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GENETIC SIMILARITY OF THE AUTOCHTHONOUS GRAPEVINE VARIETIES FROM DON REGION REVEALED BY SSR-ANALYSIS AND MAIN LEAF AMPELOGRAPHIC TRAITS

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Abstract

Native, ancient grape varieties of different cultivation regions are important part of grapevine genetic resources. Many native Don grape varieties represent a significant value for cultivation and use in breeding. The close varieties and more distant groups are distinguished on the main characteristics among the varieties of Don. The main features of the leaves of grape varieties are the key ampelographical characteristics. Currently, the study at the DNA level is considered the most informative method of plant genotyping analysis. Microsatellite markers are widely used for genotyping of grapevine varieties and rootstocks, and successfully applied in the study of the origin of varieties and the analysis of their pedigrees. We evaluated the relationship among the number of Don varieties by microsatellite genotyping. The aim was to study the genetic similarity of native Don varieties based on DNA analysis and compare the results with earlier made conclusions about relationship of varieties, and with data of the analysis of the main features of the leaves. The research was carried out on 16 varieties from the collection of the All-Russian Scientific Research Institute of Viticulture and Winemaking (Novocherkassk) and the Russian ampelographic collection (Anapa). Studied Don grapevine varieties were described ampelographically, and the main method we used in the work was the PCR. Six SSR-markers basicly recommended for *V. vinifera* fingerprinting were used. DNA was extracted from young leaves of the apical shoots of 4-5 typical bushes. Chardonnay and Cabernet Sauvignon were used as the reference cultivars. Genetic distance matrix was constructed using the coefficients (indices) similarity of M. Nei and W. Li. Based on the data of SSR-genotyping, estimation of the genetic similarity of studied varieties was performed using cluster analysis (UPGMA), and dendrograms were graphically constructed. Data on the morphological characteristics of leaves and SSR-genotyping results were analyzed by means of principal coordinates (PCA). DNA profiles of 16 local Don grapevine varieties were obtained using microsatellite loci VVMD5, VVMD7, VVMD27, VVS2, VrZAG62 and VrZAG79 with an automated genetic analyzer ABI Prism3130 («Applied Biosystems», USA). In the studied Don varieties genotypes, six (for VVS2, VVMD5, VMD7, VrZAG62) and seven (for VVMD27, VrZAG79) alleles per locus were determined. Cluster analysis allowed to divide the varieties into two main groups: one included Sibir'kovyi, Puhlyakovskii belyi, Sivolistnyi, Puhlyakovskii chernyi, Kosorotovskii and Kukanovskii cultivar, being a group of natural seedlings of Puhlyakovskii belyi, the other contained Bezymyannyi Donskoi, Plechistik oboepolyi, Staryi Goryun, Tsimlyanskii belyi, Tsimlyanskii chernyi, Tsimladar, Plechistik, Sypun chernyi, Mahrovatchik and Bessergenevskii № 7 cultivars. Interestingly, the second cluster had three subgroups. One includes varieties Bezymyannyi Donskoi, Plechistik oboepolyi, Tsimlyanskii belyi, Tsimlyanskii chernyi, Tsimladar, Plechistik, Sypun chernyi of the Tsimlyanskii group. The other contained Bessergenevskii № 7 cultivar being presumably a seedling of Puhlyakovskii belyi, and Stary Goryun of the Tsimlyanskii group. Variety Mahrovatchik (considered to be a seedling of Kokur white variety) was grouped separately. Analysis of the main features of leaves showed no differentiation according to the presumed origin of the studied varieties. As the result of SSR-analysis, most of varieties were distributed in accordance with the earlier made conclusions about their origin. Thus, the study of collections, old varieties, breeding material and introduced samples based on the complex of ampelographic traits

and SSR-markers can be considered as the most informative one.

Keywords: native gene pool, SSR-markers, ampelographic leaf traits, *Vitis vinifera* L., Don grape varieties, the genetic similarity.

Native, ancient grape varieties from different vine cultivation regions, as well as wild forms are the most important part of the world genetic resources of a cultivated plant *Vitis vinifera* L. It is autochthonous varieties, many of which do not even have a local distribution, that may be irretrievably lost. Their genotypes contain rare alleles, and are characterized by unique adaptive properties to specific areas of viticulture. For this reason, investigation of the indigenous gene pool receives special attention in all grape-producing countries [1-6].

The history of viticulture in the Don region extends back to several centuries. Local grape cultivars are diverse and specific. Many of them are of significant value both for cultivation in favourable conditions on the right bank of the Don and for breeding [7]. There is no consensus about the origin of the Don varieties of grapes, but typical local names and common traits indicate their age antiquity. Emergence of groups of varieties, close by the major morphological features, in different wine-growing districts also indicates the regional origin of most of the local Don indigenous varieties.

Based on the similarity of signs (leaves and berry form), it has been established that Kosorotovskii, Sibir'kovyi, Pukhlyakovskii Chernyi, Olkhovskii, Sivolistnyi, and Bessergenevsky № 7 cultivars are natural seedlings of the Puhlyakovsky Belyi cultivar [7]. According to A.I. Potapenko, the Pukhlyakovsky Belyi cultivar appeared on the Don at the beginning of the XIX century [8]. A.M. Aliev, in contrast, argues that a large group of grape varieties might not emerge and spread over such a short period of time [9]. According to him, the Pukhlyakovsky Belyi cultivar had been known much earlier on the Don, and belongs to the indigenous Don varieties. A group of varieties similar to the Pukhlyakovsky Belyi cultivar is not the only one. There is a much more numerous group of so-called Tsimlyanskii cultivars with similar morphological features.

When determining the origin of grape varieties of local inhabitant selection, methods for their identification are no less important. Studies have shown the effectiveness of using harmonized descriptions of grape varieties developed by the International Organization of Vine and Wine (Office International de la vigne et du vin, OIV, Paris, France) [10]. This system facilitates the evaluation of trait similarity, and thereby assisting in confirming or denying the alleged origin of the variety. The most valuable are the main features of the leaves, the formation of which is almost unresponsive to the artificial selection [7]. Depending on the shoot length and growth conditions, the size and shape of leaves vary within the same variety, but these features remain reliable ampelographic parameters.

Studies at the DNA level are considered the most informative method of plant genotyping analysis. DNA profiles complement the conventional description and agrobiological characteristics of varieties, allowing an accurate determination of the varieties, investigation of their origin, identification of synonyms and impurities in the collections. The works on the investigation of genetic diversity and identification of varieties most commonly use marker systems based on the variability of microsatellite DNA regions.

Microsatellites (simple sequence repeats, SSR) are tandem repeats of simple sequences in the DNA structure. The source of their polymorphism is a site-specific variation of the repeat length due to the difference in the number of its units [11]. Microsatellite sequences are ubiquitous in the genome of higher plants. SSR-markers are characterized by the co-dominant pattern of inheritance, high differentiation capacity and reproducibility of results. Microsatellite

markers are widely used for genotyping of grapevine varieties and rootstocks [12-16], and successfully applied in the study of the origin of varieties and the analysis of their pedigrees [17-22]. Based on the fingerprinting of grapevine varieties carried out in different laboratories, it has been established the basic standard set of SSR-markers for genotyping *Vitis vinifera* [23].

In this paper, for the first time, we evaluated the relationship of autochthonous grapevine varieties in the Don region, mainly from groups of Tsimlyanskii varieties and seedlings of the Pukhlyakovskii Belyi cultivar, based on DNA analysis.

The aim was to study the genetic similarity of the Don varieties based on the analysis of microsatellite locus polymorphism, and compare the obtained results with findings from other authors about the origin of varieties, as well as with the analysis of the main traits of a fully developed leaf.

Technique. The investigations were carried out on a set of the autochthonous Don grapevine varieties, such as Bezymyannyi Donskoi, Bessergenevskii № 7, Kosorotovskii, Kukanovskii, Makhrovatchik, Plechistik, Plechistik oboepolyi, Pukhlyakovskii belyi, Pukhlyakovskii chernyi, Sibir'kovyi, Sivolistnyi, Staryi Goryun, Sypun chernyi, Tsimladar, Tsimlyanskii belyi, and Tsimlyanskii chernyi, growing in the collection of the Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking (Novocherkassk) and the Russian Ampelographic Collection (Anapa). Chardonnay and Cabernet Sauvignon were used as the reference cultivars as their allelic composition for the studied SSR-loci was known [23]. All grapevine varieties were described based on the main ampelographic traits of a fully developed leaf, according to the methodology of testing for distinctness, uniformity and stability, proposed for grapes [9].

The main method we used was the polymerase chain reaction (PCR). Separation of PCR products was performed by electrophoresis methods, the 2 % agarose gel electrophoresis when customizing PCR parameters, and capillary electrophoresis, using an automated genetic analyzer ABI Prism 3130 (Applied Biosystems, USA) when performing the SSR-fingerprinting.

The study used SSR-markers, such as VVMD5, VVMD7, VVMD27, VVS2, VrZAG62 and VrZAG79, recommended for molecular genetic genotype certification of *Vitis vinifera* by the European Database and the GrapeGen06 project [23]. DNA was extracted from young leaves of the apical shoots of 4-5 typical bushes of the cultivar by a CTAB (cetyl trimethylammonium bromide) method [24]. The PCR mixture proportion was designed according to a basic protocol, the reaction was carried out using standard parameters, with experimentally found temperatures for annealing primer pairs [25]. Sizes of amplified fragments were determined on an automated genetic analyzer ABI Prism 3130. Results were processed in the Gene Mapper 4.1 software.

The matrix of genetic distances was constructed using similarity coefficients (indices) by M. Nei and W. Li [26]. Cluster analysis was performed by means of the unweighted pair group method with arithmetic mean (UPGMA) using the FreeTreeApplication 0.9.1.50 (ZDATv.o.s.) software. Graphical representation of dendrograms was carried out using the TreeView (Win32) 1.6.6 software.

Data on the morphological characteristics of leaves and SSR-genotyping results were analyzed by PCA method. Calculations were performed in the PAST v. 2.17c software.

Results. The studied sample set was represented mainly with grapevine cultivars belonging to two groups, which have been previously identified ampelographically, i.e. the group of Tsimlyanskii cultivars and the group of natural seedlings of the Pukhlyakovskii Belyi cultivar.

To reduce the cost of analyzes, sets for multiplex analysis were formed

after testing SSR-markers and selecting optimal PCR parameters. SSR-markers were pooled considering the size range of the amplified fragments for each locus and temperatures of annealing primer pairs, using two of the four fluorescent dyes (FAM, R6g, Rox, or Tamra) in one set. Genotyping was performed by the following marker pairs: VVS2 + VVMD7; VVMD27 + VVMD5; VrZAG62 + VrZAG79.

The results of the analysis of microsatellite locus polymorphism demonstrated that each variety had a DNA profile that was different from all other specimens. Considering the studied SSR loci in the set of the Don grapevine varieties, there were six (for VVS2, VVMD5, VMD7, VrZAG62 loci) and seven (for VVMD27, VrZAG79 loci) alleles per locus determined.

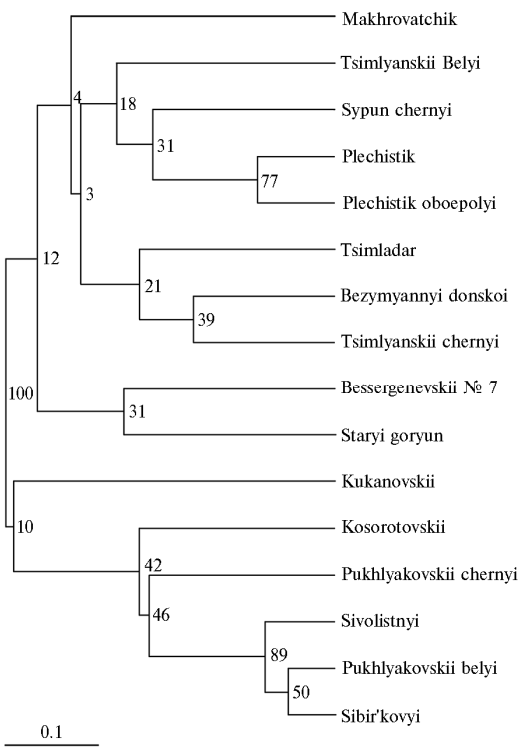

Fig. 1. Dendrogram of genetic similarity between the studied Don varieties of grapes (*Vitis vinifera* L.) based on SSR-analysis (the collection of the Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking, the Russian Ampelographic Collection).

Cluster analysis allowed dividing the cultivars into two main groups based on the fingerprinting by microsatellite loci (Fig. 1). One large group included Sibir'kovyi, Pukhlyakovskii belyi, Sivolistnyi, Pukhlyakovskii chernyi, Kosorotovskii and Kukanovskii cultivars. All of them revealed the genetic similarity, which confirmed the hypothesis of their origin from the Pukhlyakovskii belyi cultivar, with the Sibir'kovyi, Pukhlyakovskii belyi and Sivolistnyi cultivars appeared to be the most similar, based on the results of microsatellite analysis.

The second group contained the cultivars Bezymyannyi Donskoi, Plechistik oboepolyi, Staryi Goryun, Tsimlyanskii belyi, Tsimlyanskii chernyi, Tsimladar, Plechistik, Sypun chernyi, Mahrovatchik, and Bessergenevskii № 7. Interestingly, the second cluster had three subgroups. Genetic similarity can be stated for the cultivars of the so-called subgroup of Tsimlyanskii varieties, such as Bezymyannyi Donskoi, Plechistik oboepolyi, Tsimlyanskii belyi, Tsimlyanskii chernyi, Tsimladar, Plechistik and Sypun chernyi. The Plechistik and Plechistik oboepolyi cultivars appeared to be genetically the most similar. The Makhrovatchik cultivar showed similarity with the group of Tsimlyanskii varieties. The Bessergenevskii № 7 and Staryi Goryun cultivars appeared to be closer to each other than to other allegedly related cultivars, however, both of them were closer to the Tsimlyanskii group. Considering that based on ampelographic characteristics the Bessergenevskii № 7 cultivar being presumably a seedling of the Pukhlyakovskii Belyi cultivar, it may be assumed that his other parent was a variety from the Tsimlyanskii group or a variety similar to them.

More accurate conclusions can be made with the increased number of SSR-loci to be analyzed. However, even the set of microsatellite markers used in this study yielded results comparable with the findings of other researchers.

The major ampelographic leaf traits in the autochthonous Don grapevine varieties (*Vitis vinifera* L.) (the collection of the Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking, the Russian Ampelographic Collection)

Cultivar	Trait code											
	080	077-1	077-2	078-1	078-2	068	067	065	082	079	084	093
Natural seedlings of the Pukhlyakovskii Belyi cultivar												
Pukhlyakovskii belyi	3	5	3	5	3	3	4	5	2, 3	5	5	3, 2
Kosorotovskii	5	3	3	5	5	3	3	5, 7	2	5, 8	7	4
Sivolistnyi	3	5	5	5	5	3	3, 4	5, 7	2, 3	5, 6	5	2
Pukhlyakovskii chernyi	3	3	3	5	3	3	4	5, 7	2.3	5, 8	3	2
Bessergenevskii № 7	3	3	3	5	5	2, 3	4	7	1	6	5	3
Kukanovskii	5	5	3	5	3	3	4	5	2, 3	3, 5	3, 5	2
Sibir'kovyi	7	5	5	5	5	3	4	5, 7	2	5	5	2, 3
The group of Tsimlyanskii varieties												
Bezymyannyi Donskoi	3	5	3	5	5	3	4	5	2, 3	5, 8	7	2
Plechistik oboepolyi	3	3	3	7	7	3	4	5, 7	2	5, 6	7	2
Staryi Goryun	3	7	3	7	5	3	3	7	2	5	7	2
Tsimlyanskii belyi	3	5	3	7	5	3	4	5, 7	2	5, 8	3, 5	3, 4
Tsimlyanskii chernyi	3	3	3	5	5	3	4	7	2	5	7	4
Tsimladar	7	7	5	5	5	3	4	5	3, 4	8	5, 7	2
Plechistik	7, 9	5	5	5	5	3	4	7	2	5	7	2, 3
Sypun chernyi	7, 9	5	3	5	5	3	4	5, 7	2	5	5	3
Seedling of the Kokur White cultivar												
Makhrovatchik	9	7	5	7	7	3	4	5, 7	2, 4	5, 6	7	2

Note. 080 — depth of the upper lateral sinuses, 077-1 — length of an apical serration, 077-2 — length of a lateral serration, 078-1 — the apical serration length to width ratio, 078-2 — the lateral serration length to width ratio, 068 — the number of laminae, 067 — the form of the blade, 065 — the size of the blade, 082 — arrangement of the laminae of upper lateral sinuses, 079 — arrangement of the laminae of the petiolar sinus, 084 — downiness between the primary veins on the underside of the blade, 093 — the petiole length relative to the mid rib length. The figures in the table indicate the expression of a trait [10].

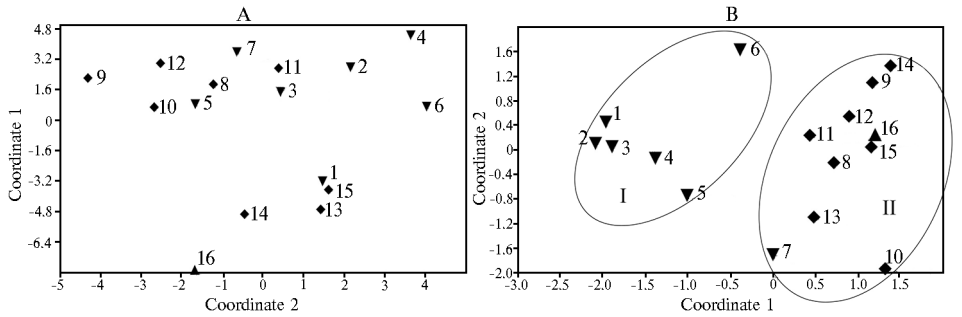

Fig. 2. PCA distribution of the studied Don varieties of grapevine (*Vitis vinifera* L.) based on the evaluation of the leaf blade traits (A) **and SSR-analysis** (B)**:** 1 — Sibir'kovyi, 2 — Pukhlyakovsky belyi, 3 — Sivolistnyi, 4 — Pukhlyakovsky chernyi, 5 — Kosorotovskii, 6 — Kukanovskii, **7** — Bessergenevsky № 7 (natural seedlings of the Pukhlyakovsky belyi cultivar); 8 — Bezymyannyi Donskoi, 9 — Plechistik oboepolyi, 10 — Staryi Goryun, 11 — Tsimlyanskii belyi, 12 — Tsimlyanskii chernyi, 13 — Tsimladar, 14 — Plechistik, 15 — Sypun chernyi (the group of Tsimlyanskii varieties); 16 — Makhrovatchik (seedling of the Kokur belyi cultivar); I and II — clusters obtained by UPGMA (the collection of the Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking, the Russian Ampelographic Collection).

The major ampelographic traits of a fully developed leaf for each cultivar were described by the index system in accordance with the methodology adopted by the International Organization of Vine and Wine (Table). The analysis of the obtained data by main features of leaves was performed by means of the PCA method. We have found no common factors consistent with the proposed origin of varieties or the results of SSR analysis, i.e. area of distribution of varieties

from a group of Pukhlyakovskii belyi cultivar seedlings significantly overlaps the area of distribution of the Tsimlyanskii group (Fig. 2, A).

Meanwhile, the results of SSR analysis demonstrated that most of varieties were distributed in the space of the principal coordinates in accordance with the earlier conclusions of their origin (see Fig. 2, B). The isolated areas I and II corresponded to clusters obtained by the UPGMA clustering.

Thus, the main features of a fully developed leaf are insufficient to evaluate the relationship of certain genotypes of grapevine. The use of microsatellite markers for these purposes is more efficient. Quite promptly, DNA markers allow drawing conclusions about the genetic similarity of the samples to confirm or rule out the information on the origin. Thus, the study of collections, old varieties, breeding material and introduced samples based on the complex of ampelographic traits and SSR-markers can be considered as the most informative one.

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