors Muccifora (0.89–1.42) or Tamas (1.88) are our studied genotypes *Sambucus nigra* the polar axis being greater (Table 2).

According to literary data, Tamas et al. (2009) and Muccifora et al. (2003) determined small size type (10–25  $\mu\text{m}$ ) in general, but our results have shown in one phenotype medium size type with average polar and equatorial axes with 26.10 and 17.29  $\mu\text{m}$ , respectively. Tsymbalyuk and Bezusko (2017) analyzed and summarized data on participation of pollen grains of *Sambucus* spp. and *Sambucus nigra* in palynofloras in the plain part of Ukraine. Authors detected a length of polar axis and equatorial diameter of pollen grains in the interval from 15.9–21.3 to 13.3–18.6  $\mu\text{m}$ , respectively (Table 2).

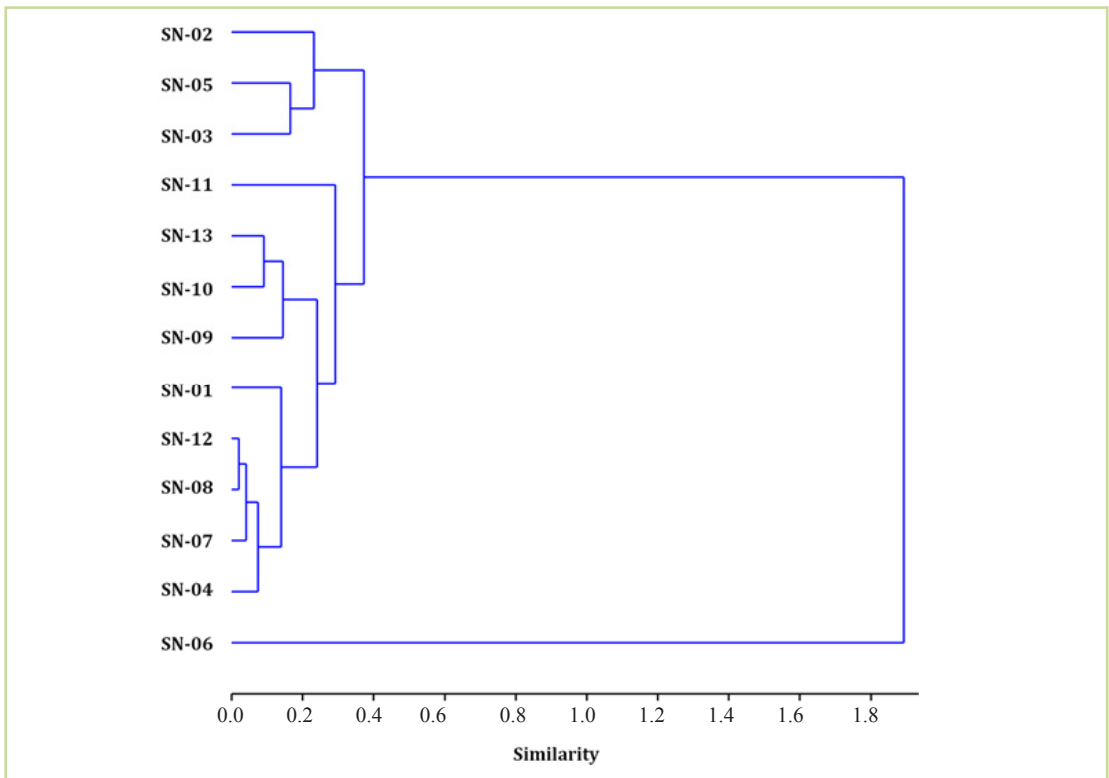
**Table 2** Literature data on pollen morphological traits in the *Sambucus nigra* L.

Characteristic	Value	Autors
<b>Polar axis (<math>\mu\text{m}</math>)</b>	16.0–24.8	Maciejewska, 1997
	25.0	Muccifora et al., 2003
	24.25	Tamas et al., 2009
	15.9–21.3	Tsymbalyuk and Bezusko, 2017
<b>Equatorial axis (<math>\mu\text{m}</math>)</b>	16.0–22.0	Maciejewska, 1997
	12.5	Muccifora et al., 2003
	12.85	Tamas et al., 2009
	13.3–18.6	Tsymbalyuk and Bezusko, 2017
<b>SI - shape index</b>	0.89–1.42	Maciejewska, 1997
	1.88	Tamas et al., 2009

Results of multi-dimensional scaling are shown in Figure 2. In Figure, it is possible to see the visual distribution the size of the pollen of the studied genotypes. The sample SN-06 (green ellipse) is with the largest pollen size.



**Figure 2** MDS plot of the similarity illustrating the length of the polar axis (A) and equatorial diameter (B) of pollen for studying samples of *Sambucus nigra* L. *Sambucus nigra* L.



**Figure 3** The dendrogram of *Sambucus nigra* L. 13 genotypes based on morphometric characteristics of pollen

Based on the cluster analysis of all 13 studied pollen characteristics, a dendrogram for the genotypes of *Sambucus nigra* was made (Figure 3). On the dendrogram, you can see that the sample SN-06 is really separated from other samples.

## Conclusions

The studying of the *Sambucus nigra* pollen via scanning electron microscope allowed to determine the most important parameters which can be used to identify the representatives of species. The detailed pollen morphological and micro-sculptural characteristics of 13 phenotypes were investigated, described and analysed by using hierarchical cluster analysis dendrogram and MDS plot. The main parameters such as the form (the pollen grains elongation, the length and the width ratio) are specific for different *Sambucus* species. Results from our analyses showed small differences among *Sambucus nigra* phenotypes from Ukraine. Some of these pollen morphological parameters can be used for identification and comparison with following analyses of *Sambucus* species phenotypes.

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