

Using modern biotechnologies for conservation of the genepool of commercial and wild grapes

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Received: November 01, 2025; Reviewed: November 30, 2025; Accepted: December 10, 2025

Plant genetic resources represent a fundamental basis for biodiversity and the sustainable development of agriculture. Their conservation is critically important for breeding, adaptation to climate change, and ensuring food security. One of the most effective approaches for the preservation and regeneration of rare and endangered plant forms is the use of *in vitro* technologies. This study aimed to develop an *in vitro* initiation protocol for commercial grape cultivars «Rizamat» and «Taifi Rozoviy», as well as the wild subspecies *Vitis vinifera* ssp. *silvestris*. Thimerosal was used as a sterilizing agent at different concentrations. The most effective treatment was 0.1 mg/L for 5-7 minutes, which ensured complete sterility and 100.0% explant survival. Two hormone-free nutrient media, MS and WPM, were tested. The MS medium demonstrated superior performance, with 90.0% bud break and up to 80.0% shoot induction. Shoots developed strong morphology and normal structure. In contrast, the WPM medium resulted in slower development and thinner shoots. Rooting was performed on a modified MS medium supplemented with 0.2 mg/L NAA. Optimal rooting occurred in shoots measuring 2.5-3.0 cm in height and bearing 2-3 well-developed leaves. These shoots developed complete and functional root systems. The developed protocol enables efficient cultivation of both cultivated and wild grape forms. It provides a valuable foundation for the production of healthy planting material and for the long-term conservation of grapevine germplasm. The results are applicable in the practice of micropropagation, grapevine biotechnology, and breeding programs in Uzbekistan and other countries with similar agro-climatic conditions.

Keywords: Grape, micropropagation, *in vitro*, production of healthy mother plants

INTRODUCTION

Plant genetic resources form the foundation of biodiversity and sustainable agricultural development. Their conservation is essential for crop breeding, adaptation to climate change, and ensuring food security.

Global climate change, agroecosystem degradation, and the expansion of viticulture into stress-prone regions require a re-evaluation of strategies for conserving and regenerating the genepool of both cultivated and wild grapevine (*Vitis* spp.). The genetic diversity of this crop determines its adaptability, resistance to diseases and pests, and underpins effective breeding and the development of competitive planting material (Abdullaev et al., 2025).

Modern viticulture in Uzbekistan, despite its scale and export potential, relies largely on grape cultivars developed in the mid-20th Century. These cultivars show limited resistance to major pathogens and abiotic stresses. At the same time, wild

grapevine forms with high resistance and valuable adaptive traits remain underexplored and insufficiently conserved (The bouquet of Uzbek winemaking: Grapes and their features, 2025).

Field collections are the traditional method for preserving vegetatively propagated grape germplasm, but they are subject to biological risks (*infections, mutations, plant senescence*) and organizational risks (*limited funding, natural disasters, loss of accessions*). These limitations emphasize the need for alternative conservation strategies (Zlenko et al., 2003).

In vitro technologies are among the most effective methods for conserving and restoring rare and endangered plant species. Cell and tissue cultures make it possible to maintain genetic material in the form of callus lines, microclones, or cell suspensions. This approach is particularly relevant for species under threat of extinction, with narrow geographic distribution or low seed productivity. Methods such as *in vitro* regeneration, cryopreservation, and micropropagation allow not only for maintaining collections in laboratory conditions but also for restoring populations in natural habitats if needed (Abdullaev et al., 2025; Zlenko et al., 2003; Murashige and Skoog, 1962).

Modern biotechnological approaches, including *in vitro* culture, offer new opportunities for the sanitation of grapevine plants, the production of planting material, and the long-term conservation of genetic diversity. *In vitro* micropropagation accelerates the production of healthy mother plants suitable for vegetative propagation and the establishment of high-yielding, disease-resistant vineyards (The bouquet of Uzbek winemaking: Grapes and their features 2025; Uzbek National Encyclopedia, 2000). These methods are also used in grape breeding to rapidly develop hybrids with valuable traits, including tolerance to biotic and abiotic stresses (Zlenko et al., 2003).

The development of effective biotechnological protocols for *in vitro* introduction of both industrial cultivars and wild grapevine forms makes it possible to:

- produce sterile and genetically uniform plants;
- accelerate the sanitation and mass propagation process;
- preserve unique genotypes in both laboratory and natural conditions;
- ensure genetic stability and integrity of collections;
- facilitate breeding progress under controlled environmental conditions (Zlenko et al., 2003; Uzbek National Encyclopedia, 2000).

Thus, integrating classical approaches with advanced biotechnological methods, particularly *in vitro* technologies, is a strategic direction in the conservation and sustainable use of plant genetic resources.

Among the fruit crops widely cultivated worldwide, grapevine (*Vitis* spp.) holds a leading position. Since ancient times, it has been used as a food crop and as a raw material for the wine industry, which accounts for its high economic importance. The genetic resources of grapevine are of strategic value for breeding and adaptation to stress conditions such as drought, high temperatures, and pathogens. Wild subspecies such as *V. vinifera* subsp. *silvestris*, distributed in the mountainous regions of Uzbekistan, are especially valuable due to their potential for conferring resistance and adaptability in new cultivars (Abdullaev et al., 2025).

Despite this potential, systematic breeding programs for grapevine in Uzbekistan are nearly absent. Industrial production still relies on cultivars developed 40-50 years ago, which limits the sector's competitiveness. Moreover, the widespread presence of plant diseases-especially fungal infections-continues to cause substantial losses in both yield and quality. These challenges underscore the urgent need for biotechnological solutions in the country's viticulture sector. Grapevine was among the first woody species for which methods of plant biotechnology and cell engineering were developed (Abdullaev et al., 2025).

Currently, Uzbekistan lacks coordinated programs for implementing advanced biotechnological tools in grapevine conservation and breeding. Addressing this gap requires a shift toward an integrated biotechnology-based strategy (The bouquet of Uzbek winemaking: Grapes and their features, 2025).

One of the key directions is the application of *in vitro* methods for the propagation and preservation of both cultivated and wild grapevine forms. Wild accessions possess a high adaptive potential and may serve as sources for breeding cultivars with resistance to drought, frost, and pathogens. However, the success of *in vitro* techniques depends largely on the initial stage of introducing field material into culture. At this critical phase, obtaining sterile and viable primary explants is essential for ensuring the success of further regeneration and micropropagation (Murashige and Skoog, 1962).

In this context, the development of robust and reproducible *in vitro* protocols for both industrial and wild grapevine genotypes represent a relevant research priority. Such protocols contribute to biodiversity conservation, enhanced crop resilience, and the modernization of viticulture in Uzbekistan.

This study aims to assess the potential of biotechnological approaches for conserving and regenerating the genepool of industrial and wild grapevine accessions, with a focus on implementing highly efficient *in vitro* systems for micropropagation, sterilization, cultivation, and rooting.

Research Aim and Objectives:

Research aim: The aim of this study is to develop optimal conditions for *in vitro* culture initiation of commercial grapevine cultivars cultivated in Uzbekistan, as well as a wild species growing under natural conditions. Special emphasis is placed on evaluating the effect of different silver compounds on the efficiency of obtaining sterile and viable explant material suitable for subsequent micropropagation.

Objectives:

- To select representative commercial grape cultivars and wild *Vitis* species adapted to the environmental conditions of Uzbekistan;
- To conduct a comparative assessment of different sterilizing agents, including silver-based compounds (*silver nitrate*, $AgNO_3$, and *silver thiosulfate*, $Ag_2S_2O_3$), based on explant viability and contamination rates;
- To determine the optimal concentration and exposure time of silver compounds that ensure maximum sterility while maintaining the regenerative potential of plant tissues;
- To refine the technological parameters for *in vitro* culture initiation, including explant type, plant developmental stage, and duration of pre-treatment;
- To establish a foundation for the development of a micropropagation protocol based on the optimized *in vitro* initiation scheme;
- To evaluate the biological response of different grapevine genotypes to the tested conditions, highlighting differences between cultivated varieties and wild forms.

MATERIALS AND METHODS

Plant material. The study employed three grapevine genotypes as initial plant material:

- «Rizamat» cultivar (*Vitis vinifera* L.) - a table grape developed by Uzbek breeders in the 1970s through hybridization of local forms «Katta-Kurgan» and «Parkent» cultivars. It is known for its high productivity, large clusters, and stable market traits.
- «Taifi rozoviy» cultivar (*Vitis vinifera* L.) - a traditional table grape widely cultivated in Central Asia. Although its precise origin remains unknown, the cultivar demonstrates drought tolerance, rich flavor, and good postharvest keeping quality.
- Wild grapevine (*Vitis vinifera* ssp. *silvestris*) - accessions obtained from the genetic collection of the Research Institute of Plant Genetic Resources. According to morphological features and local records, the age of donor plants was at least 30 years. This subspecies is considered a potential donor of stress-tolerant traits.

1. Explant selection and preparation. Apical and sub-apical shoot meristems were used as explants for *in vitro* introduction. Sampling was carried out in spring during the active growth phase. Shoots were first washed under running water and then treated with a detergent solution followed by multistep surface disinfection.

2. Surface sterilization. Silver-based agents, including silver nitrate ($AgNO_3$), were used at concentrations of 5-20 mg/L with exposure times of 5-15 minutes. Conventional disinfectants such as sodium hypochlorite ($NaOCl$, 0.5-1.0%) and ethanol (C_2H_5OH , 70%) were also employed.

3. Culture Medium. The introduction was performed on a modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with vitamins and growth regulators, including 0.2 mg/L naphthaleneacetic acid (NAA). For wild grape accessions, cysteine and activated charcoal were additionally included to reduce phenolic oxidation.

4. Culture Conditions. Explants were incubated at $25\pm 1^\circ C$ with a 16-hour photoperiod and a light intensity of 2500 lux. Viability, contamination, and signs of regeneration were assessed on days 3, 7, and 14.

5. Data Analysis. Experimental results were analyzed using one-way analysis of variance (*ANOVA*) via «MS Excel» and «Statistica 10» software packages. Statistical significance was determined by Student's t-test at a confidence level of $p < 0.05$.

Explants and Medium Composition. Nodal stem segments with a lateral (axillary) bud were used as explants. Material was harvested in the early morning during the period of active spring growth. Surface sterilization was carried out using aqueous solutions of thimerosal (sodium ethylmercurithiosalicylate, $C_9H_9HgNaO_2S$) at concentrations of 0.05-0.20% with exposure times ranging from 5 to 15 minutes, followed by rinsing in sterile distilled water. Explants were cultured on hormone-free agar-based MS media [4] containing: 1) standard macro- and micronutrients (as per original MS formulation); 2) 30.0 g/L sucrose ($C_{12}H_{22}O_{11}$); 3) 100.0 mg/L inositol ($C_6H_{12}O_6$); 4) pH adjusted to 5.6 before autoclaving.

Antimicrobial additives. To suppress endogenous microbial contamination, the culture medium was supplemented with: silver nitrate (AgNO₃) at concentrations of 1-5 mg/L, colloidal silver (Ag⁰) at volumes of 0.5-2.0 ml/l. These treatments were designed to assess the impact of silver compounds on explant contamination rates and survival.

Culture conditions and monitoring. Explants were incubated in a climate chamber under the following conditions: 1) temperature: 24±2°C; 2) photoperiod: 16 hours light / 8 hours dark; 3) light intensity: 3000 lux. Contamination was assessed visually on days 3, 10, and 30 post-inoculations. Bacterial and fungal infections were recorded separately.

Replication. For each genotype and sterilization variant, 20 explants were used. All experiments were performed in triplicate. The data were statistically analyzed using ANOVA at a significance level of p < 0.05.

Table. Efficiency of grapevine <i>in vitro</i> introduction stages.			
Stage	Parameter/Medium	Conditions	Result
Sterilization	Thimerosal	0.1 mg/ml, 5-7 min	100% sterile and viable explants
Culture medium	MS	Hormone-free	90.0% bud break, 80.0% - ≥2 shoots
	WPM	Hormone-free	84% bud break, low shoot proliferation
Shoot quality	MS	-	Vigorous shoots, green leaves, normal morphology
	WPM	-	Thin, weak shoots with reduced growth
Rooting	MS + NAA (0.2 mg/L)	Shoots 2.5-3.0 cm with 2-3 leaves	Well-developed root system formation

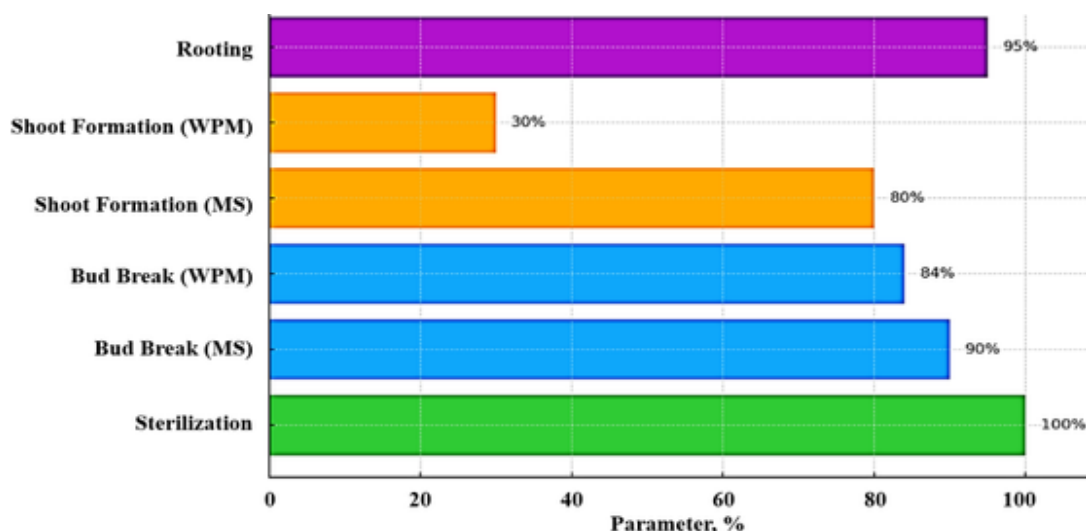


Fig. Efficiency of key stages in grapevine *in vitro* introduction.

RESULTS AND DISCUSSION

During the introduction of grapevine into *in vitro* culture, it was established that the optimal sterilization treatment consisted of using 0.1 mg/ml thimerosal for 5-7 minutes. This treatment ensured 100.0% sterility and viability of explants without signs of phytotoxicity.

A comparative analysis of MS and WPM media revealed the superiority of the MS medium. Explants cultured on MS exhibited:

- Higher bud break frequency (90.0% vs. 84.0% on WPM),
- Greater shoot proliferation rate (80.0% of explants formed two or more shoots),
- Formation of vigorous shoots with normal leaf development and internodes.

In contrast, explants grown on WPM showed slender, weak shoots with slower growth rates and signs of physiological weakness.

At the rooting stage, it was shown that using shoots of 2.5-3.0 cm in length with 2-3 well-developed leaves resulted in maximum rhizogenesis efficiency. Rooting was performed on MS medium, supplemented with 20 g/L sucrose, vitamins, and 0.2 mg/L α -naphthaleneacetic acid (NAA).

All key parameters of the *in vitro* introduction protocol are summarized in Table.

Visual representation of the main phases in the *in vitro* introduction of grapevine: (1) explant surface sterilization, (2) bud break and shoot formation on MS and WPM media, and (3) *in vitro* rooting of microshoots. The highest efficiency across all stages was achieved using MS medium supplemented with 0.2 mg/L NAA during the rooting phase (Figure).

The results demonstrated the high efficiency of thimerosal at a concentration of 0.1 mg/L for the surface sterilization of grape explants. This treatment ensured 100.0% sterile and viable explants, confirming its practical applicability for the initiation of *in vitro* culture of various *Vitis vinifera* cultivars as well as wild accessions. Comparable levels of sterility have been reported using other antiseptics; however, thimerosal proved to be less toxic and more tissue-friendly compared to many commonly used disinfectants (Abdullayev et al., 2025).

A comparative assessment of nutrient media revealed that the Murashige and Skoog (MS) medium was more effective than the Woody Plant Medium (WPM) for shoot induction. This finding is consistent with previous studies where MS medium was successfully used for organogenesis in grapes and other woody species. Shoots regenerated on MS medium exhibited favorable morphology, including normal internode length and bright green leaf coloration, indicating good physiological status of the regenerated plants.

Successful *in vitro* rooting was achieved by supplementing the culture medium with 0.2 mg/L of the auxin α -naphthaleneacetic acid (NAA). The highest rooting response was observed in shoots 2.5-3.0 cm in length with well-developed leaves. These results support previously established morphophysiological criteria for optimal rooting of grape microshoots. The use of MS medium with a reduced sucrose concentration (20 g/L) and a complete vitamin set promoted the formation of a strong root system, which is critical for subsequent *ex vitro* acclimatization.

Particular attention should be given to the successful *in vitro* establishment of the wild subspecies *V. vinifera* ssp. *silvestris*. This result highlights its potential use in breeding programs aimed at enhancing tolerance to abiotic stresses and resistance to pathogens. Wild grape genotypes are known to possess valuable traits, such as drought and disease resistance, and can serve as important parental components in hybridization schemes (Zlenko et al., 2003).

Therefore, the proposed protocol can be recommended for the mass production of pathogen-free planting material for both commercial grapevine cultivars and wild forms utilized in genetic improvement programs.

CONCLUSION

This study demonstrated the effectiveness of biotechnological approaches for the *in vitro* regeneration and preservation of both cultivated and wild grapevine genotypes. Experimental work included the optimization of sterilization parameters, selection of nutrient media, and evaluation of morphogenetic responses during shoot induction and rooting. The findings confirmed the feasibility and reproducibility of the proposed protocols. These conclusions provide scientific justification for the broader application of *in vitro* methods in grapevine sanitation, genetic resource conservation, and breeding.

1. A reproducible protocol for the *in vitro* culture initiation of industrial grape cultivars («Rizamat» and «Taifi rozoviy») and the wild subspecies *Vitis vinifera* ssp. *silvestris*, adapted to the mountainous regions of Uzbekistan, has been developed.
2. The optimal sterilization conditions- 0.1 mg/L of thimerosal for 5-7 minutes - ensured 100% explant survival and complete elimination of microbial contamination.
3. The hormone-free Murashige and Skoog (MS) medium demonstrated the highest efficiency in inducing bud break (90.0%) and shoot formation (80.0%) across all tested genotypes.
4. For *in vitro* rooting, the most effective condition was MS medium supplemented with 0.2 mg/L of NAA, using shoots 2.5-3.0 cm in length with 2-3 well-developed leaves, which resulted in consistent and stable root system development.
5. The wild subspecies *V. vinifera* ssp. *silvestris* exhibited a high regenerative capacity under *in vitro* conditions, confirming its potential as a valuable donor for breeding programs aimed at improving resistance to abiotic and biotic stresses.
6. The obtained results confirm the applicability of the developed technology for grapevine sanitation, rapid clonal propagation, establishment of stress-resilient vineyards, and integration of wild grape forms into biotechnology and breeding programs.

ACKNOWLEDGEMENTS

The authors express their sincere appreciation to the Center for Advanced Technologies (Tashkent, Uzbekistan) and the LLC “Center for the Implementation of Innovations and Technologies” (Tashkent Region, Uzbekistan) for providing institutional support, laboratory facilities, and technical assistance necessary for conducting this study. The authors also acknowledge the contributions of researchers and institutions whose previous work on grape biotechnology and genetic conservation informed this research.

ETHICAL CONSIDERATIONS

This study focuses on the application of modern biotechnological methods for plant genetic resource conservation and does not involve human participants, indigenous knowledge holders, or animal experimentation. All procedures related to plant materials were conducted in accordance with national and international guidelines for the conservation and sustainable use of plant genetic resources. Therefore, ethical approval and informed consent were not required for this research.

AUTHOR CONTRIBUTIONS

- Mirakbar Yakubov: Conceptualization of the study, methodological design, data interpretation, manuscript drafting, and correspondence.

- Fayzulla Abdullaev: Experimental design support, analysis of biotechnological approaches, critical revision of the manuscript, and validation of scientific content.

Both authors have read and approved the final version of the manuscript.

FUNDING

This research did not receive any specific funding from public, commercial, or non-profit funding agencies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this study. No financial, institutional, or personal relationships influenced the research design, data interpretation, or publication of results.

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