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Application of the Electrophoresis of Cotyledon Storage Protein and ISSR-Markers to the Identification of Hybrids Between *Sorbus sibirica* Hedl. and *Sorbocotoneaster pozdnjakovii* Pojark.[1]

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Abstract—It is shown that cotyledon protein SDS-electrophoresis and inter-simple sequence repeat DNA markers (ISSRs) can be used in identification of hybrid genotypes in *Sorbus sibirica* Hedl. and *Sorbocotoneaster pozdnjakovii* Pojark. Recombination of molecular and morphological characters was observed in hybrids, which proves close relationships of the parental genomes and, as a consequence, the potential of utilizing *Sorbocotoneaster pozdnjakovii* genotypes in breeding of *Sorbus* cultivars. The results confirm that the genus *Sorbocotoneaster* arose as a result of *Sorbus* and *Cotoneaster* hybridization.

Keywords: *Sorbocotoneaster*, mountain ash, intergeneric crosses, molecular markers

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INTRODUCTION

Ryabinokizilnik (*Sorbocotoneaster* Pojark.) is an endangered localized endemic of South Yakutia (Koropachinsky and Vstovskaya, 2012). It is included in the list of "Rare and endangered plants of Siberia" (1980), Red Data Book of the Republic of Sakha (Yakutia) (2001) and Red Data Book of the Russian Federation (2008). *Sorbocotoneaster pozdnjakovii* Pojark. is the only representative of a hybrid genus *Sorbocotoneaster* arising from spontaneous hybridization between *Sorbus* L. and *Cotoneaster* Medik. According to A.I. Poyarkova, "association of ryabinokizilnik with the plant formation characterized by the complex of Pleistocene relict plant species allows supposing that the occurrence of this intergeneric hybrid should be dated the same period" (Petrova et al., 1992, p. 75–76).

We believe that ryabinokizilnik may prove to be a donor of valuable characters in mountain ash breeding since it is characterized by a complex of criteria necessary for mountain ash: resistance to cold, stunting, early-maturing, self-fertility, lack of bitterness and astringency in the taste of fruits, and good rooting of cuttings.

However, when mountain ash hybridization is distant, seeds can be set not only as a result of true hybridization but as a result of stimulative apomixis (Liljefors, 1953, 1955; Robertson et al., 2004). Since years pass from the formation of hybrid seeds to fruiting, and considerable resources are spent, it is necessary to have rapid test methods to diagnose the seeds hybridity and carry out early breeding of seedlings. This would provide an opportunity to significantly reduce the costs connected with the complex and time-consuming seeds presowing treatment and long-term cultivation of seedlings worthless for further work.

We set the following objective: to determine the possibility of application of cotyledon storage protein SDS-electrophoresis and inter-simple sequence repeat DNA markers (ISSRs) to the identification of hybrids between *Sorbus sibirica* Hedl. and *Sorbocotoneaster pozdnjakovii* Pojark.

MATERIAL AND METHODS

Seeds and fresh or frozen and stored at –20°C leaves of *Cotoneaster melanocarpus* Fisch. ex Biytt., *Cotoneaster integerrimus* Medik., *Cotoneaster lucidus* Schlecht, *Sorbus aucuparia* L., *Sorbus sibirica* Hedl., *Sorbocotoneaster pozdnjakovii* Pojark. and the F_1 hybrids (*S. sibirica* × *S. pozdnjakovii*) were examined.

SDS gel buffer (Laemmli, 1970) adapted for the objects of research was used for extraction and electrophoretic separation of cotyledon storage proteins. To soften the seed coat before the extraction, it was placed in distilled water for 24 hours. An incision was made with a dissecting needle across the seed coat, and the germ was squeezed out through the opening.

To extract proteins, one cotyledon of the germ was ground with a pestle in a 0.2 mL Eppendorf tube in 5 μL of distilled water, the other cotyledon was ground in 5 μL of SDS extraction buffer [Tris-HCl pH 6.8, glycerol, water in the ratio of 4 : 1 : 5; 3% SDS; bromphenol blue]. Then the volumes of the water and the buffer were brought to 100 μL. The extract was vor-

[1] The article was translated by the authors.

texed for 5–10 seconds and kept at room temperature for 3–4 hours or at +4°C for 12–18 hours. After that the extract was once again vortexed for 2–3 seconds and centrifuged at 13000 rpm for 5 min. The extract was divided into 3 fractions: the insoluble residue, the upper fraction and the solution in the intermediate phase. 50–70 μL of the solution were withdrawn out of the intermediate phase. The collected extract was divided into two parts. One was mixed with 20% 2-mercaptoetanol in the 2 : 1 ratio (to denaturate the quaternary protein structure according to the variant +Me) in the 1× SDS extraction buffer in the variant with SDS-extraction and 2× SDS-extraction buffer in the variant with the water extract. To balance the concentration with the first variant the second part was mixed with 1× and 2× extraction buffer in the 2 : 1 ratio. The extracts were heated in the thermocycler at 99°C for 1.5 min, electrophoretic separation was carried out in 1 mm of 12.5% polyacrylamide gel. A standard set of protein markers (Fermentas) with the 14.4–116 kDa range was used to determine molecular weight. To identify each component with precision, we built a scale of relative electrophoretic mobility, which enables to compare the results of various tests in the uniform reference system (Agafonov and Agafonova, 1992).

The method of D.A. Puchooa was used for DNA extraction (2004). As the method was considerably modified, we list its complete version here. Grind 20 mg of fresh or frozen leaves in a ceramic mortar with 2 ml of extraction buffer containing 2% PVP-40, 5% 2-mercaptoetanol, 2% STAB, 100 mM Tris-HCl, 2 M NaCl, 20 mM EDTA. Apply 1.5 mL into a clean 1.5 mL tube, add 5 μL of RNase, vortex it for 5 seconds and incube it at 37°C for 20 min, then stir it gently and incube at 60°C for 10 min. Centrifuge at 13000 rpm for 5 min. Apply 800 μL of the supernatant into a clean tube. Add 700 μL of the mixture of chloroform and isoamyl alcohol (24 : 1), stir it gently till the emulsion becomes smooth, incube at room temperature for 15 min. Centrifuge it at 13000 rpm for 15 min, apply the supernatant into a clean tube. Repeat cleaning with chloroform. Apply 555 μL of the supernatant and precipitate DNA with 1 mL of 95% ethanol, stirring it gently until the complete dissolution of the alcohol. Centrifuge at 13000 rpm for 5 min. Pour the liquid out, wash the precipitate with the solution, containing 75% ethanol, TE, 10 mM ammonium acetate and 10 mM sodium acetate.

Then wash the precipitate with 96% ethanol. Pour out the alcohol, centrifuge at 2000 rpm for 10 s, withdraw the alcohol residue with a pipet, dry the precipitate till it loses shine. Dissolve the precipitate with 555 μL of the mixture of TE and 5 M NaCl (2 : 1), incube at room temperature for 10 minutes. Add 800 μL of chloroform (24 : 1), stir and ascertain that the undissolved part of the precipitate is floating in the solution instead of being stuck to the walls of the tube. Centrifuge at 13000 rpm for 10 min. Apply 500 μL of the supernatant into a clean tube and precipitate with 1 ml of 95% ethanol, pour the alcohol out, wash the precipitate first with 80% and then with 95% ethanol, dry it and dissolve in 200 μL TE or mQ H_2O.

PCR was carried out using C-1000 thermal cycler (Bio-Rad, USA) in a 25 μL volume. The PCR reaction consisted of 1.5 unit Taq DNA-polymerase (Medigen, Russia, 5 units/μL); 1× Taq-buffer without Mg^{2+} (Medigen, Russia); 2.3 mM MgCl; 0.8 mM dNTPs (Medigen, Russia); 0.8 mM ISSR-primer (Medigen, Russia); 2 μL of DNA solution, water mQH2O—up to 25 μL.

The amplification was carried out as follows: predenaturation at 95°C—2 min; 38 cycles—denaturation at 94°C—20 s, annealing (Ta—annealing temperature and nucleic acid sequences are given in table 2)—45 s, elongation—1.5 min; and final elongation—7 min.

The PCR products were subjected to gel electrophoresis in 1.5% agarose gel using a 1× TBE buffer at 4 V/cm voltage.

To estimate the polymorphism of the used markers and to define the level of divergency among the studied genotypes the received data was converted into the matrix of binary states, where the presence or the absence of polypeptide components or PCR-products of the same size were regarded as state 1 and 0.

Data was statistically processed using the TREECON version 1 software package (Peer, Wachter, 1994). Genetic distances were calculated according to Nei (Nei and Li, 1979), using the formula GDxy = 1–2Nxy/(Nx + Ny), where Nxy is the number of common fragments for the samples x and y, Nx and Ny are the number of fragments for samples x and y accordingly. We used the method of neighbor-joining (NJ) with bootstrap-support—100 pseudoreplications. The level of polymorphism (P) of each primer (in percents) was calculated according to the formula P = 100*Np/N, where Np is the number of polymorphous fragments—the general number of fragments.

To study the possibility of hybridization between *Sorbus sibirica* Hedl and *Sorbocotoneaster pozdnjakovii* Pojark. we chose two forms of mountain ash promising in terms of introduction and selection: TsVPR-5—*S. sibirica*, characterized by a big fruit, excellent taste and a branchy top; BK-1—an interspecific hybrid of F_1 (*Sorbus sambucifolia* (Cham. et Schlecht). M. Roem × *S. sibirica*), characterized by a big fruit, the absence of bitterness and acerbity in the taste of fruit, early maturity and low height of the tree. As the paternal plant we used a form of ryabinokizilnik (*Sorbocotoneaster pozdnjakovii* Pojark.), which can be found in the arboretum of Central Siberian Botanical Garden SB RAS and is characterized by the fineness of the taste, low height of the tree, early maturity, self-fertility, good rooting of cuttings and abundant fruitage. The pollen was extracted out of half-expanded buds, and its fertility was checked by the acetocarmine method. The hybridization was conducted according to the

Table 1. Hybridization between *Sorbus sibirica* Hedl. and F_1 (*Sorbus sambucifolia* (Cham. et Schlecht). M. Roem. × *S. sibirica*) with *Sorbocotoneaster pozdnjakovii* Pojark. and *Malus niedzwetzkyana* Dieck.

Parent plant	Pollinator	Number of pollinated flowers, p	Number of fruit set, p	Fruit set, %	Number of full seeds, p	Number of full seeds per fruit, p
S. sibirica (TsVPR-5)	*Sorbocotoneaster pozdnjakovii*	210	182	86.7	486	2.7
	Malus niedzwetzkyana	300	0	0.0	–	–
	Self-pollination	200	0	0.0	–	–
	Open pollination	200	146	73.0	505	3.5
	F_1 (*S. sambucifolia* × *S. sibirica*) (BK-1)	200	154	77.0	336	2.2
F_1 (*S. sambucifolia* × *S. sibirica*) (BK-1)	*Sorbocotoneaster pozdnjakovii*	175	61	34.9	129	2.1
	Malus niedzwetzkyana	125	0	0.0	–	–
	Self-pollination	100	0	0.0	–	–
	Open pollination	150	109	72.7	547	5.0
	S. sibirica (TsVPR-5)	132	106	80.3	379	3.6

Table 2. Primers characteristics used for the study of DNA polymorphism of the *Sorbus*, *Cotoneaster, Sorbocotoneaster* samples

Nucleotide sequence 5'–3'	Primer	Ta (°C)	Number of amplified fragments	Level of polymorphism, %	Size of DNA fragments, bp
$(CA)_6AC$	17898A	42	19 (17)	89.5	500–2500
$(CA)_6GT$	17898B	42	20 (18)	90.0	450–2250
$(CA)_6GG$	17899B	41	22 (21)	95.5	375–2000
$(GA)_6CC$	HB-10	44	26 (24)	84.6	500–2250
$(AC)_8CG$	M-1	47	24 (21)	87.5	450–2500
$(AC)_8YG$	UBC-857	55	25 (24)	96.0	350–1600

methodical recommendations of Y.S. Nesterov (*Program and Technique...*, 1972).

The hybrid seeds were kept dry at room temperature and were put for continuous stratification on 16.02.2012 at 1–3°C. The seedlings were being grown at room temperature with 16-hour lighting under an electric-discharge lamp in the course of 5 months, after which they were moved to the room with the temperature of 1–8°C and 6-hour lighting. After the period of rest was over, the seedlings were brought back to the previous regimen of growing.

RESULTS AND DISCUSSION

According to our data, in conditions of Novosibirsk in 2011 the seed fertility of chosen ryabinokizilnik was 59%, pollen fertility—92%. It should be noted that the high pollen and seed fertility of distant hybrids proves the balance of the hybrid genome and indicates a high degree of affinity between the parent species.

The results of hybridization between *S. sibirica* and F_1 (*S. sambucifolia* × *S. sibirica*) and *S. pozdnjakovii* carried out in 2011 are shown in Table 1. Since apomictic fruit set is typical of the species of the Sorbus genus depending on the individual characteristics of plants and weather conditions, (Liljefors, 1953, 1955; Robertsonet el al., 2004), in addition to the self-fertility control hybridization with Nedzvetski apple was performed (*Malus niedzwetzkyana* Dieck). Information on free-pollination is shown for comparison (Table 1).

According to our data, in 2011 in versions with self-pollination and pollen pollination of *M. niedzwetzkyana*, fruits were not set what proves self-sterility of TsVPR-5 and BK-1 and reduces the probability of stimulative apomixes. In the variants with open pollination and reciprocal hybridization of TsVPR-5 and BK-1, there was a high percentage of fruit and seed setting. This goes to prove the favorable weather conditions for hybridization (fruits do not set, or a single fruit sets under adverse weather conditions).

In combinations of TsVPR-5 and BK-1 with ryabinokizilnik fruit setting was 86.7 and 34.9%, respectively. Regarding open and reciprocal pollination, this figure was above 70%. In combinations with ryabinokizilnik compared to the control variants, the decrease of seed fertility was observed but it remained relatively high—more than two seeds per fruit.

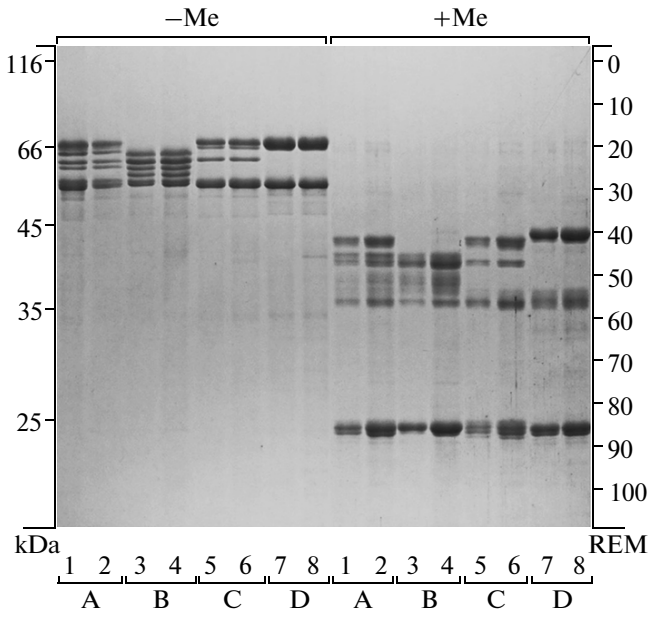

Fig. 1. SDS–electropherograms of cotyledon proteins of *S. pozdnjakovii* (A), *C. lucidus* (B), F_1 (*S. sibirica* × *S. pozdnjakovii*) (C), *S. sibirica* (D) seeds.
1, 3, 5, 7—water extracts; 2, 4, 6, 8—SDS-extracts. Polypeptide patterns of individual seeds −Me and +Me.

Thus, the results obtained on the hybridization between mountain ash and ryabinokizilnik demonstrate a high probability of hybrid seeds setting. However, on this basis it is impossible to completely exclude apomictic fruit and seed setting.

We used SDS PAAG method to determine hybrid or apomictic seed-set. Since this work was aimed at identifying the possibility of application of molecular methods, we examined only one variant of hybridization—*S. sibirica* × *S. pozdnjakovii* for a number of reasons.

It is known that plant storage proteins are classified into four solubility classes: albumin, globulin, prolamin and glutelin (Osborne, 1935). Accordingly, it was necessary to clarify the solubility class and choose the best method of extraction of cotyledon proteins. For this purpose, proteins of one cotyledon from each seed were extracted in distilled water (soluble albumins were extracted), and proteins of the other cotyledon—in SDS buffer that extracts all the above mentioned classes of proteins. The resulting electropherograms are shown in Fig. 1. Since the polypeptide patterns did not differ in composition, all extractable proteins are water soluble albumins. The variants +Me and −Me have significant differences, indicating the complex polymer structure of the part of proteins and, therefore, potentially more informative patterns of their subunits.

Since extraction in SDS-buffer makes the procedure quicker, in the following experiments all extracts were prepared in this buffer at two variants—with the addition of β-mercaptoethanol (+Me) and without it (−Me).

The Fig. 2 shows SDS-electropherograms of cotyledon proteins of hybrid seeds F_1 (*S. sibirica* × *S. pozdnjakovii*) in comparison with the parental forms and electrophoregrams of *C. lucidus* and *S. aucuparia*.

The obtained patterns of cotyledon storage proteins in hybrid seeds and seeds of parental plants confirmed the hybrid origin of seeds in the combination *S. sibirica* × *S. pozdnjakovii*, as well as hybrid origin of *S. pozdnjakovii*. Backcrossing between *S. pozdnjakovii* and *S. sibirica* leads to the formation of genotypes with significantly different albumin components which are typical of both genera. Display of components in some hybrids (samples 4, 5, 6, 10) in −Me variant that are absent in the parental genotypes (Fig. 2) can be explained by heterozygous parental forms, and in particular, by complex composition of the paternal genome of the plant.

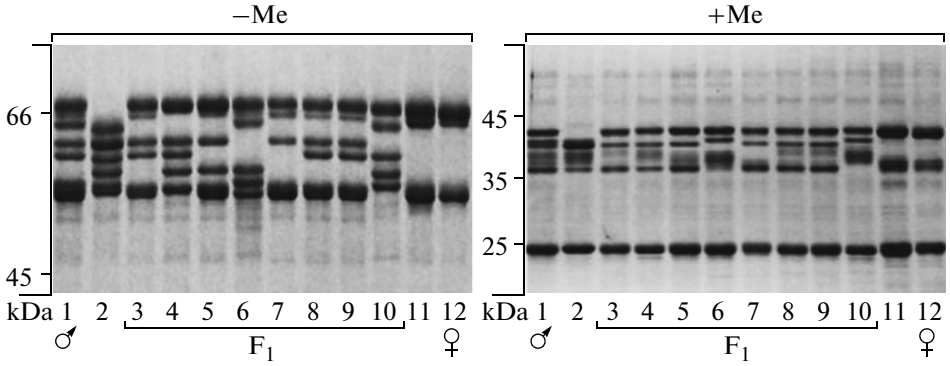

Fig. 2. SDS-electropherograms of cotyledon proteins of the hybrid seeds F_1 (*S. sibirica* × *S. pozdnjakovii*) compared to parental forms. 1—♂—male parent of *S. pozdnjakovii*; 2—*C. lucidus*; 3–10—F_1—hybrid seeds sampling; 11—*S. aucuparia*; 12—♀—female parent TsVPR-5 (*S. sibirica*). Polypeptide patterns of individual seeds −Me and +Me.

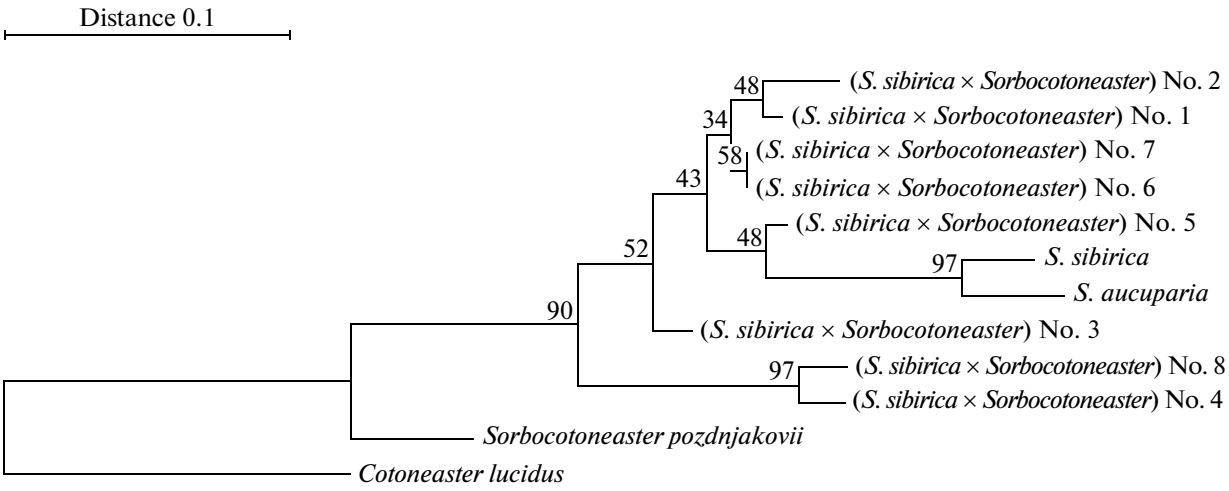

Fig. 3. Consensus NJ-dendrogram (variants −Me and + Me) based on cotyledon albumins of particular seeds *S. sibirica*, *S. pozdnjakovii*, *C. lucidus*, F_1 (*S. sibirica* × *S. pozdnjakovii*).

Top scale: genetic distances (M. Nei, W.–Li, 1979). The numbers above the junctions indicate the bootstraps.

We used in hybridization *S. pozdnjakovii* which is a complex intergeneric hybrid with an unknown number of chromosomes. According to V.N. Gladkova (1967), the chromosome set of *Sorbocotoneaster* can be $2n = 68, 85$. It is not known how many haplomes of *Cotoneaster* and *Sorbus* the hybrid genome *Sorbocotoneaster* has, and what kind of gametes it produces. For example, formation of male gametes containing haplomes of only one of the parental species of the *Cotoneaster* and *Sorbus* genus is not excluded. Taking into consideration that the basic set of chromosomes ($2n = 17$) in the Maloideae (Rosaceae) subfamily was the result of the combination ($n = 7$, 8 or 9) of ancient haplomes (Evans and Campbell, 2002), in the situation with ryabinokizilnik the variety of options for recombination is quite probable. We use the term "recombination" in a broad sense since significant restructuring associated with the activation of mobile elements, epigenetic, transcriptomic and other changes are typical of allopolyploid genomes, in addition to the crossing-over and combinatory allelic variability (Scherban, 2013). For example, as a result of such modifications appearance of new molecular and morphological attributes is possible or, on the contrary, the display of attributes that are indistinguishable or very similar to the parent.

It is not possible to judge actual phyletic connections between *Sorbocotoneaster*, *Cotoneaster*, *Sorbus* and their hybrids by the obtained data. The representative sampling of each of the three genera is needed for this purpose. Our aim was to show a specific example of applied molecular methods for rapid control of the success of hybridization. Since in this analysis parental genotypes are known, then, in our opinion, the display of polypeptide patterns, very similar to the parental one, is the evidence of successful hybridization and hence the effectiveness of the method used. There is no doubt that the method in combination with the methods of morphometric and genetic analysis will help resolving phylogenetic questions.

Based on the obtained polypeptide patterns in the variants +Me and −Me, the genetic distances were calculated and a consensus NJ-dendrogram was built (Fig. 3). The dendrogram shows that parental forms are reliably separated from the hybrids which depending on the proportion of inherit genes have an intermediate or closer to one of the parents' position. The findings suggest that well-balanced process of recombination occurs at hybridization between *Sorbocotoneaster pozdnjakovii* and *Sorbus sibirica*, which is probably caused by close genetic relationship of original forms at the level of homology of their genomes.

Germination of hybrid seeds after preliminary cold stratification in the (*S. sambucifolia* × *S. sibirica*) (BK-1) × *S. pozdnjakovii* combination was 89%; in the *S. sibirica* (TsVPR-5) × *S. pozdnjakovii* combination—72%. Significant abnormalities in the development of seedlings were not observed; the percentage of mortality was negligible. After the period of rest was over, noticeable abnormalities in the development of the seedlings were also not observed.

All hybrid seedlings combine parental characters in different proportions. They looked like *Sorbus sibirica* but had atypical densely pubescent underside and sparsely pubescent upper side of the leaf. The upper part of the leaf had a typical of *Sorbocotoneaster* asymmetric structure due to the partial or complete intergrowth of the central leaf with the upper pair of lateral leaflets (Fig. 4).

Shoots and primary ribs are more pubescent compared to *S. sibirica*. Almost all hybrid seedlings of F_1 (*S. sambucifolia* × *S. sibirica*) (BK-1) × *S. pozdnjak-*

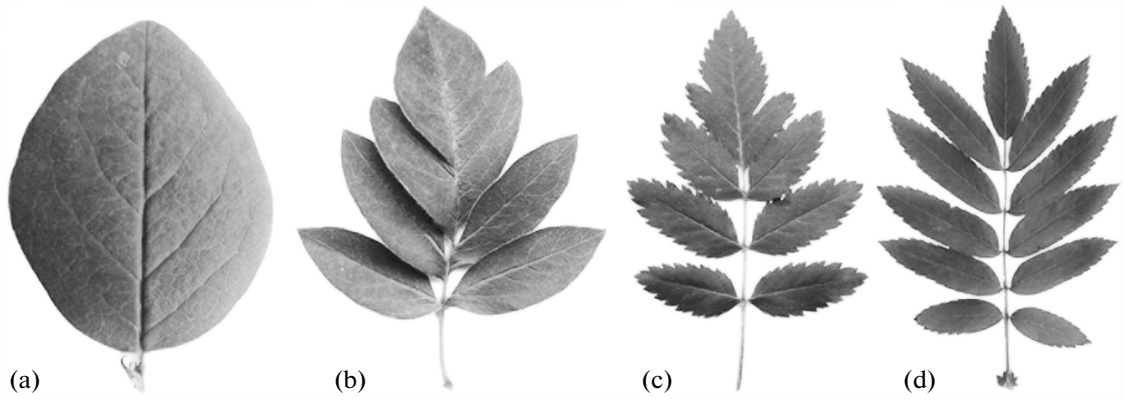

Fig. 4. Leaves (a)—*C. melanocarpus*, (b)—*S. pozdnjakovii*, (c)—F_1 (*S. sibirica* × *S. pozdnjakovii*), (d)—*S. sibirica*.

ovii) inherited a glossy surface of the leaf from *S. sambucifolia*.

Thus, a high percentage of germination of seeds produced by sexual hybridization, and developmental character of hybrid seedlings show good viability of hybrid genotypes; and the morphology of leaves confirms their hybrid origin.

However, in some cases, especially when hybridization of closely related forms occurs, morphological differences of vegetative organs may not be sufficient for the identification of hybrid seedlings. In these cases, molecular-genetic methods can be used for early diagnosis and breeding of hybrid genotypes. Inter-simple sequence repeat DNA markers (ISSRs) is one of the most common and informative methods of the analysis.

We carried out a preliminary screening of 20 ISSR primers which allowed selecting 6 most informative markers by the number of synthesized polymorphic DNA fragments that were used in further work. This resulted in the electrophoretogram of amplification products (Fig. 5).

Six electrophoregrams detected 136 fragments in size from 350 to 2500 bp, 125 of which were polymorphic i.e. they detected differences between the samples. The number of fragments amplified with one primer ranged from 19 (17898A) to 26 (HB-10), and on the average in terms of primers reached 23 (Table 2).

The level of polymorphism detected by a specific primer ranged from 84.6 (amplification with primer HB-10) to 96% (primer UBC-857), and on the average reached 90.5%.

In this experiment, as in the case of proteins electrophoresis, the behavior of certain ISSR- components did not strictly correspond to the dominant pattern of inheritance. Three amplicons were discovered which are observed in some hybrid genotypes, but absent in both parental genotypes (one in 17898B, M-1 and UBC-857 primers), and three amplicons were discovered which are observed in parental genotypes but absent in some hybrids (one in M-1 and two in UBC-857 primers). Even if these three markers are completely excluded from the analysis, the topology of the tree, and the distance between the samples remain virtually unchanged.

For ISSR-analysis we took a smaller number of hybrid samples but increased the number of *Cotoneaster* representatives. It was assumed that at least one of the *Cotoneaster* biotypes available in our collection may be close enough to ryabinokizilnik. Then, this sample could be selected as one of the parents to try artificial resynthesis of ryabinokizilnik. However, the resulting consensus NJ-dendrogram shows (Fig. 6) that all representatives of the *Cotoneaster* genus were in separate branch almost equidistant from *Sorbocotoneaster* Pojark., which together with *S. sibirica* and hybrids was removed to another branch. All artificially produced hybrids F_1 (*S. sibirica* × *S. pozdnjakovii*) occupied an intermediate position between the parental species that once again confirmed the hybrid origin of their genomes.

In conclusion, it should be noted that formation and stable reproduction of such hybrids as *Sorbocotoneaster* in the nature once again confirms the significant role of sympatric way of formation of new taxa in the apple subfamily. Originating as a result of the combination of two different morphological forms, the *Sorbocotoneaster* polyploid genome was able to perpetuate itself through apomictic reproduction which allows it to keep its new morphological type. At the same time, forming fertile pollen, *Sorbocotoneaster* does not lose the opportunity to cross with at least one of the parent species—*S. sibirica*, which promotes recombination and gene transfer between significantly different taxa in morphology and adaptive potential.

In contrast to ryabinokizilnik all other known spontaneous or artificial intergeneric hybrids involving *Sorbus* resulted from hybridization with frost sensible forms and are practically unsuitable for growing in Siberia. *Sorbocotoneaster* in this sense is unique as it is the result of hybridization of completely winter-resistant cultivars in our climatic conditions.

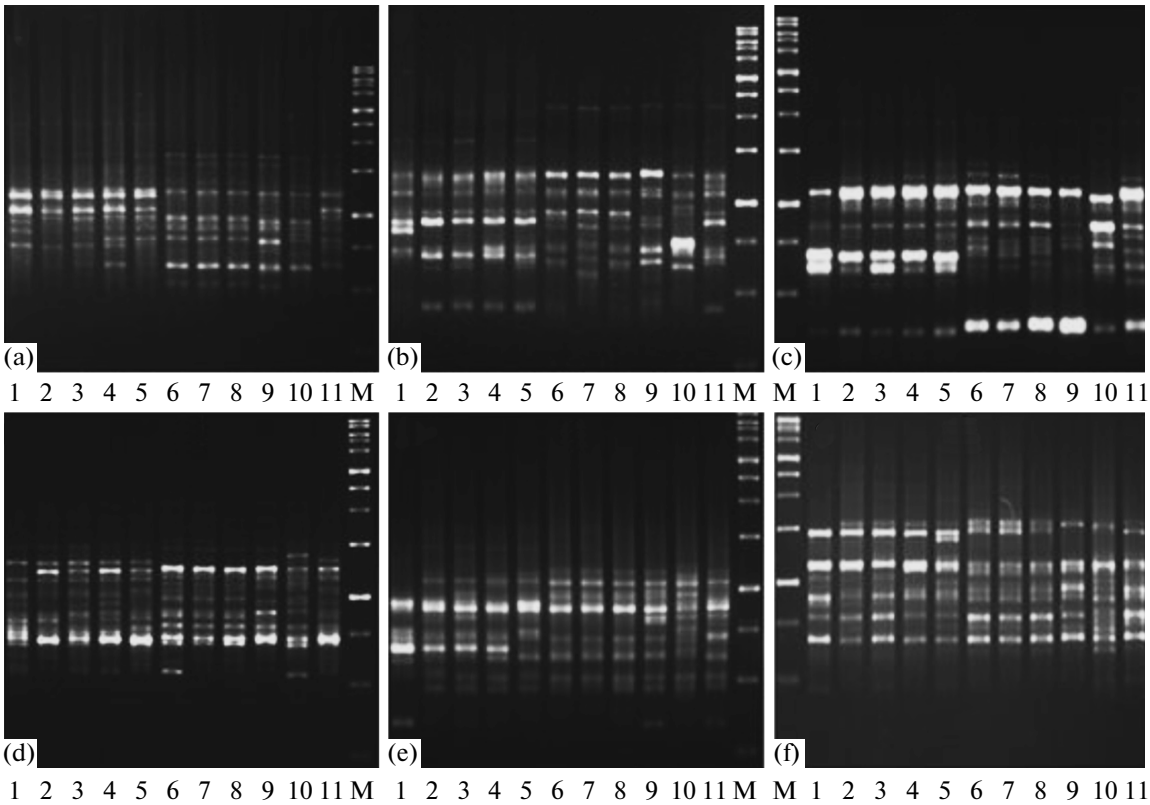

Fig. 5. Electropherograms of DNA amplification products (ISSR-PCR) of hybrid seedlings F_1 (*S. sibirica* × *S. pozdnjakovii*) (tracks 2–5) compared with the parental forms *S. sibirica* (track 1) and *S. pozdnjakovii* (track 11) and *C. integerrimus* forms (tracks 6–8), *C. melanocarpus* (track 9), *C. lucidus* (track 10). Track M—DNA marker 1kb (250–10000 bp). a—primer 17898A; b—17898B; c—17899B; d—HB-10; e—UBC-857; f—M-1.

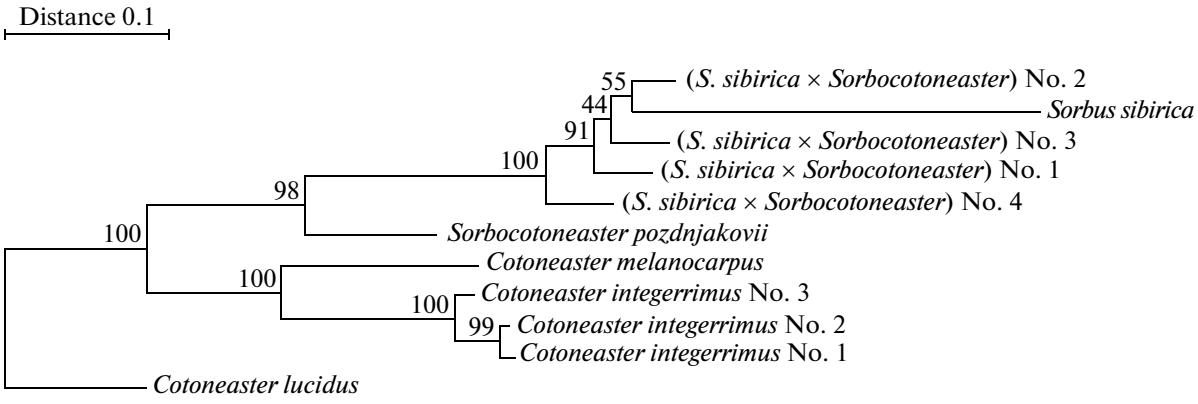

Fig. 6. Consensus NJ-dendrogram (variants –Me and +Me) based on ISSR-PCR patterns of *S. sibirica, S. pozdnjakovii*, *C. melanocarpus*, *C. integerrimus*, *C. lucidus*, F_1 (*S. sibirica* × *S. pozdnjakovii*). Top scale: genetic distances (M. Nei, W.-Li, 1979). The numbers above the junctions indicate the bootstraps.

Thus, use of *Cotoneaster* offers greats opportunities in *Sorbus* breeding in Siberia since representatives of this genus are the potential donors of essential characters which sources are very limited or non-existent. Concerning available sources, they are frost sensitive in our climatic conditions.

The present study has shown the possibility of using ryabinokizilnik in mountain ash breeding that in the future will not only allow obtaining stable Siberian *Sorbus* cultivars with a combination of valuable characters, but also enable contributing to the conservation and a more detailed study of this rare and endan-

gered genus due to its greater involvement in introduction process. Molecular-genetic methods will simplify the process and allow obtaining reliable information within a short time for monitoring the stages of introduction research.

CONCLUSION

Application of cotyledon protein SDS-electrophoresis and inter-simple sequence repeat DNA markers (ISSRs) is effective to the identification of hybrids between *Sorbus sibirica* Hedl. and *Sorbocotoneaster pozdnjakovii* Pojark.

The results obtained on the distribution of components of the cotyledons storage proteins and inter-simple sequence repeat DNA markers (ISSRs) prove that the data on the *Sorbocotoneaster* Pojark. genus arose as a result of *Sorbus* and *Cotoneaster* hybridization.

Recombination of molecular and morphological characters was observed in hybrids, which proves close relationships of the parental genomes and, as a consequence, the potential of utilizing ryabinokizilnik genotypes in breeding of *Sorbus* cultivars.

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REFERENCES

Agafonov, A.V. and Agafonova, O.V., SDS-PAGE of endosperm proteins in the members of the genus *Elymus* L. with different genomic structure, *Sib. Biol. Zh.*, 1992, no. 3, pp. 7–12.

Evans, R.C. and Campbell, C.S., The origin of the apple subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes, *Am. J. Bot.*, 2002, vol. 89, no. 9, pp. 1478–1484.

Gladkova, V.N., Cytological study of spontaneous hybridogenic genus *Sorbocotoneaster* Pojark, *Botan. Zh.*, 1967, vol. 52, no. 3, pp. 981–983.

Koropachinskii, I.Yu. and Vstovskaya, T.N., *Drevesnye rasteniya Aziatskoi Rossii* (Woody Plants of Asian Russia), Novosibirsk: Geo, 2012.

Krasnaya kniga Respubliki Sakha (Yakutii). Redkie i nakhodyashchiesya pod ugrozoi ischeznoveniya vidy rastenii i gribov (The Red Data Book of the Republic of Sakha (Yakutia). Rare and Endangered Species of Plants and Fungi), Yakutsk: NIPK Sakhapoligrafizdat, 2000, pp. 176–177.

Krasnaya kniga Rossiiskoi Federatsii (rasteniya i griby) (The Red Data Book of the Russian Federation (Plants and Fungi)), Moscow: Tovar. Nauch. Izd. KMK, 2008.

Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, 1970, vol. 227, no. 5259, pp. 680–685.

Liljefors, A., Studies on propagation, embryology and pollination in *Sorbus*, *Acta Horti Bergiani*, 1953, vol. 16, pp. 277–329.

Liljefors, A., Cytological studies in *Sorbus*, *Acta Horti Bergiani*, 1955, vol. 17, pp. 47–113.

Nei, M. and Li, W.-H., Mathematical model for studying genetic variation in terms of restriction endonucleases, *Proc. Natl. Acad. Sci. U.S.A.*, 1979, vol. 76, no. 10, pp. 5269–5273.

Osborn, T.B., *Rastitel'nye belki* (Plant Proteins), Moscow: Biomedgiz, 1935.

Peer, Van and Wachter, R.D., TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment, *Comput. Appl. Biosci.*, 1994, vol. 10, pp. 569–570.

Petrova, A.E., Nazarova, E.I., and Romanova, A.Yu., Introduction of *Sorbocotoneaster pozdnjakovii*, an endemic of South Yakutia, in *Botanicheskie issledovaniya v kriolitozone* (Botanical Studies in Permafrost), Yakutsk: YaNTs SO RAN, 1992, pp. 74–80.

Programma i metodika otdalennoi gibridizatsii plodovykh i yagodnykh kul'tur (Program and Technique of Hybridization of Fruit and Berry Crops), Nesterov, Ya.S., Ed., Michurinsk: TSGL im. I.V. Michurina, 1972.

Puchooa, D.A., Simple, rapid and efficient method for the extraction of genomic DNA from lychee (*Litchi chinensis* Sonn.), *Afr. J. April*, 2004, vol. 3, no. 4, pp. 253–255.

Redkie i ischezayushchie rasteniya Sibiri (Rare and Endangered Plants of Siberia), Novosibirsk: Nauka, 1980.

Robertson, A., Newton, A.C., and Ennos, R.A., Breeding systems and continuing evolution in the endemic *Sorbus* taxa on Arran, *Heredity*, 2004, vol. 93, pp. 487–495.

Shcherban', A.B., Reorganization of plant genome during allopolyploidization, *Vavilov Zh. Genet. Selekts.*, 2013, vol. 17, no. 2, pp. 277–285.