# Conservation of grape genetic resources in the system *in vitro*

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**Abstract.** Conservation of grape genetic resources in the system *in vitro* can be a contribution to ampelographic collections, and a source of their replenishment with healthy planting material. The goal of the study is to create a vegetative *in vitro* collection of plants of promising grape varieties and clones, optimize the conditions for maintaining samples. The basis for obtaining, cultivating, clonal micro-propagating of grape plants is the research and development of the Institute Magarach. At the moment, the collection is represented by 144 samples of grape varieties and clones. Samples of the collection are kept in a state of active growth, slow growth and true dormancy. Previous studies followed to establishment of five modes to maintain grape plants in the system *in vitro*. Changes in the parameters of individual factors affecting morphogenesis made it possible to slow down growth processes and reduce the number of re-plantings. The most promising mode to conserve samples is the fifth one (this method is patented). As a result of the research carried out in the Institute Magarach, a vegetative collection of plants *in vitro* of promising grape varieties and clones was created. It includes Crimean autochthonous varieties and varieties of the Institute Magarach breeding. The developed modes of collection conservation allow slowing down growth processes and maintaining plants in a state of true dormancy without re-planting for one to two years.

## 1 Introduction

Huge efforts of world community are recently directed to the conservation of plant genetic resources, including the varieties and hybrids of cultivated plants, each of which has its own unique characteristics. Along with the traditional methods of conservating the biological diversity of plants in natural conditions, the biotechnology methods are widely used [1-3]. In order to improve the efficiency of maintaining plant genetic collections, two approaches are currently used: conservation of collections in the form of slowly growing test-tube plant cultures under controlled conditions *in vitro*, and cryo-conservation of tissues or cell cultures of individual genotypes [4–6]. Also, an effective method to conserve plant material at low temperatures (1-4 °C) is the encapsulation in alginate balls [7]. The creation of genetic collections *in vitro* can be an important addition to ampelographic collections, and a source of their replenishment with healthy planting material. Collections *in vitro* are stored for a long time in a state of active or slow growth. Slowing down the growth processes is achieved through the use of growth-inhibiting substances, reducing the temperature, lighting, pressure,

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and other factors [9–10]. Deposition can significantly reduce the areas, labor costs and expenses for reagents and materials. The creation and maintenance of a vegetative *in vitro* plant collection contributes to healing, conservation and reproduction of promising varieties and clones of grapes.

The goal of the study: creation of a vegetative *in vitro* collection of plants of promising grape varieties and clones, optimization of conditions to conserve samples.

## 2 Materials and methods of research

The basis for obtaining, cultivating, clonal micro-propagating of grape plants is the research and development of the Institute Magarach [11, 12]. The source material in the form of lignified annual cuttings was obtained from the Ampelographic Collection and breeding plots of the Institute Magarach, Anapa Experimental Station, Crimean Experimental Station and other sources.

In order to obtain the primary explant (green shoots), the cuttings were germinated in ambient conditions. Green shoots were cut into one-or two-eye explants and placed in sample bottles for sterilization. Further operations were carried out under sterile conditions, using a laminar box for work. Shoots were sterilized with 95% alcohol for 40 seconds, then with diacid for - 8 minutes, followed by washing three times with sterilized distilled water. After mechanical operations, the shoots were planted in a nutrient MS medium containing 0.4–0.6 mg/l BAP (6-benzylaminopurine) [13]. The shoots were cultivated in the light with intensity of 1500 cd·sr/$m^2$ under conditions of a 16-hour photoperiod and a temperature of +27°C.

The shoots developed under sterile conditions were transplanted into PG medium containing 0.05-0.08 NAA (α-naphthylacetic acid) for rooting. The plants thus obtained were propagated by microcutting.

Hormone-free PG medium was used to conserve samples in the collection.

## 3 Results and discussion

The obtaining of aseptic cultures of grape varieties was associated with certain difficulties. A significant group of Crimean autochthonous varieties is represented in the Ampelographic Collection of the Institute Magarach by single bushes only, not having a good phytosanitary condition. In grapes, the genotype influence on the processes of morphogenesis is strong. Grape varieties have different potency for bud initiation at the initial stage of obtaining aseptic cultures. Limiting factors can be as follows: callus formation at the base of shoot explant, shoot necrosis, low viability and rooting of shoots, etc. For some varieties, the shoot from bud develops rapidly within a week, at the same time having a fairly high rooting ability. For others, shoots can start growth in a month and a half with slow dynamics. As a rule, in the following they take roots with difficulty. When fully developed plants are obtained in culture, their propagation is not so difficult. One of the important factors affecting the conservation of samples, is the development of inner infection, which inhibits growth followed by the loss of sample.

The collection was created in 2007 at the Institute Magarach. And by 2018, there were already about 40 specimens in it. In 2019-2020, we focused our efforts on replenishing the collection with Crimean autochthonous grape varieties. Now we concentrate on further collecting samples of varieties and hybrids of the Institute Magarach breeding in order to conserve the valuable gene pool of grapes created by different generations. At the moment, the collection is represented by 144 samples of varieties and clones of grapes.

A regulation on the vegetative collection of grape plants was prepared and approved. The first variant of the catalog with samples of the collection was developed. The samples in the catalog are divided into four separate groups as follows: Crimean autochthonous varieties; cultivars and hybrid forms bred in the Institute Magarach; rootstock varieties; introduced and common varieties (Fig. 1.). Somaclones of grape varieties (6 varieties, 16 somaclones), obtained in the process of somatic embryogenesis from cells of suspension cultures treated with colchicine, are also maintained in the collection *in vitro*. Cytogenetic analysis showed

that some of them are polyploids. Somaclones were planted in the field for study, began to bear fruits, and were used in the breeding process. Somaclones are maintained in the collection for cytological studies.

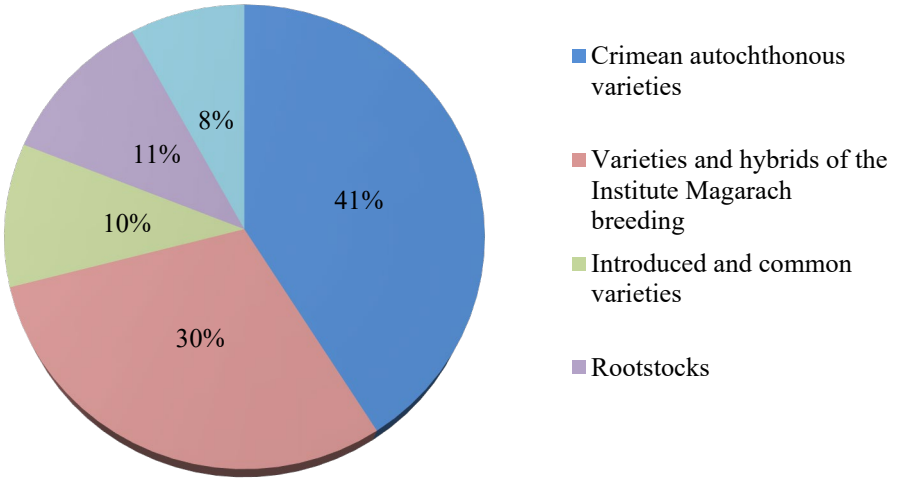


**Fig. 1.** Varietal spectrum of samples in the vegetative collection of plants *in vitro*

**Table 1.** Promising grape varieties of the Institute Magarach breeding in the vegetative collection *in vitro*

| | Name of variety | Characteristic | Usage |
|---|---|---|---|
| 1. | 'Aurora Magaracha' | Resistant to frost, mildew, phylloxera | Juices, table, sparkling and dessert wines |
| 2. | 'Akademik Avidzba' | Early ripening, showy | Fresh consumption |
| 3. | 'Alminski' | Varietal aroma | Dessert wines |
| 4. | 'Antei Magarachskiy' | Resistant to phylloxera, mildew, frost, high quality of wine | Juices, table and strong wines |
| 5. | 'Gerkules' | Showy | Fresh consumption |
| 6. | 'Granatovyi Magaracha' | High quality of wine | Dessert wines |
| 7. | 'Danko' | Frost resistant, quality of wine | Juices, dessert wines |
| 8. | 'Krasen' | Seedless | Multiuse |
| 9. | 'Liviya' | Early ripening, showy | Fresh consumption |
| 10. | 'Muscat Kryma' | Promising hybrid form | Fresh consumption |
| 11. | 'Risling Magaracha' | Resistant to phylloxera, mildew, frost, high quality of wine | Table and sparkling wines |
| 12. | 'Pamyati Golodrigi' | Quality of wine | Table, strong and dessert wines |
| 13. | 'Pervenets Magaracha' | Resistant to phylloxera, frost | Dry and dessert wines |
| 14. | 'Podarok Magaracha' | Resistant to phylloxera, frost | Brandy spirits |
| 15. | 'Safyanoyi' | Quality of wine | Multiuse, table and dessert wines |
| 16. | 'Solnechnaya Grozd' | Showy | Fresh consumption |
| 17. | Spartanets Magaracha' | Resistant to phylloxera, mildew, frost, varietal aroma | Juices, table and dessert wines |
| 18. | 'Tavkveri Magaracha' | High quality of wine | Table wines |
| 19. | 'Tsitronnyi Magaracha' | Muscadine aroma, high quality of wine | Dessert wines |

| | | | |
|---|---|---|---|
| 20. | 'Yuzhnoberezhnyi' | Showy, seedless | Fresh consumption |
| 21. | 'Yaltinskiy Bessemyannyi' | Showy, seedless | Fresh consumption |

Samples of the collection are maintained in a state of active, slow growth and true dormancy. Previous studies made it possible to establish five modes to maintain grape plants in the system *in vitro* (Table 2).

**Table 2.** Conditions for maintaining plants *in vitro* in a vegetative collection

| No. | Conditions for maintaining a collection | Characteristic |
|---|---|---|
| 1. | Light intensity of 1000 cd·sr/$m^2$ under conditions of a 16-hour photoperiod at a temperature of +25-27°C | Allows to reduce the number of re-plantings to three per year |
| 2. | Light intensity of 1000 cd·sr/$m^2$ under conditions of a 16-hour photoperiod at a temperature of +27°C, small modification of the medium | Allows to maintain plants throughout the year without re-planting, but requires certain culture vessels and an increased medium volume |
| 3. | Without additional lighting at a temperature of +10-12°C | Allows to reduce the number of re-plantings to one per year |
| 4. | Combined mode: 3 - Without additional lighting at a temperature of +10-12°C (6 months); 1- Light intensity of 1000 cd·sr/$m^2$ under conditions of a 16-hour photoperiod at a temperature of +25-27°C (3 months) | Extends the period of maintenance without re-planting to nine months due to physiological processes of restructuring the plant organism to changed cultivation conditions |
| 5. | At low temperatures +2-4°C in the dark | Allows to maintain plants from one to two years without re-planting, requiring certain conditions for transition of plants into a state of true dormancy. |

**Fig. 2.** Maintaining a collection of plant samples *in vitro* of promising grape varieties and clones in a state of true dormancy.

Changing the parameters of affecting morphogenesis individual factors led to changes in cultivation conditions of grape plants *in vitro*, and made it possible to slow down growth processes as well as to reduce the number of re-plantings during the year. The most promising for conserving samples is the mode of maintaining plants in a state of true dormancy.

A method of conserving grape micro-plants under the conditions *in vitro*, including cultivating plants on a hormone-free medium in different modes and physiological states, creating certain conditions for plants to be transferred to a state of true dormancy is modeled

in a growth chamber based on changes in the indicators of two factors: photoperiod and temperature, which allows to further conserve plants successfully at low positive temperatures (+2-4ºC) in the dark without re-planting for one to two years. This method has a patent of the Russian Federation.

The vegetative collection is annually replenished with new samples, expanding its assortment with rare and promising forms and varieties. Samples of certain varieties in the vegetative collection are used for scientific research related to the recovery from viral and phytoplasmic infections, as well as for the study of regulation mechanisms of morphogenesis processes, optimization of plant cultivating conditions, and development of effective technologies for propagating popular grape rootstocks in the system *in vitro*.

In the future, this would enable concentrating the unique genetic material of grapes on a small area, which can be used for scientific and applied research in the field of physiology and biochemistry of plants, cell biotechnology, genetic engineering, and as a source of improved grape planting material.

Thus, for the first time at the Institute Magarach, the vegetative collection of plants *in vitro* of promising grape varieties and clones, including Crimean autochthonous varieties and cultivars of the Institute Magarach breeding, was created. The developed modes of conserving the collection allow slowing down the growth processes and maintaining plants in a state of true dormancy without re-planting for one to two years.

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