Variety of wild-growing grapes of the Utrish reserve

I V Gorbunov¹, E T Ilnitskaya², A A Lukyanov¹, S S Mikhailovsky¹,
M V Makarkin², M I Pankin² and O N Bykhalova³

¹Anapa Zonal Experimental Station of Viticulture and Wine-making - Branch of
"Federal State Budgetary Scientific Institution" North-Caucasus Federal Scientific
Center of Horticulture, Viticulture, Wine-making ", 36, Pionerskiy avenue, Anapa,
353456, Russia
²Federal State Budget Scientific Institution North Caucasus Federal Scientific Center
for Horticulture, Viticulture, Winemaking (FSBSI NCFSCHVW), 39, 40 years of
Victory street, Krasnodar, 350901, Russia
³Federal State Budgetary Institution “Utrish State Nature Reserve”, 41v, Severnaya
street, Anapa, 353445, Russia

E-mail: wunsch27@mail.ru

Abstract. Recently, the importance of studying the biodiversity of the Vitaceae Juss family,
increases markedly, while new data appear on the gene pool of cultivated and wild-growing
grapes Vitis vinifera L. Modern methods of analyzing the polymorphism of populations of grape
plants, both wild-growing and cultural, are actively used. According to the literature, a number
of studies have been conducted in the Russian Federation to study native varieties and wild forms
of grapes in the Crimea, Dagestan, along the banks of the Don and other regions, but there is
very little information about Kuban wild and ancient forms of grapes. The article presents the
results of a study of wild forms of the genus Vitis L., growing in places of ancient human
settlements on the territory of the Utrish State Nature Reserve of the Krasnodar Territory. For
the first time, ampelographically and genetically samples of isolated populations
of wild grapes growing on the territory of the Utrish State Nature Reserve have been studied and described.
Several habitats of wild grapes, presumably belonging to the Vitis vinifera ssp species, have been
studied. Silvestris Gmel. or its variants var. Tipica Negr. - typically wild forest grapes. A detailed
description of phytocenoses in which the studied plant forms grow is carried out. Ecological and
geographical characteristics of their habitats are given. Samples were taken for further genetic
analysis. DNA-marker analysis of plant genotypes by microsatellite loci VVMD27, VVMD5,
VS2, VrZAG79 recommended for genotyping of grapes was performed. It was shown that some
of the samples are polymorphic to each other, while others are genetically close. When analyzing
genotypes, rare alleles were detected for the analyzed SSR loci.

1. Introduction

Wild form of grape Vitis vinifera ssp. silvestris Gmel. and its existing varieties, these are forest climbing
plants that grow by individual populations or introgressive groups of hybrids within the boundaries of
the cultivation of cultivated grapes [1].

According to world studies, a wild subspecies of cultivated grapes Vitis vinifera ssp. silvestris gmel.
is considered the ancestor of the existing varieties [2-4].
Grapes are the only species among cultivated plants of indigenous origin in Eurasia. It appeared more than 65 million years ago [4]. Historically, they began to divide it into subspecies much later, due to differences in the morphology of this plant [5]. Grapes is a unique plant, as it is the main agricultural crop, and also it has ancient historical roots with the development of human culture and civilization.

Conducted research work on the study of native grape varieties in the Crimea, Dagestan, along the banks of the Don river [6-11]. But there are no literature data on the origin of wild and native forms of grapes in the Kuban. As for the territory of the Utrish Reserve, as a unique place of the ancient Circassian settlements of the 13th-15th centuries [12], such studies are carried out for the first time.

This article shows new scientific information on the ecological conditions of growth and morphobiological characteristics of wild-growing forms of grapes found in the territory of the Utrish State Nature Reserve. In the future, it is planned to expand the geography of research on the Kuban wild plants of grapes and to identify among them sources and donors of resistance to various biotic and abiotic environmental factors. And as a result - the involvement of selected stable wild-growing forms in the further breeding process [13].

2. Objects and methods of research

Expeditionary research on the search for wild-growing forms of grapes, the study of their ecological growing conditions and the morphological and biological characteristics of vegetative and generative organs was carried out in April - November 2019 on the territory of the Utrish State Nature Reserve.

Studies of grape wild plants were carried out by route-reconnaissance method. Using the traditional methods of geobotanical research, the following were studied [14]: the topography of the habitats, the structure and floristic composition of phytocenotic communities in which wild-growing forms of grapes grow, and a brief description of the vegetation is given.

Wild grape plants were photographed in general terms, as well as captured - the apical part of the young shoot, the inner and outer sides of young and mature leaves, the bark of the central shoot (vine), etc. The morphological features of the vegetative and generative organs of wild-growing forms of grapes were studied using the ampelographic method for describing cultivated grape varieties [15]. The most variable morphological characters of the studied plants are shown. Samples were taken (the top of a young shoot) for molecular genetic analysis of wild-growing forms of grapes.

DNA samples of the studied grape forms were isolated by the CTAB method [16]. Molecular genetic studies were performed using SSR markers recommended for DNA certification of Vitis vinifera L. genotypes by the Union of Grape Genetics [17]. In this work, reference varieties were used as control when fingerprinting the samples, the allelic composition of which is known from the studied SSR loci [18]. The study was conducted on an automatic genetic analyzer ABI prism 3130 using special software Gene Mapper and Peak Scanner, which makes it possible to obtain data that meets modern international requirements for the identification of grape genotypes. Statistical processing of polymorphism data of microsatellite loci was carried out using the GenAlEx 6.5 program [19].

The purpose of the research work is the study of genetic diversity, the search and selection of new resistance donors among wild vine grapes using geobotanical, ampelographic and genetic methods.

3. Results and discussion

As a result of expeditionary studies in 2019, 10 populations of wild grapes were found on the right and left sides of the Waterfall Slit of the Navagirsky Range (table 1).

The climate type of the study area belonging to the Utrish State Nature Reserve can be defined as Mediterranean with a climate influence of moderate latitudes [20]. The average annual air temperature is 12.1-12.2 °C, the average January temperature is 2-3 °C, and July is 23.3-23.5 °C. The average annual rainfall is in the range of 570-700 mm. The height above sea level in the study area on the track section. The waterfall gap (inter-spine lowering) is 50 m.

The locations of the studied grape plants along the waterfall gap can be divided into two sections. The study area on the left side is represented by low-mountain terrain with brown leached soils,
which are occupied by mesophilic broad-leaved forests consisting of hornbeam, oak, maple and their combinations.

Table 1. Ecological and geographical characteristics of habitats of wild-growing grapes (Waterfall Slit, Utrish Nature Reserve).

| Location                       | Relief       | The steepness of the slope, deg. | Slope exposure | Vegetation type                  | Soil type          |
|--------------------------------|--------------|----------------------------------|----------------|----------------------------------|--------------------|
| Left side of the waterfall gap | low mountain | 20-25                            | SV             | fluffy oak ash forest            | brown leached      |
| Right side of the waterfall gap| low mountain | 30-35                            | SW             | pistachio-juniper woodlands      | brown carbonate    |

The habitat of wild-growing grapes on the right side of the waterfall gap also has a low-mountainous relief with brown carbonate soils of low thickness and different rockiness, where xerophytic sections of juniper sparse forests and shrub communities are represented.

The vegetation of the first and second studied sections of the waterfall gap noticeably differs from each other (table 2).

Table 2. Phytocenotic features of the studied areas.

| Location       | Tree tier              | Shrub tier                  | Grass tier                       |
|----------------|------------------------|-----------------------------|----------------------------------|
| Left side of the waterfall gap | Fluffy oak High ash Field maple Eastern hornbeam | wig-tree, Rosa canina, Wild rose canina, Sicilian sumac | English ivy, traveller’s-joy (Clematis vitalba), Carex acuta, Veronica umbrosa, Violet pleasant, Bromopsis benekenii, slender false brome |
| Right side of the waterfall gap | Wild nard Red Juniper | Butcher’s broom, Evergreen jasmine, Common ephedra | Dictamnus caucasicus L. Aegonychon purpureocaeruleum Chiy bonfire Heath false brome Bluegrass sterile |

On the southern macro slope on the left side of the waterfall gap 6 populations of grapes were found, and on the opposite slope on the right side - 4.

As you know, the time and duration of the passage of various phases by a vine plant largely depends on the climatic conditions of the area.

Winter 2018-2019 was atypically soft, almost frost-free. The minimum temperature in February of the 3rd decade of 2019 was -5.9 °C. The average temperature for the coldest month of the year February was +3.8 °C. No frost damage was noted on the collection's grape plants. Spring was warm. The beginning of sap flow was observed from the 2nd decade of March. Budding began in the 1st decade of April. May was warm with little rainfall (32 mm), and without frost, due to which flowering began in the 3rd decade of May, which is 2 weeks earlier than the average annual data. The air temperature reached 29.5 °C during the day. During the flowering of grape bushes, no rain was observed. June was hot and very arid, the amount of precipitation for the month was only 1.2 mm at an average temperature of 22.6 °C. The drought lasted all of June and early July.

In general, the sum of active temperatures for the 2019 season amounted to 4271º, significantly exceeding the long-term average (3700-4000º).

In 2019, in 1,658 wild-growing forms of grapes growing in the reserve, the beginning of bud blooming was observed from April 20 to 30. Flowering took place from June 2 to 10 under favorable conditions. Full physiological maturity was noted earlier than others on wild-growing forms on the left side of the Waterfall Slit.

Ampelographic description (study of morphological features) was carried out according to more than 40 signs of the vegetative and generative spheres of wild-growing grape forms. But only about ten of these signs are the most volatile. In particular, it was found that the shape (openness) and cobwebby
pubescence of the top of a young shoot in the studied populations of wild-growing grapes is one of the most variable morphological characters. The shape of the top of a young shoot varies in this case from slightly open to completely open [15] in plants found on the left side of the waterfall gap (figure 1).

The cobwebby pubescence of these plants on the left side of the Waterfall gap varies from rare (2 points) to thick (4 points).

On the right side of the waterfall gap, 4 populations of wild grapes were found. They are significantly different from previous plants. According to a number of phenotypic features, leaves and inflorescences are more similar to cultivated grapes. The leaves are 5 lobed, slightly dissected or whole, the upper lateral notches are not deep, the cobwebby pubescence of the lower side of the formed leaf is weaker than that of the grape shape on the port side. In these grape plants, the shape and cobwebby pubescence of the top of a young shoot is also highly variable (figure 2).

These wild-growing forms of grapes still have a number of features according to the most variable morphological characters of the vegetative and generative parts of plants (table 3).

Table 3. Morphological features of wild-growing forms of grapes (waterfall gap, Utrish Nature Reserve).

| Feature                          | Population number |
|---------------------------------|-------------------|
| Top shape of a young shoot      | 1     2  3  4  5  6  7  8  9  10 |
| Webbing pubescence of the apex  | half-open wide open slightly open half-open half-open half-open fully open slightly open slightly open wide open half-open |
| of a young shoot                | average thick average average average rare average average very thick rare |
| Bristly pubescence of internodes| Very rare average average rare very rare rare rare very rare very rare very rare |
| Leaf form                       | pentagonal round deltoid pentagonal cordate pentagonal pentagonal deltoid pentagonalpentagonal |
In order to study the genetic diversity of the found forms, a DNA marker analysis of wild grape plants was carried out.

Table 4. Results of DNA profiling of wild-growing samples at microsatellite loci.

| Sample          | VVMD27 | VVMD5 | VS2 | Vrzag79 |
|-----------------|--------|-------|-----|---------|
| Cabernet Sauvignon reference cultivar | 176    | 190   | 234 | 242     | 139    | 151    | 247   | 247     |
| Sample 1        | 180    | 190   | 230 | 234     | 151    | 153    | 251   | 259     |
| Sample 2        | 180    | 190   | 230 | 234     | 137    | 151    | 251   | 259     |
| Sample 3        | 180    | 190   | 230 | 234     | 137    | 151    | 251   | 259     |
| Sample 4        | 180    | 190   | 230 | 234     | 137    | 151    | 251   | 259     |
| Sample 5        | 190    | 190   | 232 | 232     | 143    | 155    | 249   | 251     |
| Sample 6        | 190    | 190   | 226 | 230     | 143    | 155    | 251   | 251     |
| Sample 7        | 190    | 190   | 226 | 230     | 143    | 155    | 251   | 251     |
| Sample 8        | 190    | 190   | 230 | 230     | 153    | 155    | 251   | 251     |
| Sample 9        | 180    | 190   | 230 | 242     | 137    | 153    | 251   | 259     |
| Sample 10       | 180    | 190   | 230 | 242     | 137    | 153    | 251   | 259     |
| Sample 11       | 190    | 190   | 230 | 230     | 139    | 139    | 251   | 251     |
| Sample 12       | 190    | 190   | 230 | 230     | 143    | 153    | 15 3 | 251     |
| Sample 13       | 190    | 190   | 230 | 230     | 145    | 149    | 251   | 251     |
| Sample 14       | 190    | 190   | 234 | 234     | 137    | 137    | 247   | 251     |
| Sample 15       | 190    | 190   | 236 | 236     | 151    | 155    | 251   | 251     |
| Sample 16       | 188    | 190   | 230 | 236     | 147    | 155    | 251   | 251     |
Genotyping of samples was performed at 4 microsatellite loci from the list of recommended for genotyping: VVMD27, VVMD5, VS2, VrZAG79. From Table 4 it follows that samples 2, 3, and 4 have identical DNA profiles at the studied loci, as well as samples 9 and 10. Perhaps each of these groups is the descendants of one plant. A more complete DNA profiling will clarify this question, but it can already be concluded that samples 2, 3 and 4 are genetically close forms, as well as samples 9 and 10 are genetically similar. In general, the analysis of the polymorphism of a sample of 16 samples according to microsatellite analysis showed the average level of heterogeneity: the average actual heterozygosity was at the expected level (table 5).

The VS2 locus was found as the most polymorphic: 8 different alleles were identified, the least polymorphic was VVMD 27: only three alleles. All three identified sizes of the allele of the VVMD 27 locus (188, 180, 190 nucleotide pairs) are widespread in cultivated grape varieties, according to the VIVC database, as well as the alleles identified in the locus VrZAG79. Locus VVMD5 is interesting for the combination found in 6 and 7 samples (226: 230 bp), the same state of the locus is known only in 4 ancient European and Eastern grape varieties [21]. Locus VS2 is interesting for the combination found in 9 and 10 samples (137: 153 bp); the same locus condition is known only in three European, two of which are ancient, and one American grape variety; combination 139: 139 detected in sample 11 is found only in two European wine grape varieties and in one stock (Rupestris metallicum); combination 147: 155, identified in sample 16, was previously found in only one ancient Armenian table grape variety (Khusaine belyi). Allele combinations 137: 151 and 143: 155 found in samples 2-4 and 5-7, respectively, are widespread among cultivated European grape varieties.

Table 5. Characterization of microsatellite loci in the studied sample.

| Locus    | Na number of alleles | Ne effective number of alleles | Ho observed heterozygosity | He expected heterozygosity |
|----------|----------------------|--------------------------------|-----------------------------|-----------------------------|
| VVMD27   | 3.00                 | 1.55                           | 0.44                        | 0.36                         |
| VVMD5    | 6.00                 | 3.14                           | 0.56                        | 0.68                         |
| VS2      | 8.00                 | 6.32                           | 0.84                        | 0.84                         |
| VrZAG79  | 4.00                 | 1.93                           | 0.50                        | 0.48                         |
| mean     | 5.25                 | 3.24                           | 0.59                        | 0.59                         |

4. Conclusion
For the first time ampelographically and genetically samples of isolated populations of wild-growing grapes in the territory of the state natural reserve "Utrish" unique in its natural-historical conditions (sites of ancient settlements of Circassians of XIII-XV centuries) in quantity of 10 populations which vine is not exposed to any of visually defined pathogens are studied and described. Due to the ability of wild vines to easily interbreed with the cultivated one, these valuable qualities of wild grapes will be transferred to cultivated varieties by including them as donors of resistance to biotic and abiotic stressors in the breeding process.

A morphological and biological study of grape plants and a detailed description of their environmental growing conditions have been carried out. Morphological assessment of the vegetative part among the populations of wild grape varieties studied showed that the shape (openness), web-like pubescence of the top of the young shoot and the shape of the leaf are among the most variable morphological features. For example, the openness of the crown of a young shoot varies from slightly open to completely open in plants on the left side of the waterfall gap. A web-like pubescence from medium to very dense.
Yield from the bush was not evaluated, but visually it can be said about the good productivity of these wild-growing forms. DNA-marker analysis of plant genotypes by microsatellite loci VVMD27, VVMD5, VS2, VrZAG79 recommended for genotyping of grapes was performed. In general, according to the results of microsatellite loci analysis, even with the use of only four markers, it can be noted that some of the samples from the sample are polymorphic to each other, while others are genetically close. A number of rare alleles were revealed in the analysis of genotypes for the analyzed SSR loci.

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