

Using microsatellite markers to study the pear (*Pyrus*) germplasm from the VNIISPK collection

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Abstract. Based on the analysis of SSR loci, the genetic similarity of 32 pear cultivars (*Pyrus*) from the VNIISPK collection was studied. A cluster analysis of the genetic similarity of genotypes was carried out using 19 microsatellite markers. Based on the data obtained, a dendrogram was constructed using the UPGMA method using the Jacquard coefficient and bootstrap (BP) analysis. The coefficients of pairwise genetic similarity between the varieties ranged from 0.4 (between Podgoryanka and the Bere Russkaya, Mramornaya group) to 0.85 (between Osennyaya Zheltaya and Pervomayskaya). This group had a high BP level– 100% and a close genetic distance – 0.85), and the reliability level (BP,%) was indicated on the dendrogram at a value of more than 50%. According to the clustering results, the pear samples did not form large groups with high BP, which indicated the sufficient genetic diversity of the studied cultivars. There are only three small groups with high BP. Two of them combined two cultivars with a common origin. Thus, Bere Russkaya and Mramornaya, which are descendants from the crossing of Bere Zimnya Michurina × Lesnaya Krasavitsa, joined into one cluster with BP 82. Another group with BP 92 united Viliya and Zaveya, which also have the same origin ([Belorusskaya Pozdnya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro). The third group combined the cultivars Osennyaya Zheltaya (origin is not known) and Pervomayskaya (Doch Zari × Pozdnya MOSVIR) with BP 100 and the cultivar Prosto Maria ([Belorusskaya Pozdnya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro) with BP 100 adjoins them at an extended genetic distance. There is no obvious similarity in origin in this cluster. The SSR-analysis method opens up broad prospects in studying the genetic similarity of cultivars.

1 Introduction

Pear (*Pyrus*) is one of the leading fruit crops of the *Rosaceae* family of the temperate zone. East Asia, the area of modern China and Japan, is considered the center of the primary origin of the pear, as well as many other deciduous fruit plants [1]. As of March 3, 2021, more than 150 pear cultivars were included in the State Register of Breeding Achievements

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of the Russian Federation approved for use [2]. Genetic *Pyrus* representatives are characterized by a base number of chromosomes 17 ($2n = 34$) [3].

Currently, there is a decrease in crop yields due to the reduction in the genetic diversity of modern cultivars, a decrease in immunity to diseases and pests, environmental pollution due to the use of pesticides, as well as deterioration in the quality and degradation of land resources [4].

SSR (simple sequence repeat) markers based on microsatellite polymorphism are widely used to study the genetic diversity of pears [3,5,6]. This type of markers, due to its multiallelicity, high reproducibility and polymorphism, is used for practical purposes, including checking affinity [7].

Studies of polymorphism of pear microsatellite loci were carried out by both domestic [8-10] and foreign scientists in Europe and Asia [6,11 – 14].

Suprun I. I. and co-authors [8] studied the degree of genetic similarity between autochthonous pear cultivars of the North Caucasus, genotypes of Caucasian pear from the MOSVIR collection, and cultivars of domestic and European breeding based on SSR loci. In the work of Plugatar and co-authors [10], 16 zoned and promising pear cultivars of domestic selection for the Crimea and the Southern Federal District were studied using 7 microsatellite DNA markers. Yakovin N.A. and co-authors [9] used 5 SSR markers to assess the degree of kinship between pear cultivars of Russian and Western European breeding. As a result of the data obtained, it was possible to show the genetic remoteness of *P. ussuriensis* and its descendants of the first generation from typical cultivars of *P. communis* and hybrids of *P. communis* × *P. ussuriensis* of later generations.

The Hungarian pear bank has carried out work on the systematic analysis of the genotypes of Hungarian cultivars and the development of a reliable protocol for molecular identification and interpretation of data. 88 cultivars were analyzed using eight SSR markers, which gave a total of 216 alleles. The analysis of genetic diversity was performed on the basis of the Neighbor-Joining algorithm in combination with PCA, close genetic affinity and overall high genetic diversity were found among the tested samples [12].

In Japan, the development of microsatellite markers for the Japanese pear (*Pyrus pyrifolia* Nakai) was carried out. The obtained 13 microsatellite loci showed a high degree of polymorphism in the Japanese pear [13]. At Nanjing Agricultural University (Nanjing Agricultural University, China), a wide range of pear cultivars was evaluated, according to the main SSR markers covering the entire genome. The use of markers for the study of genetic diversity, genetic relationships and the creation of the main collection of pears is demonstrated. [14].

At the Russian Research Institute of Fruit Crop Breeding (VNIISPK), breeding work with pears was started in 1949 by A.V. Parshin, and since 1956 has been conducted under the leadership of E.N. Sedov. Since 1991, the work on pear breeding has been carried out by E.A. Dolmatov with a group of employees. Currently, VNIISPK has a unique collection of pears [15].

In our study, 32 genotypes of pears (*Pyrus*) were evaluated based on the polymorphism of 19 microsatellite loci from the VNIISPK collection. The intervarietal polymorphism was studied and the genetic similarity between varietal samples was determined. The obtained results will help to select a set of informative DNA markers for effective analysis of collections and work with genetic resources.

2 Methods and material

The objects of the study were 32 pear cultivars (Table 1) from the VNIISPK genetic collection, of which the origin is not known for 3 cultivars.

A set of 19 microsatellite markers was used in the work, which includes 10 SSR markers: CH01f07a, EMPc11, CH05c06, GD147, CH01d08, CH03d12, EMPc117, CH01d09 and CH04e03, recommended by the European Cooperative Program for Plant Genetic Resources, ECPGR, [16], as well as 9 additional microsatellite markers: CH01f03b, CH01h01, CH01h10, CH02b10, CH02c11, CH04c07, CH05f06, CH-Vf1 and GD142 [17]. Primary data on the polymorphism of microsatellite loci were obtained within the framework of cooperation with the ECPGR project Pome Fruit C&E.

The work of Doyle and Doyle served as the method for DNA isolation [18]. DNA was isolated from young leaves by the STAV method. PCR analysis was carried out in a reaction mixture with a volume of 20 µl, containing 1 x PCR buffer solution, 200 µm of nucleotides, 2 µm of upstream primer, 2 µm of downstream primer, 0.3 Taq DNA polymerase units and 10 ng DNA. The amplification reaction was carried out according to the following algorithm: preliminary denaturation – 5 minutes at 95 °C; denaturation - 30 seconds at 95 °C; annealing primer - 30 seconds at a temperature selected for each marker; DNA synthesis - 30 seconds at 72°C (total 30 cycles); the last phase – elongation 10 minutes at 72°C. The separation of PCR products was carried out by capillary electrophoresis.

Based on the data, a dendrogram was constructed using the PAST program to determine genetic diversity. The dendrogram was compiled by UPGMA cluster analysis (unweighted pair group method with arithmetic mean) using Jaccard similarity coefficient and bootstrap analysis.

3 Results and discussion

With the amplification of 19 microsatellite loci in 25 varieties, there is a lack of amplification in at least one of the loci. The maximum number of non-amplified samples was found at the locus CH04c07 (22 varietal samples), CH03g07 (12 varietal samples), CH01h10 (11 varietal samples), CH01d09 (11 varietal samples), CH04e03 (10 varietal samples). Probably, the absence of the PCR product is connected with the presence of Null alleles, which is observed in microsatellite loci in apples [19], as well as in pears when analyzing microsatellite loci originally found in apples. Thus, in the work of Ferreira dos Santos and co-authors (2011) [20] on the study of the genetic diversity of pear cultivars in northwestern Spain, in five of the 19 tested microsatellite loci, a deviation of statistical parameters was revealed due to the presence of Null alleles, including in the locus CH04c07, which in our work did not amplify the largest number of samples. In the work of Yamamoto and co-authors (2001) [7], when studying the genetic polymorphism of pears using microsatellite loci analysis, Null alleles in apples were found in 4 out of 9 loci, including the CH01h10 locus.

From 8 (GD147, CH04c07) to 18 (CH01f07a) alleles were amplified on the analyzed samples, which averaged 11.7 alleles per locus. The total number of amplified alleles for 19 microsatellite markers on 32 varietal samples amounted to 223 alleles, namely: CH04c07 – 8 alleles; GD147 – 8 alleles; CH01d08 – 9 alleles; CH04e03 – 9 alleles; CH01h01 – 9 alleles; CH01h10 – 9 alleles; CH05c06 – 10 alleles; CH03d12 – 10 alleles; CH05f06 – 10 alleles; CH02c11 – 12 alleles; CH01f03b – 13 alleles; CH-Vf1 – 13 alleles; EMPc117 – 13 alleles; CH03g07 – 13 alleles; EMPc11 – 14 alleles; CH02b10 – 15 alleles; GD142 – 15 alleles; CH01d09 – 15 alleles; CH01f07a – 18 alleles.

Table 1. Plant material of pears from the VNIISPK collection.

Cultivar	Originator	Origin
Alaya	VNIISPK	(Seyanetz Yakovleva 104 × Bordovaya) × Krasavica Chernenko
Annushka	VNIISPK	2-13-23 (Russkaya Malgorzhatka × Bere Zimnyaya Michurina) × Chudesnaya Italia
Belarusskaya pozdnyaya	Belarusian Research Institute of Fruit Growing	Dobraya Luiza open pollination
Harrow sweet	Harrow RDC, Canada	William × Perdue80-51
Bere Russkaya	Rossosh Zonal Experimental Station of Horticulture	Bere Zimnyaya Michurina × Lesnaya Krasavitsa
Vesnyanka	Maykop Experimental Station VIR	Triumf Vienny × Dekanka Zimnyaya
Viliya	Institute of Fruit Growing, Belarus	[Belorusskaya Pozdnyaya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro.
Voskovaya	Cultivar of folk selection	Unknown
Voskovka	Cultivar of folk selection	Unknown
Nasha	VNIISPK, MSU, MAA	Seyanetz P. Pyrifolia open pollination
Vystavochnaya Krasnokutskaya	Krasnokutsk Station of Horticulture of UAS	Osnovyanskaya × (Bere Bosk + Sen Zhermen)
Eseninskaya	VNIISPK	Severyanka × Olivier de Serre
Zaveya	Institute of Fruit Growing, Belarus	[Belorusskaya Pozdnyaya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro.
Kokinskaya	Kokinsky stronghold of VSTISP, Bryansk	Kyre × Populyarnaya
Krasavica Chernenko	VNIIGiSPR	Doch Blankovoy × Bere Ardanpon
Kupala	Institute of Fruit Growing, Belarus Samohvalovich, Belarus	Belarusskaya Pozdnyaya × Bordovaya
Mramornaya	Rossosh zonal experimental station of horticulture	Bere Zimnyaya Michurina × Lesnaya Krasavitsa
Orlovskaya Krasavica	VNIISPK	Bergamot Novik × Lyubimitza Klappa
Orlovskaya Letnyaya	VNIISPK	Bergamot Novik × Lyubimitza Klappa
Osennaya Zheltaya	Folk selection	Unknown
Pamyati Yakovleva	VNIIGiSPR	Tyoma × Olivie de Serr
Pervomajskaya	VNIIGiSPR	Doch Zari × Pozdnyaya MOSVIR
Ploshchanskaya	VNIISPK	Severyanka × Tayushaya Krymskaya
Podarok Vysockogo	VSTISP	Tyoma × (Olivie de Serr + Bere Bosk)
Podgoryanka	Rossosh zonal experimental station of horticulture	Bere Zimnyaya Michurina × pollen mixture of European winter cultivars
Polveriya	Spontaneous hybrid	<i>Sorbus aria</i> × <i>Pyrus communis</i>
Prosto Mariya	Institute of Fruit Growing, Belarus	[Belorusskaya Pozdnyaya × (Bere Seraya × Dulya Ostzeyskaya) × Maslyanaya Ro]

Tihij don	Rossosh zonal experimental station of horticulture	Rossoshanskaya Krasivaya × Mramornaya
Tyutchevskaya	VNIISPK	11-11-163 [Severyanka × (Rossoshanskaya Rannya+ Merezhka)] × Zherve
Feeriya	VNIIGiSPR	Talgarskaya Krasavitsa × Doch Zari
Chernenko' apple and pear hybrid	VNIIGiSPR	<i>Malus domestica</i> × <i>Pyrus communis</i>
Yasachka	Institute of Fruit Growing, Belarus	Belorusskaya Pozdnyaya × Conference

The obtained data allowed us to compare the genetic similarity of pear cultivars from the VNIISPK collection. The constructed dendrogram clearly demonstrates the degree of difference and similarity of the studied samples in the polymorphism of microsatellite loci. Figure 1 shows the bootstrap support values (BP,%) for those nodes where the BP exceeds 50%. Bootstrap for other nodes is relatively low, which does not allow us to consider these branches as reliably justified.

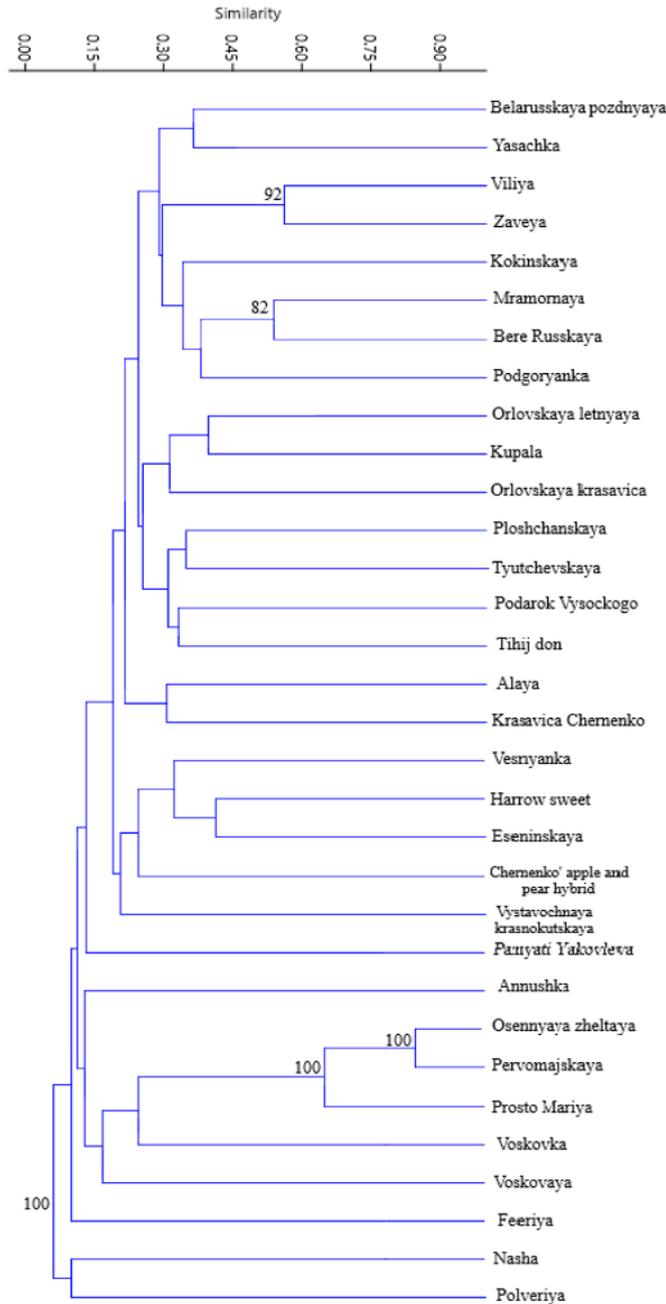


Fig. 1. A dendrogram constructed on the basis of 19 microsatellite markers characterizing the degree of genetic similarity of 32 specimens of the *Pyrus* genus.

According to the results of the UPGMA analysis, three clusters with genetic relationships between cultivars with high indications of bootstrap support (BP) were identified. One of these groups included genotypes Osennaya Zheltaya, which origin is unknown, and Pervomajskaya (Doch Zari × Pozdnya MOSVIR). This group has a high level of BP – 100% and a close genetic distance – 0.85. At a high level of BP (100%) and a further genetic distance of 0.61, Prosto Mariya ([Belarusskaya Pozdnya × (Bere Seraya ×

Dulya Ostzeyskaya] × Maslyanaya Ro) joined this group. At the borderline level of authenticity (49% and 47%), Voskovka and Voskovaya, which origin is unknown, also joined this cluster. Thus, in this cluster there is no obvious connection in pedigrees between cultivars.

At a somewhat remote genetic distance (0.56) than Osennyya Zheltaya and Pervomajskaya, Viliya ([Belorusskaya Pozdnyaya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro) and Zaveya ([Belorusskaya Pozdnyaya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro) merged – BP 92%. These cultivars have the same origin and a common originator Institute of Fruit Growing, Belarus.

Two cultivars of Bere Russkaya (Bere Zimnyaya Michurina × Lesnaya Krasavitsa) and Mramornaya (Bere Zimnyaya Michurina × Lesnaya Krasavitsa) united at a close genetic distance (0.54) into one cluster with high bootstrap support of 82%. These cultivars have the same origin and the same originator – Rossosh zonal experimental station of horticulture, Voronezh district. Thus, in two of the three clusters with high BP, the grouping of samples by polymorphism of microsatellite loci reflected the data of pedigrees.

It is interesting to note that having identical origin and a common originator (VNIISPK), Orlovskaya Krasavitsa (Bergamot Novik × Lyubimitza Klappa) and Orlovskaya Letnyaya (Bergamot Novik × Lyubimitza Klappa) did not show sufficient genetic similarity according to the results of the analysis. Pervomajskaya and Feeriya, having one common parent Doch Zari, also did not cluster together. Belorusskaya Pozdnyaya is the parent form of Usashka and Kupala, which also did not show closely related relationships by clustering the dendrogram. The reason for this may be a significant genetic heterogeneity of even closely related cultivars (which may manifest itself when crossing genetically remote parents), or insufficient resolution of the analysis. It is also impossible to exclude errors in pedigrees, which are quite often found when using the analysis of the distribution of alleles of microsatellite loci in generations. For example, when clarifying the origin of apple cultivars bred in Dresden-Pillnitz [21], according to microsatellite loci, in a number of cases, the unreliability of pedigrees both on the maternal and paternal lines was defined. Yu. Lysenko, 2021 [22] talking about breeding in dairy husbandry, argues that almost every third animal has errors in the pedigree and it is possible to find the animal's parents only through genotyping.

The work of Suprun and co-authors [8] is devoted to the study of domestic genetic collections of pears on the assessment of genetic polymorphism of modern cultivars and autochthonous forms of pears of the North Caucasus based on the analysis of microsatellite loci. According to the obtained data, a cluster analysis was carried out using the UPGMA method. The results showed that all the studied samples were divided into 2 main groups. All genotypes of the Caucasian pear and autochthonous cultivars of the North Caucasus were united in the first cluster. The second cluster was formed by the remaining analyzed pear cultivars of domestic and European breeding. The authors noted that the cultivars Bzhelyakokuzh and Skorospelka Cherkesskaya were connected into one branch of the cluster, but the genetic distance between them was significant. In the second cluster, several samples were noted, the union of which was confirmed by the origin. These are Yuzhanka, Sochinskaya Krupnoplodnaya and Verbena. Sochinskaya Krupnoplodnaya and Verbena are seedlings from open pollination of the French cultivar Bere Bosk. And Yuzhanka was obtained by pollinating Olivier de Serre with a mixture of pollen from Bere Bosk, Lesnaya Krasavitsa and Lyubimitza Klappa. Plugatar and co-authors [10] also report in general about the correspondence of the data on the origin of cultivars and the clustering observed on the basis of polymorphism of microsatellite loci. At the same time, the authors note that in order to increase the level of heterogeneity, the formation of parental pairs is promising, taking into account both the complex of phenotypic traits and the degree of their genetic similarity to ensure the greatest genetic diversity in the hybrid fund. Yakovin and co-

authors [8] also showed the relationship between SSR marker data and pedigree analysis in assessing the genetic similarity of cultivars.

4 Conclusion

In this work, using modern methods of DNA marking– analysis of microsatellite markers, the genetic similarity of 32 genotypes from the VNIISPK collection was analyzed. This study allowed us to identify several groups with a significant high degree of genetic similarity. The first cluster included the cultivars Osennyaya Zheltaya (origin unknown) and Pervomajskaya (Doch Zari × Pozdnaya MOSVIR). Prosto Mariya ([Belorusskaya Pozdnaya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro) joined this group. This group has a high level of confidence (100%) and close genetic similarity – more than 0.6. It can be assumed that Osennyaya Zheltaya may have common ancestors with Pervomajskaya and Prosto Mariya.

The second group, which united at a genetic distance of 0.54 with a confidence of 82% and has the same parental forms, is Bere Russkaya (Bere Zimnyaya Michurina × Lesnaya Krasavitsa) and Mramornaya (Bere Zimnyaya Michurina × Lesnaya Krasavitsa). Another group clustered Viliya and Zaveya with a BP of 92% at a genetic distance of 0.56. These cultivars have the same origin ([Belorusskaya Pozdnaya × (Bere Seraya × Dulya Ostzeyskaya)] × Maslyanaya Ro) and a common originator – Institute of Fruit Growing, Belarus.

According to the clustering results, the pear samples did not form large groups with high BP, which indicates sufficient genetic diversity of the studied cultivars.

In two of the three clusters with high BP, the grouping of samples by polymorphism of microsatellite loci reflected the data of pedigrees.

This study allowed us to evaluate the genetic relationships between the genotypes of pears from the VNIISPK collection.

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