STUDIES ON THE REPRODUCTIVE BEHAVIOR OF SOME ORNAMENTAL STRAWBERRY VARIETIES (*FRAGARIA X POTENTILLA*)

STUDII ASUPRA COMPORTAMENTULUI REPRODUCTIV AL UNOR VARIETĂȚI DE CĂPŞUN ORNAMENTAL (*FRAGARIA X POTENTILLA)*

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Abstract*. If for the obtention of intergeneric hybrids Fragaria x Potentilla combining ornamental value with the production of edible fruits, the only species of Potentilla used as parent is P. palustris (always as male genitor), at least four species of Fragaria were used as female genitors. Thus, there are intergeneric hybrids F. chiloensis (8x) x P. palustris, F. x ananassa (8x) x P. palustris, F. moschata (6x) x P. palustris, and F. vesca (2x) x P. palustris. As the species P. palustris is hexaploid, and the species of Fragaria used as female genitors are different in their ploidy level, from diploid to octoploid, the genetic background and ploidy of the intergeneric hybrids Fragaria x Potentilla are also different. Therefore, some major differences occur in their reproductive behavior. The studies carried out with two varieties of Fragaria x Potentilla (Pink Panda and Serenata) have shown remarkable differences between them in runnering ability, anther formation, pollen load and pollen viability. The consequences of vegetative (asexual) reproduction and/or generative (sexual) reproduction on both the propagation efficiency and phenotype stability of progenies are disscused.*

Rezumat. *Dacă pentru ținerea de hibrizi intergenerici Fragaria x Potentilla combinând valoarea ornamentală cu producerea de fructe comestibile, singura specie de Potentilla folosită ca genitor este P. palustris (întotdeauna ca genitor patern), cel puțin patru specii de Fragaria au fost folosite ca genitor matern. Prin urmare, există hibrizi intergenerici F. chiloensis (8x) x P. palustris, F. x ananassa (8x) x P. palustris, F. moschata (6x) x P. palustris, şi F. vesca (2x) x P. palustris. Intrucât specia P. palustris este hexaploidă, iar speciile de Fragaria folosite ca genitor matern au nivel diferit de ploidie, de la diploid la octoploid, fondul genetic şi ploidia hibrizilor intergenerici Fragaria x Potentilla sunt de asemenea diferite. De aceea, în comportamentul lor reproductiv apar deosebiri majore. Studiile efectuate cu două varietăți de Fragaria x Potentilla (Pink Panda şi Serenata) au arătat diferențe remarcabile între ele în privința capacității de formare de filamente şi stoloni, de formare de antere, a încărcăturii de polen şi a viabilității polenului. Sunt discutate consecințele reproducerii vegetative (asexuale) şi/sau generative (sexuale), atât asupra eficienței de înmulțire, cât şi a stabilității fenotipice a descendenților.*

Even if the intergeneric hybridization was considered unuseful in the case of species with high level of ploidy (Darrow, 1966), the advances from recent years in ploidy manipulation, improvement of techniques used for crossings and recovery of zygotic embryos resulted from distant crossings, have determined an almost radical

reconsideration of the practical usefulness of this method for the genetic improvement of cultivated strawberry.

The success gained by the *Fragaria x Potentilla* hybrid forms resulted in a rapid increasing of the breeders interest for obtaining intergeneric hybrids presenting both the important traits of *Fragaria* species and some traits of *Potentilla* (Sayegh and Hennerty, 1993; MacFarlane Smith and Jones, 2004), mainly those associated with ornamental value (Fig. 1). Therefore, numerous intergeneric varieties of *Fragaria x Potentilla* have been released in last decade.

Although an extensive range of traits, of great interest for genetic improvement, is available in the diploid, tetraploid or hexaploid species of *Potentilla*, the most attempts to incorporate directly traits from these species into the octoploid cultivated varieties of strawberry have failed. Currently, hybrid progenies can be obtained from such crossings, but they are either sterile, or with very low fertility to be used as genitors in subsequent crossings. Moreover, the restauration of fertility of *Fragaria x Potentilla* hybrids by doubling the chromosome number is inappropriate, due to the high level of ploidy.

In this context, the observations and measurements carried out by us according to the methodology for evaluation of *Fragaria* germplasm, were aiming at the characterization of the reproductive behavior of the intergeneric hybrid forms of *Fragaria x Potentilla* existing within National Collection of *Fragaria*, at the Research Institute for Fruit Growing Pitesti.

MATERIAL AND METHODS

The intergeneric hybrids of *Fragaria x Potentilla* with different origin and ploidy level, named "Pink Panda" and respectively "Serenata", have been investigated. In order to determine the reproductive behavior of these intergeneric hybrids, observations and measurements on adrosterility/androfertility, viability and germination ability of the gametes, as well as observations on fruit set and seed formation, were carried out.

Fig. 1. The ornamental strawberry (*Fragaria x Potentilla*)

All the measurements on flower morphology at the intergeneric were carried out at the moment when the plants exhibited at the maximum their characteristics and traits, according to the standardized methodology for the evaluation of *Fragaria* genetic resorces.

For the evaluation of pollen viability in the "Pink Panda" and "Serenata" varieties, the anthers excised from flowers in the stage of unopened buds just before blossoming, were fixed in Carnoy's fixative for 12 hours and then rinsed in 80% alcohol. Pollen viability was determined by microscopic observations on slides with aceto-carmine stained pollen (Zebrowska, 1995).

In order to evaluate its germination ability, the mature pollen collected from the two varieties investigated was sprinckled on solid culture media containing 1.5 g of agar, 15 g of sucrose, and 0.01 g of acid boric mixed in 100 ml of water, and maintained in normal conditions of temperature and humidity. The microscopic observations for calculating the frequency of pollen grains which germinated *in vitro* were carried out at two hours intervals for at least 8 hours, and finally after 24 hours.

RESULTS AND DISCUSSIONS

The flowers formed by *Fragaria x Potentilla* intergeneric hybrids of "Pink Panda" type have the morphology characteristic to *Fragaria* species. An interesting observation was that the plants of this type are forming two distinct groups, differing both by their overall vigor, and the diameter of flowers. However, the morphology and color of flowers formed by plants of both types is identical.

A gynodioecious reproductive system was found in the "Pink Panda" variety, characterized by the presence of both female and hermaphrodite flowers. However, the microscopic observation has revealed that if the anthers are formed, the load of viable microspores is most often low (Table 1).

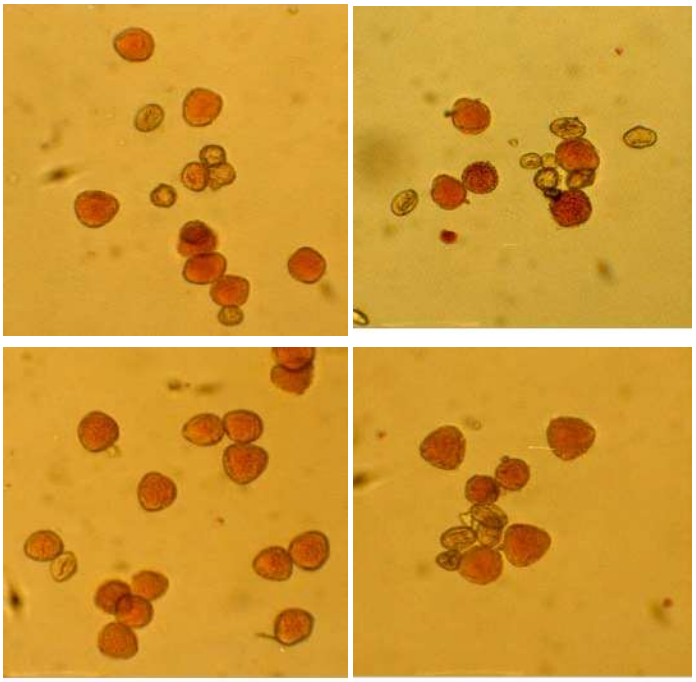

Fig. 2. Viable and non-viable (sterile) pollen formed in "Pink Panda" (left up and right up) and "Serenata" type of *Fragaria x Potentilla* (left down and right down)

The flowers formed by plants of *Fragaria x Potentilla* intergeneric hybrids of "Serenata" type have size comparable with those of "Pink Panda" type, characterized by vigor similar to the octoploid forms of *Fragaria*, but their color is red. The major difference between the flowers formed by the plants belonging to the two intergeneric hybrid forms is that those of "Serenata" types are developing anthers with normal morphology, in which viable microspores are formed. Anthers of the hermaphrodite flowers formed by the intergeneric hybrids of this type are big in size, with a length of about 2-2.5 mm, with medium to long filaments, and all of red color. They released abundantly pollen of yellow-golden color in less than 24 hours when maintained at the room temperature (20 ± 2°C).

The results of microscopic measurements have shown that *Fragaria x Potentilla* hybrids of "Pink Panda" type and respectively of "Serenata" type are significantly different in pollen size (Fig. 2). These differences have been revealed not only by the amplitude of variation in measured diameter of pollen grains, but also by their average diameter and volume, respectively. Thus, while in intergeneric hybrids of "Serenata" type (whose female parent is likely to be an octoploid species of *Fragaria*), the pollen diameter is ranging between 43 and 69 μm, in the hybrids of "Pink Panda" type (whose female parent is probably the *Fragaria vesca* species), the pollen size is ranging between 29 and 47 μm. (Table 1). As a consequence, while a frequency of 77.4% viable pollen was found in "Serenata", an average percentage of only 58.6% of viable pollen was calculated for "Pink Panda" type of Fragaria x Potentilla.

The large amplitude of variation in size of pollen grains formed by the *Fragaria x Potentilla* intergeneric hybrids can be easily explained, taking into considerations the high differences of ploidy between the parent species on the one hand, and between the *Fragaria* female parents for the investigated intergeneric hybrids, on the other hand.

Table 1

Viability and germination ability of pollen grains in "Pink Panda" and "Serenata" type of *Fragaria x Potentilla* intergeneric hybrids.

***Fragaria x Potentilla* hybrids**	**Pollen size (μ)**			**Viable pollen (%)**	**Germinating pollen (%)**	
	min.	**max.**	**average**		**after 6 hours**	**after 24 hours**
Pink Panda	29	47	39 b	58.6 b*	6 a	7 b
Serenata	43	69	56 a	77.4 a	9 a	22 a

* In each column, the mean values with different letters are significantly different (Duncan's multiple range test, $p<0.05$)

The size of pollen grains formed in anthers of investigated intergeneric hybrids and the calculated frequency of viable pollen has provided important data for establishing their origin. In this context should be mentioned the fact that even in the case when the basic number of chromosomes is identical (as is the case with species within *Fragaria* and *Potentilla* genera), the intergeneric hybrids obtained from their crossing presents

numerous abnormalities in meiosis due to the low level of chromosome homology, resulting in unbalanced distribution into gametes and implicitly in their frequent sterility.

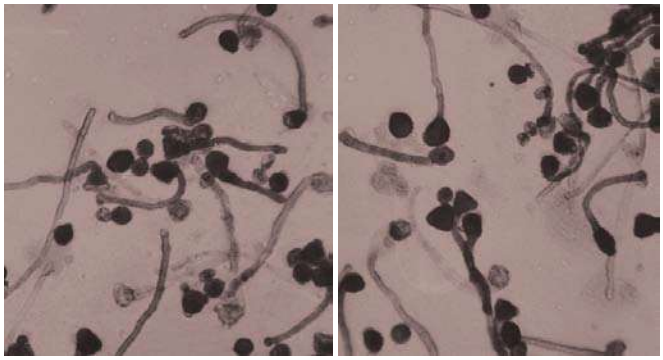

Fig. 3. Pollen germination in "Serenata" type of *Fragaria x Potentilla*.

The microscopic observations revealed that the share of viable pollen is high in *Fragaria x Potentilla* intergeneric hybrids of "Serenata" type (Fig. 2) and (when they are formed) even in those of "Pink Panda" type. Moreover, the germination ability of pollen grains is significantly higher in plants of "Serenata" type (whose female parent is considered to be the octoploid species *Fragaria chiloensis*). Thus, the calculated frequency of pollen grains forming at least an pollen tube with normal growing (Fig. 3) was 22% in hybrids of "Serenata" type compared to less than 10% in hybrids of "Pink Panda" type.

The considerable difference between the intergeneric hybrids having different *Fragaria* species as female parent in respect of fertility of the male gametes explains their different potential of fruit set. Most relevantly is the fact that the ability to set fruits is significantly higher in the *Fragaria x Potentilla* intergeneric hybrids having an octoploid species of *Fragaria* as female parent.

Observations carried out in two consecutive years on the fruit set from flowers of *Fragaria x Potentilla* intergeneric hybrids of "Pink Panda" and respectively "Serenata" type have shown that only a part of them set fruits. An interesting and surprising finding is that the frequency of fruit set is higher in hybrids of "Pink Panda" type, compared to hybrids of "Serenata" type. Thus, more than a third of the flowers from inflorescences of "Pink Panda" set fruit, while in the "Serenata" only the primary and secondary flowers from the inflorescences were found to set fruits. The number of normal achenes, containing viable embryos, is also higher in fruits formed from flowers of *Fragaria x Potentilla* intergeneric hybrids of "Pink Panda" type. As a matter of fact, as the size of fruit is decisive dependent of the number of achenes containing viable embryos, the higher size of fruits formed by the intergeneric hybrids of this type is explainable.

Since the flowers of the hybrids of "Pink Panda" type are often androsterile, the high frequency fruit set of flowers in the hybrids of this type is obviously the result of open (cross) pollination. The fruit set indicates high viability of female gametes, an essential condition for normal fruiting. Is therefore explainable the higher size and weight

of fruits in hybrids of "Pink Panda" type, as compared to those of hybrids of "Serenata" type, as it is an well known fact that the cross pollination assures always the formation of a higher number of seeds and, implicitly, formation of bigger fruits.

CONCLUSIONS

(1) The absence of anthers in many of the flowers formed by plants of "Pink Panda" type, and the low frequency of viable pollen, has great implications as, considering the fact that the plants of this type does not form runners and therefore they cannot be propagated vegetatively, the cross pollination becomes the most likely way of reproduction, which results in non-uniformity of individual plants within progenies, in which their most important characteristics (primarily the color of flowers and size of fruits) segregates.

(2) The androfertility of the *Fragaria x Potentilla* intergeneric hybrids of "Serenata" type has a major genetic consequence and practical implication, as it create the conditions for self-pollination and allows the chance of their propagation by seeds avoiding the risk of traits segregation (due to their high level of homozygosity).

(3) The differences existing between the investigated forms of *Fragaria x Potentilla* in gamete viability and fertility, resulting from their different meiotic behavior, have also an important implication in breeding. While the use as male parent of hybrids of "Pink Panda" type could be difficult or even impossible, the hybrids of "Serenata" type can be male parents in crossings with other *Fragaria x Potentilla* hybrids or with varieties of cultivated strawberry.

REFERENCES

1. Ahmadi H., Bringhurst R.S., 1989 - *Genetics of sex expression in Fragaria species*. Am. J. Bot., 78, 504-514.

2. Ashman T.L., 1999 - *Quantitative genetics of floral traits in a gynodioecious wild strawberry Fragaria virginiana: implication for the independent evolution of female and hermaphrodite floral phenotypes*. J. Hered., 83, 733-741.

3. Darrow G.M., 1966 - *The Strawberry: History, Breeding and Physiology*. Holt, Reinhart and Winston, New York, p. 3-384.

4. Delph, L.F., Galloway, L.F., Stanton, M.L. 1996 - *Sexual dimorphism in flower size*. Am. Nat., 148, 299-320.

5. MacFarlane Smith, W.H., Jones J.K., 2004 - *Intergeneric crosses with Fragaria and Potentilla. Crosses between Fragaria moschata and Potentilla fruticosa*. Euphytica, 34(3), 725-735.

6. Sayegh A.J., Hennerty M.J., 1993 - *Intergeneric hybrids of Fragaria and Potentilla*. Acta Horticulturae 348, 151-154.

7. Zebrowska J., 1995, *The viability and storage of strawberry pollen*. Plant Breed. 114(5), 469-470.