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CONSERVATION OF THE DIVERSITY OF BOTANICAL GARDEN COLLECTIONS USING IN VITRO TECHNIQUES: A CASE STUDY OF MORUS RUBRA L.

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Abstract. This article presents the results of the development of in vitro propagation protocols for species represented in the collection of the Tashkent Botanical Garden. The explants of *Morus rubra* L. from the botanical garden collection were introduced into culture using the in vitro method, with the influence of various phytohormones tested to optimize plant regeneration. For explant introduction, WPM (Woody Plant Medium) with 30% sucrose and 0.2 mg/l BAP was selected as the most effective nutrient medium. Rhizogenesis was successfully stimulated on a nutrient medium supplemented with 0.5 mg/l IBA. Following the successful development of root systems, a gradual acclimatization process was employed to help the plants adapt to greenhouse conditions. Initially, the

plants were transferred to moist vermiculite in covered containers and kept in growth chambers for 1–2 weeks under controlled environmental conditions to minimize transplant shock and promote root system strengthening. Following this, the plants were transplanted into a peat-based substrate, kept covered to maintain high humidity, and incubated in growth chambers. Over time, the covers were gradually removed to allow the plants to adjust to ambient humidity and temperature fluctuations. This gradual acclimatization process significantly improved the survival rates and ensured the successful establishment of *Morus rubra* L. plants under ex vitro conditions, demonstrating the effectiveness of the developed in vitro propagation protocol for this species.

Key words: botanical garden, *in vitro* propagation, *Morus rubra* L.

ЗБЕРЕЖЕННЯ РІЗНОМАНІТТЯ КОЛЕКЦІЙ БОТАНІЧНИХ САДУ ЗА ДОПОМОГОЮ МЕТОДИКИ *IN VITRO*: ПРИКЛАД *MORUS RUBRA* L.

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Анотація. Ця стаття представляє результати розробки методу розмноження in vitro видів, представлених у колекції Ташкентського ботанічного саду. Експланти *Morus rubra* L. з колекції ботанічного саду були введені в культуру за допомогою методу in vitro під впливом різних фітогормонів. WPM (Woody Plant Medium) із 30% сахарози та 0,2 мг/л BAP був обраний як найбільш ефективне поживне середовище для введення експлантів. Ризогенез стимулювався на поживному середовищі з ІМК (0,5 мг/л).

Для поступової акліматизації до тепличних умов рослини з кореневою системою спочатку переміщували у вологий вермікуліт у закритих контейнерах і тримали в камерах росту протягом 1–2 тижнів. Після цього рослини пересаджували в субстрат на основі торфу, утримували під накриттям і інкубували в камерах росту. З часом покриття поступово знімали, щоб дозволити рослинам адаптуватися до навколишньої вологості та коливань температури. Цей поступовий процес акліматизації значно покращив рівень виживаності та забезпечив успішне укорінення рослин Morus rubra L. в умовах ex vitro, демонструючи ефективність розробленого протоколу in vitro розмноження для цього виду.

Ключові слова: ботанічний сад, розмноження *in vitro, Morus rubra* L.

Introduction. Plant biotechnology plays a crucial role in conserving rare and endangered plant species. Many botanical gardens implement programs to establish and maintain cell and tissue culture collections. Such initiatives have been successfully developed and are actively maintained in various countries. In vitro collections of cells, organs, and whole plants, along with cryobanks, have been established to preserve plant material from diverse taxonomic groups in liquid nitrogen. These ex situ conservation methods help mitigate biodiversity loss due to habitat destruction, climate change, and human activities.

Several specimens within these collections are considered national treasures, including rare and legally protected plant species. For example, the National Clonal Germplasm Repository of the USDA in Corvallis, Oregon, safeguards 500,000 samples of economically significant, rare, and endangered plants from 10,000 species. Similarly, in Germany, more than 700 cell culture lines from 80 plant families are preserved, many of which produce pharmacologically valuable secondary metabolites [13]. Other countries, such as China

and Japan, have also invested in large-scale in vitro conservation programs, focusing on medicinal, ornamental, and food crop species to ensure their long-term availability and sustainability.

Beyond conservation, *in vitro* biotechnology is an essential tool for plant breeding, genetic improvement, and the mass propagation of economically valuable species. By using micropropagation, plants can be rapidly multiplied under sterile conditions, ensuring the production of disease-free and genetically stable clones. This technique is particularly beneficial for species that are difficult to propagate by conventional means or have low seed viability [7]. Moreover, the combination of *in vitro* culture with molecular markers and cryopreservation techniques enhances conservation strategies, allowing researchers to store germplasm long-term and revive species when needed [6].

This article presents the results of research on the development of *in vitro* microcloning protocols for propagating plants represented as a single sample at the Tashkent Botanical Garden of the Republic of Uzbekistan, using *Morus rubra* L. as an example. The study aims to optimize sterilization, culture media composition, and acclimatization procedures to facilitate the successful introduction of selected species into in vitro culture. By refining these protocols, researchers contribute to the sustainable conservation and restoration of valuable botanical specimens, ensuring their survival for future generations.

Research object. A species from the North American flora of the Tashkent Botanical garden exhibit was selected as the research object.

Morus rubra L. (Moraceae Gaudich.) is commonly known as the mulberry or fig family. This family comprises about 40 genera and over 1,100 species, including economically and ecologically significant trees, shrubs, and climbing plants. The family is primarily distributed in tropical and subtropical regions, but some species, such as Morus rubra, are adapted to temperate climates.

Morus rubra L., commonly known as red mulberry, is a deciduous tree or large shrub native to North America, particularly in the eastern and central United States. It typically grows 10–20 meters tall, with a short trunk and a broad, spreading canopy. The bark is brownish-gray and develops shallow fissures with age [12].

The object of this work. *In vitro* propagation of species from the botanical garden collection, represented by a single specimen, particularly *Morus rubra* L., which grows in the North America exhibit of the Tashkent Botanical Garden.

Materials and Methods. Different parts of the plant were used as explants for *in vitro* reproduction of the selected species to determine the most effective tissue for micropropagation and optimize regeneration efficiency. The choice of explants plays a crucial role in the success of *in vitro* culture, as different tissues exhibit varying levels of responsiveness to growth regulators and environmental conditions.

Apical and lateral buds, as well as buds from 1-year-old branches, were selected as explants due to their high regenerative potential and ability to form new shoots. These meristematic tissues are known for their rapid cell division and differentiation, making them ideal for microcloning [1].

The apical bud was cut, leaving two pairs of lateral buds intact. To promote efficient shoot formation, an oblique incision was made at the site of the opposite lower second pair of lateral buds, and one of the lateral buds was removed. This technique was employed to encourage lateral shoot development while reducing competition for nutrients and hormones.

Additionally, experiments were conducted using nodal segments, internodal stem sections, and leaf explants to assess their regeneration capacity. However, apical and lateral buds showed the highest survival rate and the most robust shoot development, making them the preferred explant sources for *Morus rubra* L.

Results and discussion. Optimization of sterilization. To develop an optimized sterilization protocol, various sterilizing agents were evaluated to determine the most effective combination for minimizing fungal and bacterial contamination of explants. The effectiveness of these agents was assessed based on their ability to eliminate contaminants while maintaining high explant viability.

The tested sterilizing agents included:

Chemical disinfectants: Sodium hypochlorite (4–6%), hydrogen peroxide (2–15%), silver nitrate (0.01%), Tween 20, ethanol (70%), and commercial disinfectants such as "Belizna" (containing 18% sodium hypochlorite) and "Domestos" sterilizing soap.

Fungicides: Difenoconazole ("Score" 250EC, 23.3% v/v), mancozeb and metalaxyl ("Ridomil Gold", 64% v/v and 4% v/v, respectively), fludioxonil ("Maxim", 9.3% v/v), and propiconazole ("Agrotilt", 25% v/v). These were tested for their efficacy in controlling fungal contamination.

Antibiotics: Streptomycin, amoxicillin, ceftriaxone, and gentamicin, applied at concentrations ranging from 1 to 4 ml/l, were used to address bacterial infections.

A total of over 30 sterilization protocols were assessed, utilizing different concentrations, exposure times, and combinations of sterilizing agents. The protocols were designed to strike a balance between effective microbial control and maintaining tissue viability. Several treatment variations were tested, including sequential washes, varying immersion durations, and combining multiple sterilizing agents[9].

Selection of the nutrient media. Ready-made nutrient media of Duchefa Biochemie B.V (https://www.duchefa-biochemie.com) production were used in accordance with the protocols by Murashige and Skoog (1962) (MS), Chu et al (1975) (N6), Gamborg et al (1968) (B5), McCown Woody Plant Medium (Lloyd and McCown, 1980) (WPM) (Lloyd and McCown, 1980), and DKW Medium (Driver and Kuniyuki, 1984; McGranahan et al, 1987). During further cultivations, antibiotics were not added to the nutrient medium since no signs of fungal or bacterial infections were observed.[2,3,5]

Selection of the phytohormones. Auxins 2.4-dichlorophenoxyacetic acid (2.4-D), indolylacetic acid (IAA), α-naphthylacetic acid (NAA), indolylbutyric acid (IBA), cytokinins kinetin (Kin), 6-benzylaminopurine (BAP), thidiazuron (TDZ), and Zeatin (Zea) were used to find optimal phytohormones for *in vitro* micropropagation[8].

Summary. Optimization of the Sterilization Protocol and *In Vitro* Micropropagation of *Morus rubra* L.

Among the tested sterilization agents, up to 80% of the explants remained viable, demonstrating the effectiveness of the optimized protocol in minimizing microbial contamination while maintaining tissue viability. The most effective sterilization protocol identified involved a multi-step treatment to ensure maximum decontamination of fungal and bacterial pathogens. The optimized sterilization procedure included the following steps: (1) Explants were initially immersed in a 25% sterilizing soap solution for 20–30 minutes to remove surface contaminants, such as dust, debris, and microorganisms. (2) The samples were thoroughly washed with distilled water to eliminate soap residues. (3) Explants were then placed in a 0.01% fungicide solution containing propiconazole (20×10^{-4}%) to target fungal spores and infections. (4) After another thorough rinse with distilled water (2–3 times), the explants were subjected to a deeper sterilization step. (5) Immersion in a 2% Belizna solution (sodium hypochlorite-based) for 15 minutes was performed to eradicate bacteria and fungi on plant surfaces. (6) Three additional washes with distilled water ensured the removal of residual sterilizing agents. (7) The explants were briefly immersed in 70% ethanol for 30 seconds, an effective step for eliminating residual pathogens and breaking down microbial cell walls. (8) Three washes with sterilized autoclaved water were performed to neutralize any remaining traces of disinfectants before the explants

were transferred to the culture medium. The sequential application of different sterilizing agents helped achieve a balance between efficient microbial decontamination and high explant survival rates, making this protocol suitable for large-scale *in vitro* propagation of *Morus rubra* L.

For the successful development of mature plants through *in vitro* micropropagation, a carefully optimized nutrient medium was selected based on the growth requirements of *Morus rubra* L. WPM (Woody Plant Medium) was identified as the most effective base medium for shoot initiation and development. Cytokinin supplementation with 6-Benzylaminopurine (BAP) at 0.2 mg/l significantly enhanced shoot formation, with superior results compared to other concentrations. BAP promoted multiple shoot induction and increased the number of axillary buds per explant. For root induction, Indole-3-butyric acid (IBA) at 0.5 mg/l was used, which facilitated robust root development, ensuring a higher survival rate during the acclimatization phase [4].

Figure 1: **Morus rubra L. Adaptation of the plant to soil.**

Adaptation. To help plants gradually adapt to greenhouse conditions, those with well-developed root systems were first transplanted into moist vermiculite inside covered containers and kept in growth chambers for 1–2 weeks under controlled temperature, humidity, and light conditions. This gradual transition helped strengthen the root system and reduce transplant shock. After this period, the plants were transferred to a peat-based mixture, kept covered to maintain high humidity levels, and incubated in growth chambers for further acclimatization. Over time, covers were gradually removed to allow plants to adjust to ambient humidity and environmental fluctuations before being fully transferred to the greenhouse (fig. 1). The stepwise acclimatization process significantly

improved the survival rates of *in vitro*-cultured *Morus rubra* L. plants, ensuring successful establishment under *ex vitro* conditions.

Conclusions. Biotechnology laboratory of the Tashkent Botanical Garden of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan: "Development of Scientific Foundations for the Sustainable Propagation of Valuable Specimens of the Botanical Garden in *In Vitro* Culture"(2023–2024), and within the framework of the state program "Establishment and Digital Documentation of the *in Vitro* Collection of the Tashkent Botanical Garden Using Innovative Biotechnology Methods scheduled for implementation from 2025 to 2029.

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CONSERVATION OF THE DIVERSITY OF BOTANICAL GARDEN COLLECTION WITH THE USE OF *IN VITRO* TECHNIQUES WITH EXAMPLE OF *PTELEA TRIFOLIATA* L. (RUTACEAE JUSS.)

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Abstract. This article presents the results of in vitro propagation of *Ptelea trifoliata* L. from the collection at Tashkent Botanical Garden. *In vitro* propagation plays a key role in conserving threatened plant species, as many species face increasing extinction risks due to environmental changes and human activities. To address this, the Biotechnology Laboratory at the Institute of Botany in Tashkent has initiated a program focused on the in vitro preservation of plant species, including those at risk of extinction. The collection includes both plants from natural habitats and specimens maintained within the Botanical Garden.

Several culture media were tested for their effectiveness in supporting the in vitro propagation of *Ptelea trifoliata*. Among the options evaluated, McCown's Woody Plant Medium (WPM) with additional vitamins from Duchefa Biochemie B.V. was identified as the most suitable for this species. WPM, specifically designed for woody plants, provided an optimal nutrient composition that promoted healthy growth and development of the plant material. This medium was further enriched with plant growth regulators, including indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), and 6-benzylaminopurine (BAP), to stimulate optimal root and shoot development.

In conclusion, this study demonstrates the successful in vitro propagation of *Ptelea trifoliata* using McCown's Woody Plant Medium supplemented with IBA, NAA, and BAP. The refined protocol enhances explant viability and facilitates the large-scale propagation of this species, making it a valuable tool for conservation and large-scale propagation initiatives. Through tissue culture techniques, this approach offers a sustainable solution to preserve plant biodiversity, ensuring that endangered species are protected for future generations.

Key words: *in vitro* propagation, botanical garden collection.

ЗБЕРЕЖЕННЯ РІЗНОМАНІТНОСТІ КОЛЕКЦІЇ БОТАНІЧНОГО САДУ З ВИКОРИСТАННЯМ МЕТОДИКИ IN VITRO НА ПРИКЛАДІ PTELEA TRIFOLIATA L. (RUTACEAE JUSS.)

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