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BIOTECHNOLOGY FOR OBTAINING HEALTHY GRAPEVINE CLONES

411.09 Plant protection

Abstract of the PhD thesis in agricultural sciences

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The thesis was developed within the Laboratory of Virology and Phytosanitary Control of Horticultural Crops of the National Institute for Applied Research in Agriculture and Veterinary Medicine (NIARAVM), Chişinău, Republic of Moldova.

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CONTENTS

CONCEPTUAL FRAMEWORK OF THE RESEARCH

Relevance and importance of the investigated problem.

In viticulture, the emergence of new types of chronic grapevine diseases, the continuous improvement of diagnostic methods, and the application of biotechnological tools for obtaining healthy plants represent rapidly evolving research directions. The main objective in this context is the development of grapevine clones with high yield potential, superior fruit quality, and free from viral, phytoplasmic infections and bacterial cancer, to introduce them into commercial vineyards as quickly as possible. To date, more than 100 infectious agents have been identified in grapevine, including viruses, phytoplasmas and bacteria, all responsible for systemic and chronic diseases [8]. As a result, the affected tissues and plants remain diseased throughout their entire life cycle, and each of their organs becomes a source of infection during vegetative propagation, leading to contaminated planting material. These diseases are transmitted through propagating and planting material, via vegetative multiplication from infected vines, as well as through the grafting process when infected scions or rootstocks are used, resulting in compromised nursery plants [11]. During grafting, multiple viruses and diseases can be combined, and their interaction is often unpredictable. The import of planting material, the exchange of propagation material between regions, or the uncontrolled introduction of new cultivars and rootstocks may significantly increase the number of diseases, which, in interaction with changing environmental conditions, can have severe consequences for viticulture.

In the Republic of Moldova, phytosanitary surveys conducted in recent years have confirmed the presence of 11 viruses affecting grapevine, as well as *Candidatus Phytoplasma solani (Ca. P. solani')*, the causal agent of Bois noir disease, and the bacterium *Agrobacterium vitis (A. vitis)*, responsible for bacterial cancer [12]. Among the viruses identified, the most frequently encountered are Grapevine leafroll-associated virus Grapevine leafroll-associated virus (GLRaV-1, GLRaV-3), Grapevine fleck virus (GFkV) and Grapevine fanleaf virus (GFLV). The most damaging viruses can lead to yield losses ranging from 20% to 70% of the total harvest. The spread of GLRaV-3 in a French vineyard resulted in an increase in the incidence of leafroll disease from 5% to 86% over a period of eight years [9]. Economic losses caused by the same virus in California (USA) have been estimated at more than 90 million USD annually [1]. In the case of GFLV, yield losses may reach up to 77%, while the economic damage is estimated at approximately 16,600 USD per hectare, making this virus a major threat to vineyards [2].

It is well known that some grapevine plants may carry pathogens in an asymptomatic form, meaning that they do not display visible disease symptoms. This phenomenon may be influenced by several factors, such as a low concentration of the pathogen, unfavorable climatic conditions, or the overall physiological state of the plant. However, even in the absence of visible symptoms, asymptomatic plants may serve as a source of infection and contribute to the spread of chronic diseases. Infected planting material (scion canes, rootstock canes, grafting cuttings, rootstock cuttings, and grafted cuttings) plays a key role in disseminating these diseases across the entire viticultural area of the Republic of Moldova. Since the control of chronic grapevine diseases through agro-technical or chemical measures is ineffective, the only efficient strategy is the production of healthy grapevine clones and their cultivation under conditions that prevent reinfection and ensure the maintenance of their phytosanitary status [10]. Phytosanitary selection is an effective method for preventing the spread of viral, phytoplasmic, and bacterial diseases by

eliminating infected individuals. This process involves a series of interconnected stages, resulting in the production of healthy clones intended for the establishment of mother plantations of higher biological categories – Pre-basic and Basic.

Background and current situation in the research domain. At the end of the 1960s, the European Union coordinated the efforts of its member states to standardize the selection and certification of grapevine planting material through Directive 68/193/EEC (1968), which regulated the marketing of vine propagating material. At present, the production process is governed by a unified legislative framework (Regulation (EU) 2016/2031, Directive 2008/90/EC, Regulation (EU) 2017/625), as well as by EPPO standards (PM 4 series), which provide detailed requirements for the maintenance and propagation of plants tested free from the main grapevine diseases. Over the years, EU legislation has undergone several amendments, and as a result, Commission Implementing Regulation (EU) 2019/2072 established the tolerance threshold for initial propagation material, basic material, certified and standard material, with regard to the viruses GFLV and ArMV, GLRaV-1 and GLRaV-3, and GFkV (applicable only to rootstocks), which is set at 0% [7].

The Republic of Moldova has taken important steps towards European and international integration by adopting a national certification program for grapevine planting material, ensuring that propagation material is free from GLRaV-1, GLRaV-3, GFLV, GFkV and GRSPV [9]. The national legislation also refers to the presence of diseases of major phytosanitary concern, such as bacterial cancer and *Flavescence dorée*. According to the national certification system, the material is classified into the following categories: Pre-basic, Basic, Certified and Standard, each with specific requirements regarding phytosanitary status.

At present, the production of grafted cuttings is based on canes collected from commercial vineyards, which means that the phytosanitary status of propagated material reflects the health condition of the country's vineyards. A recent survey of grapevine plantations in the Republic of Moldova revealed the presence of the phytoplasma disease Bois noir in all viticultural zones, with incidence levels ranging from 1% to 100%.

Phytosanitary testing carried out in the Laboratory of Virology and Phytosanitary Control of Horticultural Crops (LVPCHC) revealed a high incidence of viral diseases persisting in grapevine plants in a latent form. The viruses GLRaV-1 and GLRaV-3 were detected at rates ranging from 8–26% and 7–39%, respectively. The tests also indicated a considerable incidence of the pathogen *A. vitis*, responsible for bacterial cancer of grapevine, with infection rates varying between 26% and 44% [3].

Based on these findings, to ensure a sustainable and competitive viticulture sector, it is necessary to establish mother plantations of higher biological categories, which will serve as the basis for producing certified planting material in accordance with national and European standards and regulations. This approach is essential for reducing the risk of infection in vineyards, thereby contributing to increased productivity and longer vineyard lifespan.

The aim of the research is to improve the technology for obtaining healthy grapevine clones, based on modern methods of diagnosis, sanitation and multiplication, with the purpose of establishing a mother plantation of the Basic biological category.

To achieve this aim, the following objectives were set: determination of the spectrum of phytosanitary pathogens and the level of infection in grapevine planting material under the conditions of the Republic of Moldova; development of optimal thermotherapy regimes for the

sanitation of initial plants infected with pathogens causing chronic grapevine diseases; improvement of diagnostic methods for detecting bacterial cancer in grapevine; optimization of in vitro cultivation conditions for increasing the efficiency of micropropagation through microcutting techniques; production and accelerated multiplication of initial grapevine clones intended for the establishment of a mother plantation of the Basic biological category.

Scientific hypothesis: the application of modern biotechnological methods will significantly reduce the time required to obtain sanitized grapevine clones, thus facilitating the rapid establishment of mother plantations of the Basic biological category.

Research methodology: the research was carried out based on a complex scientific methodology, structured into successive stages of planning, organization, experimental implementation, and data interpretation, in accordance with the general and specific objectives of the study. The scientific activity focused on assessing the phytosanitary status of grapevine planting material and applying biotechnological approaches for obtaining and multiplying healthy clones intended for the establishment of mother plantations of higher biological categories.

Theoretical significance: the thesis contributes to the scientific foundation of diagnosis and treatment of viral diseases and bacterial cancer in grapevine, providing effective methods for the identification and elimination of pathogens. It also strengthens the theoretical basis for the use of in vitro culture in the accelerated multiplication of sanitized plant material.

Applied value: the initial clones obtained were used to establish a Pre-basic category mother plantation. The material produced in these plantations serves as a source for the establishment of Basic category plantations, ensuring the production of certified planting material in accordance with national standards**.**

Implementation of scientific results: **g**rafted scion and rootstock cuttings from the mother plantation of the Basic biological category are supplied to grapevine nurseries in the Republic of Moldova to produce certified propagating material and the establishment of commercial vineyards.

1 CHRONIC DISEASES OF GRAPEVINE AND THEIR HARMFUL EFFECTS

Chronic grapevine diseases such as viral, phytoplasma-related infections and bacterial cancer act systemically and persistently, meaning that the infected tissues remain diseased throughout the entire lifespan of the plant. These diseases prevent grapevine from reaching its full varietal potential by reducing its resistance to unfavorable environmental factors and increasing susceptibility to secondary pathogens. In addition, they negatively affect the quality of scion and rootstock material, decrease yield and grape quality, and reduce post-harvest storability [10]. Infected plants exhibit complex physiological alterations, including changes in gene expression, hormonal imbalance, disruptions in carbohydrate metabolism, reduced photosynthetic capacity, increased respiration rate, and disturbances in electron transport within photosystems I and II. Viruses also contribute to reduced chlorophyll levels, accumulation of sugars in the leaves, and impaired translocation of these sugars to storage tissues or grape berries. Vegetative propagation from infected vines results in contaminated planting material, which favors the spread of viruses throughout vineyards. It is well established that viruses, viroids, phytoplasmas and bacteria are the main pathogenic agents affecting agricultural crops in general, and especially those propagated

vegetatively. Grapevine, being a perennial woody crop, hosts the highest number of intracellular infectious agents among cultivated plants. Such material disseminates pathogens and their vectors over long distances, and once planted in the field, it serves as a source of inoculum at the new site, enabling secondary spread mediated by vectors. To date, more than 100 intracellular pathogens have been identified in grapevine, including 101 viruses, 5 viroids, 8 phytoplasmas and several bacterial species [8].

1.1 Research on viral and bacterial grapevine diseases in the Republic of Moldova. Biotechnological scheme for producing virus-free clones

Research on grapevine-infecting viruses in the Republic of Moldova began in the post-war period. The first viral disease identified was fanleaf degeneration (short-internode disease), described by the scientist Verderevschii D.D. Later, in 1973, Marinescu V.G. conducted an in-depth study of the Moldovan isolate of this virus, determining its thermal inactivation point in sap (50–52 °C). Using electron microscopy, he observed isometric virus particles with a diameter of approximately 28–30 nm, characteristic of the grapevine fanleaf virus. To control diseases of viral and bacterial etiology, the hot-water thermotherapy method was applied by Țurcan I.G. and Bondarchuk V.V. At the beginning of the 1980s, a technology was developed for the production of grapevine clones free from pathogens of viral origin (Fig. 1.1).

Visual selection (1 year)
↓
Provocative test in greenhouse (2 months)
→ Exclusion of positively tested plants
↓
Asymptomatic plants
↓
Herbaceous indicator tests (1 month)
→ Exclusion of positively tested plants
↓
Negatively tested plants
↓
Serodiagnosis (1 week)
→ Exclusion of positively tested plants
↓
Negatively tested plants
↓
Main testing: Graft indexing (3 years)
→ Exclusion of positively tested plants
↓
Virus-free clones

Figure 1.1 Biotechnological scheme for producing virus-free grapevine clones in the Republic of Moldova (implemented in 1980) [12]

2 MATERIALS AND RESEARCH METHODS

The research was carried out during the period 2018–2025 within the Laboratory of Virology and Phytosanitary Control of Horticultural Crops (LVPCHC) of the National Institute for Applied Research in Agriculture and Veterinary Medicine (NIARAVM). The studies were conducted under laboratory conditions, using plant material originating from vineyard plantations, greenhouse collections, and climate-controlled growth chambers. The biological material used in the study consisted of grapevine genotypes belonging to the species *Vitis vinifera L.*, family *Vitaceae*, order *Vitales*. In addition, interspecific hybrids used as rootstocks were included in the experiments.

2.1 Visual selection of grapevine plants as clone candidates

To select the initial biological material for obtaining phytosanitary grapevine clones, inspections were carried out in selection plots and vineyard plantations located in different regions of the Republic of Moldova. The identification of clone candidates was based on visual assessment of the selection vineyards at NIARAVM, commercial plantations of both European and local grapevine varieties, as well as rootstock mother plantations. The age of the evaluated vineyards ranged between 30 and over 40 years, representing a valuable resource for assessing the genetic and phytosanitary potential of the varieties. The study included grapevine genotypes belonging to three varietal categories. **Autochthonous (local) varieties**: Busuioacă de Bohotin, Busuioacă de Moldova, Copceac, Feteasca albă, Feteasca regală, Fetească neagră, Frâncușa, Negru de Căușeni, Plăvaie, Rară neagră, Telti Curuc, Zghihara de Huși; **newly bred varieties**: Apiren roz, Basarabia, Chișmiș lucistâi, Codrinschii, Legenda, Luminița, Meleag, Moldova, Muscat de Ialoveni, Onițcanskii belâi, Tudor; **european varieties**: Pinot noir, Pinot gris, Chardonnay, Cabernet Sauvignon, Merlot. The identification of clone candidates among rootstocks (Berlandieri × Riparia SO4, Berlandieri × Riparia Kober 5BB, and Riparia × Rupestris 101-14 Mgt) was carried out in the rootstock collection maintained at NIARAVM. The rootstock variety Maleque was included, as it displays a combination of traits such as tolerance to abiotic stress and high grafting compatibility. It originates from the rootstock plantation in Geamăna, Anenii Noi district.

2.2 Evaluation of the phytosanitary status of the collected grapevine material

To assess the phytosanitary status of grapevine planting material, a total of 383,350 bench-grafted vines were analysed. These vines originated from 26 commercial batches collected from nurseries located in different regions of the Republic of Moldova. The testing focused on the detection of the main viral and bacterial pathogens affecting grapevine, namely Grapevine fanleaf virus (GFLV), Grapevine leafroll-associated virus 1 (GLRaV-1), Grapevine leafroll-associated virus 3 (GLRaV-3), as well as pathogenic bacteria of the *Agrobacterium spp*. complex. The analyzed sample included 118,400 vines belonging to autochthonous varieties, 62,100 vines of newly bred varieties, and 202,850 vines of european varieties.

The phytosanitary evaluation of the candidate vines selected for clonal propagation was performed in two stages during the vegetation period. The first monitoring stage was carried out during the flowering phase, when vines showing visible symptoms of fanleaf degeneration, yellow mosaic, vein mosaic, fleck disease, or enation were excluded. The second stage involved the

elimination of plants expressing symptoms of leafroll disease, yellow mosaic, *Flavescence dorée*, wood streaking, or bacterial cancer.

Sample preparation for testing. The collected grapevine canes were mechanically fragmented, and the resulting sawdust was divided into two separate containers. In the first container, 5 mL of Liske extraction buffer was added for the isolation of *Agrobacterium vitis*. The second container was supplemented with PBST-Tween buffer (Phosphate buffered saline with Tween 20), intended for the extraction of viral antigens.

Virus testing. The biological material was tested for the presence of viruses using the serological ELISA (Enzyme-Linked Immunosorbent Assay) method, following the protocol developed by Clark and Adams, with commercial diagnostic kits provided by Agritest. The viruses analysed included GFLV (Grapevine fanleaf virus), GLRaV-1 and GLRaV-3 (Grapevine leafroll-associated viruses), GFkV (Grapevine fleck virus) and GVA (virus A)**.**

Testing for bacterial cancer. Microbiological testing was carried out to detect latent infections caused by bacteria of the *Agrobacterium spp*. complex, in particular *A. vitis* and *A. tumefaciens*. For sample preparation, lignified cane fragments were mechanically shredded, and the resulting woody sawdust was divided into sterile containers. Each container was supplemented with 5 mL of liquid Liske medium, which served as a suitable substrate for the multiplication of pathogenic bacteria present in the samples. The samples were incubated for 24 hours at 27 °C in a Boeco SI-22 thermostat. After the incubation period, 100 µL of the resulting suspension were plated onto the semi-selective Roy & Sasser medium, used for the isolation of Agrobacterium spp. [10]. The Petri dishes were incubated at 27 °C for 7 days in a Boeco SI-22 thermostat (Germany). Colony examination was performed visually and with a magnifying lens to identify morphological traits characteristic of *Agrobacterium spp*. strains, including white, convex, glossy colonies with regular margins and a red-pigmented center.

PCR testing for the detection of bacterial cancer. Molecular diagnosis was carried out using the commercial kit "PCR Agrobacterium vitis", developed by Qualiplante. The PCR reaction was performed with molecular markers targeting the *pehA*, *virD* and *virF* genes, which enable the differentiation between pathogenic and non-pathogenic strains of *A. vitis* and *A. tumefaciens*. For the analysis of PCR products, molecular weight markers of 100 bp, 200 bp, and 1 kb were used, as well as the intercalating dye ethidium bromide. Each sample was tested in duplicate, and PCR reactions were repeated to confirm the results.

2.3 Sanitation methods applied to grapevine clone candidates

Hot water treatment (HWT). For the application of this method, a laboratory thermostat LP-516, model 1387, equipped with a temperature-control system and integrated stirrer, was used to ensure uniform heat distribution within the water bath. The study of bud viability after hot water treatment was carried out on the varieties Aligoté and Pinot blanc. The biological material subjected to sanitation consisted of canes of the grapevine variety Meleag (code T2-19-25), collected from plants that had previously tested positive for the presence of bacteria belonging to the *Agrobacterium spp.* complex. The canes were sectioned into two-bud segments, labeled and grouped accordingly, and subsequently immersed in water at 50 ± 1 °C for 45 minutes. The treatment conditions were established in accordance with the technical datasheet of the

WINETWORK project and the international methodological recommendations of EPPO and OIV regarding the hot water treatment of grapevine material.

Hot air treatment. Hot air thermotherapy was applied to clone candidates of the variety Codrinschii (codes I-9-7-4 and I-9-8-1). The treatment was carried out in thermotherapy chambers of the type of KK 500 TOP+FIT, equipped with a forced-air convection system and digital temperature control, allowing constant maintenance of treatment parameters. The applied conditions consisted of exposure at 37–38 °C, in accordance with phytosanitary sanitation principles recommended by OIV and EPPO.

2.4 *In vitro* culture and accelerated multiplication of initial grapevine clones

Preparation of culture media. For the initiation, multiplication and acclimatization of in vitro grapevine cultures, two main types of culture media were used: a solid agar-based nutrient medium for the inoculation stage, and the artificial substrate "Biona-311" for the micropropagation and ex vitro adaptation stages. During the inoculation phase, a modified Murashige and Skoog (MS) medium was used, containing ½ of the standard concentration of inorganic salts, supplemented with IAA at 1 mg/L and 2iP at 0.5 mg/L, with the pH adjusted to 6.2 using a Hanna HI 5522 pH meter, and solidified with 4.5 g/L agar. In addition, the medium was enriched with 100 mg/L myo-inositol and 56 mg/L FeEDTA. All chemical compounds were dissolved in distilled water and the final volume was adjusted using a graduated cylinder.

Accelerated multiplication. Regenerated grapevine plants were multiplied by in vitro culture under aseptic conditions, under a laminar airflow hood, using plastic containers. To support the growth and development of microcuttings, the nutrient substrate "Biona-311", enriched with macro- and microelements, was used, according to the composition developed by V. Soldatov.

The regeneration and functioning of the ion-exchange substrate were ensured by the inclusion of specific concentrations of mineral salts, as follows (mg/L): K^+ – 0.27; NH_4^+ – 0.03; Ca^{2+} – 1.47; Mg^{2+} – 0.73; Fe^{3+} – 0.05; NO_3^- – 0.69; SO_4^{2-} – 0.48; $H_2PO_4^-$ – 0.054; Na^+ – 0.07; Cl^- – 0.025. The composition was supplemented with essential microelements (mg/L): Mn – 0.22; Cu – 0.064; Zn – 0.057; Co – 0.015; Mo – 0.044. The degree of nutrient substrate regeneration was monitored by measuring electrical conductivity using a Hanna HI 5522 ionometer.

2.5 Evaluation of the influence of LED light spectra on the development of grapevine plants *in vitro*

To evaluate the influence of lighting conditions on the morphogenetic and photosynthetic processes of grapevine plants in vitro, an experiment was carried out using different LED light spectra. The study was performed on the cultivar Feteasca regală, under three full-spectrum LED sources (400–700 nm). The first source was a cool-white LED (5950 ± 50 K) with a maximum photosynthetic photon flux density (PPFD) of 63 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and a maximum daily light integral (DLI) of 4.05 $mol \cdot m^{-2} \cdot day^{-1}$. The second source was a warm-white LED (3200 ± 50 K), with a maximum PPFD of 30 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and a DLI of 2.04 $mol \cdot m^{-2} \cdot day^{-1}$. The third source, referred to as the "Fito" LED (≤1700 K), generated a maximum PPFD of 25 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and a DLI of 1.64 $mol \cdot m^{-2} \cdot day^{-1}$. The emission spectra of the light sources were recorded using an MDR-23 monochromator connected to a UNIPAN RS-232 lock-in amplifier and an analogue-to-digital converter, while light intensity measurements were performed with a CEM DT-1308 lux meter.

3 PRODUCTION OF HEALTHY GRAPEVINE CLONES

3.1 Evaluation of viral infections in grapevine planting material in the Republic of Moldova

At present, no mother plantations of grafting material (scion sources) exist in the Republic of Moldova. The canes used to produce grafted rootstock cuttings are harvested from existing industrial vineyards, which means that the phytosanitary status of the planting material directly reflects the sanitary status of the vineyards from which it originates. Samples were collected from lots of cuttings grown in nurseries across the Republic of Moldova, in quantities of 5,000 units or more, from table and wine grapevine cultivars (Table 3.1). For testing, plant material samples in the form of one-year-old grafted and rooted cuttings were collected.

Table 3.1 Evaluation of Viral Infections in Grapevine Planting Material of autochthonous Varieties in the Republic of Moldova (2018–2023)

No.	Variety / Rootstock	Lot size (pcs.)	Number of samples (pcs.)	Virus-positive samples / %			Total infected samples / %
				GFLV	GLRV-1	GLRV-3	
1	Feteasca Neagră/ BxR Kober 5BB	12000	10	4/40	3/30	2/20	9/90
2	Feteasca Regală/ BxR Kober 5BB	9000	10	1/10	1/10	0	2/20
3	Feteasca Regală/ BXR SO4	9400	10	5/50	4/40	2/20	11/100
4	Feteasca Neagră/ BxR SO4	31000	30	0	0	0	0
5	Rară Neagră/ BxR Kober 5BB	29600	20	0	0	0	0
6	Feteasca Neagră/ BxR Kober 5BB	27400	25	0	0	0	0
Total:		118400	105	10 /9,5%	8 /7,6%	4 /3,8%	22 /20,9%

During the same period, viral infection testing was also carried out on planting material originating from newly bred varieties, and the corresponding data are presented in Table 3.2.

Table 3.2 Evaluation of viral infections in grapevine planting material of newly bred varieties in the Republic of Moldova (2018–2023)

No.	Variety / Rootstock	Lot size (pcs.)	Number of samples (pcs.)	Virus-positive samples / %			Total infected samples / %
				GFLV	GLRV-1	GLRV-3	
1	Moldova/ BxR Kober 5BB	5000	15	0	2/13,33	0	2/13,33
2	Viorica/ BxR SO4 cl.5	23400	20	6/30	2/20	2/10	10/50
3	Moldova/ BxR Kober 5BB	5000	10	1/10	0	3/30	4/40
4	Riton/ BxR SO4	5500	10	0	0	1/10	1/10
5	Codreanca/ BxR Kober 5BB	10000	10	0	0	1/10	1/10
6	Bianca/ BxR SO4	13200	10	1/10	0	1/10	2/20
7	Viorica/ BxR SO4	15000	15	0	4/26,7	0	4/26,7
Total:		62100	75	8 /10,7%	8 /10,7%	8 /10,7%	24 /32%

The results presented in Table 3.2 show that the planting material from newly bred varieties exhibits a higher level of infection compared to that of autochthonous varieties, and viral infections were detected in all analyzed batches. The third tested sample consisted of grapevine cuttings of european varieties, produced in viticultural nurseries in the Republic of Moldova. The results of this testing are shown in Table 3.3.

Table 3.3 Evaluation of viral infections in grapevine planting material of european varieties in the Republic of Moldova (2018–2023)

No.	Variety / Rootstock	Lot size (pcs.)	No of samples pcs.	Virus-positive samples / %			Total infected samples
				GFLV	GLRV-1	GLRV-3	
1	Saperavi/ BxR SO4	9000	10	3/30	2/20	1/10	6/60
2	Muscat Ottonel/ BxR Kober 5BB	12000	10	1/10	1/10	1/10	3/30
3	Cabernet-Sauvignon/ BxR Kober 5BB	12000	10	0	0	0	0
4	Merlot cl.348/ BxR SO4	6000	10	0	0	0	0
5	Sauvignon/ BxR SO4	8700	10	0	4/40	2/20	6/70
6	Pinot Gris / BxR SO4	35900	30	3/10	0	3/10	6/20
7	Saperavi / BxR SO4	25500	20	0	2/10	0	2/10
8	Sauvignon/ BxR Kober 5BB	12500	10	0	2/20	1/10	3/30
9	Merlot/ BxR SO4	22500	20	0	0	0	0
10	Aligote/ BxR Kober 5BB	5000	10	1/10	1/10	1/10	3/30
11	Muscat de Hamburg cl.202/ BxR Kober5BB	10000	10	0	0	3/30	3/30
12	Cardinal/ BxR 101-14	20000	20	0	0	0	0
13	Cabernet Sauvignon/ BxR SO4	23750	20	0	0	0	0
Total:		202850	190	8 /4,2%	12 /6,3%	12 /6,3%	32 /16,84%

The analysis of data presented in Tables 3.1–3.3 revealed significant differences in viral infections among grapevine planting material from autochthonous, newly selected, and european cultivars in the Republic of Moldova. The highest average infection rate was recorded in newly selected cultivars, reaching 32%. In these lots, GFLV, GLRaV-1, and GLRaV-3 were detected at equal proportions of 10.66% each. In contrast, autochthonous cultivars exhibited a lower level of viral infection; for instance, GLRaV-3 was detected in only 3.8% of the tested samples. This situation may be explained by the fact that local cultivars have only recently begun to be propagated on a larger scale, while newly selected cultivars have been more intensively used as propagation material within vineyard renewal programs, which may account for the higher infection rates observed.

The evaluation was carried out on a sample consisting of 26 lots, totaling 383,350

grapevine cuttings. Of these, 370 were tested, and 78 were found to be infected, corresponding to an infection rate of 21%, with every fifth grapevine cutting being a carrier of one or more viral infections. The test results revealed variable infection levels depending on the virus involved. The incidence of GFLV ranged from 0% to 50%, GLRaV-1 from 0% to 40%, and GLRaV-3 from 0% to 30%. The most frequently detected viruses were GLRaV1 and GFLV, with mean infection rates of 8.2% and 8.1%, respectively, while GLRaV-3 was the least common, identified on average in 6.9% of samples. It is important to note that GFLV was never detected alone, it was always found in mixed infections.

3.2 Incidence of *A. vitis* in grapevine planting material in the Republic of Moldova

During the period 2018–2023, grapevine planting material originating from viticultural farms was tested for the presence of *A. vitis* at the LVPCHC. The results of bacterial cancer testing in planting material of autochthonous grapevine varieties are presented in Table 3.4.

Table 3.4 Evaluation of *A. vitis* in grapevine planting material of autochthonous varieties in the Republic of Moldova (2018–2023)

No.	Variety / Rootstock	Lot size (pcs.)	Number of samples tested (pcs.)	Total samples infected with *A. vitis*	
				units	%
1	Feteasca Neagră/ BxR Kober 5BB	12000	10	3	30
2	Feteasca Regală/ BxR Kober 5BB	9000	10	2	20
3	Feteasca Regală/ BXR SO4	9400	10	0	0
4	Feteasca Neagră/ BXR SO4	31000	30	9	30
5	Rară Neagră/ BxR Kober 5BB	29600	20	12	60
6	Feteasca Neagră/ BxR Kober 5BB	27400	25	15	60
Total:		118400	105	41	39%

The next tested sample consisted of planting material from newly bred varieties, comprising 7 batches and 5 grapevine cultivars. The results of the examination for the presence of bacterial cancer infections in the grapevine planting material are presented in Table 3.5.

Table 3.5 Evaluation of *A. vitis* in grapevine planting material of newly bred varieties in the Republic of Moldova (2018–2023)

No.	Variety / Rootstock	Lot size (pcs.)	Number of samples (pcs.)	Total samples infected with *A. vitis*	
				units	%
1	Moldova/ BxR Kober 5BB	5000	15	0	0
2	Viorica/ BxR SO4 cl.5	23400	20	0	0
3	Moldova/ BxR Kober 5BB	5000	10	2	20
4	Riton/ B x R SO4	5500	10	4	40
5	Codreanca/ BxR Kober 5BB	10000	10	0	0
6	Bianca/ BxR SO4	13200	10	3	30
7	Viorica/ BxR SO4	15000	15	3	30
Total:		62100	75	24	32%

In the context of evaluating the presence of *A. vitis* in grapevine planting material of european varieties, batches originating from viticultural nurseries in the Republic of Moldova were examined, and the results are presented in Table 3.6. The analysed sample included 202,850 cuttings, of which 190 were tested, and 41 were found to be infected, resulting in an average infection rate of 21.6%.

Table 3.6 Evaluation of *A. vitis* in grapevine planting material of european varieties in the Republic of Moldova (2018–2023)

No.	Variety / Rootstock	Lot size (pcs.)	Number of samples tested (pcs.)	Total infected samples	
				units	%
1	Saperavi/ BxR SO4	9000	10	2	20
2	Muscat Ottonel/ BxR Kober 5BB	12000	10	1	10
3	Cabernet-Sauvignon/BxR Kober 5BB	12000	10	1	10
4	Merlot cl.348/BxR SO4	6000	10	1	10
5	Sauvignon/ BxR SO4	8700	10	2	20
6	Pinot Gris / BxR SO4	35900	30	0	0
7	Saperavi / BxR SO4	25500	20	6	30
8	Sauvignon/ BxR Kober 5BB	12500	10	2	20
9	Merlot/BxR SO4	22500	20	10	50
10	Aligote/ BxR Kober 5BB	5000	10	1	10
11	Muscat de Hamburg/ BxR Kober5BB	10000	10	3	30
12	Cardinal/ BxR 101-14	20000	20	8	40
13	Cabernet Sauvignon/BxR SO4	23750	20	4	20
Total:		202850	190	41	21,6%

Based on the data presented in Tables 3.1–3.6, it can be concluded that the grapevine planting material produced in nurseries in the Republic of Moldova is affected by various viral diseases as well as by latent bacterial cancer. Viral infections may occur either individually or in mixed combinations involving two or three viruses. The infection rate caused by viral pathogens ranges from 0 to 40%, while in the case of mixed infections the degree of contamination is considerably higher, reaching up to 100% (e.g. Feteasca Regală / B×R SO4). In addition, grapevine cuttings are also infected with latent forms of bacterial cancer, with incidence levels varying from 0 to 60%. The presence of viral diseases and Agrobacterium-induced bacterial cancer in planting material represents a serious threat to viticulture, as vineyards are initially established using infected cuttings. This negatively affects vine growth and development, reduces yield and grape quality, and shortens the lifespan of the vines. In this context, the production of healthy grapevine clones is essential to enable the transition of the Moldovan nursery sector towards the production of planting material of the Certified biological category.

3.3 Production of healthy grapevine clones through phytosanitary selection

3.3.1 Identification of clone candidates through visual field inspection

Only vines free of visible symptoms of viral, phytoplasma-associated and bacterial diseases were included in the study. A total of 109 grapevine plants were identified and marked, originating from a wide range of cultivars, including 11 newly bred varieties, 12 autochthonous varieties, 6 european varieties and 4 rootstock varieties (Table 3.7).

Table 3.7 Classification of grapevine varieties included in the study

No.	Category of grapevine varieties	Grapevine variety names
1	Newly bred varieties	Moldova, Luminița, Muscat de Ialoveni, Basarabia, Codrinschii, Meleag, Tudor, Apiren roz, Onițcanschii belâi, Legenda și Chișmiș lucistâi
2	Autochthonous varieties	Copceac, Plăvaie, Telti Curuc, Negru de Căușeni, Feteasca regală, Feteasca albă, Feteasca Neagră, Busuioaca de Bohotin, Busuioaca de Moldova, Rară neagră, Frâncușa, Zghihara
3	European varieties	Sauvignon, Merlot, Pinot nior, Chardonnay, Pinot gris, Cabernet
4	Rootstock varieties	BxR Kober 5 BB, BxR SO4, RxR 101-14, Maleque

At the end of the grapevine vegetative period, after leaf fall, canes were collected from the marked vines, and plants showing no disease symptoms were transferred to the testing stage.

3.3.2 Diagnosis of grapevine clone candidates

Analysis of infections in clone candidates of newly bred grapevine varieties. To identify the presence of infections in grapevine clone candidates, a total of 39 plants belonging to 11 newly bred varieties were analysed. Among these, 11 plants originated from 3 white-berried varieties: Luminița – 4 plants, Muscat de Ialoveni – 2 plants, and Onițcanskii belâi – 5 plants. The remaining 31 plants belonged to 8 pink- or red-berried newly bred varieties: Apiren roz – 1 plant, Basarabia – 4 plants, Chișmiș lucistâi – 1 plant, Codrinschii – 6 plants, Legenda – 1 plant, Meleag – 3 plants, Moldova – 11 plants, and Tudor – 4 plants.

Testing of the clone candidates from newly bred grapevine varieties showed that 9 out of the 42 analysed samples (21.4%) were completely free of viral infections and bacterial cancer. The following samples were confirmed healthy through repeated testing under laboratory conditions: Luminița (T2-3-40), Onițcanskii belâi (T2-9-16 and TI-9-1-4), Apiren roz (40-42), Basarabia (A-36-33), Chișmiș lucistâi (5-8-1), Legenda (2-13-6), Moldova (13-1-2) and Tudor (56-3-6).

Analysis of infections in clone candidates of autochthonous grapevine varieties. The analysis of infections in grapevine clone candidates from autochthonous cultivars was performed on 32 samples belonging to 11 grapevine cultivars, including five white-berried cultivars—Frâncușa (4 plants), Feteasca albă (1 plant), and Feteasca regală (3 plants)—and six red-berried cultivars—Busuioaca de Bohotin (5 plants), Busuioaca de Moldova (2 plants), Copceac (7 plants), Negru de Căușeni (1 plant), Rară Neagră (1 plant), and Zghihara de Huși (3 plants).

The analysis of testing results for clone candidates from autochthonous grapevine cultivars indicates that plants from white-berried cultivars exhibited a higher frequency of viral infections compared to those from red-berried cultivars, while the incidence of bacterial infections was relatively similar between the two groups, reaching 30.8% in white-berried cultivars and 33.3% in red-berried cultivars. Among the identified viruses, GLRaV-1 was detected with a higher frequency in autochthonous, white-berried cultivars (30.8%) compared to red-berried cultivars (5.2%). Infections with GLRaV-3 were detected at a rate of 23% in white-berried grapevine plants and 10.5% in red-berried plants. GFkV was diagnosed in 23% of samples originating from white-berried cultivars, compared to 13.3% in samples from red-berried cultivars. An important aspect is that GVA was detected exclusively in samples originating from autochthonous, white-berried cultivars (23%), while being absent in samples from autochthonous, red-berried cultivars, as well as in those from newly selected cultivars. The virus GFLV was not detected in the tested samples from autochthonous cultivars.

Analysis of infections in grapevine clone candidates from european cultivars. A total of 35 plants belonging to 6 european grapevine cultivars were tested, including 3 white-berried cultivars (Chardonnay – 5 plants, Pinot gris – 6 plants, Sauvignon – 2 plants) and 3 red-berried cultivars (Merlot – 3 plants, Pinot Noir – 7 plants, Cabernet Sauvignon – 12 plants).

Analysis of the testing results for clone candidates from European white- and red-berried grapevine cultivars shows that white-berried cultivars exhibit a slightly lower incidence of bacterial infections (15.4%) compared to red-berried cultivars (18.2%), indicating a similar distribution of bacterial infections between the two categories. The bacterium *A. vitis* was detected mainly in the cultivars Pinot Gris, Cabernet Sauvignon, and Pinot Noir.

More visible differences were observed in the incidence of viral infections. In white-berried cultivars, GFkV was the most frequently detected virus (30.7%), whereas in red-berried cultivars, GLRaV-3 was predominant (40.9%). This indicates a higher susceptibility of red-berried cultivars to GLRaV-3 infection, while white-berried cultivars were more affected by GFkV. In addition, GLRaV-1 was detected exclusively in white-berried cultivars (7.7%) and was not found in any sample from red-berried cultivars. No infections with GFLV or GVA were detected in any of the european cultivars tested, which reflects a relatively favorable phytosanitary status in this group.

Testing of the clone candidates from the 6 european cultivars showed that 14 out of the 35 samples (40%) were completely free of viral and bacterial infections. The healthy plants identified were: Chardonnay (1-15-5-1, 1-15-5-5, 1-15-3-4, 1-16-2-2); Pinot gris (1-65-5-4, 1-65-2-2), Sauvignon (BII-10-9-1); Cabernet Sauvignon (2-1-1, 2-1-2); Pinot Noir (1-27-11-5, 1-28-1-4, 1-27-6-3, 12-4-4); Merlot (BS-14-4-1). The results of testing the clone candidates from the three grapevine cultivar groups (newly bred, autochthonous and european) revealed notable differences in the incidence of viral infections and *A. vitis* (Table 3.8).

Table 3.8 Distribution of pathogens in grapevine clone candidates from newly bred, autochthonous and european cultivars (2018–2023)

No.	Grape cultivar	Percentage of infected clone candidates (%)					
		Newly bred cultivars		Autochthonous cultivars		european cultivars	
		White-berried	Red-berried	White-berried	Red-berried	White-berried	Red-berried
1	*A. vitis*	63,6	29	30,8	33,3	15,4	18,2
2	GFLV	0	12,9	0	0	0	0
3	GLRaV-1	0	16,1	30,8	5,2	15,4	9
4	GLRaV-3	9	12,9	7,7	10,5	0	40,9
5	GFkV	9	64,5	23	15,7	30,7	27,2
6	GVA	0	0	23	0	0	0
7	Pathogen-free material	27,3	19,3	38,5	31,6	53,8	31,8

Out of a total of 109 grapevine samples representing 25 cultivars, 31 healthy plants were identified, belonging to 23 cultivars. From each cultivar, one plant was selected and subsequently maintained as an initial clone (initial plant).

However, for certain cultivars such as Meleag, Muscat de Ialoveni and Codrinschii, all tested plants were infected. In these cases, variants carrying pathogens that are easier to eliminate were selected, and appropriate sanitation methods were applied. This step is essential to ensure the production of healthy plants for economically or scientifically valuable cultivars in which no infection-free individuals could be selected.

3.4 Sanitation of grapevine clone candidates from viral infections and bacterial cancer

The results of the performed tests demonstrated that grapevine plants which appear visually healthy may still carry latent infections caused by chronic pathogens (Table 3.9). In the case of the grapevine variety Codrinschii Pleșeni, all tested plants were diagnosed as infected with at least one pathogen. Among the six analyzed variants, all were infected with GFkV, either alone or in combination with GFLV or GLRaV-1 (e.g. Codrinschii Pleșeni I-9-1-3 and I-10-7-5), or with the bacterium *A. vitis* (variant I-11-6-1). Variant I-9-8-1 was confirmed to be infected exclusively with GFkV. For the variety Meleag, plants coded T2-19-25 and T2-19-9 tested positive for a mixed infection with GFLV and GFkV, whereas candidate T2-19-7 was diagnosed with an *A. vitis* infection. In the case of the Muscat de Ialoveni variety, variant T1-11-6 was identified as carrying a latent bacterial cancer infection, while T1-11-25 was infected with both *A. vitis* and GFkV.

Table 3.9 Evaluation of grapevine clone candidates from varieties in which all tested plants were positive for *A. vitis* and/or viruses (2018–2023)

No.	Grape cultivar	Identifier	Bacteria	Viruses				
			A. vitis	GLRaV-1	GLRaV-3	GFLV	GFkV	GVA
1	Meleag	T2-19-25	-	-	-	+	+	-
2	Meleag	T2-19-9	-	-	-	+	+	-
3	Meleag	T2-19-7	+	-	-	-	-	-
4	Muscat de Ialoveni	T1-11-25	+	-	-	-	+	-
5	Muscat de Ialoveni	T1-11-6	+	-	-	-	-	-
6	Codrinschii Pleșeni	I-9-1-3	-	-	-	+	+	-
7	Codrinschii Pleșeni	I-9-7-4	-	-	-	+	+	-
8	Codrinschii Pleșeni	I-9-8-1	-	-	-	-	+	-
9	Codrinschii Pleșeni	I-11-6-1	+	-	-	-	+	-
10	Codrinschii Pleșeni	I-10-7-5	-	+	-	-	+	-

Note: (+) – infection present; (–) – infection absent.

To obtain healthy initial plants from these grapevine varieties, two strategic approaches are recommended. The first consists in selecting new clone candidates in the following season – a classical selection method which, however, requires at least one additional year and significant time and material resources, without guaranteeing that the selected plants will be completely healthy, since infections may persist in a latent form. The second option is the application of sanitation procedures to the infected grapevine material. The most effective methods for eliminating viruses and *A. vitis* in grapevine are hot-water and hot-air thermotherapy, applied in combination with *in vitro* culture, which allows the recovery of fully sanitized plants while preserving the genetic integrity of valuable genotypes.

3.4.1 Hot-water thermotherapy for the sanitation of grapevine clone candidates

To evaluate the effect of extending the treatment duration to 45 minutes at a water temperature of 50–52 °C on the viability of grapevine buds, hot-water thermotherapy was applied to cuttings from two grapevine cultivars. Single-bud cuttings were used in the experiment. The control cuttings were kept for 45 minutes in water at 20–22 °C. After treatment, both the tested and control cuttings were maintained at 20–22 °C for 14 days. At the end of this period, the number of viable buds was assessed (Table 3.10).

Table 3.10 Effect of hot-water treatment on bud viability in grapevine propagation material

No.	Grape cultivar	Number of cuttings (pcs.)	Hot-water treatment temperature (ºC)	Exposure duration (minutes)	Number of viable buds after HWT	
					pcs.	%
1	Aligote	333	50	45	300	90,1
2	Aligote	333	51	45	302	90,7
3	Aligote	333	52	45	104	33,5
4	Pinot blank	310	50	45	252	81,3
5	Pinot blank	370	51	45	330	89,2
6	Pinot blank	320	52	45	111	34,7
7	Martor Aligote	100	-	-	-	94
8	Martor Pinot blank	100	-	-	-	93

The results obtained in this study showed that the optimal temperature for maintaining bud viability is 50 ± 1 °C, with an exposure duration of 45 minutes. At this temperature, bud viability remained high for both tested cultivars, Aligote and Pinot Blanc, ranging between 89.2% and 90.7%. In contrast, increasing the water temperature to 52 °C resulted in a significant decrease in bud viability, down to 33.5–34.7%.

Clone candidates from the cultivars Meleag (T2-19-7) and Muscat de Ialoveni (T1-11-6), infected with latent forms of grapevine bacterial cancer, were subjected to hot-water therapy [6]. Prior to treatment, the cuttings were stored in a refrigerated room at 4 °C, then acclimatized under controlled laboratory conditions at 20–22 °C for 24 hours. After acclimatization, the cuttings were sectioned into two-bud segments, labeled, and submerged in water at 24 °C for 24 hours

The actual treatment was carried out in an LP-516 laboratory thermostat, where the cuttings were maintained at a temperature of 50 ± 1 °C for 45 minutes (Fig. 3.1). After treatment, the cuttings were kept at 20–22 °C for 24 hours.

The effectiveness of the thermal treatment was verified through microbiological testing. After 5–7 days, no agrobacterial colonies were observed, indicating that the pathogen *A. vitis* had been successfully inactivated in the treated cuttings. Following the applied treatments, plants of the cultivars Meleag (T2-19-7) and Muscat de Ialoveni (T1-11-6) were confirmed to be free of both viral infections and bacterial cancer and were subsequently advanced to the next stage as initial clones, to continue the accelerated propagation process.

Figure 3.1 Hot-water treatment of grapevine cuttings in a laboratory

Within the conducted study, the effectiveness of hot water thermal treatment for controlling the pathogenic agent *A. vitis* was evaluated, as well as the impact of this treatment on the viability of grapevine material, particularly on buds. Although standard practice worldwide for the eradication of *A. vitis* involves hot water treatment at 50 °C for 30 minutes, the present study proposes the use of a temperature of 50 ± 1 °C for 45 minutes to ensure the elimination of phytoplasmas as well, especially the phytoplasma associated with Bois Noir disease.

3.4.2 Sanitation of grapevine clone candidates against viral infections

Following the diagnostic testing of grapevine clone candidates, all plants of the cultivar Codrinschii Pleșeni were confirmed to be infected with viral and bacterial pathogens. The plant Codrinschii Pleșeni I-9-7-4, infected with GFLV and GFkV, was selected for sanitation through hot-air treatment [6]. The canes were cut into two-bud cuttings and immersed in water at room temperature for 24 hours. After soaking, the upper sections of the cuttings were sealed with paraffin and planted in 5-liter pots filled with peat substrate, then transferred to a KK 500 FIT phytotronic climate chamber for rooting (Fig. 3.2).

During the rooting phase, the chamber temperature was maintained at 25 °C, with a 16-hour photoperiod and a light intensity of 4000 lux. When the cuttings developed roots and the shoots reached 4–5 internodes, the shoot tips were pinched to stimulate the formation of lateral shoots and obtain multiple apices. After the appearance of lateral shoots, the chamber temperature was gradually increased up to 38 °C, at a rate of 3 °C every three days, in order to avoid thermal shock. To prevent overheating of the root system, the pots were covered with a protective film.

Figure 3.2 Grapevine cuttings in pots covered with protective film inside the climate chamber

After 30 days of hot-air treatment at 38 °C, apical shoot tips were selected for introduction into in vitro culture. A total of 24 explants, 0.5–0.8 mm in size, were excised, surface-sterilized in a 15% calcium hypochlorite solution and inoculated onto agar medium in test tubes (40 mm diameter, 150 mm height). After 40 days of treatment, a second set of 24 apical shoot tips was collected and transferred to in vitro culture for further development.

Explant growth was carried out in the culture room under controlled conditions: 26 ± 1 °C, 85% relative humidity, and a 16-hour photoperiod. After 40 days of in vitro cultivation, the explants had developed into plants with 6–8 internodes. During this period, the regenerated plants showed uniform development and intense meristematic activity, characterized by the formation of vigorous shoots. At the end of this stage, the regenerated plants were tested to verify the effectiveness of the hot-air thermotherapy treatment (Table 3.11).

Table 3.11 Results of testing sanitized grapevine material after hot-air thermotherapy at 30- and 40-days post-treatment

Grape cultivar	Identifier	Treatment duration	Replication	Number of samples tested	Viruses	
					GFLV	GFkV
Codrinschii Pleșeni	I-9-7-4	30 zile	1	8	-	-
			2	8	-	+
			3	8	-	-
		Total:		24	-	1 (4,2%)
		40 zile	1	8	-	-
			2	8	-	-
			3	8	-	-
		Total:		24	-	-

Note: (+) – infection present; (–) – infection absent.

The plants treated with hot-air thermotherapy in climate chambers for 40 days tested 100% negative for both viruses. These results indicate that a 30-day hot-air treatment is effective for eliminating GFLV, but does not guarantee complete removal of GFkV, which showed a remaining incidence of 4.2%. Extending the treatment to 40 days resulted in the complete elimination of both viruses.

The study demonstrated that the effectiveness of thermal treatment for virus eradication depends largely on the duration of exposure to elevated temperatures, as well as on its combination with *in vitro* culture. Extending the treatment to 40 days is essential to achieve complete elimination of GFLV and GFkV, whereas shorter treatment periods do not ensure full sanitation. The clone candidate of the cultivar Codrinschii (I-9-7-4) was subjected to 40-day hot-air thermotherapy at 37 °C, after which it was confirmed virus-free, assigned the status of initial clone, and transferred to the stage of rapid multiplication via *in vitro* culture.

3.5 Accelerated multiplication of grapevine clones through *in vitro* culture

The clones obtained through phytosanitary selection and sanitized by thermotherapeutic methods were transferred to the accelerated multiplication stage. The canes that tested negative for viruses and bacterial cancer were cut into two-bud cuttings and subjected to hot-water treatment to suppress potential phytoplasma, bacterial, or fungal infections. After treatment, the cuttings were planted in peat substrate in pots and placed in climate-controlled chambers. This stage ensured the production of vigorous biological material required for the initiation of *in vitro* culture.

After 30 days, when the plants entered an active growth phase, the first collection of apical shoot tips was carried out for in vitro initiation. At this stage, green shoots were pruned back to the fourth or fifth true leaf. This operation stimulates the development of lateral shoots and increases the number of apical explants available for subsequent in vitro introduction.

The initial explants, measuring 0.8–1.0 cm, were cultured on an agar-solidified nutrient medium based on half-strength Murashige & Skoog (MS) salts, supplemented with sucrose (20 g/L) and agar (5.5 g/L). The medium was autoclaved at 121 °C for 20 minutes. Each culture tube was labeled with the cultivar name and planting date and then transferred to the culture room (Fig. 3.3).

Figure 3.3 *In vitro* culture room for grapevine micropropagation

By applying the described technology, root formation in cultivars such as Moldova, Floricica, Feteasca Neagră, Plavai, and Copceac began within 7–9 days, and after 30–35 days the in-vitro plantlets reached the developmental stage required for multiplication. In the case of seedless grape cultivars – Chișmiș moldovenesc, Chișmiș lucistii, and Apiren roz – roots were formed after 14–15 days, and the plantlets reached 6–8 internodes within 45–50 days. These results confirm the cultivar-specific development rate, with the total growth period varying between 30 and 50 days.

The regenerated grapevine plants were further multiplied through accelerated *in vitro* micro-cutting, a technique based on fragmenting the plantlets into small micro-cuttings, using nodal or apical segments (Fig. 3.4 A). Each regenerated plant was divided into 5–7 micro-cuttings, which were inoculated into containers filled with the "Biona-311" substrate (Fig. 3.4 B). Multiplication of the plants within each cultivar continued until the required number of plantlets was obtained. Once the plants grown in containers with "Biona-311" substrate reached the upper limit of the vessel, the container was removed, allowing the plants to continue their growth in the same substrate under *ex vitro* conditions (Fig. 3.4 C).

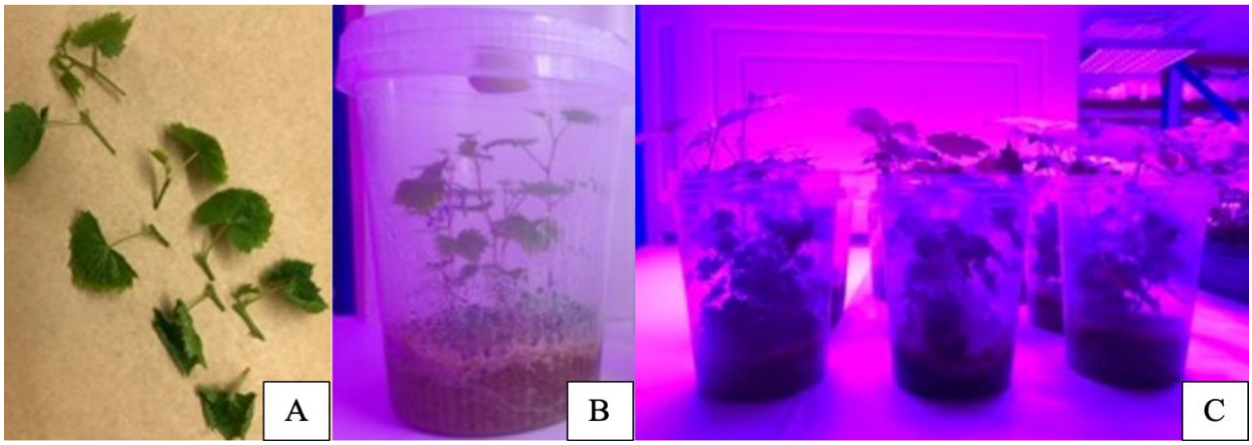


Figure 3.4 Micropropagation of grapevine on the "Biona-311" substrate

A – multiplication of the regenerated plant; **B** – development of micro-cuttings under *in vitro* conditions in containers.

As the plants continued to grow, they exceeded the limits of the container, and the leaves began to exhibit the morphological traits characteristic of the cultivar. After removing the lid, the

substrate inside the container was periodically moistened with sterile water to maintain optimal growth conditions. The adaptation of the plants to the ion-exchange substrate took place over a period of 7–14 days, with a survival rate ranging from 93% to 98%, depending on the cultivar.

After acclimatization, the plants were transplanted into multicellular trays filled with peat-based substrate. At this stage, a balanced development of the root system and a favorable morphophysiological adaptation of the plants were observed, indicating a successful acclimatization process and their readiness for transfer to the growth chamber (Fig. 3.5).

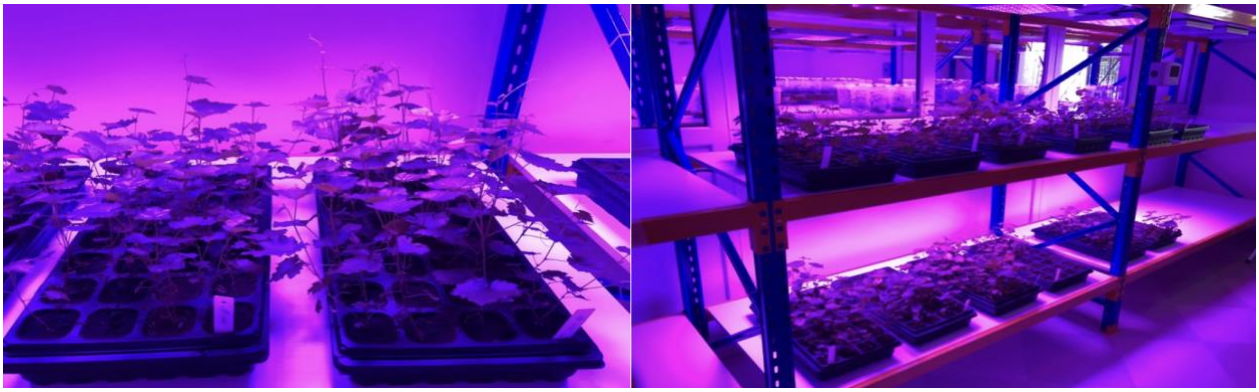


Figure 3.5 Grapevine plants during the acclimatization stage in multicellular containers in the growth chamber

The essential environmental parameters in the growth chamber, including temperature (25 ± 1 °C), humidity, and a 16-hour photoperiod, were maintained constant to ensure continuous plant development. This approach prevented physiological shock and facilitated a smooth transition to the new growth conditions without affecting the developmental rate. The favorable conditions in the culture room enabled efficient root formation in the peat substrate, ensuring vigorous growth and uniform plant development. After a period of 2–3 weeks, once the plants reached an optimal growth stage, they were transferred to the greenhouse for further development. This step allowed gradual acclimatization under semi-controlled conditions, preparing the plants for adaptation to the external environment.

In the case of transplanting carried out during the spring–summer period, the plants were transferred to the greenhouse immediately after the development of a well-formed root system in the peat substrate. When transplanting took place during the autumn–winter period, the plants were maintained in the growth chamber until early spring, to avoid exposure to unfavorable environmental conditions.

The greenhouse to which the grapevine plants were transferred from the growth chamber was equipped to ensure year-round maintenance of the plants and to support the cultivation of vegetative cuttings suitable for planting in the mother plantation. During winter, the greenhouse was heated to maintain optimal growth conditions. The minimum temperature was regulated at +5 °C during the night and between +10 and +15 °C during the day, thus ensuring proper cane maturation and promoting the natural leaf-fall process. Maintaining a stable thermal regime and good air circulation inside the greenhouse contributed to balanced plant development and reduced the risk of physiological disorders. In February, the grapevine plants were pruned to 2–3 buds to obtain a well-developed shoot.

Throughout the spring–summer period, the greenhouse was covered with a shading net and equipped with an automatic forced-ventilation system, which contributed to maintaining optimal

temperature and air humidity inside. Preventive chemical treatments were periodically applied to control fungal diseases, along with balanced foliar and root fertilization, to ensure plant health and optimal development. The greenhouse cultivation phase ensured the physiological strengthening of the plants obtained through micropropagation and their preparation for field planting. The controlled conditions enabled the production of vigorous, uniform vegetative material suitable for establishing mother plantations of higher biological category.

The *in vitro* multiplication process, carried out in culture rooms and greenhouse conditions, enabled the production of plants derived from 23 grapevine cultivars and 4 rootstocks. A total of 7,179 clonal plants were obtained, intended for the establishment of Pre-basic mother plantations.

Table 3.12 List of grapevine cultivars and clones multiplied through *in vitro* microcutting

No.	Grape cultivar	Clone	Quantity (pcs.)
1	Copceac	cl.INVV18-2	426
2	Legenda	cl.INVV21-36	325
3	Pinot noir	cl.INVV 12-44	418
4	Plăvaie	cl.INVV21-22	355
5	Telti Curuc	cl.INVV 11-55	400
6	Moldova	cl.INVV13-12	450
7	Apiren Roz	cl.INVV 40-42	393
8	Merlot	cl.INVV 14-41	103
9	Negru de Căușeni	cl.INVV21-1	207
10	Fetească regală	cl.INVV10-71	379
11	Fetească albă	cl.INVV13-11	214
12	Sauvignon	cl.INVV10-91	342
13	Chișmiș lucistâi	cl.INVV5-81	399
14	Tudor	cl.INVV56-36	364
15	Luminița	cl.INVV3-40	74
16	Busuioacă de Moldova	cl.INVV19-57HR	163
17	Busuiocă de Bohotin	cl.INVV 18-67HR	157
18	Codrinschii	cl.INVV 9-81	121
19	Codrinschii	cl.INVV9-74	65
20	Onițcanskii belâi	cl.INVV9-14	200
21	Chardonnay	cl.INVV15-55	147
22	Pinot gris	cl.INVV65-64	129
23	Meleag	cl.INVV 19-7	87
24	BxR Kober 5 BB	cl.INVV3-21	174
25	BxR SO4	cl.INVV6-42	246
26	RxR 101-14	cl.INVV11-32	291
27	Maleque	cl.INVV01MD	550
Total plants:			7179

As shown in Table 3.12, *in vitro* multiplication ensured a diverse production of graft and rootstock grapevine varieties. Compared to traditional propagation methods, such as green grafting or hardwood cutting, the *in vitro* method offers several advantages. According to studies carried out in Italy, the impact of *in vitro* culture on the phenotype of grapevine plants was investigated. The main findings indicate that, although most of the analyzed parameters did not show significant differences, plants obtained through micropropagation had a higher yield compared to plants grown from hardwood cuttings. These results are consistent with the data obtained in the present study, where the application of the *in vitro* method enabled the production of many plants in a

short period of time, while maintaining phytosanitary quality. Compared to traditional methods, the *in vitro* approach proved to be more efficient in producing high-quality planting material, reducing the time required for multiplication and ensuring the production of grapevine plants free from viruses, phytoplasmas, and bacterial cancer.

3.6 Establishment of Pre-basic and Basic mother plantations

To minimize the risk of infections and to ensure optimal conditions for the development of sanitized plants, the Pre-basic mother plantation was established on land where grapevines had not been cultivated for the past 20 years. This preventive measure helps reduce the risk of contamination with soil-borne pathogens, such as *Xiphinema index*, which can persist in the soil for long periods.

The rooted grapevine cuttings used for the establishment of the Pre-basic mother plantation were planted at a spacing of 3 meters between rows and 1.4 meters between plants within the row (Fig. 3.6). This planting layout was selected to ensure optimal vine development while facilitating subsequent maintenance operations. The chosen spacing improves air circulation and provides uniform light exposure, both of which are essential factors for healthy vine growth.

Figure 3.6 Grapevine cuttings in the vegetative stage obtained through *in vitro* microclonal propagation. A – plants rooted in peat substrate and grown in the laboratory greenhouse; **B** – plants cultivated in the Pre-basic mother plantation.

Thus, the grafted grapevine cuttings grown and strengthened in the greenhouse were planted in the Pre-basic mother plantation, where clones of 23 scion varieties and 4 rootstock varieties are currently grown on an area of 3.05 ha [3]. The grapevine canes obtained from the Pre-basic mother plantation were subsequently used to produce grafted cuttings, which were used to establish the Basic mother plantation. This plantation covers an area of 12 hectares and includes clones of 29 grapevine varieties, intended both for wine production and for fresh consumption. Each year, the assortment of the mother plantations is supplemented with new table and wine varieties that meet the current requirements of viticulture and winemaking.

Soil fertilization was carried out periodically with organic and mineral fertilizers, according to agrochemical recommendations, to maintain nutritional balance. To ensure the phytosanitary status of the plantations, preventive treatments against fungal and bacterial diseases were applied, using approved plant protection products. The implementation of these measures contributed to the harmonious development of the vines and to maintaining a consistent vigor of the clones, ensuring uniform growth, complete wood maturation.

Given the current phytosanitary situation, characterized by a high level of infection with leafroll-associated viruses (GLRaV-1, GLRaV-3), grapevine fanleaf virus (GFLV), grapevine fleck virus (GFkV), *A. vitis*, as well as '*Ca. Phytoplasma solani'* — the phytoplasma responsible for Bois Noir disease, the application of an integrated biotechnology for obtaining sanitized grapevine clones is proposed (Fig. 3.7).

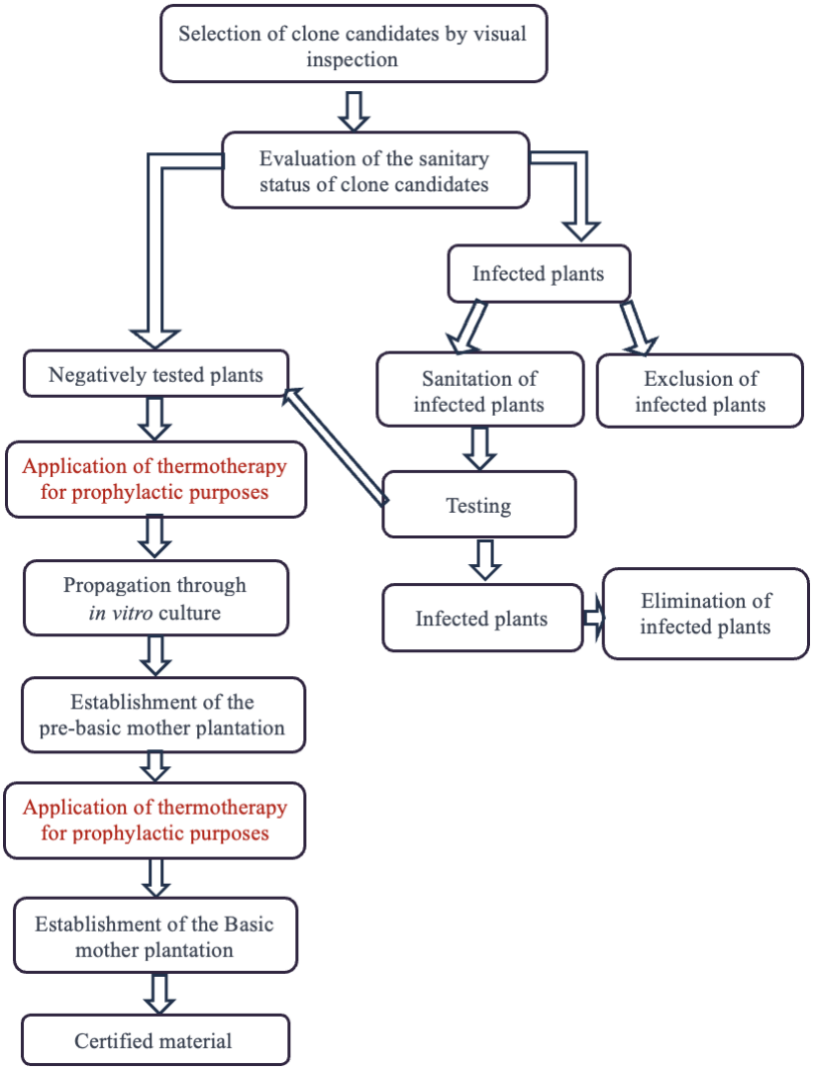


Figure 3.7 Biotechnological scheme for obtaining sanitized grapevine clones

The biotechnological scheme presented highlights the logical succession of the stages of selection, diagnosis, sanitation, and multiplication applied in the process of obtaining healthy grapevine clones. The comparison between the classical method and the biotechnology proposed in this study is shown in Figure 3.8.

The traditional method, developed in the 1980s, involved a long sequence of stages: visual selection of clone candidates, biological testing by indexing (up to 3 years), thermal sanitation, and vegetative multiplication, resulting in a total duration of 9–10 years. In contrast, the method proposed in this study applies modern diagnostic techniques, which reduce the testing period to 2–10 days, while *in vitro* culture enables the complete cycle of obtaining healthy clones to be achieved in only 3 years.

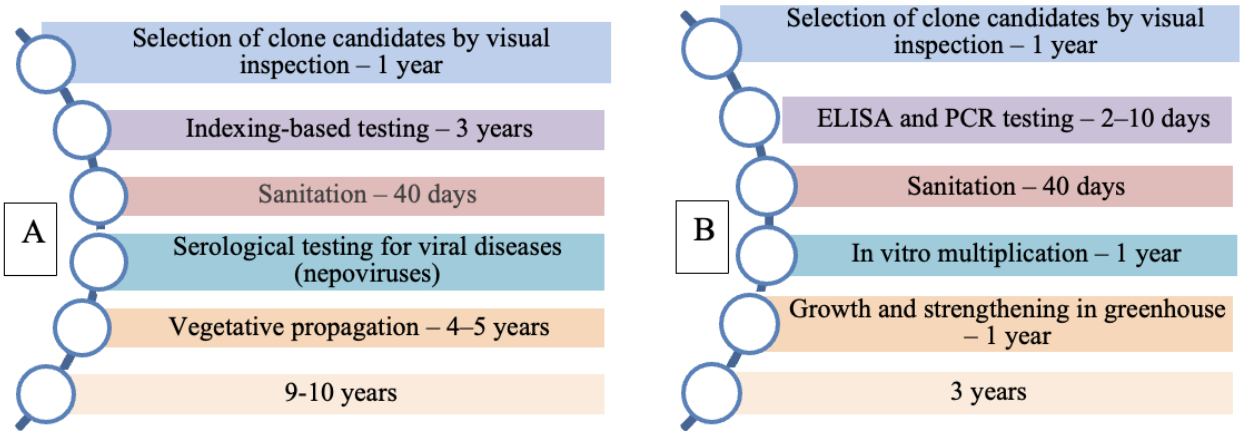


Figure 3.8 Comparison of technologies for obtaining vegetative grapevine plants intended for establishing a Pre-basic mother plantation. A – method applied in the 1980s; **B** – modernized biotechnology developed within the present study.

Thus, for the first time in the Republic of Moldova, a complex biotechnology has been developed and tested, based on modern methods of diagnosis, sanitation, and accelerated multiplication through *in vitro* culture, which enables the production of initial grapevine clones and the establishment of Pre-basic and Basic mother plantations within a significantly reduced timeframe

4 OPTIMIZATION OF TECHNOLOGICAL ELEMENTS FOR THE PRODUCTION OF SANITIZED GRAPEVINE CLONES

4.1 Evaluation and implementation of molecular diagnostic methods for bacterial cancer in grapevine material

In the Republic of Moldova, the identification of pathogenic bacteria from the Agrobacterium complex was, until recently, carried out using classical microbiological methods and bioassays on indicator plants. These methods require 5–7 days for genus-level identification and up to 3–5 weeks to confirm pathogenicity. In addition to being time-consuming, they may also be inefficient, as they involve a high degree of subjective interpretation. Currently, molecular methods such as polymerase chain reaction (PCR) are used worldwide, offering higher sensitivity and specificity compared to traditional techniques.

In this context, six grapevine plants from cultivars and elite selections, aged over 30 years, were selected from the experimental vineyards of the NIARAVM collection. From visually healthy vines, showing no external signs of tumors or tissue deformities, one-year-old canes were collected. The sampled cane sections were debarked and cut into 3–5 mm fragments. After plating, the bacterial isolates were incubated for 7 days at 27 °C to assess the presence of colonies characteristic of *Agrobacterium spp*. The colonies showed uniform growth across the entire surface of the medium, without the formation of inhibition zones or contamination, and exhibited the characteristic morphology of bacteria from the *Agrobacterium spp*. complex: white, convex, glossy appearance with a red-pigmented center.

For the detailed analysis of agrobacteria isolated from grapevine planting material, the Triplex PCR (end-point) method was applied, which enables the simultaneous identification of the genus and assessment of the pathogenic potential of the strains [5]. This method targets three

essential molecular markers: the *pehA* gene, used as a species-specific marker for the identification of *A. vitis* and for its differentiation from *A. tumefaciens*, through a specific DNA fragment of 466 bp; the *virF* gene, which encodes the synthesis of nopaline- and octopine-type opines associated with Ti plasmids involved in tumor induction, generating a PCR fragment of 382 bp; and the *virD* gene, a marker responsible for the production of vitopine, another compound characteristic of Ti plasmids and associated with the pathogenicity of the strains, with an amplified fragment of 320 bp. The use of this detection system allows not only the confirmation of the species *A. vitis* or *A. tumefaciens*, but also the identification of the Ti plasmid type present in the isolate (Fig. 4.1). All isolates showed a 466 bp band, confirming that they belong to the species *A. vitis*. The results obtained from PCR testing of the analyzed plants demonstrate that this molecular technique enables rapid and reliable diagnosis of the pathogenicity of agrobacteria from the genera *Allorhizobium vitis* and *Agrobacterium tumefaciens*, through the direct detection of the *pTi* plasmid.

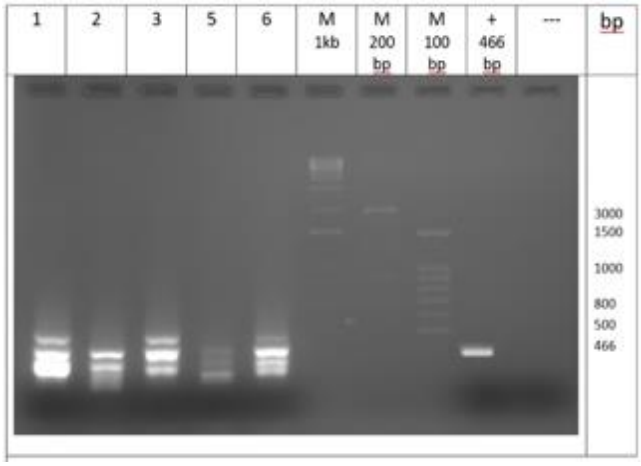


Figure 4.1 Electropherogram obtained after PCR for the identification of *A. vitis* and *A. tumefaciens*
M – molecular weight marker (1 kb, 200 bp, 100 bp);
• – positive control for the pehA fragment (466 bp).

4.2 Influence of LED light spectra on the development of grapevine plants in *in vitro* culture

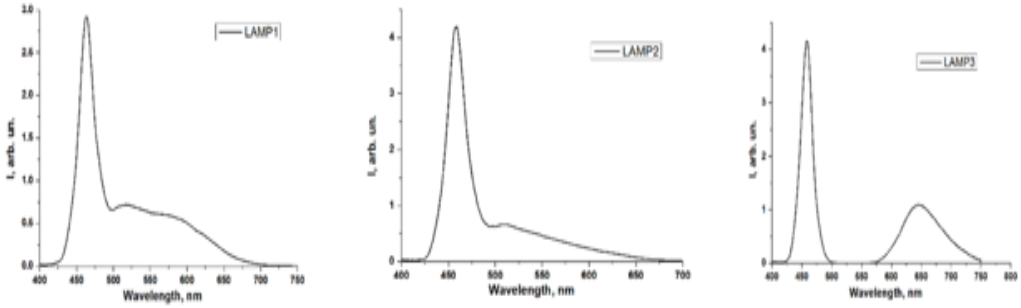
This study aimed to evaluate the effect of different LED light spectra on the regeneration and development processes of grapevine in *in vitro* culture, with the objective of identifying optimal conditions for the accelerated multiplication of sanitized clones [4]. The experiment focused on analysing the performance of plants grown under three types of LED light sources with distinct spectra, under controlled laboratory conditions (Table 4.1).

Table 4.1 Photometric measurement results of the lighting sources used

No.	Lighting source	Measured color temperature, K	PPFD max, $\mu mol \cdot s^{-1} \cdot m^{-2}$	DLI max, $mol \cdot m^{-2} \cdot d^{-1}$
1	„Cool-white”	5950±50K	63	4.05
2	„Warm-white”	3200±50K	30	2.04
3	„Phyto”	≤1700 K	25	1.64

The analysis of the emission spectra of the lighting devices used in the experiment revealed important characteristics of the composition of photosynthetically active radiation (PAR, 400–700 nm), which is responsible for activating the photochemical processes essential for plant development. Grapevine plants efficiently absorb spectral components in the blue region (430–470 nm) and the red region (630–680 nm), where photosynthetic pigments, especially chlorophylls, show maximum absorption activity. Although chlorophyll absorbs predominantly in the violet-blue region, the processes associated with carbon fixation during photosynthesis reach maximum efficiency in the presence of red light.

In this study, the emission spectra of the light sources used were determined through photometric measurements under controlled laboratory conditions (Fig. 4.2). The analysis of the distribution of photosynthetically active radiation (PAR) at the culture surface revealed a non-uniform dispersion of values depending on the distance from the source. This phenomenon can be explained by the inverse square law, according to which radiation intensity decreases proportionally to the square of the distance. The emission spectra obtained for the three lighting sources are shown in Figure 4.2.



A B C

Figure 4.2 Emission spectra of the lighting sources used

A – „Warm-white”**; B** – „Cool-white”; **C** – „Phyto”

For the light source „Warm-white”, a maximum emission peak was observed in the blue region of the spectrum, at a wavelength of approximately 465 nm (Fig. 4.3 A). The remaining spectral components — green, yellow, orange, and red (480–675 nm) — were also present, but at a much lower intensity, representing about 20–25% of the maximum level. This distribution indicates a clear predominance of blue radiation, which may positively influence the physiological processes involved in the initial regeneration of *in vitro* plantlets.

The spectrum of the „Cool-white” light source shows a similar profile, with a peak in the blue region at 460 nm. The light intensity in the green–yellow range (550–600 nm) is even lower compared to the „Warm-white” source, indicating a more limited spectral distribution in this region (Fig. 4.3 B). The „Phyto” source exhibits a completely different spectrum (Fig. 4.3 C). In this case, light components within the 500–575 nm range, corresponding to green and yellow wavelengths, are completely absent. The spectrum is dominated by two regions: a maximum peak in the blue zone ($\lambda \approx 465$ nm) and a second, lower peak in the red zone ($\lambda \approx 650$ nm).

Although the „Cool-white” and „Warm-white” light sources showed the highest values for color temperature (up to 6000 K), PAR flux and PPFD (up to 63 $\mu mol \cdot s^{-1} \cdot m^{-2}$), the best effect on root formation during the first 14–30 days of culture was obtained under „Phyto” illumination. This spectrum most effectively stimulated the rhizogenesis process. This result is also supported by the scientific literature, which indicates that combined blue–red radiation promotes root system development. Illumination with the „Cool-white” spectrum favored the development of the aerial part, with plants showing larger leaf surface and regular internodes – traits important for balanced growth. Illumination with the „Warm-white” spectrum induced callus formation and negatively affected the morphology of the vitroplants, which may indicate a stress response and partial inhibition of normal development, confirming the sensitivity of plants to light quality. In conclusion, the use of the „Phyto” spectrum is recommended for initiating rooting.

4.3 Selection of nutrient media for *in vitro* regeneration and accelerated multiplication, and *ex vitro* acclimatization

According to the classical Skoog–Miller model, the ratio between auxins and cytokinins determines the direction of the regeneration process: higher auxin concentrations stimulate root formation, while an excess of cytokinins promotes shoot development.

Based on these premises, the present study investigated the influence of different concentrations of the phytohormones IAA (indole-3-acetic acid) and 2iP (6-(γ,γ-dimethylallylamino) purine) on the regeneration of grapevine explants under controlled laboratory conditions. Four nutrient media variants were evaluated, all formulated on MS medium with the mineral salt concentration reduced to half strength (½ MS). Each variant was supplemented with different combinations of phytohormones, as follows: Variant 1 – 1 mg/L IAA; Variant 2 – 2 mg/L IAA; Variant 3 – 1 mg/L IAA + 0.3 mg/L 2iP; Variant 4 – 2 mg/L IAA + 0.6 mg/L 2iP. These combinations were selected according to the Skoog–Miller model. Ninety grapevine plants grown under *in vitro* culture conditions were analyzed. Evaluations were performed after 40 days of cultivation (Table 4.6).

Table 4.6 Results of the influence of phytohormones on the growth and development parameters of grapevine *in vitro*

Variant of medium	Rooted plants (%)	Developed plants (%)	Average height (cm)	Internodes (no.)	Leaves (no.)	Lateral shoots (no.)	Root dry mass (g)	Shoot dry mass (g)
1	100.0±0.0	50.0±3.2	7.62±0.5	8.0±0.4	10.2±0.6	0.0±0.0	0.90±0.08	0.33±0.03
2	100.0±0.0	50.0±2.8	8.20±0.6	7.8±0.3	12.0±0.7	2.2±0.2	0.90±0.09	0.40±0.05
3	100.0±0.0	80.0±4.1	7.00±0.4	5.5±0.2	10.3±0.4	2.7±0.3	0.46±0.04	0.74±0.06
4	100.0±0.0	63.3±3.6	7.20±0.5	8.3±0.3	9.8±0.5	3.5±0.4	0.34±0.03	0.56±0.05

Note: Values are expressed as mean ± standard deviation, calculated from 3 replicates.

The results obtained confirm that the hormonal formulation based on 1 mg/L IAA and 0.3 mg/L 2iP is optimal for stimulating regeneration and balanced morphological development of grapevine vitroplants, demonstrating its applicability within the technology for obtaining sanitized clones. The differences identified between the tested variants highlight the importance of adjusting the phytohormone ratio according to the genotype, to ensure efficient regeneration.

GENERAL CONCLUSIONS

1. Following the phytosanitary testing carried out between 2018 and 2023, it was determined that the grapevine planting material used in the Republic of Moldova is contaminated with pathogenic agents of viral and bacterial etiology. The most frequently detected viruses were Grapevine fleck virus, Grapevine leafroll-associated virus 3 and Grapevine leafroll-associated virus 1, with their prevalence varying depending on the grapevine group and berry color. Infections caused by a single virus showed an incidence ranging from 0 to 40%, while mixed infections exhibited a significantly higher incidence [3] (section 3.1).

2. Latent infections with *A. vitis* were detected at a high frequency, particularly in the case of autochthonous, white-berried grapevine varieties, with infection rates ranging from 0% to 60%. The presence of healthy vines represents an important prerequisite for identifying authentic

individuals with the potential to be used for clone establishment and subsequent multiplication aimed at producing healthy grapevine planting material [3] (section 3.2).

3. It has been demonstrated that hot-water treatment at 50 ± 1 °C for 45 minutes enables the sanitation of grapevine clone candidates infected with *A. vitis* and, when applied prophylactically, prevents the development of phytoplasma-associated diseases. Hot-air treatment proved effective in eliminating Grapevine fanleaf virus (GFLV) and Grapevine fleck virus (GFkV). When combined with *in vitro* propagation within the phytosanitary selection process, these treatments allow the production, within one year, of grapevine clones free from viral, phytoplasma and bacterial infections [6] (sections 3.4.1–3.4.2).

4. It was established that the PCR method enables rapid and reliable detection of the pathogenicity of *A. vitis* and *A. tumefaciens* strains within a short time frame, through the identification of the *pTi* plasmid as a virulence marker. This technique can be successfully applied to accelerate the phytosanitary selection process and the production of healthy grapevine clones [5] (section 4.1).

5. The *in vitro* cultivation conditions for grapevine were optimized by adjusting the composition of the nutrient medium, selecting an efficient artificial substrate for the acclimatization stage, and adapting the light spectrum in the culture chamber. The administration of growth regulators at concentrations of 1 mg/L IAA and 0.3 mg/L 2iP promoted regeneration, growth, and development of the vitroplants. The use of the "Biona-311" substrate during acclimatization significantly reduced the adaptation period (10–15 days), achieving a survival rate of 95–100%. Among the tested lighting variants, the „Phyto" spectrum (T ≤ 1700 K) efficiently stimulated root system formation, the „cool-white" spectrum (6000 K) supported leaf and internode development, while the „warm-white" light (T ≤ 3000 K) ensured a balanced growth of both aerial and root organs [4] (sections 4.2–4.4).

6. The accelerated *in vitro* propagation method was implemented within the phytosanitary selection process, which enabled the production of 7,179 clonally derived plants used to establish a Pre-basic mother plantation. At present, this plantation includes sanitized clones of 23 graft grapevine cultivars and 4 rootstock cultivars, cultivated on a total area of 3.05 ha [3] (section 3.5).

7. Based on the results obtained regarding the distribution of viral, phytoplasma-associated and bacterial pathogens in vineyards of the Republic of Moldova, a complex technology for obtaining healthy grapevine clones was developed. This technology includes a logical sequence of stages, namely: visual identification of apparently healthy vines; phytosanitary testing to detect latent infections; application of hot-water treatment to the selected initial plants, both for sanitation and prophylactic purposes; accelerated multiplication of healthy plants through *in vitro* micro-cutting; and the establishment of Pre-basic and Basic category mother plantations intended for the production of certified grapevine propagation material (sections 3.3–3.6).

PRACTICAL RECOMMENDATIONS

To reduce the risk of transmission of phytoplasma and bacterial infections, it is advisable that scion canes harvested from commercial vineyards be subjected to hot-water treatment at 50 ± 1 °C for 45 minutes immediately prior to grafting.

For the sanitation of grapevine cuttings infected with phytoplasmas or *Agrobacterium vitis* (the causal agent of bacterial cancer), the application of hot-water therapy at 50 ± 1 °C for 45 minutes is recommended.

Given the absence of effective chemical means for controlling viral, phytoplasma-associated, and bacterial (crown gall) diseases of grapevine, it is recommended that productive vineyards be established exclusively with healthy planting material belonging to the Certified biological category.

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LIST OF SCIENTIFIC WORKS RELATED TO THE THESIS

of Ms. Marcela Dubceac

PhD Candidate, National Institute for Applied Research in Agriculture and Veterinary Medicine

2. Scientific journal articles

1. 1. **DUBCEAC, Marcela**; LOSMANSCHII, Constantin. The influence of LED light spectra on the *in vitro* growth of grapevine plants. *Romanian Journal of Horticulture*, vol. 4, 2023, pp. 35–42. DOI: 10.51258/RJH.2023.03. DOI: https://doi.org/10.51258/RJH.2023.03 (Journal indexed in DOAJ)

2. **DUBCEAC, Marcela**. Methods for sanitation of grapevine protoclonal material in the process of phytosanitary selection. *Acta et Commentationes*, *Natural Sciences*, 2023, no. 2, pp. 74–80. 74 DOI: https://doi.org/10.55505/sa.2023.2.09 ((Journal indexed in DOAJ)

2.3 In journals included in the National Register of scientific journals:

3. **DUBCEAC, Marcela**; HAUSTOV, Evghenii; BONDARCIUC, Victor. Implementation of the PCR method for the identification of pathogenic strains of *All. vitis* causing bacterial crown gall of grapevine. *Știința Agricolă / Agricultural Science*, 2024, vol. I, pp. 48–54. (**Category B** , indexed in DOAJ)

4. **DUBCEAC, Marcela**; BONDARCIUC, Victor. Evaluation of the influence of phytohormones IAA and 2iP on grapevine (*Vitis vinifera*) development in *in vitro* culture. *Pomicultura, Viticultura și Vinificația*, 2024, no. 2(92), pp. 6–14. (National scientific journal, **Category C**, indexed in AGRIS)

5. BONDARCIUC, Victor; SULTANOVA, Olga; **DUBCEAC, Marcela**. *In vitro* micropropagation – a crucial stage in the establishment of grapevine mother plantations. *Pomicultura, Viticultura și Vinificația*, 2024, no. 1(91). ISSN 1857-3142. (National scientific journal, Category C, indexed in AGRIS)

6. **DUBCEAC, Marcela**; BONDARCIUC, Victor, SULTANOVA, Olga, HAUSTOV, Evghenii Establishment of a Pre-base biological category mother plantation of *Vitis vinifera* through phytosanitary selection. *Pomicultura, Viticultura și Vinificația*, 2023, no. 1(89). ISSN 1857-3142. (**Category C**)

7. **DUBCEAC, Marcela**. Evaluation of optical parameters of containers used in grapevine in vitro culture: light transmission and radiation uniformity. Pomicultura, Viticultura și Vinificația, 2025, no. 2.

3. Articles in conference proceedings and other scientific events

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10. **DUBCEAC, Marcela.** Effect of light spectrum on the in vitro multiplication of grapevine. In: Proceedings of the 75th Scientific Conference of Students, Master's and PhD Students. 2022.

5. Other works and domain-specific achievements

11. **DUBCEAC, Marcela.** Establishment of the nuclear stock (pre-base) of the *V. vinifera* genus through phytosanitary selection in the Republic of Moldova. In: *20th Congress of the International Council for the Study of Virus and Virus-like Diseases of Grapevine*, Thessaloniki, Grecia, 2023, pp. 167–169.

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ANNOTATION

Marcela Dubceac, „Biotechnology for Obtaining Healthy Grapevine Clones" PhD thesis in Agricultural Sciences, Chișinău, 2026

Structure of the thesis: Introduction, 4 chapters, conclusions and recommendations, a bibliography with 196 references, 10 annexes, 91 main text pages, 27 figures, and 35 tables. The obtained results have been published in 13 scientific papers.

Keywords: grapevine, viral diseases, bacterial cancer, diagnosis, healthy grapevine clones, thermotherapy, *in vitro* multiplication, grapevine mother plantations.

Field of research: agricultural sciences.

The purpose of the work: improvement of the technology for obtaining healthy grapevine clones, based on modern methods of diagnosis, sanitation, and propagation, aimed at establishing mother plantations of biological category Base.

Research objectives: to determine the spectrum of phytosanitary pathogens and the degree of infection of grapevine planting material in the conditions of the Republic of Moldova; to develop optimal thermotherapy regimes for the sanitation of grapevine plants infected with chronic pathogens; to improve diagnostic methods for bacterial crown gall; to optimize *in vitro* culture conditions for accelerated clonal multiplication through microcutting; to obtain and multiply initial clones of European, local, and newly selected grapevine varieties for the establishment of Base category mother plantations.

Scientific novelty and originality: for the first time in the Republic of Moldova, a complex technology for obtaining sanitized initial grapevine clones was developed and implemented. It integrates the stages of pathogen diagnosis, sanitation, accelerated *in vitro* propagation, and production of planting material for the establishment of mother plantations of the biological category Base.

An important scientific problem solved: the development, testing, and validation of an integrated technology based on advanced biotechnological tools for obtaining and multiplying sanitized grapevine clones, in accordance with national and European regulations on certified planting material. The proposed technology includes stages of phytosanitary selection, high-precision virological and bacterial diagnostics, sanitation treatments, *in vitro* micropropagation under optimized conditions, followed by acclimatization and the establishment of mother plantations of higher biological categories. This constitutes a scientifically grounded solution for modernizing the national system of grapevine planting material production.

The theoretical significance of the work: the research contributes to the scientific foundation of grapevine disease diagnosis and treatment, offering effective tools for identifying and eliminating viral and bacterial pathogens. It also supports the theoretical basis for using *in vitro* culture in the accelerated propagation of sanitized plant material.

The practical significance of the work: the obtained healthy clones were used to establish a Pre-base mother plantation. The plant material from these plantations serves as a source for founding Base category plantations, ensuring the production of certified planting stock in accordance with national standards.

Implementation of scientific results: Grafted and rootstock cuttings from the Base category mother plantation are delivered to grapevine nurseries in the Republic of Moldova to produce certified planting material and the establishment of commercial vineyard.

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BIOTECHNOLOGY FOR OBTAINING HEALTHY GRAPEVINE CLONES

411.09 Plant protection

Summary of the PhD Thesis in Agricultural Sciences

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