Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 73, No 10, 2020

BIOLOGY

Botany

USING SCANNING ELECTRON MICROSCOPY TO CHARACTERISE PLUM (*Prunus domestica* L.) GENOTYPES

Milena Đorđević[#], Radosav Cerović[*], Dragan Nikolić[**], Sanja Radičević, Ivana Glišić, Nebojša Milošević

(*Submitted by Academician V. Golemansky on April 9, 2020*)

Abstract

Pollen grain micromorphological characterisation of four plum (*Prunus domestica* L.) genotypes from two different breeding programmes (Fruit Research Institute, Serbia, and Hohenheim, Germany) was performed using scanning electron microscopy (SEM). The studied characteristics were pollen size and shape, colpus length, mesocolpium width as well as exine characteristics. All genotypes had tricolpate pollen grains and striate exine ornamentation, with more parallel longitudinal ridges. The most significant differences among the analysed genotypes concerned the length of the pollen grain and exine sculpturing. The genotypes 'Pozna Plava', 'Presenta' and 'Čačanska najbolja' had the largest pollen grains (> 57 µm), whereas 'Hanita' showed a smaller pollen size, below 54 µm. 'Pozna Plava' had perprolate pollen in the equatorial view (L/W ratio 2.06), while the other three genotypes had prolate shaped pollen. By analysing the pollen morphological characteristics of the plum varieties in this paper, primarily the shape, size, length of colpi and, most importantly, the sculpture of the exine, each variety showed a specificity which can be used together with other morphological characteristics for description and characterisation.

Key words: European plum, pollen grains, morphology, exine characteristics, sculpture, SEM

[#]Corresponding author.

This work was conducted with the financial support of Ministry of Education, Science and Technological Development of the Republic of Serbia, Agreement No 451-03-68/2020-14/200215.

DOI:10.7546/CRABS.2020.10.08

Introduction. Systematically, *Prunus domestica* L. (European plum) belongs to the family *Rosaceae* L., subfamily *Prunoideae*, and is the most significant fruit species in Serbia. It is mostly used for various forms of industrial and domestic processing as well as for fresh consumption [1].

To ensure high fruit set, it is important to know the quality of pollen genotypes that are planted in the orchard. Also, studying the characteristics of pollen from specific genotypes and cultivars is important for breeding programmes [2]. Differences in pollen grain size and surface ultrastructure are a useful tool for distinguishing species and cultivars of fruit trees [3]. Pollen grain at the time of maturity has three principal strata, an outer (exine) wall, an inner wall (intine) and a pollen coat consisting of components that fill the sculptured cavity of the pollen exine [4]. However, subsequent research excludes the possibility of determining varieties based on the sculpturing of the exine, and this determination can be applied only at the species level [5].

So far in several *Prunus* genotypes: plum [5], apricot [6], peach and nectarine [3], sour cherry [7], sweet cherry [8,9] micromorphological characterization has been done using SEM.

Exines contain pores and ridges and, based on their layout, the ornamentation of the exine can be meshed, granular, grooved, striated, spined or smooth [10]. The exine also varies in the number, distribution and architecture of the apertures that interrupt it, since apertures are diverse across taxa, families, species and even within a single plant [11].

The intention of the present work is to describe the pollen morphology of four plum genotypes, from two different breeding programmes (Fruit Research Institute, Serbia and Hohenheim, Germany), growing in the western region of Serbia. The major plant breeding programme in the Fruit Research Institute, Čačak, was established to breed new cultivars of European plum, using autochthonous and introduced genotypes as the parental combinations within the planned hybridisation. This programme produced 'Čačanska Najbolja' and 'Čačanska Lepotica', which were recognised in 1975. Both genotypes derived from the crossing combinations 'Wangenheims Frühzwetsche' × 'Požegača'. At the University of Hohenheim, 'Hanita' was the first plum cultivar, introduced in 1991, from the crossing of 'President' × 'Auerbacher'. This cultivar showed sharka symptoms on few leaves only, while fruit were not affected. 'Presenta' was introduced as a late-ripening variety, derived from the crossing combinations 'Ortenauer' × 'President' [12].

We applied SEM to develop an identification tool for germplasm characterisation and preservation as well as for breeding.

Material and methods. To study the pollen grains of four plum genotypes ('Čačanska Najbolja', 'Pozna Plava', 'Hanita' and 'Presenta') growing in the experimental plum orchard near Čačak, Serbia, fresh pollen material from living plants was used. The flower buds of all analysed genotypes were taken at the later balloon stage. Sepals and petals were removed, and anthers were col-

lected into petri dishes and kept at room temperature until anther dehiscence. Dry pollen grains were placed into glass bottles with silica gel and stored in a refrigerator at +4 °C until required for SEM evaluation.

For the SEM study, pollen grains were mounted using the brush directly on metallic stubs, using double-sided adhesive tape, and coated with gold in a sputtering chamber (BAL-TEC SCD 005 Sputter Coater). Observation of the prepared samples was carried out with a scanning electron microscope (SEM) JEOL JSM-7100F (Tokyo, Japan) at a magnification of 2000× for the whole pollen grain and at 15 000× for the section of the exine pattern. For each genotype, 30 pollen grains were observed to determine the pollen size, and the following morphological characteristics were analysed: polarity, symmetry, aperture (number, length and breadth width, mesocopium width), pollen grain shape (polar and equatorial), pollen size (polar diameter – length, equatorial diameter – width, length/width ratio) and characteristics of exine (ornamentation, number of muri at 10×10 µm, width of the muri and grooves). We followed the terminology of ERDTMAN [13].

Data were statistically analysed using analysis of variance (ANOVA). The means were separated using Fisher's LSD test for $P \leq 0.05$.

Results. Pollen grains of all analysed genotypes were symmetric, isopolar and trizonocolpate (with three germinal furrows or colpi). Colpi were positioned at the meridian, along the pollen grain, and symmetrically arranged at a distance of 120° in the equatorial view (Fig. 1). The mean of the equatorial diameter ranged from 28.22 µm in 'Hanita' up to 29.84 µm in 'Čačanska Najbolja' (Table 1). The shape of pollen grains was mainly prolate (P/E = 1.92–1.98), except for the 'Pozna Plava' genotype, which had perprolate pollen grains (P/E = 2.06). In the polar

Table 1

Chief pollen grain characteristics of the analyzed plum genotypes

Cultivars	P	E	P/E
'Pozna Plava'	60.14 ± 0.50a*	29.11 ± 0.47ab	2.06 ± 0.038a
'Čačanska Najbolja'	57.66 ± 0.38b	29.84 ± 0.38a	1.92 ± 0.020b
'Hanita'	53.99 ± 0.27c	28.22 ± 0.30b	1.92 ± 0.021b
'Presenta'	58.42 ± 0.49b	29.59 ± 0.29a	1.98 ± 0.019ab
Cultivars	LC	WC	WM
'Pozna Plava'	50.47 ± 0.49a	2.53 ± 0.16ns	18.98 ± 0.30a
'Čačanska Najbolja'	48.54 ± 0.50b	2.42 ± 0.12ns	16.82 ± 0.75bc
'Hanita'	44.93 ± 0.38d	2.18 ± 0.14ns	15.84 ± 0.65c
'Presenta'	49.77 ± 0.53c	2.25 ± 0.19ns	17.79 ± 0.52ab

*The values are statistically significant at $P \leq 0.05$ by LSD test; ns – non significant. Mean values followed by the different lower-case letters in column represent significant differences; P – length of polar axis; E – length of equatorial diameter; P/E – ratio of polar axis and equatorial diameter; LC – length of colpi; WC – width of colpi; WM – width of mesocolpi. All measurements in µm

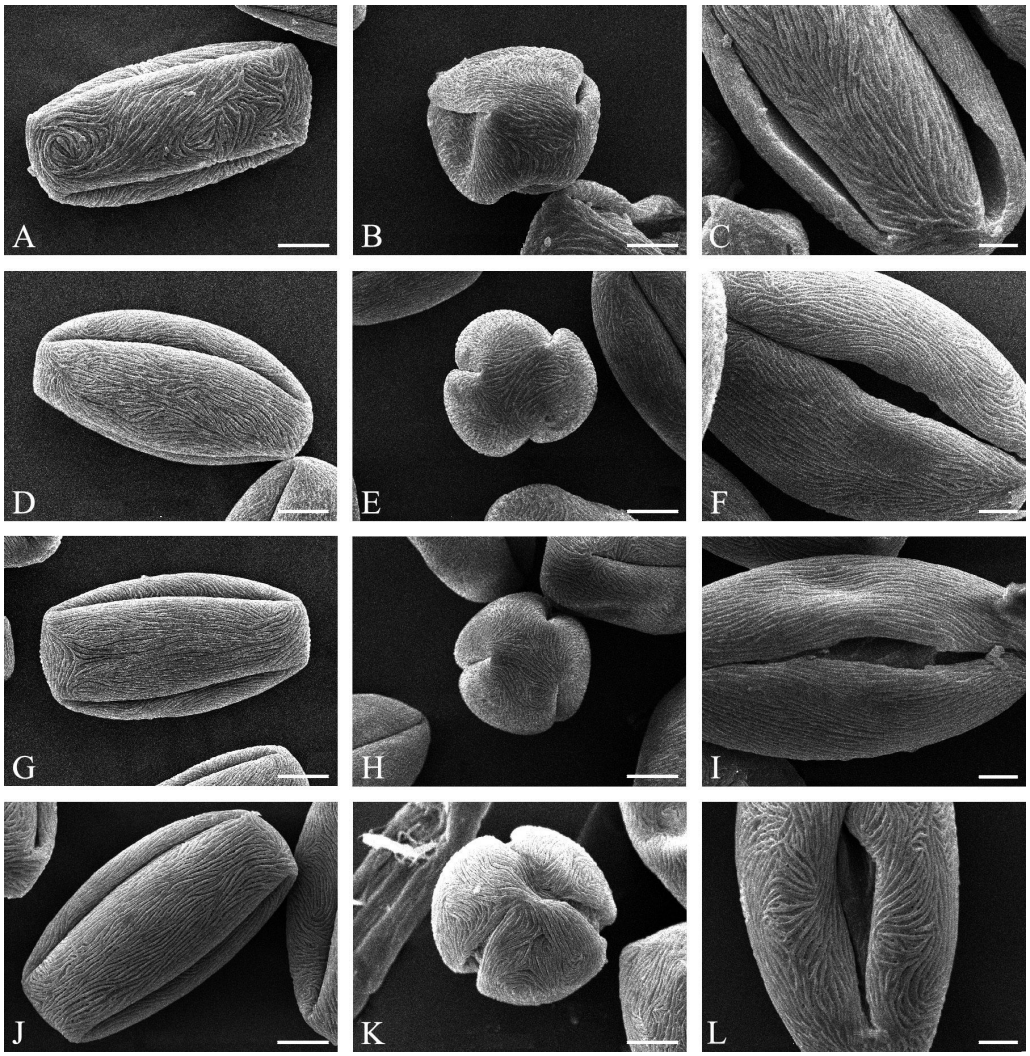

Fig. 1. Pollen shape and look of colpi analyzed genotypes: A–C. 'Pozna Plava'; D–F. 'Čačanska Najbolja'; G–I. 'Hanita'; J–L. 'Presenta'. Scale bars – 10 µm (A, B, D, E, G, H, J, K), 5 µm (C, F, I, L)

view, pollen shape was triangular-obtuse-convex, while in the equatorial view, it was elliptic-accuminate-acute. All pollen grains were tricolpate.

The highest length (µm) and width (µm) of colpi, as well as the highest mesocolpium width (µm), were determined in 'Pozna Plava' genotypes, while the lowest values were obtained for the 'Hanita' genotype (50.47, 2.53, 18.98 and 44.93, 2.42, 16.82, respectively).

Regarding exine sculpture, the tectum surface ornamentations in all analysed genotypes were with muri and separated with grooves (striate) (Fig. 2).

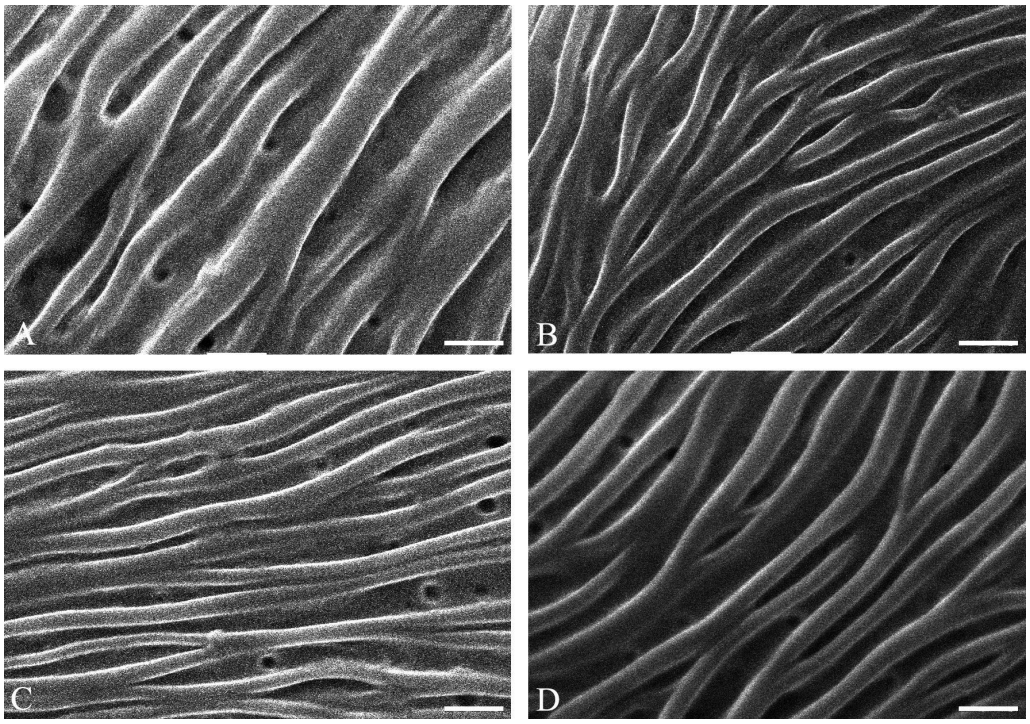

Fig. 2. Egine patterns of analysed genotypes: A. 'Pozna Plava'; B. 'Čačanska Najbolja'; C. 'Hanita'; D. 'Presenta'. Scale bars – 1 µm

Muri width ranged between 0.39 ('Hanita') and 0.63 µm ('Pozna Plava') (Table 2). The muri in all analysed genotypes covered the surface of the pollen grain and were parallel to the colpi, separated by shorter (0.34 µm in 'Hanita'and 'Presenta') or larger grooves ('Pozna Plava' and 'Čačanska Najbolja', 0.55 and 0.44 µm, respectively). Near the colpi and at the end, muri were sometimes curved and branched; this exine formation was most pronounced in 'Pozna Plava'

T a b l e 2

Tectum surface ornamentations values in analyzed genotypes

Cultivars	WM	WG	Number of muri in 100 µm^2
'Pozna Plava'	0.63 ± 0.014a	0.55 ± 0.015a	10.33 ± 0.38c
'Čačanska Najbolja'	0.49 ± 0.011b	0.44 ± 0.008b	14.33 ± 0.44b
'Hanita'	0.39 ± 0.007d	0.34 ± 0.009c	16.66 ± 0.09a
'Presenta'	0.43 ± 0.01c	0.34 ± 0.01c	16.33 ± 0.17a

*The values are statistically significant at $P \leq 0.05$ by LSD test; ns – non significant. Mean values followed by the different lower-case letters in column represent significant differences; WM – width of muri; WG – width of grooves. All measurements in µm

and 'Presenta' genotypes. The number of muri at an area of 100 µm^2 ranged from 10.33 ('Pozna Plava') to 16.66 ('Hanita'). In all genotypes, small-sized perforations were noticed.

Apart from the length of the polar axis, all other analysed pollen morphological characteristics showed the highest values in the 'Pozna Plava' genotype, while in 'Hanita', except from the number of muri in 100 µm^2, the other analysed characteristics showed the lowest values.

Discussion. Pollen grain size was less than 0.1 mm and varied significantly among plant species. The pollen of entomophilic plants, in addition to being larger than the pollen of anemophilic plants, is characterised by the exine being differently sculptured and with varying ornamentation [14]. So far, results have shown that the quality, morphology and architecture of pollen grains, along with the chemical composition, are strongly influenced by high temperatures [15].

In this paper, pollen length ranged from 53.99 to 60.14 µm, while pollen width ranged from 28.22 to 29.84 µm. Our data agreed with those reported by [16] and [5]. Based on the obtained values, all pollen grains can be classified as large grains. Based on the obtained values of the L/W ratio, pollen grains of the genotypes 'Čačanska Najbolja', 'Hanita' and 'Presenta' are prolate (L/W $\leq$ 2), while the pollen grain of the genotype 'Pozna Plava' is perpolate (2.06). Pollen grain length in species of the family *Rosaceae* varies from 32.10 to 59.45 µm, with a width from 22.2 to 35.20 µm, depending on the species. According to measurements on *Prunus domestica*, pollen grains have a flattened spherical shape [17], although most authors describe it as a prolate shape [3, 16]. In other *Prunus* species, according to L/W ratio, prolate shape of pollen was determined in apricot [18], peach [10], while in sweet cherry it was described as prolate [9] and as perprolate [8].

Analysis of colpi showed that they extended the length of the grain, ranging from 44.93 ('Hanita') to 50.47 ('Pozna Plava'). This result is in accordance with the previous finding that colpus length spans 80–90% of the distance between poles [19].

Analysing pollen morphology in a number of individuals of the genus *Prunus*, different authors outline the significance of examining exine sculptures, the most variable morphological characteristic of the pollen surface [16, 18]. The highest variability of the tested parameters was observed in pollen of plum genotypes in the study of the micro-morphological characteristics of pollen in 13 autochthonous varieties and three substrates within four species of *Prunus* in Sicily (apricot, cherry, plum, peach) [3]. Pollen grains of 'Pozna Plava' had the largest width of muri and ridges and therefore the smallest number of muri per 100 µm^2 of equatorial area, while the 'Hanita' variety had the smallest width of muri and grooves and the highest number of muri per 100 µm^2 of equatorial area. In the genotype 'Čačanska Najbolja', width of the muri and grooves was approximately the same, while in the genotype 'Presenta', the width of the muri was slightly larger than the width of the furrow. The longest and most parallel ridges, extending almost

to the poles, were noticed in the pollen grains of the genotype 'Hanita'. In the other three genotypes, the muri were short to medium long, with branching at the ends. Examining 13 autochthonous plum cultivars in the territory of Bosnia, no correlation was found between ornamentation and basic phenotypic characteristics of the examined cultivars. A positive correlation between the variability of the exine ornamentation and the fruit shape of analysed the plum varieties was observed [3]. The same authors state that the existence of high variability in the examined parameters of the analysed plum genotypes can be related to their hybrid origin.

Conclusions. Examination of the pollen grains in four plum genotypes showed differences in length, width, length of the colpi and tectum surface ornamentation. Pollen shape was mostly prolate to perprolate. In all analysed genotypes, pollen grain was tricolpate. Sculpturing provides a diagnostic tool by which taxa can be identified, usually at the generic level, but also frequently at the specific level. By analysing the pollen morphological characteristics of the plum varieties in this paper, primarily the shape, size, length of colpi and, most importantly, the sculpture of the exine, each variety showed a specificity which can be used together with other morphological characteristics for description and characterisation.

REFERENCES

[1] Glišić I., R. Cerović, N. Milošević, M. Đorđević, S. Radičević (2012) Initial and final fruit set in some plum (*Prunus domestica* L.) hybrids under different pollination types, Genetika, **44**, 583–593.

[2] Sharafi Y. (2011) *In vitro* pollen germination in stone fruit tree of *Rosaceae* family, Afr. J. Agric. Res., **6**, 6021–6026.

[3] Geraci A., P. V. Vicenza, M. P. Pasquale, S. R. Rosario (2012) Investigation on the pollen morphology of traditional cultivars of *Prunus* species in Sicily, Acta Soc. Bot. Pol., **81**, 175–184.

[4] Edlund A. F., R. Swanson, D. Preuss (2004) Pollen and stigma structure and function: the role of diversity in pollination, Plant Cell, **16**, S84–S97.

[5] Mićić N. (1988) Pollen morphology of plum, J. Yugoslav Pomology, **84/85**, 173–181.

[6] Asma B. M. (2008) Determination of pollen viability, germination ratios and morphology of eight apricot genotypes, Afr. J. Biotechnol., **7**, 4269–4273.

[7] Nyéki J., P. Sótonyi, Z. Szabó, E. Felhosné-Váczi, F. Csillag (1996) A scanning electron microscopy survey of pollen grains of sour cherry cultivars, Acta Hortic., **410**, 133–135.

[8] Radičević S., D. Nikolić, R. Cerović, M. Đordević (2013) *In vitro* pollen germination and pollen grain morphology in some sweet cherry (*Prunus avium* L.) cultivars, Rom. Biotech. Lett., **18**, 8341–8349.

[9] Nikolić D., D. Milatović (2016) Pollen morphology of some sweet cherry cultivars observed by scanning electron microscopy, Acta Hortic., **1139**, 369–374.
[10] Evrenosoplu Y., A. Misirli (2009) Investigations on the pollen morphology of some fruit species, Turk. J. Agric. For., **33**, 181–190.
[11] Mignot A., H. Coss, I. Dajoz, C. Leuret, J. P. Henry et al. (1994) Pollen aperture polymorphism in the Angiosperms: importance, possible causes and consequences, Acta Bot. Gallica, **141**, 109–122.
[12] Hartmann W. (1993) Plum breeding at Hohenheim, Acta Hortic., **359**, 55–62.
[13] Erdtman G. (1952) Pollen Morphology and Plant Taxonomy—Angiosperms, Stockholm, Almqvist and Wiksell, 539 pp.
[14] Iwanami Y., T. Sasakuma, Y. Yamada (1988) Pollen: Illustrations and scanning electronmicrograps, Berlin, Springer-Verlag.
[15] Hedhly A., J. I. Hormaza, M. Herrero (2008) Global warming and sexual plant reproduction, Trends Plant Sci., **14**, 30–36.
[16] Čalić D., N. Devrnja, M. Kostić, I. Kostić (2013) Pollen morphology, viability, and germination of *Prunus domestica* cv. Požegača, Sci. Hortic., **155**, 118–122.
[17] Gilani S. A., R. A. Qureshi, A. M. Khan, D. Potter (2010) Morphological characterization of the pollens of the selected species of genus *Prunus* Linn. from Northern Pakistan, Afr. J. Biotechnol., **9**, 2872–2879.
[18] Arzani K., M. A. Nejatian, G. Karimzadeh (2005) Apricot (*Prunus armeniaca*) pollen morphological characterization through SEM, using the multivariate analysis, New Zeal. J. Crop Hort., **33**, 381–388.
[19] Hebda R. J., C. C. Chinnappa (1994) Studies on pollen morphology of *Rosaceae*, Acta Bot. Gallica, **141**, 183–193.

Department for Fruit Breeding and Pomology
Fruit Research Institute
Kralja Petra I/9
32000 Čačak, Serbia
e-mail: mdjordjevic@institut-cacak.org
sradicevic@institut-cacak.org
iglisic@institut-cacak.org
nmilosevic@institut-cacak.org

* *University of Belgrade*
Innovation Centre at Faculty of
Technology and Metallurgy
11120 Belgrade, Serbia
e-mail: radosav.cerovic@gmail.com

** *Faculty of Agriculture*
University of Belgrade
11080 Belgrade-Zemun
e-mail: nikolicd@agrif.bg.ac.rs