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THE EFFECT OF STERILIZATION METHODS ON THE IN-VITRO PROPAGATION OF MULBERRY VARIETIES

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Abstract
This study examines the effectiveness of sterilization methods used for the in vitro propagation of mulberry (Morus spp.) varieties. The research was conducted at the laboratory of the "Ipak ko'chat klaster" farm, located in the Marhamat district of the Andijan region. Bud explants from "Marhamat 2017", "Balxi", "Marvarid", and "Shohtut" varieties were treated with 5% sodium hypochlorite (NaOCl) solution for 5, 7, and 10 minutes. For each variety, the number of uncontaminated explants, shoot tip condition, and viability were evaluated. The results showed that 7 minutes of sterilization provided the most favorable outcome. In addition, strict safety measures were followed to protect human health. The findings may contribute to the development of effective tissue culture protocols for mulberry propagation.

Keywords: Mulberry (Morus spp.), in vitro propagation, sterilization, sodium hypochlorite (NaOCl), contamination, shoot tip, explant, laboratory safety.

Introduction

In this study, bud explants from four mulberry (Morus spp.) cultivars—"Marhamat 2017," "Balxi," "Marvarid," and "Shohtut"—were used to assess the effect of different sterilization durations using a 5% NaOCl solution. The aim was to determine the contamination rate and the condition of the shoot apices after treatment and to identify the optimal sterilization conditions. The experiment was conducted in the spring of 2025 at the in-vitro laboratory of the "Ipak Ko'chat Klaster" farm in Marhamat district, Andijan region, Uzbekistan.

These cultivars were selected due to their agricultural significance and adaptability to in-vitro conditions, as well as their leaf and fruit quality. Healthy, vegetative-stage buds were collected from each cultivar and used as explants. Initially, explants were washed under tap water for 10–15 minutes to remove surface contaminants, then treated with a liquid soap solution for 10 minutes. After each step, explants were rinsed 3–4 times with distilled water. Subsequently, they were treated with 70% ethanol for 30 seconds and again rinsed twice with sterile distilled water.

The main sterilization process involved treating the explants with a 5% sodium hypochlorite (NaOCl) solution for three different durations—5, 7, and 10 minutes. To enhance the surface sterilization effect, 2 drops of Tween-20 or Fairy were added to the solution. At the end of each treatment, explants were rinsed 3–4 times with sterile distilled water. For each treatment group, 20 explants were used. All manipulations were performed under a laminar airflow cabinet, and explants were cultured on Murashige and Skoog (MS) nutrient medium.

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To assess contamination prior to planting, some explants were immersed in sterile water for 48 hours and monitored. Contamination was evaluated based on water clarity, color changes, or visible microbial growth.

Strict laboratory safety protocols were followed throughout the experiment. Personal protective equipment (PPE) including gloves, face masks, protective goggles, and lab coats were used. Work surfaces were disinfected with 70% ethanol. Waste solutions, particularly those containing $HgCl_2$ or NaOCl, were collected in separate containers and disposed of in accordance with environmental safety regulations.

The explants were observed for 21 days following sterilization, and the following criteria were used for evaluation:

1.Number of uncontaminated explants (visually free of contamination).

2.Condition of shoot apices (intact, partially damaged, or completely necrotic).

3.Viability (greening, callus formation, shoot development).

Results and Analysis

Explants from each of the four cultivars were treated with 5% NaOCl solution for 5, 7, and 10 minutes. Each treatment group included 20 explants. The number of uncontaminated explants and the condition of their shoot apices were evaluated:

1. Number of uncontaminated explants:

Cultivar	5 min	7 min	10 min
Marhamat 2017	8 / 20	17/ 20	18 / 20
Balxi	7 / 20	16 / 20	17 / 20
Marvarid	5 / 20	14 / 20	16 / 20
Shohtut	3 / 20	14 / 20	15 / 20

Note: Values are presented as “healthy explants / total explants.”

2. Shoot apex condition (at 7-minute treatment):

- **Marhamat 2017:** Shoot apices remained intact.
- **Balxi:** Shoot apices were active.
- **Marvarid:** Shoot apices were active.
- **Shohtut:** Some damage observed, growth was slow.

3. Analysis:

The results indicated that 5-minute treatments were insufficient, especially in the “Shohtut” cultivar, where 70% of the explants showed fungal or bacterial contamination. In contrast, the 10-minute treatment almost completely eliminated contamination but caused necrosis of shoot apices, especially in “Marvarid” and “Shohtut,” halting further development.

The most favorable results were observed in explants treated for 7 minutes. At this duration, contamination rates ranged from 20–30%, and most shoot apices remained viable. The cultivars “Marhamat 2017” and “Balxi” showed the best adaptability to this treatment. Thus, the 7-minute sterilization duration was both effective against contamination and preserved explant viability.

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Conclusion

This study demonstrated that sterilization is a critical stage in the in-vitro propagation of mulberry varieties. When explants were treated with 5% NaOCl for 5, 7, and 10 minutes, varying outcomes were observed based on the cultivar. The 5-minute treatment was insufficient for contamination control, while the 10-minute treatment, although effective in sterilization, negatively impacted explant viability due to shoot apex damage.

Optimal results were achieved with the 7-minute treatment, which maintained lower contamination levels while preserving shoot apex integrity. The “Marhamat 2017” and “Balxi” cultivars responded particularly well to this treatment, enabling vigorous regeneration. However, “Marvarid” and “Shohtut” exhibited higher sensitivity to sterilization and require more cautious handling.

Strict adherence to laboratory safety protocols proved vital in maintaining aseptic conditions and safeguarding human health. The use of PPE and proper disposal of chemical waste was an essential part of the experimental process.

The outcomes of this study lay the groundwork for refining sterilization protocols for different mulberry cultivars and can serve as a basis for developing effective strategies for large-scale, high-quality plantlet production.

References

1. George, E. F., Hall, M. A., & De Klerk, G.-J. (2008). Plant Propagation by Tissue Culture. Springer.
2. Murashige, T. & Skoog, F. (1962). A Revised Medium for Rapid Growth. Plant Physiology, 15, 473–497.
3. Roca, W. M. & Mroginski, L. A. (1991). Plant Regeneration in Tissue Culture. CIAT.
4. Hasanov, M., & Islomova, S. (2021). Effectiveness of micropropagation methods for mulberry seedlings in Uzbekistan. Agricultural Scientific Journal, 3(2), 45–49.
5. Ghosh, B., & Sen, S. (2020). Role of surface sterilization in explant preparation. Journal of Plant Sciences, 8(2), 67–72.
6. Plant Cell Technology. (2023). How to Sterilize Plant Explants.