Journal of the American Pomological Society 70(3): 149-157 2016

SEM Observations, Pollen Viability and Germination in Some Selected Plum Genotypes Cultivated in Romania

Ioana Mitre Jr.[1], Viorel Mitre[1], Erzsebet Buta[1], Rodica Pop[1], and Radu E. Sestraş[1]

Additional index words: *Prunus domestica,* pollen tube, pollen grain

Abstract

The pollen morphology, viability, germination and pollen tube growth of six wide-spread plum genotypes (*Prunus domestica*), were studied. 'Toptaste', 'Topfive' and 'Gras ameliorat' had the largest pollen grains (> 50 µm), whereas 'Jojo', 'Anna Späth' and 'Stanley' cultivars were represented by slightly smaller pollen size (45.28 - 48.41µm). Pollen shape was prolate-sheroidal (L/W ratio 1.18-1.02 µm) in the polar view and spherical-triangular in the equatorial view. Pollen viability varied with the genotype: the highest percentage was observed for 'Jojo' (40.96%) and 'Toptaste' (40.87%), the lowest was recorded for 'Gras ameliorat' (26.33%). The highest pollen germination (87.0%) at the end of 24 h incubation at 10°C was recorded for 'Stanley'. 'Anna Späth' developed the longest pollen tubes (1061.1 µm) when the pollen grains were incubated at 15°C for 24 h.

Plum is an important stone fruit species for industrial and domestic processing and for fresh consumption (Glišić, 2012; Nikolić, 2012). In Romania, plum is the major fruit species cultivated on 67,478 ha area with a yield of 424,468 tonnes (according to FAO, 2012). Romania holds the 4th place in the production of plums following China, Serbia and USA and the 2nd spot in Europe after Serbia (Coman, 2010; www.faostat.fao.org). Regarding this, many breeding initiatives are focused on developing new genotypes with favorable morphological traits, self-fertility, resistance to drought, frost and pest/diseases (Sharafi, 2013). To carry our breeding research, determination of the viability and germination potential of pollen is one of the most important factors. Conserving and cryopreserving pollen of the highest viability and germination potential from desirable genotypes/cultivars is important for the further improvement of valuable plums cultivars (Ćalić, 2013). Breeders, as well as growers, are often interested in the pollen viability status of their crops (Pline, 2002; Cosmulescu, 2015) to facilitate breeding programs.

Many studies evaluated pollen grain quality through morphological observations (mainly pollen size determination), staining for viability estimation and *in vitro* germination analyzes (Ćalić, 2013; Nikolić, 2012). Scanning electron microscopy (SEM) can provide information about the surface of pollen. This method is used to identify, discriminate and classify different genotypes (Komai, 2014). Pollen viability is crucial in breeding programs, in understanding the sterility issues, while providing important data for taxonomy, systematics and evolutionism (Ćalić, 2013; Nikolić, 2012; Sharafi, 2011b).

Knowledge of pollen germination potential is instrumental not only in setting the value of a cultivar as pollinator in infertile combinations, but also in establishing the biological condition of pollen as a result of the influence of some exogenous factors (preservation, pesticides, virus and fungal diseases). Generally, in order to obtain successful pol-

[1] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Horticulture, 3-5 Mănăştur 400372, Romania, Fax. +40-264-593.792, ioana_mitre@yahoo.com, mitreviorel@yahoo.com, ebuta2008@yahoo.com, prodica64@yahoo.com

[2] rsestras@usamvcluj.ro (corresponding author)

lination, the pollen must have a germinability of 30% (Cordea, 2014).

Viability and germination percentage depends on genotypes and are strongly influenced by temperature, humidity and physiological processes (Petrişor, 2012) with direct impact on yield (Glišić, 2012; Sorkheh, 2011b). Several staining method were used and tested to determine, pollen viability of plums and other species (Bolat, 1999; Ćalić, 2013; Tosun, 2007). However, there are few studies in the literature where pollen viability was evaluated using potassium iodide staining.

The aim of this communication is to describe pollen morphology and to determine the pollen viability and germination rate of six plum genotypes ('Jojo', 'Toptaste', 'Topfive', 'Anna Späth', 'Stanley' and 'Gras ameliorat'). In addition, the effect of temperature, incubation time upon pollen germination and pollen tube length was studied.

Materials and Methods

The experiment was conducted at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. Pollen grains of six plum genotypes ('Jojo', 'Toptaste', 'Topfive', 'Anna Späth', 'Stanley' and 'Gras ameliorat') were studied. In early April 2015, 10 white flower buds (balloon stage) were harvested from 15 trees of each genotype, and brought to the laboratory where anthers (pre-anthesis stage) were extracted. Petals and sepals were separated and the anthers were wrapped in tracing paper and left to dry for 24 hrs at room temperature (20-24ºC), which allowed the anther to dehisce. Pollen grains thus collected were used immediately.

SEM observations. SEM studies were conducted to highlight any morphological differences in pollen of the six plum genotypes. For the pollen grain morphological characterization different aspects were studied: shape, length, width and number of colpi in the polar and equatorial view, as reported by Ćalić (2013). Scanning electron micrograph images were taken at 140 x and 900 x using VEGA SEM LSU microscope (TESCAN, Ltd.). Pollen grains were placed directly on the microscope stubs and were studied by 2D and 3D details. Ten pollen grains were observed for each genotype.

Confocal microscope (Zeiss CLSM 710 Confocal Laser Scanning Microscope, supported by Zeiss ZEN 2009 software) was used to find depth information of pollen ornamentation. Small quantities of pollen grains were placed by a brush on the microscope slide. Ten pollen grains per genotype were observed. Exine architecture of pollen grains were taken at 10,000 x.

Pollen viability. Pollen viability was estimated after the anthers were kept in a Carnoy's solution for 2 hrs, after which they were washed in 80% ethyl alcohol. Potassium iodide (25%) stain was used to detect pollen viability or sterility (Bolat, 1999; Cordea, 2014). Five Petri dishes were used per genotype. The pollen grains of each genotype were observed in 4 fields (microscopic area) per Petri dish (20 fields/genotype). In each field, 40-60 pollen grains were counted, therefore a total of 4800 to 7200 grains were observed. The brown pollen was considered viable, and the colorless unviable. The percentage of viable pollen was determined with a digital microscope (LCD Aigo Digital Microscope EV5610).

Pollen germination. To assess germination, pollen grains were cultured in a medium containing 1.5 g agar, 15 g sucrose and 100 ml distilled water heated until the mixture become transparent (but not boiled), according to the methodology describe by Cordea (2014) and used in the same studies by Radičević (2013) and Firmage (2001). Solidification of medium occurs in 10-15 min depending on the prepared layer thickness. When the medium became cold, the pollen grains were scattered uniformly with a brush on the solid medium. Five Petri dishes was used for each genotype. The pollen grains of each genotype were observed in 4 fields (microscopic area) per Petri dish (20 fields/genotype). In each field, 30-45 pollen grains

were counted and a total of 3600 to 5400 pollen grains were observed. Germination was evaluated one, three and 24 hrs after sowing, by recording the total number of germinated and non-germinated grains (Cordea, 2014; Sestraş, 2004), using a digital microscope (Aigo Digital Microscope EV5610 - Beijing Research Institute of Precision Instrument Aigo Co., Ltd). To determine the effect of temperature, pollen was tested at 10, 13, 15 and 20ºC. A pollen grain was considered germinated when the pollen tube was equal to or greater than the diameter of the pollen grain (Sorkheh, 2011a; Wang, 2005).

Pollen tube length. Pollen tube length (PTL) was observed as the germination capacity in the same medium at various temperatures (10, 13, 15 and 20ºC) and time exposures (1, 3 and 24 hrs). PTL was recorded directly with an ocular micrometer fitted to the eyepiece of the microscope based on the micrometer scale (µm). Mean pollen tube length was calculated as the average length of 15 pollen tubes measured randomly on four microscopic areas per Petri dish. The microscope area was 50.24 mm^2.

Statistical analysis. Experimental design was a completely randomized design and the treatment structure was a factorial with six temperature/time combinations and six genotypes (plum cultivars), and five replicates per each variant. A 2-way ANOVA, including the interaction term, had illustrated that the interactions were significant; an LSD was used to compare temperature/time combinations within each genotype. A one-way ANOVA for each genotype was performed in Excel, and Duncan's New Multiple Range Test (Sestras et al., 2012), was used to compare genotypes within each temperature/time combination.

Results and Discussion

SEM observations. Results described in this study regarding the pollen morphology and exine sculpturing are similar to the results reported by Arazani (2005), Ćalić (2013), Gilani (2010), Youshihiro (1989) for pollen of the genus *Prunus*. Morphological observations of the genotypes are presented in Figures 1 and

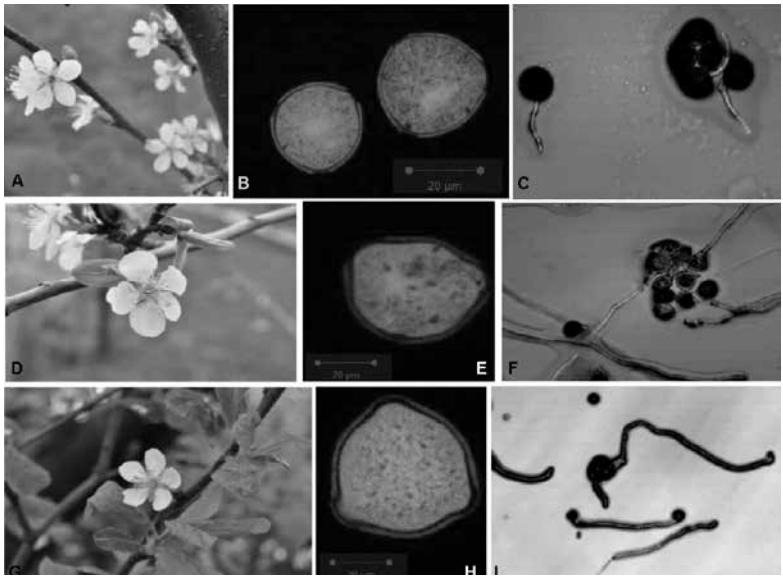


Fig. 1. (A, B, C). Mature flowers, pollen grain shape and germination in 'Jojo'. (D, E, F) Mature flowers, pollen grain shape and germination in 'Toptaste'. (G, H, I) Mature flowers, pollen grain shape and germination in 'Topfive' (Bars=20 µm)

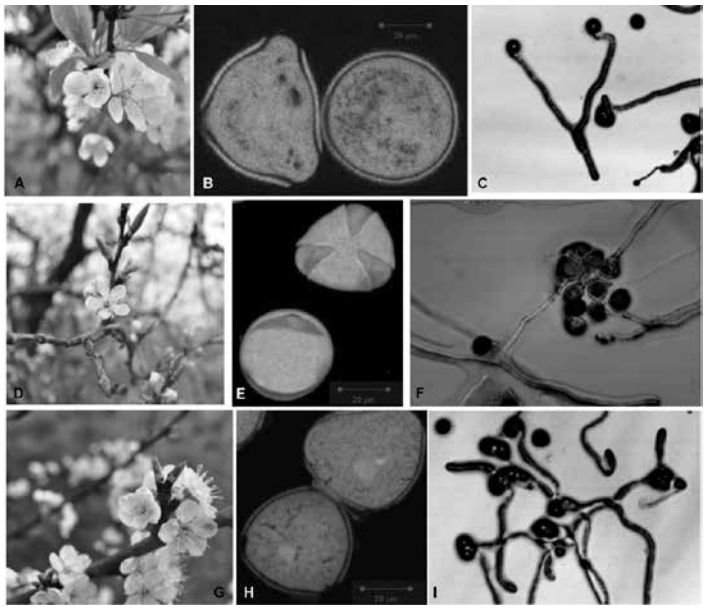


Fig. 2. (A, B, C). Mature flowers, pollen grain shape and germination in 'Stanley'. (D, E, F) Mature flowers, pollen grain shape and germination in 'Anna Späth'. (G, H, I) Mature flowers, pollen grain shape and germination in 'Gras ameliorat' (Bars=20 µm)

2. All had isopolar, symmetrical and tricolporate pollen grains. 'Toptaste', 'Topfive' and 'Gras ameliorat' had large pollen (> 50µm), whereas 'Jojo', 'Anna Späth' and 'Stanley' had medium pollen size (48.41-45.28 µm). Microscopic analysis reveals that the pollen shape was prolate-sheroidal in the polar view and spherical-triangular in the equatorial view. The length – width in the polar view was 1.18-1.02 µm. Polar diameter (length) varied from 45.26 µm ('Anna Späth') to 55.45 µm ('Topfive'). The horizontal distance (width) was between 38.18 µm ('Anna Späth') and 54.17 µm ('Topfive'). The length – width ratio in the equatorial view was 0.84 µm and 0.97 (Table1). The exine surface was striate confirmed by Ćalić (2013) with three colpi as reported by Gilani (2010) (Fig. 1 and 2).

Pollen viability. Pollen viability is important in the pollination process and seed production (Rodriguez – Riano, 2000). For most species, the viability deteriorated more rapidly if strict storage conditions were not maintained (Adhikari, 1998; Kearns 1993).

Previous results showed that pollen viability estimation depends on staining method, genotypes and environmental changes (Cavusoglu, 2013; Sorkheh, 2011a). Pollen viability differed for various genotypes (Bayazit, 2011; Sorkheh, 2011a) of plum and almond.

In the current study, pollen viability varied among the six genotypes. Pollen viability was significantly higher (<38%) for 'Jojo', 'Toptaste' and 'Anna Späth's than for the other genotypes (26.3 to 34.4%). 'Gras ameliorat' had the lowest viability (26.33%) and 'Stanley' had the lowest germination.

Pollen germination. Temperature is the important factor controlling plant growth and development and could affect pollen performance. Pollen germination temperature is a major factor in fertilization success and implicitly in crop production (Sorkheh, 2011b).

Temperature ranges and the influence of temperature on germination was studied for different plant species, including almond (Sorkheh, 2011b), sweet cherry (Radičević,

Table 1. Morphological characteristics (length, width and L/W ratio) of pollen grains for six plum genotypes (μm)

Morphological traits (μm) of pollen	Plum genotypes					
	Jojo	Toptaste	Topfive	Anna Späth	Stanley	Gras ameliorat
Length	48.41	54.25	55.45	45.26	45.28	52.13
Width	41.36	52.03	54.17	38.18	40.90	45.71
L/W ratio polar view	1.17	1.04	1.02	1.18	1.10	1.15
L/W ratio equatorial view	0.85	0.95	0.97	0.84	0.90	0.87

2013; Tosun, 2007), plum (Sharafi, 2013), medlar (Cavusoglu, 2013), pecan (Peng, 2015) and sour cherry (Blidariu, 2008). The optimum temperature required for pollen germination was about 15°C to 20°C for apricot, sour cherry and sweet cherry (Bolat, 1999).

The results of pollen germination (%) at various time exposures and temperatures are summarized in the Table 2. The current study demonstrates that the pollen's optimum germination temperature was 20°C for 24 h, for four of the six genotypes. Pollen germination at 20°C for 24 h ranged from 39.2% ('Topfive') to 85.6% ('Jojo'), whereas some authors (Botu, 2002; Glišić, 2012) reported lower values. Lower temperatures were more effective for 'Stanley' and shorter exposure time was effective for 'Gras ameliorat'.

In this study, the higher germination percent (87.0%) was recorded for 'Stanley' (10°C, 24 h) followed by 'Anna Späth' with 84.5% (20°C, 24 h). 'Anna Späth' is able to germinate rapidly only in one hr after sowing, at a temperature of 20°C (61.8%), but the means were not significantly different than other genotypes. 'Gras ameliorat' achieved a low germination percent (9.20%) at the same exposure time and temperature level. 'Gras ameliorat' had a high germination level (58.7%), if the exposure time was longer (3 h) at the same temperature (20°C).

'Anna Späth' had the higher germination rate (78.3%), followed by 'Jojo' (75.2%) and 'Stanley' (75.5%) and differences were significant. Surprisingly, at 10°C (24 h exposure) 'Stanley' achieved the highest germination percent (87.0%), germination for 'Jojo' was 67.7% (Table 2) and the remaining genotypes exceeded the minimum germination level (25-30%) according to Cordea (2014) and Nikolić (2012).

Increasing the germination temperature to 15°C had a negative influence on germination of 'Toptaste' (28.6%), 'Topfive' (39.2%), 'Gras ameliorat' (23.6%) and 'Stanley' (57.1%). 'Jojo' (75.2%) and 'Anna

Table 2. The influence of temperature for varying lengths of time on pollen germination (%) of six plum genotypes

Temp.		Pollen germination (%)[z]											
(°C)	Hrs.	Jojo		Toptaste		Topfive		Anna Späth		Stanley		Gras ameliorat	
20	1	43.8f	B	4.1e	D	7.0b	D	61.8cd	A	32.1c	C	9.2d	D
20	3	45.8e	CD	70.5a	AB	35.5a	D	80.3ab	A	68.5b	AB	58.7a	BC
13	3	4.1d	B	47.2bc	C	26.4ab	D	78.3abc	A	73.9ab	AB	24.2c	D
10	24	67.7c	B	33.8cd	C	36.1a	C	60.0d	B	87.0a	A	43.8b	C
15	24	75.2b	A	28.6d	C	39.2a	BC	65.9bcd	AB	57.1b	AB	23.6c	C
20	24	85.6a	A	59.7ab	BC	39.2a	D	84.5a	A	75.5ab	AB	56.2ab	CD
Average		**63.70**		**40.38**		**30.79**		**71.90**		**65.67**		**35.97**	

[z] Values are means of five replicates per each variant: genotype x treatment combination (temperature and time). Mean separated by different letters denote significant differences (Duncan test, $P < 0.05$) for treatment combination across rows (upper case letters) and for genotypes down columns (lower case letters).

Table 3. The influence of temperature for varying lengths of time on pollen tube length (PTL) of six plum genotypes

Temp.		Pollen tube length (μm)[z]											
(°C)	Hrs.	Jojo		Toptaste		Topfive		Anna Späth		Stanley		Gras ameliorat	
20	1	514.7b	A	447.5b	BC	428.1b	C	488.2c	AB	492.2c	AB	62.1d	D
20	3	847.6a	A	824.5a	B	620.7a	D	535.0b	E	806.8a	C	458.6b	F
13	3	169.7e	D	104.1e	E	450.8b	C	453.4c	C	578.0b	A	523.0a	B
10	24	331.6c	A	141.1d	B	79.1d	D	71.8d	E	109.4e	C	63.2d	F
15	24	246.0d	C	218.0c	CD	289.3c	B	1061.1a	A	202.0d	D	130.5c	E
Average		**421.96**		**347.05**		**373.85**		**521.95**		**437.74**		**247.52**	

[z] Values are means of five replicates per each variant: genotype x treatment combination (temperature and time). Mean separated by different letters denote significant differences (Duncan test, $P < 0.05$) for treatment combination across rows (upper case letters) and for genotypes down columns (lower case letters).

Späth' (65.9%) had an increased germination rate comparatively with 67.7%, respectively 60.0% at 10°C (Table 2). Germination was improved when pollen was exposed to 20°C for 24 hrs.

Pollen tube length (PTL). Germination and pollen tube length are the most important properties in fruit trees related to pollen quality and reproduction capacity (Sharafi 2011b; Moshtagh, 2015). According to Sharafi (2011b) for some *Prunus* genotypes high pollen germination was not associated with high pollen tube length (PTL), however for some genotypes high germination percentage was accompanied by high PTL.

Pollen tube length, in the current study, was measured at several temperature levels and time exposures (Table 3). Due to rapid development, pollen tube length at 20°C for 24 h exposure couldn't be measured. PTL varied from 62.1μm ('Gras ameliorat' 20°C, 1h) to 1061.1 μm ('Anna Späth' 15°C, 24 h). According to other studies (Sharafi, 2013) PTL varied from 353.6-642.4 μm (24 h, 24°C) in some plum genotypes. PTL measured by Sharafi (2011c) was between 70-1201 μm (24 h, 22°C) in 20 loquat cultivars. According to Moshtagh (2015) apple pollen tube length ranged between 99.65 to153.62 μm (36 h, 24°C).

In the current study, maximum pollen PTL (1061.1 μm) was observed for 'Anna Späth' at 15°C after 24 h. Ćalić (2013) recorded a maximum PTL of 822 μm (exposure 24 h) in *Prunus domestica* cv. Požegača.

'Jojo' had the significantly highest PTL (514.7 μm) at 20°C for 1 h whereas 'Gras ameliorat' had the shortest pollen tubes (62.1μm). 'Toptaste' had higher PTL (824.5 μm) after 3 h at a temperature of 20°C but exposure to 13°C resulted in the lowest PTL (104.1 μm). Similar results were reported in other studies for *Prunus persica* (Sharafi, 2011a) and *Prunus domestica* (Sharafi, 2013). 'Topfive' had the highest PTL (620.7 μm) when pollen grains are held at 20°C for 3 hrs and lower temperatures (10°C) caused shorter PTL (79.1 μm).

The highest PTL (1061.1 μm) in this study was for 'Anna Späth' when pollen were exposed to 15°C for 24 hrs. However, the most studied cultivars develop the highest PTL at 20°C, while Sorkheh (2011) found that PTL was greatest at 25°C.

If the pollen grains were kept a longer time (24 h), but at a lower temperature (10°C), the PTL of 'Anna Späth' and 'Gras ameliorat' was significantly shorter (71.8 and 63.2 μm, respectively). 'Stanley' can develop rapidly (after 3 h) the highest PTL (806.8 μm) when the pollen grains are kept on solid medium at 20°C, but in case of 'Gras ameliorat' the PTL can become higher (523.0 μm) after 3 h even if the temperature is lower (13°C). These results corresponds well with results in plum found by Ćalić (2013) and our results indi-

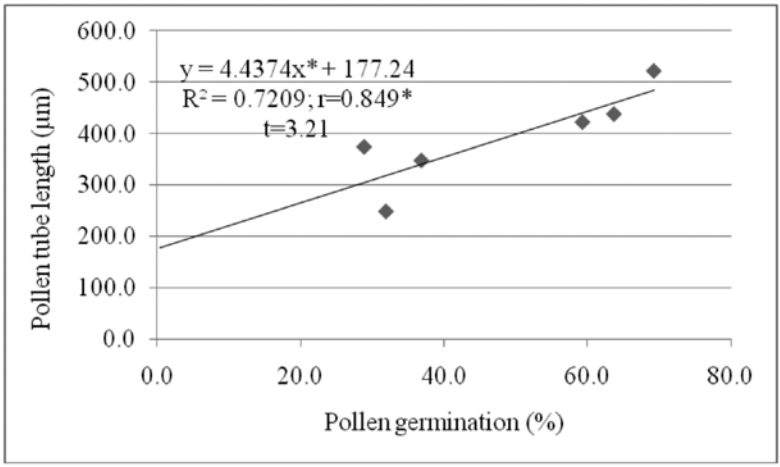


Fig. 3. Relationship between pollen tube length and pollen germination.

cate that pollen tube length can be genotype dependent.

When pooled over all six combinations of temperature and exposure time there was a significant and positive linear relationship between pollen germination and pollen tube length ($R^2 = 0.72$; Figure 3).

Conclusions

This study is important to understanding and improving plum reproduction biology, particularly pollen production, fruit set and fruit quality (Asma, 2008; Sutyemez 2011). Some size and index differences were measured among the genotypes; the pollen was generally similar in shape and exine structure. Pollen grain sizes varied depending on genotype (1.02 µm L/W for Topfive to 1.17 µm L/W for 'Jojo').

The results show that pollen germination can be influenced by temperature as found by Sorkheh (2011b). The germination rate was positively related to exposure time. Pollen germination responded to temperature differently for genotypes reported by Nikolić (2012).

Pollen of self-fertile genotypes, such as 'Jojo', 'Anna Späth', 'Stanley' can germinate rapidly (after 3 h at 20ºC), and pollen of these genotypes could germinate at 10ºC and 15ºC, respectively. For 'Stanley' the highest germination percentage (87.0%) occurred at 10ºC for 24 h.

Pollen tube length (PTL) varied significantly according to genotype and environmental conditions (exposure time and temperature). 'Anna Späth' had the highest PTL (1061.1 µm), after 24 h at 15°C. Similar PTL was recorded for 'Stanley' (578.0 µm) and 'Gras ameliorat' (523.0 µm) after 3 h at 13°C. These results can be helpful in future breeding programs.

Acknowledgements

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

Literature Cited

Adhikari, K.N., and C.G. Campbell. 1998. In vitro germination and viability of buckwheat (*Fagopyrum esculentum* Moench) pollen. Euphytica 102(1):87-92.

Arazani, K., M.A. Nejatian, and G. Karimzadeh. 2005. Apricot (*Prunus armeniaca*) pollen morphological characterization through SEM, using the multivariate analysis. New. Zeal. J. Crop. Hort. 33:381-388.

Asma, B.M. 2008. Determination of pollen viability,

germination ratios and morphology of eight apricot genotypes. Afr. J. Biotechnol. 7(23):4269-1273.

Bayazit, S., O. Çalişkan, and I. Burhanettin. 2011. Comparison of pollen production and quality characteristics of cultivated and wild almond species. Chil. J. Agr. Res. 71(4).

Blidariu, A., O. Iordanescu, R. Micu, and A. Dragunescu. 2008. Researches concerning pollen's germination and viability of some sour cherries varieties in the plain area of Banat. Bul. Hort. 65(1):505.

Bolat, I., and L. Pirlak. 1999. An investigation on pollen viability, germination and tube growth in some stone fruits. Turk. J. Agric. For. 23:383-388.

Botu, M., C. Sarpe, S. Cosmulescu, and I. Botu. 2002. The genetic control of pollen fertility, pollenizing and fruit set for the *Prunus domestica* L. plum cultivars. Acta Hort. 577:139-145.

Ćalić, D., N. Devrnja, I. Kostić, and M. Kostić. 2013. Pollen morphology, viability, and germination of *Prunus domestic cv*. Požegača. Sci. Hortic. 155:118–122.

Cavusoglu, A. and M. Sulusoglu. 2013. In vitro pollen viability and pollen germination in medlar (*Mespilus germanica* L.). Int. Res. J. Biological Sci. 2(5):49-53.

Coman, M., M. Butac, D. Sumedrea, I. Dutu, M. Iancu, C. Mazilu, and C. Plopa. 2010. Plum culture in Romania - current status and perspectives. Acta Hort. 968:25-32.

Cordea, M.I. 2014. Plant breeding - Practical works (in Romanian). ed. AcademicPres, Cluj-Napoca, Ro.

Cosmulescu, S., I. Trandafir, and V. Nour. 2015. Chemical Composition and Antioxidant Activity of Walnut Pollen Samples. Not. Bot. Horti. Agrobo 43(2):361-365.

Dalkiliç, Z., and H.O. Mestav. 2011. In vitro pollen quantity, viability and germination tests in quince. Afr. J. of Biotechnol. 10(73):16516-16520.

Firmage, H. D., and A. Dafni. 2001. Field Tests for Pollen Viability; a Comparative Approach. Acta Hort. 561.

Gasparino, E.C., M.A.V. Da Cruz-Barros, A. Chautems. 2013. Pollen morphology in Brazilian species of *Codonanthe* (Mart.) Hanst. and *Nematanthus* Schrader (Gesneriaceae). Grana. 52(4):258-274.

Gilani, S.A., R.A. Qureshi, A.M. Khan, and D. Potter. 2010. Morphological characterization of the pollens of the selected species of genus *Prunus* Linn. from Northern Pakistan. Afr. J. Biotechnol. 9(20):2872-2879.

Glišić, I., C. Radosav, N. Milošević, M. Đorđević, and S. Radičević. 2012. Initial and final fruit set in some plum (*Prunus domestica* L.) hybrids under different pollination types. Genetika 44(3):583-593.

Kearns, C. A., and D. W. Inoyue. 1993. Techniques for pollination biologists. Uni. Press, Colorado.

Komai, F., O. Kanako, I. Yuko, Y. Mitsunori, T. Osamu and K. Susumu. 2014. SEM observation of wet lily pollen grains pretreated with ionic liquid. J. Jpn. Soc. Hortic. Sci. 83(4): 317–321.

Moshtagh, F., A. Dadar, G. E. Moghadam and A. Ahmad. 2015. Investigation on pollen viability, germination, and tube growth in some apple cultivars in climate conditions of Shirvan. J. Appl. Environ. Biol. Sci. 4(12S):295-302.

Nikolić, D.T., V.S. Rakonjac, and M.M. Fotirić-Akšić. 2012. The effect of pollenizer on the fruit set of plum cultivar Čačanska Najbolja, J. Agri. Sci. 57(1):9-18.

Peng, H. Z., Q.Y. Jin, H.L. Ye, and T.J. Zhu. 2015. A novel in vitro germination method revealed the influence of environmental variance on the pecan pollen viability. Sci. Hortic. 181:43–51.

Petrişor, C., V. Mitre, I. Mitre, L. Jantschi, and C.M. Balan. 2012. The rate of pollen germination and the pollen viability at ten apple cultivars in the climatic conditions of Transylvania. Bulletin UASMV 69(1): 417-418.

Pline, W.A., K.L. Edmisten, T. Oliver, J.W. Wilcut, R. Wells, and N.S. Allen. 2002. Use of digital image analysis, viability stains, and germination assays to estimate conventional and glyphosate-resistant cotton pollen viability. Crop Sci. 42(6):2193-2200.

Radičević, S., D. Nikolić, R. Cerović, and M. Đorđević. 2013. *In vitro* pollen germination and pollen grain morphology in some sweet cherry (*Prunus avium* L.) cultivars. Rom. Biotech. Lett. 18(3):8341-8349.

Rodriguez-Riano, T., and A. Dafni. 2000. A new procedure to asses pollen viability. Sex. Plant Repro. 12:241–244.

Sestraş, R.E. 2004. Plant breeding (in Romanian). Ed. AcademicPres, Cluj-Napoca, Ro.

Sestraş, R. E., M. Ardelean, I.M. Cordea, and A.F. Sestraş. 2012. Biostatistics and Forestry Experimental Technique - Support for practical work (in Romanian). Ed. AcademicPres, Cluj-Napoca, Ro.

Sharafi, Y. 2011a. An investigation on the pollen germination and tube growth in some *Prunus persica* genotypes and cultivars. Afr. J. Microbiol. Res. 5:2003-2007.

Sharafi, Y. 2011b. Pollen viability and longevity in some selected genotypes of peach, plum, prune and sour cherry. J. Med. Plants. Res. 5(2):275-279.

Sharafi, Y., A.R. Motallebbi-Azar, and A. Bahmani. 2011c. In vitro pollen germination, pollen tube growth and longevity in some genotypes of loquat (*Eriobotria japonica* Lindl.) Afr. J. Biotechnol. 10(41):8064-8069.

Sharafi, Y., A. Ghanbari, A.M. Naji, A. Kordenaeej, and A. Rezaei. 2013. Comparison of pollen traits of

some plum cultivars of Iran. Intl. J. Agron. Plant. Prod. 4(2):314 -318.

Sorkheh, K., B. Shiran, V. Rouhi, M. Khodambashi, J.N. Wolukau, and S. Ercisli. 2011a. Response of in vitro pollen germination and pollen tube growth of almond (*Prunus dulcis* Mill.) to temperature, polyamines and polyamine synthesis inhibitor. Biochem. Syst. Ecol. 39:749–757.

Sorkheh, K., S.H. Behrouz, R. Vahid, and K. Mahmood. 2011b. Influence of temperature on the in vitro pollen germination and pollen tube growth of various native Iranian almonds (*Prunus l*. spp.) species. Trees 25:809–822.

Sutyemez, M. 2011. Pollen quality, quantity and fruit set of some selfcompatible and self-incompatible cherry cultivars with artificial pollination. Afr. J. Biotechnol. 10(17):3380-3386.

Tosun, F., and F. Koyuncu. 2007. Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations. Hortic. Sci. 34(2): 47–53.

Wang, Q., L. Kong, H. Hao, X. Wang, J. Lin, J. Šamaj, and F. Baluška. 2005. Effects of brefeldin A on pollen germination and tube growth. Antagonistic effects on endocytosis and secretion. Plant. Physiol. 139(4):1692–1703.

