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POLLEN GERMINATION AND POLLEN TUBE GROWTH *IN VITRO* IN QUINCE CULTIVARS

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Abstract

Pollen germination and pollen tube growth *in vitro* in eight quince cultivars (‘Leskovacka’, ‘Vranjska’, ‘Morava’, ‘Pazardzijska’, ‘Hemus’, ‘Asenica’, ‘Portugal’ and ‘Triumph’) were studied in the three-year period (2010-2012). Agar-plate method with three different concentrations of sucrose (10%, 15% and 20%) was applied. Statistically significant differences between quince cultivars were found in terms of pollen germination and pollen tube length. The highest germination rate was found in 15% sucrose (78.72% in average). Slightly lower germination was found in 20% sucrose (78.52%), while the lowest germination was determined in 10% sucrose (73.76%). Among quince cultivars, the highest germination rates were found in ‘Triumph’ and ‘Asenica’, and the lowest in Leskovacka. Increasing the concentration of sucrose increased the pollen tube length (from 1435.8 μm in 10%, followed by 1790.7 μm in 15% to 2077.5 μm in 20%). Pollen tube length in 20% sucrose concentration ranged from 1770.3 μm (‘Vranjska’) to 2574.4 μm (‘Asenica’). Based on obtained results, the sucrose concentrations of 15% and 20% can be recommended as optimal for pollen germination of quince cultivars.

Key words: *Cydonia oblonga, sucrose, pollen germination, pollen tube length*.

Introduction

Pollen germination and pollen tube growth are the most important characteristics that determine the quality of pollen. The quality of pollen is a very important factor for successful fertilization, fruit set and yield in quince (*Cydonia oblonga* Mill.).

Pollen viability varies between individual cultivars within the same fruit species (Stösser et al., 1996). In addition to the cultivar, pollen viability depends of rootstock on which the cultivar is grafted (Kidman et al., 2014). Besides these factors, pollen viability varies depending of microclimate, locality, character of last year's vegetation, vigour, age, nutrition and health status of fruit trees (Pejkić, 1980). Pollen viability can be affected by the presence of heavy metals: Cd, Cu, Hg and Pb (Gür and Topdemir, 2008; Kiliç et al., 2009), boric acid (Imani et al., 2011; Mondal and Ghanta, 2012; Liu et al., 2013), plant growth regulators (Bolat and Pirlak, 2003; Tosun and Koyuncu, 2007) and the fungicides (Yi et al., 2003; Kargar and Imani, 2011). Also, the quality of pollen depends on the time of taking, methods of collecting (Stanley and Linskens, 1974), the length and mode of its storage (Aslantas and Pirlak, 2002; Perveen and Khan, 2008). The germination of pollen varies depending of temperature (Pirlak, 2002; Sorkheh et al., 2011; Milatović and Nikolić, 2014).

One of the most important factors affecting to the pollen germination is sucrose concentration (Dalkiliç and Mestav, 2011; Sutyemez, 2011). The optimum concentration of sucrose for testing the pollen germination varies between different fruit species and it is ranging from 10 to 20% (Mahanoğlu et al., 1995; Milatović and Nikolić, 2007; Mert, 2009; Dalkiliç and Mestav, 2011; Sutyemez, 2011). However, the concentration of sucrose over 20% may have an inhibitory effect on pollen germination (Bolat and Pirlak, 1999).

Knowledge of pollen functional ability is a very important parameter in fruit breeding for the choice of male parent in hybridization. In addition, this parameter is very significant in the production practices for the selection of appropriate pollenisers when planting of quince orchards, because pollenisers, among other parameters need to be distinguished by good pollen germination.
The aim of this study was to determine the pollen germination and pollen tube length in eight quince cultivars, in order to evaluate suitability of these cultivars as potential pollenisers. Also, testing of nutrient media with different concentrations of sucrose aimed to determine the optimal concentration for testing pollen germination and pollen tube length.

Material and methods

Investigations were carried out in the collection orchard of quince at the Experimental Station ‘Radmilovac’ of the Faculty of Agriculture in Belgrade during a three-year period (2010-2012). The orchard was established in the spring of 1999, with the planting space of 4.5 x 3 m. The study comprises eight cultivars of quince: ‘Leskovacka’, ‘Vranjska’, ‘Morava’, ‘Pazardzijska’, ‘Hemus’, ‘Asenica’, ‘Portugal’ and ‘Triumph’. The examined cultivars were grafted on the rootstock ‘Quince MA’.
For examination of pollen germination branches with flower buds in the ‘balloon’ phase were taken and carried to the laboratory. In order to collect pollen from the flower buds anthers were isolated in Petri dishes. They are stored at room temperature (20±2^{o}C) for 24-48 h to dry and to release the pollen. Then, the pollen of each cultivar was sown with fine brushes in Petri dishes (9 cm diameter) on the previously prepared nutrient medium consisting of three different sucrose concentration (10%; 15% and 20%) and 0.7% agar-agar.
Petri dishes with a sowed pollen were observed under light microscope ‘Leica DM LS’ (Leica Microsystems, Wetzlar, Germany), for counting of germinated pollen grains. The experiment was done in three repetitions, each including at least 300 pollen grains. Pollen is considered as germinated if the length of pollen tube was larger than the diameter of the pollen grain.
Pollen tube length was measured from images taken under the microscope ‘Leica DM LS’ using ‘Leica IM 1000’ program. From each cultivar 60 pollen tubes were measured.
The data were statistically analyzed using three-factor analysis of variance (ANOVA). In the results expressed in percentages, the arcsin square-root data transformation was performed. The significance of differences between the mean values was determined using the Tukey’s test for significance level p <0.05. Data analysis was performed using the statistical software package STATISTICA, Version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and discussion

Test of pollen germination *in vitro* is one of the main indicators of pollen functional ability (Figure 1). The average pollen germination for all cultivars in the three-year study period was quite high and amounted to 77.00% (Table 1). It has significantly varied between the examined cultivars and ranged from 65.12% (‘Leskovacka’) to 84.74% (‘Triumph’). Pollen germination at a concentration of 10% sucrose was highest in ‘Triumph’ cultivar (82.40%) and lowest in ‘Leskovacka’ cultivar (62.61%). ‘Leskovacka’ cultivar had lowest pollen germination at sucrose concentrations of 15% (64.62%) and 20% (68.12%), while the ‘Asenica’ cultivar had highest pollen germination in sucrose concentrations 15% (86.43%) and 20% (87.58%). In addition to ‘Asenica’ and ‘Triumph’ cultivars, high pollen germination had ‘Pazardzijska’ and ‘Morava’ cultivars, which is consistent with the findings of Stančević (1986) and Stančević (1990). Pollen germination between quince cultivars examined in our work was much less varied in relation to the one established by Sharafi (2011a) in Iran, in which the pollen germination ranged from 45.4% to 82.3%. This is due to genetic differences between tested cultivars.

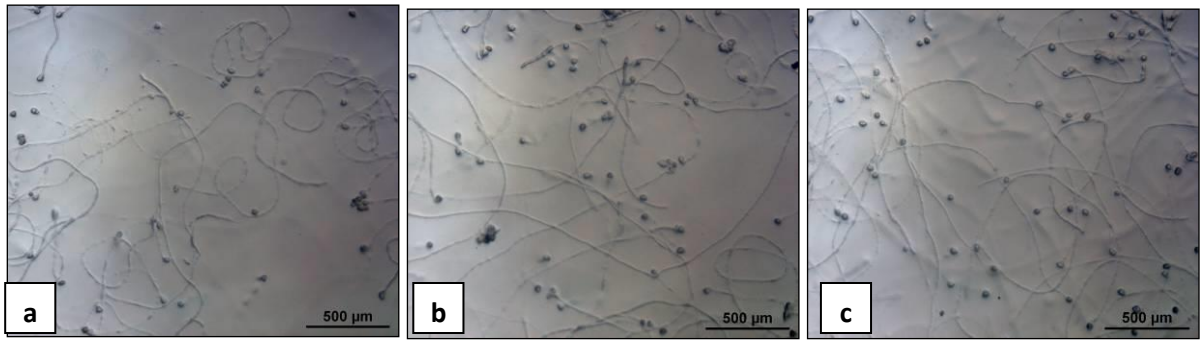

Figure 1. Pollen germination of Triumph cultivar at three different sucrose concentrations: a) 10%, b) 15%, c) 20%.

In addition to the cultivars, pollen germination differed significantly between investigated concentrations of sucrose, which is in accordance with the results Dalkiliç and Mestav (2011). The highest germination rate was found in 15% sucrose (78.72% in average). Slightly lower germination was found in 20% sucrose (78.52%), while the lowest germination was determined in 10% sucrose (73.76%). These results partially agree with those obtained by Çetin and Soylu (2006), where the highest pollen germination in quince cultivars was found in 10% and 15% sucrose solution. Pollen germination rate also varied depending on the concentration of sucrose in the Japanese pear (Okusaka and Hiratsuka, 2009), apricot (Mahanoğlu et al., 1995; Milatović and Nikolić, 2007; Asma, 2008), sweet and sour cherry (Bolat and Pirlak, 1999; Sutyemez, 2011) and walnut (Mert, 2009).

Table 1. Pollen germination (%) of investigated quince cultivars (average 2010-2012).

Cultivar/Year		Sucrose concentration			Mx
		10%	15%	20%	
‘Leskovacka’		62.61	64.62	68.12	**65.12 d**
‘Vranjska’		68.75	71.86	75.45	**72.02 c**
‘Morava’		74.41	82.60	80.61	**79.21 b**
‘Pazardzijska’		74.14	80.76	78.75	**77.88 b**
‘Hemus’		76.76	80.47	77.27	**78.17 b**
‘Asenica’		79.38	86.43	87.58	**84.46 a**
‘Portugal’		71.64	77.22	74.36	**74.41 c**
‘Triumph’		82.40	85.81	86.02	**84.74 a**
Year	2010	80.26	83.13	83.00	**82.13 b**
	2011	80.61	85.79	84.69	**83.70 a**
	2012	60.41	67.24	67.87	**65.17 c**
Sucrose concentration		**73.76 b**	**78.72 a**	**78.52 a**	**77.00**

The pollen germination in our study was significantly different between years, but it was a more balanced in the first two years, amounting to over 82%. However, it was about 15-20% lower in 2012 and amounted to 65.17%. It can be connectted with the influence of low temperatures on the pollen germination in this year.

Pollen tube length was significantly different among quince cultivars, sucrose concentrations and years of study (Table 2), which is in line with the results Milatović and Nikolić (2007). An average length for all cultivars was 1768.0 µm, and it varied between cultivars from 1577.5 µm (‘Hemus’) to 2015.8 µm (‘Asenica’). At a sucrose concentration of 10%, pollen

tube length ranged from 1236.1 μm (‘Hemus’) to 1674.2 μm (‘Portugal’). ‘Hemus’ cultivar had shortest pollen tubes (1411.9 μm) in 15% sucrose solution, and the longest pollen tube lenght had ‘Asenica’ cultivar (2072.0 μm). At a sucrose concentration of 20% lowest pollen tube lenght had ‘Vranjska’ cultivar (1770.3 μm), and highest ‘Asenica’ cultivar (2574.4 μm). Pollen tube lenght in our work was significantly higher compared to the results obtained by Sharafi (2011 a, b), at the quince cultivars in Iran, what is result of genetic differences between these two groups of cultivars.

Table 2. Pollen tube length (μm) of investigated quince cultivars (average 2010-2012).

Cultivar/Year		Sucrose concentration			Mx
		10%	15%	20%	
‘Leskovacka’		1543.2	1898.5	2011.9	**1817.9 ad**
‘Vranjska’		1375.0	1797.2	1770.3	**1647.5 bcde**
‘Morava’		1371.5	1593.9	1926.2	**1630.5 cde**
‘Pazardzijska’		1528.0	1839.6	2154.0	**1840.5 ab**
‘Hemus’		1236.1	1411.9	2084.5	**1577.5 e**
‘Asenica’		1401.0	2072.0	2574.4	**2015.8 a**
‘Portugal’		1674.2	1735.2	1957.2	**1788.9 bcd**
‘Triumph’		1357.3	1977.1	2141.5	**1825.3 ac**
Year	2010	1774.0	2121.5	2300.4	**2065.3 b**
	2011	1964.5	2407.4	2798.8	**2390.2 a**
	2012	568.8	843.1	1133.2	**848.4 c**
Sucrose concentration		**1435.8 c**	**1790.7 b**	**2077.5 a**	**1768.0**

Increasing the concentration of sucrose increased the pollen tube length (from 1435.8 μm in 10%, followed by 1790.7 μm in 15% to 2077.5 μm in 20%). This trend was evident in all cultivars except the ‘Vranjska’ cultivar, in which the pollen tube length was the highest at a concentration of 15% sucrose. However, Bolat and Pirlak (1999) were determined the highest pollen tube length at sucrose concentrations of 10% and 15% at some cultivars of apricot, sweet and sour cherry. This indicates that the optimal concentration of sucrose for testing of pollen tube growth depends of fruit species.

Depending on meteorological conditions, the pollen tube length has differed by years. It was the highest in 2011, and the lowest in 2012. The occurrence of late spring frosts in April 2012 reduced the pollen tube length two to three times compared to the previous two years. This effect was present in all tested cultivars, except for ‘Asenica’ cultivar at sucrose concentrations of 15% and 20%.

Conclusion

Based on the results of three year trials, it can be concluded that the pollen germination and pollen tube length differed significantly depending on the cultivar, year and the concentration of sucrose. ‘Triumph’ and ‘Asenica’ cultivars were characterized with highest and ‘Leskovacka’ cultivar with lowest pollen germination. All studied cultivars had high pollen germination, so they can be recommended as a potentially good pollenisers in new commercial plantations of quince. Based on obtained results, the sucrose concentrations of 15% and 20% can be recommended as optimal for pollen germination of quince cultivars.

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References

Aslantas R., Pirlak L. (2002). Storage of strawberry pollen, Acta Horticulture, 567: 227-230.

Asma B.M. (2008). Determination of pollen viability, germination ratios and morphology of eight apricot genotypes, African Journal of Biotechnology, 7(23): 4269-4273.

Bolat I., Pirlak L. (1999). An investigation on pollen viability, germination and tube growth in some stone fruits, Turkish Journal of Agriculture and Forestry, 23(4): 383-388.

Bolat I., Pirlak L. (2003). Effects of three plant growth regulators and boric acid on pollen germination and tube growth in apricot (Prunus armeniaca L.), Bangladesh Journal of Botany, 32: 53-56.

Çetin M., Soylu A. (2006). Standart ayva çesitlerinin döllenme biyolojisi üzerinde arastirmalar (Studies on the fertilization biology of standard quince cultivars (Cydonia oblonga Mill.), Bahçe, 35(1-2): 83-95.

Dalkiliç Z., Mestav O. (2011). In vitro pollen quantity, viability and germination tests in quince, African Journal of Biotechnology, 10(73): 16516-16520.

Gür N., Topdemir A. (2008). Effects of some heavy metals on in vitro pollen germination and tube growth of apricot (Armeniaca vulgaris Lam.) and cherry (Cerasus avium L.), World Applied Sciences Journal, 4(2): 195-198.

Imani A., Barzegar K., Piripireivatlu S., Masomi S.H. (2011). Storage of apple pollen and in vitro germination, African Journal of Agricultural Research, 6(2): 624-629.

Kargar M.H., Imani A. (2011). Effects of fungicides on pollen germination peach and nectarine in vitro, African Journal of Plant Science, 5(11): 643-647.

Kidman C.M. Dry P.R., McCarthy M.G., Collins C. (2014). Effect of rootstock on nutrition, pollination and fertilisation in 'Shiraz' (Vitis vinifera L.), Vitis, 53(3): 139-145.

Kiliç S., Çavuşoğlu K., Kiliç M. (2009). Taşıtların sebep olduğu kurşun (Pb) kirliliğinin kayısı (Prunus armeniaca cv. Şekerpare)'da polen çimlenmesi ve polen tüpü gelişimi üzerine etkileri (The effects of lead (Pb) pollution caused by vehicles on the pollen germination and pollen tube growth of apricot (Prunus armeniaca cv. Sekerpare), Biological Diversity and Conservation, 2(3): 23-28.

Liu L., Huang L., Li Y. (2013). Influence of boric acid and sucrose on the germination and growth of areca pollen, American Journal of Plant Sciences, 4: 1669-1674.

Mahanoğlu G., Eti S., Kaşka N. (1995). Correlations between pollen quality, pollen production and pollen tube growth of some early ripening apricot cultivars, Acta Horticulturae, 384: 391-396.

Mert C. (2009). Temperature responses of pollen germination in walnut (Juglans regia L.), Journal of Biodiversity and Environmental Sciences, 3(8): 37-43.

Milatović D., Nikolić D. (2007). Klijavost polena sorti kajsije (Prunus armeniaca L.) (Pollen germination in apricot cultivars (Prunus armeniaca L.), Savremena poljoprivreda, 56(6): 108-114.

Milatović D., Nikolić D. (2014). Uticaj temperature na klijavost polena i rast polenovih cevčica sorti višnje (The effect of temperature on pollen germination and pollen tube growth of sour cherry cultivars), Journal of Agricultural Sciences, 59(1): 45-52.

Mondal S., Ghanta R. (2012). Effect of sucrose and boric acid on in vitro pollen germination of solanum macranthum dunal, Indian Journal of Fundamental and Applied Life Sciences, 2(2): 202-206.

Okusaka K., Hiratsuka S. (2009). Fructose inhibits pear pollen germination on agar medium without loss of viability, Scientia Horticulturae, 122: 51-55.

Pejkić B. (1980). Oplemenjivanje voćaka i vinove loze (Fruit and grapevine breeding), Naučna knjiga, Beograd.

Perveen A., Khan S.A. (2008). Maintenance of pollen germination capacity of Malus pumila L., (Rosaceae), Pakistan Journal of Botany, 40(3): 963-966.

Pirlak L. (2002). The effects of temperature on pollen germination and pollen tube growth of apricot and sweet cherry, Gartenbauwissenschaft, 67(2): S. 61-64.

Sharafi Y. (2011a). Study of pollen germination in pome fruit tree of Rosaceae family in vitro, African Journal of Plant Science, 5(9): 483-488.

Sharafi Y. (2011b). Investigation on pollen viability-longevity in Malus pumila L., Pyrus communis L., and Cydonia oblonga L., in vitro, Journal of Medicinal Plants Research, 5(11): 2232-2236.

Sorkheh K., Shiran B., Rouhi V., Khodambashi M. (2011). 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.

Stančević A. (1986). Dunja, mušmula i oskoruša (Quince, medlar and service tree), Nolit, Beograd.

Stančević A. (1990). Morava - a new quince cultivar, Jugoslovensko voćarstvo, 24(93): 11-16.

Stanley R.G., Linskens H.F. (1974). Pollen: biology, biochemistry, menagement, Springer-Verlag, Berlin, Heidelberg, New York.

Stösser R., Hartman W., Anvari S.F. (1996). General aspects of pollination and fertilization of pome and stone fruit, Acta Horticuturae, 423: 15-22.

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

Tosun F., Koyuncu F. (2007). Kirazlarda (Prunus avium L.) çiçek tozu çimlenmesi ve çiçek tozu çim borusu gelişimi üzerine bazi kimyasal uygulamalarin etkileri (Effects of some chemical treatments on pollen germination and pollen tube growth in sweet cherries (Prunus avium L.), Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 20(2): 219-224.

Yi W., Law S.E., Wetzstein H.Y. (2003). Pollen tube growth in styles of apple and almond flowers after spraying with pesticides, The Journal of Horticultural Science and Biotechnology, 78(6): 842-846.