

## 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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**Анотація.** У статті наведено результати розмноження *Ptelea trifoliata* L. *in vitro* з колекції Ташкентського ботанічного саду. Методи *in vitro* значно підвищують потенціал збереження видів рослин, що знаходяться під загрозою зникнення. Останніми роками все більше видів рослин знаходяться під загрозою зникнення через зміни навколишнього середовища та діяльність людини. У відповідь Біотехнологічна лабораторія Ботанічного саду при Інституті ботаніки в Ташкенті, Узбекистан, ініціювала програму розмноження *in vitro* для збереження великої колекції видів рослин, у тому числі тих, що знаходяться під загрозою зникнення. Ця колекція включає як зразки з природних середовищ існування, так і рослини, які зберігаються в Ботанічному саду.

Серед оцінених живильних середовищ найбільш відповідним було визначено McCown's Woody Plant Medium (WPM – Wood Propagation Media, M0220) з вітамінами від Duchefa Biochemie B.V. Середовище було збагачене індол-3-масляною кислотою (IBA), 1-нафтилоцтовою кислотою (NAA) та 6-бензиламінопурином (BAP) для стимуляції оптимального росту та розвитку. Цей вдосконалений протокол покращує життєздатність експлантів та сприяє ефективному *in vitro* мікророзмноженню, що підтримує зусилля щодо збереження та масового розмноження рослин.

**Ключові слова:** розмноження *in vitro*, колекція ботанічного саду.

**Introduction.** The conservation of biological diversity is one of the primary objectives of botanical gardens worldwide. These institutions play a crucial role in preserving plant genetic resources, protecting rare and endangered species, and maintaining biodiversity. All conservation activities related to plant species and varieties are guided by key international agreements and frameworks, including the Convention on Biological Diversity (1995, 2006), the International Botanical Gardens Plant Conservation Program (2000), and the Global Strategy for Plant Conservation (2002), among others [10]. These initiatives emphasize *ex situ* conservation strategies, which involve the preservation of plant genetic material outside its natural habitat [1].

One of the most effective strategies for preserving plant biodiversity is the establishment of an *in vitro* genetic bank, where plant samples are maintained under low-temperature, slow-growth conditions. This method ensures the long-term storage of plant genetic material while minimizing genetic drift and maintaining the integrity of rare and valuable plant species. Compared to traditional vegetative propagation methods, *in vitro* conservation offers several advantages, including:

Independence from climatic conditions, enabling year-round conservation and propagation,

The ability to establish aseptic cultures using minimal explant material, reducing disturbances to wild populations,

Efficient space utilization and reduced labor costs, making it a cost-effective solution for large-scale plant conservation [2].

Modern biotechnological approaches, particularly clonal micropropagation, significantly enhance both large-scale production and the conservation of plant species in *in vitro* collections. This technique is particularly valuable for economically significant perennial crops and for the preservation of rare and endangered species that are difficult to propagate via seeds due to dormancy, low germination rates, or genetic instability. The development of effective clonal micropropagation techniques is a fundamental prerequisite for the establishment of *in vitro* gene banks, ensuring the preservation of valuable plant genetic resources for future generations [5].

*In vitro* propagation offers many benefits over traditional methods, especially in the conservation of endangered species. The ability to propagate plants in a controlled environment ensures that genetic material is preserved and can be used for reintroduction into natural habitats. Furthermore, large numbers of plants can be produced quickly, aiding both conservation and research. The protocol developed for *Ptelea trifoliata* is an important contribution to the conservation of this species and provides a foundation for similar efforts with other endangered plants.

The combination growth regulators played a significant role in ensuring successful *in vitro* micropropagation. IBA and NAA, both auxins, promoted root initiation, while BAP, a cytokinin, facilitated shoot proliferation. This hormone mixture was carefully optimized to ensure the highest viability and growth of explants from *Ptelea trifoliata*, leading to the effective development of new plantlets.

The propagation process began with the collection and sterilization of explants, which were then cultured on the optimized WPM medium. Explant viability was a critical factor for the success of the propagation process, and the refined protocol developed in this study improved explant survival rates. As a result, a higher number of viable plantlets were produced from a single explant, allowing for large-scale propagation in a controlled environment. This protocol, therefore, has significant implications for conservation efforts, as it enables the propagation of *Ptelea trifoliata* at a much larger scale and at a faster rate than traditional methods.

This article presents the results of *in vitro* propagation of *Ptelea trifoliata* L., a single-specimen plant from the Botanical Garden collection. The study explores the potential of *in vitro* propagation as a tool for the conservation of unique and threatened plant species, demonstrating its effectiveness in maintaining genetic diversity and supporting long-term conservation goals. The findings contribute to ongoing efforts in plant biotechnology and conservation science, highlighting the role of tissue culture techniques in sustainable biodiversity management [8].

**The object of this work.** The object of this work is the *in vitro* propagation of *Ptelea trifoliata* L. (hoptree or water ash) from the collection of the Tashkent Botanical Garden. This study focuses on developing and optimizing a tissue culture protocol to promote the conservation and large-scale propagation of *P. trifoliata*, a species that faces environmental pressures and threats to its natural populations. The research aims to identify the most suitable culture medium and hormonal combinations to enhance explant viability, promote optimal growth, and support the conservation of this and potentially other endangered plant species.

**Materials and Methods.** This study focuses on plant species that are represented by a single specimen within the collection of the Tashkent Botanical Garden named after Acad. F.N. Rusanov, under the Institute of Botany, Academy of Sciences of the Republic of Uzbekistan.

*Ptelea trifoliata* L., commonly known as common hoptree, wafer ash, stinking ash, and skunk bush, belongs to the Rutaceae (citrus) family. It is native to North America, with distribution across Canada, the United States, and Mexico. This species is a deciduous shrub or small tree characterized by alternate, trifoliate leaves.

Dilutions of *P. trifoliata* are used in homeopathic medicine, where they are considered highly potent. The root bark possesses anthelmintic, antibacterial, antiperiodic, and tonic properties and is often combined with other medicinal compounds to enhance efficacy. The leaves are traditionally applied to treat wounds, including intestinal injuries.

This species is an aromatic shrub or small tree with a rounded crown and a slender, crooked trunk bearing interwoven, ascending branches. When crushed, the bark, foliage, and twigs emit a slightly lemon-like, musky odor. The leaves are trifoliate and deciduous, with each leaflet attached to a petiole up to 2 inches long, while the terminal leaflet measures up to 2.5 inches in length. The leaflets are obovate, with the midrib of lateral leaflets positioned off-center. The foliage is dark green in summer and turns yellow in autumn. The flowers are small, greenish-white, and appear in clusters among the leaves in April. The fruit is a distinctive wafer-like samara, with broad wings measuring approximately 7/8 inch long by 3/4 inch wide. The species exhibits morphological variation, with leaflets differing in size and shape across different populations (fig. 1).

The species derives its common name from its historical use, where its bitter fruit was reportedly used as a substitute for hops in brewing beer. Additionally, the bitter root bark, similar to other aromatic barks, has been traditionally utilized in herbal medicine. Notably, *P. trifoliata* represents the northernmost New World member of the Rutaceae family.

Method. Classical microcloning methods were used in this research (Butenko, 1964; Kalinin, 1980). As an analogue, the micropropagation protocols of Khajehyar et al. (2024).

Different plant parts were used as explants for microclonal propagation of species: 1. Apical and axillary buds. Annual branches (annual shoots) were used as explants, which were cut from plants in different seasons throughout the year and placed in the refrigerator for 1-7 days [9].

When choosing sterilizing agents when developing a protocol for sterilization of explants of the studied species, solutions of sodium hypochloride (4-6%), hydrogen peroxide (2-15%), silver nitrate (0.01%), Tween20, ethanol (70%) were tested. Belizna sterilizing solution, “Domestos” sterilizing soap, fungicides difenoconazole (Skor), mancozeb and metalaxyl (Ridamill Gold 72%), fludioxonil (Maxim), propiconazole (Agrotilt), antibacterial agents streptomycin, amoxicillin, gentamicin, etc. were used. Thirty protocols with various combinations and concentrations of sterilizing agents were tested [10].

As a nutrient medium, we used ready nutrient media by Duchefa Biochemie B.V (<https://www.duchefa-biochemie.com>): nutrient medium Murashige and Skoog (1962), Chu et al. (1975), Gamborg et al. (1968), McCown Woody Plant Medium (WPM) (Lloyd G. and McCown, 1980) and Wollosovič (1979). Antibiotic agents (streptomycin, amoxicillin and gentamicin) were added to the nutrient medium, after which the nutrient medium autoclaved at 2 atm, 126°C for 20 minutes. Nutrient media were poured into 25 ml jars. When optimizing the nutrient medium, various concentrations of sucrose were used (10%, 15%, 20%, 25%, 30%, 35%) [3,6].

**Results and discussion.** A series of experiments were conducted to determine the most suitable nutrient medium for in vitro propagation of *Ptelea trifoliata* L. Among the tested media, McCown Woody Plant Medium (WPM – Wood Propagation Media, M0220) with vitamins from Duchefa Biochemie B.V. was selected as the optimal formulation. This medium has a classical composition of macro- and microelements and contains glycine (2.00 mg/L), myo-inositol (100.00 mg/L), nicotinic acid (0.50 mg/L), pyridoxine hydrochloride (0.50 mg/L), and thiamine hydrochloride (1.00 mg/L). At each stage of microclonal propagation, the medium was supplemented with sucrose (30,000 mg/L) and agar (7,000 mg/L).

Annual shoots of *P. trifoliata* were collected from mature trees and stored under cold stratification conditions in a damp gauze cloth at +5°C. The shoots were then segmented into 7–10 cm sections and subjected to a two-stage sterilization process:

Preliminary washing: The cuttings were washed under running water, placed in a soap solution, and rinsed with distilled water (2–3 times).

Sterilization under laminar flow conditions: The explants were treated sequentially in the following solutions:

Belizna (sodium hypochlorite) with Tween 20

Distilled water rinse

Fungicide propiconazole solution (“Agrotilt”)

Silver nitrate solution 70% ethyl alcohol for 60 seconds

Final rinse with distilled water

Microcuttings were prepared in a laminar flow hood before being introduced into in vitro culture. The sterilized shoots were processed by removing the apical bud while retaining two pairs of lateral buds. An oblique incision was made at the base of the second pair of lateral buds, and one of the lateral buds was excised.

For culture initiation, explants were placed on a nutrient medium supplemented with 6-benzylaminopurine (BAP) to induce shoot development. The incubation was conducted under a 16-hour photoperiod (light) and 8-hour dark cycle at a controlled temperature of 23–24°C. Throughout all stages of subculturing, the microcuttings were positioned in



Figure 1: **In vitro** growth of *Ptelea trifoliata* L.

the culture container such that the lowest part of the explant was submerged at a depth of 0.5 mm in the nutrient medium, ensuring that the lower bud was also in contact with the medium.

The study successfully optimized an in vitro propagation protocol for *Ptelea trifoliata* L., identifying WPM supplemented with sucrose and as the most effective nutrient medium. The two-stage sterilization method significantly reduced contamination levels, enhancing explant survival rates. Additionally, the inclusion of 6-benzylaminopurine (concentration) facilitated efficient shoot induction under controlled environmental conditions.

The optimal phytohormone options for in vitro culture induction efficiency in WPM medium were as follows:

1. BAP (0.2 mg/l) + NAA (0.05 mg/l)
2. BAP (0.2 - 0.5 mg/l)

The following phytohormones were applied to induce root formation.

1. BAP (0.1 mg/l) + IBA (0.05 mg/l)
2. IBA (0.5 mg/l)
3. IBA (1 mg/l)

Adaptation to soil: peat, sand, humus were mixed in a ratio of 1:1:1. They were kept in a phytotron for 1 – 2 weeks at a temperature of 22°C, 75-80% relative humidity (Rh), 16/8 photoperiod, and 1,000 – 1,300 lux illumination. The relative humidity was gradually reduced to 60%. The plants were transferred from the phytotron to greenhouse conditions.

These findings provide a reliable approach for *ex situ* conservation and mass propagation of *P. trifoliata*, contributing to its preservation and potential horticultural applications.

Summary. *Sterilization Protocol*: Among the tested sterilization agents, up to 80% of the explants remained viable. The most effective sterilization method involved sequential treatments as follows:

Immersion in 25% sterilizing soap solution for 20–30 minutes, followed by thorough rinsing with distilled water.

Treatment with 0.01% fungicide solution containing propiconazole ( $20 \times 10^{-4}\%$ ), followed by 2–3 washes with distilled water.

Immersion in 2% Belizna (sodium hypochlorite) solution for 15 minutes, followed by three washes with distilled water.

Treatment with 70% ethanol for 30 seconds, followed by three final washes with autoclaved sterilized water.

*Optimal Nutrient Media for In Vitro Micropropagation:*

For the development of mature plants of *P. trifoliata* L., the Woody Plant Medium (WPM) was selected.

The medium was supplemented with indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), and 6-benzylaminopurine (BAP) to promote optimal growth and development.

This optimized protocol enhances the viability of explants and supports efficient *in vitro* micropropagation for conservation and mass propagation efforts.

**Conclusions.** This research was conducted within the framework of the state program of the 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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