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Study of genetic variability of *Ribes* L. representatives grown in Belarus

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Abstract

The research aims to study the genetic variability of *Ribes* L. representatives grown in Belarus, and to reveal a set of DNA-markers for its DNA-identification. We formed a set of SSR-markers that possess rather high diagnostic value and allow identifying of black currant and gooseberry varieties at a molecular level and can be recommended for DNA-identification of those cultures.

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Introduction

The currants breeding now is focused on fruits production with high adaptive capacity, potential yield and resistance to the most common pests and diseases (1). The knowledge of the genetic relationships of crossing pairs is necessary for the effective selection process. The accurate characterization of *Ribes L.* with using only morphological characteristics is difficult because the phenotypic descriptions are susceptible to the environmental effects, which complicates the identification. Thereby it is increasingly important to develop an efficient and reliable methods of genetic diversity assessing of varieties by molecular markers using. Different types of the markers were developed for the genetic characterization of *Ribes* L. representatives: RAPD (2-4), AFLP (5-7), ISSR (3, 8). However, each of these identification systems has the certain problems with the reproducibility in different laboratories. In 2002, the scientists from the Scottish Crop Research Institute proposed the set of 12 microsatellite markers (5). This type of markers has several advantages: high level of polymorphism of SSR loci, codominant inheritance, relative simplicity of data interpretation and reproducibility in different laboratories, which allowed the SSR markers to be widely used to assess the genetic polymorphism, to study the phylogenetic relationship, to construct the genetic maps, etc. The SSR markers were used to assess the genetic diversity of the European representatives of the genus Ribes L. (9-11). Polymorphism of black currant cultivars from the collection of the Russian Research Institute of Fruit Crop Breeding was evaluated with using of 14 microsatellite loci (12). Nowadays, however, there is no universal technique of DNA fingerprinting of the *Ribes* L. representatives, the varieties of Belarusian selection remain unstudied. The genetic potential they possess and which set of markers is effective for their identifying are not established.

Materials and Methods

Plant material and DNA extraction

For the genetic diversity analysis of black currant and gooseberry, grown in the Belarus, the samples collection, including different varieties, was formed. It includes both the varieties of Belarusian selection and the varieties from Russia, Germany, Poland and other countries cultivated in Institute for Fruit Growing, Samokhvalovichy. DNA was extracted

from 0.2 g of leaf tissue using the Genomic DNA Purification Kit (Thermo scientific).

SSR amplification

For the Ribes L. representatives analysis 7 SSR-markers were used (http://www.fruitbreeding.co.uk/RibesGenomicsSSRs.asp). Primers were grouped into two sets; each of the forward primers was labeled with one of the fluorochromes: FAM, HEX, ROX, or R6G. PCR was performed in 20 µl reaction volumes, containing 50 ng of DNA, 0.2 µm of each reverse and forward primer, 200 µm dNTPs, 1.5 mM $MgCl_2$, 1×NH_4 reaction buffer and 1 U *Taq* polymerase. Thermocycling consisted of 5 min at 94°C: 35 cycles of 30 s at 94°C 45 s at 50°C and 45 s at 72°C and a final extension step of 5 min at 72°C. The PCR-products were separated by electrophoresis on 3500 Genetic Analyzer (Applied Biosystems).

Data analysis

The number of alleles per locus (Na), the expected (He) and observed (Ho) heterozygoteses, number of effective alleles (N_e) and Wright's fixation index (F) were determined using GenAlEx 6.1. Discrimination power of the markers were calculated as PD= 1 – $\Sigma(g_i)^2$ where g_i – the frequency of occurrence i- genotype. The dendrogram was drawn on the UPGMA algorithm using the program TRECON.

Results

The genetic variability of 65 *Ribes* L. representatives (60 black currant varieties, 4 gooseberry varieties and 1 *Ribes alpinum* sample) was estimated with using 7 microsatellite markers, covering various genome areas and located on the different chromosomes. The least polymorphic loci were e3-B02 and g1-A01. The number of detected alleles was 6 and 5 respectively. The loci g1-K04 revealed 8 alleles, the loci g1-E03 and e1-001 – 10 and 9 respectively. The maximum alleles number were identified in the loci g2-G12 and g1-M07 – 11. In total among the 65 Ribes L. representatives 60 polymorphic alleles were identified using 7 SSR markers. The average number of alleles per locus was 8.6. The varieties of Belarusian selection was less polymorphic (an average of 4.7 alleles per locus) than the varieties of foreign selection (an average of 7.1 alleles per locus). The same parameter among the European representatives of the Ribes L. genus was determined 10.4 with using 11 SRR-markers (11). The average number of alleles per locus with using 14 markers among 27 varieties of black currant from the collection of the VNIISPK was 4.9 (12). In our study, the number of alleles is different from the number of alleles obtained in previous studies, both upwards and downwards, because the samples differing in volume and genotypes composition were used. 17 rare alleles were revealed (observed in 2% of samples and less). The number of rare alleles was from 1 to 4 depending on the marker. One of the main sources of rare alleles were the gooseberry varieties ('Berendey', 'Malahit', 'Ravolt', 'Severnuy kapitan'). Different diversity parameters were analyzed for 7 SSR loci (**Table1**).

For some alleles, the frequency of occurrence was very high. Thus, the locus e3-B02 with allele 161 bp length was present in the genome in 58 % of the samples, the locus g1-K04 with allele 292 bp length were found in 52% of samples. The total alleles number determined by these markers, was 6 and 8, respectively. Among black currant cultivars the maximum number of unique genotypes (52) was identified with the markers g2-G12 and e1-001, the minimal – with markers e3-B02 and g1-A01 (33 and 35 respectively). The average number of unique genotypes for the 7 markers was 43.1. The discriminatory power of the markers was quite high – from 0.67 for the marker e3-B02 to 0.95 for the marker g2-G12, the mean PD value for 7 markers was 0.84, indicating a high diagnostic value of the selected SSR markers.

The expected heterozygoty (*He*) varied from 0,562 to 0,878, the mean value H_e - 0,730. The observed heterozygoty (H_o) varied from 0,508 for the *e3-B02* loci to 0,80 for the *e1-001* loci, the mean value *Ho* was 0,66. The effective alleles number (N_e) was from 2,3 for the *e3-B02* loci to 8,2 for the *g2-G12* loci. The mean value for all locuses was 4,6. The obtained data indicates the high enough heterozygosity degree of blackcurrant varieties.

Table 1. Statistical characterization of the 7 SSR loci studied in 65 *Ribes* genotypes

SSR locus	Size (bp)	No. alleles	H_e	H_o	N_e	F	No. unique genotypes	Proportion of unique genotypes	PD
g2-G12	191 – 197	11	0,878	0,738	8,2	0,16	48	0,74	0,95
e1-001	142 – 166	9	0,806	0,80	5,2	0,01	52	0,8	0,91
g1-M07	200 – 230	11	0,846	0,769	6,5	0,09	52	0,8	0,95
e3-B02	151 – 183	6	0,562	0,508	2,3	0,1	33	0,51	0,67
g1-A01	209 – 241	5	0,576	0,538	2,4	0,1	35	0,54	0,71
g1-E03	207 – 270	10	0,793	0,646	4,8	0,18	42	0,65	0,91
g1-K04	284 – 300	8	0,648	0,6	2,8	0,07	40	0,62	0,77
mean value		8,6	0,730	0,657	4,6	0,10	43,1	0,67	0,84

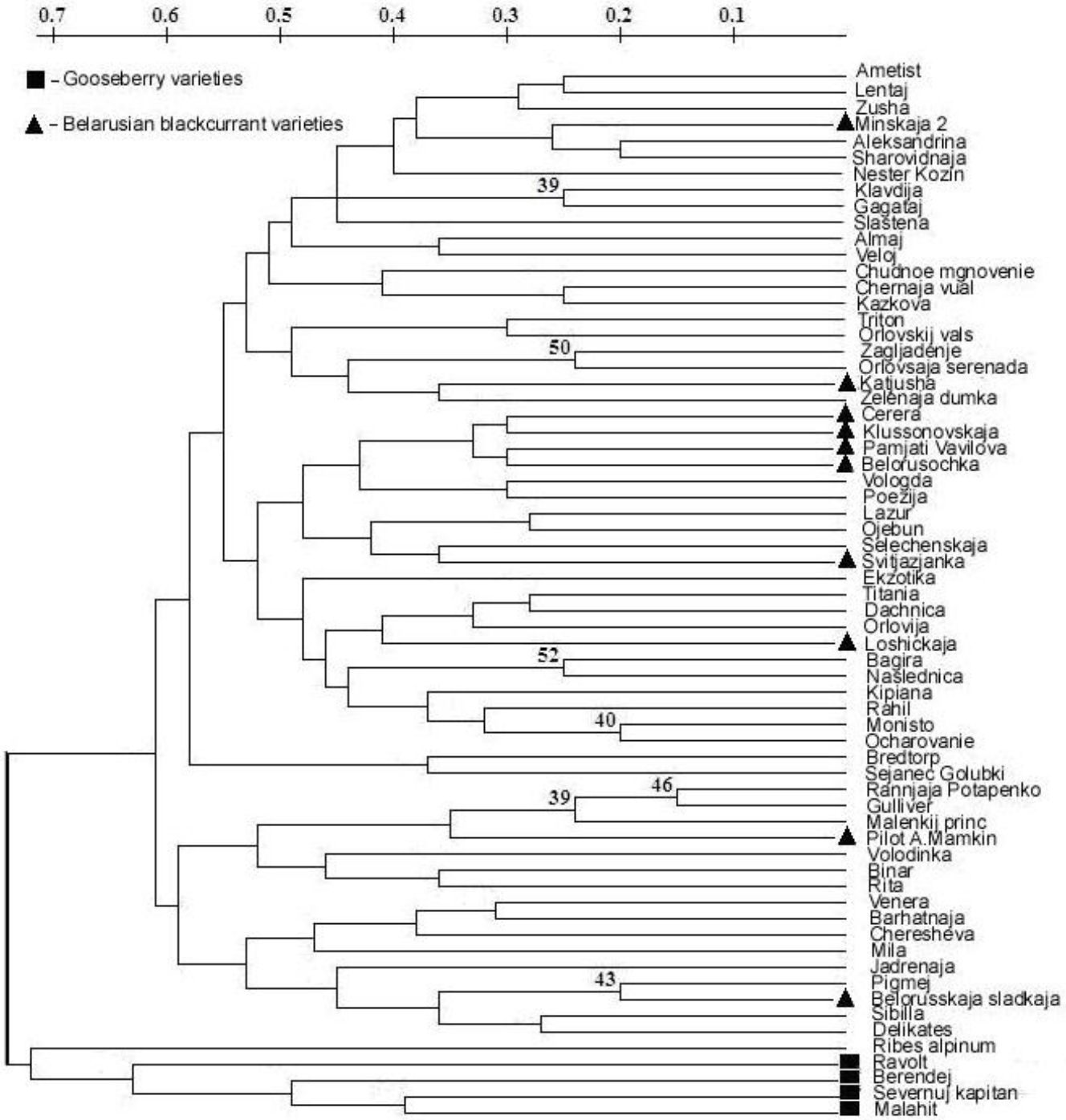

Figure 1. UPGMA dendrogram of 65 Ribes accessions based on alleles at 7 SSR loci.

The genetic similarity of the samples was estimated by the UPGMA cluster analysis.. The cluster analysis on the basis of estimated genetic distances among *Ribes* varieties was carried and the dendrogram was formed (**Fig. 1**). As shown from dendrogram, all studied varieties had a unique allele.

The genetic distances between the samples ranged from 0.15 to 0.75. The gooseberry varieties (Berndey, Malahit, Random, Severnuy Kapitan) were located at a great distance and formed a separate cluster, which also includes *Ribes alpinum*. The blackcurrant cultivars were quite genetically diverse and formed separate clusters, which included the cultivars of both Belarusian and foreign selections. One of the clusters comprised a group of Belarusian cultivars ('Cerea', 'Klussonovskaya', 'Pamyati Vavilova', "Belorusochka') that has 'Paulinka' cultivar in its pedigree. 'Cerea', 'Klussonovskaya' and "Belorusochka' cultivars came from the same crossing combination ('Paulinka'× "Pilot A. Mamkin'). 'Pamjati Vavilova' cultivar was developed by use of 'Paulinka'×"Belarusskaja sladkaja' crossing combination. Generally, modern Belarusian blackcurrant cultivars are genetically similar to foreign cultivars. This is a selection process trend, which aims to combine the best qualities of local and foreign cultivars in new cultivars.

The obtained results demonstrate that Ribes L. representatives grown in the Republic of Belarus, are characterized by high microsatellite loci diversity. This conclusion is consistent with the results obtained in the study of varieties of European and Russian breeding (9, 11, 12). A high variety of SSR loci alleles in the Ribes L. genome allows us to use a minimal set of 7 SSR markers for identification and certification the varieties included in the State register of varieties and wood-shrubby breeds of the Republic of Belarus, as well as other varieties of different breeding origin. When developing a method of DNA identification for other crops, characterized by lower genetic diversity, a much larger number of markers are used. For example, to identify barley varieties the set of 17 SSR markers was used (13), for corn – 51 (14), for almond – 16 (15). The set of 7 SSR markers allowed the identification Ribes L. genotypes. The level of informativeness of each marker, the frequency of alleles occurrence among the varieties, as well as the convenience of visualization and analysis of amplification products in this set of markers was took into account. The method of SSR analysis using a specified set of markers can be successfully applied for Ribes ssp. identification at the molecular level.

Conclusion

On the base of the analysis of polymorphism of SSR alleles we formed a set of 7 markers, allowing genetic identification of cultivars of black currant. Markers located on different chromosomes of the genome of Ribes L., which gives the opportunity to assess the polymorphism of different areas. Information values for the selected markers have high enough values in comparison with those described in the literature. The average number of alleles per locus, defined by means of the given collection, was 8.6. The average number of unique genotypes per marker among the 65 samples is 43.1. The value of discriminatory power for all markers is high and the average was 0.84. Thus, the proposed set of SSR markers can be used to identify cultivated in the Republic of Belarus Ribes L. representetives, copyright breeding institutions, the preservation of unique collection material, etc.

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