Embryological studies on *Fragaria x ananassa* Duch. after pollination by three species of *Potentilla*

KATARZYNA NIEMIROWICZ-SZCZYTT

Department of Genetics and Breeding of Horticultural Plants,
Faculty of Horticulture, Warsaw Agricultural University (SGGW-AR),
Nowoursynowska 166, 02-766 Warszawa, Poland

(Received: July 20, 1983. Revision accepted: January 13, 1984)

Abstract

Embryological studies were carried out after pollinating four female varieties of *Fragaria ananassa* Duch. (Freja, Sonja, Miebe Schindler, Reine des Precoc) with three species of *Potentilla* (*P. geoides*, *P. rupestris*, *P. fruticosa*). The aim was to elucidate the manner in which matroclinous progeny arises after pollination of the strawberry with *Potentilla* pollen. In addition to meiotic, apo- and diplosporous embryo sacs were formed in the Freja variety. On the average, in the four studied varieties, 49% of the ovules contained more than one embryo sac. At the most, five embryo sacs developed, but only two of them attained full maturity. Pollen from *Potentilla* germinated on the stigma of the strawberry and the pollen tubes grew through the style to the ovule. Symptoms of fertilization were not found in the strawberry ovules after pollination with *Potentilla*, however, after pollination with the Redgauntlet *F. x ananassa* variety, extra nucleoli or chromatin grains were found in the ovule. False polyembryony was found in the Miebe Schindler (16 cases), Sonja (2 cases) and Freja (1 case) varieties. The germination of two plants from one seed was observed, which shows the possibility of full development of twin embryos. The low germination ability of the seeds was caused by abnormalities in the development of the embryos.

Key words: apospory, diplospory, false polyembryony, twin embryos

INTRODUCTION

Questions about the way in which embryo sacs are formed in maternal forms, as well as about the formation of embryos arising from pollination by different species of *Potentilla*, arose in connection with experiments being conducted on obtaining intergeneric hybrids by crossing

F. x ananassa x *Potentilla* spp. Certain traits of the progeny indicated that at least part of it arose through apomixis. Until now it had been accepted (Shangin-Berezovskiy 1962, Asker 1971, Hughes and Janick 1974) that polyhaploids and aneuploids which are formed after pollination of the strawberry with *Potentilla* pollen could arise as the result of apomixis, and that plants of the same ploidy as the maternal form also through endomitosis. Asker (1971) suggests that part of the matroclinous plants could arise as the result of pseudogamy, and also notices morphological differences among plants from one maternal clone. However, none of the authors named above ran embryological studies on the maternal forms which could substantiate such suppositions. This author wrote a review article in order to become acquainted with the results of embryological studies on apomixis in the *Rosaceae* family (Niemirowicz-Szczytt 1982). Presently, questions connected with the species *Fragaria x ananassa* Duch. will be discussed.

In the multicellular secondary archesporium of the strawberry, numerous (1-6) embryo sacs can be formed, both as the result of meiosis and from aposporous initials (Rudloff 1930, Solntzeva 1957, Berezenko 1979, Koloteva et al. 1978). Formation of diplosporous initials was not found. The percent of ovules with two or more embryo sacs found in two consecutive years in the Kolkoznaya and Lyavonika varieties was 20.3 and 38.8%, respectively (Solntzeva 1957). It was pointed out that parts of the intertwined embryo sacs can fuse, analagous nuclei can differ in size and the polarization of the embryo sac can be changed as well (Rudloff 1930, Solntzeva 1957, Konstantinov 1966, Berezenko 1979).

The presence of numerous embryo sacs is connected with the occurrence of false polyembryony. These processes have been found in many strawberry varieties; in addition to those mentioned above, in the Yasna, Kiyowska Ranna, Koralowa 100, Misovka (Berezenko 1979), Grenadier (Koloteva et al. 1978) and in forms not described in detail (Rudloff 1930). On the basis of the ploidy of the offspring obtained from the strawberry after intergeneric pollination, many authors (Aalders 1964, Bauer and Bauer 1967, Sukhareva 1976) indicate the existence of pseudogamy. Other examples of apomictic embryo development e.g. adventive embryony (Solntzeva 1957) and the development of the embryo in the presence of unfused polar nuclei in spite of previous pollination (Berezenko 1979) or lack of it (Koloteva et al. 1978) were described on the basis of single slides. Studies done up until now indicate, however, that the frequency of apomictic processes is low, but there is a lack of numerical data. It is therefore considered that these processes do not play an important role in the reproduction of strawberries, but cannot be excluded.

MATERIAL AND METHODS

In order to study the development of the generative organs, archesporium and embryo sac, 10-20 flower buds from four female varieties of *F. x ananassa* (Freja, Sonja, Mieke Schindler, Reine des Precoce) and one variety with perfect flowers (Redgauntlet) were fixed in weekly intervals from April to mid-May. The material was divided into five classes on the basis of bud length (1-2, 2-3, 3-5 and over 5 mm), in order to study the relationship between the length of the bud and the development of the stamen and pistils.

In order to study the process of fertilization and development of embryos, pollination in 16 combinations was done under conditions of isolation. Each of the 4 female clones mentioned above was pollinated consecutively by four paternal types (*F. x ananassa* cv. Redgauntlet, *P. geoides*, *P. rupestris*, *P. fruticosa*). Five pollinated flowers from each combination were fixed after 2, 6, 24 hrs and 3 and 10 days. Both buds and flowers were fixed in FAA (80% ethyl alcohol, formalin and acetic acid 9 : 0.5 : 0.5) and stored in 80% alcohol. Paraffin sections, 10 μ m thick, were cut. The slides were stained with Heidenhain's hematoxylin mod. Darlington. More advanced embryo stages were additionally stained with ruthenium red.

Growth of pollen tubes into the style was observed in crushed samples stained with a 0.1% aniline blue solution in 0.1 N potassium phosphate (K_3PO_4) using a fluorescence attachment to a Zeiss Docuval microscope. Photographs were taken through the same microscope.

RESULTS

DEVELOPMENT OF THE GENERATIVE ORGANS OF THE FLOWER BUD

In order to catch the developmental stages of the archesporium in ovules and anthers, buds of different sizes from 4 female strawberry varieties and 1 variety with perfect flowers were studied. Attention was given to the degree of development of the receptacle and the pistils developing in it as well as to how far the development of anthers had advanced; their primordia are found in every young bud, regardless of its sex (Table 1). The development of the anther in female varieties stops at the stage of archesporial tissue or meiocytes, therefore, before the differentiation of embryo sacs takes place. Degeneration begins in buds 3-5 mm in length. In buds of that same length in the bisexual variety, quick meiotic divisions and the formation of tetrads are taking place. In buds longer than 5 mm, mature pollen grains are already found.

Table 1

Degree of anther and pistil development in relation to the bud length in four female and one bisexual variety of *F. x ananassa*

Variety of <i>F. x ananassa</i>	Sex		Length of bud, mm			
			1-2	2-3	3-5	5 (begining of flowering)
Freja	♀	A	undifferentiated	5-6 l. pist., carpels	6-10 l. pist., arch.	10-12 l. pist., meiocytes, initials, young embryo sacs
		B	—”—	archespore	arch. degeneration	degeneration
Sonja	♀	A	2-4 l. pist.	5-6 l. pist., carpels	7-8 l. pist., ovules	9-10 l. pist., arch.
		B	archespore	meiocytes or 4 n. aggregates	meiocytes degener., tapetum vacuoliz.	degeneration or drying up
Mieze Schindler	♀	A	undifferentiated	2-3 l. pist., carpels	5-7 l. pist., ovules	8-10 l. pist., arch.
		B	—”—	epidermis	arch. degener.	arch. vacuoliz.
Reine des Precoc	♀	A	—”	1-2 l. pist., carpels	3-7 l. pist. ovules	6-10 l. pist., arch.
		B	—”—	epidermis	arch. degener.	arch. vacuolization
Redgauntlet	♂	A	—”—	5 l. pist., carpels	6-7 l. pist., ovules	10-12 l. pist., arch.
	♀	B	—”—	archespore	meiocytes or tetrads	mature pollen grains

A — receptacle (comments refer to the first layer of pistils at the base of the receptacle); B — anthers; l. pist. — layer of pistils; n — nucleus; arch. — archespore.

A dependence can be seen between the number of presently differentiating pistils and the degree of development of the pistils of the first layer, which is at the base of the convex receptacle. When 5-7 layers of pistils develop, ovules differentiate in the eldest. The ovules' centers are formed by 4 rows of cells similar in size. When the number of layers of pistils reaches 10-12, a primary or secondary archesporium is found in the eldest ovules. In the majority of the studied varieties, at the moment in which the first flowers bloom (beginning of April), meiotic divisions are taking place in the secondary archesporium; cells in synizesis and the initials of the embryo sac are found. The variety Freja (the earliest of those studied) is an exception, here, when the first flowers begin to bloom, embryo sacs are already found in the lowest layer of pistils.

DEVELOPMENT OF THE ARCHESPORIUM AND EMBRYO SAC

In the description of the archesporium it was attempted to pin-point the dependence between the numbers of calotte layers and the stage of archesporium development (Table 2). When the second and next calotte layers appear under the epidermis, the cells of the archesporium enter into the first meiotic phases. As a rule, it is one cell, found in the central layers of the archesporium, closest to the chalaza, but in the strawberry, both cells in synizesis as well as in the stage of the I and II meiotic division were found equally frequently in the central and side layers. In the parietal cells (1-2) which are found above the cells of the archesporium, no signs were found that they are capable of turning into initials of the embryo sac. If we accept that the cells in the synizesis stage (black bunches of chromatin threads next to the nucleolus) have entered into the premeiotic stage, then the culmination of their appearance (max. 11) is seen at 4-8 calotte layers. On average, 4-6 cells are found in synizesis, a stage preceding the first meiotic division (Fig. 1). The moment tetrads and young one- or two-celled embryo sacs arise, the number of cells in synizesis falls, and parietal layers of the archesporium are gradually digested. In the Freja variety, at 9-13 calotte layers, young embryo sacs were found, and from the 45 studied ovules, diplosporous initials were found in 15 of them, in 8 — aposporous initials. In two cases, in one nucellus both apo- and diplosporous initials were found at the same time. These cells were seen in the presence of single meiotic figures or young uni- and dinucleated embryo sacs. Two tetrads in one archesporium were rarely found.

Observations on the position and number of embryo sacs were carried out on fixed material, after one and three days from pollination of the female varieties, also taking into account ovaries with two ovules (Table 3). The highest number of ovules with two or more embryo sacs

Table 2

Average number of cells in synizesis and secondary archespore cells in young *F. x ananassa* ovules

Variety of <i>F. x ananassa</i>	Number of studied ovules	Number of calotte layers	Average number of cells in synizezis	Average number secondary archespore cells			Comments
				width	height	thickness	
Freja	45	2-3	4	4	3	3	meiocytes, initials, young embryo sacs, (from 6-13 calotte layers) are seen in the most developed ovules
	33	4-5	6	4	3	3	
	10	6-8	6	4	3	3	
	35	9-13	4	3	3	3	
Sonja	10	5-8	6	4	3	3	
Mieze Schindler	12	4-7	3	3	2	3	
Redgauntlet	10	2-3	4	4	3	3

Fig. 1. Secondary archesporium with cells in synizesis and initials

was found in the Miebe Schindler variety (c. 70% of the studied ovules). The lowest, only c. 24% such ovules, was found in the Reine des Precoce variety. On average, 49% of the ovules in 4 female varieties contained multiple embryo sacs. In those nucelluses in which multiple (to 5) embryo sacs are developing, usually only two reach full development. The remaining sacs degenerate at the two- or four-nucleus stage. They were observed in the chalazal part (Fig. 8) and their position and color indicated that they were aposporous. Uni- and dinucleated sacs (Fig. 6) in the chalazal part were still found there during the early stages of embryo development. Fully developed embryo sacs (most often two) were found on a similar level and were often intertwined, but due to cytoplasmic strands connecting the egg apparatus, polar nuclei and antipodals, it was easy to recognize the sac to which each element belonged. In a few cases, transposition of one cell in the egg apparatus was seen e.g. the egg cell in respect to the synergids (Fig. 5). In the developed embryo sacs of one nucellus, there were differences in the diameter of analogical nuclei, which was easily seen by comparison of polar nuclei in the same stage of development.

The highest percentage of ovules with numerous embryo sacs was found in the Miebe Schindler and Freja varieties. Here too, was the highest percentage (8-14%) of composite ovaries found (Fig. 2). In each such ovule, two or three embryo sacs were developing. Composite ovaries probably easily degenerate because the development of embryos was

Table 3

Number (n) of fused ovules and number of ovules with multiple embryo sacs in one nucellus in 4 female varieties of *F. x ananassa* in 1980 (to the 3rd day after pollination)

Variety	Number of studied ovules	Ovules with 2 fused nucelluses		Ovules with embryo sacs						Max. number of embryo sacs in one nucellus
				1		2		2		
		n	%	n	%	n	%	n	%	
Freja	343	48	14.0	157	45.8	157	45.8	29	8.4	5
Sonja	276	5	1.8	172	62.3	74	26.8	30	10.9	5
Mieze Schindler	307	26	8.5	81	26.4	188	61.2	38	12.4	3
Reine des Precoce	232	2	0.9	175	75.4	54	23.3	3	1.3	3
Total	1158	81	7.0	585	50.5	473	40.8	100	8.6

Fig. 2. Ovary with one ovule (a) and two ovules (b)

found only once in the Mieze Schindler x *P. reptans* combination, but they were found among the ovules which dried up 10 days after pollination.

FERTILIZATION AND EMBRYO DEVELOPMENT

Table 4 presents a diagram of the pollinations done to observe the process of fertilization and embryo development, in which the octoploid *Fragaria x ananassa* (female variety) and the octoploid, bisexual *F. x ananassa* cv. Redgauntlet (control) and diploid species of *Potentilla* were used. In the material fixed 24 hr after pollination, 3 cases of ovules with signs of fertilization were found among the 831 studied. This gave no grounds for any conclusions and that is why the next year (1981),

Fig. 4. *Potentilla geoides* pollen tubes in the pistil tissue of *Fragaria* × *ananassa* cv. Freja. Photographed in fluorescent light

Fig. 5. Egg apparatus in the strawberry embryo sac. The egg cell's position is shifted in respect to the synergids

Fig. 6. Uni- and dinucleated embryo sacs in the chalazal part of the nucellus

Fig. 7. Strawberry egg cell 32 hours after intervarietal pollination. Extra chromatin grains in the nucleus

Fig. 8. Strawberry ovary from an unpollinated flower (10 days after blooming). Two fully formed, degenerating embryo sacs. Uninucleated embryo sacs in the chalazal part

Fig. 9. Two-celled embryos of *Fragaria* × *ananassa*. A degenerated egg apparatus in the neighboring embryo sac

Fig. 10. An atypical embryo on a long suspensor, formed in the *Fragaria* × *ananassa* cv. Freja × *Potentilla geoides* combination

Figs. 11, 12, 13. Twin strawberry embryos in different developmental stages. Differentiation in the size of the twins

Fig. 14. Germination of *Fragaria* × *ananassa* seeds obtained after pollination with *Potentilla*. The arrow points twin seedlings. Characteristic two primary roots

taking into account the suggestions given by other authors (Solntzeva 1961, Beridze 1964, Berezenko 1970) and my own observations, additional pollinations were carried out. One combination with *Potentilla* (Freja x *P. geoides*) and one between the strawberry varieties Freja x Ostara were chosen. The flowers were fixed 32 and 48 hours after pollination and the slides were prepared as given. After pollination between varieties, among 86 studied ovules, 25 (29%) showed signs of fertilization. In the nuclei of the egg cells there were additional nucleoli or — more darkly stained — chromatin grains (Fig. 7) in the presence of the first endosperm nuclei. In the combination with *P. geoides*, first endosperm nuclei were observed in 12 (c. 10%) ovules from the 122 studied, but signs of fertilization in the egg cell were missing. It should be added that both in the control as well as in all of the combinations with *Potentilla*, growth of pollen tubes into the tissue of the pistil was observed (Fig. 4).

Three days after pollination, the fastest development of the endosperm and proembryo was in the Mieke Schindler and Reine des Precoce varieties, slower development was in the Sonja and Freja varieties (Table 4). On the basis of the percentage of ovules with embryos, it can be concluded that in the Mieke Schindler variety, initiation of embryo development is more frequent than in other varieties pollinated by *Potentilla*. Comparing the paternal type plants, (excluding the control) it can be seen that the best stimulators were *P. geoides* and *P. rupestris*. At the same time, these are species producing large amounts of pollen whereas *P. fruticosa* has much smaller amounts of it.

The most highly advanced development of embryos (2-8 cells) was seen in the Mieke Schindler x *P. rupestris* combination where 3 cases of ovules with singular centers containing 2 embryos were found among the 26 studied. There was one such case in the Mieke Schindler x Redgauntlet combination. Each of the embryos was developing in a separate embryo sac and was surrounded by its own endosperm. More developed (usually by one division) was the embryo in the centrally positioned embryo sac, closest to the apical part of the ovule. If only one embryo was developing, another embryo sac was usually found nearby (Fig. 9). The reason why more twin embryos were not found 3 days after fertilization was their retarded development (compare Tables 4 and 5).

Ten days after pollination (Table 5) an astonishingly high proportion of ovules with embryos was found in all 16 combinations. Empty ovules were probably eliminated during preparation. In addition to ovules in which an embryo is developing, ovules with numerous embryo sacs in one center persist for the longest period of time and retain the largest size. Egg cells and polar nuclei (Fig. 8) are still retained in the embryo sac 10 days after full blooming. The latter often group together and

Table 4

Signs of embryo development 3 days after pollination of four female varieties *F. x ananassa* by a bisexual variety *F. x ananassa* cv Redgauntlet and three diploid *Potentilla* species

Maternal forms <i>F. x ananassa</i>	Ovules of paternal forms														
	Redgauntlet			<i>P. geoides</i>			<i>P. rupestris</i>			<i>P. fruticosa</i>			total		
	stud.	embryo dev.		stud.	embryo dev.		stud.	embryo dev.		stud.	embryo dev.		stud.	embryo dev.	
	N	N	%	N	N	%	N	N	%	N	N	%	N	N	%
Freja	10	—	—	27	13	48.1	22	—	—	10	—	—	69	13	18.8
Sonja	21	9	42.9	20	—	—	13	—	—	16	—	—	70	9	12.9
Mieze Schindler	20	13	65.0	26	14	53.8	26	19	73.1	20	4	20.0	92	50	54.3
Reine des Precoce	24	20	83.3	17	8	47.1	38	11	28.9	17	2	1.8	96	41	42.7
Total	75	42	56.0	90	35	38.9	99	30	33.3	63	6	9.5	327	113	34.5

stud. — studied, embryo dev. — embryo development.

Table 5
Development of embryos in various combination 10 days after pollination

Maternal forms				Paternal forms														
<i>F. x ananassa</i>				<i>F. x ananassa</i> cv. Redgauntlet			<i>P. geoides</i>			<i>P. rupestris</i>			<i>P. fruticosa</i>			total		
				ovules														
				stud.	with embr.		stud.	with embr.		stud.	with embr.		stud.	with embr.		stud.	with embr.	
				n	n	%	n	n	%	n	n	%	n	n	%	n	n	%
Freja				20	15	75.0	10	7	70.0	23	12	52.2	28	5	17.8	81	39	48.1
Sonja				20	20	100.0	20	11	55.0	23	11	47.8	10	4	40.0	73	46	63.0
Mieze Schindler				27	26	93.3	25	23	92.0	23	23	100.0	19	19	100.0	94	91	96.8
Reine des Precoce				14	8	57.1	21	15	71.4	20	16	80.0	22	9	40.9	77	48	62.3
Total				81	69	85.1	76	56	73.6	89	62	69.6	79	37	46.9	325	224	68.9

stud. — studied, with embr. — with embryo.

4-, 6- and 8-nuclei aggregates can often be observed (Figs. 3a, b, c). Ten days after pollination, the most developed embryos are found in the control combination (a slight domination of the heart stage over the globular stage), whereas in the *Potentilla* combinations, the globular stage prevails. The percentage of ovules with embryos was also higher in the control, although it reached 100% in some of the Miebe Schindler combinations with *P. rupestris* and *P. fruticosa*.

Fig. 3. Groups of polar nuclei: a and b — 3 days after pollination, c — 10 days after pollination

If the influence of the paternal form (excluding the control) is taken into consideration, then it is seen that the highest number of embryos was formed in the combinations with *P. geoides* (73.6%), next with *P. rupestris* (69.6%) and the least with *P. fruticosa* (46.9%). However, it should be noted that in the combination with *P. geoides*, the embryos exceptionally frequently developed abnormally. The abnormalities lay most often in the irregular cell divisions of the suspensor, that is, slanted initiation of walls, a suspensor composed of one giant cell, unproportionally long (Fig. 10) or short suspenders, giant nuclei of suspensor cells. Degeneration of cells in the globular or heart stage embryo could also be observed, which manifested itself by the vacuolization of cells and weak staining of nuclei and cytoplasm. For example, in the combination Sonja $\times$ *P. geoides*, 7 days after pollination (extra combination) 10 out of 13 embryos were described as abnormal, and 10 days after pollination, 8 out of 11 embryos were considered abnormal or degenerating.

When the influence of the maternal form is taken into account, the Miebe Schindler variety should be considered the best both in respect to the numbers of singular and twin embryos (4 cases of false polyem-

brony in the combination with *P. geoides* and 3 with *P. rupestris* (Figs. 11, 12)). In respect to the ability of 2 embryos to develop in one center, the Sonja variety is in second place (2 cases), in third — Freja (1 case). False polyembryony was not observed in the Reine des Precoce variety. The largest number of atypical embryos was in the Sonja variety, which may indicate earlier disclosure of developmental abnormalities in the embryo. These abnormalities probably arise later in other varieties, and are the reason for the generally poor viability of the seeds. On average, 0.75% of the seeds obtained by pollinating with *Potentilla*, germinate (Niemirowicz-Szczytt 1984a). This can be confirmed by additional studies (17 days after pollination) in the Miebe Schindler $\times$ *P. rupestris* combination. Here, 19 embryos were in the globular stage (highly retarded), 29 in the heart stage (with 14 degenerating) and one in the torpedo stage (its twin was in the heart stage — Fig. 13). It may be assumed that the retarded globular embryos and the degenerating heart stage ones will not be capable of further development. Embryos from open pollination are fully grown after 20 days (Yurceva 1966). In the combination mentioned above, there was a total of 5 seeds with twin embryos in one nucellus and one seed with 2 embryos in separate, but connected nucelluses.

In the Sonja $\times$ *P. geoides* combination, in one case in 650, a 4-cell-proembryo was observed in the presence of unfused polar nuclei.

DISCUSSION

Studies on the development of the generative organs of the strawberry flower bud allowed the possibility of self-pollination to be excluded in female varieties, since the anthers degenerate in the early stages of development and fully developed pollen grains are never observed. This has essential bearing on the intergeneric crossing of *Fragaria* and *Potentilla* because it guarantees the purity of the progeny. In studies on the development of the embryo sac, this author showed the existence of aposporous and diplosporous initials in the Freja variety. Until now, such types of cells were not noted in the strawberry. Aposporous and diplosporous embryo sacs contain gametes with an unreduced number of chromosomes, which in turn is the highly probable cause of octoploid, matroclinous plants arising from the intergeneric crossing of *Fragaria* $\times$ *ananassa* with *Potentilla* spp. (Niemirowicz-Szczytt 1984b).

Growth of pollen tubes into the stylar tissue and their penetration into the ovule was found in all combinations with *Potentilla*. It is slightly retarded compared with the control, but nonetheless, stimulates the development of fruit. The first signs of fertilization in the control were found 24-32 hours after pollination. Those were the enlargement of the

egg cell in the presence of chromatin grains in the nucleus and usually 2-4 endosperm nuclei. According to Berezenko (1970), these are the symptoms of fertilization. In the combinations with *Potentilla*, there were no such changes in the egg cells. The development of embryos took place in all combinations with *Potentilla*, but was, however, different depending on the maternal form and the species of *Potentilla* used for pollination (Tables 4 and 5). In the three studied female varieties, Freja, Sonja and Miebe Schindler, false polyembryony, which depends on the development of twin embryos in one nucellus or in the nucelluses of two fused ovules, was demonstrated. Earlier, this type of apomixis, but in other varieties, was indicated by other authors (Solntzeva 1957, Yurceva 1966, Beridze 1968, Koloteva et al. 1978, Berezenko 1979). The highest number of twin embryos was found in the Miebe Schindler variety (16 out of the 19 found), and one case of two embryos in two fused ovules. The false polyembryony showed by this author may be functional to some degree, since twin seedlings were among the germinating seeds (Fig. 1). The penetration of *Potentilla* pollen tubes into the pistils of the octoploid strawberry, many cases of false polyembryony, the obtaining of many tetraploids as the result of intergeneric pollination (Niemirowicz-Szczytt 1984b), suggest the existence of parthenogenetical development of embryos in connection with the pseudogamous development of seeds. This was signaled earlier by the ploidy of the offspring of interspecific crosses (Bauer 1960, Aalders 1964, Sukhareva 1976 and 1978). It can be suggested that the tetraploid plants obtained by this author (Niemirowicz-Szczytt 1984b) arose as the result of haploid parthenogenesis, whereas the octoploid plants, of diploid parthenogenesis. Calculating the ratio of 76 such plants to 99,194 seeds obtained from intergeneric crossing (Niemirowicz-Szczytt 1984a, b) gives a figure of 0.07% viable plants arising from stimulated parthenogenesis. The reason for very weak germination of the seeds from intergeneric crosses were abnormalities in early stages of embryo development. Embryos with cells containing nuclei of different size can develop into mixoploid plants, which were found in the progeny obtained in this study (Niemirowicz-Szczytt 1984a, b). The case of the 4-celled proembryo in the presence of unfused polar nuclei (1 out of 650 ovules) can be considered an example of autonomous embryo development. However, a larger number of cases would be necessary.

Berezenko's (1979) opinion that apomixis does not play an important role in the reproduction of *F. × ananassa* in the case of intraspecific pollination, may be accepted as true. It should, however, be added that due to the pollination of the strawberry by *Potentilla* species, matroclinous plants with a differing degree of ploidy, and a predominance of tetraploids (Niemirowicz-Szczytt 1984b) can be ob-

tained through apomixis. The results obtained by this author contradict those of Staudt (1970) which state that apomixis does not take place in the Freja and Miebe Schindler varieties. However, his supposition that the disclosure of apomixis depends on the choice of specific components of the cross, may be true.

Acknowledgment

I would like to thank Prof. Romana Czapik for introducing me into the embryology of *Rosaceae* and for the discussion on the results of the study.

REFERENCES

- Aalders L. E., 1964. Production of maternal type plants through crosses to apomictic species. *Nature* 204: 101-102.
- Asker S., 1971. Some viewpoints of *Fragaria* × *Potentilla* intergeneric hybridization. *Hereditas* 67: 181-190.
- Bauer R., 1960. Grundlagen und Methoden der Züchtung bei der Gartenerdbeere (*Fragaria ananassa* Duch.). *Z. Pflanzenzüchtung* 44: 403-430.
- Bauer R., Bauer A., 1967. Neue Wege in der Erdbeerzüchtung. *Erwerobstbau*. 9: 83-85.
- Berezenko N. P., 1970. Some problems of biology of fertilization and embryogeny in *Fragaria grandiflora* Ehrh. *Ukr. Bot. Zhurn.* 27: 593-600.
- Berezenko N. P., 1979. Abnormal phenomena in the development of ovules, female gametophyte and embryo in *Fragaria grandiflora* Ehrh. *Ukr. Bot. Zhurn.* 36: 16-20.
- Beridze R. K., 1964. K izucheniyu embriologii roda *Fragaria* L. *Tr. Tbilis. Bot. Inst.* 23: 235-239.
- Beridze R. K., 1968. Poliembrioniya u ziemlaniki. *Mater. Vsesoyuz. Symp. Embriol. Rast. Moskva*. pp. 32-34.
- Hughes H. G., Janick J., 1974. Production of tetrahaploids in the cultivated strawberry. *Hort. Sci.* 9: 442-444.
- Koloteva N. I., Zubov A. A., Zhukov O. S., 1978. Cytoembryological data on the stimulation of apomixis in strawberry. *Biul. Gl. Bot. Sada AN-SSSR.* 109: 65-68.
- Konstantinov A. V., 1966. Razvitye dopolnitelnykh zarodyshchevykh meskov i yavlenye poliembrionii u *Fragaria ananassa*. In: *Eksperimentalnaya Botanika*. Kolos, Minsk. pp. 43-48.
- Niemirowicz-Szczytt K., 1982. Apomiksja w rodzinie różowatych. *Wiad. Bot.* 26: 19-28.
- Niemirowicz-Szczytt K., 1984a. The results of intergeneric pollination of *Fragaria* × *ananassa* Duch. and *Fragaria virginiana* Duch. by *Potentilla* species. *Acta Soc. Bot. Pol.* 53: 443-454.
- Niemirowicz-Szczytt K., 1984b. Morphological and cytological evaluation of progeny obtained from pollination of *Fragaria* × *ananassa* with *Potentilla* spp. *Acta Soc. Bot. Pol.* 53: 455-468.
- Rudloff C. F., 1930. Entwicklungsphysiologische Studien in der Gattung *Fragaria*. *Gartenbauwissenschaft* 3: 79-99.

- Shangin-Berezovskiy G. N., 1962. O materynskom nasledovanii u zemlaniki. Tr. Inst. Gen. AN-SSSR. 26: 68-84.
- Solntzeva M. P., 1957. Osobiennosti stroyeniya zarodishchego meska *Fragaria grandiflora* Ehrh. i yavlenie poliembriinii. Dokl. AN-SSSR. 116: 866-870.
- Solntzeva M. P., 1961. Nekatorye dannye o procesie oplodotvoreniya u *Fragaria ananassa* Duch. Bot. Zhurn. 46: 371-377.
- Staudt G., 1970. Test for apomixis. Hort. Res. 10: 148-150.
- Sukhareva N. B., 1976. Ob apomiksii u ziemlaniki. In: Apomiksii i yego znacheniiye dlya evolyucii i sel. Nauka, Novosibirsk. pp. 152-164.
- Sukhareva N. B., 1978. Znacheniiye nieredukcii gamet u *Fragaria*. In: Apomiksii u rastenii i zhyvotnykh. Nauka, Novosibirsk. pp. 118-128.
- Yurceva N. S., 1966. Embriologicheskiye prichyny piestichnoy sterilnosti u sadovoy ziemlaniki. Dokl. Mosk. S-Ch. Akad. im. K. A. Timiriazeva 125: 95-98.

*Badania embriologiczne Fragaria × ananassa Duch. po zapyleniu
trzema gatunkami Potentilla*

Streszczenie

Przeprowadzono badania embriologiczne czterech odmian żeńskich (Freja, Sonja, Mieke Schindler, Reine des Precocé) *Fragaria* × *ananassa* Duch. po zapyleniu trzema gatunkami *Potentilla* (*P. geoides*, *P. rupestris*, *P. fruticosa*). Celem pracy było wyjaśnienie sposobu powstawania potomstwa matroklinalnego po zapyleniu truskawki pyłkiem *Potentilla*. U odmiany Freja obok mejotycznych, formowały się apo- i diplosporowe woreczki zalążkowe. Średnio u czterech odmian badanych, 49% zalążków zawierało więcej niż jeden woreczek zalążkowy. Maksymalnie rozwijało się 5 woreczków zalążkowych, ale tylko dwa z nich osiągały pełną dojrzałość. Pyłek *Potentilla* kiełkował na znamieniu truskawki i łagiewki przerastały szyjkę słupka do zalążka. W komórkach jajowych truskawki po zapyleniu *Potentilla* nie stwierdzono oznak zapłodnienia, natomiast po zapyleniu odmianą truskawki Redgauntlet F. × *ananassa* w jądrze komórki jajowej widoczne były dodatkowe jąderka lub grudki chromatynowe. Poliembrionia rzekoma była notowana u odmian Mieke Schindler (16 przypadków), Sonja (2 przypadki), Freja (1 przypadek). Obserwowano kiełkowanie dwóch roślin z jednego nasienia, co świadczy o możliwości pełnego rozwoju zarodków bliźniaczych. Słaba siła kiełkowania nasion była spowodowana zaburzeniami w rozwoju zarodków.