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Research Article

Incompatible pollen tubes in the quince style and their impact on fertilization success

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Abstract

Self-incompatibility presents one of the challenges in modern fruit production. It can be correlated with a lower yield of self-incompatible cultivars which also demand the planting of the pollinisers. The aim of this work was to investigate the phenomenon of incompatibility and its influence on fertilization success in quince (*Cydonia oblonga* Mill.), using the cultivars 'Leskovacka', 'Vranjska', 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph'. Incompatible pollen tubes were determined by using fluorescence microscopy. In two types of pollination (self-pollination and open pollination) pollen tubes showed signs of incompatibility, mostly in the upper third of the style. The most common sign of incompatibility is the formation of swelling at the tip of a pollen tube. Also, sometimes twisted, bifurcated, and short and thickened pollen tubes along their entire length are formed. The incompatibility was significantly more pronounced in the self-pollination than in the open pollination variant in all tested cultivars. The highest number of incompatible pollen tubes in both pollination types was present in the cultivar 'Pazardzijska', while the lowest number was present in the cultivars 'Leskovacka' and 'Vranjska'. The appearance of incompatibility affected the degree of fertilization in quince. The results showed that only 'Leskovacka' and 'Vranjska' are self-compatible, while other studied cultivars ('Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph') are self-incompatible. The results provide a good background for the future research of reproductive biology and also for adequate management of the quince orchards.

Keywords: *Cydonia oblonga*; fluorescence microscopy; open pollination; self-(in) compatibility; self-pollination

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Introduction

Self-incompatibility in flowering plants is a phenomenon of great evolutionary significance, which prevents self-fertilization and promotes cross-fertilization (Nettancourt, 2001; Frankling-Tong and Franklin, 2003). Gametophytic incompatibility is present in many fruit tree species, including quince. It is controlled by one gene with a large number of alleles called incompatibility alleles or *S* alleles. Fertilization occurs only in cases of compatible pollination when the pollen grain contains different *S* alleles than those in the pistil (Newbigin *et al*., 1993).

The wall of the pollen tube consists of two main layers of polysaccharides: the inner "callose" wall, which contains predominantly β-1,3-glucan, and the outer wall, which contains predominantly arabinan. The inner glucan layer fluoresces intensely when stained with fluorochrome dyes, such as aniline blue. This reveals transverse callose walls in pollen tubes containing sperm cells and other cytoplasmic elements of the pollen grain (Evans and Hoyne, 1982; Rae *et al*., 1985). Greater accumulation of callose in the tips of pollen tubes inhibits their growth through the style, which may show a marked difference between incompatible and compatible pollen tubes (Nettancourt, 2001). The distribution and amount of callose in the walls of pollen tubes vary depending on the species and external factors. It is sometimes distributed along the entire length of the tube, while sometimes it is localized in densely distributed plugs. It was also found that the amount of callose is much higher in self-pollination compared to controlled pollination (Halász and Hegedûs, 2006).

The way of the growth of incompatible pollen tubes at the beginning is similar to that of pollen tubes in compatible pollination, but at some stage, the growth becomes irregular. The walls become thicker and the tip thickened, while the growth of compatible tubes remains unchanged along the entire length of the style, so it can reach the ovary (Newbigin *et al*., 1993). The most significant sign of incompatibility is the formation of characteristic swellings at the tips of pollen tubes during their growth through the style (Nikolić and Milatović, 2010; Milatović *et al*., 2010). In addition to the expanded tip, other signs of incompatibility can be observed, such as the formation of loops and bifurcation of the pollen tube (Milatović *et al*., 2013a).

Self-incompatibility is most common in fruit tree species of the genus Prunus, especially in cherries and almonds (Milatović and Nikolić, 2007). Worldwide, there is almost no research addressing with the problem of self-incompatibility in quince, especially with more reliable methods, which include, among others, the method of fluorescence microscopy. In contrast to quince, many researchers have studied self-incompatibility with this method in various other fruit tree species. Thus, using the fluorescent microscopy method, has been used to determine incompatibility in apples (Adachi *et al*., 2009), pears (Jacquemart *et al*., 2006; Sanzol and Herrero, 2007), Japanese quince (Kaufmane and Rumpunen, 2002), European plums (Nikolić and Milatović, 2010), Japanese plums (Ontivero *et al*., 2006; Jia *et al*., 2008), apricots (Andrés and Durán, 1998; Milatović and Nikolić, 2007; Milatović *et al*., 2013a, b), cherries (Lansari and Iezzoni, 1990), almonds (Certal *et al*., 2002; Dicenta *et al*., 2002; Alonso and Sociasi Company, 2005a; Čolić *et al*., 2010).

In fruit growing practice, self-incompatible cultivars are undesirable because they cannot be grown in a single cultivar orchard, so it is necessary to provide suitable pollinisers. Also, self-incompatible cultivars often give lower yields, because fruit setting in them depends on the amount of pollen transfer from other cultivars (Milatović and Nikolić, 2007). The aim of this work was to examine the occurrence and characteristics of incompatible pollen tubes in the style of quince in self-pollination and open-pollination variants, in order to examine its impact on fertilization success.

Materials and Methods

Study area

The research was conducted at the experimental field “Radmilovac” of the Faculty of Agriculture - University of Belgrade, Serbia (Figure 1). The distance (airline) from the centre of Belgrade (Serbia) is 11.5

km (southeast); 320 km southwest of Cluj-Napoca (Romania). The climate is temperate continental, with an average annual temperature of 11.7 °C, an average temperature during the growing season (April-October) of 17.5 °C and a total annual precipitation of 693.9 mm.

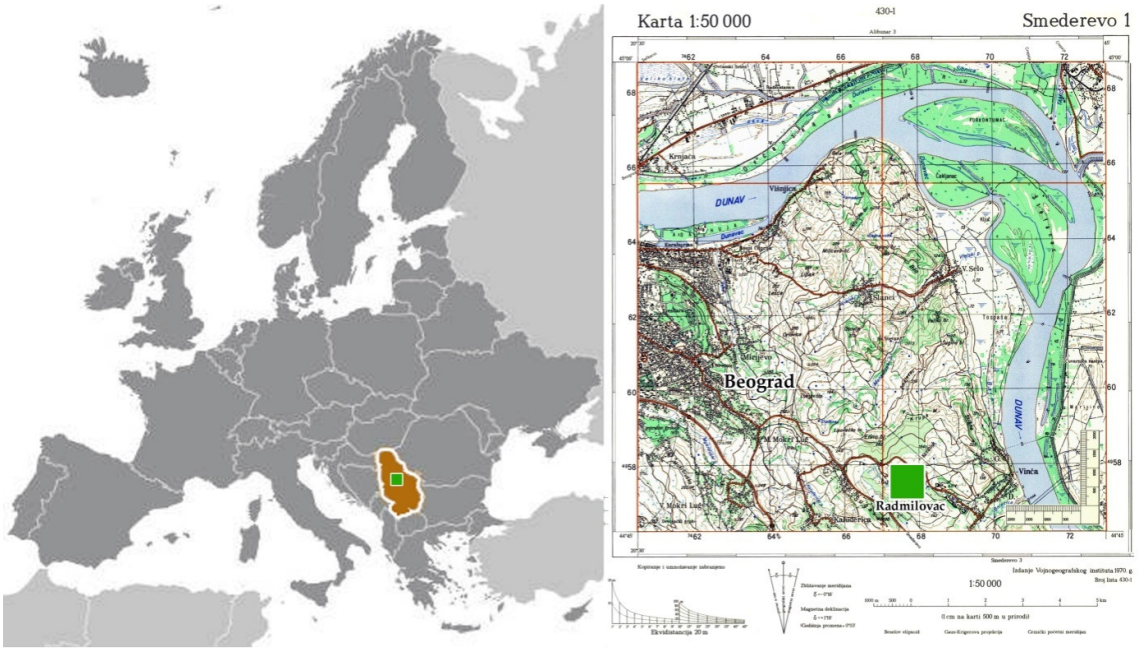

Figure 1. The study area: Experimental filed "Radmilovac" of the University of Belgrade - Faculty of Agriculture, Serbia (44°45'22.0"N 20°35'09.1"E / 44.756109, 20.585872)

Plant material

Plant material was provided from the quince collection of the Experimental farm "Radmilovac" (the University of Belgrade, Faculty of Agriculture) (Figure 2). Eight cultivars of the quince (*Cydonia oblonga* Mill.) were examined: 'Leskovacka', 'Vranjska', 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph'. All cultivars were grafted on MA quince rootstock and planted at 4.5 × 3 m distance. The study was done at a time when plants reached their full production potential.

Pollination and pistil fixation

Two variants of pollination were examined - self-pollination and open-pollination. In the variant of self-pollination, the perianths and anthers were removed just before the flowering time. During full flowering (2 days after removing the perianths and anthers), each cultivar was hand pollinated with its own pollen. Isolation bags were not used because the flowers were emasculated and consequently not visited by insects. In addition, the use of isolation bags can increase the temperature inside the bag and lead to low fertilization (Vuletin Selak *et al*., 2011). On the same day when self-pollination was performed, branches with flowers in anthesis in the open pollination variant were marked for sampling. The fixation of the pistils was done on the 2nd, 4th and 6th day after pollination. The FAA fixative (70% ethanol: glacial acetic acid: formaldehyde = 90:5:5 v/v/v) was used for fixation and the fixed pistils were stored at +4 °C.

Figure 2. Quince orchard in "Radmilovac" of the University of Belgrade - Faculty of Agriculture, Serbia

Staining and microscopic observation of pistils

Sample preparation for microscopic observation was done by pistil softening in 8N NaOH for 12 hours. After that, the staining was done with 0.1% aniline blue dissolved in 0.1 N K_3PO_4 (Kho and Baër, 1971). Before microscopic observation, the style was separated from the ovary, which was cut with the razor blade along the suture. Each stigma and style of the flower were examined. The microscopic observation was done with a fluorescent microscope Leica DM LS (Leica Microsystems, Wetzlar, Germany) with filters A (wavelength 340-380 nm) and I3 (wavelength 450-490 nm). For both types of pollination, 30 pistils per variant and per cultivar were observed.

The number of incompatible pollen tubes was determined and expressed as a percentage of the total number of pollen tubes in the upper third of the style. The results are presented as the average number of the three fixation periods. In addition, the percentage of fertilization, i.e., the number of pistils with pollen tubes reaching the nucellus on the sixth day after pollination, was determined.

Results

When studying the growth of pollen tubes through the pistil of quince cultivars, the presence of pollen tubes with typical signs of incompatibility was found in both pollination types (self-pollination and open pollination). These incompatibilities occurred mostly in the upper third of the style, much less in the middle third, and least in the lower third of the style, where their growth ceased. The most characteristic sign of incompatibility was the formation of typical swellings at the tips of pollen tubes due to a higher accumulation of callose (Figure 3). During the examination, other signs of incompatibility were also observed, such as the appearance of short and thickened pollen tubes along the entire length of the pistil (Figure 4) and the formation of twisted and bifurcated pollen tubes (Figures 5 and 6). The number of incompatible pollen tubes

was significantly different among pollination variants (Table 1). It was more expressed in the self-pollination variant.

Table 1. Analysis of the variance for percentage of the incompatible pollen tubes and fertilization percentage of the quince cultivars

Sources of variation	Percentage of the incompatible pollen tubes				Fertilization percentage			
	SS	df	MS	F	SS	df	MS	F
Cultivar (A)	1.805	7	0.258	0.92866ns	15615.64	7	2230.81	11.1021**
Variant of pollination (B)	1.444	1	1.444	5.20162*	8983.18	1	8983.18	44.7068**
Year (C)	0.100	2	0.050	0.1804 ns	461.62	2	230.81	1.1487 ns
A x B	0.488	7	0.070	0.25101ns	2219.07	7	317.01	1.5777 ns
A x C	0.767	14	0.055	0.19721ns	2430.94	14	173.64	0.8642 ns
B x C	0.572	2	0.287	1.0319 ns	58.46	2	29.23	0.1455 ns
A x B x C	0.629	14	0.046	0.16192ns	952.74	14	68.05	0.3387 ns
Error	26.650	96	0.278		19289.80	96	200.94	

* p<0.05; ** p<0.01; ns Not significant

In the self-pollination variant, the highest number of incompatible pollen tubes in the style (Table 2) was found in the cultivar 'Pazardzijska' (5.75%), and the lowest in the cultivars 'Leskovacka' (1.62%) and 'Vranjska' (2.01%).

Table 2. Occurrence of the incompatible pollen tubes (%) in relation to the total number of pollen tubes in different regions of the style in quince cultivars in self-pollination variant

Style region[1]	Incompatibility type	Cultivar /Year							
		'Leskovacka'				'Vranjska'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	1.03	1.42	1.17	1.21	0.85	2.23	0.66	1.25
	Twisted pollen tube	0.00	0.00	0.17	0.06	0.00	0.00	0.05	0.02
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stm	Swellings at the tips	0.46	0.13	0.26	0.28	0.26	0.90	0.09	0.42
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.11	0.06	0.04	0.07	0.07	0.72	0.19	0.33
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		1.60	1.61	1.64	1.62	1.18	3.85	0.99	2.01

Style region[1]	Incompatibility type	Cultivar /Year							
		'Morava'				'Pazardzijska'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	1.44	3.29	2.02	2.25	7.81	4.19	4.63	5.54
	Twisted pollen tube	0.12	0.60	0.08	0.27	0.00	0.15	0.00	0.05
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stm	Swellings at the tips	0.72	0.22	0.21	0.38	0.37	0.05	0.05	0.16
	Twisted pollen tube	0.12	0.00	0.00	0.04	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.00	0.15	0.00	0.05	0.00	0.00	0.00	0.00
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		2.40	4.26	2.31	2.99	8.19	4.39	4.68	5.75

Style region[1]	Incompatibility type	Cultivar /Year							
		'Hemus'				'Asenica'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	3.53	3.86	4.11	3.83	3.92	5.93	2.98	4.28
	Twisted pollen tube	0.12	0.00	0.04	0.06	0.07	0.04	0.04	0.05
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01
Stm	Swellings at the tips	2.07	0.26	0.35	0.90	0.91	0.23	0.62	0.59
	Twisted pollen tube	0.06	0.00	0.00	0.02	0.07	0.00	0.04	0.04
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.24	0.05	0.00	0.10	0.00	0.15	0.00	0.05
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		6.03	4.18	4.50	4.90	4.98	6.35	3.71	5.01
Style region[1]	Incompatibility type	Cultivar /Year							
		'Portugal'				'Triumph'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	2.17	2.51	1.31	1.99	5.63	3.17	3.83	4.21
	Twisted pollen tube	0.40	0.16	0.28	0.28	0.07	0.00	0.00	0.02
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stm	Swellings at the tips	1.77	0.84	0.97	1.19	1.61	0.00	0.76	0.79
	Twisted pollen tube	0.40	0.04	0.06	0.17	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.40	0.40	0.00	0.27	0.00	0.00	0.00	0.00
	Twisted pollen tube	0.08	0.04	0.06	0.06	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		5.22	3.98	2.68	3.96	7.31	3.17	4.58	5.02

[1] Style region: Stu: upper third of the style; Stm: middle third of the style; Bs: base of the style

Table 3. Occurrence of the incompatible pollen tubes (%) in relation to the total number of pollen tubes different regions of the style in quince cultivars in open pollination variant

Style region[1]	Incompatibility type	Cultivar /Year							
		'Leskovacka'				'Vranjska'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	0.51	1.37	1.15	1.01	0.28	1.82	0.61	0.90
	Twisted pollen tube	0.13	0.04	0.14	0.10	0.08	0.08	0.08	0.08
	Bifurcation of pollen tube	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.00
Stm	Swellings at the tips	0.00	0.08	0.04	0.04	0.24	0.55	0.27	0.35
	Twisted pollen tube	0.00	0.00	0.11	0.04	0.08	0.04	0.00	0.04
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.00	0.08	0.04	0.04	0.08	0.30	0.00	0.13
	Twisted pollen tube	0.00	0.00	0.04	0.01	0.00	0.04	0.00	0.01
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		0.64	1.60	1.51	1.25	0.75	2.84	0.96	1.51
Style region[1]	Incompatibility type	Cultivar /Year							
		'Morava'				'Pazardzijska'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	0.73	3.21	1.74	1.89	1.70	5.12	3.24	3.35
	Twisted pollen tube	0.00	0.08	0.03	0.04	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.04	0.00	0.01	0.00	0.04	0.00	0.01
Stm	Swellings at the tips	0.16	0.13	0.43	0.24	0.08	0.04	0.27	0.13
	Twisted pollen tube	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0,00

	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.04	0.13	0.03	0.07	0.00	0.00	0.19	0.06
	Twisted pollen tube	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		0.93	3.68	2.24	2.28	1.78	5.21	3.69	3.56
Style region[1]	Incompatibility type	Cultivar /Year							
		'Hemus'				'Asenica'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	0.84	1.25	3.82	1.97	0.49	1.76	1.80	1.35
	Twisted pollen tube	0.00	0.09	0.00	0.03	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stm	Swellings at the tips	0.39	0.04	0.45	0.29	0.23	0.29	0.18	0.23
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.39	0.04	0.36	0.26	0.13	0.11	0.12	0.12
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		1.61	1.43	4.64	2.56	0.86	2.17	2.10	1.71
Style region[1]	Incompatibility type	Cultivar /Year							
		'Portugal'				'Triumph'			
		2010	2011	2012	Mx	2010	2011	2012	Mx
Stu	Swellings at the tips	1.14	0.59	1.27	1.00	1.17	2.33	1.75	1.75
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.01
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stm	Swellings at the tips	0.55	0.43	0.28	0.42	0.16	0.43	0.58	0.39
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.01
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bs	Swellings at the tips	0.14	0.32	0.04	0.17	0.16	0.34	0.24	0.25
	Twisted pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Bifurcation of pollen tube	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ		1.82	1.35	1.59	1.59	1.48	3.19	2.58	2.42

[1] Style region: Stu: upper third of the style; Stm: middle third of the style; Bs: base of the style

The highest number of incompatible pollen tubes in the self-pollination variant was found in the upper third of the style in all cultivars. The most characteristic sign of incompatibility in the upper third of the style in all tested cultivars was the appearance of swellings at the tips of pollen tubes, which were most pronounced in cultivar 'Pazardzijska' (5.54%), and least pronounced in cultivars 'Leskovacka' (1.21%) and 'Vranjska' (1.25%).

Compared to the upper third of the style, the number of incompatible pollen tubes with swelling at the tips was lower in the middle third of the style and ranging from 0.16% ('Pazardzijska') to 1.19% ('Portugal'). The decrease in the number of incompatible pollen tubes with swellings at the tips continued towards the base of the style, so that no signs of incompatibility were observed in the lower part of the style in the cultivars 'Pazardzijska' and 'Triumph'. The reason for this is that in these two cultivars, the growth of the pollen tubes stopped mainly in the upper third of the style.

The occurrence of twisted pollen tube growth was most pronounced in the upper third of the style, but was much less pronounced compared to the swellings at the tips. They occurred to the greatest extent in the cultivars 'Portugal' (0.28%) and 'Morava' (0.27%), and in the least (0.02%) in the cultivars 'Vranjska' and 'Triumph'. The occurrence of bifurcation of the pollen tube (0.01%) in the upper third of the style was also observed in the variety 'Asenica'.

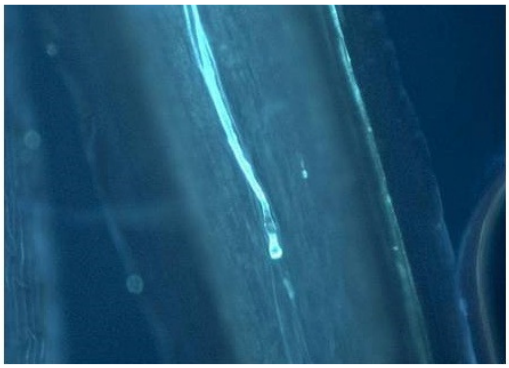

Figure 3. Pollen tube with swelling at the tip in the middle third of the style of the 'Hemus' cultivar - self-pollination

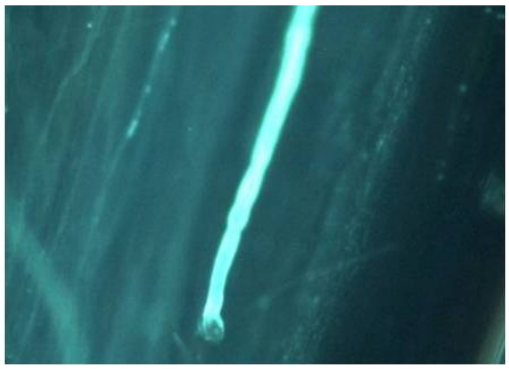

Figure 4. Thickened pollen tube along its entire length in the upper third of the style of the 'Pazardzijska' cultivar - self-pollination

Figure 5. Twisted pollen tube in the upper third of the style of the 'Portugal' cultivar - self-pollination

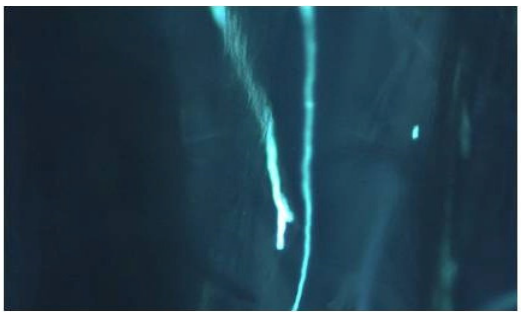

Figure 6. Pollen tube with a bifurcation in the upper third of the style of the 'Triumph' cultivar - self-pollination

The occurrence of incompatibility was less pronounced in the open pollination variant (Table 3) compared to self-pollination. The number of incompatible pollen tubes in the open pollination variant was highest in the cultivar 'Pazardzijska' (3.56%), and the least in the 'Leskovacka' cultivar (1.25%). The largest number of incompatible pollen tubes was found in the upper third of the style, as in the case of self-pollination, with swelling predominating at the top of the pollen tube. Also, in the upper third of the style, the highest number of pollen tubes with swelling at the tips was observed in the cultivar 'Pazardzijska' (3.35%) and the lowest in the cultivars 'Vranjska' (0.90%) and 'Leskovacka' (1.01%).

Going towards the middle and lower part of the style, the number of incompatible pollen tubes decreased in all cultivars. Thus, the highest number of incompatible pollen tubes with swellings at the tips was found in the cultivar 'Portugal' (0.42%) in the middle third and in 'Hemus' (0.26%) in the lower third of the style, and the lowest number (0.04%) was found in the cultivar 'Leskovacka' in both parts of the style.

The occurrence of twisted pollen tubes was most pronounced in the upper third of the style in all cultivars, but to the greatest degree in the 'Leskovacka' cultivar (0.10%), while no twisted pollen tubes were observed on the pistils of cultivars 'Pazardzijska', 'Asenica' and 'Portugal'. In cultivars 'Leskovacka', 'Morava' and 'Pazardzijska', the occurrence of bifurcation of the pollen tube in the upper third of the pistil was determined in 0.01% of the pollen tubes.

The percentage of fertilization, i.e., the number of pistils with pollen tubes reaching the nucellus on the 6th day after pollination, differed significantly between cultivars and pollination variants (Table 1). It was higher in all cultivars in the open pollination variant (Table 4).

Table 4. Fertilization percentage - number of pistils with pollen tubes reaching the nucellus on day 6 after pollination (%)

Cultivar	Self-pollination				Open pollination			
	2010	2011	2012	Mx	2010	2011	2012	Mx
'Leskovacka'	42.86	40.74	36.67	40.09	44.00	43.33	40.63	42.65
'Vranjska'	5.88	8.33	30.00	14.74	15.38	10.00	32.00	19.13
'Morava'	0.00	0.00	3.33	1.11	18.18	29.03	33.33	26.85
'Pazardzijska'	0.00	3.23	0.00	1.08	30.43	23.08	17.86	23.79
'Hemus'	0.00	0.00	0.00	0.00	18.18	13.04	24.24	18.49
'Asenica'	0.00	0.00	0.00	0.00	4.00	12.50	25.81	14.10
'Portugal'	0.00	0.00	0.00	0.00	8.33	21.43	23.53	17.76
'Triumph'	0.00	0.00	0.00	0.00	26.09	20.69	15.15	20.64

In the self-pollination variant, the highest percentage of fertilization was found in the 'Leskovacka' (40.09%) and 'Vranjska' (14.74%) cultivars. In the 'Morava' and 'Pazardzijska' cultivars, a very low percentage of fertilization was found (1.11% and 1.08%, respectively). However, no signs of fertilization were registered in the other cultivars. In the open pollination variant, the highest percentage of fertilization was also determined in the 'Leskovacka' cultivar (42.65%), and the lowest in the 'Asenica' cultivar (14.10%).

Discussion

An earlier study on the same cultivars included a detailed analysis of the number of pollens tubes in the pistil and style, but also of the dynamics of pollen tube growth by pistil regions. Based on these results, 'Leskovacka' and 'Vranjska' were found to be self-compatible, while other cultivars ('Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph') were self-incompatible (Radović *et al*., 2020). The study of incompatible pollen tubes in quince is a continuation of previous studies with the aim of analysing in detail the phenomenon of incompatibility in quince in two pollination modes (self-pollination and open pollination).

In our study, the occurrence of incompatible pollen tubes was found in both pollination modes, but to a much higher extent in the self-pollination variant compared to the open pollination. Our results are in agreement with those of Đorđević *et al*. (2014). The authors studied the European plum cultivar 'Čačanska lepotica' in three pollination variants (self-pollination, cross-pollination and open pollination) and found the highest number of incompatible pollen tubes in the variant with self-pollination.

Incompatible pollen tubes occurred most frequently in the cultivar 'Pazardzijska' and least frequently in the cultivar 'Leskovacka'. The most characteristic sign of incompatibility in our work was the formation of swellings at the tips of pollen tubes due to a larger accumulation of callose. These phenomena were most pronounced in the upper third of the style, which was experimentally confirmed in pears (Sanzol and Herrero, 2007), plums (Nikolić and Milatović, 2010) and sour cherry (Tobutt *et al*., 2004). During the growth of pollen tubes through the style, in addition to the formation of swellings at the tips, the formation of twisted tubes and bifurcation of the pollen tube was also noticed, but to a much lesser degree, which is in accordance with the results of Milatović *et al*. (2013a). This was also observed in plums (Nikolić and Milatović, 2010), and almonds (Čolić *et al*., 2010).

In apricot, it was established that pollen tubes stop their growth most often in the lower half of the style, i.e., at about 3/4 of the length of the style (Andrés and Durán, 1998; Milatović and Nikolić, 2007; Milatović *et al*., 2013a). In contrast, Čolić *et al*. (2010) found that in almonds, pollen tubes stop their growth just below the stigma or in the upper quarter of the style.

The cultivar is considered self-compatible if the pollen tubes during self-pollination reach the ovary in at least 25-30% of the pistils (Lopez *et al*., 2001). Based on the above, the cultivars 'Vranjska' and 'Leskovacka' are classified as self-compatible, while the other cultivars are classified as self-incompatible (Radović *et al*., 2020). Milatović and Nikolić (2007) and Milatović *et al*. (2013b) consider as self-fertile those apricot cultivars in which at least one pollen tube reached the pistil in most of the analysed pistils. Similarly, Alonso and Sociasi Company (2005b) consider almond cultivars in which pollen tubes in more than 50% of the pistils have reached the base of the pistil as self-fertile, and those with less than 25% of the pistils to be non-self-fertile. However, the authors consider cultivars in which the pollen tubes reached the base of the style in 25-50% the pistils as uncertain in terms of self-fertility.

Many authors reported great differences between quince cultivars in terms of their self-fertility (Booth and Klimenko, 2001; Akbari and Qorbani, 2011). Similarly, quince cultivars in our study behaved differently with respect to the degree of self-fertility. The cultivars 'Leskovacka' and 'Vranjska' had the lowest number of incompatible pollen tubes and the highest percentage of self-fertilization.

Incompatibility is a phenomenon that greatly affects the success of fertilization. In practice, it creates problems for producers, because such cultivars cannot be grown in mono cultivar plantations. Therefore, appropriate pollinisers must be provided, which will enable abundant fruit set and high yields (Dicenta *et al*., 2002). It is considered that for high yields in quince, more than 20% of the fruit must be set (Szabó *et al*., 1999; Benedek *et al*., 2000).

Conclusions

Analysis of the growth of pollen tubes in the pistil showed the occurrence of incompatibility in quince. It was expressed to different degrees depending on the genotype and pollination variant. In all tested cultivars, this phenomenon was significantly more pronounced in the self-pollination variant. The incompatibility was most pronounced in the upper third of the style. The most characteristic sign of incompatibility was the formation of a swelling at the tip of the pollen tube. The appearance of incompatibility affected the degree of fertilization in quince. In the self-pollination variant, the highest percentage of fertilization was observed in the cultivars with the least number of incompatible pollen tubes ('Leskovacka' and 'Vranjska'). On the other

hand, the most unfavourable results in terms of fertilization were found in the cultivars with the most pronounced incompatibility. Based on these data, the cultivars 'Leskovacka' and 'Vranjska' can be considered as self-compatible, while the cultivars 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph' are self-incompatible.

Authors' Contributions

Conceptualization: A.R., D.N., D.M., I.R., D.Z., V.S., B.D.; Designed the experimental set-up: D.M., D.N. Pollination and pistil fixation; staining and microscopic observation of pistils: A.R. Statistical analysis: D.Z., V.S., B.D. Data analysis: I.R., V.S., B.D. Writing - original draft: A.R., D.N., D.M., I.R., D.Z., V.S., B.D.; Writing - review and editing: A.R., D.N., D.M., I.R., D.Z., V.S., B.D. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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