

*Contribuții Botanice, XLI, (2), 2006*  
Grădina Botanică “Alexandru Borza”  
Cluj-Napoca

## THE PHENOTYPICAL AND CYTOGENETICAL CHARACTERIZATION OF SOME ORNAMENTAL VARIETIES OF *FRAGARIA* WITH UNKNOWN ORIGIN

Anca ȘUȚAN, Aurel POPESCU  
Universitatea Pitești, str. Târgul din Vale, nr. 1, RO-110040 Pitești  
e-mail: ancasutan@yahoo.com

**Abstract:** Under the circumstances of commercial success of intergeneric hybrids *Fragaria* x *Potentilla* with ornamental value, our study had the main purpose to establish the origin of intergeneric forms *Fragaria* x *Potentilla* existing in the National Collection of *Fragaria* from **Research Institute for Fruit Growing, Pitești – Mărăcineni**. Knowing the origin of these forms is an important issue for their use in hybridizations with commercial varieties of strawberry, because many varieties and selections present in commercial growing have as a parent, from *Fragaria* genus, one of the species *F. x ananassa*, *F. chiloensis* or *F. vesca*.

Since the ploidy level of intergeneric hybrids *Fragaria* x *Potentilla* could be different, depending on their origin, an important aim of our study was to establish the ploidy level of these intergeneric hybrids with normal fertility.

**Keywords:** intergeneric, hybrids, phenotype, ploidy, origin.

### Introduction

The genus *Fragaria*, including over 20 species and subspecies (Staudt, 1989; Staudt, 2001; Mabberley, 2002) with a large geographical distribution and an impressive differentiation on the aspect of the ecological adaptation, it is found today among the priorities of sustained and complex programmes of breeding.

The *Potentilla* genus – which consists of a great number of species, their number exceeding 500 (Mabberley, 1997) – it was and still is, one of the genera raising serious taxonomical controversies, implying that the genera *Fragaria*, *Comarum*, *Tormentilla* and *Sibbaldia*, distinct genera nowadays, should be included together in the *Potentilla* genus.

*Potentilla palustris* is one of the parents of the intergeneric hybrids released in the USA as ornamental cultivar of *Fragaria*. The first one was released in 1989 under the name “Frel”, and the second one was released in 1991 under the name “Serenata”.

During the last decade, the range of intergeneric hybrids increased considerably, numerous other ornamental cultivars or selections of *Fragaria* being added to “Frel” and “Serenata”, that combine the ornamental value given by the beauty of flowers and the prolonged period of flowering (May – October) with the production of edible fruits. *Fragaria* x *Potentilla* intergeneric hybrids known under the names “Pink Panda”, “Lipstick” și “Red Ruby”, enjoyed the biggest commercial success, which allowed them the constant presence in the catalogues and on the lists of varieties multiplied and commercialized by the nurseries and famous companies producing planting material from USA, Australia, Canada, and the countries from Western Europe.

### Material and Methods

The biological material investigated was represented by the intergeneric forms *Fragaria* x *Potentilla* existing in the National Collection of *Fragaria* from the Research Institute for Fruit Growing Pitești-Mărăcineni. Since the preliminary observations on their phenotype showed that the investigated intergeneric hybrids *Fragaria* x *Potentilla* must have different origin, they were

divided in two distinct groups. One of them include those hybrids that have as a female parent one of the octoploid species of *Fragaria*, and the other one include hybrids that have as female parent the diploid species *F. vesca* or *F. vesca* var. *semperflorens*.

The foliar characteristics analyzed were the length of the leaf petiole, the total length of the leaf and of each of the folioles. The analysis of floral characteristics involved the measurement of the length of the inflorescences and of the flowers' diameter, as well as determination of the number of flowers in the inflorescence.

The measurements and determinations were performed at the moment of expression at their maximum level of the phenotypical characters analyzed, according to the standardized methodology for the evaluation of the genetic resources of *Fragaria*.

Determination of the size of pollen grains formed by anthers of investigated intergeneric hybrids, the assessment of their viability, and observations on some microscopic morphological traits (stomata length and density, number of chloroplasts per stomata) that are used as indirect indicators of the ploidy level, were also carried out as means of providing important data for establishing their origin.

Because of the low frequency of metaphase cells in root tips used for chromosome counts, and also because of the large number of very small chromosomes (most of the chromosomes are punctiform) in each meristematic cell, determination of chromosomes number in the investigated intergeneric hybrids was proven to be very difficult.

### Results and Discussions

The measurements performed in accordance to the working protocol for the characterization of the genetic resources of *Fragaria* have shown that between the intergeneric hybrids *Fragaria* x *Potentilla* there are major differences in the length of the leaf petiole, as well as in the total length of the leaf and of each foliole. Thus, the intergeneric hybrid plants that have an octoploid species of *Fragaria* as a female parent and *P. palustris* as a male parent, were found to have leaves with a petiole length ranging between 262 and 308 mm, while the plants that have a diploid species of *Fragaria* (most likely *F. vesca*) as a female parent, were found to have leaves that have the petiole length ranging between 176 and 226 mm (Tab.1). Directly correlated to the amplitude variation in this trait, the average length of the petiole was 288 mm in the intergeneric hybrid plants from the first group and respectively 197 mm in those from the second group.

Significant differences among the hybrid plants *Fragaria* x *Potentilla* were observed regarding the length of the central foliole, a characteristic used currently in the morphological characterization of the genetic resources of *Fragaria*. Thus, the calculated average length was 95 mm for the plants from the first group and respectively 82 mm at those from the second group (Tab. 1).

Among the intergeneric hybrids that have *F. x ananassa* or *F. chiloensis* as female parent and respectively those that have *F. vesca* or *F. vesca* var. *semperflorens* as female parent, there are obvious differences regarding the thickness of the leaves, as well as the intensity of pigmentation, that are much bigger for the plants from the first group, confirming indirectly the higher level of ploidy of their female parent, in comparison to those of the species of *Fragaria* which have been at the origin of those in the second group.

Similarly, the measurements concerning the length of the inflorescences, the number of flowers per inflorescence and the diameter of flowers have marked out significant differences among the investigated intergeneric hybrid plants. Thus, intergeneric hybrid plants whose female parent was presumably an octoploid species of *Fragaria* were found to have inflorescences with a length ranging between 258 and 324 mm (Tab. 1), while the hybrid plants that have the species *F. vesca* as a female parent were characterized by inflorescences having a length ranging between 226 and 306 mm. Directly correlated with the amplitude variation, the average length of

## PHENOTYPICAL AND CYTOGENETICAL CHARACTERIZATION OF SOME 131 ORNAMENTAL VARIETIES OF *FRAGARIA*

the inflorescences was 285 mm in the hybrid plants from the first group and respectively 27 mm in those from the second group.

**Table 1: Variation of some phenotypical characters for the intergeneric hybrids *Fragaria x Potentilla* and their possible female parents**

Species	Length of the leaf petiole* (mm)	Length of the central foliole* (mm)	Breadth of the central foliole* (mm)	Maximum length of the inflorescence (mm)	Number of flowers per inflorescence	Diameter of flower* (mm)	Number of anthers per flower
<i>Fragaria</i> (8x) x <i>Potentilla</i>	308	95	66	324	6-13	33	28-38
<i>Fragaria</i> (2x) x <i>Potentilla</i>	226	82	45	306	5-9	28	14-22
<i>Fragaria x ananassa</i>	334	128	76	346	7-28	46	34-46
<i>Fragaria chiloensis</i>	312	92	72	244	6-16	38	38-42
<i>Fragaria vesca</i>	242	64	38	265	6-10	26	16-20
<i>Fragaria vesca</i> var. <i>semperflorens</i>	268	86	54	282	5-14	22	16-20

Intergeneric hybrids *Fragaria x Potentilla* were forming inflorescences over a period of three months, this being a characteristic inherited from *P. palustris*, which is absent in every of the octoploid species of *Fragaria*, but present in the variety *semperflorens* of the *F. vesca* species.

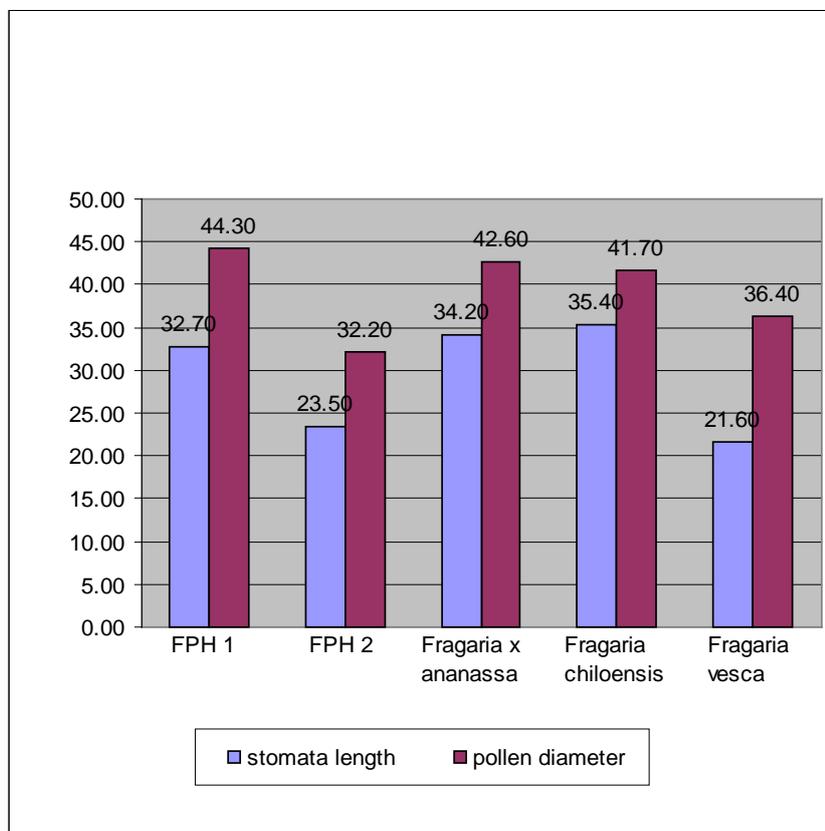
The number of flowers per inflorescence was significantly higher in the intergeneric hybrids whose female parent must have been an octoploid species of *Fragaria*, ranging between 6 and 13, as compared to those having the diploid species *Fragaria vesca* as female parent, to which the number of flowers per inflorescence varied between 5 and 9 (Tab. 1). The difference between the average number was found to be even more relevant, with a value of 9.4 for the hybrids from the first group and respectively 7.6 for those from the second group.

The comparison of the average diameter of the flowers revealed some other significant differences among the investigated intergeneric hybrids, as the average value of the flower diameter was 33 mm for the plants from the first group, while for the hybrids from the second group this value was 28 mm.

Both for the amplitude variation of stomata length and their average size, were significant differences among the investigated intergeneric hybrids *Fragaria x Potentilla*, allowing us to make a clear distinction between hybrids that have as female parent an octoploid species of *Fragaria*, respectively a diploid species. Thus, the intergeneric hybrids from first group are characterized by stomata that have the length ranging between 24.3 and 37.8  $\mu\text{m}$ , while the intergeneric hybrids from second group are characterized by stomata that have the length ranging between 18.9 and 29.7  $\mu\text{m}$  (Fig. 1). Directly correlated to the amplitude variation of this characteristic, the average length of stomata (indirect characteristic for the ploidy level) is 32.7  $\mu\text{m}$  for the intergeneric hybrids from the first group and respectively 23.5  $\mu\text{m}$  for the hybrids from second group.

Due to the relation between the ploidy level and stomata density, and also between stomata size and their frequency, there are significant differences between the intergeneric hybrids *Fragaria x Potentilla* from the two investigated groups. As compared to the intergeneric hybrids that have the diploid species *Fragaria vesca* as female parent, in which the stomata

density (measured per microscope field of vision) was 21.4, a corresponding value of only 16.9 was found for the intergeneric hybrids that have one of the octoploid species of *Fragaria* as female parent.



**Fig 1: Average values of stomata length and pollen diameter for the intergeneric hybrids *Fragaria x Potentilla* and their possible female parents**

The chloroplast number assessed through microscopic observation, allowed differentiation between hybrids from the two groups and provided further information about the octoploid species that must have been female parent, due to the highest values of this characteristic in *F. chiloensis*. It is a relevant fact that the average number of chloroplasts per stomata was 26.9 in the intergeneric hybrids from the first group (Fig.1), which is more closer to the number calculated for *F. chiloensis* (28.4 chloroplasts per stomata) in comparison with those calculated for *F. ananassa* species (23.7 chloroplasts per stomata).

Significant differences were found also between the size of pollen grains within the intergeneric hybrids from the two investigated groups (Fig. 2). Thus, the intergeneric hybrids from the first group were found to have the pollen diameter ranging between 24 and 59  $\mu\text{m}$ , while the intergeneric hybrids from the second group showed a pollen diameter ranging between 19 and 46  $\mu\text{m}$ . This large amplitude of variation in the size of pollen grains could be easily explained taking into consideration the large differences between the ploidy level of parental species on the one hand, and the large differences between the ploidy level of *Fragaria* species (that can be female parents for the *Fragaria x Potentilla* intergeneric hybrids) on the other hand.

Microscopic observations on the metaphase cells in squash preparations revealed that intergeneric hybrids *Fragaria x Potentilla* existing in the National Collection of *Fragaria* from the Research Institute for Fruit Growing Piteşti-Mărăcineni, are heptaploids ( $2n=7x=49$ ), respectively tetraploids ( $2n=4x=28$ ).

## PHENOTYPICAL AND CYTOGENETICAL CHARACTERIZATION OF SOME 133 ORNAMENTAL VARIETIES OF *FRAGARIA*

Because the only species of *Potentilla* representing the male parent for the intergeneric hybrids *Fragaria x Potentilla* is the hexaploid species *Potentilla palustris* ( $2n=6x=42$ ), the heptaploid level of ploidy at hybrids from first group revealed that the other parent belonging to the *Fragaria* genus must have been an octoploid species, either *F. ananassa* or *F. chiloensis*. On the other hand, the tetraploid level of intergeneric hybrids from the second group is indicating that their female parent should be considered *Fragaria vesca* ( $2n=2x=14$ ).

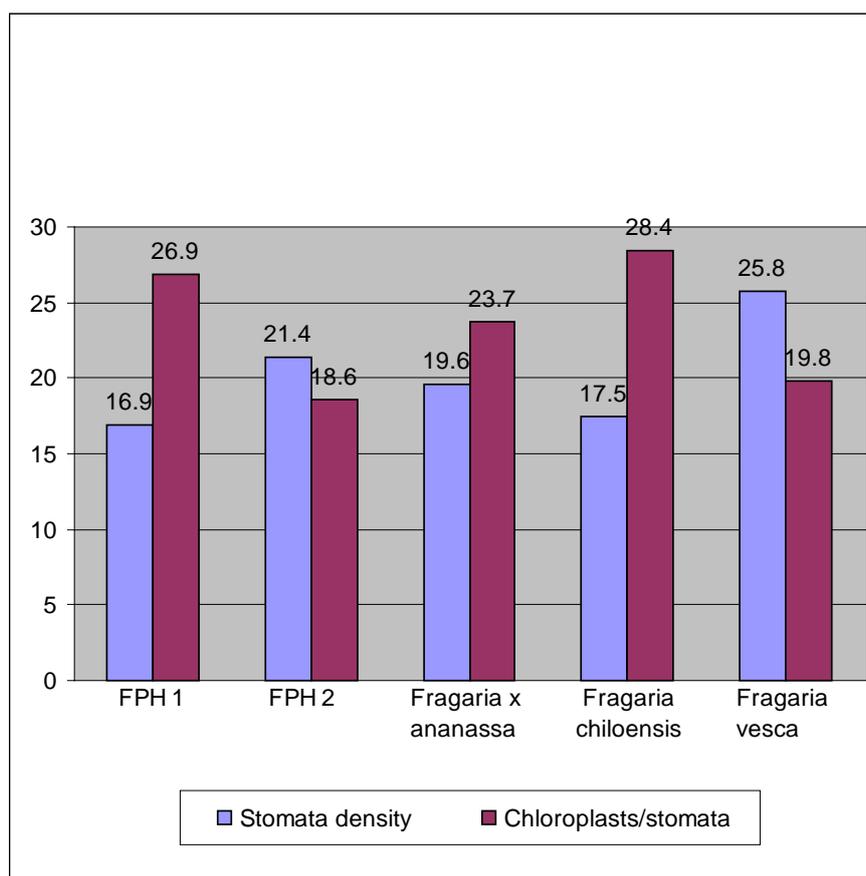


Fig. 2: Average values of stomata density and number of chloroplasts per stomata for the intergeneric hybrids *Fragaria x Potentilla* and their possible female parents

### Conclusions

The observations and measurements carried out in the field according to the standardized methodology for the evaluation of the *Fragaria* genetic resources, have shown that in the National Collection of *Fragaria* from the ICDP Pitești, there are two different groups of intergeneric hybrids *Fragaria x Potentilla*, having different species of *Fragaria* at their origin. One group includes heptaploid intergeneric hybrids that have had an octoploid species of *Fragaria* as female parent, and the other includes tetraploids, that have had the species *F. vesca* as female parent.

The knowledge of the origin of these intergeneric hybrids will allow the choice of a proper strategy for their use for further breeding. Intergeneric hybrids *Fragaria x Potentilla* that have an octoploid species of *Fragaria* as female parent have a biological and commercial value superior to those originating from a cross involving *F. vesca*, one of the characters conferring them superiority being the very high capacity of producing runners, that allows their vegetative propagation and avoiding of segregation of the progeny.

## REFERENCES

1. Ahmadi, H., Bringham, R.S., 1992, Breeding strawberry at the decaploid level, *J. Amer. Soc. Hort. Sci.* **117** (5): 856-862.
2. Barrientos, F., Bringham, R.S., 1974, Interspecific *Fragaria* and intergeneric *Fragaria x Potentilla* amphiploids in strawberry breeding, *Proc. XIX Intl. Congr. Hort.*, Warsaw: 326.
3. Ellis, J.R., 1960, *Fragaria – Potentilla* intergeneric hybridization and evolution in *Fragaria*, Symp. Exp. Taxon, *Proc. Linn. Soc.* **173**: 99-106.
4. Erikson, T., Donoghue, M.J., Hibbs, M.S., 1998, Phylogenetic analysis of *Potentilla* using DNA sequences of nuclear ribosomal internal transcribed spacer (ITS), and implications for the classification of Rosoideae, *Plant Syst. Evol.*, **211**: 155-179.
5. Evans, W.D., 1974, Evidence of a crossability barrier in diploid x hexaploid and diploid x octoploid crosses in the genus *Fragaria*, *Euphytica*, **23**: 95-100.
6. Evans, W.D., Jones, J.K., 1967, Incompatibility in *Fragaria*, *Can. J. Gen. Cyt.*, **9**: 831-836.
7. Geibel, M., 2002, Genetic resources in strawberry in Europe, *Acta Horticulturae*, **567** (1): 26-35.
8. Jelenkovich, G., Wilson, M.L., Harding, P.J., 1984, An evaluation of intergeneric hybridization of *Fragaria* ssp. x *Potentilla* spp. as a means of haploid production, *Euphytica*, **33**: 143-152.
9. Maberley, D.J., 2002, *Potentilla* and *Fragaria* reunited, *Telopea*, **9** (4): 793-801.
10. MacFarlane Smith, W.H., Jones, J.K., 1985, Intergeneric crosses with *Fragaria* and *Potentilla*. I. Crosses between *Fragaria moschata* and *Potentilla fruticosa*, *Euphytica*, **34**: 725-735.
11. Niemirovitz-Szytt, K., 1984, The result of intergeneric pollination of *Fragaria x ananassa* Duch. and *Fragaria virginiana* Duch. by *Potentilla* species, *Acta Soc. Bot. Pol.*, **53**: 443-454.
12. Owen, H.R., Miller, A.R., 1993, A comparison of staining techniques for somatic chromosomes of strawberry, *HortScience*, **28** (2): 155-156.
13. Staudt, G., 1962, Taxonomic studies on the genus *Fragaria*, *Can. J. Bot.*, **40**: 869-886.

**STUDIUL FENOTIPIC ȘI CITOGENETIC AL UNOR VARIETĂȚI ORNAMENTALE  
DE FRAGARIA CU ORIGINE NECUNOSCUTĂ**

**(Rezumat)**

În condițiile în care hibridii intergenerici *Fragaria x Potentilla* cu valoare ornamentală înregistrează un incontestabil succes comercial, studiul efectuat de noi a avut ca obiectiv principal stabilirea cu exactitate a originii formelor intergenerice *Fragaria x Potentilla* existente în Colecția Națională de *Fragaria* de la Institutul de Cercetare – Dezvoltare pentru Pomicultură Pitești – Mărăcineni. Cunoașterea originii acestor forme este o condiție importantă pentru utilizarea lor cu succes în hibridări cu soiuri comerciale de căpșun (cu sau fără o prealabilă etapă de consangvinizare prin autopolenizare), știut fiind faptul că diferitele soiuri și selecții existente în circuitul comercial au ca genitor, din cadrul genului *Fragaria*, una dintre speciile *F. x ananassa*, *F. chiloensis* sau *F. vesca*.

Deoarece s-a raportat că majoritatea hibridilor intergenerici *Fragaria x Potentilla* sunt heptaploizi, condiție care implică sterilitatea lor, un obiectiv important al studiului efectuat de noi l-a constituit și stabilirea nivelului de ploidie al acestor forme hibride intergenerice, întrucât acestea manifestă fertilitate normală, ceea ce exclude ploidia impară.