

MICROCLONAL PROPAGATION OF THE SPECIES FROM THE COLLECTION OF THE BOTANICAL GARDEN: *ACER PLATANOIDES* L. “CRIMSON KING” (ACERACEAE JUSS.)

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Acer platanoides L. is widely used in ornamental landscaping throughout the steppe zone of Ukraine and in Uzbekistan with a temperate continental climate. Due to its striking dark purple foliage, it creates a striking contrast in gardens, parks and urban green spaces. As a shade tree, it provides aesthetic appeal and excellent canopy cover, where it affects cooling, regulating the microclimate, making it a popular choice for streets, residential yards and public spaces. Its resistance to pollution, compacted soil and urban conditions makes it particularly suitable for urban environments, where it is often planted along roads and in public parks. Microclonal propagation of the ornamental species *Acer platanoides* L. “Crimson King” was achieved through indirect organogenesis and microcutting. Indirect organogenesis was induced on leaf petioles using DKW medium with 2,4D (0.5 mg/l) + Kin (0.5 mg/l) or 2,4D (0.5 mg/l) + BAP (0.5 mg/l), followed by subculturing onto BAP-supplemented medium (0.01–1.0 mg/l). Microcutting was performed on hormone-free WPM medium with 20 % sucrose, then subcultured onto BAP (0.01–1.0 mg/l) before transferring to a medium containing NAA (2 mg/l) + BAP (0.5 mg/l) + TDZ (0.5 mg/l).

Key words: *Acer platanoides* L., in vitro, arid climatic conditions, steppe zone, microclonal culture.

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Мікроклональне розмноження видів з колекції ботанічного саду: *Acer platanoides* f. “Crimson King” (Aceraceae Juss.)

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Acer platanoides L. широко використовується в декоративному озелененні по всій степовій зоні України та в Узбекистані з помірно-континентальним кліматом. Завдяки яскравому темно-пурпуровому листю створює різкий контраст у садах, парках та міських зелених насадженнях. Як тіньове дерево, воно забезпечує естетичну привабливість і чудове покриття для навісу, де впливає на охолодження, регулюючи мікроклімат, що робить його популярним вибором для вулиць, житлових дворів і громадських просторів. Стійкість до забруднення, ущільненого ґрунту та міських умов робить його особливо придатним для міського середовища, де його часто висаджують уздовж доріг і в громадських парках. Мікроклональне розмноження декоративного виду *Acer platanoides* L. «Crimson King» було досягнуто шляхом непрямого органогенезу та мікроживцювання. Непрямий органогенез індукували на черешках листків за допомогою середовища DKW з 2,4D (0,5 мг/л) + Kin (0,5 мг/л) або 2,4D (0,5 мг/л) + BAP (0,5 мг/л) з наступним пересівом на середовище з додаванням BAP (0,01–1,0 мг/л). Мікропрепарування проводили на безгормональному середовищі WPM з 20 % сахарози, потім субкультивували на БАП (0,01–1,0 мг/л) перед перенесенням на середовище, що містить NAA (2 мг/л) + БАП (0,5 мг/л) + TDZ (0,5 мг/л).

Ключові слова: *Acer platanoides* L., in vitro, посушливі кліматичні умови, степова зона, мікроклональна культура.

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Introduction

Woody plantations play an important role in creating comfortable living conditions for the population, as they stabilize the microclimate, neutralize industrial emissions. However, in the presence of climate change in technogenically polluted regions, woody plants are exposed to high temperatures, frequent droughts on the one hand and anthropogenic load on the other, which leads to various disorders in the functioning of the plant organism (Miller 2002; Bobrova et al. 2010; Harfouche et al. 2014; Suslova et al. 2025). As a result of disorders in the growth and development of trees, they weaken, are damaged by pests, diseases, premature aging and withering (Savosko et al. 2018; Kvitko et al. 2021; Kvitko et al. 2022; Kulbachko 2024; Ivanchenko et al. 2016; Lykholat et al. 2024).

Acer platanoides L. is a species widely distributed in Europe and southwestern Asia. It grows in deciduous and mixed forests, one of the edificators of broad-leaved forests. The crown is oval, in adult trees in single plantings it is sprawling and dense. The wood is distinguished by its strength and elasticity, which allows branches to grow horizontally and occupy space around the trunk. In pure plantings it creates a strong shading effect, preventing the development of vegetation of the lower tiers. Only young trees are shade-tolerant. The tree is very decorative with its crown and foliage, used in single, group, alley plantings (Diduch et al. 2000; Suslova et al. 2024; Manko 2015; Manko et al. 2016). *Acer platanoides* is generally resistant to pests, but is often affected by fungi (maple leaf spot), which cause leaf spot. Also, this type of woody plant is not susceptible to hybridization with other species of the genus *Acer* L., and has clearly pronounced specific characteristics, which helps to easily distinguish it from other members of the genus.

Species of the genus *Acer* occupy one of the leading places in landscaping urbanized areas of the steppe zone of Ukraine because they are fast-growing, wind- and drought-resistant, undemanding to soil fertility, and resistant to industrial pollution. This gives them a significant advantage in creating park dendrocenoses in settlements of industrial regions of the steppe zone. It is also widely used to create artificial woody plantations in technogenic ecotopes (Savosko et al. 2018; Kvitko et al. 2022; Kulbachko et al. 2024, Driver, Kuniyuki 1984; Chu et al. 1975).

Acer platanoides L. is one of the ornamental species cultivated in the Tashkent Botanical Garden. In this regard, the Biotechnology Laboratory of the F.N. Rusanov Tashkent Botanical Garden of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan is conducting research on its *in vitro* propagation and adaptation to garden conditions. Botanical gardens and arboreturns are vital for the preservation of plant biodiversity. *Ex situ* conservation not only preserves species, but also increases their populations and expands their natural range.

A separate sign of the impact of pollutants on the assimilation apparatus is a decrease in the area of the leaf blade. Due to the peculiarities of the pigment apparatus, leaves adapted to intense lighting usually have a higher photosynthetic potential, are able to dissipate most of the light energy and are resistant to photoinhibition (Murchie, Horton 1997; Meir et al. 2002; Lichtenthaler et al. 2007; Chu 1978; Bobrova et al. 2010). This is ensured by the operation of the xanthophyll cycle, localized in the light-harvesting complex (SHC) of photosystem II (PS II) (Modiga et al. 2019; Varga et al. 2025). The light regime significantly affects the content and ratio of chlorophylls (Chl) *a/b* and carotenoids (Car), which reflects a change in the biosynthesis and accumulation of SHC II. It should be noted that the leaf blade is the most sensitive and informative organ of the plant organism, therefore its parameters are often used for bioindication of environmental pollution. In plants that accumulate pollutants, the growth and development of leaf blades is disrupted.

Purpose of the work: to improve understanding of the adaptation processes of the *Acer platanoides* species in technogenic ecotopes of the steppe zone, a pigment analysis of leaf blades of representatives of the species was carried out for use in studies of microclonal propagation of the ornamental species *Acer platanoides* “Crimson King” in the arid conditions of the in tracontinental climate of the city of Tashkent.

Materials and methods

The object and material of the study were representatives of the genus *Acer* L. species *Acer platanoides*, and the decorative form *A. platanoides* “Crimson King”, sharp-leaved plane maple. Deciduous, slow-growing tree, up to 15 m tall. The crown is spherical, smooth. The leaves of large currents, purple, do not change color during the

growing season. The trunk is straight, dark brown, with well-pronounced longitudinal grooves. Also, the materials of the study were leaf plates of woody plants of the genus *A. platanoides* for pigment analysis.

Microclonal propagation of the ornamental species *A. platanoides* “Crimson King” was achieved by indirect organogenesis and microcuttings on the basis of the Biotechnology Laboratory of the F.N.Rusanov Tashkent Botanical Garden of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan, where research on *in vitro* propagation and adaptation is carried out.

Sterilization. Various sterilization agents were tested, including sodium hypochlorite (4–6 %), hydrogen peroxide (2–15 %), silver nitrate (0.01 %), ethanol (70 %), and fungicides like propiconazole (Agrotilt, 25 % v/v) and fludioxonil (Maxim, 9.3 % v/v). Over 30 protocols were evaluated to minimize fungal and bacterial contamination. The most effective method achieved 90 ± 2 % sterility for *A. platanoides* using gentamicin (0.6 % v/v) and streptomycin (0.2 % v/v), respectively. Media (50 ml) were sterilized at 2 atm., 126 °C for 20 minutes.

Nutrient Media Selection. Ready-made nutrient media of DuchefaBiochemie 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 and Eveleing (1968) (B5), McCown Woody Plant Medium (Lloyd, McCown 1980) (WPM) (Lloyd, McCown 1980), and DKW Medium (Driver, Kuniyuki 1984; McGranahan et al. 1987; Chu 1978). Antibiotics were used only at the initial micropropagation stage. Sucrose concentration optimization showed 20 % as optimal for *A. platanoides*. Nutrient media contained essential micro- and macroelements, phytohormones, sucrose, and 7 g/l agar.

Phytohormone Selection. Auxins 2,4-dichlorophenoxyacetic acid (2,4D), indolylacetic acid (IAA), α -naphthylacetic acid (NAA), indolylbutyric acid (IBA), cytokinins kinetin (Kin), 6-benzylaminopurine (BAP), thidiazuron (TDZ), and Zeatin (Zea) were tested. Callusogenesis was induced on DKW, MS, and WPM media using 2,4D (0.5 mg/l) + Kin (0.5 mg/l) or 2,4D (0.5 mg/l) + BAP (0.5 mg/l).

Adaptation to Soil. Plants were acclimated in a vermicompost : peat : sand (1:1:1) mix at 22 ± 2 °C, 90 % RH, and a 16/8 photoperiod for 1–2 weeks, gradually reducing RH to 40%. Adapted plants were transferred to greenhouse conditions.

For pigment analysis of the leaf blade of *A. platanoides*, leaves were collected on the territory of the Dovhyntsev Arboretum in the vicinity of Kryvyi

Rih within the maple-ash woody plantations of the abandoned *ex situ* botanical arboretum (Didukh et al. 2000) in the summer of 2024. Pigments from the leaves were extracted with acetone with the addition of CaO₃ and separated by paper chromatography using a mixture of hexane and ethanol (16: 1 by volume). Pigment bands (lutein, violaxanthin, neoxanthin) were extracted from the chromatograms with 100 % ethanol, β -carotene and chlorophylls – with 100 % acetone. The total chlorophyll (Chl) and carotenoid (Car) contents were determined in acetone extract of leaves on a SF-46 spectrophotometer as described previously (Arkus et al. 2005; Fomishyna et al. 2009; Banas et al. 2011; Nikolopoulos et al. 2008; Mund et al. 2010), using the extinction coefficients given in Lichtenthaler (1987). The pigment contents were expressed per unit dry mass.

The research was conducted in 3-4-fold replication, the results were statistically processed, the standard deviations did not exceed 5 %. Statistical data processing was performed using Microsoft Office Excel and Statistica 6.0.

Results and discussion

Despite the available information about the successful propagation of a number *Acer* L. species by using seeds, obtaining a large amount of planting material for *A. platanoides* “Crimson King” was extremely difficult. As a result of a series of experiments, we have developed protocol for microclonal propagation of *A. platanoides* “Crimson King”. Two micropropagation pathways have been identified: propagation with microcuttings and indirect organogenesis.

Protocol 1: Micropropagation via Microcuttings

Micropropagation of *A. platanoides* “Crimson King” involved sterilization, *in vitro* culture induction, growth, rooting, and soil adaptation. Annual shoots were stored at +5 °C for 1–7 days, segmented (7–10 cm), and sterilized in two stages: (I) washing (6–15 h), soap treatment (10–15 min), and distilled water rinse; (II) disinfection in a laminar flow hood using “Belizna” (30 %) + Tween 20 (7 min), fungicides (5 min), silver nitrate (2–3 min), and ethanol (70 %, 60 sec).

Explants were placed on WPM medium with 20% sucrose. Gentamicin (0.6 %) was used initially but omitted in later stages. Subculturing onto WPM with BAP (0.01–1.0 mg/l) and a hormone-free medium promoted lateral shoot growth (2–4 cm). After two subcultures, growth accelerated by 30 % on WPM with NAA (2 mg/l) + BAP (0.5 mg/l) + TDZ (0.5 mg/l). Shoots (2–5 cm) were transferred to WPM with sucrose (20 g/l) and IAA (0.5 mg/l)

for rooting, developing within a month. Greenhouse incubation ensured 90–100 % regeneration, though soil adaptation success was limited ($10 \pm 3 \%$).

Protocol 2: Indirect Organogenesis

Indirect organogenesis occurred in sterilized leaf petioles, leaf blades, and stems, with the highest callusogenesis on petioles ($90 \pm 2 \%$). Callus formation was most intense on DKW, MS, and WPM media. Callus turned dark red with morphogenic structures after multiple subcultures. Seedling development was induced on DKW medium with BAP (0.01–1.0 mg/l), with optimal results after 2–3 subcultures over six months.

Rooting was achieved on DKW medium with IAA (0.5 mg/l), followed by gradual acclimation in a phytotron (90 % to 40 % RH, $24 \pm 2^\circ\text{C}$, 1,000–1,300 lux). After closed soil adaptation, 60 % of seedlings survived in open soil.

A number of enzymes are involved in the dynamic regulation of pigment content during adaptation to external conditions, including chlorophyllase (Chlaze, CLH; chlorophyll – chlorophyllide – hydrolase 3.1.1.14), whose function is to catalyze the initial stage of degradation of Chl0 from Ar0 (Todorov et al. 2023; Harfouche et al. 2014). Despite the fact that the hydrolytic properties of Chlaza have been widely studied *in vitro*, its role and activity *in vivo* remain unclear.

The established indicators of *A. platanoides* leaves in different climatic conditions have not been studied sufficiently (Todorov et al. 2003; Sytykiewicz et al. 2013). To date, the dependence of Chlaza activity on plant lighting conditions remains poorly studied. The results of pigment analysis of *A. platanoides* leaf blades in conditions of industrial pollution of the steppe zone are given in Table 1.

Comparative study of Chlaza activity, content and ratio of pigments in *A. platanoides* plants from different regions of the study requires further in-depth research to clarify the vital state and degree of adaptation of the species in arid conditions (Ivanchenko et al. 2016; Varga et al. 2025; Modiga et al. 2019; Kvitko et al. 2024; Miller et al. 2002).

Conclusion

Microclonal propagation of the decorative and marketable species *A. platanoides* “Crimson King”

by microcuttings showed low results of adaptation of the regenerated plants to soil conditions ($10 \pm 2 \%$), while microcloning of this species by indirect organogenesis showed high results of the adaptability of the seedlings to open soil conditions ($60 \pm 2 \%$). Regenerated plants obtained by microcuttings are morphologically different from seedlings developed by indirect organogenesis. For *A. platanoides* “Crimson King”, microcuttings were introduced to *in vitro* culture on a hormone-free nutrient medium WPM with sucrose 20 % and subculture to a nutrient medium WPM containing BAP (0.01–1.0 mg/l) and further to NAA (2 mg/l) + BAP (0.5 mg/l) + TDZ (0.5 mg/l). Rhizogenesis was induced on a nutrient medium WPM with IAA (0.5 mg/l). Indirect organogenesis was induced on leaf petioles on DKW nutrient medium with phytohormones 2.4D (0.5 mg/l) + Kin (0.5 mg/l), as well as 2.4D (0.5 mg/l) + BAP (0.5 mg/l). A well-formed callus with distinct morphological structures were subcultured to the DKW nutrient medium with BAP (0.01–1.0 mg/l) to induce organogenesis and obtain branching seedlings; rhizogenesis was induced with the phytohormone IAA (0.5 mg/l).

Based on this, it is relevant to study the vital state of woody plants of park dendrocenoses under modern conditions of existence in an urban-technogenic environment in order to optimize the use of park-forming species of woody plants.

Acknowledgments

This research was conducted within the framework of the state program of the Biotechnology Laboratory of the Tashkent Botanical Garden, Institute of Botany, 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). Additionally, it was carried out 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.

Acknowledgements The Laboratory Ecology Photosynthesis of Institute of Plant Physiology and Genetics NAS Ukraine, Kyiv, Ukraine.

Table 1. Indicators of pigment content (mg/g dry mass), their ratio and Chlaza activity (mg Chl/g dry mass) in *A. platanoides* leaves (15.05–06.06.2024)

Number of experimental plots	Chl a	Chl b	C Chla+b	Kar	Chl a/b	Car/Chl
Plot 1 (<i>in vitro</i>)	$7.27 \pm 0,06$	$2.78 \pm 0,08$	$10.05 \pm 0,85$	$6.53 \pm 0,05$	$2.60 \pm 0,79$	$0.12 \pm 0,98$
Plot 2	$4.34 \pm 0,04$	$1.83 \pm 0,59$	$6.17 \pm 0,63$	$3.35 \pm 0,23$	$2.36 \pm 0,41$	$0.10 \pm 0,85$
Plot 3	$4.49 \pm 0,16$	$1.93 \pm 0,58$	$6.42 \pm 0,75$	$3.51 \pm 0,51$	$2.32 \pm 0,02$	$0.10 \pm 0,39$

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