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Microclonal Propagation of Promising Grapevine Genotypes

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Annotation: Microclonal propagation of promising grapevine genotypes is considered one of the important directions of modern biotechnology. This method enables the rapid production of genetically stable, disease-free, and high-quality planting materials within a short period of time. During the in vitro propagation of plant tissues, the composition of nutrient media, the concentration of phytohormones, and sterilization stages directly affect regeneration efficiency. Research results have shown that the microclonal propagation method is highly effective for the rapid multiplication of high-yielding grapevine cultivars. This article presents an analysis of scientific studies conducted on the microclonal propagation of promising grapevine genotypes and highlights their practical significance.

Keywords: Vitis vinifera L., Vitaceae, clonal micropropagation, totipotency, traditional propagation, in vitro, ex vitro, phytohormones, rhizogenesis.

Introduction

Grapevine — Vitis vinifera L. — is one of the most important fruit crops cultivated by humanity since ancient times and belongs to the Vitaceae family. This perennial liana-like plant is widely

distributed in temperate, subtropical, and tropical climatic regions and serves as an important raw material source for the food, winemaking, pharmaceutical, and processing industries [1-2]. Grapevine possesses extremely rich genetic diversity, and as a result of breeding processes, thousands of cultivars and genotypes have been developed worldwide. This creates opportunities to select high-yielding and high-quality varieties adapted to different ecological conditions [3-4].

Currently, the growing demand for food products, climate change, soil salinization, and the widespread occurrence of plant diseases increase the necessity of developing new and promising grape genotypes [5]. In particular, excessive accumulation of salts in the soil negatively affects the growth, development, and productivity of grape plants. In addition, unfavorable weather conditions during flowering and berry formation — such as low temperatures, excessive humidity, and prolonged rainfall — create favorable conditions for the development of diseases, leading to reductions in both yield quantity and fruit quality [6-7]. Therefore, the development of stress-resistant, high-yielding, and high-quality grape genotypes, as well as their rapid propagation, has become an important scientific and practical issue.

Traditional vegetative propagation methods help preserve the genetic characteristics of grape cultivars; however, their main disadvantages include low propagation rates and the risk of spreading viruses and other phytopathogens [8]. For this reason, the development and practical application of modern biotechnological approaches based on clonal micropropagation technologies are of great importance. Clonal micropropagation is based on the totipotency of plant cells and allows the rapid production of genetically uniform, healthy, and high-quality planting materials under in vitro conditions [9-10].

Scientific advances in plant tissue culture, especially after the development of the Murashige and Skoog medium, significantly accelerated the development of micropropagation technologies [11]. At present, meristem, nodal, leaf, and petiole explants are widely used for in vitro propagation of grapevine. Research findings indicate that meristem culture is one of the most effective methods for obtaining virus-free plants [12]. Furthermore, the mineral composition of nutrient media, the ratio of plant growth regulators, and sterilization procedures directly influence regeneration efficiency [13].

In recent years, integrating micropropagation technologies with advances in molecular genetics and genomics has expanded the possibilities for selecting and rapidly propagating promising grape genotypes. The use of molecular markers for assessing genetic diversity, identifying genotypes with high regeneration potential, and developing disease-resistant cultivars has significantly improved breeding efficiency [14-15]. Therefore, research on the microclonal propagation of promising grape genotypes is considered one of the most important biotechnological approaches for the production of high-quality and certified planting materials in modern viticulture [16].

Methodology

The research was carried out under in vitro laboratory conditions using promising grapevine genotypes of Vitis vinifera L.. Healthy and disease-free donor plants were selected as the primary source of explants. Young nodal segments and apical meristems were isolated and used as explants for microclonal propagation experiments.

The collected explants were initially washed under running water for 15–20 minutes in order to remove surface contaminants. Subsequently, the plant materials were treated with detergent solution and sterilized under aseptic conditions using 70% ethanol for 30 seconds followed by sodium hypochlorite solution for 10–15 minutes. After sterilization, explants were rinsed three to four times with sterile distilled water to eliminate chemical residues.

Sterile explants were cultured on Murashige and Skoog medium supplemented with sucrose and agar. Different concentrations and combinations of plant growth regulators, including cytokinins and auxins, were applied to evaluate their effects on shoot induction and regeneration efficiency. The pH of the nutrient medium was adjusted to 5.7–5.8 before autoclaving.

Cultures were maintained in a growth chamber at a temperature of 24–26°C under a 16/8 h light/dark photoperiod. Observations were conducted regularly to assess contamination frequency, shoot regeneration, multiplication rate, and root formation. Regenerated shoots were transferred to rooting media containing auxin supplements for in vitro rhizogenesis.

Rooted plantlets were gradually acclimatized under ex vitro conditions in sterile peat and perlite substrate mixtures. During acclimatization, humidity and temperature were carefully controlled to ensure successful adaptation of regenerated plants to greenhouse conditions.

The obtained data were analyzed comparatively to determine the most effective nutrient medium composition and growth regulator combination for rapid and efficient microclonal propagation of promising grapevine genotypes.

Results and Discussion

The conducted experiments demonstrated that the microclonal propagation method is highly effective for the rapid multiplication of promising grapevine genotypes of Vitis vinifera L. under in vitro conditions. The success of culture establishment largely depended on the type of explant, sterilization efficiency, and nutrient medium composition. Among the tested explants, nodal segments and apical meristems showed the highest survival and regeneration rates, while leaf explants exhibited relatively lower morphogenic responses.

Sterilization treatments significantly influenced contamination frequency. The combined application of 70% ethanol and sodium hypochlorite solution effectively reduced microbial contamination and ensured the establishment of healthy aseptic cultures. However, excessive sterilization exposure negatively affected explant viability, indicating the importance of optimizing sterilization duration for grape tissue culture.

The experiments also revealed that the composition of the Murashige and Skoog medium and the ratio of plant growth regulators played a crucial role in shoot induction and multiplication efficiency. Media supplemented with cytokinin-dominant combinations stimulated active shoot proliferation and increased the number of regenerated shoots per explant. In contrast, higher auxin concentrations promoted root initiation and enhanced in vitro rhizogenesis. These findings are consistent with previous studies reporting the positive influence of auxin and cytokinin balance on grapevine regeneration processes.

Differences in regeneration potential among grapevine genotypes were also observed. Some genotypes exhibited rapid shoot formation and vigorous growth, whereas others showed slower development or callus formation without complete regeneration. Such variations confirm that regeneration capacity is strongly genotype-dependent, and universal protocols may not be equally effective for all grape cultivars.

Rooted plantlets were successfully acclimatized under ex vitro conditions. Most regenerants adapted well to greenhouse environments and continued normal vegetative growth after transplantation. Nevertheless, several plantlets exhibited temporary stress symptoms during the acclimatization stage, mainly due to sudden environmental changes. Gradual adaptation and controlled humidity conditions improved survival rates considerably.

The obtained results confirm that microclonal propagation provides an efficient approach for producing genetically uniform, disease-free, and high-quality planting materials within a short period of time. This technology is especially important for the rapid multiplication of elite

grapevine genotypes, conservation of valuable germplasm resources, and improvement of modern grape breeding programs. Furthermore, the integration of tissue culture techniques with molecular and biotechnological approaches may contribute to the development of stress-resistant and highly productive grape cultivars in the future.

Conclusion

Microclonal propagation of promising grapevine genotypes represents an effective and modern biotechnological approach for the rapid production of healthy and genetically stable planting materials. The conducted study demonstrated that in vitro culture techniques based on nodal segments and apical meristems provide high regeneration efficiency and allow the successful propagation of Vitis vinifera L. genotypes within a relatively short period of time.

The obtained results confirmed that sterilization procedures, nutrient medium composition, and the balance of plant growth regulators significantly influence shoot proliferation, rhizogenesis, and overall regeneration success. Cytokinin-rich media stimulated active shoot multiplication, whereas auxin supplementation promoted efficient root formation. In addition, differences observed among grapevine genotypes indicated that regeneration capacity is strongly genotype-dependent and requires optimization of culture conditions for each cultivar.

The successful acclimatization of regenerated plantlets under ex vitro conditions further demonstrated the practical applicability of microclonal propagation technology for viticulture. This method not only ensures the production of disease-free and high-quality planting materials but also contributes to the conservation of valuable grape germplasm resources and the acceleration of breeding programs.

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