Siberian gene pool of steppe cherry polyploids (*Prunus fruticosa* Pall.): cytomorphological estimation and prospects for breeding

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Abstract. The first spontaneous cherry polyploids were detected in the Altai gene pool of Russia and inside of the interspecific hybrids of *Prunus fruticosa* Pall. only. To date, polyploid genotypes with a complex of economically valuable traits and of various origins has preserved in the collection of FASCA. Collection includes: 1) F_1 of spontaneous origin; 2) F_2 obtained from hybridization of the first spontaneous hexaploid genotypes; 3) hybrids induced *in vitro*. These hybrids have a genetic origin from steppe cherry crossings with *P. cerasus* L., *P. maackii* Rupr., *P. serrulata* Lindl., *P. canescens* Bois., *P. incisa* Thumb., etc. Hexaploid amitotic clone lines were obtained by of 0.1% trifluralin treatment. The cellular mechanism of polyploids occurrence is the male and female unreduced gametes functioning. The study of microsporogenesis in 38 hybrids has revealed the 17 genetic producers of unreduced male gametes. According to the ploidy level of seedlings, the 8 sources of unreduced female gametes were selected. The morphophysiological characteristics of mature pollen were studied and the patterns for the preliminary selection of polyploids were revealed. The main strategy for the cherry breeding in the 21th century is the creation of polygenome hybrids with increased adaptation and high fruit quality.

1 Introduction

Remote hybridization and allopolyploidy played a crucial creative role in the evolutionary speciation of stone fruits of the *Prunus* L. genus (with obtaining the genotypes resistant to abiotic and biotic factors). The polyploid row of cherry species includes genotypes with a chromosome number from 2n = 16 to 2n = 48 [1]. Steppe cherry (*Prunus fruticosa* Pall.) is recognized as the most winter-hardy tetraploid and an allopolyploid species. Therefore, this concrete cherry species was used as the basis for the strategy of adaptive varieties development for the severe climatic conditions of Siberia [2].

In the climate conditions of Western Siberia a cherry breeding is carried out for about a hundred years. In Altai, the 17 varieties, based on the steppe cherry genome, were created. To date, the Altai gene pool of cherries (stored in the Breeding Department of the Siberian Horticultural Research Institute named after M.A. Lisavenko, briefly NIISS) is considered

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as the most significant in the Russian Federation. It has included more than 100 varietal samples and 4070 hybrids from 255 families. The collection contains more than 40 steppe cherry polyploids, including 17 hybrid hexaploids. The six selected forms and the single elite form are characterized by a complex of economically valuable traits [3].

Allopolyploidy not only contributes to the restoration of diploid and triploid hybrids fertility and productivity, but also changes the certain genes quantitative ratio, and through the other regularity the Mendelian trait inheritance creates a variation of features and various types of neoplasms in the morphogenetic process [1, 4]. At the beginning of 20 th century Nikolay I. Vavilov, the founder of the genetic approach to the cultivated plants, emphasized that significant achievements of Ivan V. Michurin, Niels E. Hansen and other outstanding breeders are associated with the combination the traits of three to four and even more species of stone fruits species in one cultivar [4]. In the first half of the 21 th century, Siberian scientists are planning to transfer the steppe cherry culture up to polygenome tetraploid and hexaploid levels [3, 5]. Moreover, in order to restore of interspecific diploid and triploid cherry hybrids reproductive fertility, it is promising to use an induction of chromosomes doubling by amitotic agents in *in vitro* culture. This work was carried out at the NIISS Biotechnology and Cytology Laboratory. For the first time in the world the hexaploid clone lines from sterile triploid hybrids *P. fruti*cosa x *P. canescens* and *P. fruticosa* x *P. serrulata* were induced by mutagen trifluralin treatment [6]. The original triploid material was transmitted to the NIISS gene pool by Vladimir S. Simagin (Central Siberian Branch of the SB RAS).

The sexual process is the main mechanism for increasing of genome variability, realizing in the genetic progeny in all possible combinations of traits, valuable to the breeder [4]. This implies the necessity to manage process to correct the parental pairs selection, taking into account the characteristics of their sexual or asexual reproduction. The cellular mechanism of the occurrence of spontaneous polyploids is the functioning of unreduced male and female gametes. The study of chromosome numbers and meiosis characteristics in the parental forms and in their seedlings assists to identify the most optimal ploidy level for breeding of each species, to identify the unreduced gametes genetic sources and to select the parent pairs for crosses.

The objectives of the scientific work, carried out at NIISS in 2003-2019, were: (i) cytomorphological study of reproductive characteristics for steppe cherries polyploid hybrid genotypes of different ploidy levels and of different genetic origin; (ii) selection of valuable genotypes with a complex of economically valuable traits for breeding.

2 Material and methods

Selection of promising and elite cherry forms was carried out according to the common technique [7].

As a material for cytological studies, reproductive organs and tissues of hybrids and polyploids growing in the field from the gene pool collection of the Federal Altai scientific center for agrobiotechnology (Barnaul) were used. Mitosis in meristems and meiosis in anthers were studied after fixation with acetic alcohol (1:3) on squashed preparations stained with acetic hematoxylin. To calculate the number of chromosomes, the pretreatment of vegetative buds with a mixture of 8-hydroxyquinoline and paradichlorobenzene (1:1) was used [9]. Acetocarmine staining was used to identify the degree of fertility and the average diameter of mature pollen grains. The viability of pollen grains was determined by germination on an artificial nutrient medium containing 1% agar-agar, 15% sucrose and 0.001% boric acid [10]. The amount of multi-aperture pollen was counted after staining with basic fuchsin [11]. All the data were analyzed with Microsoft Office Excel 2007 application package.

3 Results and discussion

To date, polyploid hybrid cherry genotypes of different origin with a complex of economically valuable traits has preserved in the collection of FASCA. Collection of promising selective forms includes: F_1 – of spontaneous origin (12-4-10, 1040-05-21, 1071-05-26, 13-66-9, 5-98-277, 8-83-46, 89- 95-48, 10-97-286); F_2 – obtained from directed hybridization of the first spontaneous hexaploid genotypes (1067-05-13, 1068-07-2); clonal lines obtained *in vitro* by the action of an amitotic agent (12-1-1, 6x and 12-1-2, 6x).

Samples of cherries from collection are conditionally divided into genetic groups according to their origin. For homoploid and heteroploid crossings the following genotypes are recommended: 1) wild-growing species with a high degree of adaptation to environmental stress factors (*P. pensylvanica*, *P. maximowiczii*, *P. maackii*); 2) tetraploid varieties and hexaploid hybrids obtained from steppe cherry and sour cherry *P. cerasus* crossings, relatively resistant to coccomycosis; 3) triploid and pentaploid hybrids of steppe cherry with pseudocerasus species, winter-hardy and resistant to fungal diseases; 4) tetraploid and hexaploid "cerapaduses" (hybrids of steppe cherry with sour cherry and Manchuria cherry *P. maackii*); 5) hexaploid clonal amitotic lines from crossings of steppe cherries with gray-leaf cherry *P. canescens* (12-1-1) and with Japanese cherry *P. serrulata* (12-1-2) *in vitro* obtained. Hybrids with *P. maackii*, *P. canescens*, and *P. serrulata* are very promising, as, together with other positive qualities, they show a rich in biologically active composition of fruits and produce high quality processed products.

Spontaneous tetraploid (4x = 32), pentaploid (5x = 40), hexaploid (6x = 48) and heptaploid (7x = 56) hybrid steppe cherry genotypes have arised as a result of functioning of female and male unreduced gametes. Among 37 studied species, varieties and hybrids of steppe cherry, the 19 genetic sources of unreduced male gametes were selected. As a result of a microsporogenesis, they formed from 5.9 to 33.7% of dyads and triads of microspores (table 1).

Table 1. Cytological characteristics of microspores meiosis stage (%) in *Prunus fruticosa* representatives (2005–2017)

Genetics groups (level of ploidy)	Total of forms	Total of meiocytes with disturbances	Total of tetrads	Total of dyads + triads
Wild-growing species (2x, 4x)	7	3.9–15.0	85.0–96.1	0–5.9
Varieties and selections (4x)	8	15.9–82.0	18.0–81.2	0.2–33.7
P. maackii hybrids (4x)	6	54.6–46.7	58.1–86.4	0–28.5
P. maackii hybrids (6x)	5	32.4–83.9	16.1–68.4	3.3–30.4
Other hybrids (4x)	3	13.4–80.5	19.5–86.6	0.2–11.9
Other hybrids (5x)	5	47.7–0.2	8.9–53.3	4.5–10.6
Other hybrids (6x)	2	42.6–65.4	34.6–45.4	0.5–10.2
Other hybrids (7x)	1	82.1	17.9	24.2

Genetic sources with chromosome non-reduction of female gametes are primarily identified among triploid hybrids (12-1-1, 12-1-2, 12-4-17, 12-3-20, MC x SC), which being surrounded by tetraploid genotypes produced the pentaploid and heptaploid seedlings. In hexaploid hybrids 3-66-9 and 10-97-286, unreduced ovules can develop without fertilization by apomixis reproduction, giving hexaploid progeny along with pentaploid one, being around of the same tetraploids. Hexaploid 5-98-277, obtained from crossing of steppe and sour cherries, resulted in the development of 2 octoploid seedlings (2n = 64).

The hexaploid level in hybrids in comparison with the tetraploid one is characterized by a higher degree of pollen fertility and viability – an average of 52-56% and 16-22%, respectively (table 2).

Table 2. Cytomorphological characteristics of pollen grains in cherry representatives of *Prunus* L. (2005–2019)

Genetics groups (level of ploidy)	Pollen fertility (%): average min.–max.	Pollen viability (%): average min.–max.	Pollen with added apertures (%): average min.–max.	Pollen diameter (μm): average min.–max.
Wild-growing forms of *P. fruticosa* (4x)	86.0 45.0–93.0	27.4 3.9–43.4	0.5 0–2.0	39.6 31.5–51.6
Varieties, hybrids of *P. fruticosa* x *P. cerasus* (4x)	27.4 10.0–72.6	16.6 4.2–42.4	3.4 1.8–13.4	45.9 36.9–60.0
Hybrids of *P. fruticosa* x *P. cerasus* (6x)	52.4 15.4–81.1	29.7 9.0–53.0	56.7 33.1–78.3	48.4 40.2–57.6
Selection specimens of *P. maackii* (4x)	74.5 60.0–89.0	17.8 5.7–26.4	3.7 0.2–7.5	44.8 38.2–54.0
Hybrids $F_{3,4}$ of *P. fruticosa* x *P. maackii* (4x)	27.5 3.3–52.6	14.5 1.8–47.6	42.5 3.9–74.3	46.9 33.9–60.0
Hybrids $F_{3,4}$ of *P. fr*uticosa x *P. maackii* (6x)	55.5 8.1–76.6	21.9 4.2–41.5	47.6 7.2–87.8	48.2 38.4–62.4
Other hybrids (3x)	4.4 0.7–9.7	1.8 0.4–2.8	49.8 7.8–89.7	–
Other hybrids (5x)	34.0 8.1–49.9	7.6 3.6–17.0	–	–

Some hybrids of *P. fruticosa* with *P. maackii* (89-95-53, 11-85-39) exhibited genetically determined sterility of pollen grains.

In hexaploid amitotic clonal lines of hybrid cherries, which were induced *in vitro*, the pollen fertility is approximately by 8 times higher than that characteristic in initial triploid clonal lines (86.0 and 10.0% on average, respectively). The average diameter of fertile pollen grains in triploid lines does not significantly differ from ones in hexaploid lines (47.1 and 46.7 μm). That indicates its unreduced status (n = 24) in a significant number of microspores.

A complex of multi-aperture pollen quantity with a set of other criteria (fertility and size of pollen grains, morphology of vegetative and generative organs) can be used for previously detection of cherry genotypes of a certain ploidy level. A feature of the pollen grain diameter can be used to determine whether a particular hybrid belongs to a particular genetic group. For example, this refers to the identification of hybrids with *P. maackii* genome as well as to reveal the polyploid genotypes within a population with mixed ploidy ($P < 0.05$).

As a result of study 20 genetic sources were selected by the complex of cytological and economically useful traits including hybrids with a different number of chromosomes – from triploids to hexaploids. In addition to characteristic of high resistance to unfavorable abiotic and biotic environmental factors, they differ in significant morphological polymorphism: in terms of blossom and fruits ripening, in the fruit shape and color, in the plant habits, in the shape of the crown and leaves. The main strategy for the cherry breeding for the 21 th century is the creation and the development of polygenome hybrids and clonal lines, induced by *in vitro* (tetraploids and hexaploids containing genes from three to four

species belonging) with increased adaptation to adverse environmental factors and high fruit quality.

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