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UNUSUAL GROWTH OF POLLEN TUBES IN THE OVARY OF QUINCE (*Cydonia oblonga* Mill.)

Aleksandar Radović[1]✉, Dragan Nikolić[1], Radosav Cerović[2], Dragan Milatović[1], Boban Đorđević[1], Gordan Zec[1]

[1] University of Belgrade, Faculty of Agriculture, 11080 Belgrade, Serbia

[2] University of Belgrade, Innovation Center of Faculty of Technology and Metallurgy, 11120 Belgrade, Serbia

ABSTRACT

This paper presents the results of a three-year research (2010–2012) of unusual growth of pollen tubes in the ovary in eight cultivars of quince in two pollination variants (self-pollination and open-pollination). Unusual behavior of pollen tubes growth could be seen in all parts of the ovary, and it was most often manifested by larger or smaller branching and the formation of bundle in the ovary, without signs of further penetration of pollen tubes to the ovule. In addition, in a small number of cases, branching of the pollen tubes, as well as bypassing micropyle and forming swellings at the tips of the pollen tube was noticed. There were also the cases where the pollen tube filled embryo sac forming a bundle in it. The occurrence of unusual growth of pollen tubes in the quince ovary was primarily dependent on the genotype and pollination type. This phenomenon was more expressed in open-pollination than in self-pollination variant in all examined cultivars. Leskovacka cultivar was characterized by the highest percentage of unusual growth of pollen tubes in both variants of pollination, as follows: 13.23% (self-pollination) and 15.89% (open-pollination).

Key words: pollen tube growth, fluorescence microscopy, self-pollination, open pollination, ovule

INTRODUCTION

A very important factor for the successful growing of fruit trees is the knowledge of the cultivar's degree of fertility. From the aspect of production practice and breeding, self-fertile cultivars are of the highest value, because when growing partially self-fertile and self-incompatible cultivars it is necessary to provide adequate pollenisers [Nikolić and Milatović 2010]. The degree of fertility is affected by various factors. One of them is the unusual growth of pollen tubes in the ovary, viz. the irregular direction of pollen tube growth to the ovule. Most often such growth manifests itself by forming a swellings or branching pollen tubes in the obturator region, locula of the ovary or in the micropyle and nucellus [Cerović 1996, Herrero 2003, Hedhly et al. 2009, Đorđević et al. 2010]. Normally, such pollen tubes are stopped in these ovary regions, so that the fertilization process does not occur.

Some structures of the ovary, especially obturator and micropyle of the ovule may have an impact on the dynamics, speed and direction of pollen tube growth towards the ovule [Cerović et al. 1999, Herrero 2000, Đorđević et al. 2010]. The most significant impact on the growth of pollen tubes in the ovary has the embryo sac, or some of its elements [Herrero 2000]. It has been established that synergids actively

✉radovicaleksandar@yahoo.com

secrete attractants responsible for directing the pollen tube to the female gametophyte or moving spermatic cells that results at the joining of male and female gametes [Higashiyama et al. 2001, 2003, Punwani and Drews 2008].

Besides the genotype, environmental conditions also have a significant influence on pollen tube growth in fruit trees. Among environmental conditions, air temperature is the most important factor that influence on its growth [Cerović and Ružić 1992, Hedhly et al. 2005, De Ceault and Polito 2010].

The aim of this study was to investigate the occurrence of the unusual growth of pollen tubes in the quince ovary in variants of self-pollination and open-pollination, in order to fully understand fertilization process in this species of fruit trees.

MATERIALS AND METHODS

Plant material. 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 × 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.

Pollination and pistil fixation. The tests were performed under field conditions in two variants of pollination (self-pollination and open-pollination). In self-pollination variant, just before the flowering emasculation of flowers in all eight cultivars and removing the perianths and anthers was performed. At the beginning of full flowering (two days after the emasculation), hand pollination of emasculated flowers of each cultivar with its own pollen was carried out. At the same day when the self-pollination was carried out, the branches with flowers for testing of open-pollination were selected, where the flowers were left to freely develop. In the three successive terms (2, 4, and 6 days after pollination) the fixation of the pistils was performed, in the FAA fixative consisting of 70% ethanol, glacial acetic acid and formaldehyde in relation to 90:5:5 parts by volume. The fixed material was stored in a refrigerator at +4°C until staining.

Staining and microscopic observation of pistils. For the study of pollen tube growth in the ovary we performed their staining with 0.1% aniline blue dissolved in 0.1 N K_3PO_4 [Kho and Baër 1971]. Preparations for the experiment included separation of the style from the ovary and then the ovary was cut longitudinally with a razor blade along the suture to detect a penetration of pollen tubes in the ovule [Cerović 1997]. The examination of pistils was carried out using a fluorescent microscope Leica DM LS (Leica Microsystems, Wetzlar, Germany) with the use of filters A (wavelength 340–380 nm) and I3 (wavelength 450–490 nm).

From each cultivar, for both types of pollination, 30 pistils were analyzed. The percentage of the ovaries with the unusual growth of pollen tubes was expressed as average for all terms of fixation and displayed over the years and variants of pollination.

Statistical analysis. 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. Data analysis was performed using the statistical software package STATISTICA, Version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA).

RESULTS

Unusual growth of pollen tubes could be seen in all parts of the ovary, and most often were manifested by larger or smaller branching and the formation of bundle in the ovary, without signs of further penetration of pollen tubes to the ovule (fig. 1 a, b). In addition, in a small number of cases pollen tube branching in the ovary was noticed (fig. 1 c), as well as bypassing micropyle and forming swellings at the tips of the pollen tubes (fig. 1 d, e). The occurrence where the pollen tube fills an embryo sac forming a bundle of embryos was also noticed (fig. 1 f). In these cases fertilization does not occur, although the pollen tubes are present in different parts of the ovary, and even in the embryo sac.

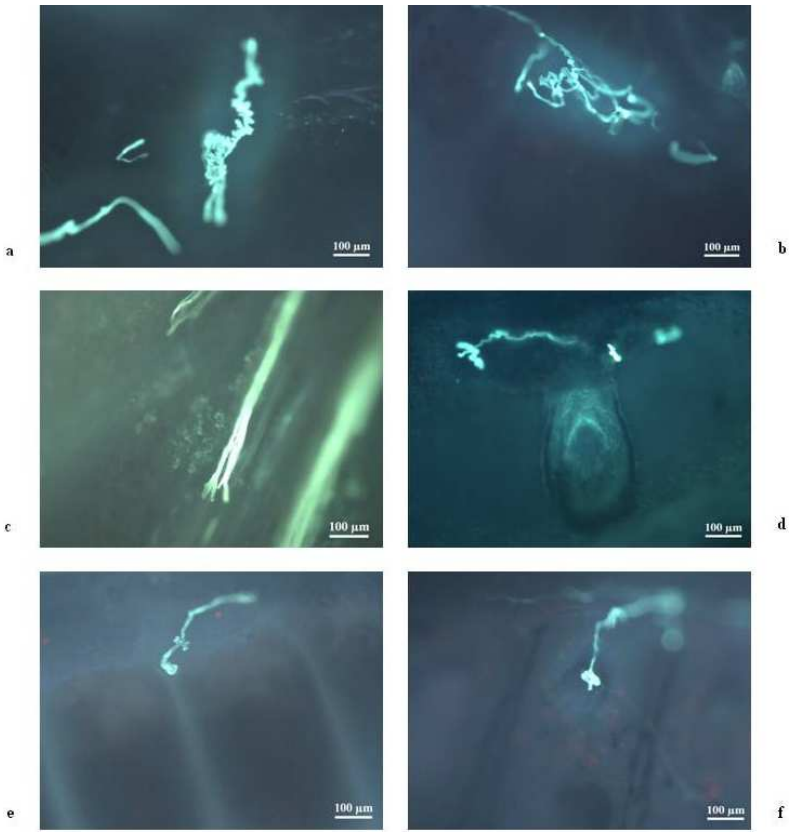
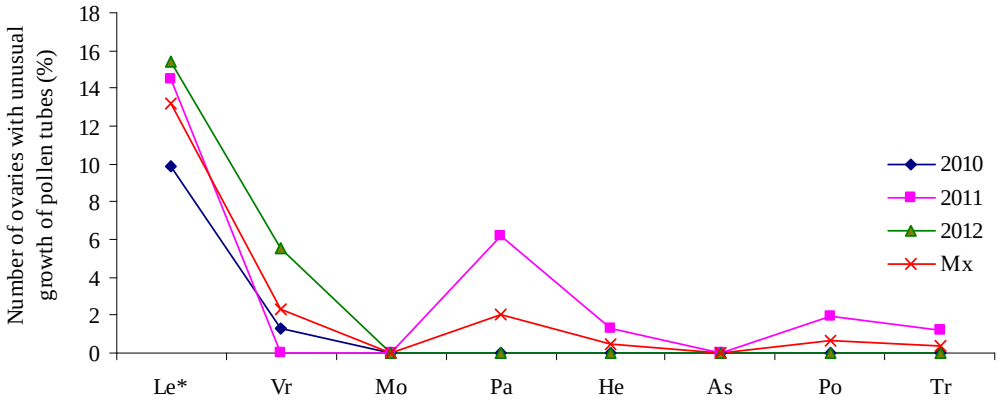


Fig. 1. Unusual growth of pollen tubes in the ovary of quince in the variants of self-pollination (a, c, e), and open-pollination (b, d, f): a) Leskovacka cultivar – tortous growth of pollen tubes, b) Pazardzijska cultivar – the boundle of pollen tubes, c) Leskovacka cultivar – branching of the pollen tubes, d) Hemus cultivar – pollen tube bypassing the micropyle, e) Vranjska cultivar – pollen tube bypassed the micropyle forming swellings at the tip, f) Hemus cultivar – pollen tube fills embryo sac forming a bundle

Table 1. Analysis of varianse for percentage of ovaries with the occurrence of unusual growth of pollen tubes in cultivars of quince

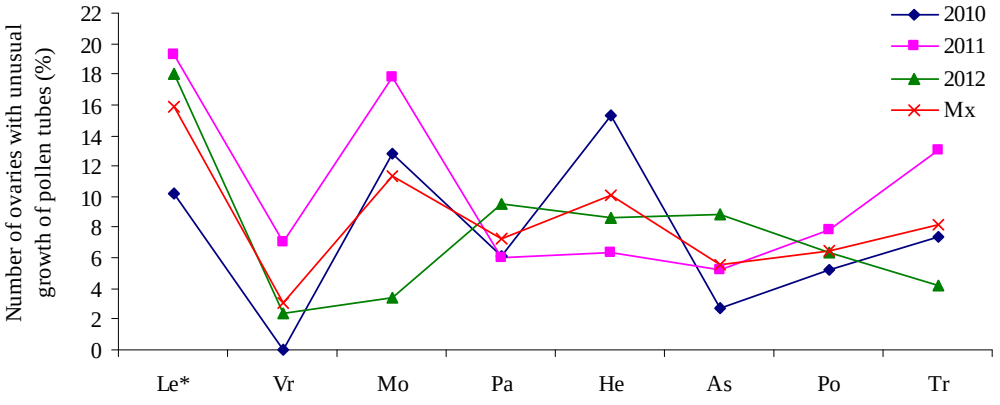
Sources of variation	SS	df	MS	F
Cultivar (A)	1786.288	7	255.184	3.803**
Variant of pollination (B)	1423.071	1	1423.071	21.210**
Year (C)	87.116	2	43.558	0.649ns
A × B	364.142	7	52.020	0.775ns
A × C	473.258	14	33.804	0.504ns
B × C	33.328	2	16.664	0.248ns
A × B × C	460.628	14	32.902	0.490ns
Error	6440.995	96	67.094	

** $p < 0.01$, ns Not significant



*Le-Leskovacka; Vr-Vranjska; Mo-Morava; Pa-Pazardzijska;He-Hemus; As-Asenica; Po-Portugal; Tr-Triumph

Fig. 2. The percentage of ovaries with the occurrence of unusual growth of pollen tubes in cultivars of quince in the variant of self-pollination



*Le-Leskovacka; Vr-Vranjska; Mo-Morava; Pa-Pazardzijska; He-Hemus; As-Asenica; Po-Portugal; Tr-Triumph

Fig. 3. The percentage of ovaries with the occurrence of unusual growth of pollen tubes in cultivars of quince in the variant of open-pollination

The occurrence of the unusual growth of pollen tubes in the ovary differed significantly depending on the cultivar and variant of pollination (tab. 1). Thus, in the variant of self-pollination the unusual growth of pollen tubes was the most observed in Leskovacka cultivar – in 13.23% ovaries (fig. 2). However, in other cultivars this phenomenon was much less noticeable.

In contrast to self-pollination, the occurrence of the unusual growth of pollen tubes was significantly more noticeable in the variant of open-pollination (fig. 3). As in the variant of self-pollination, the unusual growth of pollen tubes was the most frequent in Leskovacka cultivar (in 15.89% of ovaries) also in the variant of open-pollination. In addition to the Leskovacka cultivar, this phenomenon was often observed in Morava (11.39%) and Hemus (10.07%) cultivars. On the other hand, the occurrence of the unusual growth of pollen tubes was the

least noticeable in cultivar Vranjska (3.11% of ovaries).

Unlike the cultivars and variants of pollination, the occurrence of the unusual growth of pollen tubes in the ovary was not different between years of research (tab. 1). However, in most of the cultivars this phenomenon was the most pronounced in 2011, in both variants of pollination (figs 2, 3). In this year, irregular growth of pollen tubes was the most present in the Leskovacka cultivar (14.47% of the ovaries in self-pollination and 19.32% of the ovaries in open-pollination).

DISCUSSION

By the analysis of pollen tube growth in the ovary, unusual growth was determined and quantified in our research. It was most frequently characterized by atypical, undirected growth, as well as frequent changes in the direction of pollen tube growth in the ovary, which were consistent with the findings of Herrero [2003]. Some authors have found that the occurrence of the unusual growth of pollen tubes in the ovary was more emphasized in self-pollination when compared to open-pollination [Cerović 1997, Đorđević et al. 2010]. In contrast to these results, in our work the unusual growth of pollen tubes was more emphasized in open-pollination compared to self-pollination. The reason for this is primarily the occurrence of incompatibility reaction in the style, where a very small number of pollen tubes arrived to the ovary after self-pollination. Otherwise, the occurrence of a larger number of the ovaries with signs of the unusual growth of pollen tubes was the most common in Leskovacka cultivar which can be explained by genotype specificity related to this phenomenon.

In our experiment, unusual growth of pollen tubes indirectly indicated the presence of mechanism which regulating pollen tube growth in the ovary. Also, in some plant species, as one of the causes of the unusual growth of pollen tubes is the formation of flowers with abnormalities of embryo sacs, such as, partial degeneration of synegids, forming three synergids or deformation of synergids [Kaufmane and Rumpunen 2002]. In some other plant species have been established the existence of the mechanism and the appropriate attractant, responsible for control the final stages of pollen tube growth in the ovary locule [Márton et al. 2005, Dresselhause and Márton 2009]. Research in this direction in fruit trees, and thus in quince, could accurately explain the phenomenon of the unusual growth of pollen tubes in the ovary and the complex interaction between male gametophyte and female sporophyte.

CONCLUSIONS

The phenomenon of the unusual growth of pollen tubes in the ovary of the examined quince cultivars lead to the lack of fertilization in a certain percentage of ovules, which as a result has a lower fruit set, and lower yields. Therefore, during the establishment of commercial quince orchards one must take into the account this problem in the biology of quince fertilization, particularly in the selection of appropriate cultivars for growing.

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REFERENCES

Cerović, R. (1996). Unusual behaviour of growing pollen tubes in the ovary of sour cherry. Acta Hort., 423, 171–176.

Cerović, R. (1997). Biologija oplodnje višnje. Zadužbina Andrejević, Beograd.

Cerović, R., Ružić, Đ. (1992). Pollen tube growth in sour cherry (*Prunus cerasus* L.) at different temperatures. J. Hort. Sci., 67(3), 333–340.

Cerović, R., Vujičić, R., Mićić, N. (1999). Localization of polysaccharides in the ovary of sour cherry. Gartenbauwissenschaft, 64, 40–46.

De Ceault, M.T., Polito, V.S. (2010). High temperatures during bloom can inhibit pollen germination and tube growth, and adversely affect fruit set in the *Prunus do-*

mestica cultivars 'Improved French' and 'Muir Beauty'. Acta Hort., 874, 163–168.

Dresselhaus, T., Márton, M.L. (2009). Micropylar pollen tube guidance and burst: adapted from defence mechanisms? Curr. Opin. Plant Biol., 12, 773–780.

Đorđević, M., Cerović, R., Nikolić, D. Radičević, S. (2010). Unusual behavior of growing pollen tubes in the ovary of plum culture (*Prunus domestica* L.). Arch. Biol. Sci., 62, 137–142.

Hedhly, A., Hormaza, J.I., Herrero, M. (2005). The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. Plant Biol., 7(5), 476–483.

Hedhly, A., Hormaza, J.I., Herrero, M. (2009). Flower emasculation accelerates ovule degeneration and reduces fruit set in sweet cherry. Sci. Hort., 119, 455–457.

Herrero, M. (2000). Changes in the ovary related to pollen tube guidance. Ann. Bot., 85, 79–85.

Herrero, M. (2003). Male and female synchrony and the regulation of mating in flowering plants. Philos. Trans. Royal Soc. B, Biol. Sci., 358, 1019–1024.

Higashiyama, T., Yabe, S., Sasake, N., Nishimura, Y., Miyagishima, S., Kuroiwa, H., Kurowia, T. (2001). Pollen tube attraction by synergid cell. Science, 93, 1480–1483.

Higashiyama, T., Kuroiwa, H., Kuroiwa, T. (2003). Pollen-tube guidance: beacons from the female gametophyte. Curr. Opin. Plant Biol., 6, 36–41.

Kaufmane, E. Rumpunen, K. (2002). Sporogenesis and gametophyte development in *Chaenomeles japonica* (Japanese quince). Sci. Hortic., 94, 241–249.

Kho, Y.O., Baër, J. (1971). Fluorescence microscopy in botanical research. Zeiss Inform., 76, 54–57.

Márton, M.L., Cordts, S., Broadhvest, J., Dresselhaus, T. (2005). Micropylar pollen tube guidance by egg apparatus 1 of maize. Science, 307, 573–576.

Nikolić, D., Milatović, D. (2010). Examining self-compatibility in plum (*Prunus domestica* L.) by fluorescence microscopy. Genetika, 42, 387–396.

Punwani, J.A., Drews, G.N. (2008). Development and function of the synergid cell. Sex Plant Reprod., 21, 7–15.