POLLEN VIABILITY IN QUINCE CULTIVARS

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Abstract
Pollen viability of eight quince cultivars ('Leskovacka', 'Vranjska', 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph'), was studied in the two-year period (2011-2012). Testing of pollen viability was performed using two methods: the staining of pollen with acetocarmine (indirect method) and pollen germination *in vitro* with sucrose and agar-agar (direct method). Studied cultivars differed significantly in terms of pollen viability. The lowest percentage of stained pollen grains was detected in 'Leskovacka' cultivar (70.29%) and the highest in the cultivars 'Asenica', 'Hemus' and 'Triumph' (over 90%). Similarly to that, the lowest percentage of pollen germination was obtained in 'Leskovacka' cultivar (62.86%) and the highest in the cultivars 'Morava', 'Asenica', and 'Triumph' (over 80%). With the exception of 'Portugal' cultivar, the values of pollen viability determined by staining with acetocarmine were higher for 3-15% compared to the pollen germination *in vitro*. However, values obtained using these tho methods are highly positively correlated. On the basis of obtained results, the both methods can be recommended as reliable tests for pollen viability of quince, although priority should be given to the method of pollen germination *in vitro*, because it is more accurate. All tested cultivars are distinguished for high pollen viability, and can be successfully used as male parents in hybridization. In addition, they also can be recommended as a good pollenisers when are planting new quince orchards.

Key words: *Cydonia oblonga*, pollen staining, pollen germination *in vitro*, breeding.

Introduction

Achieving high yields and obtaining high quality fruits are main goals in production of each fruit species, including quince. In order to obtain high yields, successful pollination and fertilization are necessary. In these processes, one of the key factors is the pollen viability. Pollen viability was primarily determined by genotype. It varies between different cultivars of the same species of fruit trees (Stösser et al., 1996), and depends on rootstock on which the cultivar is grafted (Kidman et al., 2014). In addition to genetic factors, some environmental factors may also influence pollen germination (Sorkheh et al., 2011). The external factors influencing pollen germination are temperature (Chagas et al., 2008; Radović et al., 2016a), boric acid (Liu et al., 2013), plant hormones (Sotomayor et al., 2012; Radović et al., 2016b), fungicides (Kargar and Imani, 2011) and the presence of heavy metals (Gür and Topdemir, 2008). Two methods are most often used to test the pollen viability: pollen staining (indirect method) and pollen germination *in vitro* with sucrose and agar-agar (direct method). Pollen staining is a quick and cheap method in assessing pollen viability. However, Pearson (1984) states that pollen germination *in vitro* is a better indicator to evaluate pollen viability than the pollen staining. Knowledge of pollen viability is very important for breeding and production of quince. For breeders it is very important to know the functional ability of pollen before crossing. For producers it is important for the choice of appropriate pollenisers when planting new quince orchards. The aim of this study was to examine pollen viability of eight quince cultivars in order to determine the cultivars with the highest pollen viability. They will be recommended as a potentially good pollenisers when planting of quince orchards and as parents for breeding work.

Material and methods

Investigations were carried out at the Experimental field "Radmilovac" of the Faculty of Agriculture, University of Belgrade (Serbia) during the two-year period (2011-2012). The subject of this research was eight quince cultivars: 'Leskovacka', 'Vranjska', 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph'. The orchard was established in the spring of 1999, with the planting space of 4.5 m × 3 m. The examined cultivars were grafted on the rootstock 'Quince MA'. For examination of pollen viability branches with flower buds in the 'balloon' phase were taken and carried to the laboratory. In order to collect pollen anthers were isolated from the flower buds in Petri dishes. They are stored at room temperature (20ºC) for 24-48 h to dry and to release the pollen. Testing of pollen viability was performed using two methods: the staining of pollen with acetocarmine (indirect method) and pollen germination *in vitro* with sucrose and agar agar (direct method). Pollen viability was tested immediately after the anthers drying and pollen release. For the pollen staining assay, 1-2% acetocarmine solution was used. One to two drops of acetocarmine were placed on the microscope slide, and the pollen was added using special needles. Immediately after placing, pollen was covered with a cover slip and observed under light microscope 'Leica DM LS' (Leica Microsystems, Wetzlar, Germany). The experiment was done in three repetitions. In each repetition at least 300 pollen grains were analyzed. Pollen grains that were colored in pink were considered vital, while pollen grains that lack vitality were not colored at all. From the ratio of the number of colored and uncolored pollen grains, the pollen vitality was calculated. For testing of pollen germination *in vitro*, the pollen of each cultivar was sown with fine brushes in Petri dishes (9 cm diameter) on the previously prepared nutrient medium consisting of 15% sucrose and 0.7% agar-agar. After 24 hours at 20ºC, Petri dishes with a sowed pollen were observed under the light microscope 'Leica DM LS', 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. The obtained results were processed statistically using the two-factorial analysis of variance. Percentage data were subjected to arcsin square root transformation before the statistical analysis. Tukey's test (5%) was performed for means comparison. Correlations among the parameters were determined by correlation-regresion analysis and Pearson's correlation coefficients. Data analysis was performed using the statistical software package 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and discussion

Pollen staining is a quick method in assessing its viability. Vital pollen grains are pink, unlike non-functional pollen grains which do not colored at all (Figure 1). The number of stained pollen grains was quite high and significantly varied between the investigated quince cultivars. It was in the range from 70.29% in the 'Leskovacka' cultivar to 94.04% in the 'Asenica' cultivar (Table 1). In addition to the 'Asenica' cultivar, high levels of pollen staining (over 85%) were also found in the cultivars 'Morava', 'Pazardzijska', 'Hemus' and 'Triumph'. The high values of pollen staining (over 90%) were also found in some quince cultivars in Turkey (Dalkiliç and Mestav, 2011).

Table 1. Pollen staining with acetocarmine of quince cultivars (%).

Cultivar/Year	2011	2012	Mx
Leskovacka	62.81	77.78	70.29 e
Vranjska	75.57	82.43	79.00 d
Morava	86.11	87.90	87.00 bc
Pazardzijska	81.10	83.47	82.29 cd
Hemus	95.20	89.28	92.24 ab
Asenica	94.40	93.67	94.04 a
Portugal	74.73	78.94	76.83 de
Triumph	88.83	92.20	90.52 abc
Mx	82.34 b	85.71 a	-

In addition to the cultivar, the pollen staining was varied considerably between the years studied. It was slightly higher in 2012. The 'Leskovacka' cultivar had the lowest value of pollen staining in both years (62.81% - 2011 and 77.78% - 2012), while the highest values in 2011 were determined for the 'Hemus' cultivar (95.20%), and in 2012 in the 'Asenica' cultivar (93.67%).

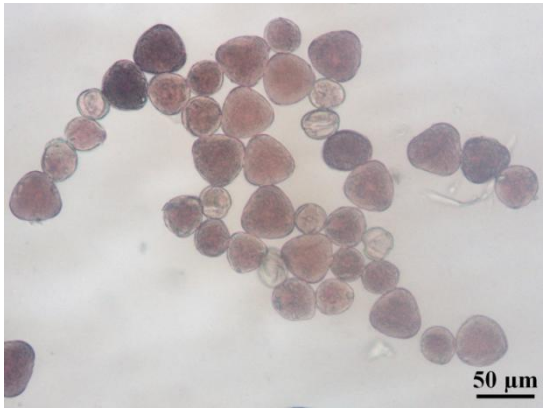

Figure 1. Pollen staining with acetocarmine of Portugal cultivar.

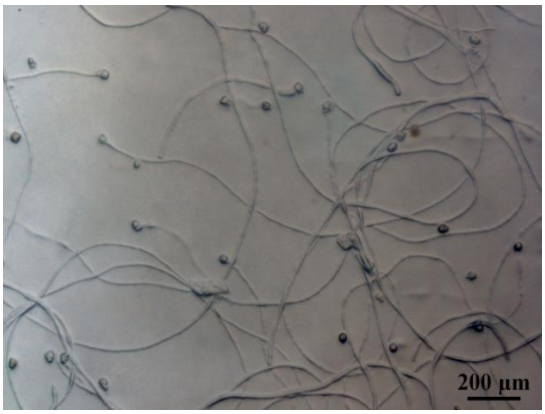

Figure 2. Pollen germination *in vitro* of Morava cultivar.

Test of pollen germination *in vitro* is one of the main indicators of pollen viability (Figure 2). Pollen germination was significantly different among the quince cultivars. In accordance with the staining of pollen, the highest pollen germination was found in 'Morava' and 'Asenica' cultivars (84.34% and 84.19%). In addition to the 'Morava' and 'Asenica' cultivars, high pollen germination had 'Triumph' cultivar (81.82%) (Table 2). On the other hand, as with the staining of pollen, pollen germination rate was the lowest in the 'Leskovacka' cultivar (62.86%). The variability of pollen germination in our work was in the range determined by Dalkiliç and Mestav (2011) in the quince cultivars in Turkey.

Table 2. Pollen germination *in vitro* of quince cultivars (%).

Cultivar/Year	2011	2012	Mx
Leskovacka	75.33	50.39	62.86 d
Vranjska	83.78	53.36	68.57 cd
Morava	90.22	78.46	84.34 a
Pazardzijska	89.89	61.61	75.75 bc
Hemus	88.81	64.70	76.75 abc
Asenica	86.03	82.35	84.19 a
Portugal	80.66	75.08	77.87 ab
Triumph	91.65	71.99	81.82 ab
Mx	85.80 a	67.24 b	-

Pollen germination significantly differed between years. Depending on the cultivar, it was for 4-30% lower in 2012. Different meteorological conditions during the development of pollen between the years studied were most likely contributed to this. Of the meterological factors, the greatest influence on pollen germination has the temperature (Pirlak, 2002; Milatović and Nikolić, 2014). In both examined years, the smallest pollen germination was determined in the 'Leskovacka' cultivar (75.33% - 2011 and 50.39% - 2012), and the highest in the cultivars 'Triumph' (91.65% - 2011) and 'Asenica' (82.35% - 2012). Pollen staining and pollen germination *in vitro* were in a strong positive correlation (r = 0.80). That is in line with previous studies (Khatun and Flowers, 1995). Therefore, both these methods can be recommended as reliable in testing of pollen viability. However, the pollen germination *in vitro* should be preferred because it is stricter and more reliable.

Conclusions

The staining of pollen with acetocarmine and pollen germination *in vitro* are very important methods in assessing pollen viability in quince. These two methods were highly positively correlated. Pollen viability significantly varied among the studied quince cultivars. It was the lowest in 'Leskovacka' cultivar, and the highest in the cultivars 'Morava', 'Asenica', and 'Hemus'. Generally, all tested cultivars are characterized by high pollen viability, and can be successfully used as male parents in hybridization and as a good pollenisers when planting new quince orchards.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia through the project TR 31063.

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