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PECULIARITIES OF THE SEED GERMINATION OF AGAMOSPERMIC SEEDLINGS *Fragaria* × *ananassa* Duch. IN DIFFERENT BREEDING SYSTEMS

***S.O. Baturin*[1], *E.V. Ambros*[2]**
[1] *Institute of Cytology and Genetics, Siberian Brunch of the Russian Academy of Sciences, Novosibirsk 630090, Russia, e-mail: SO_baturin@mail.ru*
[2] *Central Siberian Botanical Garden, Siberian Brunch of the Russian Academy of Sciences, Novosibirsk 630090, Russia, e-mail: ambros@ngs.ru*

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Summary

The seeds germination dynamics of parthenogenetic progenies of the cultivar Purpurovaya *Fragaria* x *ananassa* Duch. (2n = 8x = 56) were compared during inbreeding, autbreeding and agamospermia. It was shown that the revealed differences are caused by genotypic conditions. The seed germination of parthenogenetic progenies is much higher during inbreeding and autbreeding, than the one of original cultivar from which they were breeding. It is possible to use offsprings of agamospermetic origin succesfully in garden strawberry breeding due to high seed germination.

Key words: garden strawberry, germinating capacity, seed germination rate, inbreeding, outbreeding, agamospermy, selection.

Garden strawberry *Fragaria* × *ananassa* Duch. (2n = 8 × = 56) is one of fruit crops occupying largest areas in the world. The assortment of strawberry is being constantly updated owing to implementation of science-based breeding programs (1). The main breeding technology is hybridization aimed at seeds obtained in different combinations of crosses. Under favorable conditions, garden strawberry performs a sexual (zygotic) way of seed formation with double fertilization. In this case, seeds (achenes) develop after self-pollination (autogamy) or cross-pollination (xenogamy). T.S. Fadeev defined F. × *ananassa* as a crop with facultative cross-pollination (2).

It has been shown that strawberry seeds germinate non-simultaneously and their germination rates depend on genotypic characteristics of species (3, 4). Seeds obtained by cross-pollination (outbreeding) demonstrated a very good germination rate (3). At the same time, it has been reported about relatively low germination rates and partial death of seedlings in several studies (5-7). The frequency of these symptoms is determined by action of lethal genotypic factors typical for outbreeding or inbreeding (2). Multiplicity of systems and ways of seed formation in F. × *ananassa* provides a wide genetic variability in seed progeny, which is necessary for primary selection. Along with zygotic formation of seeds, garden strawberry demonstrates azygotic way (agamospermy) (8-11). *Fragaria* has a typical agamospermy the pseudogamy type, which occurs under adverse conditions of pollination and fertilization difficulties (10). Agamospermic descendants retain maternal number of chromosomes and show the genetic variability sufficient for selection of promising seedlings - candidates for cultivars (10, 11). They can be also successfully used in breeding programs as one of parental forms, because their genotypic status corresponds to seedlings obtained by self-pollination (12).

The purpose of this work - to study germinating capacity of achenes in garden strawberry agamospermic descendants obtained by inbreeding, outbreeding and agamospermy.

Technique. Experiments were carried out in the experimental field of the Institute of Cytology and Genetics SB RAS (Novosibirsk). The maternal forms of *F.* × *ananassa* were octoploid samples of agamospermic origin derived from cv Purpurovaya: Yu-541 and Yu-545 – the result of heterogeneous pollination with pollen of silverweed (*Potentilla anserina*, 2n = 4 × = 28), D-344 – the descendant from intervarietal pollination with pollen of musk strawberry (*F. moschata*, 2n = 6 × = 42) subjected to X-ray irradiation (186 Gy). These three samples showed substantial biomorphological resemblance with maternal form and some individual features as well (13).

In controlled crosses, inflorescences were covered with transparent food cellophane packages with a cotton wool pad wrapped around the base of inflorescence to prevent the entry of insects-pollinators. During an outbreeding, unopened flowers were castrated by tweezers and then pollinated with pollen of the experimental sample *F.* × *ananassa* – the hybrid cultivar-line L-1-17-2 (2n = 56). The pollen was collected from unopened flower buds and dried at 20-22 °C without access of direct sunlight. Pollination was performed one-fold by soft brush. Agamospermic seeds were obtained as a result of diploid parthenogenesis (10, 11) induced by heterogeneous (intergeneric) pollination of castrated and isolated flowers. The pollinator - a local ecotype of silverweed (*P. anserina* L., 2n = 4 × = 28); the pollen was isolated from flower buds and dried at a 20-22 °C without access of direct sunlight. The control – seeds of garden strawberry cv Purpurovaya formed after open pollination.

Seeds were germinated in Petri dishes on wet filter paper at 20-23 °C after the preliminary stratification at 2-3 °C for 4 months. Survival rates of seedlings were accounted in 30 days after planting them in soil (open ground).

Statistical analysis was performed using the criterion χ^2 (14).

Results. In the samples Yu-545 and Yu-541, the pollen of *P. anserina* L. provided a statistically significant decrease in seed germinating capacity compared with that at self-pollination (Table 1).

1. Characteristics of seed germination in samples of *Fragaria* × *ananassa* Duch. cv Purpurovaya at different variants of pollination (laboratorial experiment)

Variant of pollination	Seeds			χ^2
	total, pcs.	germinated, pcs.	germinated, %	
D-344 × PA	473	308	65,1	1,7
D-344 S	48	42	87,5	
Yu-545 × PA	80	12	15,0	24,0
Yu-545 S	40	36	90,0	
Yu-541 × PA	371	54	14,6	38,6
Yu-541 S	38	29	76,3	

Note: description of samples – see "Technique", PA — *Potentilla anserina*; S — self-pollination; $\chi^2_{0,05}$ = 3,84.

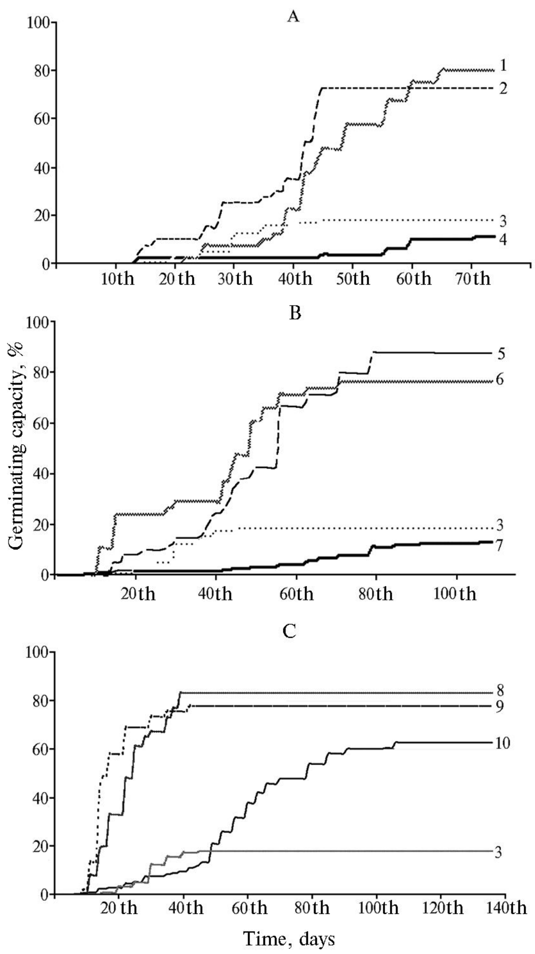
It was possible, that seeds obtained by intergeneric hybridization didn't germinate owing to postzigotic mortality of hybrid embryo (5, 6). The sample D-344 also manifested at heterogeneous pollination the lower germination rate than that at self-pollination, but these differences were not reliable (χ^2 = 1,7). In other words, the number of germinated seedlings of hybrid (intergeneric) origin is quite small of completely absent.

2. Mortality of seedlings in samples of *Fragaria* × *ananassa* Duch. cv Purpurovaya at different variants of pollination (laboratorial experiment)

Variant of pollination	Seeds, pcs.	Dead seedlings		χ^2
		pcs.	%	
D-344 × PA	473	206	43,5	0,72
D-344 S	48	26	54,2	
Yu-545 × PA	80	9	11,3	1,90
Yu-545 S	40	9	22,5	
Yu-541 × PA	371	48	12,9	0,14
Yu-541 S	38	4	10,5	

Note: see Table 1.

This assumption was confirmed by comparison of death rates of seedlings at different variants of pollination (Table 2). For both heterogeneous and self-pollination, proportions of non-viable seedlings were almost similar, therefore, juvenile mortality occurred as manifestation of lethal genetic factors with no connection with hybrid (intergeneric) origin of seedlings.



The dynamics of seed germination in samples of garden strawberry cv Purpurovaya Yu-545 (A), **Yu-541** (B) **and D-344** (C) **obtained at different variants of pollination:** 1, 6 and 8 — self-pollination; 4, 7 and 10 — heterogeneous pollination with pollen of *Potentilla anserina* L.; 2, 5 and 9 — cross with the line L-1-17-2; 3 — control (cv Purpurovaya, open pollination) (laboratorial experiment).

The minimum germinating capacity was observed in seeds obtained by heterogeneous pollination (agamospermic origin) (see Fig.) - germination rates in the samples Yu-545, Yu-541 and D-344 amounted to, respectively 11,3, 12,7 and 62,6%. The seeds produced by inbreeding and outbreeding showed significantly higher germination rates and a common dynamics of germination. At the same time, the detected differences in germination rates at inbreeding and outbreeding were found to be significant. For Yu-545, Yu-541 and D-344, χ^2 equaled to, respectively, 33,0, 18,1 and 26,8 while the threshold values of χ^2 were, respectively 11,0, 12,6 and 9,5.

Seeds from the cross Yu-545 × L-1-17-2 (outbreeding) germinated within 46 days, seeds from self-pollination of Yu-545 - 68 days (see Fig., A) at a slightly higher proportion of germinated seeds (80,0%) compared with the variant with outbreeding (73,0%). The combination Yu-545 × *P. anserina* (heterogeneous pollination) had a very low germinating capacity (11,3%) and extended period of germination (up to 78 days).

For another tested maternal form – the sample Yu-541, the seeds obtained by self-pollination germinated slower than at outbreeding (see Fig., B). The proportion of germinated seeds was higher at outbreeding (87,5%) than at inbreeding (76,4%). Heterogeneous pollination of Yu-541 resulted in seeds with lowest germinating capacity (12,7%) and extended period of germination (110 days).

In contrast to Yu-545 and Yu-541, the sample D-344 manifested faster germination of seeds at different variants of pollination (see Fig., C): at inbreeding - 39 days, at outbreeding - 42 days (germinating capacity - respectively, 83,0 and 77,7%). At heterogeneous pollination of D-344, germinating capacity of seeds was significantly higher than that of Yu-545 and Yu-541 (62,6%) while retaining the extended period of germination 107 days.

It is a well-known fact, that seed reproduction of garden strawberry requires specific conditions for germination of achenes as they often have low germination rates and extended germination period even with preliminary short-term treatment with cold (4). Additional activities (long-term stratification, scarification with concentrated sulfuric acid, seed germination in vitro, etc.) improve germinating capacity of seeds (4, 15, 16). In this experiment, seed germination rate increased owing to reconstruction of genotype of cv Purpurovaya using agamospermy. In *F.* × *ananassa* cultivars and hybrids, agamospermy occurs as meiotic diplospory (10). In experiments on the induced agamospermy in *F.* × *ananassa*, it was found the possibility to identify genotypic structure of polyploid maternal plant by segregation of discrete parameters in agamospermic progeny (12). The authors earlier have shown similarities of genetic variation at inbreeding and in parthenogenetic offspring of garden strawberry (10, 11). In this regard, parthenogenetic descendants have different genotypes manifested in their phenotypic variation. Plants of agamospermic origin (maternal form – cv Purpurovaya) after sexual reproduction (outbreeding and inbreeding) formed the achenes with germinating capacity exceeding 73,0% while in Purpurovaya it was only 17,9%. It is possible that improved germination of achenes in agamospermic samples was provided by homozygotization of the maternal genotype. In this case, seeds carrying lethal genetic factors don't germinate, or they do, but seedlings die soon after germination, as it was observed in this experiment. Viable seedlings of parthenogenetic origin showed the nature of seed reproduction close to pure-bred lines. Thus, a singly performed agamospermy - the reconstruction of variety genotype - significantly increased the efficiency of seed reproduction in parthenogenetic progenies, which suggests the prospects for their use in new direction of breeding work – creation of garden strawberry remontant varieties propagated by seeds (17).

So, achenes of garden strawberry obtained by inbreeding, outbreeding and agamospermy showed different germination rates owing to their genotypic differences. At inbreeding and outbreeding, germination of seeds in agamospermic progeny was much higher than that in the initial maternal variety. When pollination with heterogeneous pollen (*P. anserina*), resulting agamospermic seeds demonstrated slow germination process and low germinating capacity, and, consequently, a small number of agamospermic seedlings. High germination rates of achenes in *Fragaria* × *ananassa* samples of agamospermic origin suggest using them in selection of remontant strawberry propagated by seeds.

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