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THE POLLEN MORPHOLOGY OF DIFFERENT *IRIS* L. SPECIES FROM ROMANIA

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Abstract

In order to have proper identification of Iris species, many scientific works are referring to the morphological features of pollen grains as adequate sources of information about the origin and variability concerning the different populations of Iris.
According to several taxonomic works, in Romania are 17 (18) species of Iris. There is a lack of information about the morphology of pollen in Iris species from our country. SEM analyses of pollen from five populations of Iris species from the Botanical Garden of the University of Agronomic Science and Veterinary Medicine of Bucharest revealed that four of them had reticulated exine, respectively Iris germanica, I. pseudacorus, I. variegate, and I. suaveolens and one had gemmated exine - Iris pumila. The pollen morphological description presented in this study may be of systematic significance to Iris species, enabling species distinction.

Key words*: exine; morphology; pollen; Roumanian Iris species; scanning microscopy.*

INTRODUCTION

The pollen grains, as gametophytes, provide protection for male gametes during dispersal, against mechanical and environmental damage. Their particular shape, surface coating, and sculpturing reflect the adaptations of each species during evolution as vectors of grain transport and adhesion to stigma surfaces (Knox, 1984).

The Iris species have a polymorphism due to their flower structure and reproduction type, which can facilitate the emergence of hybrids (Colasante et al., 2021). The diversity of the characters at every level (morphological, cytological, biomolecular, etc.) made the identification of the Iris species a complex task. These issues, are described also by Dembicz et al., 2018, regarding *I. pumila* and was found a high fragmentation and a high genetic diversity across all investigated *I. pumila* populations in the Kherson region, Ukraine.

Therefore, it is important to find the right methods for assessing the origins of Iris species populations. Pollen grains analysis with scanning electron and optic microscopy are among the methods promoted to evaluate the origins and phylogeny of the species (Colasante et al., 2021). Using light and scanning microscopy methods, Dönmez et Pinar (2001) describe pollen clypeata types' morphological peculiarrities in subgenus *Scorpiris* species from Turkic countries. In order to investigate the pollen morphology of *Iris* species in Croatia and to contribute additional data on species with a wider distribution than the European one, Mitić et al. (2013) analyzed pollen grains from 20 species of Iris employing scanning microscopy. The exine ornamentation of 42 cultivars observed with a scanning microscope allowed, the determination of the systematic, with a focus on cross-breeding, inside *I. barbata* species (Zhang et al., 2021). A study presented in 2022 on pollen micromortphological peculiarities has helped to establish a good delimitation between Korean Iris species (Choi et al., 2022). It is clear from the results of this study that pollen exine ornamentation plays a role in the systematics of *Iris* species.

The most recent work on Romanian vascular plants shows 18 species of the *Iris* genus in the country (Sârbu et al., 2013).

There is not much information about the pollen morphology and micromorphology of the species from Romania. This study focuses on the morphology of pollen grains from some Iris species cultivated in the botanical garden of our university, conducted using scanning microscopy methods.

MATERIALS AND METHODS

The pollen grains analyzed are from five Iris species cultivated in the botanical garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Four out of these five species are found in different habitats in our country (Sârbu et al., 2013): *Iris suaveolens* - rare in xerophytic grasslands and rocky places in the steppe and wooded steppe; *I. pumila* - often, in grasslands, rocky coasts, or grassy rocky ground, from steppe to beech floor; *I. variegata* - common in grasslands, forest fringes, or on sandy soils from steppe to beech floor; *I. pseudacorus* - common in marshes, meadows, or reedy swamps from steppe to beech and spruce floor. *I. germanica* is a cultivated species occasionally found in the wild.
The fresh pollen grains were collected from anthers and dried at room temperature at the beginning of the anthesis. Observations were made on dried grains in the Research Center for Studies of Food Quality and Agricultural Products. The pollen grains were powdered on SEM stubs and observed directly under the scanning electron microscope (SEM) FEI Inspect S 50 using low vacuum mode. All the measurements were made in the dehydrated stage.
The detailed description of the pollen grains follows Halbritter et al., 2018, pollen monography.
The polar axis and equatorial diameter ratio allowed for the classification of pollen into a shape class, according to Rahmawati et al., 2019.
For each parameter, mean values, standard deviation, coefficient of variation, and Pearson correlation at $p \leq 0.05$, were calculated using Microsoft Excel 2019.

RESULTS AND DISCUSSIONS

Iris species disperse their pollen as isolated grains (monads). The pollen grains are monoaperturate and sulcate, with an elongated aperture located distally (Figure 1).

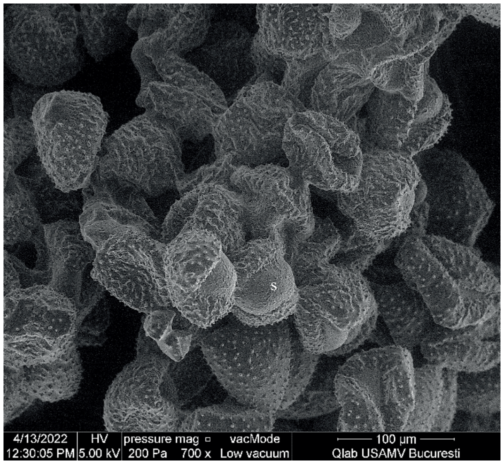

Figure 1. *Iris pumila* - isolate grains, with sulcus (s)

Iris germanica L.
Species of Mediterranean origin with a blooming period of May and June.
In dry conditions, grains are wedge-shaped with a sunken aperture (Figure 2). The ornamentation of exine is of a heterobrochate type, with a reticulated pattern and lumina of different sizes. In each lumina are free-standing columellae.

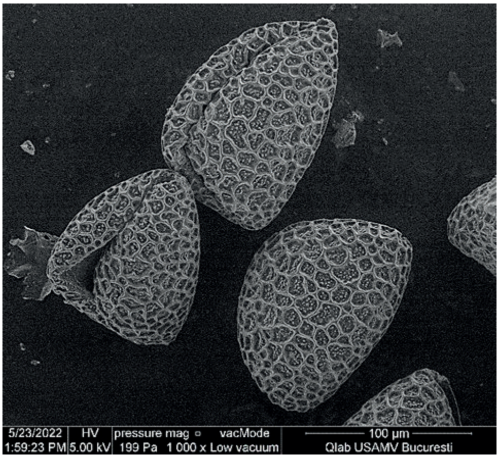

Figure 2. *Iris germanica* - dry pollen grains; free-standing columella in the lumina of the network

Iris pumila L.
Originated in the Ponto-Pannonian-Balkan regions. The blooming period is April to May.
Dry pollen grains are wedge-shaped with a sunken aperture (Figure 1). Exine ornamenttation is a clavate-gemmate type (Figure 3).

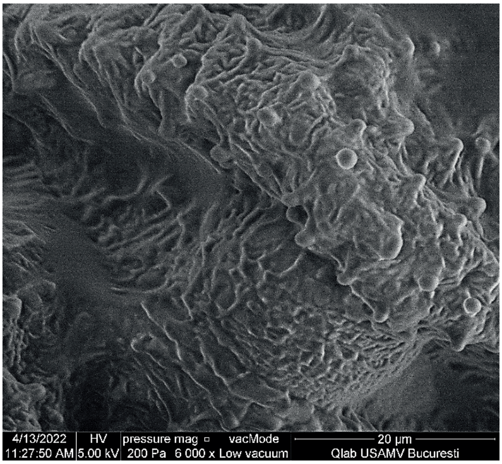

Figure 3. *Iris pumila* - exine ornamentation

Iris pseudacorus L.

Is a European species with a blooming period from May to July.

Pollen grains that are dry are typically boat-shaped with reticulated ornamentation of the exine. Network laminae decrease towards the aperture area (heterobrochate type). The aperture is sunken with infolded edges (Figure 4).

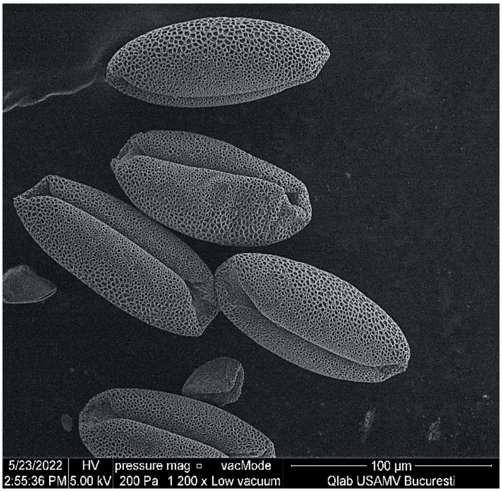

Figure 4. *Iris pseudacorus* - dry pollen grains boat-shaped

Iris suaveolens Boiss. et Reut.

Est Balkan-Anatolian element. Blooming periods last from March to April.

Dry pollen grains are wedge-shaped with heterobrochate exine. There are few free-standing columellae visible (Figure 5).

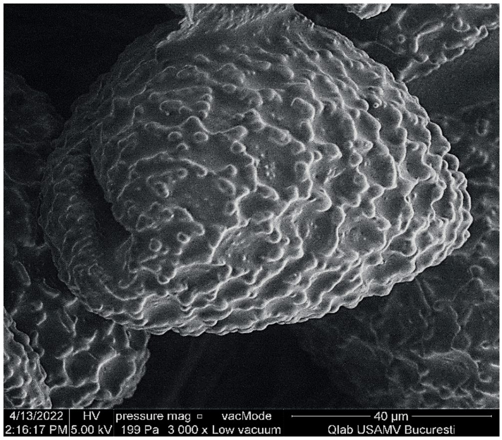

Figure 5. *Iris suaveolens* - heterobrochate exine

Iris variegata L.

Ponto-Central European-Balkan element with the blooming period in May and June.

Dry pollen grains are wedge-shaped. The ornamentation of exine is of a heterobrochate type with free-standing columellae. The aperture is sunken (Figure 6).

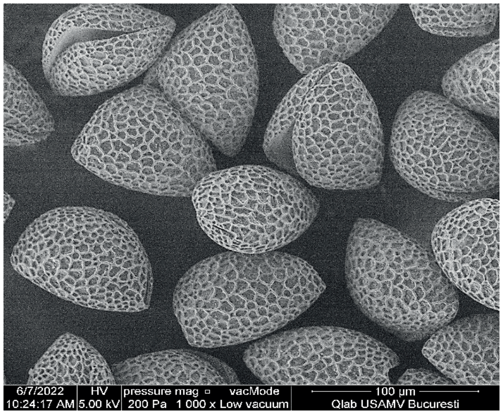

Figure 6. *Iris variegata* - Heterobrochate dry pollen grains

A reticulate pattern of exine was also found in Iris subgenera by Choi et al., 2022, and Dönmez, & IşIK , 2008.

Analyzing the pollen size, a higher polar axis length variability was found in *I. germanica*, followed by *I. pumila*. The lowest variability was found in *I. pseudacorus* species. *I. germanica* presented the highest values from all species, being 41.344 µm higher than *I. pumila*. The dry pollen size varied between 63.860 µm for *I. pumila* to 137.900 µm for

I germanica. For equatorial axis length, higher variability was found in *I. pseudacorus* species, followed by *I. pumila,* and the lowest was found in *I. germanica*. *I. germanica* having the highest equatorial axis length with 35.731 µm more than *I. pseudacorus*. Equatorial axis length varied between 35.77 µm for *I. pseudacorus* and 86.42 for *I. germanica.*

For P/E, also *I. pseudacorus* variety showed the highest variability, followed by *I. pumila*, like in equatorial axis lengths, but the lowest ratio was found in *I. suaveolens*. *I. pseudacorus* presented the highest values, with 1.032 µm then *I. suaveolens*. P/E varied between 0.999 µm for *I. suaveolens* and 3.008 µm for *I. pseudacorus* (Table 1).

Table 1. Pollen morphology of Iris species

Iris varieties	Polar axis length ± SD (µm)	CV%	Equatorial axis length ± SD (µm)	CV%	P/E ± SD (µm)	CV%
Iris germanica	120.797±16.01	13.254	81.933±4.86	5.926	1.478±0.20	13.753
Iris pseudacorus	102.547± 4.90	4.781	46.202±6.78	14.677	2.271±0.40	18.014
Iris variegata	85.003± 7.26	8.547	56.631±4.45	7.861	1.514±0.22	14.367
Iris suaveolens	84.989±6.04	7.110	68.981±6.44	9.340	1.239±0.11	9.151
Iris pumila	79.453±8.04	10.118	60.085±8.45	14.064	1.341±0.18	13.549

SD - standard deviation, CV - coefficient of variation, P/E - ratio of polar axis length to equatorial axis length

I. pseudacorus presented the highest values, with 1.032 µm then *I. suaveolens*. (Table 1).

Our results showed that the Iris species studied have a high pollen shape, equatorial axis, and polar axis length variability.

Analyzing the Pearson correlation, for polar axis length, was found a moderate negative relationship between *I. germanica*, *I. pseudacorus,* and *I. suaveolens*

Table 2. Correlation among *Iris* species and pollen polar axis length

Polar axis length (µm)	*I. germanica*	*I. pseudacorus*	*I. variegata*	*I. suaveolens*	*I. pumila*
I. germanica	1				
I. pseudacorus	0.355	1			
I. variegata	0.369	-0.215	1		
I. suaveolens	-0.600	-0.633	0.290	1	
I. pumila	0.414	-0.151	-0.106	0.080	1

r < 0.05

A very weak positive correlation was found between *I. suaveolens* and *I. pumila* (Table 2).

Regarding equatorial axis length, a strong relationship was found between *I. germanica* variety, *I. variegata,* and *I. pumila*. A strong relationship was found also between *I. germanica* and *I. variegata* (Table 3).

Dönmez & IşiK in 2008, in their study, found that Iris pollen grains were medium to large in size, the measurements varied between 45-163 µm for polar axis length and 33-163 µm for equatorial axis length, our findings are in accordance with their study.

A moderate positive relationship was found between I. *suaveolens* and *I. pumila* related to the ratio between polar axis length and equatorial axis length (Table 4). P/E is an important parameter that could vary depending on the environmental conditions (Güvenet al., 2014).

Table 3. Correlation among Iris species and pollen equatorial axis length

Equatorial axis length (µm)	*I. germanica*	*I. pseudacorus*	*I. variegata*	*I. suaveolens*	*I. pumila*
I. germanica	1				
I. pseudacorus	-0.186	1			
I. variegata	0.787	-0.144	1		
I. suaveolens	-0.441	-0.095	-0.247	1	
I. pumila	0.831	-0.552	0.719	0.162	1

r < 0.05

Table 4. Correlation among *Iris* species and the ratio between polar axis length and equatorial axis length of pollen

P/E (µm)	*I. germanica*	*I. pseudacorus*	*I. variegata*	*I. suaveolens*	*I. pumila*
I. germanica	1				
I. pseudacorus	0.072	1			
I. variegata	0.563	-0.079	1		
I. suaveolens	0.505	-0.612	0.292	1	
I. pumila	-0.002	-0.730	0.497	0.666	1

Wang & Dobritsa, 2018 in their study specified that there is a correlation between pollen surface morphology and plant pollination. It was observed that pollinators can influence the patterns and decorations from pollen surfaces, whereas plants pollinated with the help of wind or water have a smoother pollen surface, making the pollen more aerodynamic. These differences in patterns might influence pollen hydrodynamic and pollen-stigma interaction. Lumina and muri from pollen exine, also have a very important role in differentiation between species.

The measurements on lumina (Table 5) width, showed that *I. suaveolens* have the largest width of 8.406 µm, the largest being of 10.610 µm, and the smallest of 5.690 µm due to heterobrochate exine. Between *I. suaveolens* and *I. germanica*, the values for lumina width, are close, the difference between them being 0.042 µm. *I. pseudacorus* had the smallest lumina of 0.883 µm, the largest being 1.416 µm and the smallest of 0.117 µm, with network laminae decreasing towards the aperture.

I. germanica was found to have the highest length of lumina (11.246 µm), having a maximum length of 14.940 µm and a minimum of 8.378 µm. *I. suaveolens* with 13.790 µm maximum length and with a minimum of 8.014 µm. The smallest lumina was found in *I. pseudacorus* with 2.711 µm the maximum length and 0.989 µm minimum length.

Table 5. Lumina characteristics of Iris species

Iris varieties	Width ± SD (µm)	CV%	Length ± SD (µm)	CV%
I. germanica	8.364±1.494	17.866	11.246±1.999	17.773
I. pseudacorus	0.883±0.339	38.448	1.574±0.496	31.496
I. variegata	4.180±1.081	25.852	7.756±1.674	21.582
I. suaveolens	8.406±1.359	16.169	10.384±1.923	18.514

SD - standard deviation, CV - coefficient of variation

The results, related to lumina measurements, showed that Iris species have irregular-shaped lumina, and we found variability between the studied Iris species.

Our results are in concordance with those of Mitić et al. 2013, that found that on the basis of karyological results, *I. germanica* pollen type derived from the *I. pumila* pollen type, and also *I. sibirica* from Bjelolasica Mountain, have possible parents from the primitive subgenus *Limniris* and the other possible parent is a taxon from the series *Pumilae*, so *Pumilae* can be more primitive.

CONCLUSIONS

Among the studied species we found variations in pollen morphology related to the shape, size, and ornamentation of the exine.

Iris germanica, *I. variegata,* and *I. suaveolens* have the exine heterobrochate with lumina with free-standing columellae, *I. pseudacorus* has the exine heterobrochate, but without free-standing columellae.

I. pumila has the exine ornamentation clavate-gemmate type.

I. germanica was found to have the highest length of lumina, and the smallest lumina length was found in *I. pseudacorus*.

I. germanica presented the highest values for polar axis length and equatorial axis length from all studied species.

I. pumila presented the lowest polar axis length and also the ratio between the polar axis length and equatorial axis length of pollen.

A strong relationship between *I. germanica* variety, *I. variegata,* and *I. pumila*, was found, and also between *I. germanica* and *I. variegata*.

Our study of the morphological aspects of the Iris species pollen, offers an important characterization of the species from our country, helping in their differentiation.

The results of this study bring new information on the characterization of pollen, contributing to conservation and genetic improvement.

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