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Features of microclonal propagation of plants of genus *Cercis* L.

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Abstract. Due to the complexity of traditional vegetative propagation of *Cercis* L., it is necessary to use *in vitro* biotechnology to obtain a regenerative aseptic culture with preservation of decorative features. The study aimed to develop methods of microclonal propagation of *Cercis siliquastrum* "Alba" and *Cercis canadensis* L. For this purpose, plant material was collected at different periods of the growing season from plants growing in Kyiv. Statistical and biotechnological methods were used in the study. As a result of the study, two sterilisation modes were tested. The study determined that the efficiency of explant sterilisation and regeneration is influenced by the season of isolation. Using 70% ethyl alcohol and 1% silver nitrate, the sterilisation efficiency of the explants inserted in May was as follows: 20.0 ± 1.8% for *Cercis siliquastrum* "Alba" and 31.3 ± 3.2% for *Cercis canadensis* L. The explants were introduced into *in vitro* culture on hormone-free nutrient medium according to the WPM (Wood Plant Medium) prescription. Aseptic explants were further subcultivation on WPM with 0.4 mg/l BA (N6-Benzyladenine) and 0.3 mg/l 2iP (6-(γ, γ-Dimethylallylamino)purine) and 0.25 mg/l NAA (1-Naphthylacetic acid). The multiplication factor for *in vitro* shoots on WPM with 0.4 mg/l BA was: 9.4 ± 3.5 for *C. canadensis* and 9.7 ± 2.9 for C. siliquastrum "Alba". The use of WPM with 0.3 mg/l 2iP and 0.25 mg/l NAA stimulated active regeneration of in vitro shoots with this multiplication factor: for *C. canadensis* 5.0 ± 1.5 and *C. siliquastrum* "Alba" 6.5 ± 1.5. The research obtained shoots and *in vitro* plants of *Cercis* L. for further use in the landscaping of settlements

Keywords: in vitro plant tissue culture; aseptic explants; in vitro shoots; regeneration; morphogenesis

Introduction

Traditional methods of vegetative propagation of plants of the genus *Cercis* L. are often ineffective due to the complexity of rooting and low regeneration capacity, which limits the mass distribution of these valuable ornamental species in gardening and landscape design, and therefore, the use of microclonal propagation methods is relevant. The study of the peculiarities of microclonal propagation of plants of the genus *Cercis* L. is necessary due to the complexity of traditional vegetative propagation of these ornamental tree species, which limits widespread use in gardening and landscape design. Plants of this genus are valued for high decorative value, due to bright flowering and spectacular leaf colour, but cuttings or propagation by layering is often ineffective due to low rooting percentage and poor regeneration capacity. The use of *in vitro* microclonal propagation methods can solve the problem of preserving and propagating valuable *Cercis* L. genotypes, providing healthy and homogeneous planting material in large quantities. Studying the processes of sterilisation, optimisation of the composition of the culture medium and the regenerative capacity of explants can be used to develop an effective biotechnological scheme for obtaining aseptic cultures, stimulate active shoot formation and ensure successful rooting of regenerated plants.

The provision of high-quality planting material was considered a determining factor in the creation of decorative and sustainable green spaces in urban environments, but traditional methods of propagation of *Cercis* L. plants were ineffective due to the complexity of rooting and the risk of losing donor traits. N. Nimavat & P. Parikh (2024) noted that generative propagation was accompanied by genetic instability and splitting of decorative

characteristics of seedlings, which would negatively affect the preservation of valuable plant traits and limit the use of this method for *Cercis* L. L.A. Koldar (2016) noted a low level of rooting of cuttings of *Cercis griffithii* Boss, which ranged from 3.5-6%, which confirmed the problematic use of traditional vegetative propagation for this genus.

In this regard, A. Ram & D. Thomas (2024) substantiated the feasibility of using microclonal propagation as an effective alternative, enabling the mass production of homogeneous and healthy planting material with the preservation of all valuable donor traits in a short time. Similar conclusions were demonstrated by V.I. Voytovskaya *et al.* (2020), describing the peculiarities of the sterilisation of different types of *Rhododendron* L. explants and further propagation *in vitro*. The authors proved that the optimisation of sterilisation regimes is a key step in successful microclonal propagation, as it ensures the production of aseptic cultures with a high level of viability. The researchers emphasised that microclonal propagation methods made it possible to preserve valuable decorative traits inherent in donor plants and to ensure mass production of homogeneous planting material, which is unattainable with generative and traditional vegetative propagation. A. Eisold *et al.* (2024) considered the possibilities of using microclonal propagation to grow ornamental woody plants with valuable decorative properties. The authors noted that traditional methods of propagation are often accompanied by significant difficulties due to low rooting rates and loss of varietal traits, while *in vitro* technology ensured the stability of genetic characteristics and improved many plants in a short time. The researchers concluded that microclonal propagation is feasible and effective as an alternative to traditional methods in ornamental nursery production for growing high-quality planting material.

P. Chmielarz *et al.* (2023) emphasised that the effectiveness of microclonal propagation depended largely on the genotype of donor plants and physiological age, demonstrating the possibility of successfully producing micropropagules even from *Quercus robur* trees up to 800 years old. O. Chornobrov & S. Bilous (2021) and O. Chornobrov *et al.* (2023) demonstrated that *in vitro* technologies ensured the preservation of valuable genotypes and the production of high-quality plant material for nursery purposes.

At the same time, J. Nath *et al.* (2024) emphasised the importance of developing effective explant sterilisation protocols and incorporating the growth characteristics of woody plant tissues to obtain viable aseptic micropropagules with high regeneration capacity. Thus, the analysis of scientific papers confirmed the relevance of microclonal propagation as an effective tool for obtaining high-quality planting material of *Cercis* L. plants suitable for use in horticulture and landscape design.

The study aimed to develop a technology for microclonal propagation of *Cercis siliquastrum* "Alba" and *Cercis canadensis* L.

Materials and Methods

The research was conducted at the Plant Biotechnology Laboratory of the Separated Subdivision of National University of Life and Environmental Sciences of Ukraine "Boyarka Forest Research Station", from May to October 2024. Trees growing on the territory of the Fomin Botanical Garden (*Cercis siliquastrum* "Alba") and on the territory of the nursery of the Department of Forest Restoration and Forest Melioration of the Education and Research Institute of Forestry and Landscape-Park Management aged 25-30 years and 10-15 years, respectively, were used as donor plants. Annual shoots were harvested from the donor plants. To reduce transpiration and loss of turgor,

the shoots were placed in a flask of water and refrigerated. Sterilisation and introduction into culture were carried out the day after the shoots were harvested. Cultivation of plant material, sterilisation of laboratory glassware and instruments were performed according to generally accepted methods (Melnychuk *et al.*, 2003; Kushnir & Sarnatska, 2005).

In the laboratory, shoots were cut into cuttings 3-5 cm long. Two sterilisation modes were tested. The general sterilisation process for both modes was washing in soapy water with the addition of a few drops of TWIN-80 surfactant for 20 minutes. A magnetic mixer was used for better washing in soapy water. After that, the cuttings were rinsed with running water for 15 min. Subsequently, the cuttings were filled with sterile distilled water and transferred to a laminar flow box. In the first sterilisation regime, the main sterilising agents were 70% ethyl alcohol and 1% $AgNO_3$. The shoots were immersed in an ethyl alcohol solution for 30-60 s, followed by immersion in a 1% silver nitrate solution for 7-8 min. After sterilisation, they were washed three times in sterile distilled water for 10 min in each portion. After that, the explants were kept on filter paper and cut into 1.0-1.5 cm long pieces. The explants were then planted on WPM (Woody Plant Medium). The results of sterilisation and subcultivation of aseptic viable explants were recorded on day 28 of cultivation.

The second stage of introduction took place in late June and early July. The cuttings were harvested from the same donor trees as for the first introduction. As the shoots were harvested later, they were already semi-lignified, so the second *in vitro* introduction required a different sterilisation regime. The process for the laminar flow box is identical to the first case, with the difference being an increase in the duration of the ethanol soaking and the use of hydrogen peroxide (H_2O_2). After washing the micropropagules in soapy water and rinsing them in running water, they were sterilised in ethyl alcohol for 1-2 min, followed by transfer to a 1% silver nitrate solution for 10 min, after which the cuttings were washed in sterile distilled water for 1 min and then immersed in a 35% hydrogen peroxide solution for 3-4 min. After sterilisation, the explants were washed in sterile distilled water three times for 10 min. The explants were divided into smaller parts and cultured in WPM medium with 1.0 mg/l GA (Gibberellic acid) and 0.1 mg/l TDZ. Explants were counted on day 90 of cultivation. The sterilisation efficiency was defined as the ratio of sterile viable explants to the total number of explants introduced *in vitro* (Melnychuk *et al.*, 2003):

$$K=\frac{A}{L}\cdot 100\%, \quad (1)$$

where: K – sterilisation efficiency, %; A – number of aseptic viable explants, pcs; L – total number of explants inserted, pcs.

The sterile explants were further subcultured on two variants of WPM medium: 0.4 mg/l BA (variant 1) and 0.3 mg/l 2iP and 0.25 mg/l NAA (variant 2). Transplantation of sterile explants was started on day 32 of cultivation. The plant material was cultivated according to the conventional method in a light room at a temperature of 24 ± 1°C and illumination of 2.0-3.0 klx at a 16-h photoperiod and relative humidity of 70-75%. The hormone-free WPM medium was used as a control. Biotechnological (microclonal propagation) and statistical methods (mean, standard deviation, and one-factor analysis of variance) were used using Microsoft Excel. One-factor analysis of variance (ANOVA) was used to analyse the effect of sterilisation mode on efficacy. The experiments were conducted with four replications.

Results and Discussion

When accounting for the explants introduced into the first sterilisation regime in the *in vitro*

culture, the following were obtained: non-viable and non-sterile explants, non-sterile viable and sterile viable explants. The efficiency of the first sterilisation regime was 20 ± 1.8% for *C. siliquastrum* "Alba" and 31.3 ± 3.2% for *C. canadensis*. The study determined that among all explants of *Cercis siliquastrum* "Alba", 24% were infected with biota and were not viable (Fig. 1). Among all explants, 56% were infected, of which 36% did not show signs of growth inhibition (Fig. 2, Fig. 3).

Notably, 4% of explants formed a root 1.52.0 cm long on day 28 of cultivation on hormone-free WPM (Fig. 4).

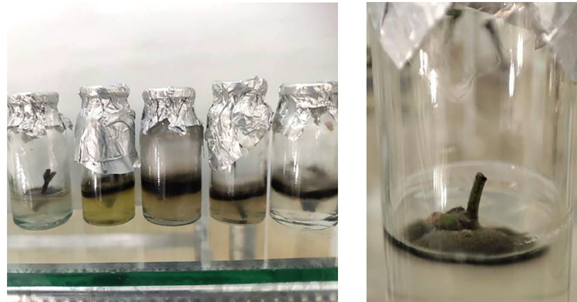

Figure 1. Non-sterile and non-viable explants of *Cercis siliquastrum* "Alba" *in vitro*
Source: authors' photo

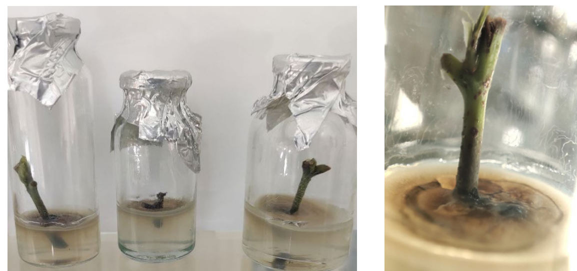

Figure 2. Non-viable explants infected with biota
Source: authors' photo

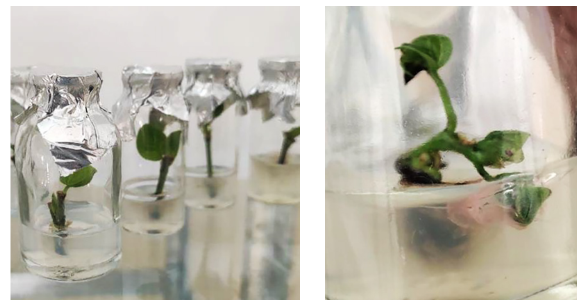

Figure 3. Viable infected explants
Source: authors' photo

Figure 4. Aseptic explant of *Cercis siliquastrum* "Alba" with root
Source: authors' photo

For *C. canadensis*, the counts revealed that 9.3% of explants were not viable and not sterile; 59.4% were viable but not sterile. A summary of the research findings is shown in Table 1.

Notably, viable explants released secondary metabolites (Fig. 5).

The effectiveness of this sterilisation regime for *C. siliquastrum* "Alba" was 15.8 ± 1.4% (8.8% of them formed shoots, 7.0% showed formation of callus tissue on the sections). Notably, 28.1% of explants were sterile but not viable. For *C. canadensis*, the sterilisation efficiency was 20 ± 3.8% (some explants formed callus tissue) (Fig. 6).

Table 1. Efficiency of sterilisation of explants of *Cercis* L. plants

Species, cultivar	Date of entry	Accounting date	Explants sterilisation mode	Culture medium	Sterilisation efficiency (mean ± standard deviation), %
***Cercis siliquastrum* "Alba"**	21.5.24	21.6.24	soap solution, TWIN-80 (20 min), running water (10 min), 70% C_2H_5OH (30-60 s), 1% $AgNO_3$ (7-8 min), washing with distilled water for 10 min (three times)	hormone-free WPM	20.0 ± 1.8
	28.6.24	2.10.24	soap solution, TWIN-80 (20 min), running water (10 min), 70% C_2H_5OH (1-2 min), 1% $AgNO_3$ (10 min), H_2O (1 min), 35% H_2O_2 (3-4 min), H_2O 10 min (three times)	WPM with 1.0 mg/l GA, 0.1 mg/l TDZ	15.8 ± 1.4
***Cercis canadensis* L.**	21.5.24	21.6.24	soap solution, TWIN-80 (20 min), running water (10 min), 70% C_2H_5OH (30-60 s), 1% $AgNO_3$ (7-8 min), rinsing with distilled water for 10 min (three times)	hormone-free WPM	31.3 ± 3.2
	21.6.24	2.10.24	soap solution, TWIN-80 (20 min), running water (10 min), 70% C_2H_5OH (1-2 min), 1% $AgNO_3$ (10 min), rinsing with distilled water for 1 min, 35% H_2O_2 (3-4 min), H_2O 10 min (three times)	WPM, 1.0 mg/l GA, 0.1 mg/l TDZ	20.0 ± 3.8

Source: compiled by the authors

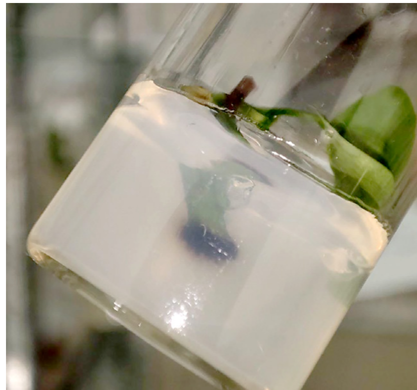

Figure 5. Isolation of secondary metabolites with a sterile explant
Source: authors' photo

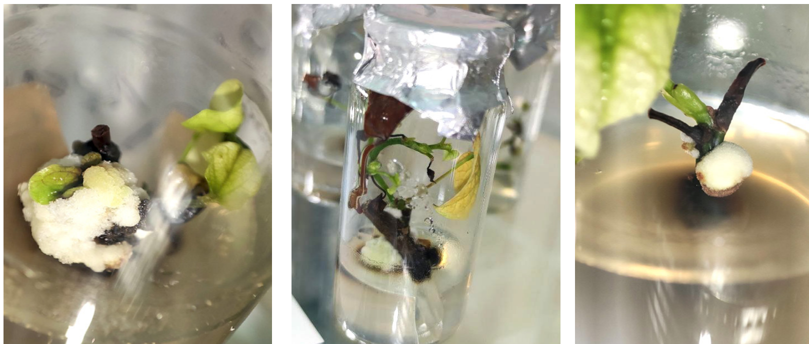

Figure 6. Callus tissue on explants of *Cercis canadensis* L.
Source: authors' photo

Among all explants, 25% were not sterile (20% of which were viable), 5% of sterile and non-viable, and the rest were not sterile and non-viable. According to the results of a one-factor analysis of variance (ANOVA), the effect of explant sterilisation on efficiency is statistically significant at the level of 5% ($F > Fcrit$, $P < 0.05$) (Table 2).

Table 2. Results of one-factor analysis of variance for experimental plants

***Cercis siliquastrum* "Alba"**						
Source of variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between GROUPS	22.78125	1	22.78125	7.326142	0.035261	5.987378
***Cercis canadensis* L.**						
Between Groups	228.98	1	228.98	7.169815	0.036651	5.987378

Note: *df* – number of degrees of freedom; *MS* – variances; *F* – calculated value of the Fischer criterion; *P-value* – calculated value of the minimum substantiality; *Fcrit* – critical value of the Fischer criterion
Source: compiled by the authors

Among the scientists who were involved in the introduction of *Cercis* plants into *in vitro* culture were L.A. Koldar & M.V. Nebikov (2007). A stepwise sterilisation during the *in vitro* introduction, using sodium hypochlorite (2.5% NaClO), mercury dichloride (0.1% $HgCl_2$) and silver nitrate (1% $AgNO_3$) as sterilising agents, was employed in the study. According to the results, mercuric dichloride showed high sterilisation efficiency in 74-90% of sterile explants, of which 53-78% were viable. This figure decreased significantly after exposure to silver nitrate and amounted to about 20-30% of viable explants, which is quite similar to the results obtained in our study. In contrast to L.A. Koldar & M.V. Nebikov (2007), the current study paid more attention to sterile viable explants and based on this, made conclusions about the effectiveness of sterilisation. After all, sterile non-viable explants have no value in further reproduction. For example, researchers W. Dai *et al.* (2005) used a stepwise sterilisation with two main sterilising agents: 70% ethyl alcohol and 0.6% sodium hypochlorite. The effect of culture medium components on the regeneration ability is shown in Table 3.

Table 3. *In vitro* regeneration ability of shoots of plants of the genus *Cercis* L.

No.	Species, cultivar	Composition of the nutrient medium	Number of shoots per 1 explant, pcs	Length of shoots, cm	Multiplication factor	Characteristics of shoots on the 90th day of cultivation
1	2	3	4	5	6	7
1	*Cercis canadensis* L.	WPM, 0.3 mg/l 2iP, 0.25 mg/l NAA	2.0 ± 1.1	2.5 ± 0.9	5.0 ± 1.5	Callus of granular structure was formed, from light brown to brownish brown in colour; the colour of leaf blades varied from pale yellow to yellow-green, and there were brown areas on the edges of leaf blades.
2		WPM, 0.4 mg/l BA	3.1 ± 2.1	3.0 ± 1.7	9.4 ± 3.5	The callus tissue is white cream to light brown in colour. Explants formed callus tissue with a diameter of 0.5 to 2 cm. The callus was localised both on the surface of the medium and in the explant node. The release of secondary metabolites and the death of the tops of some shoots were observed. The colour of the leaf blades varied from pale yellow with green veins to green yellow.

Table 3, Continued

No.	Species, cultivar	Composition of the nutrient medium	Number of shoots per 1 explant, pcs	Length of shoots, cm	Multiplication factor	Characteristics of shoots on the 90th day of cultivation
1	2	3	4	5	6	7
3	*Cercis siliquastrum* “Alba”	WPM, 0.4 mg/l BA	1.3 ± 0.5	5.3 ± 1.2	9.7 ± 2.9	The callus tissue is formed with a granular structure from light brown to brown with white spots. A small amount of callus was formed. The leaf blades were green or light green in colour, with light brown spots on the edges of some leaves.
4		WPM, 0.3 mg/l 2iP, 0.25 mg/l NAA	1.5 ± 0.5	5.8 ± 1.4	6.5 ± 1.5	Callus tissue is not formed or is formed in rather small quantities, brown in colour. Leaf blades are light green in colour. Secondary metabolites were detected in small amounts.

Source: developed by the authors based on the Nature Reserve Fund of Ukraine (n.d.)

Viable aseptic explants from hormone-free WPM were subcultured on WPM supplemented with 0.3 mg/L 2iP and 0.25 mg/L NAA. A callus was formed from the part of the leaf blade that touched the surface of the medium, which may indicate that this species is capable of reproducing using leaf blades. A callus was also formed on the basal section of the shoots. In both cases, the callus is brownish-brown in colour. Two shoots, 3 and 4 cm long, are formed from the bud. The leaf blades are pale yellow in colour, with brown areas from the edge.

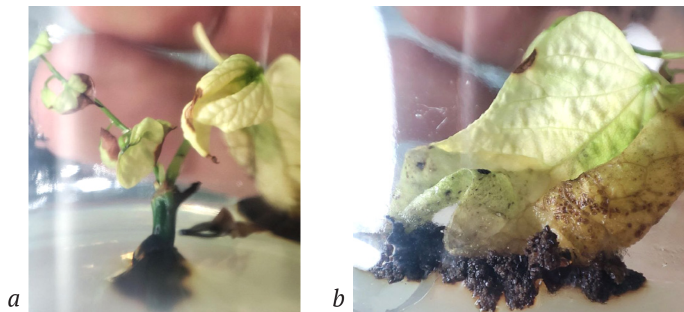

Figure 7. Aseptic shoots of *Cercis canadensis* L.
Note: a – 90th day of cultivation, b – callus tissue formed from the leaf blade
Source: authors’ photo

Subcultivation was performed from hormone-free WPM to WPM supplemented with 0.4 mg/l BA. This explant regenerated callus with significant intensity on the shoots section and in the bud axils of brown and light brown colour, respectively. Also, the formation of white callus tissue in the node area was noted, demonstrated in the images (Fig. 8). As noted by X. Yu *et al.* (2025) in the study on the propagation of *Quercus suber* L., the best substance to reduce the browning of explants was polyvinylpyrrolidone (PVP). The best medium for microclonal propagation was WPM supplemented with trace elements and vitamins per MS.

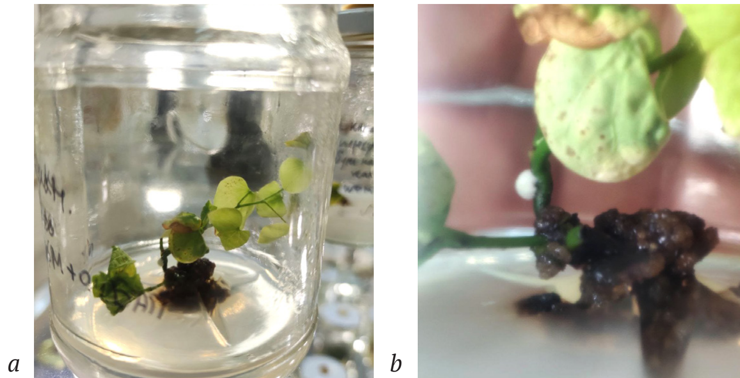

Figure 8. Aseptic culture of *Cercis canadensis* L. (a) and the resulting callus tissue (b)
Source: authors' photo

Notably, this explant intensively secreted secondary metabolites, as well as formed a significant number of shoots. It is possible to assume that an increase in the number of subcultivations of plant material will encourage the development of more shoots on explants (Fig. 9).

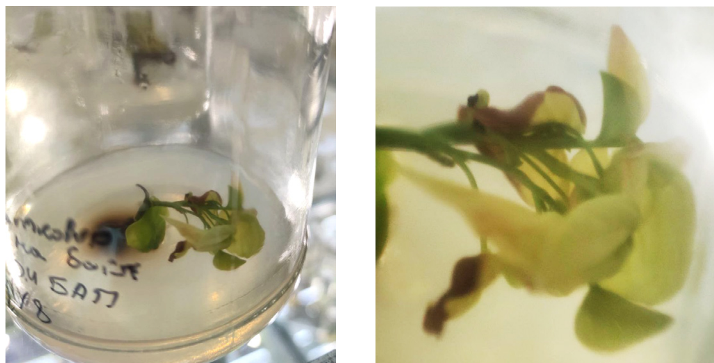

Figure 9. Aseptic shoots of *Cercis canadensis* L.
Source: authors' photo

According to S. Yusnita *et al*. (1990), BA had a positive effect on the efficiency of shoots formation from axillary buds. The study noted that with each subsequent subcultivation, the

number of newly formed shoots increased significantly. The highest number of shoots was achieved after three subcultivations on a nutrient medium with a concentration of 20 μM (4.5 ppm) BA. Further increases in the number of subcultivations or BA concentration did not give significant results. It can be observed that the shoots formed a significant number of shoots (7-9 pcs), has a productive vegetative part without significant signs of depression, but the tops die off (Fig. 10). This phenomenon is typical for experimental plants in the environment, therefore it is assumed that it is a species-specific feature that persists in *in vitro* culture. J.M. Nielsen *et al.* (1993) claimed that the effect of BA and TDZ on explants of *Miscanthus sinensis* Anderss. In terms of root formation, the proportion of chlorotic shoots and size were the same at identical concentrations of phytohormones. However, as noted by the authors, BA induced a significantly higher number of axillary shoots than TDZ.

Figure 10. Shoots of *Cercis canadensis* L. on WPM with 0.4 mg/l BA
Source: authors' photo

The explant of *C. siliquastrum* "Alba" formed brown and white-brown granular callus tissue, as well as a rather powerful shoot. Furthermore, the vegetative parts were in good condition: the leaf blades were green or light green in colour, but one leaf blade showed pronounced signs of depression and was visually dying. The brownish-brown colour of the culture medium in the basal cut zone should be noted, which may indicate the release of secondary metabolites. Little callus tissue was formed (about 0.7 cm in diameter) (Fig. 11).

Figure 11. Aseptic culture of *Cercis siliquastrum* "Alba"
Source: authors' photo

Oliinyk *et al.* (2017) investigated plants under stress, which occurs when plants are introduced into *in vitro* culture, can synthesise phenolic compounds that are rapidly oxidised, polymerised and cause tissue necrosis. The study recommended the use of gallic acid to reduce tissue auto-intoxication by secondary metabolites. To reduce the negative impact of secondary metabolites, W.A. Mackay *et al.* (1995) recommended the use of activated carbon in the WPM medium at a concentration of 0.1% due to its high adsorption capacity.

Root formation was recorded in 4% of *C. siliquastrum* "Alba" explants on hormone-free WPM on day 28 of cultivation. The shoots was subcultured with the root on WPM with 0.4 mg/l BA. During the examination, it was found that a callus (about 0.7 cm in diameter) was formed in the zone of root regrowth, and no second-order roots were detected. There is a darkening around the roots, which may be secondary metabolites released during the life of the explant. Two shoots 5-7 cm long each were formed (Fig. 12).

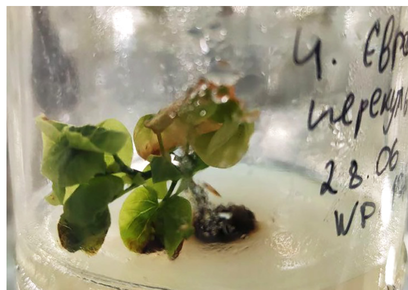

Figure 12. *In vitro* plant *Cercis siliquastrum* "Alba"
Source: authors' photo

C. canadensis shoots cultivated for 135 days on hormone-free WPM medium were 3-5 cm long (explants were infected, visually normal, no necrosis or vitrification was observed, and no regeneration inhibition was recorded) (Fig. 13). The infected shoots of *C. siliquastrum* "Alba" did not show any inhibition in regeneration ability (Fig. 14).

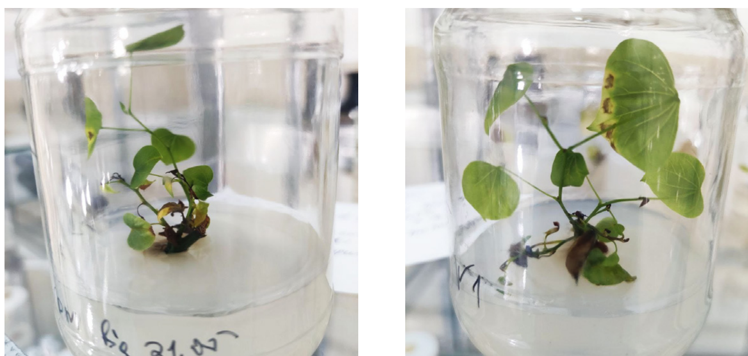

Figure 13. Shoots of *Cercis canadensis* L. at 135 days (hormone-free WPM)
Source: authors' photo

Figure 14. Infected viable shoots of *Cercis siliquastrum* "Alba"
Source: authors' photo

As a result of these studies, aseptic viable shoots and plants of *C. canadensis* and *C. siliquastrum* "Alba" were obtained *in vitro*. Microclonal propagation of plants of the genus *Cercis* was addressed in several studies. When optimising the sterilisation protocol for deciduous plants, the scientists used a stepwise sterilisation using the following substances: ethanol (70%), sodium hypochlorite (5%) and silver nitrate (2%). To improve sterilisation, the authors of the research paper pre-treated the shoots with a fungicide solution: spraying and soaking. The best result in terms of sterilisation efficiency was shown by shoots that were pre-soaked for 24 hours in a fungicide, and then sterilised with ethyl alcohol for 1-2 minutes, followed by keeping in a sodium hypochlorite solution for 10 minutes and finally transferred to a silver nitrate solution for 9-10 minutes. During the propagation of *Sansevieria trifasciata* Prain. K. Panneerselvam *et al.* (2024) used two-stage sterilisation. They note that at the first stage, it was not possible to optimise the sterilisation of leaf explants due to excessive surface infection, therefore, triple sterilisation with ethanol (70%) with different exposure times was utilised. As Ukrainian scientists K. Yevpak & M. Bublyk (2024) determined, the sterilisation efficiency was also affected by the time of *in vitro* introduction. Explants introduced in winter had the highest sterility rate (about 90%), while explants introduced into *in vitro* culture in summer had a sterility rate of only 21%. When *Aronia melanocarpa* (Michx.) Elliott was propagated by D. Abduganiyeva *et al.* (2024) from Uzbekistan, the cuttings were washed in running water to remove solid particles and dust. The scientists also used stepwise sterilisation. The initial explants were sterilised in a solution of domestos (10%) for 30 min, followed by washing 7 times in distilled water, and the shoots were additionally kept in a fungicide solution for 20 min, followed by transferring the explants to a solution of ethanol (70%) for 2 min to remove the residual disinfectant. As a result of this sterilisation protocol, the scientists obtained about 46% of sterile explants.

In particular, L.A. Koldar & M.V. Nebikov (2007) studied the effect of different variants of MS (Murashige & Skoog) nutrient media on the adventitious regeneration of *Cercis* plants (Murashige & Skoog, 1962). The study analysis concluded that the best nutrient medium is a modification of MS with the addition of

2.0 mg/l BA (N^6-Benzyladenine), 0.5 mg/l 3-IAA (3-Indoleacetic acid), and 0.05 mg/l 2, 4-D (2,4-Dichlorophenoxy)acetic acid). The reproduction rate was 3.4 and 11.4 for the first and second passage, respectively. The *in vitro* propagation of *Cercis glabra* Pamp. An emphasis on obtaining polyploid viable plants was conducted by J. Nadler *et al.* (2012). They studied the effect of oryzalin on the induction of polyploidy by different methods of explant treatment.

American scientists led by Wenhao Dai also propagated *Cercis canadensis* L. *in vitro*. They sterilised the explants using a 70% ethanol, followed by transferring the explants to a 0.6% sodium hypochlorite for 15 minutes, containing 3 drops of liquid soap per 100 ml. The shoots were then washed three times in distilled sterile water and blotted dry in sterile paper towels. The actual micropropagation was carried out on 3 nutrient media: MS, DKW (Driver & Kuniyuki Walnut) and WPM (Wood Plant Medium) with the addition of cytokinins, BA and TDZ (Thidiazuron) (McCown & Lloyd, 1981; Driver & Kuniyuki, 1984; Dai *et al.*, 2005). Analysing the works of foreign scientists who were directly involved in the propagation of *Cercis* L. plants, it is possible to conclude that the most common medium was the MS medium variants. For example, when propagating *Cercis yunnanensis* Hu et Cheng, E. Cheong & M.R. Pooler (2003) compared the effect of different growth regulators and explant types on shoot formation and regeneration *in vitro*. The results indicate that the best shoot formation rate was achieved when using MS medium supplemented with 6-BA alone or in combination with TDZ. The multiplication factor was about 3 units. At the same time, when using a hormone-free nutrient medium, the multiplication factor was almost half, namely 1.6 shoots.

In addition to microclonal propagation, *Cercis* was propagated by traditional methods of vegetative propagation. However, R.L. Geneve (1991) noted that stem cuttings of this plant genus are inefficiently propagated, so summer budding is often used. However, according to the author's research, this method is quite expensive due to the fact that the survival rate of budding is less than 50%. Therefore, the study recommended the use of microclonal propagation. Although there are many publications on microclonal propagation of *Cercis* plants, several factors affect the explant *in vitro*, which is why the method was developed individually for each plant.

Thus, the results of the study demonstrate the high potential ability of *Cercis canadensis* and *C. siliquastrum* "Alba" explants for microclonal propagation under *in vitro* cultivation. The dependence of the morphogenetic activity of explants on the number of subcultivations, the type of growth regulators and the composition of the culture medium was established, which is confirmed by both empirical data and literature. These results provide a scientific basis for optimising micropropagation protocols for *Cercis* species, incorporating species characteristics and cultivation conditions.

Conclusions

As a result of the research, a methodology for microclonal propagation of *Cercis canadensis* L., *Cercis siliquastrum* "Alba" plants was developed, aseptic regenerative shoots and plants suitable for further replication and adaptation to environmental conditions were obtained. Using 70% ethanol with an exposure time of 30-60 s and subsequent immersion of explants in a 1% silver nitrate solution for 7-8 min, the sterilisation efficiency was as follows: *C. canadensis* L. 31.3 ± 3.2% and *C. siliquastrum* "Alba" 20.0 ± 1.8%. An effective culture medium for the introduction of explants of the studied plants is hormone-free WPM.

A correlation between the effectiveness of sterilisation and the time of *in vitro*

introduction of plant material was revealed. The best time for harvesting cuttings for propagation is May, in the phase of active growth. The efficiency of the sterilisation of explants introduced into the *in vitro* culture in May was almost 1.5 times higher than that of those introduced in late June and early July. The study determined that the mode of sterilisation of shoots of experimental plants significantly influenced the efficiency. To obtain a significant number of shoots and callus tissue of *C. canadensis* and *C. siliquastrum* "Alba", it is advisable to use WPM nutrient medium with the addition of 0.4 mg/l BA. The study determined that the experimental explants formed shoots on hormone-free nutrient medium, but the formation of callus tissue did not occur. As a single phenomenon, root formation was detected in explants of *C. siliquastrum* "Alba" on the 28th day of cultivation on hormone-free WPM.

The release of secondary metabolites in the area around the basal cut of shoots was observed. Since secondary metabolites can adversely affect the micropropagation process, it is recommended to use sorbents, such as activated carbon, which is characterised by high adsorption properties, to reduce this effect. The study determined, employing one-factor analysis of variance (ANOVA), that the sterilisation efficiency of plants of the genus *Cercis* L. is influenced by the sterilisation mode with a statistically significant difference. The regularity that the number of subcultivations of plant material directly affects the shoot formation was revealed.

Further research could address the identification of the biota in non-sterile explants, studying its effect on the organism of plants of the genus *Cercis* L. and investigating the peculiarities of adaptation of plants to environmental conditions.

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Conflict of Interest

None.

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Особливості мікроклонального розмноження рослин роду *Cercis* L.

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Анотація. У зв’язку зі складністю традиційного вегетативного розмноження *Cercis* L., актуальним є застосування біотехнології *in vitro* для отримання регенераційно здатної асептичної культури зі збереженням декоративних ознак. Мета даного дослідження полягала у розроблені методики мікроклонального розмноження рослин *Cercis siliquastrum* ‘Alba’ та *Cercis canadensis* L. Для цього відібрано рослинний матеріал в різний період вегетації з рослин, що зростали в умовах м. Київ. У роботі використані статистичні та біотехнологічні методи. У результаті проведеного дослідження було апробовано два режими стерилізації. Встановлено, що на ефективність стерилізації та регенерації експлантів впливає сезон ізоляції. За використання 70 % етилового спирту та 1 % нітрату срібла ефективність стерилізації експлантів введених в травні становила: 20,0 ± 1,8 % для *Cercis siliquastrum* ‘Alba’ та 31,3 ± 3,2 % для *Cercis canadensis* L. Експланти вводили у культуру *in vitro* на безгормональне живильне середовище за прописом WPM (Woody Plant Medium). Асептичні експланти в подальшому субкультивовували на WPM з додаванням 0,4 мг/л BA (N6 –Benzyladenine)

та 0,3 мг/л 2iP (6-(γ, γ-Dimethylallylamino)purine) й 0,25 мг/л NAA (1-Naphthylacetic acid). Коефіцієнт розмноження для мікропагонів, культивованих на WPM з 0,4 мг/л BA становив: 9,4 ± 3,5 для *C. canadensis* та 9,7 ± 2,9 для *C. siliquastrum* 'Alba'. Застосування WPM з 0,3 мг/л 2iP і 0,25 мг/л NAA стимулювало активну регенерацію мікропагонів з таким коефіцієнтом розмноження: для *C. canadensis* – 5,0 ± 1,5 та *C. siliquastrum* 'Alba' – 6,5 ± 1,5. Здійснені дослідження дали змогу отримати мікропагони і рослини-регенеранти *Cercis* L. для наступного використання озелененні населених пунктів

Ключові слова: культура тканин рослин *in vitro*; асептичні експланти; мікропагін; регенерація; морфогенез