Received: 30.06.2023; Revised: 20.10.2023; Accepted: 22.11.2023

UKRAINIAN JOURNAL OF FOREST AND WOOD SCIENCE

Journal homepage: https://forestscience.com.ua/en
Ukrainian Journal of Forest and Wood Science, 14(4)

UDC 631.53:57.085:631.526.3:582
DOI: 10.31548/forest/4.2023.103

Post-aseptic adaptation and *ex vitro* propagation of Ukrainian cultivars of *Paulownia* Sieb. et Zucc.

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Abstract. Plantation forestry using highly productive and fast-growing plants involves the use of high-quality improved planting material for genetic constancy, which is solved by microclonal reproduction. However, the high survival rate and stability of plants obtained *in vitro* are realised as a result of post-aseptic adaptation of regenerants. Therefore, the research aims to improve the

Suggested Citation:
Matskevych, V., Yukhnovskyi, V., Kimeichuk, I., Urliuk, Yu., & Tupchii, O. (2023). Post-aseptic adaptation and *ex vitro* propagation of Ukrainian cultivars of *Paulownia* Sieb. et Zucc. *Ukrainian Journal of Forest and Wood Science*, 14(4), 103-121. doi: 10.31548/forest/4.2023.103.

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survival techniques of paulownia regenerants of the Ukrainian varieties "Feniks" and "Enerdzhy" *ex vitro*. The study was conducted in December 2021 on the Ukrainian paulownia varieties "Feniks" and "Enerdzhy", cultivar "Feniks". The survival rate, development of regenerants, and their damage by facultative saprophytic pathogens differed on substrates of organic (Eco Plus, La Flora, Jiffy, coconut peat, cotton wool) and mineral (vermiculite, perlite, sand) origin. The biological products "Rise P" and "Prestop" proved to be effective for the control of pathogenic microorganisms on substrates of organic origin. The technological feasibility of using perlite substrate is substantiated. The pathophysiology of *ex vitro* regenerants of paulownia under Fusarium is described. The thickness of the films used for sheltering influenced the regeneration, engraftment, microbial damage, and temperature of the wet chambers. Growing regenerants with covering films of different thicknesses showed a high survival rate in the variants with stretch films of 10 and 23 μm thickness. In the case of using polyethene films with thicknesses of 60, 80 and 100 μm, a sharp increase in temperature was observed, which was 48, 53 and 65^{0}C, respectively. At these high temperatures, most of the regenerants died, after which they were colonised by facultative saprophytes, and the tissues were completely macerated within two days. The optimal ratio of juvenility reduction and the acquisition of adaptive traits is characteristic of the second, third and fourth generation *ex vitro*. The expediency of propagation by stem cuttings up to the fourth generation, which preserves the regenerative ability of shoot cuttings, was substantiated. The practical results of the research are a protocol for growing paulownia plants *in vitro* and *ex vitro* on different types of substrates for use by institutions engaged in paulownia propagation.

Keywords: explant; regenerant; rhizogenesis; survival; juvenility

Introduction

Modern rapid development of society is impossible without the development and implementation of the latest technologies. In particular, in forestry, this means the introduction of plantation technologies for growing fast-growing valuable species, varieties, and clones of plants, i.e., the transition from extensive to intensive forestry methods (Kaletnik *et al.*, 2021). At the same time, climate change and the ever-growing demand for timber necessitate the urgent creation of high-performance fast-growing plantations from tree species that are adapted to new conditions, resistant to pathogens and climatic stress, and have valuable wood. These requirements are met by hybrid varieties, clones of *Paulownia*. The plant is resistant to high temperatures in summer and low temperatures in winter, and it grows rapidly and develops well. Ukraine has developed varieties that meet the requirements of the State Register of plants suitable for distribution in Ukraine (2023).

V. Matskevych *et al.* (2019) determined that microclonal propagation and post-apoptotic adaptation methods are used for the rapid introduction of Ukrainian paulownia varieties into production. *In vitro*, under heterotrophic nutrition and conventional methods of microclonal propagation using exogenous sources of hydrocarbons and hormones, plant objects reach a juvenile state. Juvenilisation is valuable because it increases the regeneration potential. However, according to A. Podhaietskiy *et al.* (2020), at the stage of adaptation, regenerated plants in this state are vulnerable to adverse *ex-vitro* non-static conditions.

The adaptation of regenerated plants *in vitro* to conditions "outside the glass" (*ex vitro*) is called post-applied adaptation. At this stage, technological methods should be aimed at a gradual, stress-free transition of different levels of endogenous regulation of plant growth and development from organelles and cells to the organismal level. Under such conditions, mixotrophic nutrition with a predominance of heterotrophic plant objects will be transformed into autotrophic nutrition through the activation of photoassimilating systems. V. Matskevych *et al.* (2019) noted that the water exchange system is also reformatted, including the balance between absorption and transpiration. Therefore, after aseptic cultivation, *in vitro*, plants must undergo a post-aseptic "reset", i.e. post-aseptic adaptation.

M. Abbasi *et al.* (2020) show great potential for renewable energy production in Iran, as scientists have substantiated favourable conditions for growing paulownia in an area of about 160,000 km^2. On the other hand, the results of the calculations show that the use of the Z-score reduces the error of weather forecasts by 8%, so the assessment of land suitability can be carried out more accurately. As a result, this robust method can prevent loss of agricultural productivity due to the selection of unsuitable locations.

The research of M. Jakubowski (2022), conducted on paulownia hybrids, showed significant differences in the growth dynamics of individual clones in their response to local environmental and climatic conditions. For example, the yield of dry biomass in the second year of cultivation ranges from 1.5 t/ha to 14 t/ha. This diversity is manifested not only in growth characteristics but also in wood properties and possibilities of its use (Magar *et al.*, 2016; Pożoga *et al.*, 2019).

The water productivity of paulownia and poplar in Kyrgyzstan was studied by N. Thevs *et al.* (2021). These studies were conducted to identify the possibility of additional load on water resources of the introduced paulownia (*Paulownia tomentosa* × *fortunei*) compared to three-year-old poplar (*Populus deltoides* × *nigra*) plants. Poplar increased stem biomass by 5.4 kg with an average water consumption of 4.18 l/day (water productivity 6.79 g/l). The stem biomass of paulownia increased by 4.8 kg at an average flow rate of 2.36 l/day (water productivity 11.90 g/l). Therefore, N. Thevs *et al.* (2021) concluded that the reproduction of paulownia will not put more pressure on the water resources of Central Asia than poplar. This refutes the negative feedback that paulownia is an invasive species and has a negative impact on other tree species.

Biotechnological production of planting material takes more than half of both labour and material resources. Therefore, it is necessary to develop technological methods for using plants *in vitro* only as mother plants for their further propagation using less costly methods in post-aseptic conditions of closed ground. To grow paulownia planting material for industrial purposes, it is necessary to develop technological methods of paulownia propagation as a result of post-aseptic adaptation. Since *in vitro* production is highly costly in the whole technological process, it is important to improve the adaptation of regenerants to *ex vitro* conditions.

The research aims to develop technological methods for simultaneous post-aseptic adaptation and propagation of Ukrainian paulownia varieties based on the results of morphophysiological studies.

Materials and Methods

The research was carried out on the Ukrainian varieties of paulownia "Feniks" and "Enerdzhy". The "Feniks" variety is characterised by fast growth rates and large leaves up to 1.0 m in diameter. The "Enerdzhy" variety has a slightly slower growth rate, but it is characterised by higher winter hardiness (24-26°C) and slightly denser wood. The explants used were cuttings harvested in December 2021 at the Plant Biotechnology Research Laboratory of

Bila Tserkva National Agrarian University. The age of the mother trees used for the study was 6-8 years. Shoots were taken in the middle part of the tree on the south side. The cuttings were taken only from healthy shoots and cut early in the morning to ensure the maximum amount of moisture in them. For each of the varieties, 360 shoot cuttings were taken. Cutting explants were cultivated *in vitro* according to the generally accepted methodology on Murashige and Skoog medium (Japan), which contains salts and a mixture of vitamins (Matskevych *et al.*, 2019). This medium is widely used for growing plant tissues. It has proven to be effective in the culture of tissues derived from monocots and dicots. This medium was developed to support callus and cell growth in suspension culture and regeneration of shoots and seedlings from explants (Kushnir & Sarnatska, 2005). Cuttings explants were cultivated *in vitro* for up to 30 days in containers with a total volume of 250 ml (Fig. 1). Then they were transplanted into closed-ground conditions on artificial substrates: peat, perlite, vermiculite, etc.

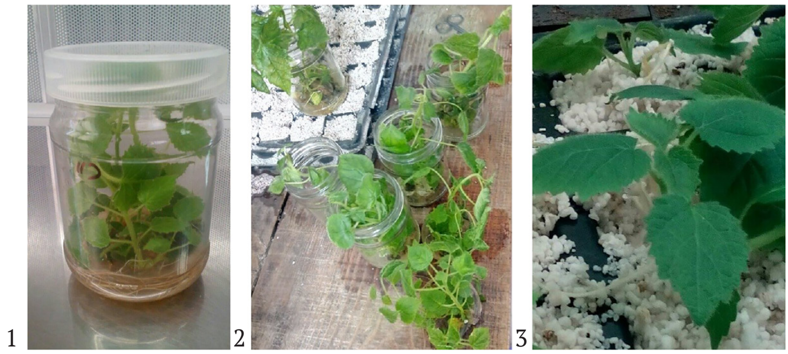


Figure 1. Cultivation of paulownia *in vitro* and *ex vitro* in research
Note: 1 – *in vitro* plant; 2 – planting *in vitro* plants in cassettes with substrate; 3 – *ex vitro* regenerant
Source: authors' photo

The mineral part of the Murashige and Skoog (MS) medium was added to the substrates. Cuttings were carried out for six generations *ex vitro* using the overlay method. Adapted plants *in vitro*, from which cuttings were taken for the first green cuttings, were conditionally considered as "generation 0". For successful adaptation of paulownia plants *in vitro*, their survival rate was analysed on the following substrates: universal substrate Eco Plus (produced in Ukraine), substrate LaFlora KKS-2 (produced in Latvia), peat loose substrate Jiffy, Jiffy tablets (produced in Estonia), coconut substrate Grond Meester UNI (produced in Sri Lanka), cotton wool, vermiculite, perlite, sand (produced in Ukraine).

Since it is necessary to maintain a turgid state in green cuttings to preserve moisture, they were planted in a humid chamber. For the first two weeks, the chamber was covered with a film lid. In the third week, the lid was gradually lifted to reduce the humidity from 95% to 65%. The temperature in the wet chambers was maintained at 24°C during cultivation. The chambers were ventilated twice a day for 15-20 minutes.

After rooting of green cuttings and regeneration of plants from them, they were re-cut or transplanted into cassettes with 500 ml cells, which were used for planting in the field or for sale. To combat pathogenic organisms (facultative saprophytes), two types of preparations were selected – "Rise P" (manufactured by Lallemand Plant Care SAS, France) and "Prestop" (Verdera OY, Finland), which were used to treat the substrate. The *Bacillus amyloliquefaciens* IT45 bacteria contained in "Rise P" were isolated from natural soils and selected by researchers from hundreds of other PGPR bacterial strains due to their agronomic properties: colonisation of the rhizosphere, secretion of compounds that are beneficial for the growth and nutrition of the host plant. The "Rise P" product is available as a wettable powder and provides benefits for a variety of crops.

"Prestop" is a biological fungicide for the control of fungal pathogens, in particular: grey rot (*Botrytissp.*), didymella (*Mycosphaerella*), fusarium, and Rhizoctonia. Clothianidin is an active ingredient from the neonicotinoid class that is systemically distributed throughout the plant and provides long-term protection against pests - for more than 30 days. The product has been tested for safety in the EU (Baibakova *et al.*, 2019). The biological products "Rise P" and "Prestop" were applied by spraying the substrate and plants in cassettes. The concentration of the solutions was 0.01%.

Organic substrates based on peat and coconut can be food not only for plants but also for undesirable organisms: saprophytic and pathogenic fungi, and insect larvae. In particular, peat substrates and coconut substrates were characterised by the colonisation of larvae of the mushroom mosquito *Mycetophilidae*, identified by the results of analyses carried out by the State Institution "Volyn Regional Phytosanitary Laboratory". Therefore, for plant objects, an additional measure was the use of Aktara (manufactured by Syngenta, Switzerland).

Plants were grown for two weeks in a humid chamber with 4 replications throughout the experiment. This is partly in line with the use of sugar-free medium technology (Photoautotrophic Micropropagation) or the New Micropropagation and Transplant Production System. These methods involve the absence of exogenous sources of carbohydrates, but instead intensify their synthesis by photoassimilation and affect photosynthesis simultaneously by increasing the light intensity (11,000 lx or more) and increasing the CO_2 content in the air (Kozai *et al.*, 2005). Wet chambers for *in vitro* plant adaptation are characterised by different volumes both in the root zone and in the air space around the plants. Among the most commonly used in production, two types were selected: 1 – micro-greenhouse cassette; and 2 – hydroponic trough with a lid (Fig. 2). Their dimensions are 140×60×8 cm (0.0192 m^3) and 100×60×20 cm (0.12 m^3), respectively.

Figure 2. Wet chamber – hydroponic trough with lid

Source: authors' photo

In the first variant, 60 planting cells were placed in 0.0192 m^3, which was an average of 0.00032 m^3 per cell. In the second variant, 150 cells (2.5 cassettes of 60 cells each) were placed in a volume of 0.12 m^3, which averaged 0.0008 m^3. That is, the volume per plant with the same area was 2.5 times larger (0.0008/0.00032 = 2.5). To study the effect of the wet chamber coating material, thin films with a thickness of 10-100 microns were used. The study was conducted following the requirements of the Convention on Biological Diversity (1992).

The mean biometric values X, their error m and correlation analysis were calculated using the Statistica 8.0 software package. The reliability of the difference in the mean values of the obtained data was assessed by the Student's t-test (Oghirko & Galayko 2017). The series of studies was conducted using the "step-by-step" principle. Its essence was that a series of consecutive experiments were carried out with regenerants that had better growth rates and were involved in the next stage of research, i.e., the variant of the previous experiment was the control in the next one.

Results and Discussion

Under *in vitro* conditions, regenerants develop a special anatomical structure that requires post-applied adaptation. This includes the creation of moist chambers with a gradual controlled decrease in air humidity and a moisture-absorbing and at the same time breathable substrate, which helps to develop the root system. More than 20 types of substrates and various combinations are used in indoor conditions (Matskevych *et al.*, 2019; Podhaietskiy *et al.*, 2020). The analysis of plant survival on different substrates (Table 1) showed that sand is not a technologically acceptable substrate for adaptation of both *in vitro* plants (0 generation) and for planting green cuttings of the 2nd generation. The survival rate on sand was only 2-7% higher than the least significant difference ($NIR_{0.5}$).

Table 1. *In vitro* survival of paulownia plants and green cuttings, %

Substrate	"Feniks" Variety		"Enerdzhy" Variety	
	***in vitro* plants**	**second-generation**	***in vitro* plants**	**second-generation**
Eco Plus	19	61	16	59
La Flora	35	73	22	64
Jiffy	44	72	41	66
Jiffy pills	69	71	56	65
Coconut peat	33	72	27	71
Cotton wool	51	72	60	69
Vermiculite	49	75	42	66
Perlite	64	84	61	71
Sand	7	11	6	8
$HIP_{0.5}$	5	4	5	6

Source: compiled by the authors

The decrease in the survival rate of "Feniks" plants *in vitro* on different substrates was distributed in the following order: Jiffy pills, perlite, cotton wool and vermiculite, which was 69%, 64%, 51% and 49%, respectively. The *in vitro* survival of "Enerdzhy" plants in descending order was recorded in perlite (61%), cotton wool (60%), Jiffy tablets (56%) and vermiculite (42%); the second-generation plants of this variety had the best survival in perlite (71%) and coconut peat wool (69%), followed by vermiculite and Jiffy loose peat (66%). Green cuttings regenerated 3-6 adventitious roots in the second week (Fig. 3).

Figure 3. Root formation in green cuttings of second-generation paulownia *ex vitro*
Source: authors' photo

In vitro, the survival rate of plants compared to green cuttings of the 2nd generation was lower in both varieties of paulownia. This is due to both anatomical features (condition of stomata, structure of integumentary tissues) and the time regime for restarting trophic and hormonal determinants. Among the peat substrates compared, Jiffy pills were the best in terms of survival rates. On this substrate, plants of the "Feniks" variety *in vitro* survived in the amount of 69%, and of the "Enerdzhy" variety – 56%. These figures were higher than when the same Jiffy substrate was used, but not in the format of tablets with a mesh, but simply poured into cassette cells. In addition to the properties of the substrate itself, greater aeration of the root zone affected the plant organisms (Fig. 4). In green cuttings of the 2nd generation, the survival rate was higher, but this substrate was inferior to perlite in terms of survival rate in both varieties.

a  *b* 

Figure 4. Development of second-generation paulownia regenerants on different substrates
Note: *a* – Jiffy pills; *b* – cotton wool
Source: authors' photo

It should be noted that in addition to the influence on the presence of air, water, and the specifics of nutrient retention, substrates have different colours, and therefore different absorption of light energy and heating. Among the substrates compared, perlite and cotton wool had a white colour. The biological characteristics of the varieties also had an impact on their survival and regeneration. The "Feniks" variety had higher survival rates than the "Enerdzhy" variety. This pattern was typical for regenerants of all generations in subsequent experiments. On peat substrates, in addition to lower survival rates, both cuttings and regenerated plants were affected by fungal pathogens that cause diseases such as Fusarium and blackleg (Table 2, Fig. 5). Damage and damage rate increased with increasing temperature above 24°C, increasing humidity and decreasing time of ventilation of wet chambers.

Table 2. Affection of plants on different substrates by fungal pathogens, %

Substrate	"Feniks" Variety		"Enerdzhy" Variety	
	in vitro plants	of the second-generation	*in vitro* plants	of the second-generation
Eco Plus	63	31	74	39
LaFlora	45	33	22	34
Jiffy	14	12	21	27
Jiffy pills	19	17	16	15
Coconut peat	29	13	37	19
Vermiculite	0	0	0	0
Perlite	0	0	0	0
Cotton wool	0	0	0	0
Sand	100	79	100	94
$HIP_{0.5}$	5	4	5	6

Source: compiled by the authors

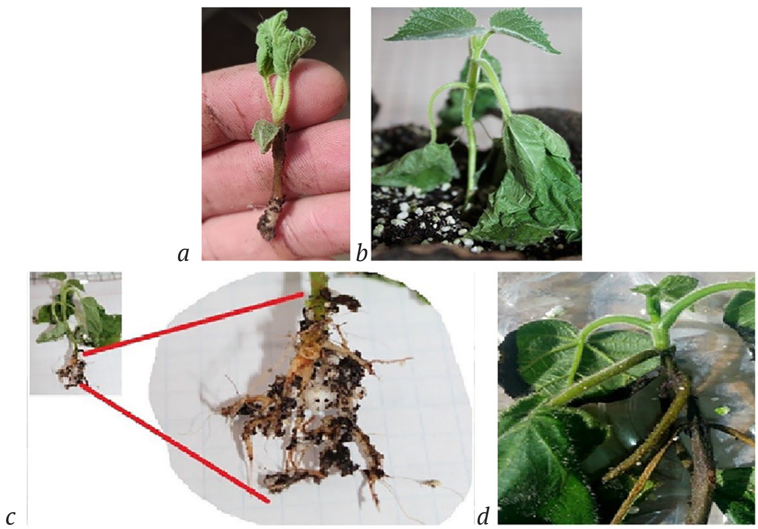


Figure 5. Damage of green cuttings and regenerated plants of "Enerdzhy" on peat substrate
Note: *a* – the first symptoms of wilting of the cutting; *b* – the first symptoms of wilting of the regenerate; *c* – the affected root system of the regenerate; *d* – stem necrosis
Source: authors' photo

In the case of Fusarium infection, the symptoms appear on the lower part of the shoots. The lower leaves are the first to wilt and droop due to vascular occlusion. According to observations, this wilting feature is what distinguishes infection with this fungus from wilting caused by moisture deficiency. Affected plants have a smaller root system, yellow brown in colour. Root hairs are the first to die, followed by small peripheral roots. In the case of a lack of water in the substrate, the upper leaves wither first due to loss of turgor. Young organs are more susceptible to dehydration. With a slight moisture deficit, the root system remained unchanged in size and colour. Regenerants without signs of damage grown on perlite substrate are shown in Figure 6. The root is white, and the lateral roots "grow" into the perlite particles. After 15 days, a well-developed root system, growing and intertwining in the cell of the cassette, holds all the perlite and does not lose its shape when transplanted into larger containers.

a *b*

Figure 6. Condition of regenerated "Enerdzhy" plants on perlite substrate
Note: *a* – healthy white root system; *b* – root growth in the cassette cell
Source: authors' photo

In addition to purely pathogenic organisms, facultative saprophytes were also colonised on substrates of organic origin (Fig. 7). They had no direct impact on the regenerants in the studies conducted. However, over time, in cases of death of these microorganisms, the regenerants were inhibited. In particular, the height of plants and the percentage of survival decreased. To combat them, the trial established the effectiveness of "Rise P" and "Prestop" preparations. The effectiveness of the preparations was found to be both preventive and therapeutic.

Figure 7. Use of "Rise P" and "Prestop" for the control of facultative saprophytes
Source: authors' photo

Thus, the use of peat substrates and coconut substrate was inferior to vermiculite and perlite in terms of survival rate and was a favourable environment for the development of facultative saprophytes, pathogenic fungi and insects. Therefore, perlite substrates were used for further research. The use of perlite changes the strategy of controlling facultative heterotrophic parasites that are unable to develop on mineral substrates. Table 3 shows the survival rate and growth rates of regenerants in wet chambers of different volumes.

Table 3. *In vitro* survival and height of regenerants in wet chambers on the 15th day of cultivation

Wet chamber variant	"Feniks" Variety		"Enerdzhy" Variety	
	the survival rate of regenerants, %.	increase in shoot height, mm	the survival rate of regenerants, %.	increase in shoot height, mm
1 (40×60×8 cm)	61	56	49	37
2 (100×60×20 cm)	78	75	63	43

Source: compiled by the authors

The effect of chamber volume on the survival rate of both cultivars under the same conditions was found. In the case of the "Feniks" variety, the number of regenerants that survived increased from 61 to 78% with increasing chamber volume. In the "Enerdzhy" variety, the number of regenerated plants that took root increased from 49 to 63%. The stem growth rate, i.e. the difference in the height of the regenerant before planting and the height on the day of the survey, also increased.

The better survival rate with a larger air volume is due to greater gas exchange, and less temperature and humidity fluctuations. When comparing the varieties, the "Feniks" variety had a higher survival rate and shoot growth compared to the "Enerdzhy" variety. To adapt the cassette greenhouses to a larger volume on the farm, they were modified by replacing them with arcs with film material. This allows for an increase in the volume of air above the plants. And later, hydroponic troughs are used both in experiments and in production conditions (Suryawanshi, 2021).

Influence of the covering material of the wet chamber. To reduce the dehydration of plant objects in wet chambers, it is necessary to seal them. In particular, covering the top of the chamber with transparent lids that transmit light well and ensure humidity maintenance. The effectiveness of different films on post-septic adaptation was compared (Table 4). In the control, in the absence of the film, all regenerants died from dehydration.

Table 4. *In vitro* survival and height of regenerants in wet chambers on the 15th day of cultivation

Wet chamber variant	"Feniks" Variety		"Enerdzhy" Variety	
	of regenerated plants survived, %.	increase in shoot height, mm	of regenerated plants survived, %.	increase in shoot height, mm
Control without film	0	0	0	0
Transparent stretch film 10 μm	79	56	63	39
Transparent stretch film 23 μm	78	55	63	37
Polyethylene film 60 μm	41	59	21	33
Polyethylene film 80 μm	33	38	18	11
Polyethene film 100 μm	9	27	5	12
$HIP_{0.5}$	4	5	4	3

Source: compiled by the authors

The covering material compared (different types of film) differed primarily in thickness. Depending on the variant, this indicator ranged from 10 to 100 microns. When using stretch films with a thickness of 10 and 23 microns, the temperature increased from 22 to 26 and 27°C. A sharp increase in temperature was observed in the variants with thicknesses of 60, 80 and 100 μm (Fig. 8).

Despite the high heat resistance of paulownia, the use of films and, accordingly, chambers with a temperature of 48°C and higher led to significant losses among tender plants *in vitro* (Fig. 9).

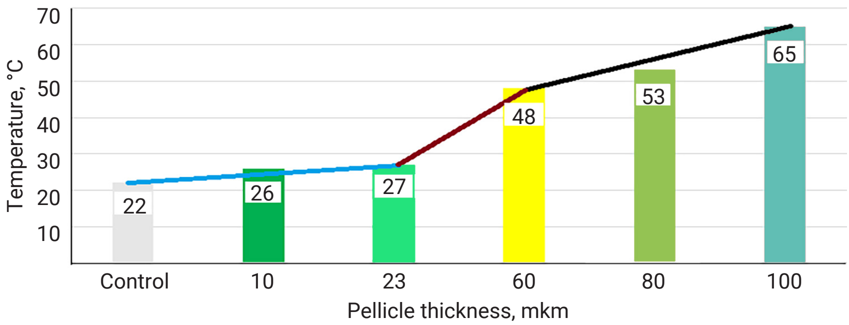
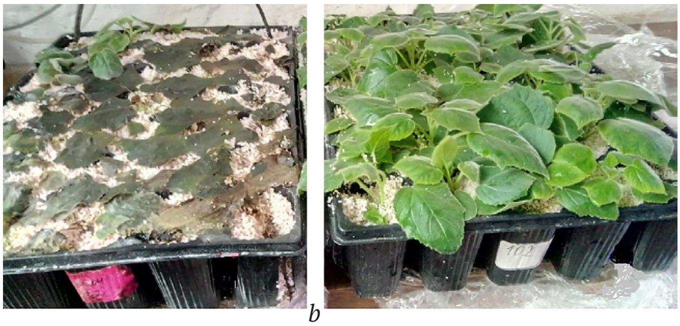


Figure 8. Wet chamber temperature at different film thicknesses
Source: compiled by the authors

1 2

Figure 9. Damage of plants *in vitro* by high temperatures and colonisation by thermophilic bacteria on the variant "Polyethylene film 80 μm"
Note: 1 – general view of greenhouse cassettes; 2 – maceration of damaged regenerant tissue
Source: authors' photo

Studies by A. Ivanyuk *et al.* (2019) on the effect of variable temperature on the germination of *Paulownia tomentosa* Steud. seeds also showed that lowering the temperature causes a decrease in germination energy, laboratory, and absolute seed germination, and increases the average seed dormancy. Therefore, it is important to find the optimal temperature regime for growing regenerants. At high temperatures, most regenerants died. The dead plant objects were colonised by facultative saprophytes, in particular thermophilic bacteria. Regenerants that were more adapted, including *ex vitro* plants of the third generation, also died (Fig. 10). The plant tissues were observed to be completely macerated within two days.



a *b*

Figure 10. Damage of *ex vitro* plants of the 3rd generation of "Feniks" cultivar by high temperatures and colonisation by thermophilic bacteria on variants

Note: 1 – polyethylene film 80 μm; 2 – transparent stretch film 23 μm

Source: authors' photo

An attempt to reduce the temperature in the room where the wet chambers with a film thickness of 60-100 μm were located led to an excessive increase in humidity in the chambers and the formation of a significant amount of dew condensation. There was no difference in the change in chamber humidity between films with thicknesses of 10 and 23 μm. However, the thicker film was observed to be denser under production conditions. As a result, its service life was extended from 1 month with a thickness of 10 μm to 4 months with a thickness of 23 μm. Czech researchers J. Kadlec *et al.* (2021) concluded that simple soil cover is the most effective protection of the root system from frost when growing paulownia in the open field. At the same time, a nonwoven bandage is the best protection for the aboveground parts of the plants.

Effect of the number of generations of green cuttings donors on explant regeneration. Cultivation of artificial nutrient media with a high content of stimulatory hormones, in particular, cytokinins, causes the effect of juvenilisation. Changes in juvenility over several successive passages and post-appliance adaptation affect several indicators during regeneration: explant survival, biometric dimensions of regenerated vegetative organs (stem, leaf, root), which was also confirmed by V. Matskevych *et al.* (2019), A. Podhaietskiy *et al.* (2020).

Survival rate. Tests on the "Feniks" and "Enerdzhy" varieties revealed that *in vitro* survival rates of 64 and 61% were observed, respectively (Table 5, Fig. 11). Moreover, the survival rate of "Feniks" increased up to the third-generation *ex vitro*. In the "Enerdzhy" variety, growth is observed up to the second generation. In the sixth generation, the survival rate decreased to 36% in "Feniks" and 32% in "Enerdzhy".

Table 5. Regeneration rates and parameters of vegetative organs of green cuttings of paulownia *ex vitro* on the 15th day of cultivation depending on generation

Indicator	Generation							Pearson's correlation coefficient
	0*	1	2	3	4	5	6	
"Feniks" Variety								
Survival rate,	64	78	84	92	72	48	36	0.75
Shoot height, mm	51	68	77	74	71	62	56	0.78
Leaf blade diameter, mm	22	58	93	97	89	84	72	0.62
Root system length, mm	26	41	67	61	54	31	16	0.88

Table 5, Continued

Indicator	Generation							Pearson's correlation coefficient
	0*	1	2	3	4	5	6	
"Enerdzhy" Variety								
Survival rate,	61	66	71	65	60	49	32	0.78
Shoot height, mm	43	55	69	66	58	51	38	0.92
Leaf blade diameter, mm	17	37	51	49	46	31	27	0.77
Root system length, mm	13	22	29	21	17	12	9	0.85

Note: * "Generation 0 - in *vitro plants*"
Source: compiled by the authors

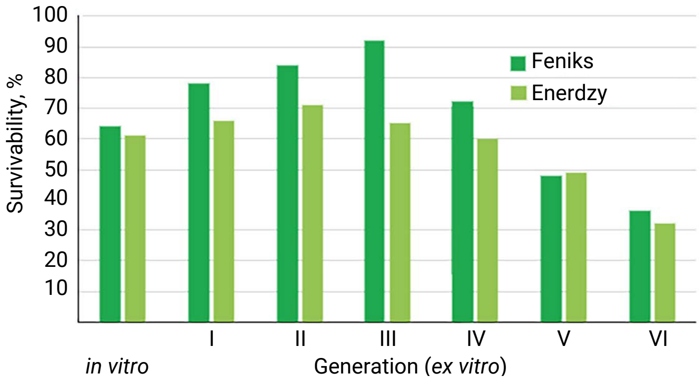


Figure 11. Explants survival rate in different generations of green cuttings donors, %
Source: compiled by the authors

Studies have shown that an increase in survival rate is associated with improved adaptation of plants to *ex vitro* conditions. A decrease in survival is associated with a gradual loss of juvenility. Thus, the optimal ratio of juvenility reduction and the acquisition of adaptive traits is characteristic of the second, third and fourth *ex vitro* generations. In this physiological state, two centres of the body's hormonal axis are actively functioning in regenerants: 1 – the apical meristem as the centre of auxin synthesis; 2 – the root meristem as the centre of cytokinin synthesis. With each subsequent passage, with the loss of juvenility and approaching the generative period of ontogeny, the activity of stimulatory hormones decreases (Keara & Wigge, 2014; Podhaietskiy *et al.*, 2020).

Size of vegetative organs. With each subsequent generation, not only the survival rate but also the size of the vegetative organs, including the stem, changed. The size of the root system was in natural correlation with the size of photoassimilating organs (Table 5). At the stage of adaptation of two species of Paulownia (*Paulownia hybrid* and *Paulownia tomentosa*), M. Mohamad *et al.* (2021) used peat and sand in different proportions as a soil mixture. The results showed that paulownia seedlings successfully survived (100%) in a soil mixture containing peat and sand (1:2, volume/volume). The highest values of seedling height and number of leaves/seedlings were recorded in this mixture. It was found that one of the signs of the loss of juvenility by plants is an increase in the size of the teeth on the leaf blades. A clearer sign of cuttings unsuitable for grafting was the formation of a void in the middle of the stem, which is characteristic of the fourth and older generations (Fig. 12).

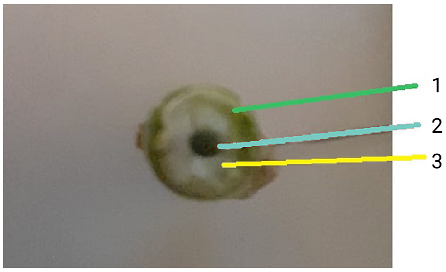


Figure 12. Stem void formation in 5th generation regenerants of "Feniks" cultivar
Note: 1 – cambium, cortex; 2 – parenchyma; 3 – cavity
Source: authors' photo

By studying the cell morphology and anatomical composition of green paulownia wood (a hybridisation of *Paulownia elongata* × *Paulownia fotunei* and tropical *Paulownia* spp.) as a raw material for the woodworking industry, N. San *et al*. (2016) found that the physical and mechanical properties of 3-year-old green paulownia have the same properties as 7-11-year-old *P. fortunei*. This confirms the optimal age of paulownia plants for cuttings.

Seedlings of different physiological ages. The loss of juvenility and intervention in the plant organism by cutting a part of the shoot changed the physiological age of both explants and their donors. Cutting of cuttings of the next generation from mother plants showed that shoot buds of mother plants formed the next order of branches, from which cuttings can also be harvested.

The induction of *in vitro* organogenesis of different explants originating from short-term stored seeds of *Paulownia elongata* and long-term stored seeds of *Paulownia elongata* x *fortunei* was studied by V. Gyuleva (2010). The nodal segments of three-month-old plants germinated from seeds under controlled conditions were used as primary explants. After induction of shoots and growth on Murashige and Skoog medium enriched with 0.3 mg/l 6-benzylaminopurine (BAP), isolated nodal and leaf segments were used for the active propagation stage. Of the different concentrations of BAP tested for propagation, the best effect was observed in a medium with 1.0 mg/l for nodal segments as explants of both species. The growth regulator Thidiazuron at a dose of 10 mg/l had a similar effect on the regeneration capacity of leaf segments grown on MS medium. These concentrations provoked the best effects related to the multiplication ratio, the average length of the newly formed bud and the overall shoot morphology. A 100% rooting rate *in vitro* and 90% adaptation to greenhouse conditions of the studied plants were achieved.

The ontogeny of the organism as a whole, organogenesis, histogenesis, and cell development occur in clear systemic correlations. In particular, one organ influences another. This is one of the determinants of the activation of certain genes or combinations of genes during the life cycle. For example, the branches of one tree are of different quality not only in terms of calendar but also in terms of physiological age. It is believed that the higher the order of branches, the older they are physiologically, despite their lower calendar age. There is a theory of physiological age in plant physiology. The physiological age of a branch of a certain order is the sum of the calendar ages of the branches of the previous branching, of which this branch is a continuation (Dolzhitska & Panchuk 2010). Considering this circumstance, a technique has been developed that allows inducing flower buds in three-month-old paulownia seedlings (Fig. 13). Such plants can be useful for ornamental gardening and breeding purposes.

Figure 13. Biological clock effect – laying flower buds in three-month-old seedlings
Source: authors' photo

This method is based on cutting off the tops of branches of one order to stimulate the growth of branches of the next order. In this way, branching is stimulated, which is typical for three- or four-year-old plants. It is at this biological age that paulownia begins to lay flower buds. Thus, the Ukrainian varieties of Paulownia "Feniks" and "Enerdzhy" adapt well to selected environments of organic and mineral origin. However, the best results were obtained on vermiculite, perlite, and cotton wool substrates, where the regenerants were not affected. The post-aseptic adaptation of regenerants in closed ground conditions using different types of covering material showed the effectiveness of using stretch films with a thickness of 10 and 23 μm, which form the optimal temperature regime.

Conclusions

The studies of post-applied adaptation of Ukrainian paulownia varieties "Feniks" and "Enerdzhy" revealed different effects of organic and mineral substrates on the survival rate, development of regenerants, and their damage by facultative saprophytic pathogens. On peat substrates with lower survival rates, cuttings and regenerated plants were affected by fungal pathogens such as Fusarium and blackleg, and their harmfulness and damage rate increased with increasing temperature above 24°C, increasing humidity and decreasing the time of ventilation of wet chambers. The biological characteristics of the varieties also influenced their survival and regeneration. Regenerants of the "Feniks" variety had higher indicated indicators compared to the "Enerdzhy" variety in all generations. Plant survival ranged from 64 to 81% depending on the variety and generation of regenerants. These values were higher than when the same Jiffy substrate was used, but not in the format of tablets with a mesh, but simply poured into cassette cells. The number of regenerants that took root increased with the increase in the volume of cassette cells. On the perlite substrate, regenerants were not affected by Fusarium. Biological products "Rise P" and "Prestop" proved to be effective for the control of pathogenic microorganisms on substrates of organic origin.

The coating material used in the form of polyethene and stretch films differed in thickness and, depending on the variant, ranged from 10 to 100 microns. When using stretch films with thicknesses of 10 and 23 microns, the temperature increased from 22 to 27°C.

A sharp increase in temperature was observed under polyethene films with thicknesses of 60, 80 and 100 microns, which were 48, 53 and 65°C, respectively. At these high temperatures, most of the regenerants died, after which they were colonised by facultative saprophytes, in particular thermophilic bacteria. It was found that plant tissues were completely macerated within two days. The obtained results of the research will allow us to identify the optimal varieties of paulownia and select a substrate that allows us to obtain the maximum survival rate and yield of healthy planting material for use in plantation forestry of forestry enterprises of the State Enterprise "Forests of Ukraine".

Acknowledgements

None.

Conflict of Interest

None.

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Постасептична адаптація та розмноження українських сортів *Paulownia* Sieb. et Zucc. *ex vitro*

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Анотація. Плантаційне лісовирощування з використанням високопродуктивних і швидкорослих рослин передбачає застосування високоякісного оздоровленого садивного матеріалу генетичної константності, що вирішується міклоклональним розмноженням. Проте висока приживлюваність і стійкість рослин, отриманих *in vitro*, реалізується в результаті постасептичної адаптації регенерантів. Тому вдосконалення прийомів приживлюваності регенерантів павловнії українських сортів ‘Feniks’ і ‘Enerdzhy’ *ex vitro* стало метою цієї роботи. Дослідження поводилось в грудні 2021 року на українських сортах павловнії ‘Feniks’ і ‘Enerdzhy’. Сорту ‘Feniks’. Приживлюваність, розвиток регенерантів, їх пошкодження факультативними сапрофітними патогенними мікроорганізмами різнилися на субстратах органічного (Eco Plus, La Flora, Jiffy, кокосовий торф, бавовняна вата) та мінерального (вермикуліт, перліт, пісок) походження. Для контролю патогенних мікроорганізмів на субстратах органічного походження ефективними виявилися біопрепарати ‘Rise P’ та ‘Prestop’. Обґрунтовано технологічну доцільність застосування перлітового субстрату. Описано патофізіологію регенерантів павловнії *ex vitro* при фузаріозі. На показники регенерації, приживлювання, ураження мікроорганізмами, температуру вологих камер впливала товщина плівок, які використовувалися для укриття. Вирощування регенерантів з покривними плівками різної товщини показало високу їх приживлюваність у варіантах із стреч плівок товщиною 10 і 23 мкм. У випадку застосування поліетиленових плівок товщиною 60, 80 і 100 мкм, відмічене різке зростання температури, що становило 48, 53 і 65°C відповідно. За цих високих температур більшість регенерантів гинули, після чого заселялися факультативними сапрофітами, і тканини повністю мацерувалися упродовж двох діб. Оптимальне співвідношення зниження ювенільності і набуття пристосувальних ознак властиве другому, третьому та четвертому поколінню ex vitro. Обґрунтовано доцільність розмноження стебловими живцями до четвертого покоління, за яких зберігається регенераційна здатність пагонових живців. Практичними результатами досліджень є створений протокол вирощування рослин павловнії *in vitro* та *ex vitro* на різних видах субстрату для використання установами, які займаються розмноженням павловнії

Ключові слова: експлант; регенерант; ризогенез; приживлюваність; ювенільність