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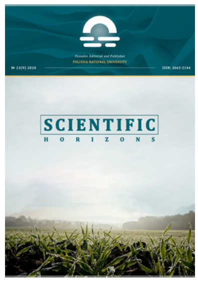

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Peculiarities of plant adaptation of interspecific hybrid *Betula ex vitro*

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Abstract. Microclonal propagation as one of the methods of biotechnology allows obtaining genetically homogeneous plants during the year from a minimum amount of donor material. Adaptation of plants *ex vitro* to environmental conditions is the final and important stage of microclonal propagation. The purpose of the study is to determine the optimal mode of plant adaptation of triploid interspecific hybrid *Betula ex vitro* to environmental conditions. Biotechnological (microclonal propagation) and statistical (arithmetic mean, standard error, one-way analysis of variance (ANOVA)) methods were used for research. The study was carried out in the research of Plant Biotechnology Laboratory of the Separated Subdivision of National University of

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Life and Environmental Sciences of Ukraine "Boyarka Forest Research Station" during 2019-2023. According to research, the MS nutrient medium with the addition of 0.25 mg·l^{-1} of kinetin stimulated the active proliferation of microshoots and root system. Optimum plant development took place on the nutrient medium *vitro* for a 25-30-day cycle of cultivation, which contributed to successful adaptation to environmental conditions. The proportion of adapted *Betula ex vitro* hybrid plants on agroperlite substrate was more than 60% for 30 days. Significant plant viability (more than 80%) was obtained under the conditions of preliminary exposure of the plant root system in auxin solution (1.0 mg/l IAA, 1.0 mg/l NAA, 1.0 mg/l IBA) for 25-30 minutes followed by daily spraying of leaves with 20% glycerin for 15-20 days. Adapted plants had typical pigmentation without signs of chlorosis and vitrification. In the spring, the survival rate of plants under environmental conditions was more than 80% and indicated a high level of adaptability after *in vitro*. The developed protocol for the adaptation of the *Betula ex vitro* hybrid allows obtaining high plant survival in environmental conditions. The practical value of the research is obtaining plants adapted to environmental conditions *ex vitro,* which in the future can be used to qualitatively enrich the range of artificial plantings in settlements

Keywords: culture *in vitro*; birch; microclonal propagation; substrate, viability

INTRODUCTION

Microclonal propagation is considered as a component of intelligent management of natural resources, which emphasizes the use of high-quality plant material *in vitro.* Application of technologies *in vitro* allows you to save and multiply valuable plant genotypes, create a plant bank, carry out cryopreservation, improve health and obtain plants with the necessary quality parameters. Such technologies are an effective way of obtaining high-quality plant material of forest-forming species. Obtained plant tissues *in vitro* – a model for genetic and cellular engineering. In this context, microclonal propagation of the triploid interspecific hybrid *Betula ex vitro,* which has a high growth rate and resistance to adverse environmental factors, is an extremely urgent task. Such propagation allows obtaining genetically homogeneous plants throughout the year from a minimum amount of donor material. Adaptation of woody plants *ex vitro* as the final and important stage of microclonal propagation shows how much the protocol *in vitro* is effective and can be used in large volumes for mass cultivation and high-quality enrichment of artificial plantations.

In the world practice of research with tissue culture of woody plants *in vitro* aimed at the study of plant material's juvenileness and the expression of target genes; intraspecific reaction of plants to LED lighting; development of a protocol for microclonal propagation, study of the regenerative capacity of tissues under the action of the components of the nutrient medium; determination of the anatomical, biochemical and physiological state of tissues; study of plant adaptation *ex vitro.*

In particular, the authors of M. Zeps *et al.* (2022) studied the intraspecific response of plants *in vitro* silver birch (*Betula pendula* Roth.) and clones of hybrid aspen (*Populus tremuloides* Michx. × *Populus tremula* L.) from the eastern Baltic to LED artificial lighting (LED). It was established that the studied light treatments did not affect the density of stomata and the secondary cell wall of xylem in birch stems, as well as the length of stomata, stem radius and phloem width of hybrid aspen. In the study, scientists B. Krivman *et al.* (2023) evaluated the expression of the target genes responsible for the juvenile microRNAs during the micropropagation of white birch, and also determined the factors affecting the juvenile genotypes and their different morphogenic ability *in vitro.*

Researchers S. Välimäki *et al.* (2021) studied the regenerative capacity *of in vitro* of dormant plant buds *of Ulmus laevis* Pall. and *Ulmus glabra* Huds. after cryopreservation. The authors also studied tissue initiation on DKW nutrient medium with the addition of growth regulators and tested the rooting of microshoots during short-term induction in a liquid nutrient medium followed by adaptation. P. Chmielarz *et al.* (2023) developed a protocol for microclonal propagation of 800-year-old *Quercus robur* L. plants using WPM and QL nutrient medium based on previous studies of M. Quorin & P. Lepoivre (1977).

Scientists J. Martins *et al.* (2022) investigated the effect of BA and kinetin (6-furfurylaminopurine) in WPM nutrient medium on the anatomical, biochemical and physiological condition of *Q. robur* shoots. Authors N. Dimitrova *et al.* (2021) note that plant adaptation *ex vitro* is a rather important stage that generally determines the success of micropropagation. It was established that the biostimulant Lumbrical (1:16, v:v), which was added to the substrate (peat, perlite, 1:1; peat-perlite, 1:1, v/v), increased the efficiency of plant adaptation *ex vitro Pyrus communis* L., improved vegetative growth and photosynthetic characteristics. H. Ribeiro *et al.* (2022) to adapt microshoots of walnut hybrid rootstock 'Paradox' (*Juglans hindsi* × *Juglans regia*) cl. 'Vlach' used rhizogenesis *ex vitro* using a substrate (coconut fibre, perlite, vermiculite, 80:10:10%). Researchers M. Capuana *et al.* (2022) established that for the adaptation of *Salix acmophylla* (Boiss.) *in vitro,* it is advisable to use an autoclaved substrate consisting of garden soil, peat, and sand (3:2:1).

N. Sharma *et al.* (2023) investigated various strategies (hydroponics, photoautotrophic acclimation, biotization) to achieve successful acclimation of plants *ex vitro*. Authors O. Chornobrov & O. Tkachova (2020) note that to prevent dehydration of *Fragaria vesca* L. *ex vitro* effectively apply the method of daily spraying of leaves with 30% glycerin solution for 30 days. They also note the influence of the length of time plants are kept in conditions of high relative humidity (85-90%) on the efficiency of adaptation of *F. vesca,* because its sudden change is a stress for plants.

Analysis of the literature indicates that the effectiveness of the adaptation of woody plants *ex vitro* to environmental conditions depends on a complex of internal and external factors, and quite often a significant percentage of waste is recorded. This, in turn, determines the need to develop an individual adaptation protocol and carefully select cultivation conditions for successful plant survival *ex vitro*. That is why the goal of the study was to develop an optimal protocol for the adaptation of plants of the triploid interspecies hybrid *Betula ex vitro* to environmental conditions. Research objectives: 1) to analyse the effect of various substrates on the efficiency of adaptation of hybrid plants; 2) to establish the viability of hybrid plants under the conditions of keeping the root systems in different auxin solutions; 3) to investigate the effect of different concentrations of glycerol on the growth characteristics of hybrid plants.

MATERIALS AND METHODS

The research was carried out at the Plant Biotechnology Laboratory of the Separated Subdivision of National University of Life and Environmental Sciences of Ukraine "Boyarka Forest Research Station" during 2019-2023. Aseptic regenerating plants of the triploid interspecific hybrid *Betula* were used for the research. This hybrid was obtained at the Scientific and Research Institute of Forestry "Silava" (Latvia) by artificial pollination of a suspended birch with downy birch pollen. The *Betula* hybrid has a high growth rate and resistance to adverse environmental factors. Plants *in vitro* were cultivated on MS nutrient medium according to T. Murashige & F. Skoog (1962), with the addition of growth regulators of the cytokinin type of action according to the method of M. Melnychuk *et al.* (2003), V. Kunakh (2005), S. Park (2021). 100 $mg \cdot l^{-1}$ myo-inositol, 30 $g \cdot l^{-1}$ were added to the nutrient media sucrose and 7.0-7.3 $g \cdot l^{-1}$ microbiological agar. The indicator of acidity of the environment (pH) was adjusted to the level of 5.7-5.8. The plant material was cultivated in a light room at a temperature of 24±1°C and illumination of 2.0-3.0 klx with a 16-hour photoperiod and a relative humidity of 70-75%. Aseptic conditions were created according to methods generally accepted in biotechnology (Slater *et al.*, 2003; Smith, 2012; Clark & Pazdernik, 2015).

Regenerants with a cultivation cycle of 25 days were adapted to the conditions of *ex vitro* in a stepwise manner, which consisted of adaptation to the conditions of the adaptation room and environment. Plants were removed from the test tubes with tweezers, the root system was washed from the remnants of the nutrient medium in tap water with subsequent transfer to 0.001% $KMnO_4$ (2-3 min). Plants were planted in plastic containers with a volume of 0.25 l, 1 pc. in the following substrates: river sand; coarse-grained agroperlite; horse peat, agroperlite, vermiculite (1:1:1); coconut fibres; coconut fibres, agroperlite (1:1). After planting, the plants were sprayed with water. Regenerants were fed once every 30 days with a solution of macro- and microsalts for ½ MS. The plants were kept in conditions of high relative humidity (HRH) (85-90%) for 3-5 days. HRH was determined using a digital thermohygrometer. Planting material was placed in glass containers. The main methods of gradual adaptation of plants were performed according to the method of H. Kushnir & V. Sarnatska (2005).

The HRH was gradually reduced to the level of 60-70% by means of artificial ventilation. Plants were sprayed with water when signs of wilting appeared. To increase the number of transplanted plants, the root system was kept for 15-20 minutes in a solution of auxins (1.0 mg/l IAA (indole-3-acetic acid), 1.0 mg/l NAA (1-naphthylacetic acid), 1.0 mg/l IBA (indole-3-butyric acid). Plants not treated with auxins were used as a control. We determined the effect of concentrations of glycerol solution (10%, 20%, 30%, 60%) on the morphometric parameters of plants, which were previously kept for 15-20 minutes in auxin solution and planted on an agroperlite substrate. Spraying of leaves with glycerin solution was carried out for 15-20 days. Plants were grown in an adaptation room under Osram Fluora phytolamps (illumination 3.0-4.0 klx, 16-hour photoperiod) at a temperature of 21±2°C. Plant viability was recorded after the appearance of new leaves (30 days of adaptation).

60 days after adaptation, the plants were transplanted into containers with a volume of 0.33 l in a substrate (agroperlite, coconut fibres, peat, 2:1:1). Starting from May and ending in September, container culture of plants lasts 2-8 hours. was in environmental conditions. In autumn, the plants were transplanted into containers with a volume of 1.0-1.5 l in a substrate (peat, agroperlite, coconut fibres, vermiculite, 4:1:1:1). In autumn 2021, the plants were planted in the environment. In winter, at temperatures below -10°C, plants *ex vitro* covered with agrofibre. Plant viability was determined in the spring.

Biotechnological methods, such as microclonal propagation, as well as statistical approaches, including arithmetic mean, standard error, and one-way analysis of variance (ANOVA), were used for the research. Statistically, the experimental data were processed using the MS Excel analysis package. Results are presented as M±m (M – arithmetic mean, m – standard error). To analyse the effect of substrates, auxins, and glycerol on the efficiency of plant adaptation, a one-way analysis of variance (ANOVA) was performed. When conducting the research, the requirements of the Convention on Biological Diversity (1992) were observed.

RESULTS AND DISCUSSION

Active proliferation of explants by activating the growth of existing meristems, forming the root system, and obtaining *Betula* hybrid plants was obtained on MS with the addition of 0.25 mg/l kinetin (Fig. 1, a). According to the results of the study, the MS nutrient medium modified with 0.25 mg/l kinetin (6-(furfuryl-amino)purine) simultaneously stimulated the development of the microshoots and the roots of interspecific hybrid *Betula plants* (Fig. 1, b). On the 30th day of cultivation, plants were obtained *in vitro* with the following morphometric parameters: the share of regeneration-capable explants – 93.4±2.5%, microshoot length – 5.8±0.3 cm, the share of rooted microshoots – 96.4±1.6%, root length – 4.2±0.3 cm. Plants *in vitro* had pigmentation characteristic of the hybrid, signs of chlorosis and vitrification were not fixed (Fig. 1, c).

The substrate is of great importance for the survival of regenerating plants, and the efficiency of adaptation depends on its correct selection (Table 1).

Figure 1. *Betula interspecific hybrid plants in vitro on the nutrient medium and adaptation to conditions ex vitro*
Note: *a) plant in vitro; b) roots of plants, 30 days of cultivation; c) plants in a culture room on MS with the addition of kinetin; d) ex vitro plants on agroperlite substrate in glass containers in an adaptation room; e) plants ex vitro in distilled water with auxins; birch container culture, 63 days (f) and 95 days of adaptation (g); h) plants ex in vitro under environmental conditions*
Source: *photographed by the authors*

Table 1. *Efficiency of plant adaptation of interspecific hybrid Betula ex vitro to the conditions of the adaptation room and their growth on substrates, 30-day cultivation*

Version	Composition of substrates	The efficiency of plant adaptation *ex vitro* [1], %	The beginning of the formation of new leaves, era	Linear growth[2]	Pigmentation of shoots
1	river sand	0	–	–	–
2	agroperlite	64.0±5.1	14-18	active	green

Table 1, Continued

Version	Composition of substrates	The efficiency of plant adaptation *ex vitro* [1] %	The beginning of the formation of new leaves, era	Linear growth[2]	Pigmentation of shoots
3	horse peat, agroperlite, vermiculite (1:1:1)	27.0±3.0	35-38	weak	light green
4	coconut fibres	32.0±4.1	35-38	weak	light green
5	coconut fibres: agroperlite (1:1)	42.0±3.0	25-30	average	-//-

Notes: *1. mean ± standard error; 2. linear growth: active (more than 3 cm), medium (2.0-2.9 cm), weak (less than 1.9 cm), absent (–)*
Source: *developed by the O. Chornobrov*

Insignificant plant growth was recorded on substrates containing peat, agroperlite, and vermiculite (option 3); coconut fibres (option 4); coconut fibres, agroperlite (option 5). Such variants of substrates contributed to weak and medium growth of plants, which mostly had light green pigmentation. To adapt plants *ex vitro* for each genotype, it is necessary to select the substrate individually. According to the research results, agroperlite substrate was optimal for the growth and survival (more than 60%) of *Betula* hybrid plants *ex vitro* (Fig. 1, d). The composition of the substrate had a significant effect on the viability of plants (Table 2).

Table 2. *Final results of one-way analysis of variance plants for Betula interspecies hybrid plants ex vitro in the conditions of the adaptation room*

Analysis of variance						
Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F critical*
Effectiveness of adaptation of plants on different substrates						
Between groups	11653.75	3	3884,5833	64.0756	3.92	3.2389
Viability of plants due to the endurance of roots in auxins						
Between groups	3800	3	1267	38	4.43	4.0662
Liveability plants by spraying with glycerin solution						
Between groups	5625	3	1875	17.3077	0.0007	4.0662

Notes*: SS – level of reliability; df – the number of degrees of freedom; MS – variances; F is the estimated value of Fisher's test; P-value – calculated value of minimum significance;* F_{crit}*. is the critical value of Fisher's test*
Source: *developed by the O. Chornobrov*

To increase the number of viable plants of the hybrid *Betula ex vitro* the roots before planting in the substrate was kept for 25-30 minutes in auxin solutions (IAA, NAA, IBA, Fig. 1, e) (Table 3).

Table 3. *Characteristics of plant growth of hybrid Betula ex vitro in the conditions of an adaptation room for roots in auxins on an agroperlite substrate, 30 day*

Auxin	Survival of plants *ex vitro*[1] %	The beginning of the formation of new leaves, day	Linear growth[2]	Pigmentation plants	Other signs
IAA	53.3±3.3	22-25	average	light green	signs of chlorosis
NAA	26.7±3.3	28-30	weak	-//-	-//-
IBA	56.7±3.3	22-25	average	-//-	-//-
IAA, NAA, IBA	76.7±3.3	18-20	active	green	no signs of chlorosis and vitrification were detected

Notes: *1. mean ± standard error; 2. linear growth: active (over 2.0 cm), medium (1.0-1.9 cm), weak (less than 0.9 cm), absent*
Source: *developed by the O. Chornobrov*

Maintenance of plant roots *Betula* hybrid *ex vitro* in different versions of auxin solutions reliably influenced their viability. The difference in survival rates of regenerants with the use of different auxins was statistically significant ($F_{calc.}$=38, $F_{crit.}$=4.0662; $F_{calc.}$>$F_{crit.;}$ Table 2). Under the conditions of the use of several auxins, more than 70% survival rate, active growth was obtained, the plants had pigmentation typical for the hybrid without signs of chlorosis and vitrification. For to prevent dehydration of regenerants, various methods are used, in particular, spraying with a glycerin solution. The results of the effect of glycerin concentrations on the viability of plants (the root systems were pre-treated with a mixture of auxins and planted in an agroperlite substrate) are shown in Table 4.

Table 4. *Growth characteristics of hybrid Betula ex vitro for spraying with glycerin solution on agroperlite substrate, 30 day*

Glycerin concentration, %	The viability of plants in the conditions of the adaptation room[2], %	The beginning of the formation of new leaves, era	Linear growth[3]	Pigmentation plants	Other signs
K [1]	76.7±3.3	18-20	active	green	no signs of chlorosis and vitrification were detected
10	5 6.7±6.7	22-25	average	green	-//-
20	83.3±3.3	16-21	active	-//-	-//-
30	36.7±8.8	22-25	average	light green	signs of chlorosis
60	26.7±3.3	26-31	weak	-//-	-//-

Notes. *1. control; 2. mean value ± standard error; 3. linear growth: active (over 2.0 cm), medium (1.0-1.9 cm), weak (less than 0.9 cm), absent*
Source: *developed by the O. Chornobrov*

Based on the results of the research, a reliable influence of the concentration of glycerol on the viability of *Betula ex vitro* hybrid plants was established. In the case of using a 20% solution of glycerin, more than 80% of viable *Betula* hybrid plants with active growth and new leaves were obtained. The plants had the typical hybrid morphology and pigmentation (Fig. 1, f, g). The plants in the containers adapted well to the environmental conditions and were characterized by active growth: at the beginning of May, the height of the plants was 34.4±1.3 cm, the proportion of adapted ones was more than 95.2±1.8% (Fig. 1, h). Hybrids planted in open ground in autumn showed 86.0±1.7% survival rate in spring, which indicated a high level of adaptation of plants *in vitro* to environmental conditions.

So, the artificial solid universal nutrient medium MS was used for the initiation and regeneration of *Betula* hybrid explants. R. Rathwell *et al.* (2016) investigated the regeneration of *B. lenta* plants also on solid nutrient medium, but preferred WPM. O. Chornobrov *et al.* (2019) introduced into culture *in vitro* endangered plant *Salix retusa* L. and *Salix alpine* Scop. using nutrient medium, MS with the addition of 0.25-0.50 mg/l kinetin and 2.0 g/l of activated carbon (regeneration frequency over 80%). The selection of different basic environments is determined by the study of tissues of different genotypes *of Betula in vitro* and, accordingly, their different necessity in inorganic and organic substances.

R. Rathwell *et al.* (2016) used ½ DKW with the addition of auxin (20 μM IBA) for active rhizogenesis of *B. lenta* plants with 80% of rooted microshoots. L.K. Simola (1985) for obtaining *B. pendula* plants *in vitro* used a nutrient medium with the introduction of cytokinins and auxins, in particular 5-10 mg/l zeatin and 0.1-0.2 mg/l NAA. S. Välimäki *et al. (2021)* used semi-solid or liquid nutrient medium ½ DKW with 3-5 mg/l IBA for 2-3 days in the dark for rooting microshoots of *U. laevis* and *U. glabra*. According to the results of this study, the proportion of rooted microshoots of interspecific hybrid *Betula* on nutrient medium with cytokinin was more than 90%. Plants of different genotypes are cultivated on different types of nutrient media and use different consistencies.

According to the results of this study, the relatively low survival rate of plants of interspecies hybrid *Betula* on such types of substrates (peat, agroperlite, vermiculite; coconut fibres; coconut fibres, agroperlite) is due to the stress that occurs during the transition from heterotrophic to autotrophic nutrition. Each genotype of plants involves the use of an individual approach to the selection of the substrate for adaptation. In particular, according to L.K. Simola (1985) it was found that peat humic substrate is optimal for the survival of *B. pendula* plants*;* according to N. Dimitrova *et al.* (2021) for *P. communis* plants, it is – peat-perlite substrate; according to M. Capuana *et al.* (2022) for *S. acmophylla* – a mixture of garden soil, peat, and sand; according to S. Välimäki *et al.* (2021) for *U. laevis* and *U. glabra* – light sphagnum peat. According to the results of this study, it is advisable to adapt *Betula ex vitro* hybrid plants on an agroperlite substrate (over 60% survival rate).

To increase the survival rate of woody plants, various methods are used, in particular, auxins, biostimulants, glycerin, etc. are used. In particular, according to the results of this study, more than 70% survival rate was obtained using the procedure of keeping the root system of *Betula* interspecies hybrid plants in auxin solution (IAA, NAA, IBA). Research by N. Dimitrova *et al.* (2021) showed the positive effect of using biostimulants, in particular, Lumbrical, to improve vegetative growth and photosynthetic characteristics of *P. communis ex vitro* plants.

Authors S. Välimäki *et al.* (2021) tend to improve the adaptation of *U. laevis* and *U. glabra* recommend the use of short-term induction of microshoots in a nutrient medium with auxin, followed by planting in sphagnum peat in mini-greenhouses. The proportion of rooted and adapted plants was 18% for *U. laevis* and 64% for *U. glabra* at 41 days. It is also noted that the use of a liquid nutrient medium reduces injury to the roots and is less time-consuming, as it does not need to be washed from the remains of the nutrient medium.

O. Chornobrov & E. Tkachova (2020) established in the study the expediency of spraying *F. vesca ex vitro* with a 30% solution of glycerin for 30 days, the treatment of which reduced the dehydration of plants and made it possible to obtain more than 70% survival rate. According to the results of this study, in the case of applying the following procedure: using a 20% glycerin solution, keeping the roots in auxin solution followed by planting on an agroperlite substrate, more than 80% of viable plants of the *Betula* interspecies hybrid were obtained. For comparison, the indicator of survival of plants *ex vitro* for other species is: *B. lenta* – 37% for R. Rathwell *et al.* (2016); *B. pendula* – 95% for L.K. Simola (1985); *U. laevis* – 18% and *U. glabra* – 64% for S. Välimäki *et al.* (2021); *S. acmophylla* – 90% according to M. Capuana *et al.* (2022); walnut hybrid rootstock 'Paradox' – 84% by H. Ribeiro *et al.* (2022).

In general, the efficiency of plant adaptation *ex vitro* depends on a complex of internal and external factors, therefore a careful selection of the protocol, which includes the technology of microclonal propagation and acclimatization, is the key to the success of obtaining viable plants. The procedure for the adaptation of a triploid interspecific *Betula* hybrid was developed in this study *ex vitro* provides a new tool that allows obtaining high viability of healthy planting material under environmental conditions and provides a platform for further research.

CONCLUSIONS

Betula interspecies hybrid has been developed *ex vitro* using agroperlite substrate, auxins and glycerin solution, which allows obtaining more than 80% survival in environmental conditions. The optimal conditions for the proliferation of microshoots and the roots were the use of nutrient medium MS with the addition of 0.25 mg/l kinetin during a 30-day cultivation cycle.

The composition of the substrate had a significant effect on the viability of *Betula* hybrid plants *ex vitro* in the conditions of the adaptation room. Weak growth of plants was recorded on substrates containing horse peat, agroperlite, and vermiculite; coconut fibers; coconut fibres, agroperlite. According to research, the agroperlite substrate is optimal for growth and survival (over 60%) of plants. Endurance of root systems of *Betula* hybrid plants *ex vitro* in auxin solutions reliably affected survival. Under the conditions of keeping plant roots in a mixture of 1.0 mg/l IAA, 1.0 mg/l NAA and 1.0 mg/l more than 70% received IBA within 25-30 minutes survival with active growth and typical pigmentation.

A reliable influence of experimental concentrations of glycerol on the viability of the hybrid was established, *Betula ex vitro*. In the case of daily spraying of plants 20% glycerin solution for 15-20 days, more than 80% were obtained of viable plants. Spring viability of hybrid plants *Betula ex vitro,* planted in autumn under environmental conditions, was more than 80%. Further research can be directed to a detailed study of the genetic and molecular mechanisms that underlie the adaptation of the hybrid *Betula ex vitro* to variable environmental conditions. Additional studies may include the analysis of physiological and morphological features of these plants under the influence of various factors, such as soil conditions, climatic conditions and competition with other species.

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CONFLICT OF INTEREST

None.

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Особливості адаптації рослин міжвидового гібриду *Betula ex vitro*

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Анотація. Мікроклональне розмноження як один із методів біотехнології дозволяє одержати генетично однорідні рослини упродовж року з мінімальної кількості донорного матеріалу. Адаптація рослин *ex vitro* до умов довкілля – заключний і важливий етап мікроклонального розмноження. Мета дослідження – визначення оптимального режиму адаптації рослин триплоїдного міжвидового гібриду *Betula ex vitro* до умов довкілля. Для досліджень використовували біотехнологічні (мікроклональне розмноження) та статистичні (середнє арифметичне, стандартна похибка, однофакторний дисперсійний аналіз (ANOVA)) методи. Дослідження здійснено у науково-дослідній лабораторії біотехнології рослин Відокремленого підрозділу Національного університету біоресурсів і природокористування України «Боярська лісова дослідна станція» упродовж 2019-2023 р. За дослідженнями живильне середовище MS з додаванням 0,25 $мг \cdot л^{-1}$ кінетину стимулювало активну проліферацію мікропагону і кореневої системи. На живильному середовищі відбувся оптимальний розвиток рослин *in vitro* за 25-30-добовий цикл культивування, що сприяв успішній адаптації до умов довкілля. Частка адаптованих рослин гібриду *Betula ex vitro* на агроперлітовому субстраті становила понад 60 % на 30-добу. Значну приживлюваність рослин (понад 80 %) одержано за умов попереднього витримування кореневої системи рослин у розчині ауксинів (1,0 $мг \cdot л^{-1}$IAA, 1,0 $мг \cdot л^{-1}$ NAA, 1,0 $мг \cdot л^{-1}$ IBA) упродовж 25-30 хв із наступним щоденним обприскуванням листків 20 % гліцерином упродовж 15-20 діб. Адаптовані рослини мали типову пігментацію без ознак хлорозу та вітрифікації. Навесні приживлюваність рослин в умовах довкілля становила понад 80 % і свідчила про високий рівень пристосованості після *in vitro*. Розроблений протокол адаптації гібриду *Betula ex vitro* дозволяє одержувати високу приживлюваність рослин в умовах довкілля. Практичною цінністю дослідження є одержання адаптованих до умов довкілля рослин *ex vitro*, які в подальшому можуть використовувати для якісного збагачення асортименту штучних насаджень населених пунктів

Ключові слова: культура *in vitro*; береза; мікроклональне розмноження; субстрат; приживлюваність