Flowering and Fruit Set in Japanese Quince (*Chaenomeles japonica*)

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SUMMARY

The amount of flowers with defective pistils, drop of flowers after pollination, initial fruit set, drop of fruitlets and final fruit set were investigated for Japanese quince (*Chaenomeles japonica*). Pollen germination, pollen tube growth and fertilisation were studied at cross- and self-pollination. Embryo development was followed from fertilisation to mature fruit. The percentage of flowers with defective pistils was very high in most of the genotypes studied. This phenomenon seemed to be influenced by both environmental and genetic factors. In compatible (cross-) combinations, pollen tubes reached the base of the ovary within 2–5 days, and fertilisation took place 4–8 days after pollination. In incompatible combinations, pollen germination was slow, pollen tubes grew slowly and were often completely arrested in the style. The effective pollination period was estimated to be 3–5 days. The average initial fruit set was 21.1% (range 0–83.3%), whereas the final fruit set decreased to 10.4% (range 0–37.9%). During embryo development some deviations from normal development were observed, which may influence final fruit set.

INTRODUCTION

The species Japanese quince (*Chaenomeles japonica*) belonging to the subfamily *Maloideae* (*Rosaceae*) was introduced in Europe as late as 1869 (Weber 1964). Japanese quince soon became an appreciated ornamental plant, but was also developed as a fruit crop in some countries. It is currently cultivated as such in Latvia, and also in Lithuania. Japanese quince is cross-pollinated and, when propagated by seeds, the plants become extremely heterogeneous. No varieties are yet available but plant breeding programmes are making progress (Rumpunen 2002).

It is well established that in many important fruit crops of the family *Rosaceae*, fruit set and yield are strongly dependent on genotype and genotype interactions. Fruit set is influenced by *e.g.* pollen production, pollen viability and pollen germination capacity, pollen tube growth through the style and incompatibility reactions, embryo sac viability, crop adaptation, availability of pollen vectors and environmental conditions. In particular, the temperature during flowering is often crucial. Potential fertility depends on the number of normally developed male and female generative organs and is directly associated with the effective pollination period, which in turn is limited by the senescence of the ovules and pollen tube growth (Williams 1970). Cool temperatures delay pollen tube growth, fertilisation and embryo development so long that the ovules in many cases begin to degenerate before fertilisation has occurred (*e.g.* in prune, Thompson & Liu 1973).

In a study on pollination, pollen tube growth and fertilisation it has been shown that Japanese quince

has a strong gametophytic self-incompatibility system (Kaufmane & Rumpunen 2002b) which prevents self-pollination and promotes cross-pollination. Previously, sporogenesis and gametophyte development have also been studied in Japanese quince (Kaufmane & Rumpunen 2002a). The present study was planned to further investigate flowering and fruit development of Japanese quince. In particular the phenomenon of imperfect flowers was studied and the process leading from pollination, via embryo development, to final fruit set was studied in detail.

MATERIALS AND METHODS

Studies on Japanese quince plants took place at Dobele HPBES, Latvia, during 1999–2001. The selected genotypes had been propagated from seed and were planted in the field during spring 1992. For each observed genotype, 70–420 flower buds were enclosed in pollination bags to prevent cross-pollination. In 2000, the selected genotypes D2-1, D2-133, D2-135 and D2-229 were pollinated with pollen of the superior selections 'Abava', A-19, A-20 and A-15, which had been selected in a test plantation near Jelgava. During the flowering period the number of flowers with visually normally developed pistils, the number of flowers with defective pistils, drop of flowers after pollination and initial fruit set were recorded. During fruit development drop of fruitlets was studied, and the final fruit set at harvest was recorded.

Fluorescence microscopy

Pollen germination, pollen tube growth and fertilisation were studied by fluorescence microscope (Leica DMLS) for controlled self-pollination and cross-pollination of selected genotypes. The fluorescence method used is one of the basic methods currently available (Knox & Williams 1986, Kaufmane & Rumpunen 2002b). From each combination, pistils were picked every 24 hours, from the first to the seventh or tenth day after controlled pollination. The pistils were put in fixative FAA (80% ethanol: 37% formaldehyde: 100% acetic acid in proportions 8:1:1) for 24 hours, rinsed in tap-water for 4 hours, softened for 8–10 hours in 8N NaOH, and rinsed again for 4–6 hours in tap-water and distilled water. The pistils were then stained in 0.1% water-soluble aniline blue and 0.1 N K_3PO_4 for 12–24 hours. Instant samples were prepared in a droplet of the same staining solution or a drop of glycerine. For each genotype, the ovary with enclosed ovules was separated from the base of the style during the sample preparation procedure. The samples were then covered with cover slips and softly crushed. In each of the five styles, pollen tube growth was followed to the ovules under the microscope.

Pollen viability

Pollen viability was tested *in vitro* on a medium containing 1% agar and 15% sucrose, at 23–25 °C. For each genotype, 3 replicates with approximately 100 pollen grains were counted.

Embryogenesis

The complete embryogenesis process was studied in two genotypes, pollinated with a pollen mix. Fixation and preparation of pistils and fruitlets were carried out as described in Kaufmane & Rumpunen (2002a). Thus, buds were fixed with Carnoy fixate I (96% ethanol: chloroform: acetic acid, in proportions 6:3:1) for 2–4 weeks. The buds were then washed with 95% ethanol (3 x) and stored in 70% ethanol at 5 °C until preparation. At the start of preparation, the buds were washed in 95% ethanol and 100% butanol successively (2 x 2–3 hours) and stored overnight in butanol. The following day, the buds were kept for 2–3 hours in solutions of xylol and butanol in proportions 1:3, 1:1 and 3:1 successively, and stored overnight in the last solution. The buds were then treated with pure xylol for 24 hours (2 x), placed in a mixture of xylol-paraffin and kept at 37 °C for 5–6 days to allow the xylol to evaporate. Then buds were kept at 56–57 °C in pure paraffin for 1–2 days. Buds were sectioned (12–16 μm), treated with xylol

(2 x 20 min), with butanol and xylol (1:1, 10 min), and with pure butanol (2 x 20 min). Samples were then washed with 95% ethanol and 50% ethanol for 10 min successively and finally with distilled water (3 x 10 min).

For sample staining, the samples were treated with 4% $Fe(NH_4)_3$ for 16 hours and washed (one hour in running tap water and 3 x 10 min in distilled water). The samples were stained with Heidenhain's haematoxylin (1% in distilled water, 30 min) and again washed as previously described. Samples were then bleached with 2% of $Fe(NH_4)_3$ as long as necessary (checked under microscope) and washed in distilled water. Samples were then treated with 25%, 50% and 75% ethanol successively (5 min). Light-green staining solution (1% light-green in 95% ethanol) was added to samples of late stages to improve contrast. Samples were then washed with 100% butanol (2 x). Again the samples were treated with xylol and butanol in proportions 1:3, 1:1 and 3:1 successively (2–3 hours) and then with 100% xylol (2 x 24 hours). Finally, the samples were imbedded in balm and examined by microscope (Leica DMLS).

RESULTS AND DISCUSSION

Temperature during flowering

The Japanese quince plants started to bloom on May 8 to May 15 in 1999, very early on April 28 to May 1 in 2000, and an extended start of flowering from May 9 to May 30 in 2001. Flowering continued for 15–22 days, depending on the genotype and temperature.

During flowering in 1999, the average air temperature was 9.9 °C. In the first to third weeks of May the average temperature and precipitation were lower than usual, but for the remainder of the month the temperature was higher. The lowest temperature was -2 °C on May 13, and the highest was 27.5 °C on May 29.

During flowering in 2000, the average temperature was 16.0 °C in the last 10 days of April, *i.e.* 8.7 °C higher than usual, but the highest temperature was 26.7 °C on April 29. These conditions promoted a rapid start of flowering. However, in the first 10 days of May temperature fell drastically. The average temperature was then 9.7 °C, with the highest temperature on May 10 (22 °C), and the lowest temperature on May 2 (-3.5 °C), when some pistils were damaged by frost. Frost (-1 °C) was also observed in the night of May 13. Nevertheless, these conditions only slightly decreased the total number of functional flowers because the duration of flowering of Japanese quince was in general quite long. A few days of spring frost therefore did not damage all flowers. The average temperature in May 2000 was 12.0 °C, with only little precipitation during flowering.

In April 2001, the average temperature was lower than normal (6.9 °C). These conditions delayed start of flowering of Japanese quince. The average temperature in May was 11.9 °C, with the highest temperature on May 1 (27 °C) and the lowest temperature on May 23 (-0.1 °C).

Imperfect flowers

The percentage of flowers with visually defective pistils, *i.e.* pistils with very short reduced styles, partially undeveloped stigmas and undeveloped seed-buds, is shown for seven genotypes of Japanese quince in Figure 1. The average percentage of flowers with visually defective pistils was high in 1999 (65%) and 2001 (55%). For genotype D2-135 it was very high in all three years (> 70%). Nevertheless, the true percentage of non-functional flowers could be even higher, because it was noticed that some pistils with normally developed styles had undeveloped or partly developed seed-buds in the ovary. These flowers could not possibly be fertilised.

The reason for the very high number of imperfect flowers found in some genotypes of Japanese quince is still not known. It is possible that the phenomenon is caused by unfavourable temperature during flower bud development, but there also seem to be clear genetic factors involved. However, the available number of perfect flowers should allow a high yield of fruits, if they are sufficiently pollinated.

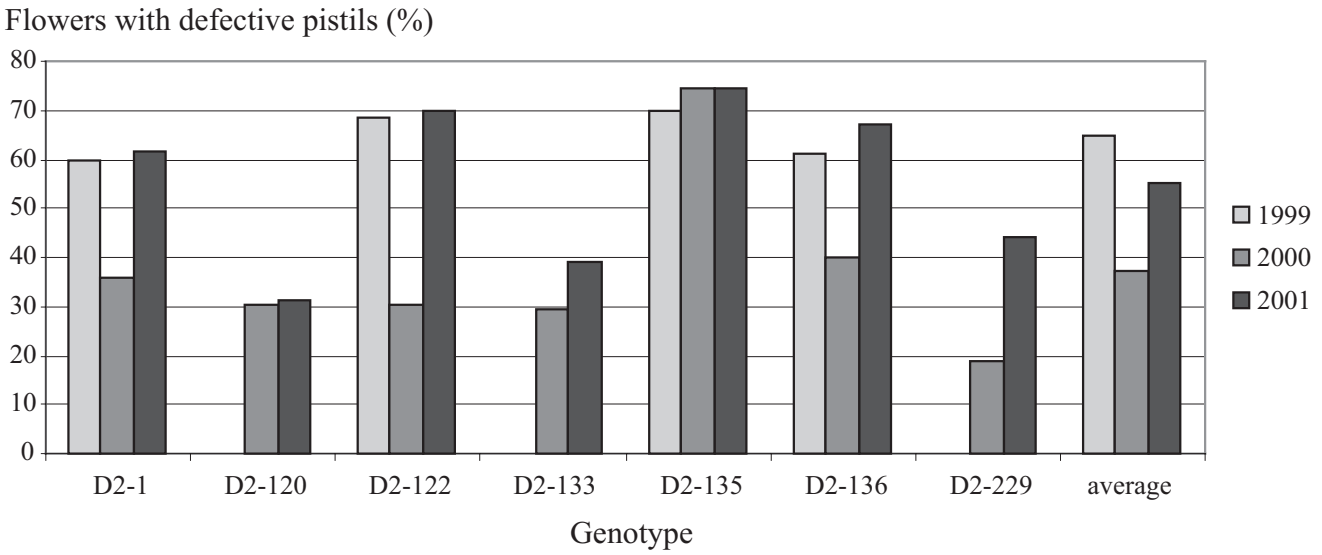

Figure 1. Percentage (%) of flowers with defective pistils in seven Japanese quince (*C. japonica*) genotypes studied during 1999–2001.

Drop of flowers, drop of fruitlets, and initial and final fruit set

Drop of flowers after pollination and initial fruit set depended mainly on the amount of flowers with defective pistils. Fruit set also depended on the compatibility among genotypes crossed (Table 1). The percentage of flowers that dropped after pollination was 10.0–100%. All flowers with defective pistils and a large proportion of flowers with visually normally developed pistils dropped after pollination. The initial fruit set was 0–83.3%, and the final fruit set was 0–37.9%. At self-pollination the final fruit set was 0–6.5%, indicating a slight tendency for self-fertility, but the fertility of the resulting seeds was not checked.

Table 1. Flowers with normally developed and defective pistils, flowers dropped after pollination, initial fruit set, dropped fruit lets and final fruit set at controlled self- and cross-pollination of superior selections of Japanese quince (*C. japonica*) in 2000.

Seed parent	Pollen parent	Pollinated flowers (*n*)	Normal pistils (%)	Defective pistils (%)	Dropped flowers (%)	Dropped fruitlets (%)	Initial fruit set (%)	Final fruit set (%)
D2-1	D2-1	50	72.0	28.0	88.0	12.0	8.0	4.0
	'Abava'	40	70.3	29.7	47.5	52.5	15.0	37.5
	A-15	29	82.8	17.2	55.2	44.8	6.9	37.9
	A-19	51	41.2	58.8	96.1	3.9	2.0	2.0
D2-133	D2-133	51	78.4	21.6	98.0	2.0	2.0	0.0
	'Abava'	61	39.3	60.7	90.2	9.8	8.2	1.6
	A-19	28	57.1	42.9	100.0	0.0	0.0	0.0
	A-20	30	100.0	0.0	10.0	90.0	63.3	26.7
D2-135	D2-135	40	10.0	90.0	100.0	0.0	0.0	0.0
	'Abava'	27	77.8	22.2	66.7	33.3	14.8	18.5
	A-19	41	0.0	100.0	100.0	0.0	0.0	0.0
	A-20	50	14.3	85.7	96.0	4.0	2.0	2.0
D2-229	D 2-229	31	87.1	12.9	77.4	22.6	16.1	6.5
	'Abava'	23	95.7	4.3	39.1	60.9	47.8	13.1
	A-19	30	96.7	3.3	10.0	90.0	83.3	6.7
	A-20	32	90.6	9.4	21.9	78.1	68.7	9.4

Table 2. Pollen viability (as percentage germinating pollen grains) tested *in vitro* for pollen-parents of Japanese quince (*C. japonica*) used in the hybridisation trial.

Genotype	Pollen viability (%)[1]
Abava	39.7 a
A-19	50.3 ab
A-15	57.7 bc
A-20	64.5 c

[1]Different group letters indicate significance between groups (at the significance level 0.05, Fischer's protected LSD)

Pollen viability, pollen germination and pollen tube growth

Pollen viability was in general good and pollen germination varied between 39.7 and 64.5% (Table 2) for pollen parents.

In a compatible combination, a high percentage of the pollen grains germinated, germination was fast, and pollen tubes grew rapidly in a dense cluster through the style. Most of the pollen tubes reached the base of the ovary within 2–5 days. Fertilisation of ovules took place 4–8 days after pollination, depending on the genotype.

In an incompatible combination a low percentage of pollen grains geminated, germination was slow, the pollen tubes grew slowly and they were often completely arrested in the style. Callose was accumulated along the tube and at its end and the percentage of fertilised ovules was very low.

In defective pistils germination of pollen grains was extremely low, about 1.5%. A few pollen tubes grew into the ovary, but seed-buds were undeveloped and fertilisation could not take place.

The growth of pollen tubes in the styles at cross-pollination and self-pollination is illustrated in Figure 2 for the genotype D 2-1. The retarded pollen tube growth at self-pollination is evident.

Fertilisation and embryogenesis

In Japanese quince, double fertilisation takes place. In a compatible combination fertilisation was usually finished on the 4th–7th day after pollination and the fastest pollen tube growth to the embryo sac was 2–3

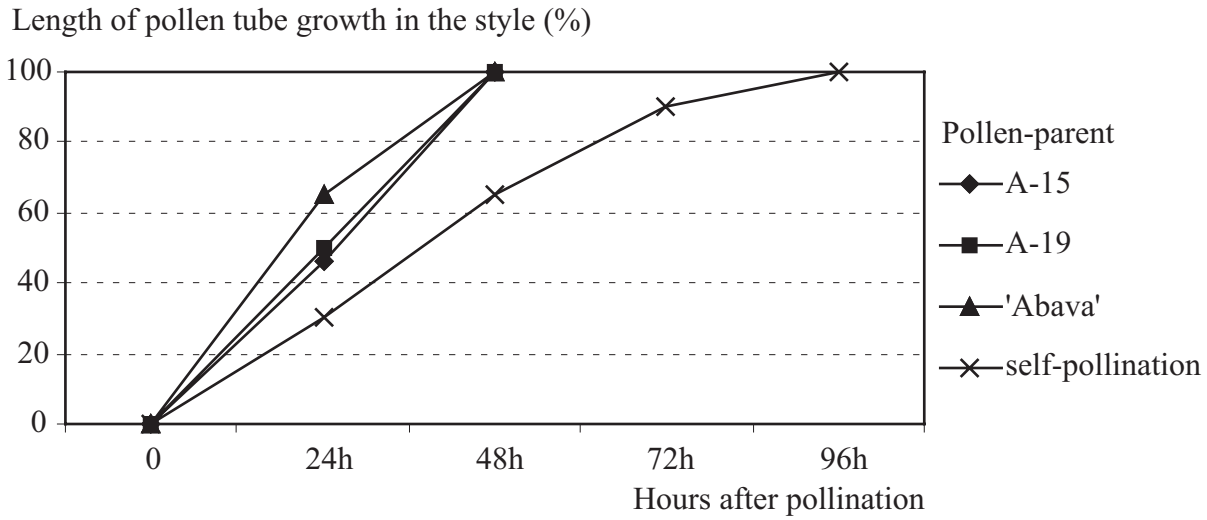

Figure 2. Length of style reached by the longest pollen tube (average) at different time intervals after pollination of Japanese quince (*C. japonica*) genotype D2-1.

days. The embryo sac was ripe 2–4 days after anthesis and viable until 5–7 days after anthesis. Thus, the effective pollination period could be estimated to be 3–5 days in Japanese quince. This is similar to data previously reported (Kaufmane & Rumpunen 2002b).

The pollen tube entered the embryo sac when the embryo sac was already fully developed. The sperm were delivered close to the egg apparatus, most commonly into one of the synergids, which disintegrated soon after that. The other synergid usually remained intact for some time, sometimes until the formation of a multicellular embryo. After the release from the pollen tube, one of the sperm moved towards the nucleus of the ovule. The other sperm moved to one of the polar nuclei or to the central nucleus if the nuclei had merged. The fertilisation in Japanese quince followed the so-called premitotic type. Thus, the gametes merged before the first mitosis of the zygote. The membrane thickness of the fertilised ovule (zygote) increased markedly and the nucleus was enlarged.

The fertilised diploid ovule rested for 1–2 days. Then a two-cell embryo formed, in which the basal cell was larger than the apical cell, and the embryo continued to develop from the apical cell (Figure 3A). A tetranuclear embryo was then formed, where the cells were situated in a row (Figure 3B). Next, rapid cell division took place, which resulted in a multicellular embryo. The embryo was first pole-shaped, then balloon-shaped (Figure 3C). The embryo then grew rapidly, and cell differentiation started. Initially two bulges formed at the top of the balloon-shaped embryo (Figure 3D), which constituted the beginning of the seedbeds. These elongated and the vascular system differentiated, the primordial rootlets developed and somewhat later, between both seedbeds, a budlet formed containing the germs of the true cotyledons (Figure 3E).

The triploid endosperm in Japanese quince formed after fertilisation of the polar nuclei. Sometimes following fertilisation both polar nuclei merged, forming a secondary nucleus, or this merging happened at the same time as fertilisation. Almost immediately after fertilisation a synchronic division of the nucleus began (Figure 3F). When more than 10 endospermic nuclei had developed, the zygote became active and started to divide. When the differentiation of embryonic tissue began, the development of protoplasm started in the region of micropyle around the endospermic nuclei. The nuclear endosperm was then transformed into cellular endosperm (Figure 3G). When the seedbeds had reached their normal size, the endosperm slowly degenerated. Thus, the fully developed seed of Japanese quince did not contain endosperm.

In Japanese quince, seed development took 3.5–4 months. The critical periods during embryogenesis, when most deviations occurred, were during fertilisation (4–8 days after pollination) and during the first 30 days after fertilisation. These events coincided with drop of flowers immediately after flowering and drop of fruitlets, which occurred 1–1.5 months after pollination. The fruitlets that did not fall during this period usually developed into normal fruits.

Deviations during embryogenesis

During embryogenesis a number of deviations were observed, of which a part could result in a drop of fertilised flowers and fruitlets.

In 15–20% of ovaries two nucelli were found. However, this deviation cannot be considered to be a reason for obligate sterility, because normal embryo sacs were found in each of these nucelli and more than one pollen tube may enter through the micropyle. It was also observed that in early stages of embryo development such complex ovaries sometimes contained two embryos.

Deviations were also noticed for the development of integuments. In some cases only the inner or the outer integument developed. This phenomenon was accompanied by deviations in the embryo sac, which sooner or later degenerated, and no fertilisation could take place.

More than one embryo sac was sometimes present in the ovary. These were found in the beginning of the female gametophyte development, when more than one of the numerous archesporial cells developed. In this case several embryo sacs were observed, of which one was usually better developed. This

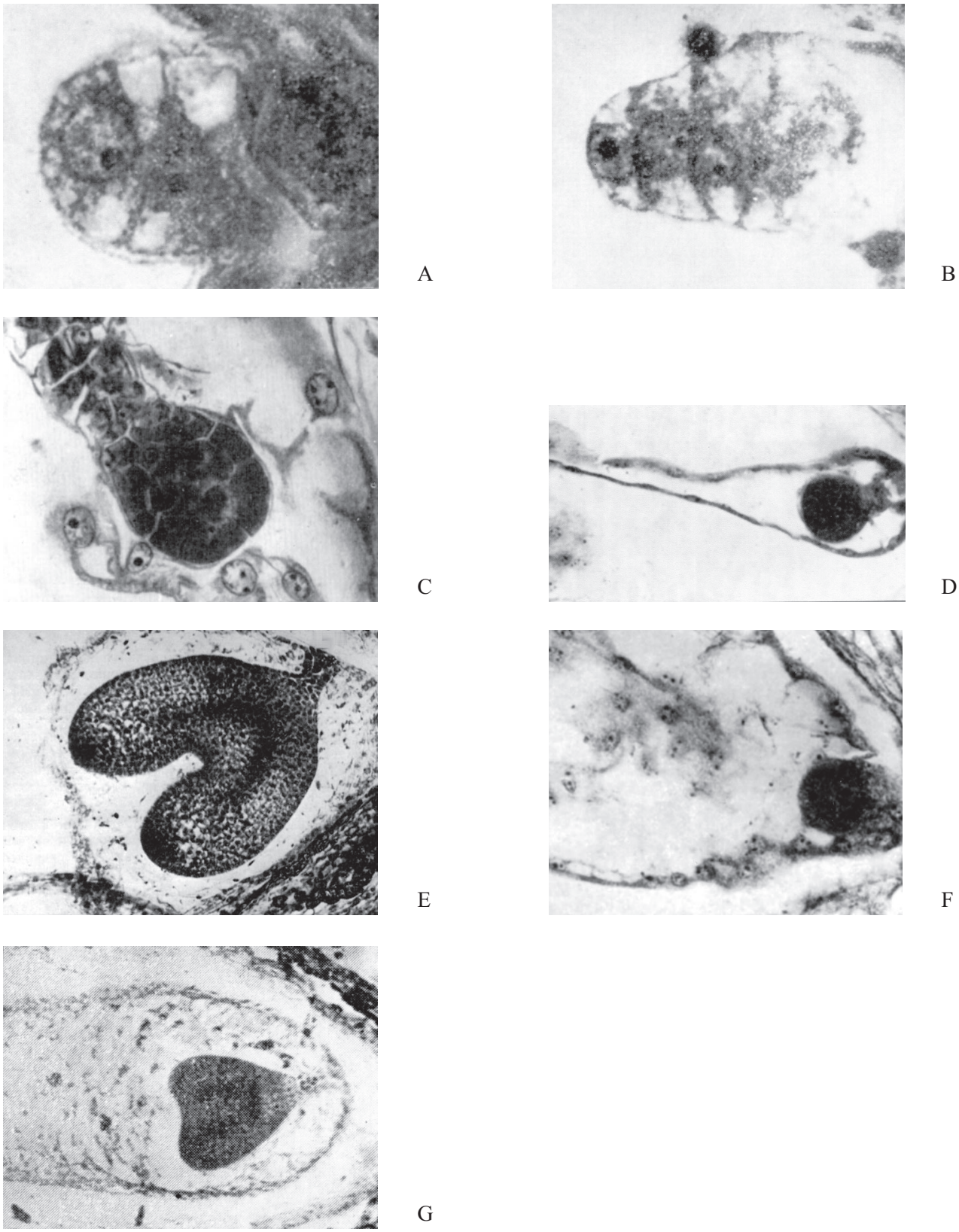

Figure 3. Embryogenesis of Japanese quince (*Chaenomeles japonica*): A) two-cell embryo with larger basal cell, B) tetranuclear embryo, C) ballon-shaped embryo, D) ballon-shaped embryo and nuclear endosperm, E) embryo with developing cotyledons, rootlets and vascular system, F) development of nuclear endosperm, G) cellular endosperm.

deviation could perhaps be considered to be an adaptation to unfavourable environmental conditions, because when one embryo sac perishes, another could start to develop and fertilisation may occur (Kaimahan & Krilova 1984). Another deviation noticed was the presence of two ovules in the egg apparatus, leading to the formation of two embryos. In addition, three and more synergids were sometimes found in the egg apparatus and sometimes also 3–4 polar nuclei. Such ovaries usually degenerated.

Persistence of antipodial cells was also observed. If antipodal cells persisted they enlarged and gradually filled the embryo sac. Eventually the ovaries degenerated.

Quite often embryo development aborted. In dropped fruitlets, a mass of homogeneous cells, a plug-like formation, or just a few cells in a tangled ball was formed instead of a multicellular, normally developed embryo. The reasons for abortion at different stages could be unfavourable weather conditions during embryo formation (*e.g.* low temperature in the second half of May and beginning of June, which is common in Latvia), or an incompatible combination at fertilisation. For two genotypes studied, this type of embryo abortion was noticed in 18–22% of the total number of dropped fruitlets in 2000, and in 32–36% of dropped fruitlets in 2001.

In a flower with a defective pistil there was usually no normal embryogenesis at all, and no complete embryo sac was formed. The embryo sac lacked the ovule or synergids, and instead a thick mass of undifferentiated cells developed.

CONCLUSION

Pollen viability for Japanese quince was normal as inferred from germination tests *in vitro*. A high self-incompatibility system prevails, which was confirmed both by controlled pollinations in the field, the study of pollen tube growth by fluorescence microscopy, and counting of final fruit set. The high amount of defective pistils commonly found in Japanese quince plants is probably not the single most important factor that influences yield. Instead, the compatibility between seed parents and pollen parents seems most important. The deviations found during embryo development in a normally developed pistil were not sufficiently frequent to decrease yield.

To ensure good fruit set and high yield, Japanese quince plants must be cross-pollinated. None of the genotypes studied showed satisfactory fruit set during all three years when self-pollinated.

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