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**SHORT COMMUNICATION**

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# Hybridity of a plant created in a combination of crossing of (*Vaccinium uliginosum* L. × *V. vitis-idaea* L.) × *Oxycoccus macrocarpus* (Aiton) Pursh at the tetraploid level

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## Abstract

The aim of the study was to determine the hybridity of the $F_1$ generation of *Vaccinium cf. uliginosum* × *V. vitis-idaea* × *Oxycoccus macrocarpus* created through the consecutive crossing of some common berry species of the family Ericaceae (bog whortleberry, cowberry and marsh cranberry) at the tetraploid level. Certain aspects of phenology and morphometric parameters of vegetative organs of the supposed hybrid and parent plants were analysed using traditional methods of comparative analysis. Molecular genetic assay, including random amplification of polymorphic DNA, simple sequence repeat and sequencing, were also used. Comparison of the phenological and morphometric features of the experimental plant and its parents allows suggesting that in the combination of crossing of (*V. uliginosum* × *V. vitis-idaea*) × *O. macrocarpus* (the cultivar Searles), a three-species hybrid was created. The allelic variants, specific for *V. uliginosum, V. vitis-idaea* and *O. macrocarpus*, were detected in the *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* genotype. A next-generation sequencing approach is suggested for estimating the share of the genomes of *Vaccinium* spp. in the formation of the interspecies hybrid.

## Key words

consecutive crossing, DNA markers, Ericaceae, hybridisation, sequencing, tetraploids

## Introduction

A positive result from breeding using a remote hybridisation technique may not be achieved using only paired mating. An increase in the combining ability in order to breed cultivars possessing desired characteristics is also achieved through consecutive crossing, i.e. involvement of new species in the crossing with a two-species hybrid. However, it should be borne in mind that the possibility of achieving the breeding potential of interspecies hybridisation of berry (fruit) species is largely conditioned by the crossing at the tetraploid level allowing the production of fertile plants.

The complication of creating a new genotype in a consecutive combination of the tetraploid crossing of (*V. uliginosum* × *V. vitis-idaea*) × *O. macrocarpus* (the



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cultivar Searles) was evident even from the beginning (Fig. 1).

**Figure 1.** Form *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* (the cultivar Searles) (age – 15 years, July 2017)

A low level of genetic compatibility of the interbreeding plants is objectively conditioned by their distance within the system. According to modern judgements, bog whortleberry and cowberry, which are parent species of the plant resulting from cowberry--blueberry crossing (the maternal component), are representatives of the genus *Vaccinium*, but marsh cranberry (the paternal component) belongs to the genus *Oxycoccus* (Mirek et al. 2002). Both of these genera represent the family Ericaceae. At the same time, there is an opinion among taxonomists that these species are not so distant within the system. It means *V. uliginosum*, *V. vitis-idaea* and *O. macrocarpus* are members of different subgenera (*Vaccinium* and *Oxycoccus*, respectively) of the genus *Vaccinium* (https://en.wikipedia.org/wiki/Vaccinium).

Moreover, since the tetraploidy of the *O. macrocarpus* plants used in the experiment, as shown later in the "Material and methods" section, is artificially induced (Lehmushovi et al., 1993), the probability of genetic imbalance in meiosis, as well as the impossibility of their effective use as pollinators, is great.

In relation to these circumstances, the following idea is valid: in the combination of (*V. uliginosum* × *V. vitis-idaea*) × *O. macrocarpus,* there exist not true but so-called false or pseudo-hybrids, which either appear on stimulation of fission of the egg (apomixis) by the paternal gamete or are the products of self-fertilisation. We shall emphasise that in both cases, the plants develop strictly matrilineally (Kartel et al. 1999).

Note that the possibility of uncontrolled consecutive crossing (anemophilous and entomophilous pollination) in this case was minimised – immediately after the controlled pollination, a light frame with fabric stretched on it was placed over the experimental mother plant. To prevent self-fertilisation, mechanical castration (removal of the anthers from buds) was performed.

The aim of the research was to determine the hybridity of the $F_1$ generation of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus*.

This is necessary in order to determine what kind of plant was created and to specify the directions of further research more reasonably.

## Material and methods

The object of the research resulted from the long-running introductory and hybridisation experiments started in 1992. The experimental form itself was created by the crossing of the parent plants (*V. uliginosum* × *V. vitis-idaea*) (mother) and *O. macrocarpus* (father) in May 2003.

In the 1990s, the above-mentioned plants, as well as the forms of the original parent species *V. uliginosum* and *V. vitis-idaea* were in the collection of Gantsevichi experimental farm enterprise (EFE) of the Central Botanical Garden of the National Academy of Sciences of Belarus. More than 25 years after the beginning of the experiment, one of the authors has preserved the plants *V. vitis-idaea*, *O. macrocarpus* and one of the forms of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* in his personal collection. Results of research on the latter are presented in this article.

*V. uliginosum* is a forest ecobiomorph, a natural tetraploid (2n = 48), and it was selected in the mid-1990s for its high yield in the area of Gantsevichi.

*V. vitis-idaea* are natural forms identified in 1992 among the natural flora in the area of the village Ust-Omchug (Magadan Region, Russia) (Marozau 1995b; Morozov 1996). These plants were noticed because of some of their morphological and phenological features (see Results and discussion), and in connection with

this, the suggestion was made that they represented a tetraploid micro-population (2n = 48). Later, this hypothesis was confirmed by the results of karyological analysis (Marozau 1995a; Marozau 1995 b). Normally, *V. vitis-idaea* is a diploid (2n = 24).

#### *V. uliginosum* × *V. vitis-idaea*

Since 1996, several dozen forms of these plants have been created as a result of paired mating at the tetraploid level.

#### *O. macrocarpus*

*O. macrocarpus* is a tetraploid form of the cultivar Searles (2n = 48). In the 1990s, several cuttings of this plant were received from the Finnish researchers Lehmushovi, Hokkanen and Hiirsalmi. The authors created this genomic mutant by the action of colchicine solution upon the seedlings of seeds (Lehmushovi et al. 1993). Normally, *O. macrocarpus*, which is an aboriginal species of North America, is a diploid (2n = 24). The plant was propagated using a traditional vegetative method; its study was started under conditions of an introductory experiment (Morozov et al. 2009).

In the present research, traditional methods of comparative analysis of certain aspects of phenology and morphometric parameters of the vegetative organs of the supposed hybrid and parents are used, as well as methods of analysis based on molecular genetic approaches.

Samples for genetic analysis (leaf blades) were collected from individual plants belonging to the following *Vaccinium* taxa: *V. uliginosum* (growing in the forest stand near Gantsevichi EFE), *V. vitis-idaea*, *O. macrocarpus* and *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* (see Material and methods).

Samples were placed in 70% alcohol and preserved at 4°C for 3 days for primarily removing alcohol-/water-soluble polymerase chain reaction (PCR) inhibitors from the tissues. Total DNA extraction from the plant tissues was carried out using the cetyl trimethylammonium bromide (CTAB) protocol (Padutov et al. 2007).

Molecular genetic assay of the plant *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* was performed using two methodological approaches: a) utilising separate loci, characterised by multi-allelic diversity. The loci of the rDNA-operon (internal transcribed spacer-1 [ITS1], 5.8S ribosomal RNA [rRNA] and ITS2), highly polymorphic simple sequence repeat (SSR) and expressed sequence tag (EST) loci. SSR- and EST-loci were species-specific (according to the literature data: Wang et al. 2010; Zhu et al. 2012; Mayer et al. 2014) to *V. uliginosum*, *V. vitis-idaea* and *O. macrocarpus*; b) utilising a large number of dispersed genomic loci, characterised by low allelic diversity. Random amplification of polymorphic DNA (RAPD) markers (diallelic system) were used. To obtain reliable results by RAPD, the following requirements were met during the research: exclusion of minor bands, triple replication of PCR results and the absence of DNA of fungal and bacterial endophytes in the leaves (White 1990).

PCR analysis was performed using DreamTaq™ DNA polymerase mixes (Thermo Fisher Scientific, Waltham, MA, USA) with the parameters corresponding to each of the types of primers used (Padutov et al. 2007). Analysis of the RAPD-PCR products was performed using 1.4% agarose electrophoresis. Sequencing was performed using ABI Prism 310 (Thermo Fisher Scientific) according to the BDT Sequence Kit v.1.1 manufacturer's protocol. Obtained sequences were processed using the Basic Local Alignment Search Tool (BLAST) in the GenBank database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/genbank/accessed 01.08.2017). SSR assay was performed using the genetic analyser ABI Prism 310 and Gene Mapper 4.0 software (Thermo Fisher Scientific).

## Results and discussion

In this article, we do not consider the question of the true hybridity of the plant's maternal component. It should be discussed in detail based on genetic analysis. Unfortunately, over the course of >20 years after the beginning of the experiment, the very form of *V. uliginosum* × *V. vitis-idaea,* which – through consecutive crossing – resulted in the creation of the supposed three-species hybrid, was not preserved.

We cannot claim that all of the plants created as a result of the hybridisation experiment aimed at creation of the maternal component were indeed hybrids, since they were largely different, primarily in habitus and in some other morphological features. For a number of reasons, including the successful interspecies cross-

ing conducted earlier, for instance, in a combination at the diploid level of *V. vitis-idaea* × *O. macrocarpus*, we did not isolate bog whortleberry plants immediately after the manipulations of artificial pollination (Marozau 1993; Morozov 2005).

Two facts needs to be emphasised which, in our opinion, testify that the maternal component of the supposed three-species hybrid was indeed a two-species hybrid.

1. In the set of characteristics of the plant created as a result of pollination of *V. uliginosum* × *V. vitis-idaea* by the pollen of *O. macrocarpus*, one of the most defining properties of a tetraploid *V. vitis-idaea* of Magadan origin became obvious – re-blooming (repeated fruiting) in the first half of August (Fig. 2). During the floristic expedition to the Magadan region in 1992, we succeeded in revealing a tetraploid micro-population of *V. vitis-idaea* L. in a natural environment primarily due to the phenomenon of re-blooming of this species (Marozau 1995b). Introductory studies of this plant in the southern part of Belarus showed a consistent stability of re-blooming and its prevalence over spring blossoming (Morozov 1996).

We had been recording the re-blooming of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* annually in the first half of August each year over a period of 7 years (2010–2017), regardless of whether the weather conditions of the growing season (warm August) contributed to it. According to the visual assessment of the supposed hybrid, during all the years of observation, re-blooming had been much more abundant than the spring blossoming (the same happened to the Magadan cowberry). The percentage of flowers developed during spring blossoming and during re-blooming was 20/80. Thus, in our opinion, the phenomenon of re-blooming (repeated fruiting) of this plant results not from abnormal weather patterns, yet it is a phenotypically well-expressed, special genetic feature inherited from cowberry.

**Figure 2.** Re-blooming (repeated fruiting) of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* (August 15, 2017)

Note that re-blooming is also typical for *V. uliginosum* (Yevtukhova 1990). In this regard, uncontrolled crossing needs to be considered, when bog whortleberry is pollinated not with the pollen of cowberry, but with that of some other species (cultivar). Various cultivars of *V. corymbosum* that do not re-bloom (fruit repeatedly) grew on the experimental site. In this case, the re-blooming of the supposed three-species hybrid (Fig. 2) resulted from the influence of bog whortleberry (it is not typical for marsh cranberries to re-bloom). It should be noted that, according to Yevtukhova (1990), re-blooming is more an exception than a natural phenomenon for *V. uliginosum*. Even under the conditions of cultivation, the re-blooming (repeated fruiting) of bog whortleberry does not dominate the spring blossoming, in contrast to the case with the tetraploid cowberry (Morozov 1996) and the plant created as a result of the consecutive crossing of (*V. uliginosum* × *V. vitis-idaea*) × *O. macrocarpus*. According to Yevtukhova (1990, p. 8), bog whortleberry 'reblooms at the expense of reproductive buds unused during the spring blossoming which are lower on the shoots of branches...' At the same time, both tetraploid cowberry and *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* re-bloom as a result of the breaking of newly formed buds at the shoot tips (see Fig. 2) but not because of the breaking of buds unused during spring blossoming.

Obligatory re-blooming, its dominance over spring blossoming and other special features (such as localisation of flower buds on shoot tips), which are identical for the tetraploid *V. vitis-idaea* of Magadan origin and for *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus,* allow us to make an assumption about the true hybridity of the maternal component.

2. Previous research has revealed the possibility of crossing of cowberry (mother) and marsh cranberry at the diploid level (Marozau 1993). Unfortunately, all the plants produced (10 forms) were sterile – they bloomed abundantly, even individual berries appeared,

but they had no seeds inside. The same result was earlier obtained by Christ (1977). We also created a sterile hybrid in the process of crossing of diploid cowberry (mother) and tetraploid fenberry (*O. palustris* L.) (Morozov 1999). Note that the latter is very close to *O. macrocarpus* within the system. All these facts testify both directly and indirectly to the possibility of combination of the genetic material of cowberry and marsh cranberry in one genotype. And if the hybridity of the plant *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* is proved, this, in our opinion, will prove the hybridity of the maternal component (*V. uliginosum* × *V. vitis-idaea*). Because only the presence of the genetic material belonging to *V. vitis-idaea,* which is compatible (as shown above) with *O. macrocarpus*, in the maternal component could ensure the success of the consecutive crossing. In the literature that we have studied, there is no information about natural or artificial hybrids of *V. uliginosum* and *O. macrocarpus*. And this reflects that they are much more distant within the system than *V. vitis-idaea* and *O. macrocarpus*.

In order to prove the hybridity of the supposed three-species hybrid using traditional methods, it is necessary 1) to identify the evidence of certain characteristics caused by the hereditary influence of the father (*O. macrocarpus*) and 2) to show that it does not develop strictly matrilineally.

One evidence of hybridity can be obtained through analysis of the assimilation organs. The closer these parameters of the experimental form are to those of marsh cranberry, the higher the possibility is that hybrids are created. And, on the contrary, the more similar they are to the cowberry–blueberry hybrid, the lower is the possibility. In our opinion, the most evident are the results of the analysis of the least labile leaf shape factor (length/width). Confirmation of this can be found in the literature. According to Marcysiak (2012), hereditary signs that characterise the shape of a plant organ are less susceptible to change under the influence of the external environment than its dimensional characteristics. As already noted, the maternal form (*V. uliginosum* × *V. vitis-idaea*) is not preserved. Therefore, to prove the hybridity through the analysis of the morphometric parameters of the leaves, we use the data obtained in 2007, which is based on a comparison of the parent plants directly used in the crossing (Morozov and Morozova 2007).

As can be seen (Tab. 1), the leaf parameters of marsh cranberry and the supposed three-species hybrid practically coincide. For a more justifiable assertion about the reliability of the difference between the indicators studied, we analysed the value of the standard deviation *t* determined in the process of a pair-wise comparison of their average number (Tab. 2). It was revealed that at a 0.99 confidence level, which was high for such studies, the supposed three-species hybrid does not have statistically authentic differences from marsh cranberry in two of the four studied morphometric parameters of leaves – leaf area and shape factor.

We also analysed how the value of the leaf shape factor of the original parent species (used in paired mating and consecutive crossing) and the supposed three-species hybrid *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* was distributed. As can be seen, the empirical distribution of the values of this factor, shown in Figure 3, is overlapped in 50% of cases of the supposed hybrid and marsh cranberry. There were no cases of overlapping of this factor with the original parent form of bog whortleberry and cowberry. Analysis of the distribution pattern of the values of the leaf shape fac-

**Table 1.** Parameters of leaves of parents and form *Vaccinium cf. uliginosum* × *V. vitis-idaea* × *Oxycoccus macrocarpus*

| Plant number | Length (cm) | | Width (cm) | | Area ($cm^2$) | | Length-to-width ratio | |
|---|---|---|---|---|---|---|---|---|
| | $\overline{x} \pm s_{\overline{x}}$ | V (%) | $\overline{x} \pm s_{\overline{x}}$ | V (%) | $\overline{x} \pm s_{\overline{x}}$ | V,% | $\overline{x} \pm s_{\overline{x}}$ | V (%) |
| Mother *V. uliginosum x V. vitis-idaea* | 3.2±0.08 | 12.8 | 1.2±0.04 | 17.8 | 2.6±0.15 | 31.9 | 2.8±0.07 | 12.9 |
| Father marsh cranberry *Oxycoccus macrocarpus* | 1.6±0.03 | 10.4 | 0.7±0.02 | 14.8 | 0.8±0.03 | 22.1 | 2.3±0.04 | 9.3 |
| Form *V. cf. uliginosum* × *vitis-idaea* × *macrocarpus* | 1.8±0.05 | 14.3 | 0.8±0.02 | 13.9 | 0.9±0.05 | 26.6 | 2.4±0.04 | 9.8 |

tor indicates the similarity between the supposed hybrid and marsh cranberry.

**Table 2.** Reliability of the difference in the average parameters of the leaves of the paternal form and form *V. cf. uliginosum* × *vitis-idaea* × *macrocarpus* according to t-criterion (t theoretically = 2.58, P = 0.01)

| Number of plants under comparison (see * Tab. 1) | Actual value of *t* –criterion | | | |
|---|---|---|---|---|
| | length of a leaf | width of a leaf | area of a leaf | length-to-width ratio |
| 2↔3 | 3.5 | 2.7 | 1.9 | 1.7 |

Thus, the similarity of certain features of the assimilatory organs belonging to the supposed three-species hybrid to those of *O. macrocarpus* is reliably ascertained, which is caused by the presence of the genetic material belonging to the latter species in the genotype under study and the influence of *O. macrocarpus* on the phenotype.

It is also determined that the supposed three-species hybrid is characterised by a very significant time gap between the beginning of the development of the vegetative organs and the development of generative organs (spring blossoming), accounting for 10–15 days or more. This time gap is also typical for marsh cranberry. The period of time between the beginning of shoot growth and budding of the latter species is about 3.5 or 4 weeks. Note that this feature of pheno-rhythmics does not correspond to the development of the named phases of seasonal development that the parent plants on the maternal line undergo. The budding of bog whortleberry and cowberry begins about 3–5 days after the blooming of vegetative buds, which is typical for the maternal component *V. uliginosum* × *V. vitis-idaea*.

Utilisation of a set of species-specific primers for PCR amplification of the EST and SSR loci (presented in previous papers [10–12]) showed that all used loci were detected in the investigated *Vaccinium* species. It suggests that this approach is not applicable for the analysis of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* because, initially, separation and identification of taxa based on species-specific PCR is impossible. Results of the PCR amplification of the fragment of the gene *611 dehydrin COR11* (using primers specific to *V. vitis-idaea* [11]) are presented in Figure 4. As seen, all three species (*V. uliginosum*, *V. vitis-idaea* and *O. macrocarpus*) had one major band in the expected area, which indicates the lack of strong primer specificity to *V. vitis-idaea* species. It was also impossible to use band size as a marker for species diagnostics.

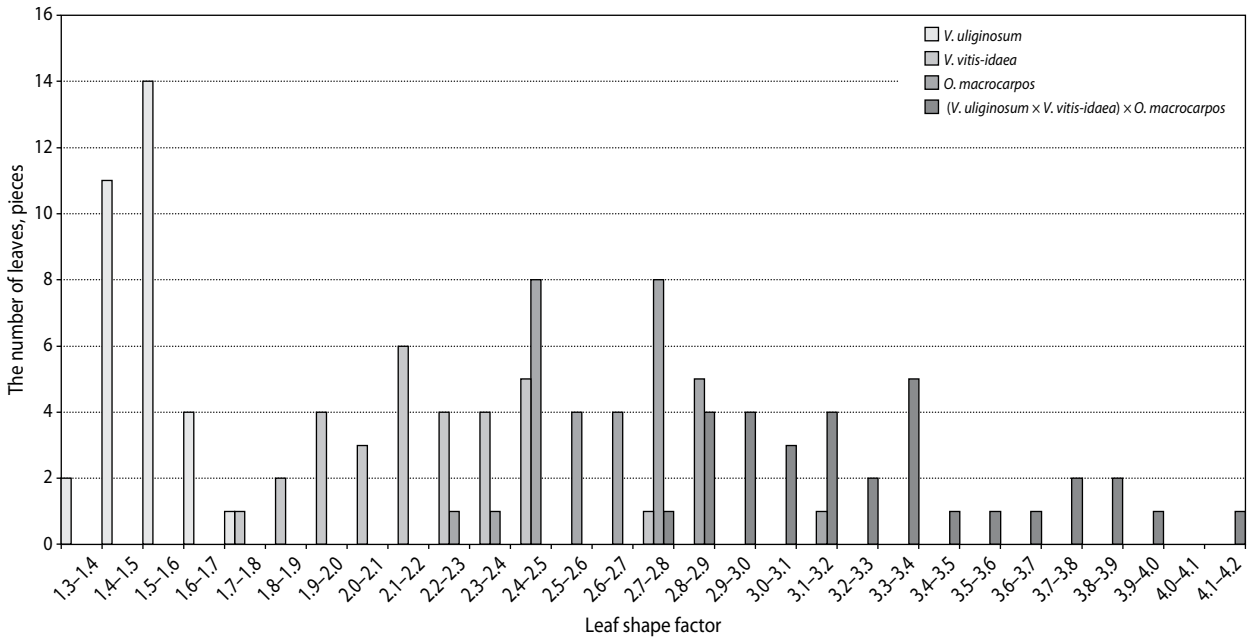


**Figure 3.** The distribution of the leaf shape factor value of the original parent species and the supposed hybrid *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus*

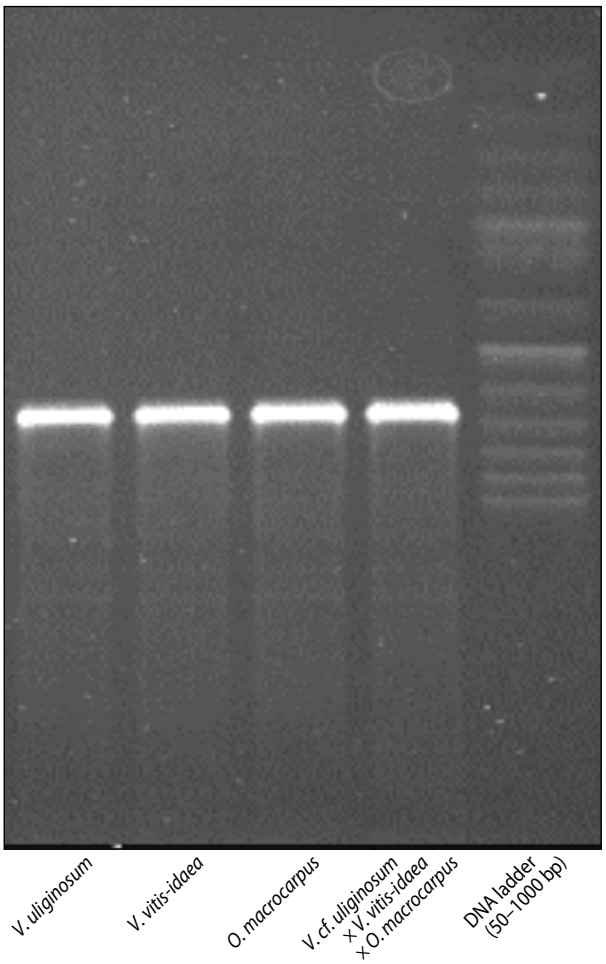


**Figure 4.** Results of polymerase chain reaction amplification of the *611 dehydrin COR11* locus for *Vaccinium* taxa

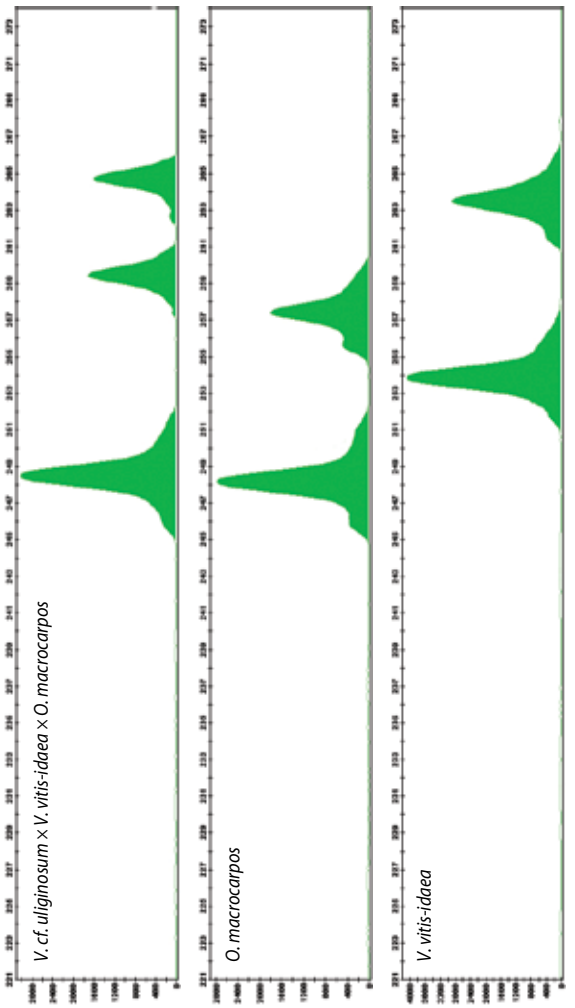


**Figure 5**. Results of SSR assay of plant samples (Vm52682 marker)

The SSR assay has shown that the species-specific microsatellite markers used from literature [10–12] were also not unique to the taxa, and they were found in *V. uliginosum*, *V. vitis-idaea* and *O. macrocarpus*. At the same time, the SSR alleles of the studied species had species-specific sizes, which could be used in the preliminary screening of plant samples. Thus, Figure 5 shows that *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* had *O. macrocarpus* species-specific allele in its genotype.

The same results were obtained for the remaining SSR loci, e.g. the presence of *V. uliginosum* species-specific allele in *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* was noted for the VuCT7 marker. Direct DNA paternity testing (by SSR fingerprinting) for *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* was not possible, because the genetically analysed plants (*V. uliginosum*, *V. vitis-idaea* and *O. macrocarpus*) were not the individuals that were crossed.

One more DNA assay was based on species-specific single-nucleotide polymorphism (SNP) identification in the rDNA loci of the hybrid *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* (Fig. 6).

The obtained results showed that all nucleotide positions with species-specific SNPs in *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* genotype are heterozygous. Thus, the hybrid nature of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* is indicated (Fig. 7).

The subtraction of the nucleotide sequence of *V. uliginosum* (♀ initial parent plant) from the genotype of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* showed that the remaining sequence could, according to the NCBI GenBank database, equally belong to both

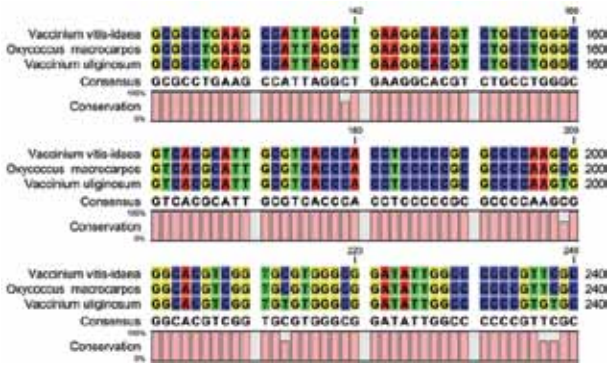


**Figure 6.** Species-specific single-nucleotide polymorphism in 5.8S rRNA and ITS2 loci of *V. uliginosum*, *V. vitis-idaea*, *O. macrocarpus* (fragment of alignment)

*V. vitis-idaea* and *O. macrocarpus*. Nevertheless, the level of similarity in both cases did not exceed 98%. In addition to these species, according to the information provided by NCBI GenBank, *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* could – with equal or lesser probability – include a combination of haplotypes of *V. corymbosum*, *V. angustifolium*, *V. angustifolium* × *V. corymbosum*, etc.

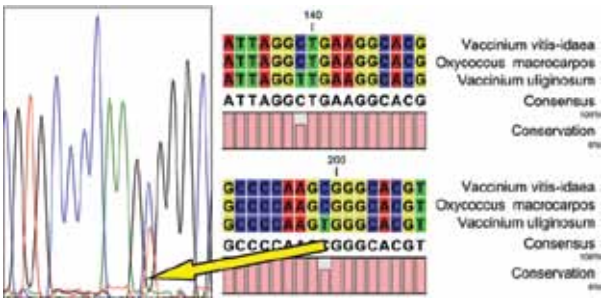


**Figure 7.** Identification of species-specific single-nucleotide polymorphisms in *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* genotype

The complexity and falsity of interpretation of rDNA sequencing results may be caused by the paralogous nature of rDNA (these loci are localised in several chromosomes and could be different in the nucleotide structure; they are amplified in a cell selectively).

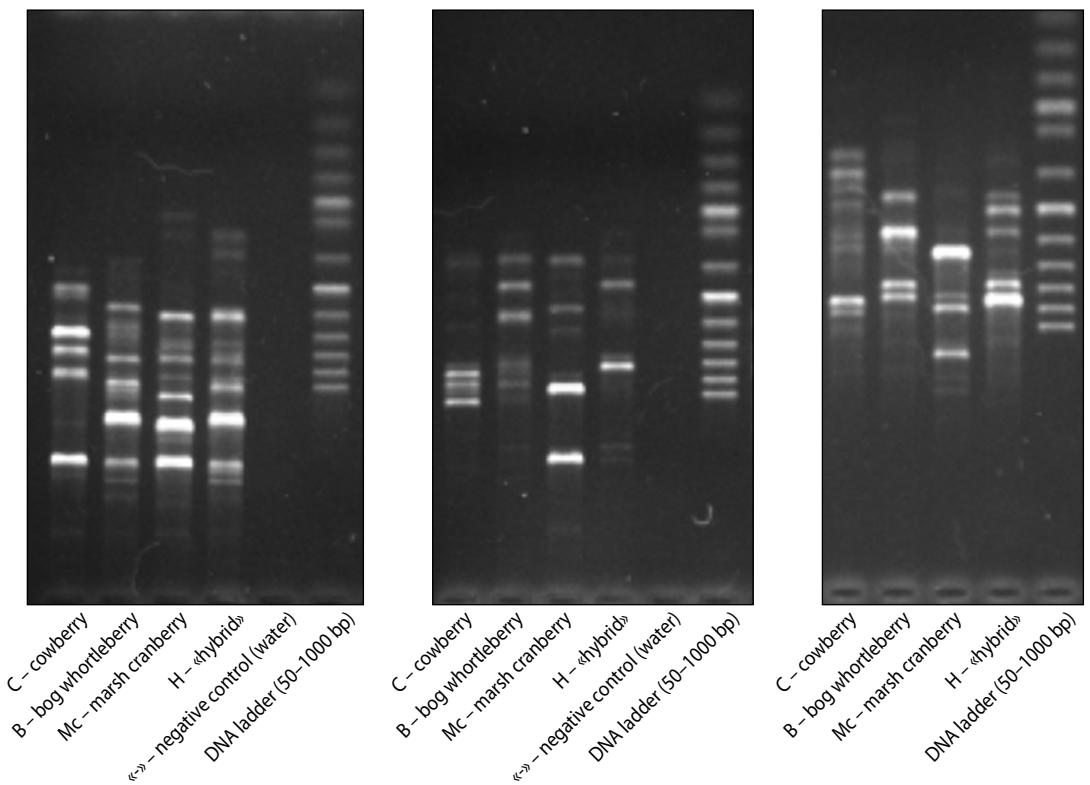


**Figure 8.** Results of random amplification of polymorphic DNA assay of four plant taxa

One more possible error is the presence/absence of species-specific SNPs of *V. uliginosum* (maternal species), *V. vitis-idaea*, *O. macrocarpus*, *V. corymbosum*, *V. angustifolium* and others (which may be caused by the geographic variability of rDNA loci of *Vaccinium* species) in the NCBI GenBank database, as well as the lack of analysis of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* parent individuals, which were directly crossed.

The multilocus approach of the assay was based on RAPD, which allows assessing the general pattern in the structure of the genome of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus*.

Results of RAPD fingerprints of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* showed the greatest similarity to two species – *V. uliginosum* and *O. macrocarpus*. *V. uliginosum* species-specific bands were »35% among the total loci of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus*; *O. macrocarpus* species-specific bands were noted in »25% of the set. About 8% of loci were not found in the reference samples of *V. uliginosum* and *O. macrocarpus* species, but these were similar to the reference sample of *V. vitis-idaea* (Fig. 8). The results of the partial summed concordance of the RAPD profile of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* and that of the reference samples of *Vaccinium* species can be explained by intraspecific variability – the difference of the reference samples from the parent plants used in the crossing; the distribution and conjugation of hybrid chromosomes (of parental individual *V. uliginosum* × *V. vitis-idaea*) in the process of meiosis during gametogenesis; introgressive hybridisation between species of the genus *Vaccinium*, which may cause the presence of loci borrowed from other species in the genome structure of various species.

## Conclusion

The comparative analysis of some aspects of the phenology and morphometric parameters of vegetative organs of the hybrid and parent plants allows assuming that during the combination of crossing of (*V. uliginosum* × *V. vitis-idaea*) × *O. macrocarpus* (the cultivar Searles), a three-species hybrid was created.

The plant created is fertile. Preliminary results show that, on average, about 13 seeds emerge in one berry (the latter are small, usually pear-shaped, black and without wax coating). The seeds have germination capacity of about 10%. Shoots and seedlings of the plant have extremely low (<1%) viability. Nevertheless, several plants aged 1 year belonging to the $F_2$ generation have now been produced. One of them is characterised by strong vertical growth; it has begun to bush actively. The plant also has horizontal shoots, and, even at this age, the roots contact the surface of the substrate (Fig. 9). The growth rates of other plants are much lower; the formation of horizontal shoots prevails.

**Figure 9.** A 1-year-old plant of the $F_2$ generation *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* (March 19, 2018)

Thus, the supposed hybrid *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* has a fertile female gametophyte. It was proved by obtaining the $F_2$ generation through self-fertilisation. Individual plants of this generation are characterised by high growth rates in the first stage of life. It is also revealed that for *O. macrocarpus,* the plant under study has about 25% of amplified loci similar in size. This is second only to the base plant *V. uliginosum*. Based on the results obtained, it would be logical to continue the research in the following areas.

1. To create a rich base of the $F_2$ generation of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* for as-

sessing the amplitude of variability of economically important features and the subsequent selection of valuable genotypes.

2. To perform back-crossings of $F_1$ of *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* with *O. macrocarpus* (the cultivar Searles), as well as with other genetically related species of the genus *Oxycoccus*, for instance, with selected forms of *O. palustris*, for the creation and subsequent selection of valuable genotypes.
3. To carry out next-generation sequencing, with the subsequent sorting of genome fragments peculiar to one or another species of the genus *Vaccinium,* in order to finally determine the structural organisation of the *V. cf. uliginosum* × *V. vitis-idaea* × *O. macrocarpus* genome (according to the list of species).

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