The Kuban grapes wild forms growing on the Red Forest nature reserve territory

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Abstract. Modern scientific literature analysis on the Vitaceae Juss. Family biodiversity study showed that this issue is relevant today and is being studied by many scientists in our country more intensively than before, although not so deeply. For example, there is some information on indigenous varieties and grapes wild plants from Crimea, Dagestan, Don and other regions. Unfortunately, there is almost no scientific information on the Kuban wild forms and autochthons. This article contains a part or, one might say, a new large research work beginning on the Kuban grapes wild plants study, studied on the Krasnodar Territory, namely in the Red Forest state nature reserve. At the same time, their ecological and geographic growing conditions were studied with a phytocenosises detailed description. Found isolated populations of grapes, hypothetically could be Vitis vinifera ssp. Silvestris Gmel. or its variants var. Tipica Negr. (wild forest grapes). Wild-growing grapes samples genotypic was carried out for 7 microsatellite loci. The samples genetic similarity analysis according to the microsatellite analysis data revealed the close and most distant genotypes among the wild forms studied sample.

1. Introduction
Wild grapes – *Vitis vinifera ssp. silvestris* Gmel. and its variations are forest climbing plants growing in separate populations or even single plants [1].

According to the world research literature data, the cultivated grapes wild-growing subspecies (*Vitis vinifera ssp. Silvestris* Gmel.) is the existing cultivars' ancestor [2-4].

Grapes are considered the cultivated plants only species that have a Eurasian aboriginal origin, which appeared about 65 million years ago [5]. If you look into history, then much later it was divided into subspecies and this was due to the grapes morphological characteristics [6].

Grapes aborigines and wild plants from viticulture different geographical zones are this culture gene pool and selection most valuable material. Their study, along with molecular genetic analyzes, makes it possible to more deeply reveal the grapes' origin question, more or less close or distant genotypes.
In the literature, you can find scientific information regarding the Crimean, Dagestan, Don and other grapes aboriginal forms [7-11]. However, there is practically no information on the wild or autochthonous Kuban forms. On the state nature reserve Krasny Les territory such studies are being conducted for the first time.

Thus, this article reflects the new scientific research results, namely, the ecological, wild grapes biological and morphogenetic features in the Red Forest natural reserve.

2. Objects and methods of research
The ecological and biological study, as well as grapes wild forms morphological and genetic characteristics, was carried out during expeditions conducted in the 2020 spring and autumn on the Red Forest state nature reserve territory.

Research methods - route-reconnaissance, traditional geobotanical [12]. Studied: the habitats' relief, the phytocenoses, vegetation structure and floristic composition. At the same time, the vegetative morphological grape wild plants' parameters and generative spheres were studied [13]. The wild-growing grapes studied plants more variable morphological characteristics were identified.

The studied forms of grapes DNA samples were isolated by the CTLB method [14]. Molecular genetic studies were carried out using highly polymorphic SSR markers, which are recommended for DNA certification of genotypes *Vitis vinifera* L. by the union of grape geneticists and the less polymorphic marker GF 09-46 [15]. Reference varieties [16] were used as a control in the samples genotypic. The study was carried out on an automatic genetic analyzer ABI prism 3130 using special software Gene Mapper and Peak Scanner. This allows obtaining data that meets the modern world requirements for the grape genotypes identification. Microsatellite loci polymorphism data Statistical processing was carried out using the GenAlEx 6.5 program [17]. Cluster analysis based on the microsatellite genotypic results was performed by the pairwise intragroup unweighted mean (UPGMA) method using FreeTreeApplication 0.9.1.50 (ZDAT vos) and subsequent construction of a dendrogram in the TreeView (Win32) 1.6.6 program [18].

The work aim is to study the grape wild plants genetic diversity using geobotanical, ampelographic and genetic methods.

3. Results and discussion
The Red Forest state natural reserve has an area of more than five thousand hectares. This is a Krasnodar Territory flat part large forest area. It is, in fact, a forest remnant, which previously framed in a Kuban River wide strip the right bank to its delta. Today it is a small island washed by this river waters for six kilometres. Forest phytocenosis are of natural origin and belong to the forest types fresh and moist group.

The main forest-forming species here is the Petiolate oak. Also, woodlands consist of high ash, common hornbeam, field maple, Tatar maple, Caucasian pear, Eastern Apple, and elms. The Petiolate oak is 60-100 years old. The average diameter of the trunks is 24-28 cm, the maximum diameter is 44 cm. Average height - 24.0-28.5 m. Fullness - 0.7 units. Bonitet - 2.

The reserve forest area some part was replaced by high ash. The ash formations age is also 60-80 years old, but there are also older ones. Average height - 23.0-29.0 meters, trunk diameter - 18-28 cm, bonitet - 1. As part of the phytocenosis, white poplar and white willow are isolated.

In the undergrowth are widely distributed-male dogwood, southern svidina, single-seed hawthorn, common hazel, black elderberry, rosehip, blue BlackBerry, cherry plum, etc. Among the vines, forest grapes and ordinary hops can be noted.

The grassy tier total projection coverage is 30%, in the canopy windows - up to 70%. The herbaceous layer consists of forest violets, the purple sparrow, euphorbia amygdaloid, of the city avens, cleavers, etc.

As an expeditions' result, grapes wild-growing forms five populations were found, which differ from each other in vegetative and generative spheres morphological characteristics. More than forty morpho-characters have been studied, 10 of which are the most variable. For example, the shape of the top (crown) of a young shoot varies in this case from slightly open to completely open. The leaf underside
cobweb pubescence ranges from sparse (2 points) to dense (4 points), and the formed leaf bristly pubescence is from very rare to medium. The leaf shape is also variable to a large extent both at the interpopulation and endogenous levels - deltoid, round, pentagonal (figure 1).

![Leaf shapes](image1.png)

**Figure 1.** The leaf blade shape in the Red Forest investigated wild plants: a) pentagonal, b) round, c) deltoid.

The upper side cuts depth varies significantly from medium to very deep, which is also expressed at the endogenous level. There were formed leaves on one grape plant with an almost whole leafy rounded blade and a dissected pentagonal or deltoid.

The studied wild-growing forms productivity study was assessed visually, while bunches with berries were observed in significant quantities, which indicates their good productivity (figure 2).

![Bunches with berries](image2.png)

**Figure 2.** Bunches with berries on the studied wild forms of grapes (July third decade).

The bunches of the studied wild-growing forms of grapes are small, loose, sometimes pea. The berries are small, at biological maturity they are black, maroon or black with a reddish tint. The berry has a sweet-tart taste, but pleasant freshness. Ripening - September second and third decades.
The damage presence to grapes wild populations by pests and diseases was not visually detected. In the future, these plants can be tested to identify new sources or donors of resistance to various biotic and abiotic environmental factors, including pathogenic microflora.

In 2020, samples genotypic from the grape wild plants studied populations was carried out at 7 microsatellite loci (table 1).

Table 1. The grapes wild plants samples DNA profiles from the Red Forest reserve.

| Sample | Microsatellite loci identified alleles, bp |
|--------|------------------------------------------|
|        | VVS2 | VVMD5 | VVMD7 | VVMD27 | VVMD28 | VrZag79 | GF09-46 |
| 1      | 157  | 230   | 239   | 190    | 236    | 251     | 407     |
| 2      | 157  | 234   | 239   | 190    | 264    | 251     | 425     |
| 3      | 141  | 230   | 239   | 180    | 236    | 249     | 395     |
| 4      | 151  | 232   | 239   | 190    | 254    | 249     | 425     |
| 5      | 141  | 230   | 239   | 174    | 236    | 251     | 395     |

As a result, it was found that forms 3 and 4 are identical at all seven analyzed loci. Hypothetically, we can talk about the vegetative offspring of the same genotype or genetically close samples, identical in the studied loci.

In general, the studied sample is quite diverse. The seven analyzed loci actual heterozygosity (Ho) values exceeded the expected heterozygosity (He) values (table 2).

Table 2. Characteristics of microsatellite loci in the studied sample.

| Locus   | Na    | Ho    | Ne    | He    |
|---------|-------|-------|-------|-------|
| VVS2    | 5.000 | 0.800 | 4.167 | 0.760 |
| VVMD5   | 5.000 | 0.800 | 3.846 | 0.740 |
| VVMD7   | 2.000 | 0.000 | 1.471 | 0.320 |
| VVMD27  | 5.000 | 0.800 | 3.125 | 0.680 |
| VVMD28  | 5.000 | 1.000 | 3.571 | 0.720 |
| VrZag79 | 4.000 | 0.200 | 2.381 | 0.580 |
| GF09-46 | 3.000 | 0.800 | 2.941 | 0.660 |

Note: Na = No. of Different Alleles, Ho = Observed Heterozygosity, Ne = No. of Effective Alleles, He = Expected Heterozygosity.

The identified alleles number per loci varies from 2 (VVMD7) to 5 (VVS2, VVMD5, VVMD27, VVMD28). The alleles three types were identified at the GF09-46 loci and four at VrZag79.

The samples genetic similarity analysis according to the microsatellite analysis data showed that sample 5 significantly differs from all other found samples (figure 3). On the dendrogram, the sample separated into a separate branch with 100% confidence.
Figure 3. The wild-growing grapes (Red Forest, 2020) samples genetic similarity dendrogram.

It can also be noted that samples 2, 3, and 4 have a greater genetic similarity to each other compared to sample 1.

Having studied the sample 5 DNA profile allelic composition in the VIVC Database, we assume that sample 5 may carry the American grape species genplasm [19]. For example, the allele is 266 bp. at the VVMD5 locus, according to the International Database data, it is found only in interspecific hybrids. Also the allele 165 bp. the VVS2 locus was not detected in any genotype V. vinifera and was previously detected only in interspecific forms with the American species participation. It is possible that sample 5 was introduced by accident by humans or birds.

4. Conclusion

As a result of expeditionary research in the Red Forest state nature reserve, 5 wild grapes isolated populations were found. These forms were studied and described for the first time according to the growth ecological-geographical conditions and the grape plants vegetative and generative spheres morphological characteristics. When studying the wild-growing grapes plant organs morphology, some indicators’ significant variability was found, such as a young shoot crown openness and its cobweb pubescence degree, the bristly degree and the formed leaf underside cobweb pubescence, the leaf blade shape and corrugation, etc.

The studied wild forms yield study was evaluated visually, while clusters with berries were observed in a significant amount, which indicates their good productivity. In this case, the grapes studied wild-growing forms bunches are small, loose; the berries are also small, mostly black with a sweet-tart taste. The damage presence by pests and diseases is not visually detected.

The plant genotypes DNA marker analysis for microsatellite loci VVMD7, VVS2, VVMD5, VVMD27, VVMD28, GF09-46, VrZag79 was carried out. As a result, two samples were identified with identical DNA profiles at the analyzed loci, which indicates their genetic similarity high degree. And one of the specimens differs significantly from other forms and, possibly, is a seedling from hybrid forms with American grape varieties.
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