UDC 575.630
https://doi.org/10.2298/GENSR1701105C
Original scientific paper

HORSE CHESTNUT POLLEN QUALITY

Dušica ĆALIĆ, Ljiljana RADOJEVIĆ

Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia

Ćalić D. and Lj. Radojević (2017): *Horse chestnut pollen quality*.- Genetika, Vol 49, No. 1, 105-115.

Pollen quality of horse chestnut, expressed as pollen productivity, viability and germination was studied. Anthers of horse chestnut genotypes had pollen production from 3.66 to 5.06 x 10^3 pollen grains per anther, depending of genotype. Also, pollen of horse chestnut Ah1-Ah4 genotypes showed different viability (from 56 to 68%), after staining with fluorescein diacetate. Pollen germination of Ah1-Ah4 genotypes varied from 50-66% on basic medium. Inclusion of polyethylene glycol-PEG from 10%, 15% and 20% v/w increased pollen germination. The best results were achieved on medium with the largest PEG concentration. On these medium 76-91% pollen grains were germinated, depending of genotype. The best pollen quality, for all tested parameters, had genotype Ah2. Knowledge about morphology, production, viability, in vitro germination, tube growth as well as pollen: ovule ratio can be of great importance for future pollen biology studies.

Key words: Aesculus, pollen morphology, pollen:ovule ratio, pollen tube growth, pollen viability

INTRODUCTION

European horse chestnut (*Aesculus hyppocastanum* L.; Sapindaceae: Hippocastanaceae) is an attractive ornamental plant. Horse chestnut is a relict and endemic species of the Balkan Peninsula (STEVANOVIĆ and RADOJEVIĆ, 1993; PRADA *et al*., 2011) and important medical plant (ĆALIĆ *et al*., 2003; ĆALIĆ-DRAGOSAVAC *et al*., 2010).

Corresponding author: Dušica Ćalić, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia, Tel.: +381-11-2078-366; fax: +381-11-2761 433, E-mail address: calic@ibiss.bg.ac.rs

Seeds of horse chestnut are the main source of escine, which is used in the treatment of peripheral blood vessels. Pollen quality which includes pollen morphology, pollen production, pollen: ovule ratio, pollen viability, pollen germination and tube growth, is an important component of fertilization success in seed-producing plants.

Pollen production has been an important process in the field of plant reproductive biology. For example, the amounts of pollen produced by a plant and anther position (HARDER and BARRETT, 1993) are a critical component of male reproductive function (KEARNS and INOUYE, 1993). Counting pollen can also be an important part of plant–pollinator studies, such as quantifying the amount of pollen that is removed and carried by pollinators (THOMSON and GOODELL, 2001).

Many studies have indicated that viability, germination and tube growth of pollen grains varied significantly according to species and cultivar (HEDLY *et al*., 2004; DU *et al.*, 2006; ATLAGIĆ, *et al.*, 2009; SHARAFI, 2011; SOUZA *et al.*, 2015). Pollen performance is clearly affected by the genotype (VULETIN SELEK *et al*., 2013) which is why pollen development and morphology are often used by taxonomists and paleobotanists to clarify the classification and identity of plant species (MERT, 2009).

A variety of factors influencing pollen performance such as temperature (KOUBOURIS *et al.*, 2009; ACAR and KANANI, 2010; BECK-PAY, 2012; CEROVIĆ *et al.*, 2014), sucrose (ALCARAZ *et al.*, 2011; DEVRNJA *et al.*, 2012), polyethylene glycol-PEG (SAKHANOKHO and RAJASEKARAN, 2010; ĆALIĆ *et al.*, 2013) were tested. Furthermore, the ratios of pollen grains to number of ovules (P: O ratios) have been shown to correlate with the mating system of a plant. CRUDEN (1977), MUCHHALA *et al.* (2010) and PANG and SAUNDERS (2015) were demonstrated that in general, xenogamous species had higher P: O ratios than predominantly selfing species and conclude that xenogamous species had higher P: O ratios while autogamous species had lower P: O ratios. This pollination efficiency hypothesis is based on the argument that maximum seed set in xenogamy plants would require more pollens grains as result of inefficient pollen transfer.

Knowledge about morphology, production, viability, *in vitro* germination, tube growth as well as pollen: ovule ratio can be of the most importance for understanding not only the basic characteristics of horse chestnut pollen but also its pollination biology.

The aim of the present study was to determine and compare pollen production, pollen: ovule ratio, pollen viability, pollen germination and tube growth capacity of *A. hippocastanum* genotypes.

MATERIALS AND METHODS

Plant materials

The study was conducted on flowers from the lower branches of four genotypes of *A. hyppocastanum* (voucher N_0. Ah1; Ah2, Ah3, Ah4). The samples were collected from 125-year old trees growing in the Botanical Garden ‘‘Jevremovac’’ of the Belgrade University. For experiments, flowers at blooming phase were used.

Scanning electron microscopy

Anther and pollen of Ah1-Ah4 genotype samples were collected from fresh flowers, and processed for *scanning* microscopy (SEM) without the usual fixation and dehydration procedures. Anthers and pollen grains were placed directly on the stubs, and were covered with a thin layer of gold (ion sputtering coating) in a BALETECSCD 005 Sputtering Device, imaging at 15 kV, using

a JSM-6390 LV (JEOL, Tokyo, Japan) *scanning* electron microscope. *Scanning* electron micrograph images were taken on magnification from 45× to 13000× (Fig. 2a-e). The measurements were made in micrometer (μm).

Pollen count and pollen : ovule ratio

Average number of pollen grains per anther, flower and inflorescence was counted on 180 randomly chosen flowers and 30 inflorescences. Six flowers per inflorescence were analyzed for each genotype.

The drying and dehiscence of anthers in vial were obtained first at room temperature, then in a stove at 50°C for 6 hours. Too many pollen grains stuck to the endotheciums, so that it was necessary to free most of the grains by triturating carefully the anthers with a glass rod. Two ml of 0.002 % aqueous solution of a detergent (Tween 20, Sigma) were poured in each vial and a uniform suspension of grains was obtained after shaking.

The chambers of the "Bürker" haemocytometer were filled with drops of the suspension with micropipette.

Also, for content screening of anthers, anthers were cut longitudinally and stained with 1% carmine solution prepared in 45% acetic acid (Fig. 1d). Acetocarmine treated anthers were observed under DMRB microscope from Leica (Wetzlar, Germany).

The counts of grains in each of the 9 small chambers were averaged and multiplied *x* 100. This procedure was repeated six times per vial.

The lower portion of the corolla tube was cut off and split open with a scalpel to reveal the ovary. The ovary was then placed under a light magnifier, and the ovules were carefully removed with two dissecting needles. Subsequently, ovules were spread out and counted. For each flower, the pollen: ovule ratio (P: O) was determined by dividing the number of pollen grains per anther by the number of ovules. For each genotype, 180 randomly chosen flowers were used.

Pollen viability in vitro

To study pollen viability *in vitro*, anthers were sampled at late balloon stage. Pollen viability was precisely detected with fluorescein diacetate following HESLOP-HARRISON and HESLOP-HARRISON (1970). A fresh fluorescein diacetate (FDA) in acetone at a concentration of 2 mg mL^{-1} was added diluted by 0.5 M sucrose solution (1:1) up to saturation. One droplet was deposited on each pollen sample. Slides were mounted with a coverslip and observations were made with a Zeiss Axiovert fluorescent microscope equipped for reflected-light fluorescence with a UV mercury lamp. Digital images of the microscope view were obtained with a digital camera (AxioCamMRc3, Carl Zeiss), after 24- and 48 hours of FDA staining.

In addition viable, semi-viable and dead pollen numbers and their percentages were determined. Viable pollen was dyed in green, semi viable pollen dyed in light green and dead pollen was not dyed.

In vitro pollen germination and tube growth

For the pollen germination test, the drop technique was used (ĆALIĆ *et al*., 2013). A ”basic” liquid medium containing 1.2 M sucrose, 0.3 g L^{-1} calcium nitrate [$Ca(NO_3)_2$], 0.10 g L^{-1} boric acid (H_3BO_3), 0.1g L^{-1} potassium nitrate (KNO_3), and 0.2 g L^{-1} magnesium sulfate ($MgSO_4 \cdot 7H_2O$) was used. The effect of polyethylene glycol (PEG) concentrations (10, 15, and 20%, w/v) on pollen germination and tube growth was evaluated. PEG-free medium was used as a

control. For the germination test with the PEG-based media, both pollen germination and pollen tube growth were recorded. Counts of germinated pollen grains were made under a light microscope (DMRB microscope from *Leica* Wetzlar, Germany) after 24 h.

Mean pollen tube length was calculated as the average length of 200 pollen tubes measured from each slides after 24 h. Three replicates (slides) were used for each genotype. A pollen grain was considered to have germinated when pollen tube length equaled or exceeded the grain diameter. Germination percentage was determined by dividing the number of germinated pollen grains by the total number of pollen grains per field of view and multiplying by 100. To measure pollen tube growth, samples were prepared as described above, and the slides were observed under the Leica (Wetzlar, Germany) light microscope equipped with a camera.

Statistical analysis

The measurements of the diameter, viability, nuclei status and germination were taken on 600 pollen grains for each genotype. The means were separated using FISHER's LSD post hoc test for $P \leq 0.05$. Statistical analysis was performed using OriginPro 8 (OriginLab Corporation) and Statistica 8 (StatSoft, Inc.).

RESULTS

Characteristics of inflorescence

The flowers of horse chestnut were arranged in pyramidal inflorescence. This means that multiple flowers were grouped together into clusters. The flowers of *A. hippocastanum* had white spot (Fig. 1a). These flowers were produced in spring in erect inflorescences. All genotypes of *A. hippocastanum* had inflorescences up to 30 cm length.

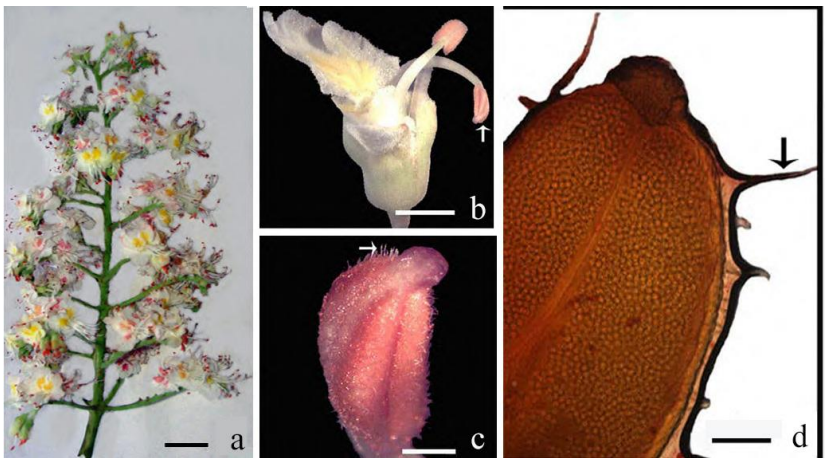

Figure 1. Inflorescence, flower, anthers and pollen morphology of A. hyppocastanum photographed under a binocular magnifier and light microscope. (a) Inflorescence morphology; (b)Detail flower morphology photographed under a binocular magnifier; (c) Anther morphology; (d) Lengthwise dissection of acetocarmine stained anther. Darker (viable) colored and lighter (nonviable, arrow) pollen grains. Anther hairs (arrow). Scale bars for (a)= 3 cm, (b)= 2 cm, (c)= 3 mm, (d)= 250 μm

Anther and pollen morphology

Anther morphology of horse chestnut is shown in Fig. 1b, c, 2a. Lengthwise dissection of acetocarmine stained anthers showed that pollen grains were densely packed into the anther (Fig. 1d).

Anther hairs were noticed on anthers of all *A. hyppocastanum* genotypes (Fig. 1b-d; 2a-c). The pollen of all genotypes was tricolporate while the shape of pollen was circular in the polar view and elliptical in the equatorial view (Fig. 2c, d). The number of colpi (furrows) was three while the spines were absent. Exine ornamentation of mature pollen grains was striate (Fig. 2d, e). Pore of exine, diameter from 0.05 to 0.15 μm were roundish or oval. Four perforations (Fig. 2e) were noticed on 1 μm^2 of exine. Pollen grains within a species, not depending on the genotype, have the same morphology.

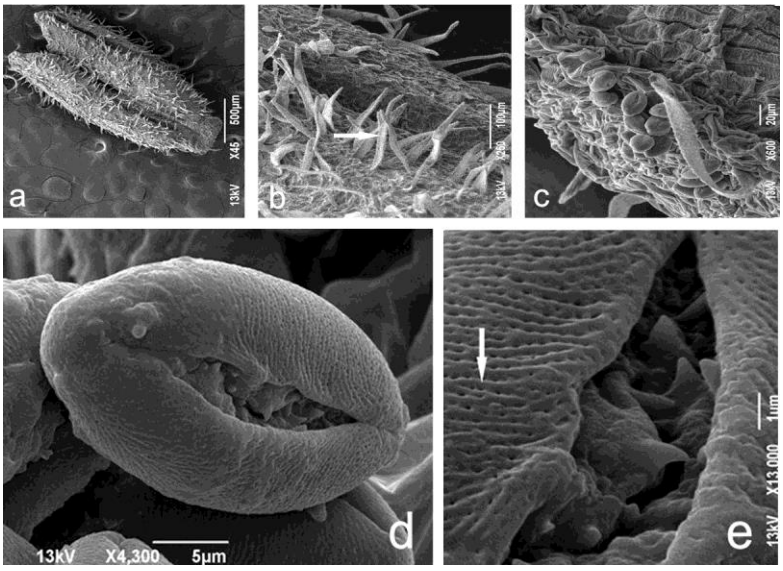

Figure 2. Scanning electron micrographs of anther and pollen morphology of A. hyppocastanum. (a) Anther morphology; (b) Detail of anther surface; (c) Pollen grains in different views; (d) Equatorial view of pollen grain with a specific exine ornamentation; (e) Striate detail of exine with pores. Anther hairs and perforation (arrow)

Pollen production and pollen: ovule ratio

Each genotype of horse chestnut was significantly different compared by pollen number per anther and pollen number per flower. Genotypes were not significantly different compared by the number of flowers per inflorescence. Genotypes Ah1 and Ah2 had larger number of flowers per inflorescence, but lower production of pollen grains per anther, flower and inflorescence (Table 1). The pollen production in anthers of *A. hyppocastanum* genotypes varied from 3.66-5.06 x 10^3, depending of genotype (Table 1).

P: O ratio varied from 610 to 844, while the best performing was genotype Ah2. However, no significant differences in ovule number and number of anthers per flower were found between analysed genotypes (Table 1).

Table 1. Number of flowers/inflorescence (F/I), ovules/flower (O/F), pollen grains/anther (PG/A), pollen grains/flower (PG/F), anthers/flower (A/F) and P:O ratio/flower (P:O) of Aesculus hyppocastanum

Species	Gen.	F/I	O/F	PG/A	PG/F	A/F	P:O
A. hippocastanum	Ah1	205 a	6.0 a	4.38 x 10^3 b	3.07 x 10^4 ab	6.78 a	830 a
	Ah2	208 a	6.0 a	5.06 x 10^3 a	3.48 x 10^4 a	6.88 a	844 a
	Ah3	199 a	6.0 a	3.66 x 10^3 c	2.50 x 10^4 b	6.82 a	610 c
	Ah4	197 a	6.0 a	4.16 x 10^3 b	2.87 x 10^4 ab	6.91 a	693 b

**Same letters show no significantly difference among species and genotypes of each column ($p<0.05$)*

Pollen viability in vitro

We noticed viable, semi-viable and dead FDA treated pollen grains in *A. hyppocastanum* after 24- and 48 hours. After 24 h, depending of genotypes, pollen viability varied from 56 to 68%. The best results showed Ah2 genotype (Table 2). However, differences between 24 and 48 hour pollen viability rates were statistically not significant. Therefore, 24 hours may be enough for detection of pollen viability.

Table 2. Pollen viability of A. hyppocastanum after 24- and 48 hours

Species	Genotype	Viable		Semiviable		Dead	
		24h	48h	24h	48h	24 h	48h
A. hippocastanum	Ah1	62 ab	63 ab	6 ab	5 ab	7 a	7 a
	Ah2	68 a	68 a	7 a	7 a	5 ab	5 ab
	Ah3	56 b	57 b	4 b	3 b	3 b	3 b
	Ah4	59 b	60 b	2 b	1 c	6 a	6 a

**Same letters show no significantly difference among species and genotypes of each column ($p<0.05$)*

In vitro pollen germination and tube growth

Pollen germination varied from 50-66% on basic medium, depending of genotype (Table 3). Inclusion of polyethylene glycol-PEG from 10%, 15% and 20% v/w increased pollen germination. The best results were achieved on medium with the largest concentration of about 20% PEG. On this medium 76-91% pollen grains of *A. hippocastanum* were germinated, depending of genotype. The best germination had genotype Ah2.

PEG had the same impact on pollen tubes elongation as well as on pollen germination in all genotypes (Table 4, Fig. 3). So, the highest pollen tube length (1188 μm) had Ah2 genotype on medium with 20% PEG.

Table 3. Germination of A. hyppocastanum pollen on different PEG concentration after 24h

Species	Genotype	Germination (%) PEG concentration (%)			
		0	10	15	20
A. hippocastanum	Ah1	59 ab	62 b	73 ab	85 ab
	Ah2	66 a	69 a	77 a	91 a
	Ah3	50 c	55 c	66 b	76 c
	Ah4	54 b	60 b	70 b	80 b

**Same letters show no significantly difference among species and genotypes of each column (p<0.05)*

Table 4. Tube growth of A. hyppocastanum pollen on different PEG concentration after 24h

Species	Genotype	Tube growth (μm) PEG concentration (%)			
		0	10	15	20
A. hippocastanum	Ah1	114 b	208 b	604 b	963 ab
	Ah2	178 a	313 a	712 a	1189 a
	Ah3	75 d	110 c	433 d	764 c
	Ah4	91 c	179 bc	568 c	878 bc

**Different letters show significantly difference among species and genotypes of each column (p<0.05)*

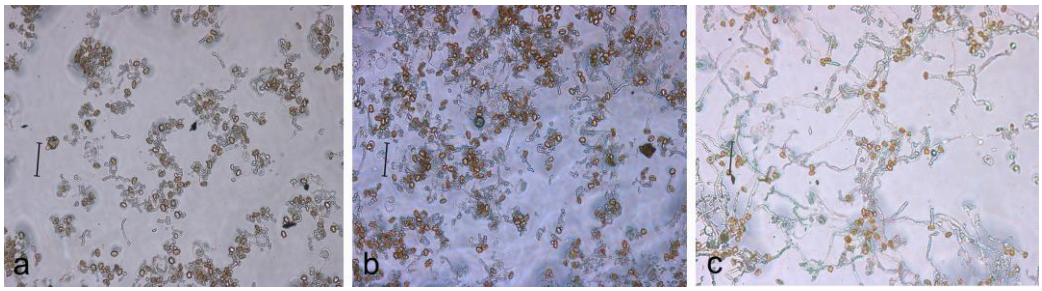

Figure 3. Morphology of A. hyppocastanum germinating pollen on medium with 10% (a), 15 (b) and 20 % (c) sucrose. Scale bars= 200 μm

DISCUSSION

SEM images showed that these pollen grains are tricolporate with a well-defined exine with specific architecture and ornamentation. In general, pollen grains of terrestrial taxa have well developed and defined exine (MARTINSSON, 1993; OSBORN and PHILBRICK, 1994; COOPER *et al.*, 2000). The role of the exine is to protect the male spore and gametophyte from desiccation and other dangers of sub-aerial dispersal.

Our measurement of equatorial axis, the polar axis and diameter of the pollen grains of *A. hyppocastanum* genotypes are similar with previously reported results for the genus *Aesculus* (HEATH, 1984; POZHIDAEV, 1995; KONYAR, 2012).

Such morphological characteristics of pollen can have implications for pollen germination, pollen viability as well as pollination mechanisms. TANAKA *et al.* (2004) concluded that the selective pressures acting on the pollination mechanisms have resulted in various exine sculptures that are adapted to the different pollination mechanisms in entomophilous and anemophilous plants.

Genotypes were significantly different in pollen production per anther, unlike for flower and inflorescence, as they contained the same anther number per flower and similar number of flowers per inflorescence. These results are supported by previously published findings on horse chestnut (SZKLANOWSKA and STRZALKOWSKA, 2000; WERYSZKO-CHMIELEWSKA *et al.*, 2012) and walnut (SŰTYEMEZ, 2007). Pollen viability and germination varied between horse chestnut genotypes, as was found for oak, almond and peach genotypes (GÓMEZ-CASERO *et al.*, 2004; IMANI *et al.*, 2011).

There is some data about of acidity and detergent effects on in vitro horse chestnut pollen germination and tube growth (PAOLETTI, 1992). In our research, the use of PEG is proved very effective in stimulating of horse chestnut pollen germination. However, all *A. hippocastanum* genotypes showed significant differences in pollen germination and pollen tube elongation. Indeed, highest PEG (20 %) concentration ensured almost complete pollen germination (91 %) of Ah2 horse chestnut genotype. Previous studies showed that PEG functions as an osmoticum and improves *in vitro* pollen germination and tube growth by preventing tube bursting (SAKHANOKHO and RAJASEKARAN, 2010; ĆALIĆ *et al.*, 2013).

The P: O ratio has traditionally been used as a rough estimator of plant breeding systems. It has been shown that plant breeding systems are associated with particular floral traits. In this study, we determined the P: O in horse chestnut and explored relationships between P: O and pollen presentation and pollination mechanisms. This species showed high P: O ratios. Horse chestnut, on the basis of P: O ratio may be classified as facultative xenogamous according to CRUDEN (1977). It is common for xenogamous (outcrossed) hermaphroditic flowering plants to produce more flowers and ovules than fruits and seeds (CRUDEN, 1977, 2000; BAWA and WEBB, 1984; MUCHHALA *et al.*, 2010; PANG and SAUNDERS, 2015; LI *et al.*, 2016). Accordingly, horse chestnut produced many more flowers than fruits. Our results demonstrate that P: O variability is determined by pollination mechanism in horse chestnut species.

The presented horse chestnut pollen study could be a test model for pollen quality as essential component for successful fertilization in seed-producing plants.

ACKNOWLEDGMENTS

This work is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. 173015).

Received May 09th, 2016
Accepted December 25th, 2016

REFERENCES

ACAR, I. and V.G. KANANI (2010): The effects of temperature on in vitro pollen germination and pollen tube growth of *Pistacia* spp. Scientia Horticulturae, *125*: 569-572.

ALCARAZ, M.L., M. MONTSERRAT, J.I. HORMAZA (2011): In vitro pollen germination in avocado (*Persea americana* Mill.): Optimization of the method and effect of temperature. Scientia Horticulturae, *130*: 152-156.

ATLAGIĆ, J., A. MARIJANOVIĆ-JEROMELA, R. MARINKOVIĆ, S. TERZIĆ (2009): Pollen grain traits of oil species from the Novi Sad collection. Genetika, *41*: 263-270.

BAWA, K.S. and C.J. WEBB (1984): Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. American Journal of Botany, *71*: 736-751.

BECK-PAY, S.L. (2012): The effect of temperature and relative humidity on *Acacia mearnsii* polyad viability and pollen tube development. South African Journal of Botany, *83*: 165-171.

ĆALIĆ, D., S. ZDRAVKOVIĆ-KORAĆ, S. JEVREMOVIĆ, M. GUĆ-ŠĆEKIĆ, LJ. RADOJEVIĆ (2003): Efficient haploid induction in microspore suspension culture of *Aesculus hyppocastanum* and karyotype analysis. Biologia Plantarum, *47*: 289-292.

ĆALIĆ-DRAGOSAVAC, D., S. ZDRAVKOVIĆ-KORAĆ, K. ŠAVIKIN-FODULOVIĆ, LJ. RADOJEVIĆ, B. VINTERHALTER (2010): Determination of aescin content in androgenic embryos and hairy root culture of *Aesculus hyppocastanum* (Hyppocastanaceae). Pharmaceutical Biology, *48*: 563-567.

ĆALIĆ, D., N. DEVRNJA, I. KOSTIĆ, M. KOSTIĆ (2013): Pollen morphology, viability, and germination of *Prunus domestica* cv. Požegača. Scientia Horticulturae, *155*: 118-122.

CEROVIĆ, R., Z. PAJIĆ, M. FILIPOVIĆ, M. FOTIRIĆ-AKŠIĆ, S. RADIČEVIĆ, D. NIKOLIĆ, M. ĐORĐEVIĆ (2014): Pollen germination and pollen tube growth in ZP maize lines. Genetika, *46*: 935-948.

COOPER, R.L., J.M. OSBORN, C.T. PHILBRICK (2000): Comparative pollen morphology and ultrastructure of the Callitrichadaceae. American Journal of Botany, *87*: 161-175.

CRUDEN, R.W. (1977): Pollen–ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution, *31*: 32-46.

CRUDEN, R.W. (2000): Pollen grains: why so many? Plant Systematic Evolution, *222*: 143-165.

DEVRNJA, N., J. MILOJEVIĆ, LJ. TUBIĆ, S. ZDRAVKOVIĆ-KORAĆ, A. CINGEL, D. ĆALIĆ (2012): Pollen morphology, viability, and germination of *Tanacetum vulgare* L. Hortscience, *47*: 440-442.

DU, Y.H., S.L. ZHANG, T. JIANG, J. WU (2006): Characteristics of pollen germination and pollen tube growth of *Prunus mume* in vitro. Acta Botanica, *26*: 846-1852.

GÓMEZ-CASERO, M.T., P.J. HIDALGO, H. GARCIA-MOZO, E. DOMÍNGUEZ, C. GALA (2004): Pollen biology in four Mediterranean *Quercus* species. Grana, *43*: 22-30.

HARDER, L.D. and S.C.H. BARRETT (1993): Pollen removal from tristylous *Pontederia cordata*: effects of anther position and pollinator specialization. Ecology, *74*: 1059-1072.

HEATH, G.L.A. (1984): Hyppocastanaceae. Review Palaeobotany and Palynology, *42*: 11-114.

HEDLY, Y.A., J.I. HORMAZA, M. HERREROTT (2004): Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). American Journal of Botany, *91*: 558-564.

HESLOP-HARRISON, J. and Y. HESLOP-HARRISON (1970): Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Stain Technology, *45*: 115-120.

IMANI, A, M.H. KARGAR, S.P. PIREIVATLOU, F. ASGARI, S.H. MASOMI (2011): Evaluation of germination capacity of stored pollen of almond and peach. International Journal of Nuts and Related Sciences, *2*: 68-72.

KEARNS, C.A. and D.W. INOUYE (1993): Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA

KONYAR, T. (2012): Pollen morphology of exotic trees and shrubs of Edirne II. Journal of Japanese Society for Horticultural Science, *6*: 13-18.

KOUBOURIS, G.C., I.T. METZIDAKAS, M.D. VASILAKAKIS (2009): Impact of temperature on olive *(Olea europaea* L.) pollen performance in relation to relative humidity and genotype. Environmental and Experimental Botany, *67*: 209-214.

LI, P.S., PANG, C.C., R.M.K. SAUNDERS (2016): Contrasting pollination ecology of *Disepalum* species (Annonaceae): evolutionary loss of the floral chamber and partial breakdown of protogyny associated with a shift in pollination system. Botanical Journal of the Linnean Society, *182*: 708-718.

MARTINSSON, K. (1993): The pollen of Swedish Callitriche (Callitrichadaceae). Grana, *32*: 198-209.

MERT, C. (2009): Pollen morphology and anatomy of Cornelian cherry (*Corns mas* L.) cultivars. Hortscience, *44*: 519-522.

MUCHHALA, N., Z. BROWN, W. S. ARMBRUSTER, M.D. POTTS (2010): Competition drives specialization in pollination systems through costs to male fitness. American Naturalist, *176*: 732-743.

OSBORN, J.M. and C.T. PHILBRICK (1994): Comparative pollen structure and pollination biology in the Callitrichaceae. Acta Botanica Gallica, *141*: 257-266.

PANG, C.C. and R.M.K. SAUNDERS (2015): Floral biology and pollination ecology of *Desmos chinensis* (Annonaceae): assessing the efficacy of floral synchrony for promoting xenogamy. International Journal of Plant Sciences, *176*: 333-345.

PAOLETTI, E. (1992): Effects of acidity and detergent on in vitro pollen germination and tube growth in forest tree species. Tree Physiology, *10*:357-366.

POZHIDAEV, A.E. (1995): Pollen morphology of the genus *Aesculus* (Hyppocastanaceae). Grana, *34*: 10-20.

PRADA, D., T.M. VELLOZA, P.E. TOOROP, H.W. PRITCHARD (2011): Genetic population structure in horse chestnut (*Aesculus hippocastanum* L.): effects of human-mediated expansion across Europe. Plant Species Biology, *26*: 43-50.

SAKHANOKHO, H.F. and K. RAJASEKARAN (2010): Pollen biology of ornamental ginger (*Hedychium* spp. J. Koenig). Scientia Horticulturae, *125*: 129-135.

SHARAFI, Y. (2011): An investigation on the pollen germination and tube growth in some *Prunus persica* genotypes and cultivars. African Journal of Microbiology Research, *5*: 2003-2007.

SOUZA, E.H., F.V.D. SOUZA, M.L. ROSSI, N. BRANCALLEÃO, C.A.S. LEDO, A.P. MARTINELL (2015): Viability, storage and ultrastructure analysis of *Aechmea bicolor* (Bromeliaceae) pollen grains, an endemic species to the Atlantic forest. Euphytica, *204*: 13-28.

STEVANOVIĆ, V. and LJ. RADOJEVIĆ (1993): Natural distribution, importance and possibilities for cultivation of *Aesculus hippocastanum* L. (HIPOCASTANACEAE). Archives of Biological Sciences, Belgrade, *45*: 15P-16P.

SÜTYEMEZ, M. (2007): Determination of pollen production and quality of some local and foreign walnut genotypes in Turkey. Turkish Journal of Agriculture and Forestry, *31*: 109-114.

SZKLANOWSKA, K. and M. STRZALKOWSKA (2000): Blooming biology and pollen exposure of horse chestnut trees (*Aesculus* L.). Ann Universitatis Mariae Curie-Skłodowska, Sectio EEE, Horticulturae, *8*: 107-116.

TANAKA, N., K. UEHRA, J. MURATA (2004): Correlation between pollen morphology and pollination mechanisms in the Hydrocharidaceae. Journal of Plant Research, *117*: 265-276.

THOMSON, J.D. and K.GOODELL (2001): Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. Journal of Applied Ecology, *38*: 1032-1044.

VULETIN SELAK, G., S. PERICA, S. GORETA BAN, M. POLJAK (2013): The effect of temperature and genotype on pollen performance in olive (*Olea europaea* L.). Scientia Horticulturae, *156*: 38-46.

WERYSZKO-CHIMIELEWSKA, E., M. TIETZE, M. MICHOŃSKA (2012): Ecological features of the flowers of *Aesculus hyppocastanum* L. and characteristics of *Aesculus* L. pollen seasons under the conditions of Central-eastern Poland. Acta Agrobotanica Polonica, *65*: 61-68.

KVALITET POLENA DIVLJEG KESTENA

Dušica ĆALIĆ, Ljiljana RADOJEVIĆ

Odeljenje za Fiziologiju biljaka, Institut za Biološka Istraživanja "Siniša Stanković", Univerzitet u Beogradu, Beograd, Srbija

Izvod

Proučavan je kvalitet polena divljeg kestena, izražen kao produktivnost, vijabilnost i klijavost. Prašnici divljeg kestena produkuju od 3.66 do 5.06 x 10^3 polena po anteri, u zavisnosti od genotipa. Polen genotipova Ah1-Ah4 divljeg kestena pokazuje različitu vijabilnost (od 56 do 68%), nakon bojenja fluorescein diacetatom. Klijanje polena Ah1-Ah4 genotipova varira od 50 do 66% na osnovnoj hranljivoj podlozi. Međutim, uključivanjem polietilenglikola-PEG (10%, 15% i 20%) povećava se klijavost polena. Najbolji rezultati postignuti su na hranljivoj podlozi s najvećom koncentracijom PEG-a. Na ovoj podlozi klijalo je 76-91% polena divljeg kestena, u zavisno od genotipa. Najbolji kvalitet polena, po svim testiranim parametrima, ima genotip Ah2.

Poznavanje morfologije polena, njegove produkcije, vijabilosti, klijanja *in vitro*, rasta polenove cevi, kao i odnosa polen: neoplođeno jaje, može biti od velike važnosti za buduće studije biologije polena.

Primljeno 09. V. 2016.
Odobreno 25.XII 2016.