

POLLEN CHARACTERISTICS IN SOME *DIOSPYROS* SPECIES

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Abstract. Aim of the work was to study the general characteristics and essential morphological traits of pollen grains viz the size, shape of pollen grains and number, form and position of apertures in *Diospyros kaki* L.f., *D. virginiana* L., *D. lotus* L. species and interspecies hybrid of *D. virginiana* × *D. kaki*. Significant differences were detected among the tested species and the interspecific hybrid as well as between individual genotypes of *D. lotus*, especially in the equatorial axis.

Key words: *Diospyros kaki*, *Diospyros virginiana*, *Diospyros lotus*, pollen grains; morphological traits, hybrids

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Introduction

Genus *Diospyros* belongs to an extensive family Ebenaceae consisting of 7 genera. YONEMORI *et al.* (2000) listed over 400 species forming the genus *Diospyros* and several of these species are of economic importance. The mild climatic region is suitable for growing and use in horticulture of three persimmon species – *Diospyros kaki* L.f., *D. virginiana* L. and *D. lotus* L. (BELLINI & GIORDANI 2005).

In Slovakia, persimmon species are cultivated in botanical gardens as well as by many small growers. The present knowledge on growing of these originally exotic plants clearly show the possibility of successful cultivation in local climatic conditions, preferably in the southern part of Slovakia. Research work and most studies of these species were concentrated for several years in the Slovak University of Agriculture in Nitra in close cooperation with the National Botanic Garden of the Ukraine National Academy of Sciences in Kiev (GRYGORIEVA *et al.* 2009).

For practical reasons, concerning the use and distribution of mentioned plant species in Slovakia, it is quite important to learn the biological peculiarities connected with flowering as well as with interspecific

variability and reproduction capacity in local climatic conditions. It is known that the persimmon flowers are prevalingly unisexual, only exceptionally being bisexual. It means that the plant bears both, the male and female flowers. Sometimes the dioecism occurs. Many individuals have only female flowers and the fruits can arise without fertilization – by parthenocarpy and they are seedless. Flowering biology complexity is for these species symptomatic, and it brings questions concerning several topics like the pollen grains characteristics designated in palinology as sporeforms. Information on the genus *Diospyros* pollen grain traits occurs scarcely in a few papers (ERDTMAN 1966; KODELA 2006; GEERAERTS *et al.* 2009).

The morphological characteristics of pollen grains are applied for taxonomic classification purposes, further for phylogenetic evaluation of plant species and detection of hybrid and polyploid forms (ERDTMAN 1966; 1986; ERDTMAN & ROGER 2007).

Then aim of this work was determination of pollen grains general characteristics and basic morphological traits in selected species from the genus *Diospyros* including the variability of examined species.

Material and methods

The pollen grains morphology was studied on *Diospyros kaki*, *D. virginiana*, *D. lotus* and interspecies hybrid *D. virginiana* × *D. kaki*. We have evaluated the basic morphological characteristics of tested persimmon species pollen grains – the size and shape, position, number and form of apertures (aperture – the thinned region of the sporoderma – wall pollen grain, usually function as sites of the germination). The pollen grain size has been determined by measuring the length of polar axis (P – the straight line between the distal and proximal poles of a pollen grain) and length equatorial axis (E – pollen width, the distance between the poles in equatorial part of pollen). The shape index (SI – the ratio of the length of the polar axis and equatorial axis) allowed determination of the pollen grains shape. The pollen grains are classified according to shape index into 9 shape classes (DOSTÁL *et al.* 1966). The pollen size (length of polar axis and equatorial axis – width of pollen) and shape (SI – P/E) were measured in micrometers.

Above mentioned morphological traits were measured and evaluated on 40–60 pollen grains (in polar position) of each species. Electron microscopes Carl Zeiss EVO LS 15 and PEMMA-102 (SEMI) enabled to distinguish and identify the pollen surface – structure, special ornamentation, upmost thickness of exine – sexine and aperture membrane structure.

Morphological traits were evaluated on pollen samples collected in 2010 from female flowers during full flowering from the persimmon trees of species *D. virginiana*, cultivated in Kyiv (Ukraine), *D. lotus* – in Arboretum Mlyňany (Slovakia) and *D. kaki* – in Nitra (Slovakia). Pollen samples released from dry male flowers were further dried under laboratory conditions. For microscopic studies on morphological characteristics the dry pollen was used.

Results and discussion

Pollen grains of the genus *Diospyros* are solitary, defined as monades. This study showed

that pollen grains are radially symmetrical, isopolar and according to the localization of apertures they are zono-tricolporate.

Three compound apertures are according to distribution equidistant. The size, the shape of pollen grains and the number of apertures are documented on Fig. 1. Pollen grains possess three prolonged apertures – colpi which are narrowed at poles. Additional pole is in the colpus middle at the equatorial centre (circular equatorial aperture). This classification correlates with literature data (SOWUNMI 1995; KODELA 2006; GEERAERTS *et al.* 2009). In some pollen grains were observed annuluses – an area of the exine surrounding a pore with thickened periphery or operculum – part of exine over the pore (Fig. 1). The exine membrane in an area of the colpus is granulated. From cytological point of view the pollen grains of most *Diospyrus* are bicellular with exception of *D. kaki* and *D. lotus* with unicellular pollen grains (ПОДАДУБНЯЯ-АРНОЛЬДИ 1982). It means that generative cell division on sperm cells is accomplished in the pollen pocket.

An important morphological characteristic is the size of pollen grains. Our data showed that the pollen grains of *D. kaki*, *D. virginiana*, *D. lotus* and interspecies hybrid *D. virginiana* × *D. kaki* are from medium size to large accordingly to DOSTÁL *et al.* (1966). The length of polar axis (P) measured in the mentioned three species (Tab. 1) varied from 42.25 to 55.41 µm and the width of equatorial axis (E) was in range of 21.40–32.47 µm. The highest values of mean length of polar axis (71.19 µm) and equatorial axis (33.14 µm) was recorded in pollen of the interspecies hybrid *D. virginiana* × *D. kaki*. Differences in values of the both indicators of pollen grain size (P and E) between this hybrid and other tested species were statistically significant.

The significance of differences was observed also in the mean length of polar and equatorial axes in all analyzed species with exception of mean length of polar axis in *D. kaki* and *D. lotus*. The values of the coefficient of variation were in the range of 3.63–6.50% for polar and in the range of 6.15–7.56% – for equatorial axes. It suggests a low variability in pollen grains size of

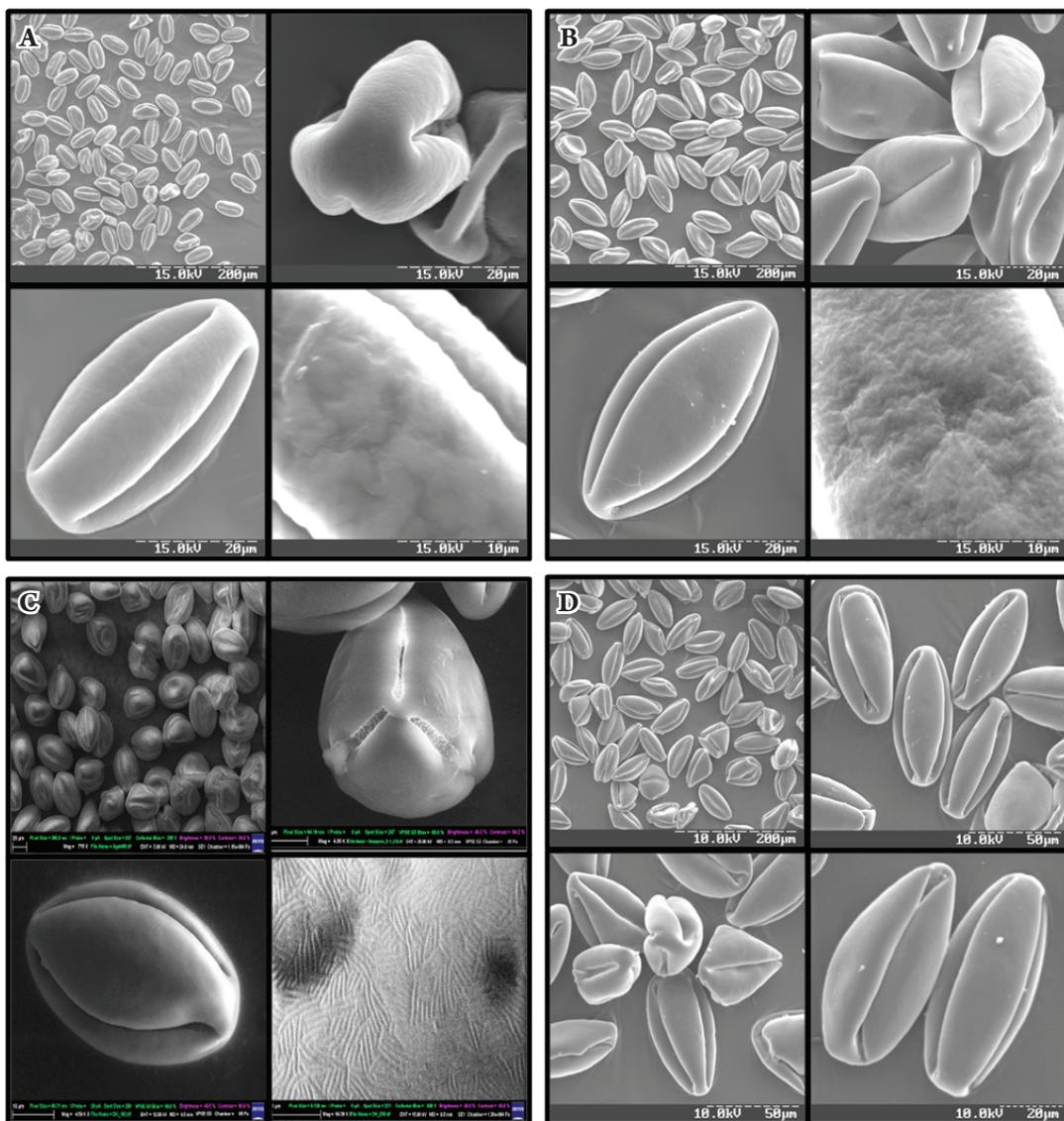


Fig. 1. Pollen grains of *Diospyros* species in different position: **A** – *D. lotus*; **B** – *D. virginiana*; **C** – *D. kaki*; **D** – *D. virginiana* × *D. kaki*.

studied species, despite statistically significant intraspecific variability, illustrated in Tab. 1 ($P = 48.00-51.39 \mu\text{m}$ and $E = 23.56-27.78 \mu\text{m}$).

Results of morphological analysis of pollen in selected genotypes of *D. lotus* from Arboretum Mlyňany (Tab. 2) confirmed a higher intraspecific variability of pollen grains size as evidenced by the values of the coefficients of variation for polar (9.76-21.64%) and equatorial (7.09-9.29%) axes. It was found,

that pollen grains size varies considerably in one individual plant even (OSTROLUCKÁ & KRÍŽO 1989). 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 1.75 in *D. kaki* to 2.18 – in *D. virginiana* (Tab. 2).

According to the values of shape index the pollen grains can be classified as prolate or prolate-spheroidal. The shape indexes

Table 1. The measured pollen morphological traits of *Diospyros* species: **min** – minimum value; **max** – maximum value; **mean** – arithmetic mean; **SI** – shape index (P/E); **SE_{mean}** – standard error; **CV** – coefficient of variation (%).

Species	min	max	mean	SE _{mean}	CV%	SI	Homogeneous groups
P – polar axis (µm)							
<i>D. kaki</i>	43.97	54.06	48.73	0.91	6.50	–	c
<i>D. lotus</i>	42.50	50.50	48.00	0.50	3.63	–	c
<i>D. virginiana</i>	49.28	55.41	51.39	0.59	4.00	–	bc
<i>D. virginiana</i> × <i>D. kaki</i>	63.17	80.54	71.19	0.48	6.30	–	a
E – equatorial axis (µm)							
<i>D. kaki</i>	24.58	32.47	27.78	0.57	7.20	1.75	b
<i>D. lotus</i>	23.97	31.17	26.06	0.56	7.56	1.84	bc
<i>D. virginiana</i>	21.40	25.94	23.56	0.41	6.15	2.18	d
<i>D. virginiana</i> × <i>D. kaki</i>	30.20	36.51	33.14	0.50	5.00	2.14	a

ranging from 1.77 to 1.83 in the genotypes of *D. lotus* confirmed certain degree of identity substantiating thus the classification to shape class of prolonged pollen grains, i.e. with the vertical axis longer than equatorial one. In comparison with the data of GEERAERTS *et al.* (2009) our results concerning the shape indexes of tested species are generally higher. On the other hand, the data shown in Table 1 and the values of shape indexes (1.28-1.33) presented in Tab. 3 verify that the pollen grains of *Diospyros* representatives are prolonged sporofomes with exception of *D. lotus* (ERDTMAN 1966; GEERAERTS *et al.* 2009). Interesting values of shape index (1.79-1.82) for *D. kaki* are reported by EVRENOSOGLU & MISIRLI (2009). However, it is consistent with our result for this species (1.75) given in Table 1.

Morphological traits variability could be influenced by the environmental factors, such as temperature, humidity and mineral nutrition. In case of pollen grain size, except the above mentioned factors, the inner factors are also of importance – especially the chromosomes number. Polyploid species are characterized by larger pollen grains with increased aperture numbers. Pollen size could be influenced even by different methodical procedures and approaches to pollen samples processing e.g. the pollen elaboration by acetolysis, using various agents like acetocarmine, KOH or processed without any chemicals, applying the dry pollen

method (OSTROLUCKÁ & KRÍŽO 1989). These factors can significantly influences on the pollen size. For example SOWUNMI (1995) reported for *D. mespiliiformis* Hochst. ex A.D.C. the mean value for polar axis length (P) of 42.4 µm (in range 39.0-46.0 µm) and the equatorial axis (E) of 31.2 µm (27.0-33.0 µm). Whereas GEERAERTS *et al.* (2009) for the same species found the mean values for the length P – 32.3 µm and E – 24.8 µm. There were found also significant different values of the mentioned traits in *D. lotus* and *D. virginiana* presented in our study as well as in the study published by GEERAERTS *et al.* (2009). The lengths of polar and equatorial axes were larger in pollen samples collected on localities of Slovakia. Nevertheless, in the paper of GEERAERTS *et al.* (2009) an extensive knowledge on pollen morphological diversity was accumulated for different persimmon species.

KODELA (2006) reports the evaluations for *D. australis* (R. Br.) Hiern., where the mean length of polar axis reached 36.9 µm and the equatorial one – 29.7 µm. Our results suggest a possible polymorphism of pollen obtained from individual genotypes of a given species grown in different ecological and geographical conditions, respectively. Colour of pollen grains is taken as a relevant trait from taxonomic point of view. The tested samples of persimmon pollen were of different yellow shades. The exine sculpture is also an important morphological trait, but its

Table 2. The measured pollen morphological traits of selected genotypes of *Diospyros lotus* from Arboretum Mlyňany. Abbreviations see in Tab 1.

Genotype	min	max	mean	CV%	SI	Homogeneous groups
P – polar axis (µm)						
DL – AM 1	10.07	63.44	44.37	21.64	–	a
DL – AM 2	26.92	54.09	46.93	9.76	–	ab
DL – AM 5	22.93	54.22	44.03	11.20	–	a
E – equatorial axis (µm)						
DL – AM 1	6.06	34.63	25.01	19.29	1.77	a
DL – AM 2	16.46	34.00	25.57	9.34	1.83	ab
DL – AM 5	12.17	30.70	24.44	7.09	1.80	b

Table 3. Literature data on pollen morphological traits in some *Diospyros* species: **P** – polar axis; **E** – equatorial axis. Other abbreviations see in Tab 1.

Plant species	axis	min	max	mean	SI	References
D. kaki L.f. – G1	P	51.6	53.7	52.6	1.79	Evrenosoglu & Misirli (2009)
	E	29.0	29.5	29.4		
D. kaki L.f. – G2	P	50.8	54.2	52.6	1.82	Evrenosoglu & Misirli (2009)
	E	28.4	29.6	28.9		
D. abyssinica (Hiern) F. White	P	–	–	22.9	1.18	Geeraerts et al. (2009)
	E	–	–	11.4		
D. kirkii Hiern	P	–	–	31.6	1.05	Geeraerts et al. (2009)
	E	–	–	30.2		
D. lotus L.	P	–	–	50.6	0.98	Geeraerts et al. (2009)
	E	–	–	51.5		
D. squarrosa Klotzsch	P	–	–	37.4	1.48	Geeraerts et al. (2009)
	E	–	–	25.3		
D. virginiana L.	P	–	–	41.4	1.33	Geeraerts et al. (2009)
	E	–	–	31.2		
	P	–	–	59.0	1.28	Erdtman 1966
	E	–	–	46.0		

structural elements could be observed in detail only by SEM. Our SEM studies have shown that the sexine of tested species (Fig. 1) is not smooth but sculptured. There is domination of finely striation (striate), scarring (scabrate) or wrinkles, more markedly in *D. virginiana*, also with occurrence of perforations. Also significantly was distinguished *D. kaki*, its sexina is irregularly baculiform. Similar characteristics of sculpture elements for some species of

Diospyros are described by GEERAERTS *et al.* (2009) and KODELA (2006).

Conclusions

Experimental studies were oriented on morphological characterization of pollen grains (sporoforms) of *D. kaki*, *D. virginiana*, *D. lotus* and interspecies hybrid *D. virginiana* × *D. kaki* with special attention paid to the size and

shape of pollen grains. Statistically significant differences in the length of polar and equatorial axes identifying their size and shape were detected between investigated taxa. According to values of shape index, the pollen grains can be included into the shape class verified as prolate or prolate-spheroidal. Pollen grains of tested species are radially symmetric and isopolar. Taking into account the apertures' shape and their number they were classified as zonicolporate.

Our observations showed that not only pollen size, but also the type and the number of apertures together with microstructural characteristic of exine sculpture are important morphological traits, which are characteristic for each species. Certain polymorphism of pollen morphological traits in *Diospyros* species was detected. Analyses of tested species oriented to pollen grains morphology are of importance from various viewpoints – evolution and taxonomy of the genus provides new information and improves the knowledge about interesting plants, which despite of their exotic origin become a fully deserved concern of the Slovakia growers' community. This is closely connected to the fact, that persimmon has an extending economical importance leading to an increased exploitation in practice especially as fruit species suitable for the branch of ecological agriculture.

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