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OPTIMIZATION OF MICROCLONAL PROPAGATION AND ADAPTATION TO *EX VITRO* CONDITIONS FOR THE HOP (*HUMULUS LUPULUS* L.) VARIETY SLOV'IANKA

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Aim. The development of a well-balanced microclonal propagation technology for the Slov'ianka hop variety is of importance for the industry since this is a widely used variety. Therefore, the aims of our research were 1) to investigate the influence of growth medium, disinfection procedures and means, hormones and other growth factors on the success of micropropagation and further multiplication of this variety and 2) to advice a standard protocol for the micropogation of the variety Slov'ianka. **Methods.** The research was conducted during 2023–2025 at the Department of Virology, Health Improvement and Reproduction of Fruit and Berry Crops of the Institute of Horticulture of the NAAS of Ukraine. Plant material for the experiments was selected in the Khmelnytskyi region and tested for the absence of pathogens in accordance with the *EPPO PM4/016(2) standard: Certification scheme for hop*. Various, culture media and variations of the standard MS culture medium, using different iron and carbon sources, auxins and cytokinins in early stages of micropropagation, use of perlite, coconut and peat and two biostimulants Helprost and Radifarm in later stages. Statistical processing of the experimental data was performed using ANOVA and correlation analysis was conducted in Excel. **Results.** For the disinfection the use of 70% ethanol for 30 seconds and a 9% sodium hypochlorite solution diluted to 0.25% for 20 minutes appeared to be the most effective. From five culture media an adapted Murashige-Skoog (MS) medium was found to be optimal for regeneration with minimum callus growth. To improve the overall condition of the plants, a dose of iron chelate FeNaEDTA (40 mg/l), and glucose (30 g/l) as a carbon source and furthermore benzylaminopurine (BAP) at a concentration of 0.1 mg/l the medium gave best results. Despite the concentrations of respectively 2.0 mg/l and 1.0 mg/l of thidiazuron and kinetin in the nutrient medium during the shoot proliferation stage, their effectiveness was non-significant and can be deleted. This was also true for gibberellic acid since the maximum concentration of 1.0 mg/l led to an increase in the distance between internodes, thereby reducing the propagation coefficient. During the induction stage of rhizogenesis in hop microshoots, the auxins indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), were tested at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/l, compared to a nutrient medium without growth regulators. Remarkably a 100% rooting of Slov'ianka variety in a hormone-free medium with high root system quality indicators — the mean number of roots was 7.9, with a mean length of 2.1. However, among the auxins under study, IBA was the most effective at concentrations of 0.5 and 1.0 mg/l, promoting rooting rates of 58% and 52%, respectively, whereas IAA and NAA resulted in rooting rates of less than 43%. IAA and NAA at 2.0 mg/l, were totally inhibitory for root formation. A high level of adaptation of rooted plants was achieved when using peat substrate «Domoflor» as a substrate — 98%; whereas the use of a two-component substrate (peat and perlite in a 3:1 ratio) slightly reduced the adaptation to 72%, and the use of perlite and coconut substrate alone, supplemented with MS macro- and micronutrients, the survival was only 27–30%. During the plant growth stage, Helprost fertilizer, containing amino acids, B vitamins, polysaccharides, and chelated microelements for root feeding after transplantation was most effective, which

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subsequently resulted in a 97% yield of standard plants. **Conclusions.** A balanced alternative protocol for the microclonal propagation of Slov'ianka hop variety has been developed, which includes efficient disinfection with ethanol and sodiumhypoclorite, optimized composition of the culture medium, methods for adapting microshoots to ex vitro conditions, and the growing of seedlings in plastic containers until they reach standard sizes.

Keywords: hop, microclonal propagation, cytokinins, auxins, adaptation.

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INTRODUCTION

Hop (*Humulus lupulus* L.) is a perennial dioecious plant of the Cannabaceae family (Acosta-Rangel et al., 2021). Due to the versatile uses of hop, the trend among producers in recent years has been to produce high-quality raw materials not only for beer production but also for the pharmaceutical and food industries. Female flowers (cones) have glandular trichomes that store and secrete lupulin and contain resins and essential oils that can be used for industrial and medical purposes (Rossini et al., 2021). In the beer brewing industry, these components are used to add bitterness, flavor, and aroma to beer, and they also serve as cofactors in several product stabilization processes, making hops an important ingredient in beverage production (Almaguer et al., 2015). Hop products made from Ukrainian aromatic hop are highly valued on the international market (Ivchenko et al., 2024; Ryzhuk & Ratoshniuk, 2020).

Ukraine has an advantageous geographical location and favorable soil and climatic conditions for growing and selling hops (Ryzhuk et al., 2018; Ryzhuk et al., 2019). Commercial hop-growing areas with a total of 420 ha are concentrated in the Polissia and Forest-Steppe regions, which, even under conditions of climate change, are characterized by sufficient moisture and are best suited for growing aromatic hops (Ryzhuk & Ratoshniuk, 2020). This enables Ukraine to develop its hop-growing industry and cultivate sufficient quantities of high-quality hop raw materials to meet its own needs and increase its export potential. However, the foundation for further industrial development lies in expanding the area of commercial hop plantations, which requires a sufficient supply of high-quality planting material.

According to Hirenko (2017), the variety Slovianka ranks third among aromatic hop plantings. It is one of the most valuable aromatic varieties of Ukrainian origin, distinguished by its high adaptability to the soil and climatic conditions of Ukraine's Polissia and Forest-Steppe regions. Its key advantages include stable yields and high levels of alpha acids and essential oils. The variety is characterized by plant uniformity, excellent cone quality metrics, and suitability for commercial cultivation (Shtanko, 2020). It is precisely the lack of sufficient quantities of high-quality planting material that limits large-scale hop production in the Mediterranean region (Liberatore et al., 2020; Iacuzzi et al., 2023), Brazil (Brighenti et al., 2023) and Ukraine (Kovaliov et al., 2016). Another problem in hop cultivation is the occurrence of viral diseases such as Apple mosaic virus (ApMV), Arabis mosaic virus (ArMV), Hop latent virus (HLV), Hop mosaic virus (HMV), American hop latent virus (AHLV), Hop latent viroid (HLVd), Cucumber mosaic virus (CMV), Cherry leaf roll virus (CLRV), which significantly reduce and impair the quality of the raw material (Dann et al., 2014; Luigi et al., 2023, Sus et al., 2020). Micropropagation methods have been used for hop (Gippert et al., 1970; Machado et al. 2018; Rajput et al., 2020) particularly for shoot regeneration (Gurriarán et al., 1999), cryopreservation (Reed et al., 2003), and virus eradication (Postman et al., 2005).

The most important goal of any successful tissue culture for a particular variety is to establish an effective protocol that encompasses all stages, from *in vitro* propagation and adaptation to *ex vitro* conditions to the plant growth stage, which can differ significantly depending on the species and variety. The effects of cytokinins, auxins, and gibberellic acid have extensively been studied (Kliuvadenko & Melnychuk, 2007; Gurriaran et al., 1999; Roy et al., 2001; Clapa & Machado et al., 2018; Liberatore et al., 2020; Harta, 2021; De-Souza et al., 2022). In hop tissue culture, different types of sugar are used as a carbon source for different hop varieties (Roy et al., 2001; Hrybovets et al., 2012; De-Souza et al., 2022; Hirakawa & Tanno, 2022). Melnychuk et al. (2022) investigated the effect of biogenic metals on improving the micropropagation of hop, specifically regarding the prevention of chlorosis in the leaves of cultivated plants. The most commonly used growth regulators in hop *in-vitro* culture are: IBA — indole-3-butyric acid (auxin), used to induce callus formation or rooting of microshoots; BAP — 6-benzylaminopu-

rine (synthetic cytokinin) that promotes cell division and induces the germination of axillary buds; GA (gibberellic acid), which elongates shoots or internodes (Taiz et al., 2017; Zimmermann, 2014).

Given the global importance of hop cultivation, a number of protocols for *in vitro* propagation have been developed over the past several decades, demonstrating success in the microclonal propagation of *Humulus lupulus* L. (Batista et al., 1996, 2000 and 2008; Roy et al., 2001; Skof et al., 2007; Peredo et al., 2009; Gatica-Arias & Weber, 2013; Liberatore et al., 2018; Machado et al., 2018; Clapa & Hârța, 2021; De Souza et al., 2021; Iacuzzi et al., 2023). A universal protocol that can be used for all species has not yet been established. For many species it is often not sufficiently known if varieties of the same species would need fine-tuning of the protocol. However, all protocols agree that *in vitro* propagation of hop plants requires limiting callus formation, as this is considered one of the factors that can lead to somaclonal variation (Peredo et al., 2009; Clapa & Hârța, 2021).

The use of growth regulators and other key components of the culture medium helps address the challenges that arise during *in vitro* explant regeneration; however, the possibilities for combinations of growth regulators are vast, leading to varied results for key indicators such as shoot, root, and callus formation (De Souza et al., 2021).

Despite significant progress in the development of micropropagation methods for hop, the optimization of culture conditions remains relevant even for genotypes for which suitable protocols have already been proposed. In particular, the variety Slov'ianka was included to develop and publish a standard protocol for the micropropagation of hop (Shtanko et al., 2020). They published a protocol with the standard Murashige & Skoog medium where three different additions of nutrients yielded good results for this variety. A patented method (Ukrainian Patent No. 120207, Kozlyk & Kovalov, 2019), describes a micropropagation methodology using meristems instead of stem cuttings for two other different varieties. However, the above mentioned protocols can never be seen as final and therefore, the aim of our study was to comparatively evaluate some more nutrient media apart from MS, furthermore the optimal concentrations of key components of the MS medium and its additions, (including sources of iron and carbon and growth regulators) for the efficient propagation and rhizogenesis of variety Slov'ianka, as well as to optimize the conditions for acclimatization and subsequent growth of regenerated plants using different growth media and biostimulants.

MATERIALS AND METHODS

The research was conducted from 2023 to 2025 at the Department of Virology, Plant Health, and Propagation of Fruit and Berry Crops at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine. The research included the following stages: 1) selection of plant material and extraction of explants from the mother plant, 2) treatment with sterilizing solutions, 3) introduction into *in vitro* culture, 4) selection of the culture medium composition for the proliferation and rhizogenesis stages, 5) adaptation to *ex vitro* conditions, and 6) further growth of seedlings in containers.

Selection of plant material and aseptic explant preparation. The study focused on Slov'ianka variety, which is currently in high demand among growers. The explants used were obtained from rhizomes (collected from five plants in the Khmelnytskyi region and cleaned of soil residues) by sprouting them in perlite under room conditions (temperature 21–24°C and relative humidity 60–75%) to produce green shoots. The plant material was tested for the absence of pathogens in accordance with the EPPO standard PM4/016(2): Certification scheme for hop. Testing was performed using ELISA and PCR methods with commercial test systems (LOEWE Biochemica GmbH, Germany). The shoots obtained after 21 days were cut into 1–1.5 cm-long segments and rinsed under running water, after which they were immersed in a 9% sodium hypochlorite solution diluted to 0.25% for 20 minutes. The disinfected plant material was subsequently rinsed with sterile distilled water. Disinfected explants were cultured on an adapted Murashige-Skoog (MS) medium (Kushnir & Sarnatska, 2005). The MS medium was prepared from a stock solution containing macronutrients and micronutrients according to Murashige & Skoog, 1962, 100 mg/l myo-inositol, 0.1 mg/l vitamin B1, 0.5 mg/l vitamin B6, 0.5 mg/l nicotinic acid, 2 mg/l glycine, 0.2 mg/l BAP, 30 g/L sucrose and 8 g/l agar (Kushnir & Sarnatska, 2005). All components of the modified MS medium were added prior to autoclaving. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH. The culture medium was sterilized by autoclaving at 121°C for 20 minutes. *In vitro* plant cultivation was

carried out in a controlled environment propagation room at a temperature of 21–24°C, under a 16/8-hour (day/night) photoperiod, and at a light intensity of 2.000–3.000 lux.

The observations of morphogenic potential were conducted weekly. In each treatment, disinfection efficiency was determined by counting healthy (viable) and infected (necrotic) explants (as the ratio of sterile to infected explants to the total number of explants, expressed as a percentage).

Microclonal propagation. *A study on the selection of a basal medium for hop regeneration*. The next stage of the study was to determine the optimal culture medium for hop propagation. Based on the analysis of previous studies on this crop, six culture media were selected to evaluate the organogenic potential of nodal explants: **MS** — Murashige & Skoog (1962), **QL** — Quoirin & Lepoivre (1977), **B5** — Gamborg et al. (1968), **W** — White (1963), SH — Schenk & Hildebrandt (1972), and **A** — Adams (1975), containing 0.5 mg/L BAP, 30 g/L sucrose, and 8 g/L agar.

Each medium variant was tested in three repeats, with 10 microshoots per variant. The observations were conducted after four weeks of cultivation, specifically regarding the regenerative capacity of the microshoots and the formation of callus tissue.

The study of the effect of different forms of iron and carbon sources in the culture medium on shoot-forming capacity. After establishing the best basal medium (in our case MS), a study was conducted to optimize its composition, specifically the forms of iron and the carbon source, since the general appearance of plants indicated an iron deficiency, and Melnychuk et al. (2022) indicated the importance of the type of carbon source for hop. Therefore, the following experimental variants were investigated: **1.** Modified basal MS + 5 mg/l Fe-EDTA (Merck/Sigma-Aldrich, Germany) (12% Fe) + 30 g/l sucrose (ARGANA Zucker GmbH, Austria); **2.** MS + + 5 mg/l Fe-EDTA (12% Fe) + 30 g/l glucose (Liaoning Yihai Kerry Strarch Technology Co., Ltd Chine); **3.** MS + 100 mg/l Fe-EDDHA (Duchefa Biochemie, Netherlands) (6% Fe) + 30 g/l sucrose; **4.** MS + + 100 mg/l Fe-EDDHA (6% Fe) + 30 g/l glucose; **5.** MS + 40 mg/l FeNaEDTA (Thermo Fisher Scientific, USA) (14% Fe) + 30 g/l sucrose; **6.** MS + + 40 mg/l FeNaEDTA (14% Fe) + 30 g/l glucose. Plant height was measured, and the multiplication coefficient was calculated as the ratio of the number of shoots to the number of explants that regenerated after one culture passage, using the formula: Multiplication coefficients = Total Number of Shoots/Number of Shoot Induced Explants (George et al., 2008). The experiment was conducted three times.

The study of the effect of cytokinin type and concentration on shoot-forming ability. The following cytokinins were used in the studies: 6-benzylaminopurine (BAP), kinetin (Kn), and thidiazuron (TDZ) (Merck/Sigma-Aldrich, Germany) at a fairly wide range of concentrations — 0.1, 0.5, 1.0, 1.5, and 2.0 mg/l. For each treatment, 18 plants were used, with 6 plants in each individual culture vessel. The propagation coefficient and plant height were determined after 35 days of cultivation. The experiment was conducted three times.

The study of the effect of gibberellic acid concentration on the number of internodes and their length. The study examined the effect of gibberellic acid (GA) (Merck/Sigma-Aldrich, Germany) at the following concentrations: 0.1, 0.5, and 1.0 mg/l. The control was modified basal MS medium, which did not contain the above-mentioned growth stimulants. For each treatment, 15 explants were used, with 5 explants in each individual culture vessel. The propagation coefficient, plant height, number of internodes, and their length were determined after 35 days of cultivation. The experiment was conducted three times.

The induction of rhizogenesis under *in vitro* conditions. To induce rhizogenesis, a series of experiments was conducted using the modified basal MS medium. Various types and concentrations of auxins were tested as rhizogenesis inducers: indole-3-butyric acid (IBA), naphthylacetic acid (NAA), and indole-3 -acetic acid (IAA) (Merck/Sigma-Aldrich, Germany) at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/l. The control was the modified basal MS medium, containing no growth regulators. For each treatment, 30 explants were used, with 10 explants in each separate culture vessel. The first root primordia began to appear after 14 days. After 40 days of cultivation, the number of rooted microshoots and the average number and length of adventitious roots formed on the plant shoots were recorded. The experiment was conducted three times.

Adaptation and further growth. Rooted microshoots that had reached a size of 4 cm or more were adapted to *ex vitro* conditions starting in end of March in trays filled with various peat mixtures. The experiment scheme was as follows: **1.** «Domoflor» peat

only (mix 3, fertilizer content, kg/m^3: 1 kg NPK 140:160:180 ml/l + microelements, pH: 5,5–6,5/ Domoflor UAB — Vilnius-Lithuania)– control; **2.** perlite + MS macro- (50 ml/l) and micronutrients (1 ml/l); **3.** coconut substrate (free fertilizer, pH: 5,6–6,8/Ceres — Sri-Lanka) + MS macro- and micronutrients; **4.** peat and perlite in a 3:1 ratio. After 60 days, plant growth and development were assessed by visual, qualitative observations for each variant.

To maintain a high level of humidity (within the range of 90–100%), the trays with the planted microshoots were covered with transparent plastic lids, which were gradually opened starting from the second week of growing. The temperature in the acclimatization room was maintained between 18° and 24°C.

After the adaptation phase and 40 days of cultivation in trays, the hops were transplanted into pots with volumes of 0.3 l and 0.5 l, and the plants were given root fertilization using Helprost (biostimulant, composition g/L, up to: macro- and microelements: K — 17.0; P — 6.5; Mn — 0.4; S — 0.35; Zn — 0.15; Cu — 0.15; B — 0.15; organic acids — 25.0; amino acids — 6.0; microorganism complex, Biotechnology company BTU, Ukraine) and Radifarm treatment (biostimulant, containing nitrogen — 3%, potassium oxide — 8%, organic carbon — 10%, and zinc — 0.1%, polysaccharides (arginine and asparagine) — 7%; organic elements — 14%; polypeptides — 11%; steroidal glycosides — 0.2%; free amino acids — 1%; vitamins (B1, B2, D, H, PP) — 0.04%. Valagro, Italy) variants, with the control receiving no fertilization. The plants were matured in a greenhouse equipped with an automatic fine-spray irrigation system and the air temperature not exceeding 24–28°C, relative humidity 90–95% for the first 7 days, 70–80% thereafter, photoperiod 16 hours light/8 hours dark, shading: 60%. At the end of the growing season, a visual assessment of the development of the root and aboveground systems was conducted based on the percentage yield of standard-quality plants.

Statistical processing of the data. The statistical processing of the experimental data was performed using analysis of variance (ANOVA); the data in the tables are presented as $x \pm SE$ (mean ± standard error). The significance of differences between mean values was assessed using the least significant difference (LSD) criterion at a significance level of $p < 0.05$. The correlation analysis of the dependence of growth regulators and their concentrations in the culture medium on the main indicators of hop plants was performed in Excel.

RESULTS

Obtaining aseptic explants. Among the tested surface-sterilization treatments, the combination of 0.25% sodium hypochlorite for 20 min and 70% ethanol for 30 s, followed by three 15-min rinses with sterile water, provided the most effective establishment of aseptic explants from rhizome-derived plants of the variety Slov'ianka. The optimized protocol was associated with a reduced incidence of tissue necrosis and enabled the successful introduction of viable explants into *in vitro* culture.

Microclonal propagation. *The selection of a basal medium for the regeneration of hop variety Slov'ianka*. MS medium exhibited the highest regeneration capacity compared to the other four growth media, at 97% regeneration, whereas the use of medium A as a basal medium yielded 63%, SH — 44%, B5 — 40%, QL — 35%, and W — only 17% **(Fig. 1)**. On the media with the lowest regeneration rates, a high degree of callus formation was observed at the base of the microshoot — 58% on QL medium and 73% on White medium. No callus was observed on the MS medium, which demonstrates the effectiveness of this medium for the *in vitro* regeneration of Slov'ianka hop variety. Furthermore, when comparing regenerated shoots on different culture media, the microshoots on MS medium were greener and looking healthier than those on other types of media. This difference may reflect different nitrogen levels in the media (MS — 60.03 mM NH_4NO_3; SH — 27.47 mM $NH4H_2PO_4$; B5 — 27.03 mM $(NH_4)2SO_4$; W — 3.33 mM $(Ca(NO_3)_2)$.

A study of the effect of different forms of iron and carbon sources in the culture medium on shoot-forming

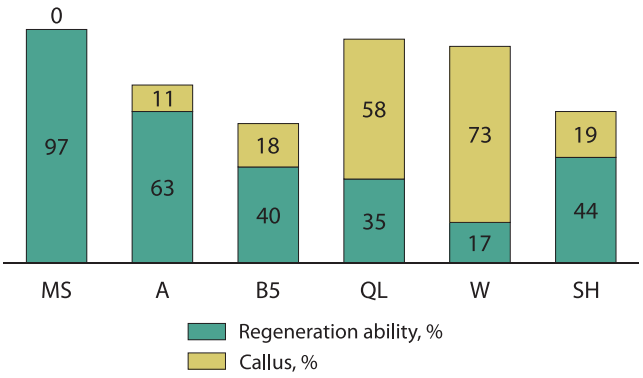

Fig. 1. The morphogenic responses of microshoots of Slov'ianka hop variety to basal media

capacity. **Table 1** presents the results of the effect of different forms of iron and carbon sources in the culture medium on plant height and the propagation coefficient. It was found that the investigated factors significantly impacted the morphogenetic parameters of the microshoots. The highest values for plant height (5.5 ± 0.45 cm) and the propagation coefficient (2.7 ± 0.11) were obtained using FeNaEDTA (40 mg/l) in combination with 30 g/l glucose. This variant outperformed the other combinations on both parameters significantly. Slightly lower, yet statistically comparable, results were observed when using FeNaEDTA (40 mg/l) with sucrose 30 g/l (5.0 ± 0.51 cm; 2.4 ± 0.26) and Fe-EDTA (5 mg/l) with glucose 30 g/l (4.8 ± 0.29 cm; 2.5 ± 0.36), which demonstrates the effectiveness of these variants for the growth and propagation of hop microshoots. The lowest values of these indicators were observed when using Fe-EDDHA (100 mg/l) regardless of the carbon source, where plant height ranged from 4.1 to 4.4 cm and the propagation coefficient was 1.9–2.0. These variants were significantly inferior to the others in terms of the set of indicators. Overall, it was found that the use of glucose as a carbon source contributed to in the investigated indicators compared to sucrose, especially in combination with FeNaEDTA (9.1% and 11.1% respectively). The form of iron had a more pronounced effect on plant height and the propagation coefficient than the carbon source. The results indicate an interaction between the factors, since the effectiveness of the carbon source depended on the form of iron in the culture medium.

We found that the application of FeNaEDTA at a concentration of 40 mg/l resulted in significantly greater plant height compared to other forms or concentrations of iron. The effect of the carbon source (sucrose, glucose) within a single iron chelate formulation was not statistically significant, that is, both carbon sources can be used for this variety, although based on the visual appearance of the plants, glucose was preferred.

The study of the effect of cytokinin type and concentration on shoot-forming ability. We found that the type of cytokinin and its concentration had a significant effect at the propagation stage. Among the cytokinins under study, BAP proved to be the most effective for this variety, promoting, on average, the best plant growth — 4.98 ± 0.31 cm, and propagation coefficient — 2.32 ± 0.21. The use of thidiazuron and kinetin reduced these indicators by 1.5 and 1.7 times, respectively.

For the proliferation and production of the largest number of Slov'ianka hop plants suitable for further cultivation, the optimal BAP cytokinin at 0.1 mg/l **(Fig. 2)**, as significantly demonstrated by the plant

Table 1. The impact of forms of iron and source of carbon in the culture medium on sprout-forming ability of hop variety Slov'ianka at 0.5 mg/l BAP

Form of iron (factor A)	Source of carbon (factor B)	Height of plants, cm	Average	Propagation coefficient	Average
Fe-EDTA (5 mg/l)	Sucrose (30 g/l)	4.5±0.50^{bcd}	4.65	2.3±0.30^{b}	2.40
	Glucose (30 g/l)	4.8±0.29^{ab}		2.5±0.36^{ab}	
Fe-EDDHA (100 mg/l)	Sucrose (30 g/l)	4.1±0.95^{d}	4.25	2.0±0.50^{c}	1.95
	Glucose (30 g/l)	4.4±0.26^{cd}		1.9±0.10^{c}	
FeNaEDTA (40 mg/l)	Sucrose (30 g/l)	5.0±0.51^{ab}	5.25	2.4±0.26a^{b}	2.55
	Glucose (30 g/l)	5.5±0.45^{a}		2.7±0.11^{a}	
Average (sucrose)		4.53	—	2.23	—
Average (glucose)		4.90	—	2.37	—
LSD_{05} (factor A)		0.52	—	0.34	—
LSD_{05} (factor B)		0.42	—	0.28	—
LSD_{05} (interaction between factors AB)		0.73	—	0.48	—

Note: * SE — standard error as compared to the average value. ** $LSD_{05(A)}$ — the least significant difference by factor A — the form of iron, $LSD_{05(B)}$ — the least significant difference by factor B — a source of carbon, $LSD_{05(AB)}$ — the least significant difference by factors of the form of iron and a source of carbon. *** different letters within one index indicate a reliable difference between the mean values at $p < 0.05$ (LSD_{05}).

height of 9.4 ± 0.512 cm and the propagation coefficient of 3.8 ± 0.26 as compared to higher concentrations and other cytokinins **(Table 2)**. An increase in BAP concentration to 0.5 mg/l or higher was accompanied by growth inhibition; plant height ranged from 5.1 to 3.1 cm, which is 1.8–3.0 times lower than at the lowest concentration (0.1 mg/l BAP), and a deterioration in the overall condition of the plants. Massive callus formation was also observed in variants with cytokinin concentrations exceeding 1.0 mg/l.

The use of thidiazuron and kinetin, regardless of their concentration, was less effective for this hop variety. The most effective treatment was adding thidiazuron in the highest concentration (2.0 mg/l) to the culture medium, resulting in a propagation coefficient of 2.1 ± 0.10 and a plant height of 3.9 ± 0.20 cm. Using kinetin at 1.0 mg/l also had a positive effect on the growth of microshoots. On average, plant height was 3.6 ± 0.17 cm, and the propagation coefficient was 1.9 ± 0.06, which did not differ significantly from the variants using 1.5 mg/l of thidiazuron and 1.5 mg/l of benzylaminopurine. Higher concentrations of kinetin had an inhibitory effect, plant height decreased by 1.2–1.7 times, and the propagation coefficient decreased nearly twice compared to the best variant (1.0 mg/l kinetin).

A two-factor analysis of variance showed a statistically significant effect of cytokinin type, its concentration, and their interaction on the morphogenetic parameters of hop plants ($p < 0.05$). A maximum propagation coefficient was obtained for BAP at 0.1 mg/l, which significantly exceeded the other variants. Higher doses were progressively inhibitory **(Table 2)**. For TDZ, optimal concentration was in the range of 1.5–2.0 mg/l, whereas for kinetin, they were observed at 0.5–1.0 mg/l. The observed significant interaction of factors confirmed the need to select the optimal cytokinin and its concentration.

The correlation analysis revealed a strong positive correlation ($r = 0.721$), demonstrating that the propagation coefficient increases along with the increase in plant height. The regression equation ($y = 0.32x + 0.45$)

Table 2. The impact of the type and concentration of cytokinin on the sprout-forming ability of Slov'ianka hop variety

Cytokinin (A)	Concentration (B)	Height of plants, cm	Average	Propagation coefficient, it.	Average
BAP	0.1 mg/l	9.4±0.51^{a}	4.98±0.31	3.8±0.26^{a}	2.32±0.21
	0.5 mg/l	5.1±0.26^{b}		2.6±0.17^{b}	
	1.0 mg/l	3.9±0.30^{cd}		2.2±0.10^{bc}	
	1.5 mg/l	3.4±0.20^{de}		2.0±0.30^{c}	
	2.0 mg/l	3.1±0.30^{e}		1.0±0.20^{d}	
TDZ	0.1 mg/l	2.2±0.20^{f}	3.10±0.26	1.1±0.21^{cd}	1.58±0.22
	0.5 mg/l	2.8±0.30^{e}		1.4±0.28^{c}	
	1.0 mg/l	3.0±0.21^{de}		1.5±0.23^{bc}	
	1.5 mg/l	3.6±0.40^{d}		1.8±0.26^{b}	
	2.0 mg/l	3.9±0.20^{cd}		2.1±0.10^{ab}	
Kn	0.1 mg/l	2.5±0.15^{ef}	2.88±0.23	1.2±0.18^{c}	1.34±0.18
	0.5 mg/l	3.2±0.20^{de}		1,6±0,20^{ab}	
	1.0 mg/l	3.6±0.17^{d}		1.9±0.06^{a}	
	1.5 mg/l	3.0±0.30^{de}		1.0±0.35^{bc}	
	2.0 mg/l	2.1±0.17^{f}		1.0±0.12^{cd}	
LSD_{05} (factor A)		0.52	—	0.31	—
LSD_{05} (factor B)		0.67	—	0.40	—
LSD_{05} (interaction between AB)		0.94	—	0.56	—

Note: * SE — standard error as compared to the average value. ** $LSD_{05(A)}$ — the least significant difference by factor A — a type of cytokinin, $LSD_{05(B)}$ — the least significant difference by factor B — a concentration of cytokinin, $LSD_{05(AB)}$ — the least significant difference by factors of cytokinins and their concentration. *** different letters within one index indicate a reliable difference between the mean values at $p < 0.05$ (LSD_{05}).

a *b* *c*

Fig. 2. The general appearance of individual Slov'ianka hop plants during the propagation stage using MS medium with optimal cytokinin concentrations: *a* — BAP 0.1 mg/l; *b* — TDZ 2.0 mg/l; *c* — Kn 1.0 mg/l

indicated a linear relationship, meaning that a 1-cm increase in plant height increases the propagation coefficient by 0.32.

The study of the effect of gibberellic acid concentration on the number of internodes and their length. Gibberellic acid in the culture medium for Slov'ianka hop plants did not result in the formation of a greater number of internodes, but it did increase the distance between them. The lowest number of internodes, 4.3, was observed with gibberellic acid at 1.0 mg/l, which is 2.5 times fewer than in the control without gibberellin (10.8 internodes). However, the distance between internodes was the greatest, 1.78 cm, as opposed to the control (0.87 cm), The highest propagation coefficient, 3.85, was obtained on a culture medium without gibberellic acid. The addition of gibberellic acid to the culture medium reduces this value by 1.3–1.8 times, depending on the concentration; the higher the concentration of the growth regulator, the lower the propagation coefficient — 2.91–2.10.

There was a very strong inverse correlation ($r = 0.957$) between the length and the number of internodes, as evidenced by the regression equation $y = –6.9113x + 16.296$ **(Fig. 3, *a*)**. A very strong direct correlation was found between the number of internodes and the propagation coefficient ($r = 0.992$), which confirms the pattern: the greater the number of internodes, the higher the propagation coefficient **(Fig. 3, *b*)**. In addition, the correlation coefficient between internode length and the propagation coefficient was $r = −0.957$ (a strong inverse relationship), meaning that as internode length increases, the propagation coefficient decreases **(Fig. 3, *c*)**. In conclusion gibberellic acid causes the lengthening of internodes, but this, in turn, is accompanied by a decrease in their number and a reduction in the propagation coefficient.

Table 3. The impact of the concentration of gibberellic acid on the number of internodes and their length at the background of 0.1 mg/l of BAP

Concentration	Internode length, cm	Number of internodes, it.	Propagation coefficient
0 (control)	0.87±0.10	10.8±0.32	3.85±0.13
0.1 mg/l	1.20±0.18	7.2±0.22	2.91±0.12
0.5 mg/l	1.53±0.23	5.7±0.10	2.34±0.17
1.0 mg/l	1.78±0.13	4.3±0.17	2.10±0.13
LSD_{05}	0.63	4.45	1.24

Note: * LSD_{05} — the least significant difference between variants at $p < 0.05$.

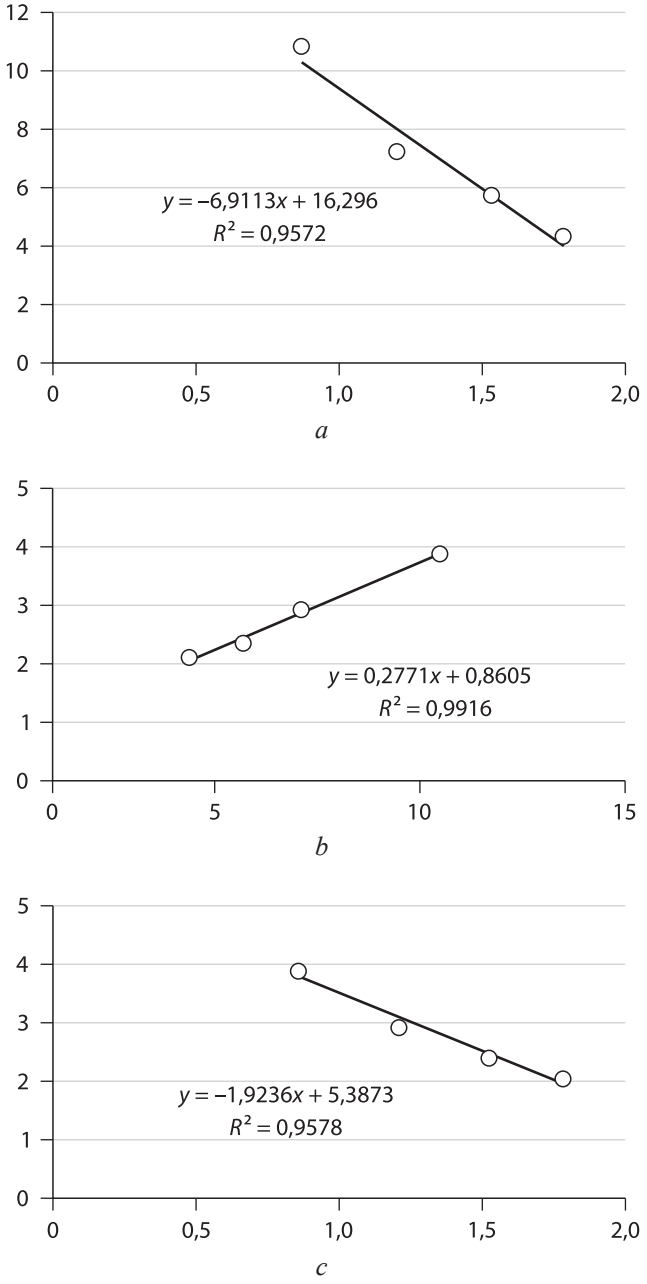

Fig. 3. The correlation relationships: *a* — between the length and number of internodes; *b* — between the number of internodes and propagation coefficient; *c* — between internode length and propagation coefficient.

The induction of rhizogenesis under *in vitro* conditions. Auxins had an inhibitory effect, whereas the control variant, a culture medium without growth regulators, resulted in 100% of microshoots rooting with a high-quality root system **(Fig. 4)**. Among the investigated auxins, IBA was the most effective at 0.5 and 1.0 mg/l, increasing rooting with 58–52%, whereas IAA and NAA ensured the only below 43%. Furthermore, IAA and NAA at 2.0 mg/l, the plants did not form roots.

The control variant produced the highest number of roots (7.9 ± 0.36), which significantly exceeded all variants with growth regulators. Among the investigated auxins, IBA (0.5–1.0 mg/l) and IAA (1.0 mg/l) were the most effective, resulting in the formation of 2.8–3.1 roots per microshoot **(Table 4)**. Increasing auxin concentrations to 1.5–2.0 mg/l led to the inhibition of rhizogenesis, which was particularly evident for IAA and NAA (complete absence of root formation at 2.0 mg/l).

The length of the root system depended significantly on the type and concentration of auxin. However, maximum values were obtained in the control without growth regulators and amounted to 2.10 ± 0.36 cm, which significantly exceeded all other tested variants **(Table 4, Fig. 5)**. However, among the auxins, the highest results were obtained with IBA at 1.0 mg/l — 1.40 ± 0.20 cm. When the auxin concentration was increased to 1.5–2.0 mg/l, a significant decrease in root length was observed within the range of 0.8–0.2 cm.

A correlation analysis between the number and length of roots revealed a strong positive correlation

Table 4. The impact of auxins on the rhizogenesis induction of Slov'ianka hop variety

Experiment variant	Number of roots, it.	Length of root system, cm
Control (w/g)	7.9±0.36[a]	2.1±0.35[a]
IBA 0.5 mg/l	3.1±0.20[b]	1.2±0.10[bc]
IBA 1.0 mg/l	2.9±0.26[b]	1.4±0.20[b]
IBA 1.5 mg/l	1.2±0.21[d]	0.8±0.17[d]
IBA 2.0 mg/l	1.0±0.20[d]	0.3±0.17[f]
IAA 0.5 mg/l	2.0±0.17[c]	1.1±0.17[c]
IAA 1.0 mg/l	2.8±0.20[b]	0.8±0.10[d]
IAA 1.5 mg/l	1.1±0.17[d]	0.7±0.17[d]
IAA 2.0 mg/l	0	0
NAA 0.5 mg/l	1.0±0.10[d]	0.4±0.17[e]
NAA 1.0 mg/l	1.1±0.10[d]	0.5±0.06[e]
NAA 1.5 mg/l	1.0±0.06[d]	0.2±0.12[f]
NAA 2.0 mg/l	0	0
LSD_{05}	0.42	0.27

Note: * SE — standard error as compared to the average value. ** LSD_{05} — the least significant difference between variants at $p < 0.05$; *** different letters within one index indicate a reliable difference between the mean values at $p < 0.05$ (LSD_{05}).

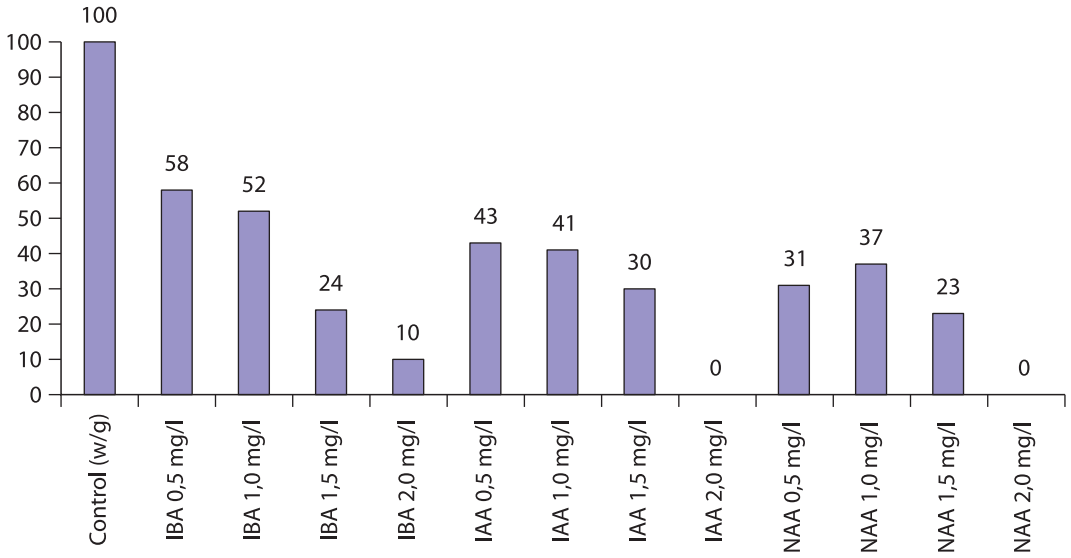

Fig.4. The efficiency of rooting Slov'ianka hop microshoots depending on auxin type and concentration

Fig. 5. Rooted plants of hop variety Slov'ianka grown on adapted MS medium without the use of growth regulators

($r = 0.813$), and the linear regression equation showed that for every additional root, the length of the root system increased by 0.25 cm **(Fig. 6)**.

The regression analysis conducted for each type of auxin confirmed a linear relationship between the investigated parameters. The most pronounced increase in length per unit of root number was observed for IBA ($y = 0.54x - 0.20$; $R^2 = 0.890$). For IAA, this dependence was less intense ($y = 0.35x - 0.10$; $R^2 = 0.771$), whereas for NAA, the highest consistency of data was observed ($y = 0.45x - 0.02$; $R^2 = 0.941$). Thus, an increase in the number of roots is accompanied by a statistically significant increase in their length, which may indicate coordinated development of the root system.

Adaptation and further growth. At the adaptation stage of rooted hop plants, the single-component substrate «Domoflor» peat for Slov'ianka, was the most effective, with 98% of the plants successfully adapted **(Fig. 7)**. The quality of the root system at the time of transplanting into plastic containers (60 days after planting for adaptation) was high: the cells contain-

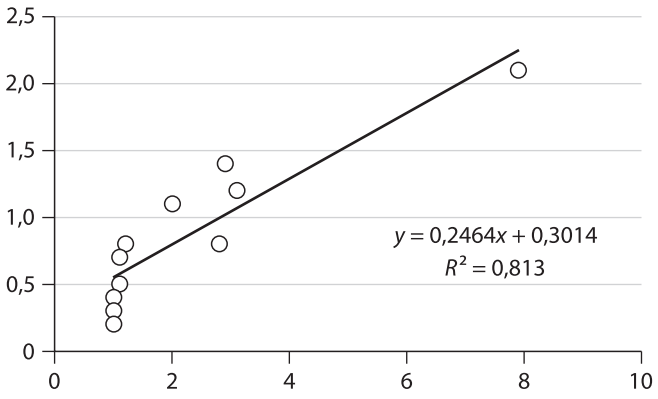

Fig. 6. The correlation between the number of roots and their length

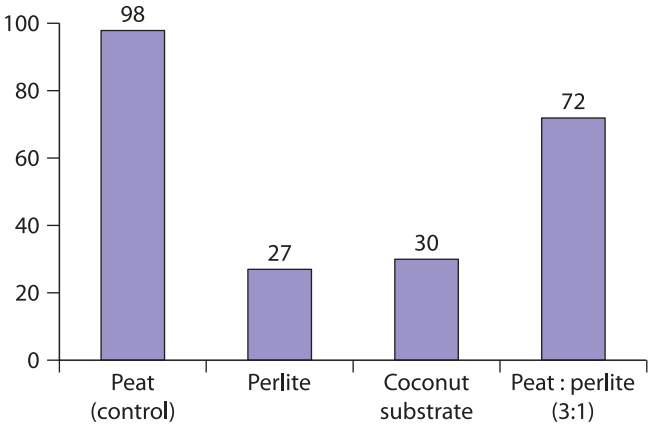

Fig. 7. The adaptation efficiency of Slov'ianka hop variety plants depending on the substrate, %

ing the substrate were completely filled with the root system, and the aboveground part of the plant was well developed **(Fig. 8)**. The use of a two-component substrate peat and perlite in a 3:1 ratio, resulted in a 72% adaptation rate. However, the use of perlite and coconut substrate alone resulted in a low level of plant adaptation to *ex vitro* conditions, at 27–30. Therefore, the selection of a substrate is crucial, as it must not only ensure a high level of adaptation but also promote plant growth and development.

After the adaptation stage, the plants were transferred into 0.3 and 0.5-liter plastic containers for further growth **(Fig. 9)**. In this stage the Helprost fertilizer was the most effective when applied at the rate recommended by the manufacturer, which is 3,5 ml/l. Furthermore, given the rapid growth of the root system during the growing season, it is recommended to use pots with a volume of at least 0.5 l; containers with a volume of 1.0 l are the optimal choice.

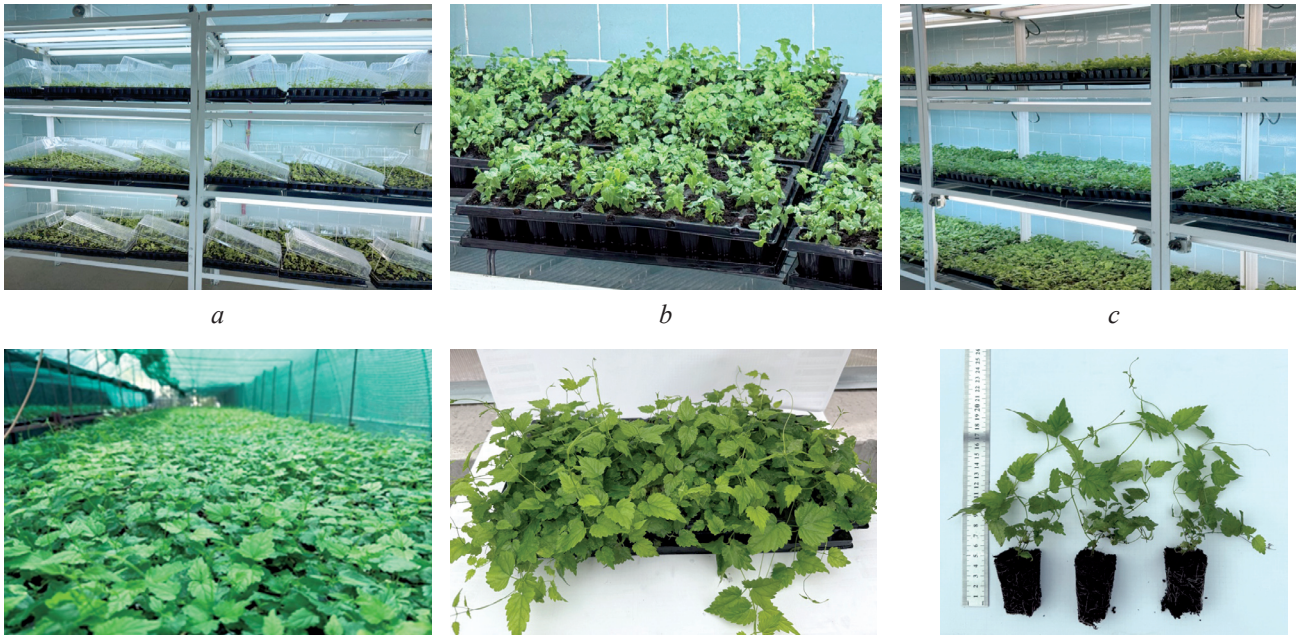

Fig. 8. The adaptation of Slov'ianka hop plants to *ex vitro* conditions: *a* — general appearance of the plants 7 days after planting them from *in vitro* conditions; *b* — after 14 days; *c–d* — further growth in trays; *e–f* — tray seedlings

a *b* *c* *d*

Fig. 9. The general view of Slov'ianka hop plants during the finishing stage: *a* — hop plants after transplanting and foliar feeding; *b* — hop plants during the further growing stage (first ten days of August); *c* — a hop seedling during the growing season, during the period of root system development and aboveground growth; *d* — a one-year-old hop seedling at the end of the growing season

DISCUSSION

As with many crops, the availability and quality of hop planting stock are considered the main limiting factors for industrial use (Kovaliov et al., 2016; Liberatore et al., 2020). In this context, rapid and effective *in vitro* regeneration methods that minimize somaclonal variation are essential for ensuring the mass propagation of commercial varieties.

The main and most common problem in initiating *in vitro* cultures is the risk of contamination of explants, especially those taken from underground organs and the success of *in vitro* culture initiation largely depends on the use of uncontaminated juvenile plant material (Singh et al., 2011; Sing, 2018; Obisesan et al., 2021). Regeneration of hop varieties in micropropagation systems was achieved via the use of various explants, primarily shoots (Roy et al., 2001a; Batista et al., 2000; Horlemann et al., 2003; Faragó et al., 2009; Mafakheri & Hamidoghli, 2019), as well as other plant organs such as leaves (Peredo et al., 2009). We utilized explants (shoot parts) obtained from plants grown under controlled conditions (in the laboratory), which also partially reduced the level of contamination by (pathogenic) microorganisms. The primary requirement for producing plants *in vitro* is maintaining working under sterile conditions. Based on our own experience and the findings of other researchers, we applied the most widely used disinfection agents. The feasibility of using commercial bleaches (specifically 20% «ACE» and 7.5% «Domestos» with a 20 min. exposure) as alternative disinfecting agents for explants was previously demonstrated in studies by Gurriaran et al. (1999) and Clapa & Harta (2021). The high effectiveness of the aseptic treatment stage is also supported by data from Machado et al. (2018), who employed sequential treatment of plant tissues with 70% ethanol for 25 s and 2% sodium hypochlorite for 20 min.

Concerning the selection of the most suitable basal growth medium analysis of the literature showed that MS medium is most preferred for a wide range of hop varieties (Clapa & Harta, 2021; Mafakheri & Hamidoghli, 2019; Santos, 2019; Fortes & Pais, 2000; Roy et al., 2001; Iacuzzi et al., 2023; Kliuvadenko & Melnychuk, 2007; Kovalyov et al., 2016; De-Sauza et al., 2022; Hirakawa & Tanno, 2022). However, some studies, notably Gurriaran et al. (1999), have demonstrated the effectiveness of the Adams (1975) medium for the hop varieties Brewers Gold and Nugget, with regeneration rates of 29% and 60%, respectively. Roy et al. (2001) found for the hop variety H138 that MS was most effective (53.9%) as opposed to W medium (27.8%), B5 medium (38.6%) and SH medium (42%). In light of this, we selected six types of media with the potential for the most effective regeneration capacity. Our results confirmed the effectiveness of MS medium for regeneration of variety Slov'ianka (97% with no callus formation), whereas W, B5, SH, and QL media were much less effective (14-44%). Adams medium with 63% performed in our hands quite similar to Gurriaran et al. (1999) for the Nugget variety (60%). It is also worth

noting that media with low microshoot regeneration capacity produced a large amount of callus, ranging from 18-73%, a finding also reported by Roy et al. (2001). Callus formation is a negative indicator that may lead to somaclonal variation, and this finding also demonstrates the ineffectiveness of the other investigated culture media for regeneration of the hop variety Slov'ianka.

The choice of iron chelate and carbon source is equally important for improving a tissue culture medium. Iron is one of the main microelements, used in microclonal propagation and plays a relevant role in many processes, including chlorophyl formation (Shibli et al., 2002). This was also found for hop (Aynalem et al., 2006; Melnychuk et al., 2022). Our results are consistent with theirs; plant height and the propagation coefficient were higher when iron chelate was used at the highest concentration. For example, Melnychuk et al. (2022), obtained a propagation coefficient of 1.1 with a plant height of 2.5 cm when using Fe-EDTA (12% Fe) at a double concentration (11 mg/l), and when using 5.6 mg/l, the values were 1.1 and 1.3 cm, respectively. In our studies, the plant height of Slov'ianka variety when using Fe-EDTA (12% Fe) at 5.0 mg/l was 4.65 cm with a propagation coefficient of 2.40. The use of FeNaEDTA (14% Fe) at 40 mg/l promoted better plant development, as evidenced by plant height of 5.25 cm and a propagation coefficient of 2.55. In contrast, the use of the Fe-EDDHA chelate (6% Fe) at 100 mg/l yielded the lowest values as compared to other forms of iron, specifically a plant height of 4.25 cm and a propagation coefficient of 1.95. However, Antonopoulou et al. (2007) reported posotive results with Fe-EDDHA in a high concentration (280 mg/l) on growth of peach root stocks.

Sugar as carbon source also plays an important role in regulating growth and development in plant tissue culture, as noted by Roy et al. (2001); De-Souza (2021) and Hirakawa & Tanno (2022) in *Humulus lupulus* L. tissue culture. However, generally both glucose and sucrose have been used successfully as carbon source in hop micropropagation Roy et al. (2001) used sucrose for variety H138. De-Souza et al. (2021) found sucrose to be the best carbon source for varieties Hallertauer Mitterlfrueh, Columbus, Chinook. Clapa & Hârța (2021), however, preferred glucose for hop varieties Cascade and H138. Thus, we checked the response of hop to glucose and sucrose for variety Slov'ianka and determined that both glucose and sucrose can be used. However, glucose had a slightly more favorable growth effect as was also found by Hirakawa and Tanno (2022) for variety Kirin-2.

We found that the type of cytokinin added to the culture medium and its concentration had a significant effect on shoot-forming capacity. As already noted by Gaspar et al. (2003), high cytokinin concentrations lead to a reduction in shoot length and conversely, low cytokinin concentrations have a stimulating effect on shoot growth, which was confirmed in our study, particularly for BAP and Kn, whereas the reaction to TDZ was positive. De-Souza et al. (2022) determined that MS medium, which did not contain growth regulators, caused only a modest growth of shoots for varieties Chinook, Columbus and Hallertauer. However, these results contradict the studies of Machado et al. (2018), in which the medium without phytohormones had the highest average index of hop plant height. Such discrepancies in the experimental data confirm the need to develop protocols for different varieties due to their variety-specific nature.

Our study found that the most effective variant was the use of BAP at a concentration of 0.1 mg/l, which promoted more vigorous shoot growth (plant height was 9.4 cm) and increased the propagation coefficient to 3.8. Clapa & Harta (2021), found that the average propagation rate of Cascade variety on a medium supplemented with 0.5 mg/l of BAP was (9.48 ± 0.78). Mafakheri & Hamidoghli (2019) recommend using 1 mg/l BAP in combination with 0.1 mg/l IAA for node segments and 0.5 mg/l BAP for shoot tips. Kliuvadenko & Melnychuk (2007), 1.0 mg/l to be effective for activating axillary meristems and promoting the growth of microshoots in the variety Kumyr and 1.5 mg/l for the Haidamatskyi variety.

Thidiazuron and kinetin were ineffective, regardless of concentration, for the proliferation of variety Slov'ianka microshoots which confirmed findings of Clapa & Harta (2021) for the variety Cascade. Moreover, Roy et al. (2001) proved that it is better for the propagation stage of H 138 hop microshoots to add 0.5 mg/l thidiazuron and 0.1 mg/l IAA to the culture medium, resulting in a propagation coefficient of 7.9 ± 1.0. Iacuzzi et al. (2023) also recommend using an MS medium with TDZ (2 mg/l) for the variety Cascade.

Excess of growth regulators in the culture medium can cause explant toxicity, leading to rapid and exces-

sive tissue division and subsequent callus formation. De-Souza et al. (2022), found that a medium containing BAP, IAA, and GA induced callus formation in 27% of the grown explants, regardless of genotype. We observed that when concentration of BAP in the culture medium increased, there was higher callus formation; however, at concentrations of 1.5 and 2.0 mg/l, the growth of microshoots slowed down, and their partial death was observed. This contradicts somewhat De-Souza et al. (2022), where BAP at 1.0 mg/l gave the highest propagation coefficients for American hop varieties and they concluded that growth regulators studied were most effective when used individually rather than in combination.

Kliuvadenko & Melnychuk (2007) noted that the optimal concentration of GA in the culture medium is 1.0 mg/l in combination with BAP at concentrations of 1.0-1.5 mg/l for the hop varieties Kumyr and Haidamatskyi. In contrast Hrybovets et al. (2012) found that a much lower concentration of 0.1 mg/l of GA in combination with BAP at 0.5 mg/l was most effective for variety Slov'ianka. Hirakawa & Tanno (2022) found that gibberellic acid (GA) promoted the formation of axillary buds in stem explants of the hop varieties Kirin-2, Cascade, and Nugget, and the application of cytokinins enhanced their growth; the highest growth rates were obtained using minimal concentrations, specifically 0.01 mg/l BAP in combination with 0.05 mg/l GA. We observed that it was even better to grow the variety Slov'ianka in absence of GA.

The balance between hormone classes (primarily auxins and cytokinins) controls plant development and determines which organ will be formed (Mercier, 2019). Auxins induce of root formation and are often used at specific concentrations during the final stage of *in vitro* plant cultivation. We found however, that it is better rooting (100%) occurred in the absence of growth regulators. and we demonstrated the inhibitory effect of auxins at 1.0–2.0 mg/l. Our findings are consistent with those of Clapa and Harta (2021) who reached 87% for variety Cascade in hormone-free MS medium (average was 6.95 ± 0.47 roots per plant, with a length of 1.13 ± 0.06. Kovalev et al. (2020) found 92-98% rooting and 3.2–3.4 roots, 2.3–2.9 cm long equally in a hormone-free medium. However, our results contradict the studies by Roy et al. (2001) and Mafakheri & Hamidoghli (2019), who found that the use of an auxin-free medium did not promote root system development; according to their data, the best approach is to use an MS medium with half the concentration of macro- and micronutrients, supplemented with IBA and IAA at concentrations of 1.0 mg/l, resulting in a rooting rate of 87–100%. The separate use of each auxin at a concentration of 1.0 mg/l promoted rooting by only 23–38% for IBA and 27–33% for IAA, whereas in our studies, these indices were 52% and 41%, respectively. Iacuzzi et al. (2023) recommend using MS medium with IAA (1 mg/l) for Cascade variety; the rooting rate was 72% in contrast, our study achieved a rate of 42%, a difference that can be attributed to variations in carbone sources within the nutrient medium and cultivar-specific characteristics. In their studies on hop rooting under *in vitro* conditions, Kliuvadenko & Melnychuk (2007) determined that for the Haidamatskyi and Kumyr cultivars, the most effective approach is the use of IBA at concentrations of 1.5 and 2.0 mg/l, resulting in rooting rates of 98% and 94%, respectively—whereas our own studies yielded only 24% rooted microshoots. Root system development metrics also differed significantly: the Haidamatskyi cultivar produced 5.7 roots (4.5 cm long) and the Kumir cultivar produced 5.8 roots (2.4 cm long), while the Slov'ianka cultivar produced only 1.2 roots with a length of 0.8 cm.

On the other hand, Machado et al. (2018) demonstrated that the best medium for hop rooting was MS medium without auxins, containing only 0.1 mg/l BAP for variety Columbus. De-Souza et al. (2022) demonstrated that a combination of auxin and cytokininat 0.02 mg/l IAA and 1.0 mg/l BAP, provided a hormonal balance favorable for auxin, promoting root formation in Chinook and Columbus genotypes. In fact, IAA impacts rooting, and the segmentation of the explant naturally leads to the accumulation of this hormone in its basal part, since auxin transportation occurs in a polar direction (De-Souza et al., 2022).

The auxin NAA did not have a positive effect on the rooting of variety Slov'ianka variety, and at 2.0 mg/l, there was no effect at all. Our findings confirmed those of Hirakawa & Tanno (2022) using variety Kirin-2 for NAA at 0.05 mg/l.

In the patented method using meristems of hop (Ukrainian Patent No. 120207) of 2019 glucose at a concentration of 20–40 g/l is used in MS as carbon source, and kinetin (0.05–0.5 mg/l) and IAA (6–15 mg/l) as growth regulators. Our study established that at a glucose concentration of 30 g/l, the average multiplication rate was 2.37, whereas with sucrose at

the same concentration, it was 2.23, a bit higher, but statistically not significant. We expanded the range of growth regulators evaluated: for cytokinins, alongside kinetin, BAP and TDZ were investigated, while for auxins IBA, IAA, and NAA were tested. The highest multiplication rate in response to cytokinins was obtained on a medium with BAP at a concentration of 0.1 mg/L (3.8 ± 0.26), whereas among the auxins tested, IBA at a concentration of 0.5 mg/L ensured the best root system development. The conclusion is that our results expanded the experimental understanding of the possibilities for optimizing the standard MS nutrient medium for the micropropagation of a specific hop genotype, variety Slov'ianka in our case.

Plant adaptation is a crucial step for successful *in vitro* propagation. Attempts to reduce the number of steps in the micropropagation process, integrating the rooting stage with the adaptation stage to cut the costs have been made, using other plant species, but were not very successful (Barrales-Lopez et al., 2015). Due to the weak root system and incomplete autotrophy of plants obtained through *in vitro* cultivation, adaptation rates are low and vary in effectiveness in the case of pineapple (Lakho et al., 2023). For hop variety Slov'ianka rooted under *in vitro* conditions, four types of substrates were used were used to evaluate adaptation. We found that peat was the best substrate for the adaptation of rooted plants (98%); the use of perlite supplemented with MS macro- and micronutrients was not effective (27%), which differed significantly from the control (98%) and contradicted the data obtained by Iacuzzi et al. (2023), who reported a 99.1% adaptation rate for Cascade variety when using perlite. Such a significant difference in plant acclimatization can possibly be explained by the genetic differences between the varieties, as well as by the addition of not only macro- and microelements but also MS vitamins to the perlite. The use of peat mixtures in our studies is not effective (72%), whereas according to Kliuvadenko and Melnychuk (2007), this figure was 93%.

CONCLUSIONS

We have adapted existing technology for the microclonal propagation of hop, specifically for the variety Slov'ianka. Our research once more confirmed the interaction between genotype and the culture medium, as it is well known that the development of the explant depends on both hormonal and genetic factors.

To achieve high disinfection rates for a limited quantity and type of plant material (rhizomes), a disinfection using 70% ethanol for 30 seconds and 9% sodium hypochlorite solution diluted to 0.25% for 20 minutes was used.

To ensure a high level of microshoot proliferation, the adapted MS medium we used should be supplemented with the following components: FeNaEDTA (40 mg/l); glucose or sucrose (30 g/l) may be used as carbon sources; among cytokinins, BAP should be used at 0.1 mg/l and gibberellic acid should not be used to elongate shoots. To induce rhizogenesis, a medium without growth regulators should be used, as it ensured 100% rooting of microshoots and high rates of root system development. During the adaptation stage, «Domoflor» peat should be used as a substrate; the adaptation rate was 98%. To reduce stress and improve plant survival after transplanting, root fertilization should be performed using the rooting agent Helprost and plastic containers with a volume of at least 0.5 l; the yield of standard plants was 97%.

Conflict of interests. The authors declare the absence of any conflict of interests.

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ОПТИМІЗАЦІЯ МІКРОКЛОНАЛЬНОГО РОЗМНОЖЕННЯ ТА АДАПТАЦІЇ ДО УМОВ *EX VITRO* ДЛЯ СОРТУ ХМЕЛЮ (*HUMULUS LUPULUS* L.) СЛОВ'ЯНКА

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Мета. Розробка збалансованої технології мікроклонального розмноження для сорту хмелю Слов'янка має важливе значення для галузі, оскільки цей сорт широко використовується у промислових насадженнях. Тому метою нашого дослідження було 1) вивчити вплив поживного середовища, процедур та засобів дезінфекції, гормонів та інших ростових факторів на успіх мікророзмноження та подальше розмноження цього сорту та 2) розробити стандартний протокол для мікророзмноження сорту Слов'янка. **Методи.** Дослідження проводили протягом 2023–2025 років у відділі вірусології, оздоровлення та розмноження плодових і ягідних культур Інституту садівництва НААН України. Рослинний матеріал для проведення експериментів був відібраний у Хмельницькій області та протестований на відсутність патогенів відповідно до стандарту *EPPO PM4/016(2): Certification scheme for hop*. Використовували різні поживні середовища та варіації стандартного поживного середовища Мурасіге-Скуга (МС), застосовуючи різні джерела заліза та вуглеводів, ауксини та цитокініни на ранніх етапах мікророзмноження, а також перліт, кокосовий субстрат і торф та два біостимулятори — Хелпрост та Радіфарм на пізніх етапах. Статистичну обробку експериментальних даних проводили за допомогою дисперсійного аналізу (ANOVA), а кореляційний аналіз — в Excel. **Результати.** Для дезінфекції найбільш ефективним виявилося використання 70% етанолу протягом 30 секунд та 9% розчину гіпохлориту натрію, розведеного до 0,25% протягом 20 хвилин. З п'яти поживних середовищ для регенерації з мінімальним ростом калюсу оптимальним було визнано адаптоване середовище МС. Для покращення загального стану рослин найкращі результати забезпечило середовище з додаванням хелату заліза FeNaEDTA (40 мг/л) та глюкози (30 г/л) як джерела

вуглеводів, а також бензиламінопурину (БАП) у концентрації 0,1 мг/л. Незважаючи на концентрації відповідно 2,0 мг/л та 1,0 мг/л тидіазурону та кінетину в поживному середовищі на етапі проліферації пагонів, їх ефективність була незначною, тому їх можна виключити. Це стосувалося і гіберелової кислоти, оскільки максимальна концентрація 1,0 мг/л призводила до збільшення відстані між міжвузлями, тим самим знижуючи коефіцієнт розмноження. На етапі індукції ризогенезу мікропагонів хмелю випробовували ауксини: індол-3-масляну кислоту (ІМК), індол-3-оцтову кислоту (ІОК), нафтилоцтову кислоту (НОК) у концентраціях 0,5, 1,0, 1,5 та 2,0 мг/л порівняно з поживним середовищем без регуляторів росту. Встановлено, що 100% укорінення сорту Слов'янка відбулося на безгормональному середовищі з високими показниками якості кореневої системи: середня кількість коренів становила 7,9, середня довжина — 2,1. Однак серед вивчених ауксинів найбільш ефективною була ІМК у концентраціях 0,5 та 1,0 мг/л, що сприяло укоріненню 58% та 52% відповідно, тоді як ІОК та НОК призвели до укорінення менше 43%. ІОК та НОК у концентрації 2,0 мг/л повністю інгібували утворення коренів. Високий рівень адаптації вкорінених рослин був досягнутий при використанні торф'яного субстрату «Domoflor» — 98%; тоді як використання двокомпонентного субстрату (торф і перліт у співвідношенні 3:1) дещо знизило адаптацію до 72%, а використання одного перліту та кокосового субстрату з додаванням макро- та мікроелементів МС призвело до приживлюваності лише 27–30%. На етапі росту рослин найбільш ефективним було добриво Хелпрост, яке містить амінокислоти, вітаміни групи В, полісахариди та хелатні мікроелементи для кореневого живлення після пересадки, що згодом призвело до 97% виходу стандартних рослин. **Висновки.** Розроблено збалансований альтернативний протокол мікроклонального розмноження сорту хмелю Слов'янка, який включає ефективну дезінфекцію етанолом та гіпохлоритом натрію, оптимізований склад поживного середовища, методи адаптації мікропагонів до умов *ex vitro* та вирощування саджанців у пластикових контейнерах до досягнення ними стандартних розмірів.

Ключові слова: хміль, мікроклональне розмноження, цитокініни, ауксини, адаптація.