ABSTRACT

of the dissertation work by Khassein Altyn on the topic "Technology for the healing and cultivation of grape varietes by *in vitro* methods", submitted for the degree of Doctor of Philosophy (PhD) in the educational program 8D08104 – Plant Protection and Quarantine.

Relevance of the research topic. Viral infections of grapevines represent a key limiting factor in the sustainable development of viticulture, as they lead to reduced productivity, deterioration of crop quality, and a shortened productive lifespan of plantations. Viral diseases are particularly dangerous because grapevines are predominantly propagated vegetatively, which allows viral pathogens to persist and accumulate in plant material and to spread through the use of infected saplings and mother plants.

Historically, viticulture in the Republic of Kazakhstan had a well-established production base. However, in subsequent years, the industry experienced a reduction in planted areas and a weakening of the specialized nursery system. At present, there is renewed interest in the establishment of new vineyards and the renewal of the varietal assortment, which necessitates the development of a sustainable system for supplying the industry with high-quality planting material with guaranteed phytosanitary status.

One of the main factors constraining the development of this sector in Kazakhstan is the shortage of high-quality planting material with low viral load. As a result, grapevine seedlings are predominantly imported from the countries of the European Union, Russia, and China. Among imported agricultural products, fruits, grapes, and their processed products constitute a significant share, with import volumes averaging more than 900 thousand tons over the past five years. However, many fruit and berry crop varieties imported from abroad are insufficiently adapted to the soil and climatic conditions of Kazakhstan. In addition, there is a high risk of introducing planting material infected with various diseases into the country. Currently, more than 600 species of phytopathogenic viruses and bacteria have been identified, and new forms continue to emerge. A notable example is fire blight, which was introduced into the republic through imported seedlings. Within seven years following the first detection of this disease, the affected area of fruit crops increased fortyfold. One of the major causes of this situation is the insufficient development of modern nursery production systems in the country capable of providing high-quality and pathogen-free planting material.

Modern biotechnological approaches based on plant tissue and cell culture techniques provide effective solutions for the recovery and accelerated propagation of commercially valuable varieties. The most effective approaches include the production of regenerants from apical meristems, the application of *in vitro* thermo- and chemotherapy, and the micropropagation of recovered plants, followed by monitoring of their phytosanitary status using serological and molecular methods. The implementation of these technologies ensures the production of standardized, virus-free planting material and reduces the risk of viral pathogen dissemination during the establishment of commercial vineyards.

In this regard, an important and timely task is to conduct a molecular assessment of the phytosanitary status of plants, identify the pathways through which detected viruses have entered the country, apply in vitro techniques for plant sanitation, and optimize protocols for microclonal propagation and *ex vitro* acclimatization of microplants. These approaches enable the production of viable virus-free planting material. The obtained results provide a scientific and practical foundation for the restoration and further development of the domestic grapevine nursery industry.

Objective of the dissertation research:

Assessment of the phytosanitary status of the studied grapevine varieties using molecular methods, determination of the pathways of introduction of the identified viruses into the country, plant sanitation based on *in vitro* techniques, establishment of plants in *in vitro* culture, and optimization of protocols for microclonal propagation and adaptation of microplants to *ex vitro* conditions in order to obtain viable and pathogen-free grapevine planting material.

Research tasks:

- to assess the presence of viral infections in the source plants of the grapevine cultivars under study using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) techniques;
- to perform a phylogenetic analysis of the major grapevine viruses in order to determine the pathways of pathogen introduction into the territory of the Republic of Kazakhstan and to evaluate their genetic diversity.
- to compare the effectiveness of *in vitro* methods for grapevine virus sanitation apical meristem culture, thermotherapy, and chemotherapy (including their combinations) - based on the following parameters: the yield of viable regenerants and the proportion of virus-negative plants.
- to confirm the phytosanitary status of the regenerants obtained after treatment using ELISA and PCR methods to generate a stock of sanitized material for further propagation.
- to optimize the *in vitro* culture protocol (sterilization, initiation, and cultivation conditions) for obtaining primary regenerants of the studied varieties.
- to optimize the micropropagation of recovered plants by selecting the type and concentration of phytohormones (gibberellins and cytokinins) and to determine regimes that ensure maximum proliferation and normal morphogenesis**.**
- to evaluate the *ex vitro* adaptation scheme for grape microplants and to determine conditions that ensure high survival rates, sustained growth, and the formation of physiologically complete plants under protected cultivation.

Research Methods.

The study employed methods of cell and tissue culture, *in vitro* microclonal propagation of plants, and cultivation of apical meristems (meristem culture), as well as thermotherapy, chemotherapy, and molecular biology techniques.

Main points to be defended:

The infection status of source grapevine plants was evaluated for the major economically important viruses using molecular diagnostic methods.

Phylogenetic analysis of the major grapevine viruses was performed to identify the probable routes of pathogen introduction into the Republic of Kazakhstan and to assess their genetic diversity.

The comparative effectiveness of various *in vitro* grapevine sanitation methods - including apical meristem culture, thermotherapy, chemotherapy, and their combinations - was experimentally established in terms of the production of viable regenerants and the reduction of viral load, as confirmed by ELISA and PCR methods.

Protocols for *in vitro* introduction and regeneration of grape plants have been optimized, ensuring the consistent production of primary regenerants suitable for subsequent clonal propagation.

Protocols for micropropagation of recovered regenerants have been optimized based on the selection of combinations and concentrations of phytohormones (gibberellins and cytokinins), ensuring increased propagation rates and the stabilization of morphogenesis.

Methods for *ex vitro* adaptation of grape microplants have been optimized and validated, ensuring high survival rates and the formation of physiologically complete plants under protected soil conditions.

Description of the main research results.

The viral load of grapevine stocks grown in various regions was assessed using enzyme-linked immunosorbent assay (ELISA) and PCR methods. The presence of viral pathogens in the grapevine material was confirmed, indicating a high phytosanitary risk and highlighting the need to develop a scientifically substantiated technology for improving grapevine health and producing virus-free planting material to prevent the spread of infections during propagation and the establishment of new vineyards.

To improve plant health with respect to viral diseases, a combination of *in vitro* methods - including apical meristem culture, thermotherapy, and chemotherapy - was optimized, and their effectiveness was comparatively evaluated. Following these treatments, the regenerated plants were retested using ELISA and PCR methods, which allowed the identification of plants that were completely or partially free of viral pathogens.

A technology for producing *in vitro* cultures of 15 grape varieties has been optimized, and a protocol for generating primary regenerated plants with reduced viral load and suitable for subsequent micropropagation has been developed. By selecting appropriate concentrations and combinations phytohormones (gibberellins and cytokinins), the most effective methods for micropropagation of healthy regenerated plants were determined, ensuring increased proliferation rates and the stabilization of morphogenesis processes.

Optimal conditions for the *ex vitro* adaptation of grape microplants obtained *in vitro* to protected soil conditions have been identified, ensuring high survival rates and the formation of physiologically complete plants**.**

The dissertation is devoted to the development, scientific substantiation, and optimization of biotechnological approaches based on *in vitro* technologies aimed at the sanitation of grape plants (*Vitis vinifera L.*) from viral infections. The infection status of the source plant material of 15 grapevine cultivars - Pobeda, Rizamat, Guzal

kara, Asiya, Baikonur, Medeu, Aisulu, Alma-Ata, Kara koz, Kyzyl tan, Alisa, Arkadia, Victoria, Bagrovy, and Alyoshenkin - was assessed using molecular diagnostic techniques. The detected viruses were subjected to phylogenetic analysis, which allowed the probable pathways of pathogen introduction into the Republic of Kazakhstan to be elucidated. A comparative evaluation of the effectiveness of sanitation methods was conducted, and protocols for obtaining regenerant plants and their subsequent microclonal propagation were optimized. Special attention was given to the adaptation of sanitized microplants to *ex vitro* conditions (protected soil) to produce viable planting material suitable for further use in nursery production.

Justification of the novelty and importance of the obtained results.

Scientific novelty of the research. The scientific novelty of the research lies in the first detection of Raspberry bushy dwarf virus (RBDV) in grapevine in Kazakhstan, as well as in the establishment and experimental validation of effective sanitation protocols for this cultivar based on the application of apical meristem culture in combination with thermo- and chemotherapy. For the first time, genotype-specific responses of the studied grapevine cultivars to 6-benzylaminopurine (6-BAP) were investigated under *in vitro* conditions, and its optimal concentrations were determined, ensuring a high regenerative potential of explants while reducing the intensity of callus formation.

Practical significance of the research. The results obtained and the developed integrated technology for producing virus-free grape saplings - including diagnostics, treatment, *in vitro* micropropagation, and *ex vitro* adaptation - can be implemented in nurseries and specialized laboratories to produce standardized, healthy planting material for establishing mother plants and commercial vineyards. The use of regenerated saplings, treated for viral diseases using biotechnological methods, enhances the phytosanitary quality of planting material, reduces the risk of viral infections, and contributes to the sustainable development of viticulture in the Republic of Kazakhstan.

Compliance with the directions of scientific development or state programs.

This dissertation is also substantiated within the framework of the program targeted funding project BR21881942 "Development of biotechnological approaches for the control of phytopathogens in order to increase the productivity of crops" (2023–2025).

Description of the doctoral candidate's contribution to the preparation of each publication.

The author of this dissertation independently defined the research goals and objectives and determined the object and methodological approach for conducting the experiments. The candidate organized and carried out a series of studies on the improvement of grapevine plant health *in vitro*, including apical meristem culture, thermo- and chemotherapy, and subsequent monitoring of the phytosanitary status of regenerated plants using ELISA and PCR methods. The author optimized protocols for *in vitro* culture and micropropagation of improved plants by selecting appropriate combinations and concentrations of growth regulators. Methods for *ex vitro* adaptation of grapevine microplants under greenhouse conditions were also developed and experimentally validated.

The experimental data were processed, statistically analyzed, and interpreted, and conclusions and practical recommendations were formulated. The author personally prepared the dissertation text, formatted the results, and published the associated research findings.

Publication of dissertation results. The main results of the dissertation were published in 6 scientific articles, including: 1 article in a journal indexed in the Scopus database with a Q3 quartile index (37%), 3 articles in scientific journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, and 2 publications in the proceedings of international scientific conferences. The author's h-index is 1.

Volume and structure of the dissertation.

The total volume of the dissertation is 139 pages. The dissertation consists of an introduction, 4 sections, conclusion and recommendations for practical application, and a list of references. The work includes 43 figures, 14 tables, and 6 appendices.The reference list includes 235 publications by domestic and international researchers.