Using wild strawberry of the Sakha (Yakutia) Republic in breeding garden strawberry (*Fragaria* × *ananassa* Duch.)

Sergey Baturin[1,*], and *Valentina* Belevtsova[2]

[1]Institute of Cytology and Genetics SB RAS, 630090 Novosibirsk, Russia
[2]M.G. Safronov Yakut Scientific Research Institute of Agriculture, 677001 Republic of Sakha (Yakutia), Russia

Abstract. In Central Yakutia, cultivating of *Fragaria* × *ananassa* varieties involves high risk of frost-killing. To create winter-resistant cultivars of garden strawberry there was performed open pollination under the conditions of redundant pollen background of local wild strawberry *F. mandshurica* Staudt. Most seedlings grown from the achenes thus developed retain specific characters of *F.* × *ananassa*. The stock of selected seedlings was established. Creation of the varieties adapted to the local growing conditions proves the selection technique to be successful.

Fragaria L. genus includes the species following the polyploidy series: $2n = 2x$, $4x$, $5x$, $6x$, $8x$, and $10x$, where $x = 7$ [1, 2]. The taxonomy of *Fragaria* L. genus is considered to comprise 20 wild species, three spontaneous interspecific hybrids species and two cultivated hybrid species [3-5]. Among those, the synthetic octoploid garden species (*Fragaria* × *ananassa* Duch., $2n = 8x = 56$) is the most widely-cultivated berry in the world [5]. In Russia, the garden strawberry is grown throughout the country being very popular with the public. This species appeared in the Royal Botanical garden in France due to occasional crossing of two octoploid species delivered from America, namely *Fragaria chiloensis* (L.) Mill. and *F. virginiana* Duchesne [6, 7]. In Russia, garden strawberry varieties brought from Western Europe have been grown since the late XVIII century; first, mainly in the gardens of monasteries and landlords and since the XX century they have become mass-cultivated [8]. That means in Russia, *F.* × *ananassa* is actually an introduced species with all the attendant problems of low adaptation to frost, especially in Siberian regions. Practically, during the XX century till nowadays, there have been numerous attempts to both enrich the taste and increase the winter-hardiness of the garden strawberry by introgression of the genetic material of Northern Eurasia wild strawberries such as *F. vesca* L. [9-12], *F. viridis* Duch. [11-14], *F. orientalis* Los. [15-18], *F. moschata* Weston [14, 17-19]. Despite the evident progress in creating winter-resistant varieties mostly by intraspecific hybridisation *F.* × *ananassa*, the varieties with high adaptability to stressors are still needed. That is particularly important for cultivation in Eastern Siberia, including the Sakha (Yakutia) Republic, where winter temperatures are extremely low. The climate in

* Corresponding author: SO_baturin@mail.ru

Central Yakutia is extreme continental with long severe winters with low snowfall and dry hot summers. The sum of active temperatures above 10°C is 1400–1600°C. The absolute minimum is -64°C, the absolute maximum is +38°C, the range of variability for temperature to demonstrate the climate continentality level comprising 102°C [20]. In such conditions, growing *F.* × *ananassa* varieties is extremely difficult and needs special protective measures in winter. In that regard, since 1990th in M.G. Safronov Yakut Scientific Research Institute of Agriculture there were initiated investigations on strawberry introduction and breeding with selection of winter-resistant samples. Essential attention was paid to inter-specific hybridisation of *F.* × *ananassa* varieties with selected samples of local wild strawberry. According to the literature, the *Fragaria* genus in Yakutia is represented by two species: *F. viridis* (insignificantly) and *F. orientalis* (predominantly) [21]. It should be mentioned, the specific belonging interpretation differs in Russian and international botanical nomenclatures referring to the *Fragaria* genus and *F. orientalis* particularly for strawberry plants growing in Yakutia, Buryatia and Zabaykalsry Krai. In all the literature but Russian, wild strawberry samples from the listed regions are considered as the species *Fragaria mandshurica* Staudt. This conclusion results from diploid chromosome level ($2n = 2x = 14$) and complete flowers of plants from Yakutia, Buryatia and Zabaykalsky Krai, on the contrary to *F. orientalis* with its tetraploid chromosome number ($2n = 4x = 28$) and dioecy in populations. More detailed arguments for the necessity to distinguish these species are presented by a prominent taxonomist of *Fragaria* genus Günter Staudt [22]. In this connection, in the paper, we will follow his recommendation to refer wild strawberry samples from the Central Yakutia to *F. mandshurica*.

In M.G. Safronov Yakut Scientific Research Institute of Agriculture, for more than 25-years have been working on selection of seedlings, which were taken from local *F. mandshurica* populations growing in 8 uluses of Central Yakutia, by winter-hardiness and productivity; that resulted in creating the varieties Pokrovskaya and Bogdalena [23, 24]. Open pollination of selected by winter-resistance varieties of *F.* × *ananassa* and selected local *F. mandshurica* samples led to obtaining Sadovo-Spasskaya, Bersenevskaya, Vladyka Zosima cultivars [24, 25]. Conditions of open pollination of alpine forest small-fruited remontant strawberry of Alexandria variety (*F. vesca* L., $2n = 2x = 14$) by pollen of selected forms from Yakut cenopopulations *F. mandshurica* allowed to breed an adaptive and productive small-fruited variety Alexandra [26]. Experiments on crossing *F.* × *ananassa* × *F. viridis* Duch. were not further enhanced due to low efficiency of hybridisation and sterility of seedlings.

The aim of the paper is to analyse the chromosome number of seed progeny of the remontant variety Moskovsky Delicates selected seedlings with pistillate flowers crossed by *F. mandshurica* selected samples under the conditions of open pollination with redundant pollen background of *F. mandshurica*.

Materials and methods

All the wild strawberry populations from the collection of M.G. Safronov Yakut Scientific Research Institute of Agriculture under the study are diploid and by their morphology match the taxonomy keys typical for *F. mandshurica* [22]. There were two ways of pollination in the experiment: directed pollination *F.* × *ananassa* (variety Moskovsky Delicates) × *F. mandshurica* and open pollination of Moskovsky Delicates with redundant pollen background of *F. mandshurica* (Fig. 1). For the study, from the seedlings of Moskovsky Delicates (*F.* × *ananassa*, $2n = 8x = 56$) there were taken the plants with pistillate flowers which do not need castration. In directed pollination *F.* × *ananassa* × *F. Mandshurica* the inflorescences were isolated by technical wrapping cellophane bags tightly fixed by twine with labels. In the isolating bags where pollination was not performed, fruit and seed development was not observed and this proves good isolation and autonomous apomixis absence.

Fig. 1. Mass blossom of *F. mandshurica* samples near the town Pokrovsk (Yakut Republic).

As the mother form there were involved seven *F.* × *ananassa* plants pollinated by *F. mandshurica* with the help of a soft brush. The pollen was collected randomly from the nearest plants and dried for 24 hours at +20-22°C. Pollination was performed during the mass strawberry blossom in the second decade of June.

For the second type of pollination, eight Mokovsky Delicates individuals with pistillate flowers were surrounded only by flowering *F. mandshurica* plants of different selections (total number more than 2000, distance of 1 to 5 m). Thus the achenes in the Moskovsky Delicates seedlings could develop only if the pollen of *F. mandshurica* was delivered to the carpel stigma by insect pollinators. After three months of stratification, the obtained achenes were germinated in Petri dishes on wet filter paper, seedlings then placed to pricking boxes with fertile soil. When 6-8 leaves developed, the seedlings were planted to the experimental open ground; the total numbers of germinated seeds were 230 in the first and 1090 in the second type of the study. The chromosome number in seedlings were counted at root tips by staining the chromosomes with lacto-propionic orcein [27].

Results and discussion

During the mass blossom of *F. mandshurica*, *F.* × *ananassa* plants successfully develop fruits and achenes (Fig. 2). Achene germination in the two parts of the experiment differed insignificantly, standing at 81.2 ± 3.2% for directed pollination of *F.* × *ananassa*, and 85.8 ± 2.4% for open pollination. The results generally characterize good germination ability of received achenes for both cases. The seeds were planted and there were obtained 91 (39.6%) and 610 (56.0%) seedlings in the two parts of the experiment, consequently. Morphological analysis of characters along with blossom and fruitage revealed two groups of seedlings in every experimental version: mother-type (*F.* × *ananassa*-type) and hybrid-type plants. In both cases, *F.* × *ananassa*-type seedlings formed developed achenes and fruits after blossom, although most hybrid-type plants were sterile or produced misshaped fruits with sporadic achenes. Selective count of chromosome number showed $2n = 56$ in mother-type plants, while in hybrid-type seedlings, the chromosome number matched the expected result of the heteroploid crossing ($8x \times 2x$), that was $2n = 5x = 35$.

Fig. 2. Fruitage of a Moskovsky Delicates plant with pistillate flowers under the conditions of redundant *F. mandshurica* pollen background.

It is noteworthy, among the off-springs in the first version of the experiment or *F.* × *ananassa* × *F. mandshurica*, there were as many as 23.9% mother-type seedlings. Heteroploid crossings in *Fragaria* often result in occurrence of matromorphic-type seedlings (mother parent chromosome number) alongside the expected hybrid ones. When the pollinated flowers are isolated, there appear $8x$ ($2n = 56$) seedlings showing no any character of a father parent due to realisation of the additional precautionary mechanism of sexual process – agamospermy in a form of pseudogamous diplospory. We have shown this mechanism to be realised in *F.* × *ananassa* cultivars only under the conditions when normal megasporogenesis and fertilisation are hindered [28]; thus variability in agamospermic progenies allows to perform selective breeding [29]. Undoubtedly, in Central Yakutia, preparation of *F.* × *ananassa* plants to blossom and the blossom process are negatively influenced by low temperatures during their vegetation. Hence a rate of seedlings of agamospermous origin is high. Under the climatic conditions of Novosibirsk, we performed controlled crossings *F.* × *ananassa* ($8x$) × *F. orientalis* ($4x$) and *F.* × *ananassa* ($8x$) × *F. moschata* ($6x$). In the first crossing, there was 1 agamospermous seedling of 104, and in the second – 2 of 129 ones, that comprises 0.95% and 1.55% of all fruit-bearing seedlings, consequently. We suppose occurrence of 1.5% agamospermous off-springs to be a typical frequency rate for the pollination and fertilization norm under the climatic conditions of Novosibirsk Oblast [28]. When blossom of *F.* × *ananassa* plants takes place under severe climatic conditions of Central Yakutia, the rate of agamospermous progenies increases manifold.

True F1 hybrids *F.* × *ananassa* × *F. mandshurica* ($2n = 35$) do not have practical value for breeding. The plants are stunted, with lowered vitality, mostly sterile. However, such true hybrids could possibly be used for obtaining decaploid plants with re-established fertility, the method proposed and tested practically in *F.* × *ananassa* ($8x$) × *F. vesca* ($2x$) crossing [9-11].

By open pollination of plants with pistillate flowers, 79.1% seedlings were *F.* × *ananassa*-type, while the rate of hybrid-type ones was 20.9%. Moreover, *F.* × *ananassa*-type off-springs could occur in two cases: either when the egg-cell with unreduced chromosome number was stimulated by *F. mandshurica* pollen to parthenogenetic development (pseudogamous diplospory), or when *F.* × *ananassa* pollen was brought to the carpel stigma by an insect pollinator. In such an experiment, it is not possible to determine exactly due to which process each *F.* × *ananassa*-type plant appeared. Nevertheless, the most productive off-springs were selected and thus first cultivars of Yakut breeding were created [24, 25]. These varieties are characterised by good taste of fruit and high winter-hardiness.

A pollen mixture of a wild species and of garden strawberry varieties to produce productive seedlings from *F.* × *ananassa* varieties with pistillate flowers has repeatedly been used by Russian breeders. Thus, selected hybrids (candidates for varieties) were created due to pollination the plants of Komsomolka and Pozdnyaya from Leopoldsghall varieties with pistillate flowers by pollen mixture comprising *F. moschata* and several *F.* × *ananassa* varieties [30, 31]. These crossings partly simulate our experiment in Yakutia with open pollination of *F.* × *ananassa* cultivars with pistillate flowers under the conditions of redundant pollen background of a wild species *F. mandshurica*. The rates of *F.* × *ananassa*-type plants obtained in the experiment with *F. moschata* are 70.7% and 77.3%, that is also similar to our results equal to 79.1%.

As a result, in Central Yakutia, germination of *F.* × *ananassa* achenes obtained by open pollination with redundant pollen background of *F. mandshurica* allows to produce the stock of *F.* × *ananassa* seedlings which enables to conduct selection and breed new varieties adapted to cultivation under climatic conditions of Central Yakutia.

Acknowledgements. The authors sincerely thank Tatyana Kolomiychuk for the translation of this article. This study was conducted according to budget project RF № № 0259-2021-0012.

References

1. M. Rousseau-Gueutin, A. Gaston, A. Aïnouche, M.L. Aïnouche, K. Olbricht, G. Staudt, L. Richard, B. Denoyes-Rothan, Mol. Phylogenet. Evol. **51** (2009)
2. O.K. Kamneva, J. Syring, A. Liston, N.A. Rosenberg, BMC Evolutionary Biology **17** (2017)
3. K.E. Hummer, N. Bassil, W. Njuguna, Wild Crop Relatives: Genomic and Breeding Resources, Temperate Fruits. (2011)
4. L. M. DiMeglio, G. Staudt, H. Yu, T. M. Davis, PLoS ONE, **9**, 7 (2014)
5. J. Hancock, Strawberries (USA: State University, 2nd ed., 2020)
6. G. M. Darrow, The strawberry. History, breeding and physiology. (Holt, Rinehart & Winston, New York, 1966)
7. A. Liston, R. Cronn, T. L. Ashman, Amer. J. of Bot. **101**(10) (2014)
8. G.F. Govorova, D.N. Govorov, Garden strawberry and musk strawberry (Moscow, publishing house MSP, 2016)
9. R. Bauer, A. Bauer, Hybridzuchtung in der Gattung *Fragaria*, Erwerbsobstbau, **21** (1979)
10. K. Trajkovski, Acta Horticult. **348** (1993)
11. H. Ahmadi, R.S. Bringhurst, J. Amer. Soc. Hort. Sci., **117**(5) (1992)
12. I. R. Rho, Y. J. Hwang, H. I. Lee, K. B. Lim, C.-H. Lee, Scientia Hort., **134** (2012)

13. P. Gruner, D. Ulrich, C. Neinhuis, and K. Olbricht, Acta Horticult. **1156** (2017)
14. G. Luo, L. Xue, R. Guo, Y.H. Ding, W. Xu, and J. Lei, Scientia Hort., **234** (2018)
15. K. Olbricht, D. Ulrich, V. Waurich, H. Wagner, D. Bicking, U. Gerischer, R. Drewes-Alvarez, X. Gong, M. Parniske, N. Gompel, L. Bräcker, N. Bölke, S. S. Lesemann, S. Buschmann, C. M. Ritz, P. Gruner, I. and Pinker, Acta Horticult. **1309** (2021)
16. N. B. Sukhareva, V. P. Klipko, Induced mutagenesis and apomixes (Novosibirsk, Nauka, 1980)
17. D. F. Petrov, N. B. Sukhareva, Remote hybridization and apomixes (Novosibirsk, Nauka, 1982)
18. N. B. Sukhareva, Genetic fundamentals of apomixes and plant selection (Novosibirsk, Nauka, 1984)
19. T. S. Kantor, Genetics, **XIX**, 12 (1983)
20. M. K. Gavrilova, Climate of Central Yakutia (Yakutsk, 1973)
21. L. V. Kuznetzova, V. I. Zakharov, Conspectus on Flora of Yakutia: Vascular plants, (Novosibirsk, Nauka, 2012)
22. G. Staudt, Bot. Jahrb. Syst. **124**(4) (2003)
23. V. I. Belevtzova, A. A. Mironova, V. N. Sorokopudov, Science News, ser. Natural Science, **9** (104), 15/1 (2011)
24. V. I. Belevtzova, V. N. Sorokopudov, Progress in modern science and education, **8**, 11 (2016)
25. V. I. Belevtzova, E. P. Vasilyeva, V. N. Sorokopudov, The Bulletin of KrasGAU, **7** (2010)
26. V. I. Belevtzova, Science and technique in Yakutia, **1** (28) (2015)
27. N. Preeda, T. Yanagi, K. Sone, S. Taketa, and N. Okuda, Scientia Hort., **114**, 2 (2007)
28. S. O. Baturin, *Experimental apomixes in garden strawberry* (*Fragaria* × *ananassa* Duch.) (Novosibirsk, 1997)
29. S. O. Baturin, N. B. Sukhareva, Genetic fundamentals of crops (Moscow, 1995)
30. E. A. Lapteva, Nature, **10** (1954)
31. S. H. Duka, Proceedings of Ukraine Research Institute of Gardening, Kiev, **35** (1959