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Morphology and ultrastructure of pollen exine in hazelnut (*Corylus avellana* L.) cultivars grown in Bosnia and Herzegovina

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Abstract

The morphology and ultrastructure of the pollen exine were studied using scanning electron microscopy (SEM) in 14 hazelnut genotypes (*Corylus avellana* L.). Pollen measurements were performed in a dry stage at magnifications ranging from 500 × to 15,000 ×, during which the following parameters were analyzed: pollen grain height and width (µm), estimated pollen grain volume (µm³), type and ultrastructure of the exine, and the frequency of microexcrescences per 10 µm². Pollen samples were collected for analysis at two locations in the Banja Luka region (Bosnia and Herzegovina), and the following cultivars were analyzed: Multiflorum, Tankoljuskasti, Tonda Gentile Romana, Merveille de Bollwiller, Avellino, Ludolph Zellernuss, Apolda, Hall's Giant, Romai I, Romai II, Istarski okrugli, Imperial de Trebizonde, Gustav Zellernuss, Jean's. The research results have indicated that the pollen grains were of the triporate type, subspheroidal in shape, with dimensions ranging from 19.91 µm in Imperial de Trebizonde to 32.29 µm in Tonda Gentile Romana. The lowest pollen volume was determined for the Tankoljuskasti cultivar (5417.40 µm3), and the highest for the Tonda Gentile Romana cultivar (12645.70 µm3). Measurements of exine ultrastructure have indicated the presence of certain differences among the observed cultivars. The available literature states that the pollen exine in cultivated hazelnut cultivars is uniform and characterized by a verrucate-type exine. In this study, differences were identified at the level of microexcrescences in the Gustav Zellernuss and Tonda Gentile Romana cultivars, in

which clustering of microexcrescences positioned on shallow reticula was observed. The question of genotypic specificities of hazelnut exine ultrastructure remains open in this study, and future research should focus on the characteristics of interspecies hybrids resulting from the crossing of different species within the genus *Corylus* (*Corylus avellana* L., *Corylus pontica* Koch., and *Corylus maxima* Mill.).

Key words: Palynology, Microexcrescences.

Introduction

Hazelnut (*Corylus avellana* L.) is a monoecious, anemophilous fruit species with a dichogamous flowering type that occurs during the autumn-winter period. The growth and development of male inflorescences and the process of microsporogenesis occur from May to September (Tiyayon and Azarenko, 2005; Mićić et al., 2022), and hazelnut is the only fruit species in the continental region that enters dormancy with mature pollen ready for release from the anther and fertilization (Mićić et al., 2022). Similar to other anemophilous fruit species, hazelnuts have a high pollen production. In a study conducted in Poland, Piotrowska (2008) reported that one catkin of a common hazel (*Corylus avellana* L.) contains an average of 66 mg of pollen, with this parameter depending on cultivar-specific characteristics and the influence of agro-ecological conditions. Hazelnut pollen belongs to the triporate type of pollen grains with circular pores (Mićić et al., 1988), while the shape of the pollen grain in the dry state, during wind dispersal, is subsphaerocytic with characteristic depressions around the germination apertures, which are situated in the equatorial plane and positioned 120º from one another (Mićić et al., 1988). The same author states that with the hydration of the pollen grain, it swells, taking the form of a polar-flattened rotational ellipsoid with depressions around its aperture. The pollen dimensions of *Corylus avellana* L. can range from 14 to 40 μm, depending on the cultivar (Mićić et al., 1988; Nikolaieva et al., 2014; Yuan et al., 2024). In other species of the *Corylus* genus, the dimensions have different values, namely, in *Corylus colurna* L. from 25 to 30 μm (Halbritter et al., 2020), in *Corylus Jacquemontii* Decne. from 31 to 32 μm (Chandra et al., 2025), and in *Corylus americana* Marsh. from 17 to 22 μm (Zhi-Duan, 1991).

The pollen grain wall consists of two membranes, the inner intine and the outer exine, which is composed of nexine and sexine. Microexcrescences, sculptural elements with various characteristics are located on the surface of pollen and play a role in the reception of pollen on the stigma (Mićić et al., 2022; Mićić et al., 2024). The exine microexcrescences in the hazelnut belong to the verrucate type. In addition to the standard morphological parameters used for identifying plant species, such as the stem, leaf, flower, and fruit, the

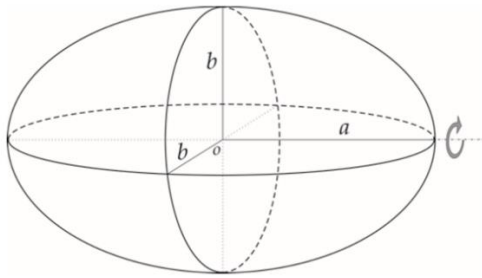
morphological characteristics of pollen are also used in the fields of palynology and paleobotany. This primarily refers to the dimensions and ultrastructural characteristics of the exine, which represent an element of plant species taxonomy. Previous research using the SEM confirmed that it is possible to identify the genus and even species based on the surface of the exine, the type and shape of the aperture, and the dimensions of the pollen (Hebda et al., 1988). Mićić et al. (1988) studied the morphological characteristics of the pollen grain and the ornamentation of the hazelnut exine, and concluded that there is a significant difference between the studied assortment, but this difference could not be used for determining the genotype. The possibility of identifying certain pollen based on morphological characteristics, such as dimensions, size, and microexcrescences, has been reported in fruit trees such as walnut (Milatović et al., 2020), almond (Vafadar et al., 2010), pistachio (Afshari et al., 2008), and other fruit species.

This study aimed to examine the morphological characteristics of pollen and the cultivar specificities of exine ornamentation in hazelnut cultivars present in the territory of B&H, which have not been previously researched under these agro-ecological conditions.

Material and Methods

The study of the morphology and ultrastructure of pollen exine was conducted by collecting pollen from 14 hazel cultivars from two locations in the Banja Luka region, northwestern Bosnia and Herzegovina (B&H). At the first location (Economy of the Agricultural Institute of the Republic of Srpska, municipality of Banja Luka, 44º48'25“N 17º12'95”E, 160 m u.s.l.), the following cultivars were collected: Multiflorum, Tankoljuskasti, Tonda Gentile Romana (TGR), and Merveille de Bollwiller. At the second location (Jaružani, municipality of Laktaši, 44º48'20" N 17º20'40"E, 265 m u.s.l.), the following cultivars were collected: Avellino, Apolda, Ludolph Zellernuss, Istarski okrugli, Halls Giant, Imperial de Trebizonde, Jean's, Gustav Zellernuss, Romai I, and Romai II (the cultivar could not be determined, but was designated as such by the collector). Pollen samples for measurements were collected in 2012 with successive collections from January to March during the flowering phenophase. After collecting the pollen, it was dehydrated in laboratory conditions and stored in hermetically sealed containers at room temperature until the time of analysis. Scanning of pollen in a dry state was performed using an SEM (JEOL-6390LV, Tokyo, Japan), and sample preparation and gold layer application on a BAL-TEC (SCD 005, Pfaffikon, Switzerland) in the Laboratory for Microscopy of the Faculty of Agriculture, University of Belgrade. Measurements were made on three samples with recording of 10 positions (individual pollen grains).

Photographs of pollen were made at different magnifications: 500× (pollen grains in groups near anthers), 2000× (shape of pollen grain), 3000× (pores on pollen grains), and 10,000 and 15,000× (exine ultrastructure and microprojections). The recorded material was processed, and a morphological characterization of the pollen grains was performed, which included measuring the following morphological characteristics: pollen height and width (μm), volume (μm3), microexcrescences representation per 10 μm2, and their form. Measurements of height and width were performed in Corel Draw X5, while the formula for the volume of an ellipsoid was used to calculate the volume of the pollen grain (Figure 1):



$$V = \frac{4 \times a \div b^2 \pi}{3}$$

Fig. 1 - The flattened ellipsoid shape and formula were used for calculating pollen dimensions

Results and Discussion

Table 1 shows the average values for pollen grain dimensions in the dry state recorded by the SEM. The smallest pollen grain height was observed in Romai II (24.84 μm), and the largest was observed in TGR (32.29 μm). The smallest width of pollen grains was recorded in the Imperial de Trebizonde cultivar (19.26 μm), and the largest in the TGR cultivar (27.37 μm). The pollen volume ranged from 5417.40 μm3 in the Tankoljuskasti cultivar to 12645.70 μm3 in the TGR cultivar.

Tab. 1 - Morphological characteristics of the pollen grain in the observed cultivars

Cultivar	Height of pollen grain (µm)	Width of pollen grain (µm)	Volume of pollen grain (μm^3)
	Mean ± SE	Mean ± SE	
Multiflorum	30.02 ± 0.30	25.27 ± 0.60	10029.40
Tankoljuskasti	26.15 ± 1.00	19.91 ± 0.68	5417.40
Tonda Gentile Romana	32.29 ± 0.46	27.37 ± 0.92	12645.70
Merveille de Bollwiller	30.10 ± 0.40	24.25 ± 1.03	9255.70
Avellino	29.61 ± 0.81	25.71 ± 0.93	10231.40
Apolda	26.99 ± 0.54	22.92 ± 0.81	7417.40
Ludolph Zellernuss	27.59 ± 0.61	22.45 ± 1.28	7268.10
Istarski okrugli	28.51 ± 0.74	24.02 ± 1.21	8605.40
Halls Giant	30.06 ± 0.98	26.74 ± 1.15	11248.30
Imperial de Trebizonde	28.04 ± 0.74	19.26 ± 1.11	5446.14
Jean's	30.21 ± 0.66	26.45 ± 0.76	11048.60
Gustav Zellernuss	27.97 ± 0.49	24.32 ± 0.81	8654.50
Romai I	25.77 ± 0.66	23.48 ± 0.79	7432.25
Romai II	24.84 ± 1.00	21.77 ± 1.20	6155.27

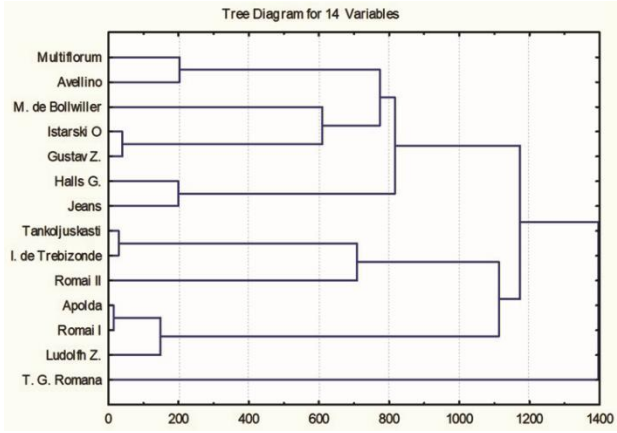


Fig. 2 - Cluster analysis of pollen grain size (volume) of the tested genotypes

Cluster analysis (Figure 2) showed the grouping of cultivars according to the average dimensions and volume of the pollen grains. All cultivars were grouped into five groups based on the genotypic specificities of pollen size:

- large pollen → TGR;

- medium-large pollen→Halls Giant, Jean's, Avellino, Multiflorum, Merveille de Bollwiller;
- medium-sized pollen → Gustav Zellernuss and Istarski okrugli;
- medium fine pollen →Apolda, Ludolph Zellernuss, Romai I;
- fine pollen → Tankoljuskasti, Imperial de Trebizonde, Romai II;

Table 2 shows the values related to the type of ultrastructure of the exine and the appearance of the average number of microexcrescences on the surface of 10 μm2 of the exine.

Tab. 2 - Type and ultrastructure of exine with the average number of microprojections in the observed cultivars.

Cultivar	Type and ultrastructure of exine	microexcrescences on 10 μm^2 of exine (M ± SE)
Multiflorum	microexcrescences in the form of slightly expressed folds	15.2 ± 0.79
Tankoljuskasti	unevenly short furrows with smaller outgrowths	14.4 ± 0.80
Tonda Gentile Romana	3 – 7 micorexcrescences connected to shallow folds	24.2 ± 0.59
Merveille de Bollwiller	microexcrescences in the form of tiny warts	30.6 ± 0.20
Avellino	microexcrescences in the form of pronounced warts	31.0 ± 2.19
Apolda	microexcrescences in the form of tiny warts	22.8 ± 1.84
Ludolph Zellernuss	microexcrescences in the form of short folds with warts	25.0 ± 3.39
Istarski okrugli	microexcrescences in the form of pronounced short folds	19.1 ± 4.20
Halls Giant	microexcrescences in the form of tiny warts	20.1 ± 0.39
Imperial de Trebizonde	microexcrescences in the form of short folds with warts	20.4 ± 1.99
Jean's	short furrows with smaller micoexcrescences	14.2 ± 1.00
Gustav Zellernuss	2 – 3 microexcrescences connected to shallow folds	24.0 ± 2.79
Romai I	microexcrescences in the form of short folds with warts	16.2 ± 0.99
Romai II	short furrows with smaller microexcrescences	17.3 ± 1.05

All cultivars were divided into three groups based on the form of microprojections (Figures 3-16) :

- cultivars with microexcrescences in the form of small or pronounced verrucate (Apolda, Avellino, Merveille de Bollwiller, Halls Giant);
- cultivars with more or less pronounced short wrinkles with microexcrescences (Gustav Zellernuss, Istarski Okrugli, Ludolph Zellernuss, Multiflorum, Romai I, TGR, Jean's);
- cultivars with short furrows and smaller microexcrescences (Romai II, Tankoljuskasti, Imperial de Trebizonde).

The average number of microexcrescences per 10 μm^2 ranged from 14.2 for the Jean's cultivar to 31.0 for the Avellino cultivar. All cultivars were divided into three groups according to the average number of microexcrescences:

- cultivars with a lower average number of microexcrescences from 14 to 20: Jean's, Tankoljuskasti, Multiflorum, Romai I, Romai II, Istarski Okrugli;
- cultivars with an average number of microexcrescences from 20 to 25: Halls Giant, Imperial de Trebizonde, Apolda, TGR, Gustav Zellernuss, Ludolph Zellernuss;
- cultivars with a high average number of microexcrescences over 30: Merveille de Bollwiller, Avellino;

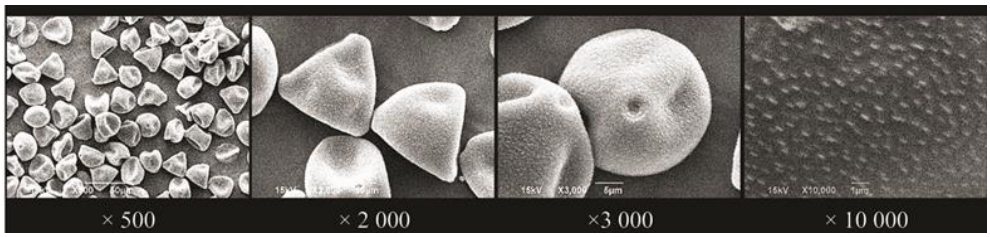


Fig. 3 - The Multiflorum cultivar

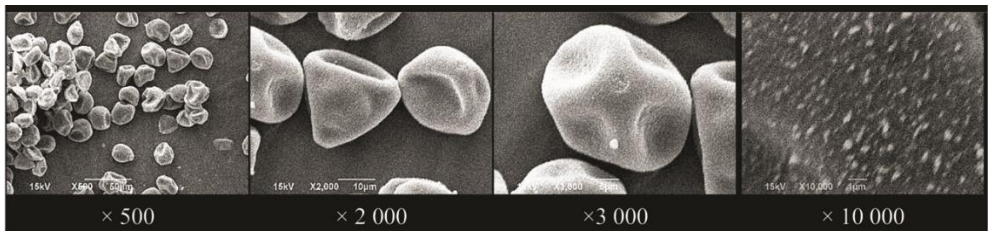


Fig. 4 - The Tankoljuskasti cultivar

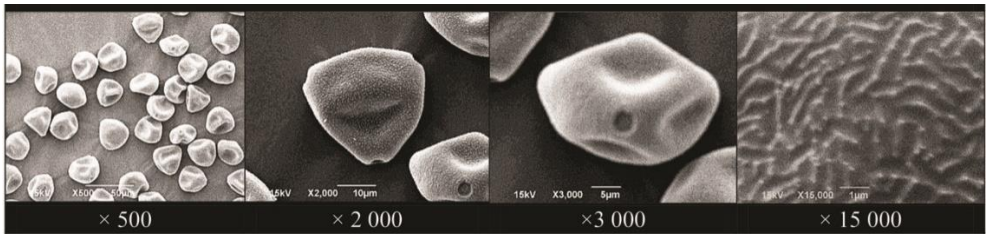


Fig. 5 - The Tonda Gentile Romana cultivar

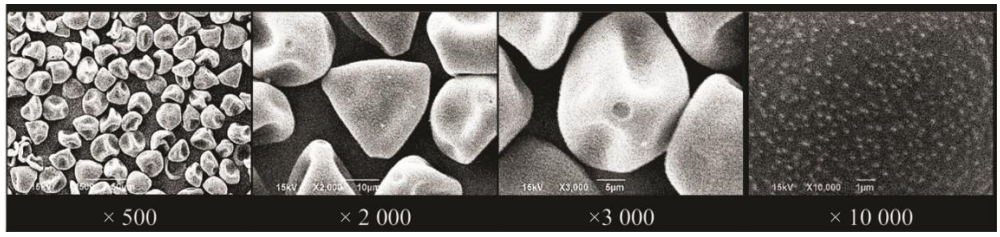


Fig. 6 - The Merveille de Bollwiller cultivar

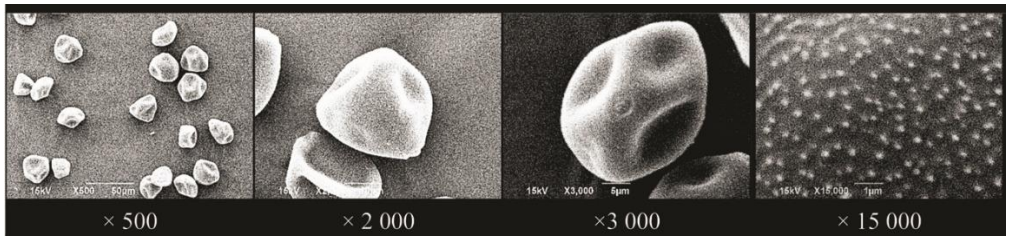


Fig. 7 - The Cultivar Avellino cultivar

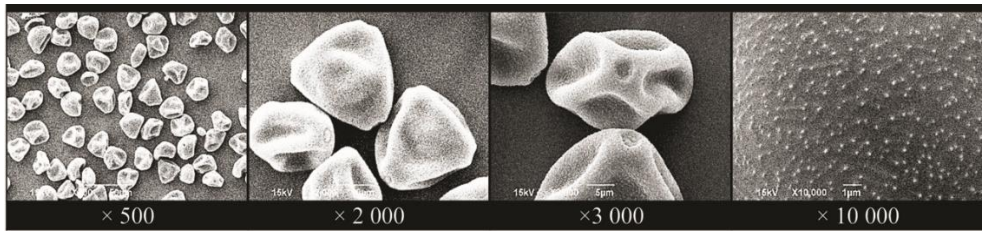


Fig. 8 - The Apolda cultivar

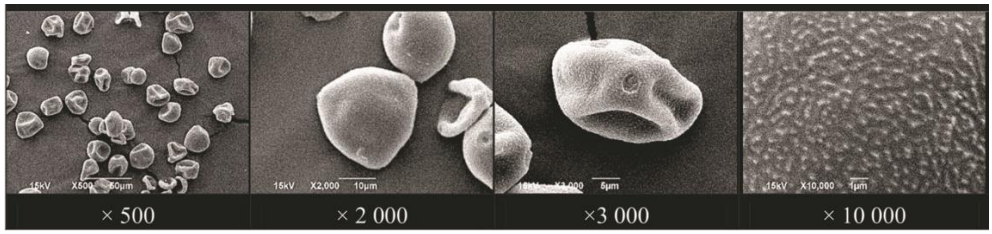


Fig. 9 - The Ludolph Zellernuss cultivar

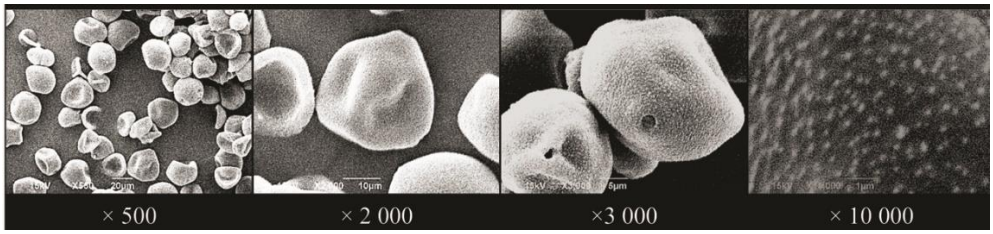


Fig. 10 - The Istarski Okrugli cultivar

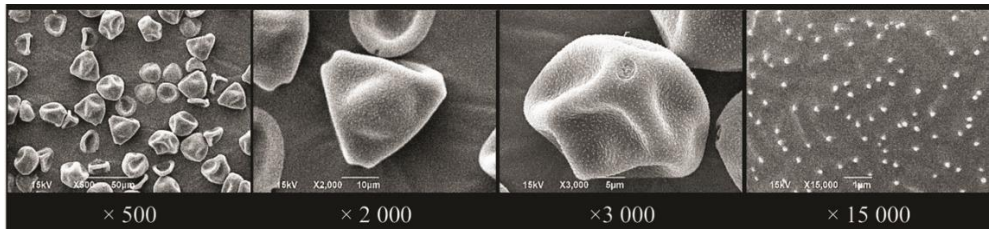


Fig. 11 - The Hall's Giant cultivar

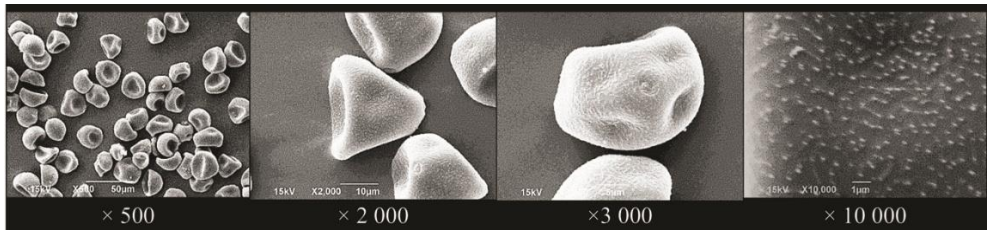


Fig. 12 - Imperial de Trebizonde cultivar

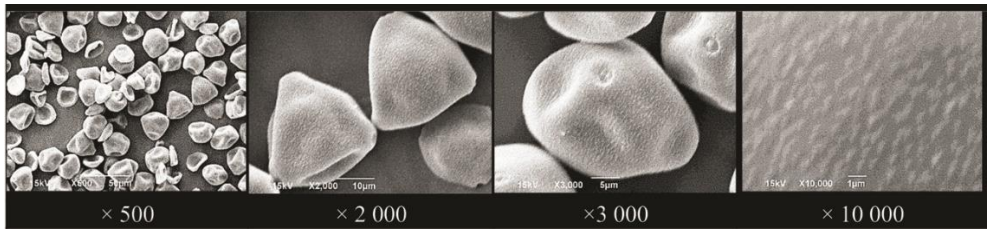


Fig. 13 - The Jean's cultivar

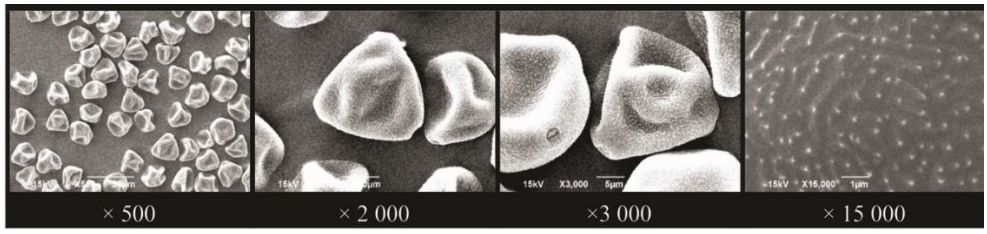


Fig. 14 - The Gustav Zellernuss cultivar

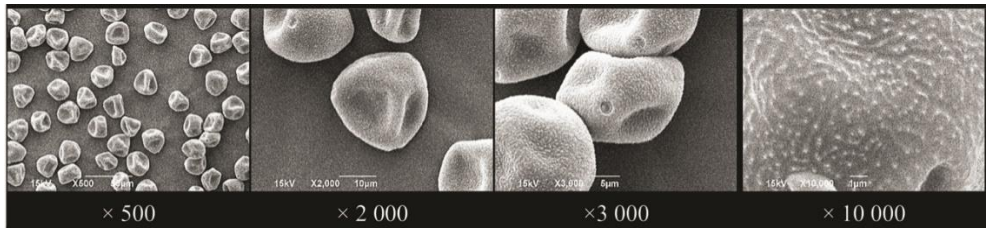


Fig. 15 - The Romai I cultivar

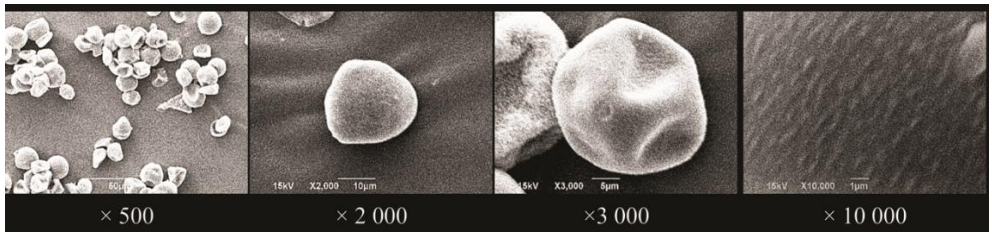


Fig. 16 - The Romai II cultivar

The pollen grains in our study were triporate and subspherical in shape, which is in accordance with previous measurements (Mićić et al., 1988;

Nikolaieva et al., 2014; Halbritter et al. 2025). The dimensions of the pollen grains (height and width) in our study in most observed cultivars were larger than those in the study by Mićić et al. (1988). Larger pollen dimensions were also recorded in the cultivars observed in both studies: Imperial de Trebizonde, Hall's Giant, and Apolda, while they were uniform for the Romai cultivar. Nikolaieva et al. (2014) reported approximately uniform pollen dimensions with our measurements, which ranged from 25 to 30 μm, and in Halbritter et al. (2025) average pollen dimension were larger. Different values were recorded for other species of the genus *Corylus*. Higher values were recorded for *Corylus jacquemontii* Decne. (Chandra et al., 2025) and Corylus colurna L. (Halbritter et al., 2020) compared to our measurements, approximately equal to *Corylus ferox* Mail. and higher values than *Corylus americana* Marsh. and *Corylus yunnanensis* Franch. (Zhi-Duan, 1991). The pollen exine of the hazelnut cultivars observed in our study was of the varucatoid type, which is in agreement with the measurements of Mićić et al. (1988) and Halbritter et al. (2025). In our research, the occurrence of microexcrescences that are positioned on shallow reticules in the Gustav Zellernuss and TGR cultivars were registered, which is quite different from other cultivars. Regarding the average number of microexcrescences per 10 μm2, our measurements showed that the average number of microexcrescences per 10 μm2 was between 14 and 30, which is significantly lower than that reported by Mićić et al. (1988), where all cultivars had an average of 25 to 41 microexcrescences per 10 μm2. The same author stated that the ornamentation of the exine was not specific enough to serve for cultivar determination, which is consistent with research on walnuts (Milatović et al., 2020).

Conclusion

The analysis of pollen morphology and exine ultrastructure in 14 hazelnut genotypes has shown certain morphological differences in terms of dimensions and density of microexcrescences on the exine surface. The TGR cultivar had the largest pollen dimensions, whereas the Imperial de Trebizonde and Romai II cultivars had the smallest pollen dimensions. The highest number of microexcrescences was recorded in the Avellino and Merveille de Bolwiller cultivars, and the lowest in the Tankoljuskasti and Jean's cultivars. In terms of the type and positioning of microexcrescences on the surface of the exine, the Gustav Zellernuss and TGR cultivars stood out, indicating certain genotypic differences that can be assumed to be related to the interspecies hybridization of the observed cultivars. In this way, the question of the genotypic specificities of the exine ultrastructure in the observed hazelnut cultivars remains open until their genotypic characterization [the question of the selection of cultivars created by

crossing species from the *Corylus* genus (*Corylus avellana* L., *Corylus pontica* Koch., *Corylus maxima* Mill.).

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Морфологија и ултраструктура егзине полена код сорти лијеске (*Corylus avellana* L.) гајених на подручју Босне и Херцеговине

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Сажетак

Истраживање морфологије и ултраструктуре егзине полена извршено је помоћу електронског скенирајућег микроскопа (СЕМ) код 14 генотипова лијеске (*Corylus avellana* L.). Мјерење полена извршено је у сувом стању при увећањима од 500 до 15000 × при чему су анализирани следећи параметри: висина и ширина поленовог зрна (µm), процјењена запремина поленовог зрна (µm^3), тип и ултраструктура егзине и заступљеност микроизраштаја на 10 (µm^2). Полен за анализу је прикупљен на две локације на подручју бањалучке регије, Босна и Херцеговина (БиХ), а анализиране су следеће сорте: Multiflorum, Танкољускасти, Tonda Gentile Romana, Merveille de Bollwiller, Avellino, Ludolph Zellernuss, Apolda, Halls Giant, Romai I, Romai II, Истарски округли, Imperial de Trebizonde, Gustav Zellernuss и Jean's. Резултати истраживања показују да су поленова зрна трипоратног типа, субсфероидног облика са димензијама од 19.91 µm код сорте Imperial de Trebisonde до 32.29 µm код сорте Tonda Gentile Romana. Најмања запремина плода утврђена је код сорте Танкољускасти (5417.40 µm^3), а највећа код сорте Tonda Gentile Romana (12645.70 µm^3). Мјерења ултраструктуре егзине указује на постојање одређених разлика које се јављају између посматраних сорти. У доступној литератури наводи се да је егзина полена код гајених сорти лијеске једнообразна и да се одликује егзином варукатоидног типа. У овом истраживању утврђене су разлике које се јављају на нивоу микроизраштаја код сорти Gustav Zellernuss и Tonda Gentile Romana код којих је уочено груписање микроизраштаја позиционираних на плитким ретикулима. Питање генотипских специфичности ултраструктуре егзине лијеске овим истраживањем остаје отворено за истраживања која треба усмјерити на питање карактеристика интерспецијес хибрида насталих укрштањем различитих врста из рода *Corylus* (*Corylus avellana* L., *Corylus pontica* Koch., *Corylus maxima* Mill.).

Кључне ријечи: палинологија, микроизраштаји

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