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Pollen morphology of some species of the genus *Amelanchier* Medik.

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Pollen grain structure is one of the diagnostic taxonomic and phylogenetic parameters. Study of morphology and morphometry of pollen grains of *Amelanchier* spp. allows found new additional diagnostic parameters of species. We determined that pollen of *Amelanchier* spp. is variable both in size and morphology. SEM investigations showed that the pollen grains various species of *Amelanchier* are prolate and perprolate, the surface sculpture and shape index of the species vary. The average length of the polar axis varied from 27.38 to 47.14 µm and the width of the equatorial axis was in the range from 14.33 to 28.95 µm. Shape index (P/E) of tested species varied from 1.77 to 2.09. The most average length of pollen was *Amelanchier spicata* (43.24 µm) and the least length was *Amelanchier arborea* (13.69 µm). The most average width pollen was *Amelanchier canadensis* (23.04 µm), and the least width was *Amelanchier arborea* (16.07 µm). Cluster analysis showed that the relationships of the tested *Amelanchier* species according to morphological features are represented by two main groups. It is evidently that *A. arborea* which has the smallest parameters is really separated from other species. *A. lamarki*, *A. spicata* and *A. canadensis* are similar according to morphometric sizes. Studies via scanning electron microscope have established characteristic differences in the morphometric and microsculptural features of pollen for each of the studied *Amelanchier* spp. which can be used to identify the representatives of species. Differences in the size of pollen grains in comparison with other authors may be the result of many environmental factors specific to the geographical area, climatic conditions of the country such as constantly increasing atmospheric temperature, alternation of rainfall with a dry season and others, which could be the subject of further research.

Keywords: *Amelanchier* spp., pollen, SEM, morphology

Introduction

The science that focuses on the study of pollen grains produced by seed and gymnosperms and all other palynomorphs is called palynology. The word palynology comes from the Greek word "palunein" meaning to sprinkle or scatter and the Latin word "pollen" meaning flour or dust. This means that palynology is the "science of dispersed dust" (Nughoro, 2018).

Palynology as a science creates many opportunities for its practical use. The study of symmetry, polarity, shape, size, structure, sculpture and apertures of spores can be very useful for many other sciences such as botany, oceanography, limnology, pedology,

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geology, paleontology, ecology, melitology, entomology, archeology, aerobiology, allergology, criminology and many others (Giveyrel et al., 2000; Radice et al., 2003; Shinwari and Khan 2004; Wrońska-Pilarek 2010; Ďurišová, 2018; Halbritter et al., 2018; Auer, 2021). Plant pollen is widely used in dietetic nutrition, in the production of medicines, bioadditives, vitamins, and in cosmetology (Hudz et al., 2017a; Hudz et al., 2017b; Nikolaieva et al., 2017). Pollen is an indicator that allows researchers to study past phytogeography, plant evolution, climate, rock and soil characteristics, air pollution levels, plant-insect relationships, and the botanical and geographic origins of bee products, among many other issues (Persson et al., 1996; Carlo and Paula, 2004; Hesse and Blackmore, 2013). The study of pollen found in sediments and sedimentary rocks allows us to obtain a lot of information about deep time, because the pollen grains are distinctive, and their outer covering known as the exine is very strong and therefore durable (De Vernal, 2009; Ďurišová, 2018; Halbtitter et al., 2018).

Amelanchier Medik. (family Rosaceae Juss.) commonly called saskatoon, saskatoonberry, sugarplum, serviceberry, juneberry, shadbush or northern serviceberry is native to temperate regions of the Northern Hemisphere, growing primarily in early successional habitats. It is most diverse taxonomically in North America, especially in the north-eastern United States and adjacent south-eastern Canada, and at least one species is native to every U.S. state except Hawaii and to every Canadian province and territory. Two species also occur in Asia, and one in Europe (Crompton and Wojtas, 1993).

Pollen morphology of selected *Amelanchier* species was described by some researchers in Texas (Weir, 1976), Pakistan (Perveen et al., 2014), Canada (Hebda et al., 1988; Hedba and Chinnappa, 1990a, b; 2014), Ukraine (Grygorieva et al., 2019), Armenia (Hayrapetyan, 2020), Poland (Hunt and Morawska, 2020).

The data of the authors on the size of the pollen grains of same plant species are variable and may vary, often depending on environmental factors or pollen treatment in the analyses. Various external factors such as air temperature, precipitation, desiccation, flood stress, drought stress, plant nutrition and effect of herbicides can influence on the size of pollen grains (Dajoz et al., 1991; Delph et al., 1997; Ejsmond et al., 2011, 2015; Yamburov et la., 2014; Mehmood et al., 2023; Wrońska-Pilarek et al., 2023).

The pollen grains adapted to different strategies have anatomical-morphological differences. The aim of this study was to compare the morphological parameters of pollen grains of the five species of genera *Amelanchier* Medik. and comparison their parameters, micromorphology and microsculpture.

Material and methodology

Pollen grains collection

Pollen grains studied on the species collected in Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine at the laboratory of the Department of Tropical and Subtropical plants (NBG). The following species were analyzed: *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem., *Amelanchier arborea* (F.Michx.) Fernald, *Amelanchier canadensis* (L.) Medik., *Amelanchier lamarckii* F.G. Schroed., *Amelanchier spicata* (Lam.) K. Koch. Freshly flowers (not opened) were collected randomly from the different *Amelanchier* spp. introduced in the condition of Kyiv at the balloon stage (May 2022). Pollen samples released from 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)

An investigation carried out at the laboratory of Department of Tropical and Subtropical plants of NBG using an electron microscope Carl Zeiss LS 15. The measurement of morphometric parameters was carried out on 50 pollen grains from each species using the AxioVision Rel. 4.8.2.0 program. The measurements were made in micrometre (μm). The length of a polar axis (P) and the equatorial diameter (E) of grain, P/E ratio were measured, and their variation was compared among studied species. The pollen shape classes (P/E ratio) were adopted according to the classification proposed by Erdtman (1952): oblate-spheroidal (0.89–0.99), spheroidal (1.00), prolate-spheroidal (1.01–1.14), subprolate (1.15–1.33), prolate (1.34–2.00) and perprolate (>2.01).

The comparative morphological studying of the pollen grains was performed according to the working rules on the SEM in the conditions of low vacuum (P = 60 Pa) with the following zooming: 500 times – during the measurements; 3000–10,000 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, sizes, and dimensions of pollen grains for *Amelanchier* species were determined by using a scanning electron microscope (SEM).

Statistical analysis

Statistical analysis was performed using SAS®9.2 software; hierarchical cluster analyses of similarity between genotypes were computed based on the Bray-Curtis similarity index; Basic statistical analyses – the minimal and maximal values of the traits, arithmetic means, and coefficient of variation (CV, %) were performed using PAST 2.17. The variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by Stehlíková (1998). All the observed traits were shown in graphic form.

Results and discussion

The complex morphological characteristics and ultrastructure of pollen grains allow determining the differences or similarities between the species. Quantitative (dimensions) and qualitative (ornamentation, colour) data of pollen have significant value in botanical (Ďurišová, 2018) and taxonomic classification, due to preserved palynological features in many plants (Grygorieva et al., 2019; Motyleva et al., 2017, 2018a, b; Horčinová Sedláčková et al., 2020, 2021a, b).

SEM investigations showed that the pollen grains species of *Amelanchier* are prolate and perprolate, 3rd furrow is meridional, in outline from the pole are three furrow – the furrows are long, narrow, smooth. The surface sculpture and shape index of the species vary. The average length of the polar axis varied from 27.38 to 47.14 µm and the width of the equatorial axis was in the range from 14.33 to 28.95 µm. Shape index (P/E) of tested species varied from 1.77 to 2.09. The most average length of pollen was *Amelanchier spicata* (43.24 µm), *Amelanchier canadensis* (41.82 µm), the least length was *Amelanchier arborea* (31.35 µm). The most average width pollen was *Amelanchier canadensis* (23.04 µm), *Amelanchier spicata* (22.96 µm), and the least width was *Amelanchier arborea* (16.07 µm).

Pollen grains of *Amelanchier alnifolia* among other species the longest from the poles, slightly pointed. Ultra-sculpture of exine is finely trickled, often perforated on all surfaces. Pollen grains of *Amelanchier arborea* long from poles, rounded. Ultra-sculpture of exine is wide streaming and less perforated then *Amelanchier alnifolia*. Ultra-sculpture of *Amelanchier canadensis* exine is pronounced streaky, dense, relief, perforation not often, close to poles. Pollen grains of *Amelanchier lamarckii* and *Amelanchier spicata* had the most equatorial diameter and more rounded shape than other species. *Amelanchier lamarckii* ultra-sculpture width streaky, sometimes intermittent, perforated on all surfaces. Ultra-sculpture of exine of *Amelanchier spicata* not perforated, intermittently finely trickle. The shape of the pollen grains and the structure detail of their surface are illustrated micrographically (Figure 1).

Other SEM studies described pollen morphology of *Amelanchier* species. *A. alnifolia* grains tricolpate, subprolate in equatorial view; sculpturing striate; ektexine and endexine about 1.5 µm thick; structure tectate. SEM shows puncta in the striate spaces.

Hebda et al. (1988) studied *A. alnifolia* pollen grains in monads, isopolar, radially symmetrical, circular to elliptic in equatorial view, amb subtriangular with apertures at angles, spherical to prolate; pores angular, distension – predominant; non-3-colpate grains – common, equatorial bridge and/or pore flaps are present; grain highly susceptible to distension, so that most grains in silicone oil preparations are fully distended 3-colporate, occasionally appearing 3-colpate if not swollen, to syncolpate, and especially in some specimens 4-colporate or pericolporate. Size (PxE) is highly variable, shape circular to prolate.

Table 1 Variability of the basic statistic parameters of pollen grains of *Amelanchier* species

Genotypes	P – polar axis (µm)				E – equatorial axis (µm)				SI (P/E)
	min	max	s_x	V%	min	max	s_x	V%	
Amelanchier alnifolia	31.52	43.27	0.28	6.59	15.67	22.37	0.38	9.07	2.09
Amelanchier arborea	27.38	37.90	0.42	7.72	14.33	24.65	0.64	11.60	1.92
Amelanchier canadensis	35.36	44.73	0.29	5.31	19.57	28.95	0.56	10.18	1.83
Amelanchier lamarki	33.00	45.43	0.40	7.21	19.40	25.10	0.41	7.55	1.77
Amelanchier spicata	35.79	47.14	0.32	5.78	19.92	26.03	0.39	7.18	1.89

min – minimal values; max – maximal values; x – average s_x – standard error the mean; V% – coefficient of variation; SI – shape index (P/E)

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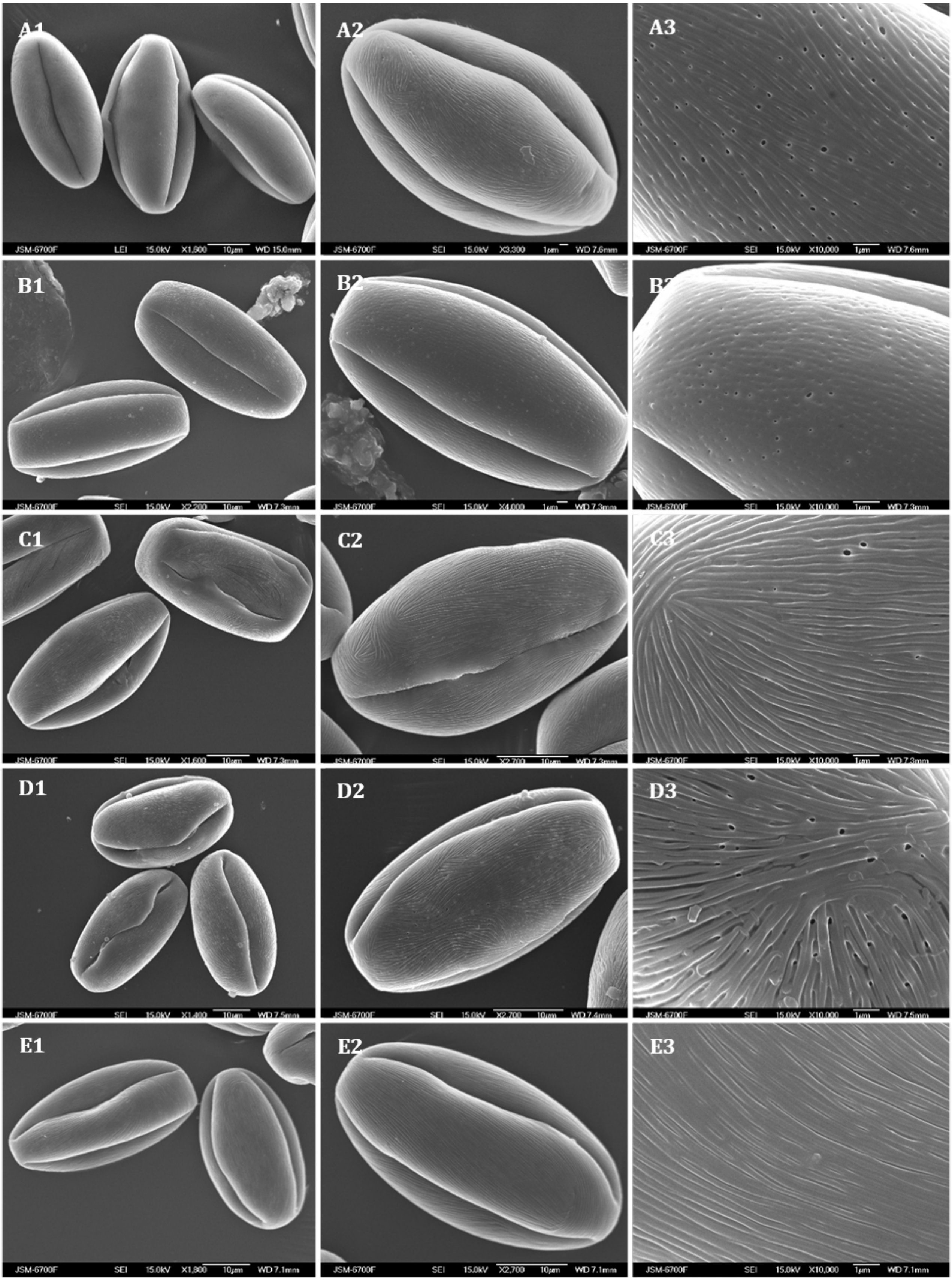

Figure 1 Pollen grains of *Amelanchier* Medik. species in different positions
Photo: Gurnenko, 2020
A – *A. alnifolia*; B – *A. arborea*; C – *A. canadensis*; D – *A. lamarki*; E – *A. spicata*; 1 figures (scale bar = 1 µm × 3,000): 2 figures (scale bar = 1µm × 5,000); 3 figures – detail of the sculpture of the exina surface in the place of the mesocolpium (scale bar = 1 µm × 10,000)

Based on cluster analysis, the relationships of the tested *Amelanchier* species according to morphological features are graphically displayed on a dendrogram (Figure 2). It is evidently that *A. arborea* which has the smallest parameters is really separated from other species. *A. lamarki*, *A. spicata* and *A. canadensis* are similar according to morphometric sizes.

Grygorieva et al. (2019) described *Amelanchier* pollen grains from Ukrainian conditions as oblong-spheroidal and small sizes with average length of the polar axis from 13.03 (*A. arborea*) to 21.37 µm (*A. spicata*) and the width of the equatorial axis from 6.55 (*A. arborea*) to 11.96 µm (*A. canadensis*). Shape index (P/E) of tested species varied from 1.85 to 2.11.

Hunt and Morawska (2020) studied pollen grains of *A. alnifolia* (Nutt.) Nutt. Ex M. Roem with following results of morphology: tricolporate, sculpture very finely striate to psilate; the striae were found to form patterns: scabrate to slightly striate (Perveen et al., 2014); sizes of polar axis 16(18.2)–25 µm, equatorial axis 16(17.7)–24 µm. Crompton and Wojtas, (1993) analysed sizes of polar 20.0–24.7 (22.5) µm and equatorial 17.1–20.9 (18.9) µm axes and authors Hebda and Chinnappa (1990a) concluded that *Amelanchier alnifolia* pollen grains exhibit systematic geographic variability and presented that dimensions is highly variable depending on source of collection and degree of swelling in silicone oil distended grains, P = (17.0) 21.0–28.0 (30.0) µm, E = (12.0) 19.0–28.0 (30.0) µm; undistended. P = (19.0) 21.0–29.0 (31.0) µm, E = (14.0) 15.0–19.0 (21.0) µm; glycerol, P =, distended 26.0–32.0 µm, E =27.5–33.0 µm; glycerol, P =, undistended 30.0–37.0 µm, E = 17.5–24.0 µm (Hebda et al., 1988).

Some authors also evaluated other *Amelanchier* species, e.g. *A. spicata* which has medium sized (26–50 µm), colporate, isopolar, oblate shape, outline in polar view is circular, dominant orientation is oblique, aperture type is colporus, aperture conditiom is colporate, tricolporate (Auer, 2021; PalDat, 2023); *A. ovalis* (= *A. rotundifolia* (Lam.) Dum.) pollen grains are 3-zonocolp-porate (poroidate), from oblong to oblate, outline in polar view rounded-triangular or rounded-3-lobed (Hayrapetyan, 2020); pollen has small sized (10–25 µm), size of hydrated pollen is 21–25 µm, shape is spheroidal, endexine very thin (Loos et al., 2021; PalDat, 2023), polar axis 14.5–22.5 µm, equatorial diameter 17.4–20.3 µm. Colpi is long, with thickened edges and with pointed, sometimes anastomosing ends (synaperturate); apocolpium diameter has 2.5–4.5 µm, mesocolpium width 12.4–14.5 µm; ornamentation of colpus membrane is granulate. Pores are not always clearly defined, elliptical, slightly elongated along the colpi, 5.1 µm × 3.5 µm. Exine 0.8–1.0 µm, columellae are not clearly defined. Exine ornamentation granulate (LM), exine ornamentation sinuosly finely striate (SEM) (Hayrapetyan, 2020); *A. utahensis* has size 21.4 (19–24) µm, shape is subprolate and spheroidal (Weir, 1976).

Various environmental factors such as geographical conditions and change of climate can influence on

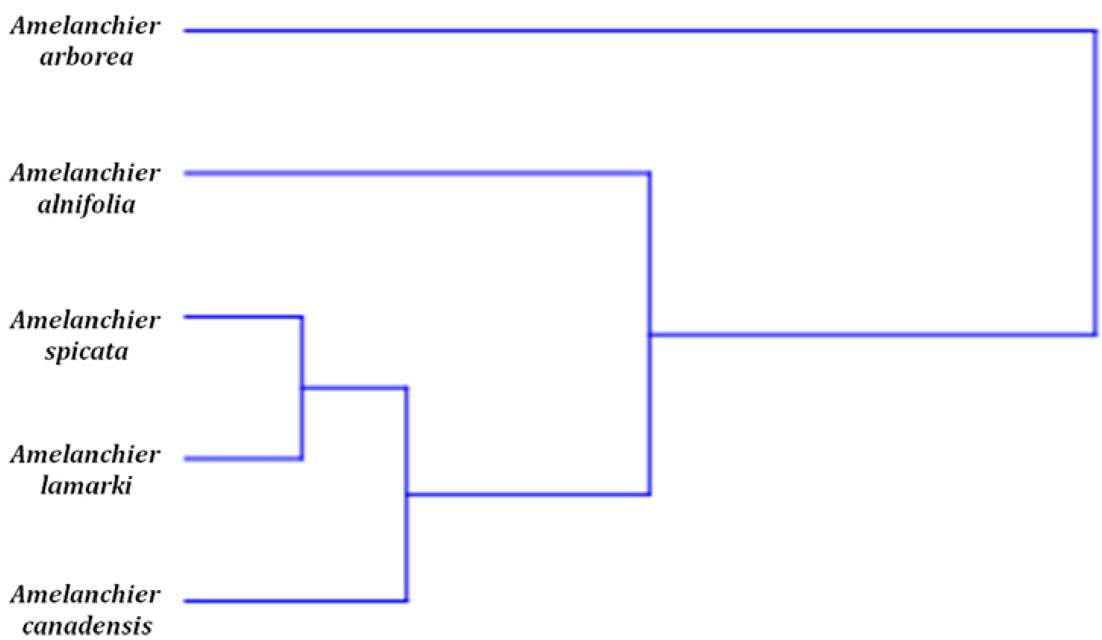

Figure 2 Dendrogram of *Amelanchier* species pollen according to morphometric features

the size of pollen grains (Dajoz et al., 1991; Delph et al., 1997; Ejsmond et al., 2011, 2015; Yamburov et al., 2014).

Ejsmond et al. (2011) studied influence of temperature increased on the pollen morphology, pollen size and pollen shape. Empirical results showed that pollen grains were larger when under intense desiccation stress, but without change pollen grain shape. Plants are known to produce fewer pollen grains but larger sizes to avoid desiccation stress during the flowering period which studied some authors (Ejsmond et al., 2011; Mehmood et al., 2023).

Yamburov et al. (2014) studied effects of drought and flood stress on pollen quality and quantity in *Clivia miniata* including pollen size, viability, germination, and number of pollen grains per anther while evaluating how stress influences these features. The study provides evidence that microsporogenesis of *Clivia miniata* is more sensitive to flooding than to drought. The studied pollen features can be ranked based on their degree of sensitivity to flooding in the following order: number of pollen grains per anther > pollen germination > pollen fertility > pollen size.

Effect of long-term herbicide influence on *Prunus serotina* pollen studied Wrońska-Pilarek et al. (2023). Pollen grains from the control variant had a longer equatorial axis, were more elongated in shape and had the largest range of exine thickness compared to the pollen from the herbicide-treated samples. The average exine thickness in the samples treated with herbicides were larger. There were differences in the P/E ranges of variability between the control and herbicide-treated samples. In the control sample the P/E ratio was 1.32–2.04 and elongated forms of pollen shapes prevailed, while in the herbicide-treated samples it ranged from 1.03 to 1.47. The share of deformed pollen grains in the herbicide-treated samples was lower than expected.

Differences in pollen grain size may also occur between different genotypes of the same species The size of the pollen grains can also vary within a single plant, the largest pollen grains are found in the best developed top inflorescences (Miter et al., 2016).

Conclusions

Studies via scanning electron microscope have established characteristic differences in the morphometric and microsculptural features of pollen for each of the studied species of *Amelanchier* spp. which can be used to identify the representatives of species. The detailed pollen morphological and micro-sculptural characteristics were investigated, described, and analysed by using hierarchical cluster analyses dendrograms. The main parameters such as the form (the pollen grains elongation, P/E ratio) are specific for different *Amelanchier* species. Results from our analyses showed differences among various *Amelanchier* pollen grains. Some of these pollen morphological parameters can be used for identification and comparison with the following analyses of *Amelanchier* species. These species require detailed palynogeographic study. Differences in the size of pollen grains in comparison with other authors may be the result of many environmental factors specific to the geographical area, climatic conditions of the country such as constantly increasing atmospheric temperature, alternation of rainfall with a dry season and others, which could be the subject of further research.

Conflict of interests

The authors declare that they have no competing interests.

Ethical statement

This article complies with all ethical standards.

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