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The tissue culture as the source for propagation of decorative species *Acer platanoides* Crimson King (Acearceae Juss.) and *Philadelphus* L. (Hydrangeaceae Dumort.)

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Abstract

Microclonal propagation of the decorative species *A. platanoides* Crimson King was achieved by microcutting and indirect organogenesis. Indirect organogenesis was observed on leaf petioles on DKW medium with 20% sucrose, phytohormones 2.4D (0.5 mg/l) + Kin (0.5 mg/l) or 2.4D (0.5 mg/l) + BAP (0.5 mg/l), and further subculture to the medium with BAP (0.01-1.0 mg/l). Propagation by microcuttings was achieved on WPM with sucrose 20%, IAA (0.05 mg/l) + Kin (0.1 mg/l) + TDZ (0.05 mg/l) or IAA (0.5 mg/l) + TDZ (0.05 mg/l), or hormone free, and further subculture to the medium with BAP (0.01 - 1.0 mg/l) following to the nutrient media with BAP (0.5 mg/l) + TDZ (0.5 mg/l), rhizogenesis was induced with the phytohormone IAA (0.5 mg/l). Microclonal propagation of the *Philadelphus* L. species was achieved on nutrient medium WPM with sucrose 30%, Zea (0.1 - 0.2 mg/l), Zea (0.2 mg/l) + NAA (0.05 mg/l), BAP (0.2 - 0.5 mg/l), BAP (0.5 mg/l) + IBA (0.05 mg/l), or on hormone-free medium. Rhizogenesis was stimulated on a nutrient medium with IBA (0.5 mg/l). The study of the antioxidant activity of callus by DPPH and ABTS methods allowed to conclude that tissue culture can be used as a source of biologically active components for pharmaceuticals and medicine needs.

Key words: botanical garden, microcuttings, callusogenesis, *A. platanoides* Crimson King, *Philadelphus* L., DPPH, ABTS.

Introduction

Botanical gardens and arboretums are important centers for the conservation of the plant biodiversity. *Ex situ* conservation of plants is not only an effective method for preserving biological diversity, but also a way to increase the abundance of a conserved taxon and expand its range of distribution.

Tashkent botanical garden was founded in 1950 on an area of 80 hectares; currently *ex situ* collection of the garden is estimated at 6,000 species, forms and varieties of plants, including more than 2,000 species of trees, shrubs and subshrubs, more than 900 species of the herbaceous plants, more than 2,100 ornamental species, more than 900 tropical and subtropical species. The *ex situ* collections of the botanical garden have been significantly enriched recent years: in 2013 the number of species was around 4,500. The total area of the modern Tashkent botanical garden is 66 hectares. It is located in the northeastern part of the city. The garden's arboretum occupies 40 hectares and consists of expositions of dendroflora of East Asia, North America, Central Asia, the Far East, Europe, Crimea, and the Caucasus.

More than 43% of the species of Tashkent botanical garden are presented in a single copy, many of them are not propagated by seeds and cuttings. Microclonal propagation methods are of great interest, sometimes a single approach allowing the unique material of a botanical garden to be propagated in a short period of time.

Tissue culture is the object of research by scientists as a source of biologically active components of many plants in botanical garden *ex situ* collections, including medicinal species.

Experimental part

Materials

Acer platanoides Crimson King, sycamore maple (Fig. 1A). Deciduous, slowly growing tree, up to 15 m tall. The crown is spherical, smooth. The leaves are large, purple, and do not change color throughout the growing season. The trunk is straight, dark brown, with well-defined longitudinal grooves.

Philadelphus sp., mock orange (Fig. 1B). Deciduous species, with a thin gray bark, leaves opposite, simple, short petiolate. The shape of the leaves ranges from ovate, oval or elongated, sparsely and shallowly serrated or almost entire, to wedge-shaped or rounded at the base and more or less pointed at the ends. The inflorescence is a raceme consisting of three to nine flowers. Due to the pleasant aroma of the flowers, the plant is incorrectly called jasmine. In our studies, the objects were the species from *Philadelphus* L. genera represented in the Tashkent botanical garden's *ex situ* collection: 1. *Ph. laxus* Schrad., 2. *Ph. lavaleyii*, 3. *Ph. lemoinei* Lemoine, 3. *Ph. levisii* Pursh., 4. *Ph. nivalis* Jacques., 4. *Ph. pubescens* Lois., and 5. *Ph. inodorus* L.

Methods

Sterilization

Different sterilization agents were used to develop the protocol, including sodium hypochloride (4 - 6%), hydrogen peroxide (2 - 15%), silver nitrate (0.01%), Tween20, ethanol (70%), "Belizna" solution with a concentration of sodium hypochloride 18%, "Domestos" sterilizing soap, fungicides difenoconazole ("Score" 250EC, 23.3% v/v), mankozeb and metalaxyl ("Ridamil Gold", 64% v/v and 4%, v/v, respectively), fludioxonil ("Maxim", 9.3% v/v), propiconazole ("Agrotilt", 25%, v/v), antibiotics streptomycin, amoxicillin, ceftriaxone and gentamicin at concentrations of 1 - 4 ml/l. More than 30 sterilization protocols were tested with various concentrations and combinations of sterilizing agents to determine the most optimal to reduce the contamination of explants with fungal and bacterial diseases.

The material with fungal contamination was transferred to the phytopathology laboratory to determine the nature of the infection. Mainly, material was infected with Penicillium or Aspergillus fungi, as well as bacterial diseases (personal communication). The most effective preparates were fungicides propiconazole ("Agrotilt", 25% v/v) and fludioxonil ("Maxim", 9.3% v/v). Both products were dissolved in distilled water and used to combat fungal contamination of the material.

The sterility of explants of *A. platanoides* Crimson King reached 90±2% on nutrient media with gentamicin (0.6%, v/v), the sterility of explants of *Philadelphus* L. species reached 80±1.6% on nutrient media with streptomycin (0.2%, v/v), amoxicillin and gentamicin were also tested, but decrease of fungal contaminations was much lower.

Nutrient media (50 ml) were poured into glass containers (jars) of 0.5 l each pre-sterilized in an autoclave BK75 (Irkutsk, Russian Federation) at 2 atm., 126°C for 20 minutes.

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). The antibiotics gentamicin (0.6%, v/v) and streptomycin (0.2%, v/v) were added only at initial stages of micropropagation of *A. platanoides* and *Philadelphus* L. species, respectively. During further cultivations, antibiotics were not added to the nutrient medium since no signs of fungal or bacterial infections were observed.

In the process of optimizing the nutrient media, sucrose was used in various concentrations (10 - 35%), the most effective solution for *A. platanoides* was a nutrient medium with a sucrose concentration of 20%, while for *Philadelphus* L. species high values of explant viability were noted at sucrose concentration 30%. Nutrient media with appropriate concentrations of micro- and macroelements, phytohormones, sucrose, agar 7 g/l, antibiotics at the initial stages of microcloning were autoclaved at 2 atm., 126 °C for 20 minutes. Fifty ml of a nutrient medium was poured into each sterilized glass container (jars). Sterilized explants were transferred to the solid nutrient media.

Selection of the phytohormones

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 used to find optimal phytohormones for *in vitro* micropropagation. In accordance with the instructions of the manufacturer for growth regulators, 10 g of 2.4D, IAA, NAA and IBA were first dissolved in a small volume of 70% ethyl alcohol, then diluted with distilled water to 10 ml; cytokinins BAP and Kin were dissolved in a small volume of 1 N alkali (KOH or NaOH) or acid (HCl), then the solution was brought to 10 ml with distilled water. TDZ was dissolved in a small volume of dimethyl sulfoxide (DMSO), then the volume was adjusted to 10 ml with distilled water.

Callusogenesis on the experimentally selected explants of *A. platanoides* Crimson King and *Philadelphus* L. species was induced on DKW, MS and WPM nutrient media with 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 the soil

Adaptation to closed soil was achieved with the composition vermicompost:peat:sand in a ratio 1:1:1 at a temperature of 22±2°C, relative humidity (Rh) 90%, photoperiod 16/8, illumination 1,000 - 1,300 lux for 1 - 2 weeks. The relative humidity value was gradually reduced to 40%. The adapted plants were transferred to open soil conditions in a greenhouse.

Antioxidant activity research

Chemical components extraction

Chemical compounds of the callus developed nutrient media with various concentrations and compositions of phytohormones were the subjects of the analyses.

Callus was dried in a “Binder” incubator (Tuttlingen, Germany) for 3 days at a temperature +50°C. The dried material was thoroughly grinded, the chemical components were extracted using 99% methanol in a ratio of 1:20 (w/v) respectively at a room temperature (twice with an interval of three days, for a total of six days). The extracts were filtered through filter paper (No. 2, Whatman Co., Maidstone, Kent, UK)

and further dried at +40°C using a rotary evaporator (Eyela N-1300, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The extracts were further dissolved in DMSO and stored at −20°C until use.

Free radical analyses DPPH

Free radical analyses with 2,2-diphenyl-1-picrylhydryl (DPPH) (Sigma Aldrich, St. Louis, MO, U.S.A.) was done according to Shah et al (2019) with small modifications. In total, 150 µl of DPPH in methanol was added to 20 µl samples of different concentrations in 96-well microplates. Ascorbic acid was used as a standard. All measurements were carried out in triplicate. After 30 minutes, the absorbance was checked at 517 nm using a UV spectrophotometer (Molecular Devices, San Jose, CA, U.S.A.).

Free radical analyses ABTS

Free radical analyses with 2,2-Azinobis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) (Sigma Aldrich, St. Louis, MO, U.S.A.) was done according to previously published methodology (Velioglu et al, 1998). The ABTS solution was prepared using 7 mM ABTS and 2.45 mM potassium persulfate, further incubated in the dark at a room temperature for 16 h. The ABTS solution was diluted with distilled water to a concentration of 0.70 ± 0.02 at 740 nm. Ten microliters of extracts of various concentrations were added to 190 µl of ABTS solution. Antioxidant activity measurements were carried out in triplicate. Ascorbic acid was used as a standard.

Statistical analysis

Data analysis was displayed with box plots to reflect the distribution, mean, and standard deviation of measurements, conducted in triplicate (n = 3). One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test with a 95% significance level ($P < 0.05$) was used for statistical analysis. All statistical analyses were performed using RStudio, version 2023.06.1.

Results and their discussions

***Acer platanoides* L. сорт Crimson King (Acearceae Juss.).** 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 (Tomov, 2017; Zhou and Mattsson, 2021; Mehbub *et al*., 2022). As a result of series of experiments, we have developed two protocols for microclonal propagation for *A. platanoides* Crimson King: Protocol 1. Microcloning by microcuttings, i.e. parts of branches with two pairs of oppositely located lateral buds. Protocol 2. Microcloning through indirect organogenesis (Fig. 2). In a comparative aspect, microcloning using indirect organogenesis was more successful compering to microcuttings.

Protocol 1. Micripropagation with microcuttings of *A. platanoides* Crimson King includes the stages of sterilization, inducing *in vitro* culture, growing, rooting, planting in closed and open soil conditions.

Annual branches (annual shoots) were used as explants, which were cut from trees and placed for a period of 1-7 days in the refrigerator in a gauze cloth at a temperature of +5°C, divided into segments of 7-10 cm long, sterilized in two stages: I. Explants were washed with running water for 6-15 hours, placed in a soap solution for 10-15 minutes, washed with distilled water 2-3 times. II. All procedures were executed in a laminar flow box Biobase BSC 1300IIA2-X (Biobase Biodusty Co., Shandong, China).

Explants were placed in a 30% “Belizna” solution with 5-7 drops of Tween20 for 7 minutes, washed with distilled water, placed for 5 minutes in a 0.1% solution of the fungicide propiconazole (“Agrotilt” preparate) or fludioxonil (“Maxim” preparate), then in a 0.01% solution of silver nitrate for 2 - 3 minutes, washed with distilled water, placed in a 70% solution of ethyl alcohol for 60 seconds, washed with distilled water.

In a laminar flow box the sterilized parts of the branches were cut in such a way that the apical bud was cut off, leaving two pairs of lateral buds, then an oblique incision was made at the site of the lower second pair of lateral buds located oppositely, and one of the lateral buds was removed, then microcuttings were placed on a nutrient medium.

Among the culture media tested, a high percentage of inducing *in vitro* culture for *A. platanoides* Crimson King was observed on WPM culture medium with 20%

sucrose. At the initial stages of micropropogation, the antibiotic gentamicin was added to the nutrient medium at a concentration of 0.6% (v/v). All subsequent stages of growing and rhizogenesis, antibiotics were not used, because no signs of fungal and bacterial diseases were observed. At all stages, incubation was carried out in a culture room at a standard photoperiod of 16/8 and temperature 24±2°C. The most optimal nutrient media for micropropogation was WPM IAA (0.05 mg/l) + Kin (0.1 mg/l) + TDZ (0.05 mg/l) or IAA (0.5 mg/l) + TDZ (0.05 mg/l), or hormone free, and further subculture to the medium with BAP (0.01 - 1.0 mg/l) following to the nutrient media with BAP (0.5 mg/l) + TDZ (0.5 mg/l)

The first subcultures were done on a nutrient medium of the same composition as used for induction of *in vitro* culture. An increased development of lateral side shoots up to 2-3 cm in length after the first subculture and up to 3-4 cm in length after the second subculture were observed. After the second subculture on WPM nutrient medium with BAP (0.01 - 1.0 mg/l), the growth of the lateral shoots accelerated up to 30±2%.

The third subculture to WPM nutrient media with BAP (0.5 mg/l) + TDZ (0.5 mg/l), the young lateral shoots of 2-5 cm long were cut off from the main branch using a sterile scalpel and were transferred to a prepared nutrient medium WPM, sucrose 20 g/l, IAA (0.5 mg/l), which allowed to stimulate root growth. At this stage, the development of the root system was observed within one month. Seedlings with a well-developed root system were transferred to the soil for adaptation. Incubation was carried out in a greenhouse at temperature 24±2°C. Regeneration was observed on 60-70% of explants, mainly from lateral buds throughout the year. Adaptation to soil conditions was observed for 10±3% of seedlings.

Protocol 2. Indirect organogenesis was observed for *A. platanoides* Crimson King on leaf petioles, leaf blades and stems, preliminarily sterilized in accordance with the protocol described above for *A. platanoides* Crimson King. The highest percentage of the callusogenesis was observed on leaf petioles (up to 90±2%), leaf blades (up to

60±1.2%), and annual shoots (up to 50±1.4%). Further development of seedlings was observed only for callus formed on leaf petioles.

High intensity of callus formation was observed on DKW, MS and WPM nutrient media; callusogenesis on 90±2% of explants. On other nutrient media, callusogenesis was less intensive. The experiments were continued on DKW nutrient medium, where the seedlings were developed more intensively.

The highest share of the explants with callus was observed on nutrient media DKW with 20% sucrose with composition of the phytohormones 2.4D (0.5 mg/l) + Kin (0.5 mg/l) - 58±4.8%, and 2.4D (0.5 mg/l) + BAP (0.5 mg/l) - 59±3.6% (Fig. 3), and further subculture to the medium with BAP (0.01-1.0 mg/l).

The color of the callus was bright red of a granular structure with morphogenic loci; after the third and subsequent subcultures the callus became dark red, morphogenic structures were characterized with dark purple color.

Subculture of the callus of dark purple color on DKW nutrient media with BAP (0.01-1.0 mg/l) induced developing of the seedlings, most intensive process was observed after second and third subcultures. During the 6 months every 4-6 weeks constant subcultures have been done to a nutrient media of the same composition and concentrations of phytohormones, which resulted in development of the seedlings on 63±3% of explants.

The rhizogenesis was described on DKW nutrient medium with IAA (0.5 mg/l). Up to 60±6% of the seedlings with a well-formed root system were adapted to closed soil conditions in phytotron with relative humidity (Rh, %) starting from 90%, gradually decreasing to 40%, with a standard photoperiod of 16/8, temperature 24±2°C, illumination 1,000 - 1,300 lux (Fig. 4).

After adaptation to closed soil conditions, the regenerated plants were transferred to open soil conditions in a greenhouse with a standard photoperiod of 16/8 and a temperature of 24±2°C. The adaptation of up to 60% of regenerated seedlings to open ground conditions was observed.

The propagation with microcuttings resulted in developing of the regenerated plants of *A. platanoides* Crimson King with thick dark green stems, non-branching, relatively large reddish leaves, while indirect organogenesis resulted in formation of the regenerated plants with thin stems of light green color, sometimes with a reddish tint, the leaves small, pink-red, intensively branched from the base. The plants regenerated from microcuttings were characterized with a low percentage of adaptation to soil conditions, while regenerated plants developed through indirect organogenesis were characterized by high adaptability to closed and then open soil conditions. The induced callus of *A. platanoides* Crimson King was characterized by a high level of antioxidant activity allowing to conclude that callus can be used as a source of biologically active components.

***Philadelphus* sp. (Hydrangeaceae Dumort.**)

The use of the selected sterilization conditions resulted in 70% success of the experiments. Annual shoots were used as explants. Microclonal propagation protocol for *A. platanoides* variety Crimson King included preliminary stratification of the explants, for *Philadelphus* L. species stratification at +5°C did not have significantly different results from without stratification. Freshly cut shoots were divided into segments of 7-10 cm long and thoroughly washed under running water, then the explants were sterilized on a shaker at 150 rpm in a solution of washing soap, followed by rinsing with distilled water. For the studied species of *Philadelphus* L., the use of the fungicide propiconazole significantly reduced fungal contamination; explants were kept in a 0.2% solution of this fungicide for 5-7 minutes, followed by rinsing with distilled water. The explants were placed for 10 minutes in a 20% solution of “Belizna” with 5 - 7 drops of Tween20, followed by washing with distilled water and sterilization for 30 seconds in a 70% solution of ethanol, further washing with distilled water. All sterilization steps, starting with fungicide treatment, were conducted in a Biobase BSC 1300IIA2-X laminar flow box (Biobase Biodusty Co., Shandong, China).

Microclonal propagation of the studied species of the genus *Philadelphus* L. is possible only by microcuttings. Callusogenesis was also induced, but the development of the seedlings from the callus was not observed.

Protocol 1. Microcloning of *Philadelphus* L. species with the use of microcuttings. i.e. parts of branches with two pairs of oppositely located lateral buds (Fig. 5).

Protocol 2. Callusogenesis on plant parts of *Philadelphus* L. species.

Protocol 1. The protocol includes the stage of sterilization, inducing *in vitro* culture, stages of growing, rooting, planting in closed soil and then in open soil conditions. Annual shoots were used as explants. The inducing of *in vitro* culture of the studied species of the genus *Philadelphus* L. became possible on WPM nutrient media with 30% sucrose. At the initial stages of micropropogation, the antibiotic streptomycin was added to the nutrient medium at a concentration of 0.2% (v/v), which reduced contamination of the explants. At subsequent stages, antibiotics were not used, because no signs of fungal and bacterial diseases were observed. At all stages, cultivation was done in a culture room at a standard photoperiod of 16/8 and temperature of 24±2°C. The optimal variants of phytohormones in the nutrient medium WPM with the share of the induction of *in vitro* culture were Zea (0.1 - 0.2 mg/l) - 65±6%, Zea (0.2 mg/l) + NAA (0.05 mg/l) - 64±4%, BAP (0.2 - 0.5 mg/l) - 68±5%, BAP (0.5 mg/l) + IBA (0.02 mg/l) -72±3%, and a hormone-free nutrient medium - 72±3%.

The first and second subcultures were processed on the same nutrient medium where *in vitro* culture of the studied *Philadelphus* L. species was initiated. After the first subculture, lateral shoots grew up to 1 - 2 cm in length, after the second subculture the lateral shoots reached a length of 5 - 6 cm.

After reaching 5-6 cm in length, the experiments were carried out in two options: 1. Lateral shoots were cut using a scalpel and planted on a nutrient medium of the same composition and with the same concentration of phytohormones. 2. Lateral shoots were

cut using a scalpel and planted on a new WPM nutrient medium containing IBA (0.5 mg/l). 3. Explants were subcultured to a new WPM nutrient medium containing IBA (0.5 mg/l).

Most of the dissected lateral shoots of 5 - 6 cm in length subcultured to the nutrient media of the same composition and with the same concentrations of phytohormones, did not survive. However, the development of roots from the base of the lateral shoots was soon observed on 95±5% of the explants with well-developed lateral shoots subcultured to a new WPM nutrient medium with IBA (0.5 mg/l). Seedlings with a well-developed root system were subcultures to closed soil conditions in phytotron with humidity starting from 90%, gradually decreasing to 40%, with a standard photoperiod of 16/8, temperature 24±2°C, and illumination 1,000 - 1,300 lux. After adaptation, the seedlings were transferred to open soil conditions in a greenhouse. The studied *Philadelphus* L. species were characterized by up to 60±3% of adaptation to open soil conditions.

Protocol 2. Callusogenesis for *Philadelphus* L. species was induced on leaf petioles and leaf blades on MS and WPM nutrient media with 2.4D (0.5 mg/l) + Kin (0.5 mg/l) or 2.4D (0.5 mg/l) + BAP (0.5 mg/l). The callus was characterized by a light yellow color, without structural elements. But plants were not developed from this callus.

Study of the antioxidant activity with DPPH and ABTS methods.

Free radicals are formed as a result of endogenous metabolic reactions, intoxication, exposure to environmental pollutants. They are the cause of the tissue destruction as a result of reactions with polyunsaturated fatty acids, polypeptides and DNA. Many diseases are resulted from the impact of free radical activity. Protective mechanisms against the destructive effects of free radicals that reduce the risk of atherosclerosis, cancer, diabetes, arthritis, rheumatism and other diseases are chemical components of an antioxidant nature. Numerous studies have convincingly shown the direct dependence of the human body’s resistance to cardiovascular and carcinogenic

diseases on the level of consumption of foods rich in antioxidants. Analysis of the ability to capture free stable radicals with DPPH (1,1 diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) methods are one of the ways to study the antioxidant activity of material of plant origin as well as callus (Hensley and Floyd, 2003; Makarova and Zyuzina, 2011).

For *A. platanoides* Crimson King and *Philadelphus* L. species, antioxidant activity were analyzed with DPPH and ABTS methods for extracts from callus induced on various nutrient media (MS, WPM, and DKW) with various compositions of phytohormones (Tables 1, 2).

For the tested culture media best antioxidant activity of *A. platanoides* Crimson King callus analysed with DPPH and ABTS was detected for callus induced on DKW. The antioxidant activity was comparatively higher for callus induced with TDZ and ZEA on WPN compering to the nutrient medium with auxins.

For *Philadelphus* L. species best results of antioxidant activity were detected for callus formed on MS nutrient medium, antioxidant activity was not detected on DKW nutrient medium.

Conclusions

1. 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±2%), while microcloning of this species by indirect organogenesis showed high results of the adaptability of the seedlings to open soil conditions (60±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, phytohormones IAA (0.05 mg/l) + Kin (0.1 mg/l) + TDZ (0.05 mg/l) or IAA (0.5 mg/l) + TDZ (0.05 mg/l), or hormone free, and further subculture to the medium with BAP (0.01 - 1.0 mg/l)

following to the nutrient media with 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).

2. Microclonal propagation of the *Philadelphus* L. species was achieved on WPM nutrient medium with 30% sucrose. The optimal compositions of the phytohormones for WPM nutrient medium were Zea (0.1 - 0.2 mg/l), Zea (0.2 mg/l) + NAA (0.05 mg/l), BAP (0.2 - 0.5 mg/l), BAP (0.5 mg/l) + IBA (0.02 mg/l) and hormone-free nutrient medium. Rhizogenesis was stimulated on a nutrient medium with IBA (0.5 mg/l). Callusogenesis was also induced, but no regeneration of seedlings on callus was observed.

3. A study of the antioxidant activity of the components extracted from the callus of *A. platanoides* Crimson King and the studied species of the genus *Philadelphus* L. showed that this data depends on the composition of the nutrient medium for callus induction and concentration of the phytohormones. These results allow to conclude that the tissue culture can be used as a source of biologically active components for pharmaceuticals and medicine (Bhatia *et al.*, 2015; Mustafina *et al*., 2024; Shahzad *et al.* 2017).

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Table 1. Indicators of antiradical activity according to the DPPH and ABTS methods for *A. platanoides* Crimson King callus

Name of the researched material	**Antioxidant activity (free radical scavenging)***	
	DPPH	**ABTS**
	EC50 (μg/ml)	
DKW TDZ 0.1 mg/l	307.09	101.44
DKW BAP 1.0 mg/l	280.5	100.4
DKW NAA0.05 mg/l + BAP 0.1 mg/l	644.97	284.43
DKW NAA0.05 mg/l + TDZ 0.05 mg/l	600	603
DKW TDZ 0.1 mg/l	580.5	594.3
WPM 2.4D 0.5 mg/l + TDZ 0.5 mg/l	638.16	-
WPM IBA 0.2 mg/l	588.1	-
WPM BAP 1.0 mg/l	641.2	-
DKW BAP 2.0 mg/l	413.36	95.58
MS 2.4D 0.5 mg/l	1816.19	501.92
DKW 2.4D 0.5 mg/l + TDZ 0.5 mg/l	524.36	158.60
DKW IBA 0.2 mg/l + TDZ 0.01 mg/l	505.2	
DKW 2.4D 0.5 mg/l + TDZ 0.5 mg/l	515.2	
DKW IBA 0.25 mg/l + TDZ 0.1 mg/l	695.32	376.57
MS 2.4D 0.5 mg/l + TDZ 0.5 mg/l	-	-
MS 2.4D 0.5 mg/l + Кин 0.5 mg/l + TDZ 0.1 mg/l	-	-
DKW 3EA 0.5 mg/l + TDZ 0.1 mg/l	241.24	255.10

*Positive control: EC50 ascorbic acid DPPH 10.84 μg/ml, ABTS 3.93 μg/ml

Table 2. Indicators of antiradical activity according to the DPPH and ABTS methods of callus for the studied species of the genus *Philadelphus* L.

Name of the researched material	**Antioxidant activity (free radical scavenging)***	
	DPPH	
	EC50 (μg/ml)	
DKW TDZ 0.1 mg/l	-	-
DKW BAP 1.0 mg/l	-	-
DKW NAA0.05 mg/l + BAP 0.1 mg/l	1287.7	1032.2
DKW NAA0.05 mg/l + ТДЗ 0.05 mg/l	987.2	543.2
DKW TDZ 0.1 mg/l	765.2	654.2
WPM 2.4D 0.5 mg/l + TDZ 0.5 mg/l	638.16	645.2
WPM IBA 0.2 mg/l	567.8	435.8
WPM BAP 1.0 mg/l	465.9	654.4
DKW BAP 2.0 mg/l	-	-
MS 2.4D 0.5 mg/l	116.19	104.2
DKW 2.4D 0.5 mg/l + TDZ 0.5 mg/l	-	-
DKW IBA 0.2 mg/l + TDZ 0.01 mg/l	-	-
DKW 2.4D 0.5 mg/l + TDZ 0.5 mg/l	-	-
DKW IBA 0.25 mg/l + TDZ 0.1 mg/l	-	-
MS 2.4D 0.5 mg/l + TDZ 0.5 mg/l	143.4	121.3
MS 2.4D 0.5 mg/l + Кин 0.5 mg/l + TDZ 0.1 mg/l	112.2	132.2
DKW ЗЕА 0.5 mg/l + TDZ 0.1 mg/l	638.16	645.2

*Positive control: EC50 ascorbic acid DPPH 10.84 μg/ml, ABTS 3.93 μg/ml



Fig. 1. A. *Acer platanoides* Crimson King. Square of the Hazrati Imom complex, Tashkent, Uzbekistan. Photo: Mustafina F.U. 05.05.2023 B. *Philadelphus inodorus* L. Flowering plant. Ulugbek village, Tashkent, Uzbekistan. Photo: Tillaev T. (https://www.plantarium.ru). 05.11.2020

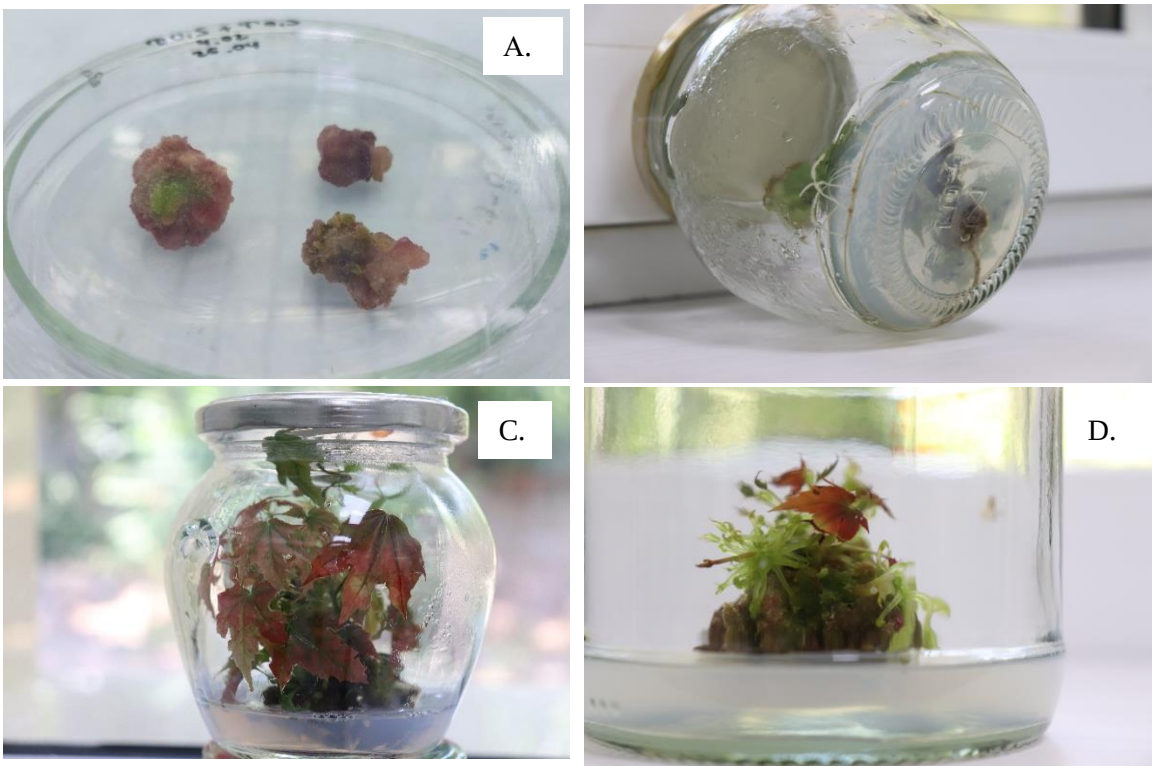


Fig. 2. *Acer platanoides* Crimson King. Indirect organogenesis. A. Callus. B. Development of seedlings on callus. C. Seedlings obtained by indirect organogenesis. D. Developed root system

В Г

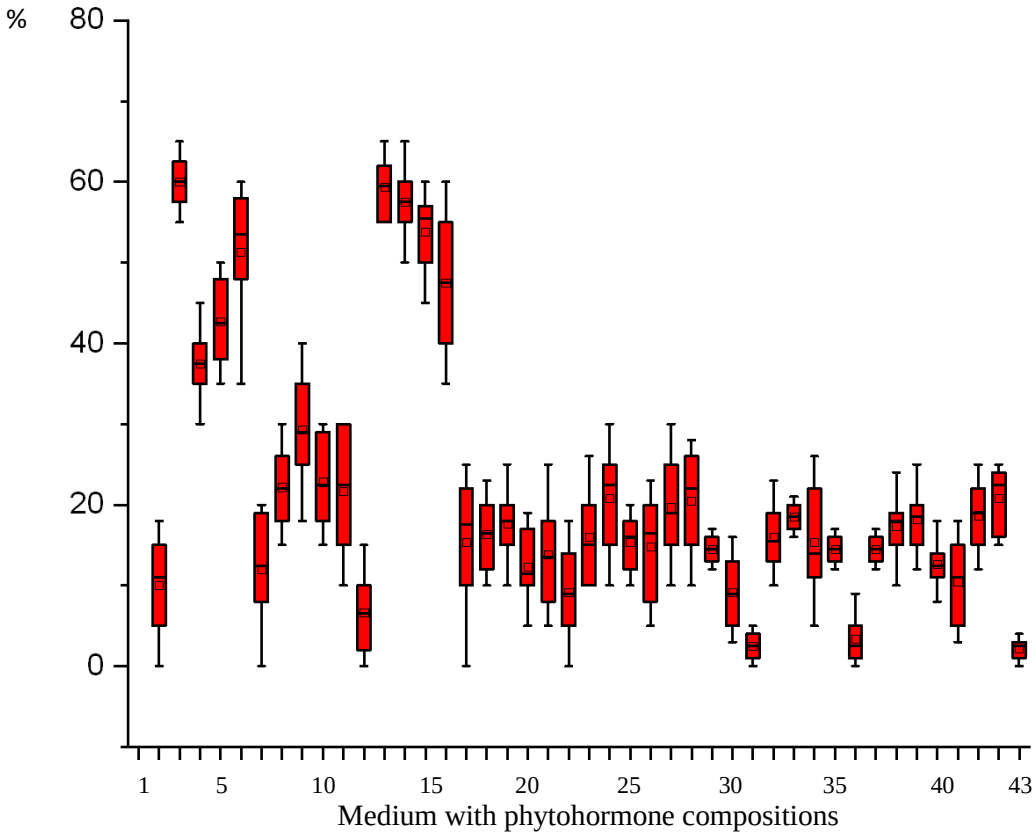


Fig. 3. Callusogenesis rate (%) on the leaflets of the *Acer platanoides* Crimson King on nutrient media DKW with phytohormones. The highest share of the explants with callus was observed on nutrient media DKW with 20% sucrose with composition of the phytohormones 2.4D (0.5 mg/l) + Kin (0.5 mg/l) - 58±4.8%, and 2.4D (0.5 mg/l) + BAP (0.5 mg/l) - 59±3.6%

1) 2.4D (0 mg/l) + Kin (0.5 mg/l); 2) 2.4D (0.5 mg/l) + Kin (0.5 mg/l); 3) 2.4D (0.75 mg/l) + Kin (0.5 mg/l); 4) 2.4D (1.0 mg/l) + Kin (0.5 mg/l); 5) 2.4D (1.5 mg/l) + Kin (0.5 mg/l); 6) 2.4D (0 mg/l) + Zea (0.5 mg/l); 7) 2.4D (0.5 mg/l) + Zea (0.5 mg/l); 8) 2.4D (0.75 mg/l) + Zea (0.5 mg/l); 9) 2.4D (1.0 mg/l) + Zea (0.5 mg/l); 10) 2.4D (1.5 mg/l) + Zea (0.5 mg/l); 11) 2.4D (0 mg/l) + BAP (0.5 mg/l); 12) 2.4D (0.5 mg/l) + BAP (0.5 mg/l); 13) 2.4D (0.75 mg/l) + BAP (0.5 mg/l); 14) 2.4D (1.0 mg/l) + BAP (0.5 mg/l); 15) 2.4D (1.5 mg/l) + BAP (0.5 mg/l); 16) IAA (0 mg/l) + Kin (0.5 mg/l); 17) IAA (0.5 mg/l) + Kin (0.5 mg/l); 18) IAA (0.75 mg/l) + Kin (0.5 mg/l); 19) IAA (1.0 mg/l) + Kin (0.5 mg/l); 20) IAA (1.5 mg/l) + Kin (0.5 mg/l); 21) IAA (0 mg/l) + Zea (0.5 mg/l); 22) IAA (0.5 mg/l) + Zea (0.5 mg/l); 23) IAA (0.75 mg/l) + Zea (0.5 mg/l); 24) IAA (1.0 mg/l) + Zea (0.5 mg/l); 25) IAA (1.5 mg/l) + Zea (0.5 mg/l); 26) IAA (0.5 mg/l) + Zea (0.5 mg/l); 27) IAA (0.75 mg/l) + Zea (0.5 mg/l); 28) IAA (1.0 mg/l) + Zea (0.5 mg/l); 29) IAA (1.5 mg/l) + Zea (0.5 mg/l); 30) NAA (0 mg/l) + Kin (0.5 mg/l); 31) NAA (0.5 mg/l) + Kin (0.5 mg/l); 32) NAA (0.75 mg/l) + Kin (0.5 mg/l); 33) NAA (1.0 mg/l) + Kin (0.5 mg/l); 34) NAA (1.5 mg/l) + Kin (0.5 mg/l); 35) NAA (0 mg/l) + Zea (0.5 mg/l); 36) NAA (0.5 mg/l) + Zea (0.5 mg/l); 37) NAA (0.75 mg/l) + Zea (0.5 mg/l); 38) NAA (1.0 mg/l) + Zea (0.5 mg/l); 39) NAA (1.5 mg/l) + Zea (0.5 mg/l); 40) NAA (0.5 mg/l) + Zea (0.5 mg/l); 41) NAA (1.0 mg/l) + Zea (0.5 mg/l); 42) NAA (1.5 mg/l) + Zea (0.5 mg/l); 43) Hormone free

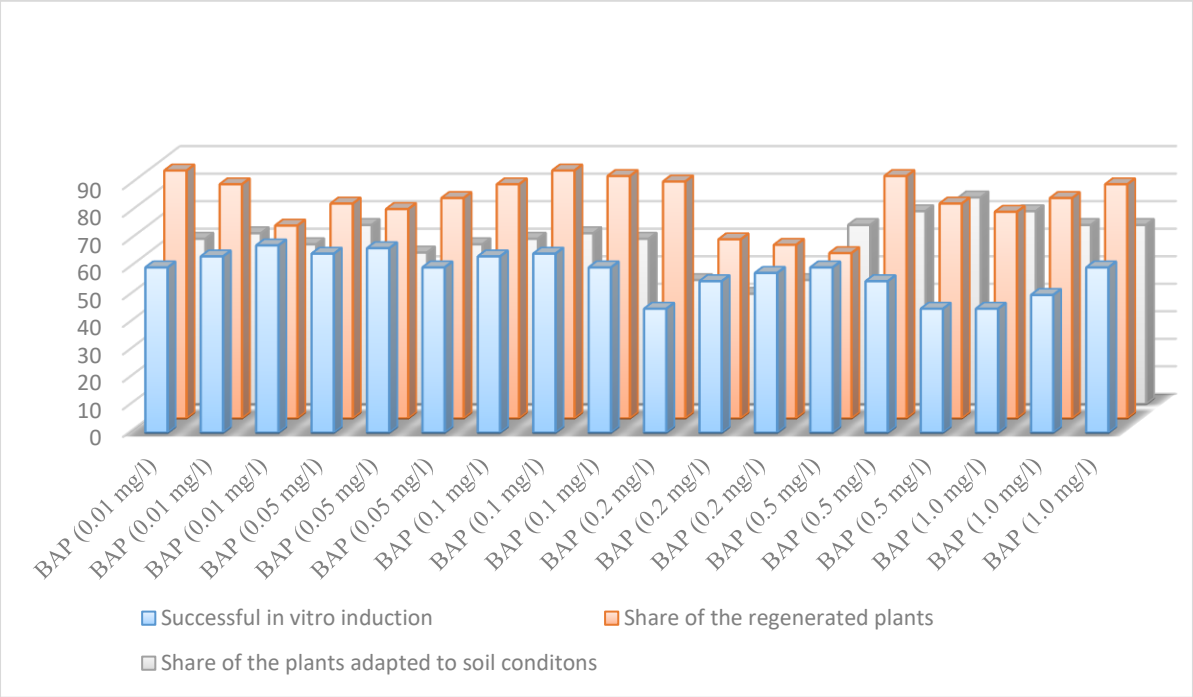


Fig. 4. The share of the regenerated and adapted to the soil plants from indirect organogenesis. Callusogenesis was indiced of the petioles of the *Acer platanoides* Crimson King on nutrient media DKW with phytohormones 2.4D (0.5 mg/l) + Kin (0.5 mg/l) or 2.4D (0.5 mg/l) + BAP (0.5 mg/l). The maximum share of the regenerated seedlins was observed on nutrient media DKW qith phytohormone (0.01-1.0 mg/l). Seedlings with a well-formed root system (60±6%) were adapted to closed soil conditions

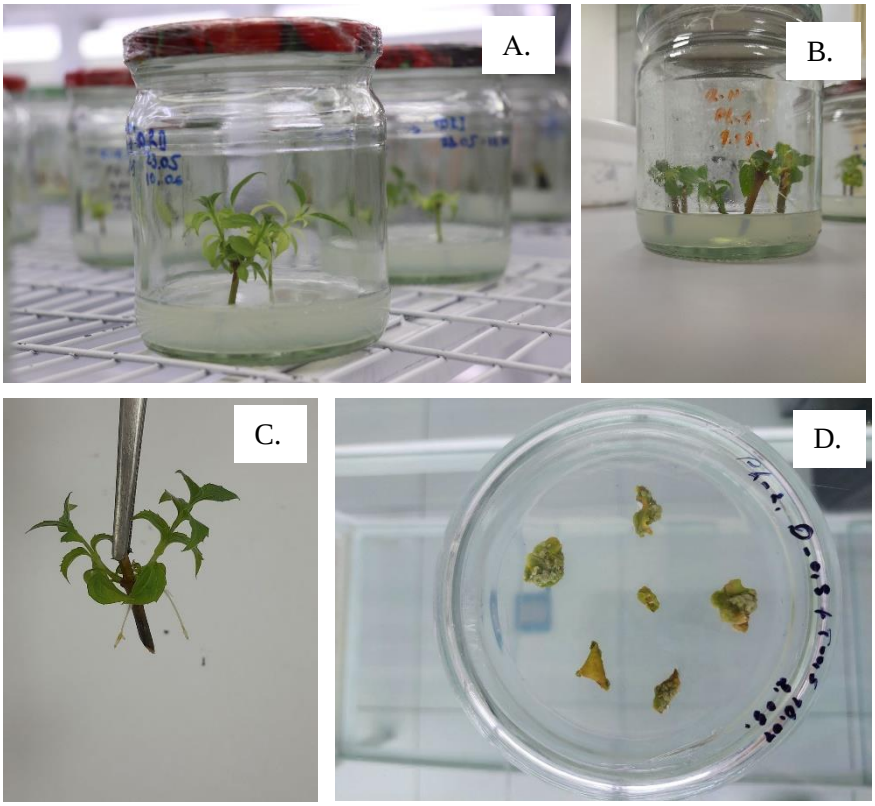


Fig.5. *Philadelphus* L. A.B. Explants introduced into culture. C. Rhizogenesis. D. Callus formed on nutrient medium 2.4D (0.5 mg/l) + Kin (0.5 mg/l)

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Abbreviations

2.4D - 2,4-dichlorophenoxyacetic acid

B5 - nutrient medium according to Gamborg et al. (1968)

DKW – nutritional medium according to Driver and Kuniyuki (1984)

KOH – potassium hydroxide

MS – nutrient medium according to Murashige and Skoog (1962)

NaOH – sodium hydroxide

WPM - Woody Plant Medium. Nutrient medium according to Lloyd and McCown (1980)

ABTS - 2,2-Azinobis (3-ethylbenzothiazoline)-6-sulfonic acid

BAP 6-benzylaminopurine

Ha - hectare

DPPH - 2,2-diphenyl-1-picrylhydryl

DMSO - dimethyl sulfoxide

IBA – indolylbutyric acid

IAA - indolylacetic acid

Kine – kinetin

L - liter

Mg - milligram

mcg - microgram

μl - microliter

Ml – milliliter

n is the number of equivalents of a given substance in 1 liter of mixture.

NAA- α - naphthylacetic acid

TDZ - tidiazuron

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