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Reproductive compatibility of *Sorbocotoneaster* with mountain ash species and varieties that are promising for breeding in Siberia

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Abstract. In Russia, in the Republic of Yakutia, there is the only point in the world where natural intergeneric hybridization of species of the genera *Sorbus* and *Cotoneaster* is observed, resulting in the formation of a new hybridogenic genus ×*Sorbocotoneaster* with a rich polymorphism of parental genera traits. For the selection of mountain ash in Siberia, it is promising to involve *Sorbocotoneaster* genotypes in artificial hybridization as sources of a complex of economically valuable traits, including winter hardiness, short stature, and self-fertility. Artificial hybridization of the tetraploid selective form *Sorbocotoneaster* with selected forms and varieties of *Sorbus sibirica*, *S. aucuparia*, *S. sambucifolia* and their hybrids allows obtaining viable hybrid offspring in combinations where *Sorbocotoneaster* is used as a pollen donor.

1 Introduction

In its ability to form interspecific and intergeneric hybrids, mountain ash is superior to many other breeds. A significant number of modern mountain ash species were formed because of interspecific hybridization, often accompanied by polyploidy. Due to apomictic reproduction, which is also common in the genus *Sorbus* L., hybrid and polyploid plants, while retaining their new morphological type, occupy vast territories. Some of them form fertile pollen and, when backcrossed with the original or related species, create a huge variety of morphological and genetic forms. For this reason, the genus Sorbus is taxonomically one of the most confusing and complex in the subfamily Maloideae C. Weber [1-3]. The genus *Sorbus* is one of the most numerous in terms of the number of interspecific hybrids, both between closely related species and between species belonging to unrelated sections [1]. On the territory of Russia and neighboring states, natural hybridization of species of the genus *Sorbus* was noted in many areas of growth [4].

The absence of reproductive barriers between many *Sorbus* species opens great opportunities for breeding this crop. For Siberia, this is relevant, since only one species naturally grows on this territory – *S. sibirica* Hedl. Even the best selective forms of this species are of little use for industrial and home gardening. The most promising sources of

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necessary economically valuable traits are *S. aucuparia* L. varieties and *S. sambucifolia* (Cham. et Schlecht.). M. Roem selected forms.

It is *Sorbus* that most often turns out to be one of the parents during intergeneric hybridization: ×*Amelosorbus* Rehd. (*Amelanchier* Medik. × *Sorbus*); ×*Crataegosorbus* Makino ex Koidz. (*Crataegus* L. × *Sorbus*); ×*Malosorbus* Browicz. (*Malus* Mill. × *Sorbus*); ×*Sorbaronia* Schneid. (*Sorbus* × *Aronia* Medik.); ×*Sorbocotoneaster* Pojatk. (*Sorbus* × *Cotoneaster* Medik.); ×*Sorbopyrus* Schneid. (*Sorbus* × *Pyrus* L.) [1, 5, 6].

The prospect of using intergeneric hybridization in the selection of mountain ash was shown by I.V. Michurin [7]. As a result of hybridization of Sorbus with Aronia, Crataegus, Mespilus I.V. Michurin received such varieties as \`Likernaya\`, \`Burka\`, \`Granatnaya\`, \`Michurinskaya dessertnaya\`. The listed varieties are quite different from *Sorbus*; however, their origin requires clarification.

Hybrid genus *Sorbocotoneaster* Pojark. with the only described species *Sorbocotoneaster Pozdnjakovii* Pojark. – endemic to South Yakutia, listed in the Red Data Books of the Russian Federation and the Republic of Yakutia [8, 9]. To date, only a few works are known concerning mainly the description of phenorhythms, seed productivity and stability of individual specimens in botanical collections [10, 11, 12, 13], a high content of carotenoids in fruits is noted [14]. The data on the numbers of chromosomes that are multiples of the basic number n = 17 (2n = 51, 64, 81) in different morphological forms are given, aneuploidy occurs [15, 16] A phytocenotic characteristic of habitats and some features of the morphology of *Sorbocotoneaster* are given [17, 18], the authors note a significant polymorphism and greater ecological plasticity of various forms of *Sorbocotoneaster*. 10 phytocenoses are described, data on the occurrence of various morphological forms of *Sorbocotoneaster* in each of them are presented. In our studies [19], the possibility of hybridization and recombination of molecular markers *Sorbocotoneaster* with *Sorbus sibirica* Hedl was experimentally shown for the first time. and interspecific hybrid F_1 (*S. sambucifolia* × *S. sibirica*). The aim of this study is to study the possibility of hybridization of the tetraploid form (2 *Sorbus* genomes plus 2 *Cotoneaster* genomes) *Sorbocotoneaster* with *Sorbus* species and varieties promising for breeding in Siberia.

2 Material and methods

The work was performed in Novosibirsk, at the Central Siberian Botanical Garden (CSBS) SB RAS based on the bioresource scientific collection "Collections of living plants in open and closed ground", UNU No. USU 440534.

The paper used the "Program and methodology for remote hybridization of fruit and berry crops" [20] and standard methods for studying fruit crops [21].

The pollen was stored in a dry form at -20 ˚C. The inflorescences were isolated until the buds opened. Self-pollination means the isolation of inflorescences from insects without artificial pollination of flowers. For comparison, the results of fruit and seed setting were recorded with free pollination (without isolation) of a given number of flowers. Hybrid seeds were germinated by laboratory method with preliminary cold stratification at a temperature of 1-3 ˚C [22, 23].

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3 Results and discussion

The tetraploid form of ×*Sorbocotoneaster* (2 genomes of *Sorbus* and 2 genomes of *Cotoneaster*) intermediate in morphology between *S. sibirica* and *C. melanocarpus* was used as a pollen donor. This form is characterized by a complex of valuable features: large fruits with good taste, short stature, early fruitfulness, self-fruitfulness, abundant fruiting, high winter hardiness.

The best selected forms of *S. sibirica* (selected form TsVPR-5) and *S. sambucifolia* (selected form B-1), the varieties \`Nevezhinskaya\` (*S. aucuparia*) and \`Sharik\`(F_1 *S. sambucifolia* × *S. sibirica*), as well as the F_1 hybrid *S. sibirica* × *S. aucuparia* were selected as the second parent.

As a result of artificial hybridization, it was possible to obtain fruits and full-fledged seeds in all combinations of crosses (table).

Table. Results of hybridization of ×*Sorbocotoneaster Pozdnjakovii* with *Sorbus* species and interspecific hybrids

No	Mother plant	Pollinator	Pollinated flowers, (pcs.)	Fruits formed, (pcs.)	Fruits formed, (%)	Average number of seeds per fruit (pcs.)
1	*S. sibirica* (TsVPR-5)	*S. pozdnjakovii*	210	182	86.7	2.7
		Free pollination	200	146	73.0	3.5
		Self-pollination	200	0	0.0	0.0
2	*S. aucuparia* \`Nevezhinskaya\`	*S. pozdnjakovii*	307	160	52.1	2.0
		Free pollination	150	120	80.0	2.9
		Self-pollination	150	0	0.0	0.0
3	*S. sambucifolia* (B-1)	*S. pozdnjakovii*	113	66	58.4	4.1
		S. sibirica	163	52	31.9	3.0
		Free pollination	81	33	40.7	2.8
		Self-pollination	73	2	2.7	0.5
4	F_1 (*S. sibirica* × *S. aucuparia*)	*S. pozdnjakovii*	200	140	70.0	3.1
		Self-pollination	150	0	0.0	0.0
5	F_1 (*S. sambucifolia* × *S. sibirica*) \`Sharik\`(BK-1)	*S. pozdnjakovii*	175	61	34.9	2.1
		S. sibirica	132	106	80.3	3.6
		Free pollination	150	109	72.7	5.0
		Self-pollination	100	0	0.0	0.0

The percentage of formed fetuses varied from 58 to 90 %, which is an extremely high indicator and indicates good reproductive compatibility of parental genotypes. In all combinations of crosses, full-fledged seeds from 2 to 4 pieces per fruit were obtained, which is also a particularly good indicator for intergenerational crosses. The hybridity of the seeds was checked by SDS-PAGE electrophoresis of spare cotyledon proteins [19], recombination of protein markers was observed in all combinations, which confirms the hybrid nature of the seeds.

Since the seeds of all species participating in hybridization are characterized by the presence of deep physiological dormancy, the hybrid seeds were subjected to cold stratification in petri dishes at a temperature of 1-3 ˚C. As a result, hybrid seedlings were obtained (figure).

The hybrid nature of seedlings is easily established visually by the morphological features of the leaves. In all species and hybrids of mountain ash included in the hybridization, the leaves are complex non-pinnate. In *Sorbocotoneaster*, the leaf combines the characteristics of a complex mountain ash leaf and a simple *Cotoneaster* leaf. This is especially evident in the structure of the upper part of the leaf, where there is an accretion of the upper leaflet with one or more lateral leaflets [5]. The hybrids synthesized by us retain this feature of the leaf structure, although to a lesser extent, since the volume of the genetic material of *Cotoneaster* is less than that of the parent genotype of *Sorbocotoneaster*.

Fig. Seedlings: **a** – ×*Sorbocotoneaster Pozdnjakovii* (left) и *Sorbus aucuparia*; **b** - F_1 *S. sambucifolia* × *S. Pozdnjakovii*; **c** - F_1 *S. aucuparia* \`Nevezhinskaya\` × *S. Pozdnjakovii*; **d** - F_1 [(*S. sambucifolia* × *S. sibirica*) \`Sharik\`) × *S. Pozdnjakovii*].

Previously, during direct hybridization of *S. sibirica* and *C. melanocarpus* [19], the resulting hybrid seedlings were clearly depressed and died at the early stages of development. In all the present combinations, most seedlings in all combinations developed normally. This is probably largely due to the balance of the hybrid genome of natural *Sorbocotoneaster* genotypes. Firstly, in nature, *Sorbocotoneaster* evolves as an independent genus for quite a long time, and secondly, we used a tetraploid genotype intermediate in morphology with equal sets of parental genomes and, accordingly, with a balanced meiosis.

All species, varieties and hybrids of mountain ash included in the hybridization are diploid compared to the tetraploid *Sorbocotoneaster* genotype. Consequently, the hybrids we synthesized should be triploid with a predominance of *Sorbus* genomes. This is an unbelievably valuable economic trait, since allopolyploid genotypes of *Sorbus* are most likely to form seeds by apomixis [2, 24], and, accordingly, form fruit yield more stably. In addition, such genotypes can be reproduced by seed without losing varietal qualities, which is also important for mountain ash, since this culture does not reproduce well by cuttings.

4 Conclusion

The possibility of using *Sorbocotoneaster* in hybridization with species, varieties and interspecific hybrids of mountain ash has been experimentally shown, which opens broad prospects in the selection of mountain ash in Siberia. Already in the first generation, it is possible to obtain hybrids with a combination of valuable *Cotoneaster* and *Sorbus* traits, which can be used as ornamental and food plants. At the same time, *Sorbocotoneaster* will act as a donor of self-fertility, increase winter hardiness, reduce the growth force, accelerate the beginning of fruiting.

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