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Morphogenesis of cell lines of plants *Lysimachia nummularia* L. at *in vitro* culture promising for the content of biologically active substances

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Abstract. *Lysimachia nummularia* L. is a plant belonging to the *Primulaceae* family, which is particularly valuable as a medicinal raw material used in folk medicine in many countries. It has excellent antibacterial and antioxidant capacity of metabolites. That is why the microclonal propagation of *Lysimachia nummularia* L. is a relevant issue. The purpose of this paper is to develop approaches to microclonal propagation of L. *nummularia*. For microclonal reproduction of *L. nummularia*, the method of activation of isolated tissues and organs already present in the plant meristem and induction of direct regeneration directly by explant tissues was used. To select highly productive cell lines of representatives of the genus *Lysimachia*, callus culture was obtained by indirect morphogenesis from stem and leaf explants. It was found that the formation of tissues and organs of *L. nummularia* into *in vitro* culture depended on the composition of the nutrient medium and the quantitative and qualitative ratio of growth regulators in it. Active proliferation of *L. nummularia* microshoots into *in vitro* culture was noted on the variants of Murashige and Skoog, and Driver and Kuniyuki nutrient medium with 6-benzylaminopurine 4.0 mg·l^{-1}, indolyl butyric acid 0.03 mg·l^{-1}, gibberellic acid 0.1 mg·l^{-1}. It was established that for microclonal reproduction, induction, and proliferation of the root system and obtaining regenerating plants of *L. nummularia*, the most effective is the use of nutrient media according to Murashige and Skoog with the addition of thidiazuron 0.5 mg·l^{-1} and 0.25 mg·l^{-1} kinetin. The optimal conditions for the induction of callusogenesis and obtaining the culture of cells and callus tissues of *L. nummularia* and its passage *in vitro* were selected. It has been shown that the modified nutrient medium of Murashige and Skoog, with 2.4-dichlorophenacetic acid 1.5 mg·l^{-1} and indole-3-acetic acid 0.2 mg·l^{-1}, is optimal for the accumulation of callus tissue biomass of *L. nummularia*, which ensured the frequency of callusogenesis for the first and second passages up to 98.0 ± 0.2%. 5 cell lines that actively synthesize stilbenoids and the highly productive LN-EE 02/19 cell line, which is capable of synthesizing and accumulating in callus tissues up to 10-12 mg·g^{-1} of myricetrin, were selectively isolated. As a result of the analysis, the callus culture cell line LN-EE 02/19 was obtained, which allows obtaining myricetrin in amounts up to 10.0-12.0 mg·l^{-1} of raw biomass. The developed protocol can be used both for *L. nummularia* plants and other representatives of the *Primulaceae* family

Keywords: tissue and organ cell culture *in vitro*, nutrient medium, explant, microclonal reproduction, callusogenesis

Introduction

Lysimachia nummularia L. is a perennial herbaceous plant which is especially valuable as a medicinal raw material used in conventional medicine in many countries. Among the herbaceous plants of the *Primulaceae* family, the genus *Lysimachia* has about 165 species. *L. nummularia* plants contain bioactive components that are actively used in medicine. Thanks to biotechnology, it is possible to grow cells, tissues, or plant organs *in vitro* on specially selected artificial nutrient media to obtain target products on an industrial scale. Therefore, developing of microclonal propagation

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protocols is of primary importance for obtaining many aseptic, genetically homogeneous regenerating plants of *L. nummularia*.

There is little information on the cultivation of various decorative and medicinal forms of *L. nummularia* in the scientific literature [1; 2]. Available publications cover the investigation of the theoretical foundations of induced morphogenesis, the role of trophic, hormonal, and physical factors in regeneration processes, and the problem of mass production of planting material of this group of plants [3; 4].

The presence of a wide range of biologically active substances, many phenolic acids, allows for obtaining analgesic, antiseptic, anti-inflammatory herbal preparations, and medicines for wound healing. Most phenolic compounds (secondary metabolites) are obtained from various parts of the plant [5-7].

In 2013, a new saponin (glycoside triterpene – 1) was discovered, isolated from the underground part of the plant, which counteracts active prostate cancer cells without affecting normal cells [8]. This compound affects damaged (affected) glioblastoma cells and exhibits moderate activity against melanoma cells [9-11].

Uniform spread growth, high decorative qualities have preconditioned the significant use of these plants in decorative gardening, as ground cover plants and in aquaculture [12; 13].

Lately, considerable attention has been paid to the adaptive potential of plants of the genus *Lysimachia*, the selection of the most promising species, and their cultivation using modern biotechnological methods for various purposes (pharmacology and ornamental horticulture, etc.) [3; 14].

The method of microclonal reproduction allows obtaining virus-free, healthy plant material, in significant quantities, genetically identical, which is relevant both for decorative horticulture and for industry [15-17].

Currently, there are few studies on tissue culture of *Lysimachia* L. plants. The works of scientists [2-4] report on attempts to reproduce representatives of this genus – *L. christinae, L. rubrinervis*, *L. nummularia* 'Aurea' *in vitro*.

A genetic collection of the genus *Lysimachia* L. was created on the territory of the Feofaniya park in Kyiv, which is a state park. A comprehensive assessment of the pharmacological value of vegetable oils of species of this genus common in Ukraine has also been developed. Such species as *L. nummularia, L. vulgaris, L. nemorum, L. punctata* were involved [18; 19].

Distinctive features of the growth and development of this herbaceous plant were investigated, especially the formation of the generative part of plants as the main source of valuable flower oils [11, 20; 21].

Lysimachia nummularia L. is found in synanthropic communities of Feofaniya Park (on the shores of ponds and certain parts of the park). The reproductive part of plants of this species is the main source of potentially valuable flower oils, so it is vital to pay attention to the most favourable environment for its formation [21; 22].

Research on the accumulation of polysaccharides in cells of the secretory epithelium is considered as a basis for creating a polymer film with bactericidal and fungicidal components, for this it is relevant to use microclonal reproduction [23].

Earlier studies were aimed at investigating the features of introducing into the culture, obtaining aseptic plants of *L. nummularia*, and identifying the inducing effect of various cultivation factors on the processes of direct and indirect morphogenesis [21; 24].

The purpose is to develop a protocol for microclonal propagation of *L. nummularia* plants to obtain cell lines producing biologically active compounds.

The main task is to determine the features of direct regeneration of *L. nummularia* plants at the initial stage of reproduction and indirect morphogenesis to identify promising cell lines.

Specifically, the originality of the conducted research lies in the specific features of de-differentiation, the use of cell selection methods to isolate cell lines producing biologically active compounds and the detection of patterns of accumulation of flavonoids, namely myricetrin and stilbenoid with high antioxidant activity in cultivated *in vitro* cell lines of *L. nummularia*.

Materials and Methods

For conducting experimental work, samples of *L. nummularia* were used, which were selected from the population of the garden art park monument (GAPM) "Feofaniya" at the beginning of the flowering of plants and during the period of mass vegetation.

Aseptic cultures were obtained from fragments of plant shoots of 3-5 cm, using stepwise sterilization, first washing in a soapy solution for 5-10 minutes (intensely stirring). Then washed under running water for 5 minutes. In a laminar box, explants were immersed in a 25% H_2O_2 hydrogen peroxide solution for 7 min and washed once in sterile distilled water for 10 min, which ensured the least contamination and the highest percentage of viable explants with a sterilization efficiency of 93% [21].

At the initial stage of cultivation of aseptic explants *in vitro*, basic nutrient media (NM) were used according to the prescription of Murashige and Skoog (MS) [25] and Driver and Kuniyuki (DKW) [26]. For the induction of morphogenesis by plant tissues *in vitro*, the NM composition was modified by adding to its composition plant growth regulators (PGR), both individually and in combination.

For microclonal reproduction of *L. nummularia*, the authors used the method of activation of isolated tissues and organs already present in the plant meristem and induction of direct regeneration directly by explant tissues. Activated carbon as an adsorbing element in the amount of 1 $g{\cdot}l^{-1}$, sucrose (30 $g{\cdot}l^{-1}$) – as a hydrocarbon source, meso-inositol 100 $mg{\cdot}l^{-1}$, agar-agar – 0.7% and two chelated forms of iron: ethylenediamine-di-2-hydroxy-phenylacetic acid (Fe-EDDHA) and ethylenediaminetetraacetic acid (Fe-EDTA), medium pH 5.6-5.7 [27; 28; 29].

Explants were grown in special rooms at 25 ± 2°C, with a 16-hour photoperiod and illumination intensity of 2000-3000 lux. With the proven methodology and effective sterilization, the beginning of the growth of primary microshoots was noted in 80% of the obtained viable explants on the Days 5-7 of cultivation (Fig. 1).

To obtain a high reproduction coefficient, it is necessary to activate the regenerative capacity of totipotent plant cells [27; 28; 30].

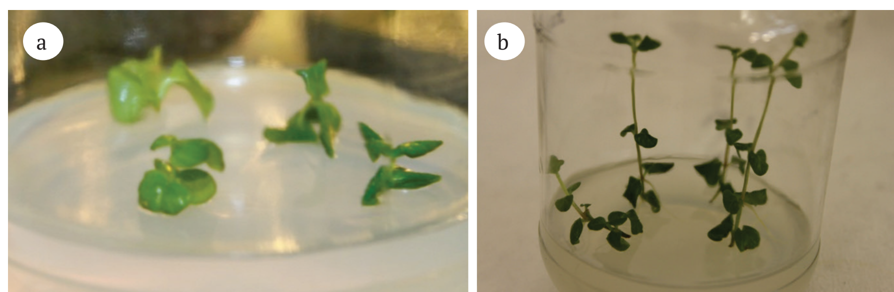

Figure 1. Formation of aseptic microshoots of *L. nummularia* on Days 7-10 of *in vitro* cultivation:
a – explants on Days 1-2; b – on Days 14-21 of cultivation

Therefore, the primary regenerated plants were subcultivated on fresh NM with different compositions and quantitative ratio of PGR and used as explants for obtaining callus culture and mass microclonal reproduction.

Results and Discussion

During microclonal reproduction, the main factor is the genotypic and species characteristics of cultivated cells, tissues, and organs, but the mineral composition of NM and the ratio of vitamins and growth regulators have a direct effect on the formation of plant cells, tissues, and organs [21; 27].

For most cultures, the synthetic analogue of cytokinin, thidiazuron (TDZ) can stimulate plant growth through its biological (cytokinin) activity. For this, it is best to use low concentrations. Compared to the effectiveness of traditional purines, growth is stimulated and the ability of tissues to intensify the synthesis and accumulation of endogenous cytokinins increases [15; 25].

Thus, in the propagation of *L. nummularia*, M. Dogan uses both solid and liquid NM, but the best results regarding the number of shoots and percentage of regeneration were found in solid NM according to the values of *in vitro* propagation. On the other hand, results regarding the best shoot length formation were noted in liquid NM [20].

M. Dogan also uses TDZ in different concentrations of 0.05-0.4 mg·l^{-1} with the addition of indolylacetic acid (IAA) to stimulate tissues of *L. nummularia* explants. It is noted that high concentrations of TDZ negatively affect the explants, inhibit their growth and development [20; 26; 31]. In turn, the authors of the paper [27] use NM supplemented with 6-benzaminopurine (BA) 0.05-1.6 mg·l^{-1} for explants of *L. nummularia*, thereby stimulating the formation of added buds directly by explant tissues. Mass microclonal propagation is stimulated by adding MS BA with α-Naphthaleneacetic acid (NAA) to the NM.

Considering previous studies [20; 26; 27], experiments were conducted to establish the efficiency of regeneration of *L. nummularia* and the course of organogenesis depending on the concentration of growth regulators of the cytokinin and fuchsin type of action in NM and the type of explant [21]. For this, the authors of this study used the method of activation of isolated tissues and organs of *L. nummularia* already present in the plant meristem and induction of direct regeneration directly by explant tissues (Table 1). The authors also modified the composition of NM, specifically by adding Ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid (Fe-EDDHA), gibberellic acid and kinetin.

Table 1. Effect of NM composition on shoot formation of *L. nummularia* explants

NM	NM composition, mg·l^{-1}	Number of explants with regenerants, pcs.	Regenerative ability, %	Number of regenerants per explant, pcs.
MS1	0.5 TDZ	10.0	100.0	5.4
MS2	1.0 TDZ	4.0	53.3	1.1
MS3	0.5 KIN	8.0	80.0	3.4
MS4	0.25 KIN	9.0	86.7	3.5
MS5	0.5 BA	3	33.3	2.2
DKW 1*	4.0 BAP, IBA 0.003, GA 0.1 benzoic acid 0.025	10	100.0	8.7
DKW 2*	Basic	3.0	30.3	1.4
DKW 3*	5.0 BA, 0.01 IBA	0	0	0
DKW 4	0.5 BA	5.0	49.6	1.0
DKW 5	1.0 BA	3.0	20.5	1.0
DKW 6	2.0 BA	0	0	0

*NM with the a ddition of Fe-EDDHA 4.8%

For this, MS and DKW NM were used with the addition of distinct groups of cytokinins and auxins to their composition: 6-benzaminopurine (BA), thidiazuron (TDZ) and kinetin (KIN), indolebutyric acid (IBA), gibberellic acid (GA), etc., both separately and in combination with each other.

As a result of the experiment, shoot genesis was

observed on stem explants. Explants cultured on NM with 0.5 mg·l^{-1} KIN formed a small number of shoots and were characterized by slow development. But when adding 0.25 mg·l^{-1} KIN and 1 g·l^{-1} of activated carbon to the composition of NM, it helped induce flowering of regenerating plants of *L. nummularia* already in 2-3 passages.

Cultivation of explants on a medium with 0.5 mg·l^{-1} kinetin caused the development of a small number of microshoots with bud meristems. Added buds formed in 60% of explants were characterized by slow development. The addition of KIN to the composition of NM at a concentration of 0.25 mg·l^{-1} and 1 g·l^{-1} of activated carbon induced the flowering effect of *L. nummularia* regenerating plants in 2-3 passages (Fig. 2).

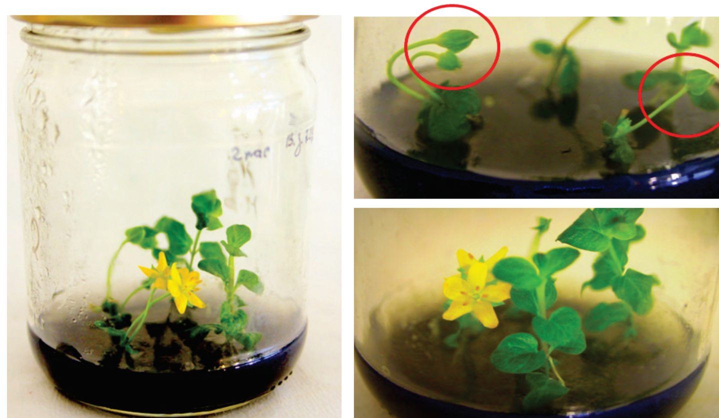

Figure 2. Flowering of regenerating plants of *L. nummularia* in *in vitro* culture

The regenerating plants of *L. nummularia*, cultivated on the nutrient media of MS and DKW with the addition of 1.0-1.5 mg·l^{-1} TDZ to its composition, had a negative effect on the explants. In some plants, reddening of leaves and their falling were noted. The formation of added microshoots was not observed. This phenomenon is characteristic when using thidiazuron [5]. High morphogenic ability and formation of the root system were noted on NM supplemented with 0.5 mg·l^{-1} TDZ. Under such conditions, the regenerative capacity and maximum growth of regenerating plants were activated, reaching almost 100% (Fig. 3a, b). The use of DKW and MS nutrient media supplemented with BA ensured little shoot formation. With the addition of 0.5 mg·l^{-1} of BA, 33.3% for MS and 49.6% for DKW were obtained for explant tissues to form microshoots.

High results were obtained on nutrient medium DKW 1*, DKW 2* with the addition of Fe-EDDHA 4.8%. Regenerating plants formed the maximum number of microshoots by activating meristems already present in the plant, but the growth rate was lower (Fig. 3c, 3d).

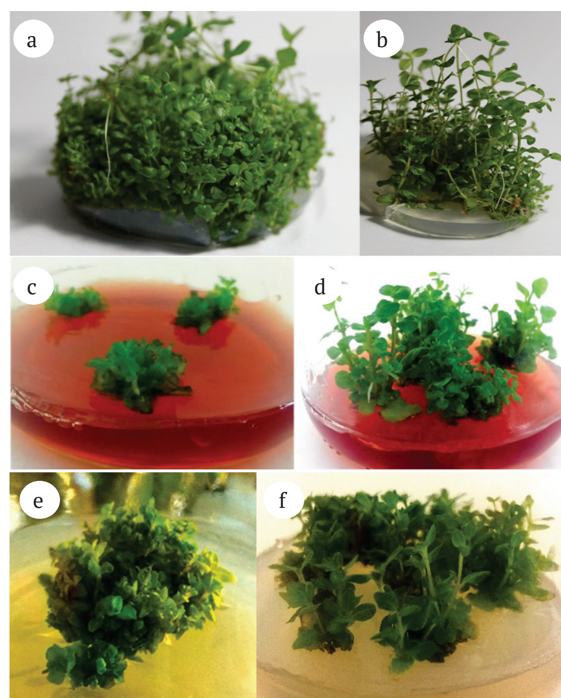

Figure 3. Morphogenesis of *L. nummularia* (a, b – direct morphogenesis on MS NM with the addition of 0.25 mg·l^{-1} kinetin; c, d – mass microclonal propagation of *L. nummularia* on NM using Fe-EDDHA 4.8% (NM DKW 1*, DKW 2*); e, f – multiplications on NM supplemented with 0.5 mg·l^{-1} TDZ)

The use of NM of this composition is best for propagation by direct morphogenesis and preservation of plants into *in vitro* culture conditions for a long time with a minimum amount of passaging.

Subsequently, the formed microshoots from distinct types of explants were passaged on fresh NM supplemented with 0.5 mg·l^{-1} TDZ (Figs. 3e, 3f) for a long time, such cultivation is effective only for 3-4 passages, then plant growth is inhibited and decreases regeneration coefficient. Taking this into account, further passages were carried out on NM according to the prescription of MS, or with the addition of 0.25 mg·l^{-1} kinetin, where the stimulation of growth factors and the induction of the morphogenic potential of explant tissues, with a high regeneration coefficient, were noted.

For the selection of highly productive cell lines of representatives of the *Lysimachia* genus, specially balanced NM was selected according to the MS prescription (Table 2).

Table 2. The influence of different concentrations of plant growth regulators on the formation of callus culture from distinct types of *L. nummularia* explants

Variant, cell lines	Growth regulators				Explant type					
					Leaf plate part			Stem part		
	2,4-D, mg·l^{-1}	BA, mg·l^{-1}	TDZ, mg·l^{-1}	NAA, mg·l^{-1}	Frequency of callus formation, %	Intensity of callus formation*	Growth index	Frequency of callus formation, %	Intensity of callus formation*	Growth index
K	0	–	–	–	–	–	–	–	–	–
1	1.0	–	–	0.1	24.3	++	1.3	13.3	+	1.2
2	1.5	–	–	0.1	85.7	++	1.4	20.0	+	1.5
3	2.0	–	–	–	56.7	++	3.6	50.0	+ +	3.2
4	2.5	–	–	–	84.7	++	1.8	30.0	+	1.7
5	3.0	–	–	–	96.2	+ + +	–	–	–	–
6	–	–	1.0	–	–	–	–	–	–	–
7	–	–	1.5	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	
9	–	1.0	–	–	–	–	–	–	–	–
10	–	2.0	–	–	–	–	–	–	–	–
11	–	2.5	–	–	16.1	+	–	–	–	–
12	–	3.0	–	–	23.4	+	–	–	–	–
13	–	1.0	–	0.1	67.6	+ + +	–	–	–	–
14	–	1.5	–	0.1	100	+ + +	1.4	83.3	+ +	3.0
15	–	2.0	–	0.2	100	+ + +	4.8	98.3	+ + +	7.2

Note: (–) – absence of callus formation, (+) – low, (++) – medium, (+++) – active

As a result, a callus culture was obtained from segments of young stems and leaf plates, which were grown on selective MS media with different ratios of hormones of cytokinin – TDZ, BA, and auxin – NAA, 2.4-D type of action.

According to the data of the experiment on NM with TDZ of different concentrations, the formation of callus culture did not occur, only 30% of the explants showed activation of axillary buds, the rest were twisted or did not undergo changes. The addition of high concentrations of BA to NM led to the intensive formation of accessory buds directly on the explant, without the stage of callus formation. Callus was formed only in some variants, not in significant quantities.

As a result of adding 2.0 mg·l^{-1} BA and 0.2 mg·l^{-1} NOK to NM and cultivation in the dark under controlled conditions for 3-4 weeks, callus cultures with a predominantly dense structure with a coarsely bumpy surface were obtained, of light green and yellow colors, which grew intensively in the depth of NM.

To investigate the possibilities of growth and accumulation of phenolic compounds, studies on the cultivation of raw callus biomass, as well as strains obtained from cultivated cells of *L. nummularia*, were continued on six variants of NM with the addition of plant growth regulators, namely: 1.5-2.0 mg·l^{-1} BA and 0.1-0.2 mg·l^{-1} NOK, 1.5-3.0 mg·l^{-1} 2.4-D (Table 3).

Table 3. Obtaining cell lines of *L. nummularia* with the content of specific metabolites

Variant, cell lines	Growth regulators				Visual observations, after 3 weeks in a thermostat
	2.4-D, mg·l^{-1}	BA, mg·l^{-1}	TDZ, mg·l^{-1}	NAA, mg·l^{-1}	
K	0	–	–	–	–
LN-EE 01/19	2.0	–	–	–	Slight formation of callus, loose yellow-white structure

Table 3, Continued

Variant, cell lines	Growth regulators				Visual observations, after 3 weeks in a thermostat
	2.4-D, mg·l^{-1}	BA, mg·l^{-1}	TDZ, mg·l^{-1}	NAA, mg·l^{-1}	
LN-EE 02/19	–	2.0	–	0.2	Active formation of biomass with a dense tumour structure, light green and white
LN-EE 03/19	1.5	–	–	0.1	Inactive formation of yellow callus mass
LN-EE 04/19	3.0	–	–	–	Active formation of callus biomass of non-morphogenic nature, light yellow with brown zones
LN-EE 05/19	–	–	0.5	–	Insignificant formation of a dense light green callus culture, with the ability to morphogenesis.
LN-EE 06/19	2.5				Slight formation of callus, loose yellow-white structure

As a result of three-week cultivation in the dark, strains of callus cultures were obtained, which were analysed for the presence of promising biologically active substances and their concentrations.

All cultured cell lines were characterized by sufficiently high production of callus biomass, but they differed in structure and colour. Three major phenolic components were isolated from six cell lines' biomass. As a result of the analysis, the most significant accumulation of active substances was the LN-EE 02/19 cell line (Fig. 4).

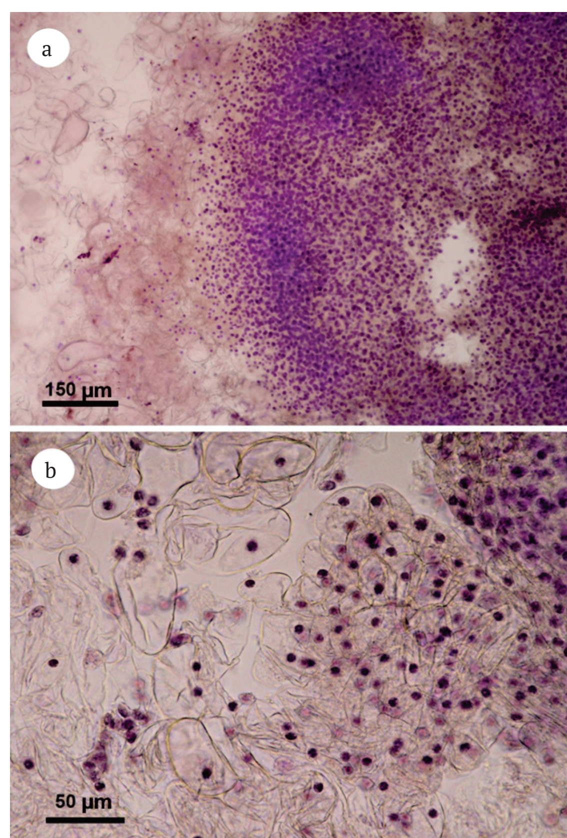

Figure 4. Cells of non-morphogenic callus of *L. nummularia* line LN-EE 02/19 – an active producer of myricetrin; DNA staining with Schiff's reagent after cold hydrolysis for 40 min with 1N HCl at 25°C

It was established that on MS nutrient media with 2.0 mg·l^{-1} BAP and 0.2 mg·l^{-1} NAA in callus and suspension culture biomass, the accumulation of the flavanol myricetrin occurs, where its amount was the largest and amounted to 9.8 mg·l^{-1} raw weight.

Cells of non-morphogenic callus had high proliferative activity and the nuclei sizewas 7-8 µm. The cells of the peripheral zone are elongated, much larger and mostly anucleate. The active zone of division is represented by small cells with an insignificant index of the ratio of the nucleus to the cytoplasm.

The ability of callus cultures to synthesize target substances under the conditions of serial passage was preserved. Among the isolated groups of secondary metabolites in the LN-EE 02/19 line, myricetrin (flavonol) was isolated (Fig. 5). The ability of the obtained *L. nummularia* cell lines to synthesize this group of compounds actively can be considered in the perspective of its use as an antiseptic agent.

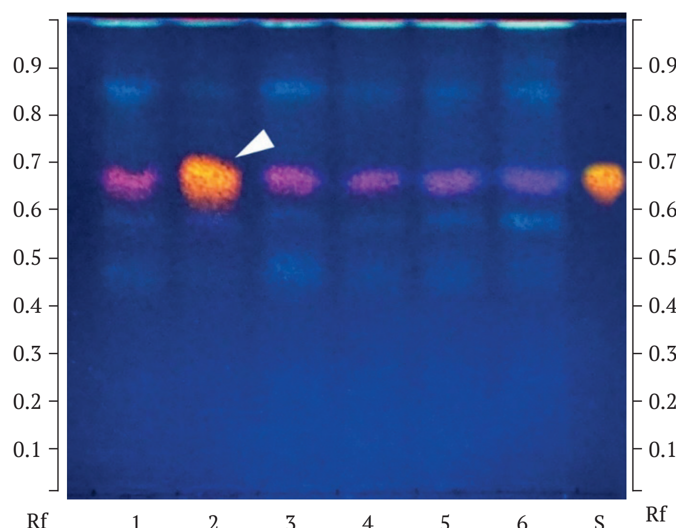

Figure 5. Chromatography of methanolic extracts of callus tissues of the investigated *L. nummularia* cell lines: 1 – LN-EE 01/19, 2 – LN-EE 02/19, 3 – LN-EE 03/19, 4 – LN-EE 04/19, 5 – LN-EE 05/19, 6 – LN-EE 06/19

As a result of the analysis, the obtained callus culture cell line LN-EE 02/19 allows obtaining myricetrin in amounts up to 10.0-12.0 mg·l^{-1} of raw biomass.

This suggests that for cultivation and obtaining the isolated cell lines, it is necessary to use NM with 2.0 BAP and 0.2 NAA in the dark for 3 weeks, and after that continue cultivation on the same medium, but under lighting conditions for 7 days The change in NM composition to lower concentrations of hormones during subsequent passages and their effect on the synthesis and accumulation of target products have not yet been studied.

The established experiments indicated that the most effective callus formationoccurs on stem segments without access to light, for 3-4 weeks, followed by their cultivation on specially selected media.

Cell lines LN-EE 01/19, LN-EE 05/191, LN-EE 06/19 were characterized by low productivity. For Days 21-28 of growth on the nutrient medium according to the MS prescription, the callus biomass yield was on average 1.7 mg per explant. In contrast to this, line 2 accumulated significantly more callus biomass under the same cultivation conditions and biologically active substances, respectively, which is promising for growing the culture in industrial conditions. The high ability of the obtained lines to morphogenesis under lighting conditions was also noted. This was in contrast to cell lines LN-EE 01/19, LN-EE 03/19, LN-EE 05/19, which were characterized by a low ability for morphogenesis, slowed growth and accumulation of a small numberof flavonoid substances.

Conclusions

It was found that organogenesis in the culture of *L. nummularia* depended on the size of the donor plant, the phenological phase of plant vegetation, the composition of NM and the amount of PGR.

The need to develop methods of microclonal reproduction lies in optimizing the composition of the nutrient medium for cultivating various explants and using methods of direct and indirect morphogenesis.

It was established that on nutrient media with 0.25 mg·l^{-1} kinetin and 0.5 mg·l^{-1} TDZ g·l^{-1}, the full realization of the morphogenetic potential of explant tissues, induction, and proliferation of the root system took place, and regenerative plants capable of adaptation were obtained.

For long-term cultivation of *L. nummularia* plants in *in vitro* culture, the hormone-free MS nutrient medium is optimal. For the active proliferation of microshoots from different types of explants of *L. nummularia* in isolated conditions, the best results were obtained using NM with Fe-EDDHA complex 4.8%, DKW NM with 4.0 mg·l^{-1} BA, 0.003 mg·l^{-1} IBA, 0.1 mg·l^{-1} GA, 0.025 mg·l^{-1} BA.

The optimal conditions for the induction of callus formation and obtaining the culture of cells and callus tissues of *L. nummularia* and its passage *in vitro* were selected. It has been shown that the modified nutrient medium of Murashige and Skoog, with 2.4-D (1.5 mg·l^{-1}) and NAA (0.2 mg·l^{-1}) is optimal for the accumulation of callus tissue biomass of *L. nummularia*, which provided the callus formation frequency for the first and second passages up to 98.0 ± 0.2%.

5 cell lines that actively synthesize stilbenoids and the highly productive LN-EE 02/19 cell line, which is capable of synthesizing and accumulating in callus tissues up to 10-12 mg/g of myricetrin, were selectively isolated.

It was established that for the intensive synthesis of myricetrin by the LN-EE 02/19 cell line, the nutrient medium of MS with 2.0 mg·l^{-1} BA and 0.2 mg·l^{-1} NAA is optimal.

The prospect of further research lies in the implementation of the developed protocols and the verification of the effectiveness of the technology on an industrial scale.

References

[1] Belmain, S. (2014). Herbivore defence compounds occur in pollen and reduce bumblebee colony fitness. *Journal of Chemical Ecology*, 40, 878-881.

[2] Zhang, H.Z., Jie, J.Q., & Lv, D. (2005).Tissue culture of *Lysimachia christinae*. *Agricultural Science and Practice,* 3, 83-85.

[3] Xu, G.F. (2007). Physiological process of drough tresistance of two *Lysimachias* pecies. *Journal of Northwest Forestry University*, 22, 12-14.
[4] Gupta, S., Kaliamoorthy, S., Jayashankar, D., Mao, A., & Soneswar, S. (2012). Micropropagation of *Lysimachia laxabaudo*. *Asian Journal of Science and Technology*, 4(12), 024-027.
[5] Grodzinsky, A.M. (Ed.). (1989). *Medicinal plants*. Kyiv: Main Editorial Office of the Ukrainian Soviet Encyclopedia.
[6] Goncharenko, I.V., Ignatyuk, O.A., & Shelyag-Sosonko, Yu.R. (2013). Forest vegetation of the Feofania tract and its anthropogenic transformation. *Ecology and Noospherology,* 24(3-4), 51-63.
[7] Johannsson, M., Winsor, J., & Stephenson, A. (1994). Genetic and environmental effects on *in vitro* pollen tube growth in Cucurbita. Pollen-pistil interactions and pollen tube growth. *An American Society of Plant Physiologists*, 12, 307-309.
[8] Simpson, S., & Raubenheimer, D. (2012). *The nature of nutrition: A unifying framework from animal adaptation to human obesity.* Princeton: Princeton University Press.
[9] Havens, K. (1995). Secondary nitrogen limitation in a subtropical lake impacted by non-point source agricultural pollution. *Environmental Pollution*, 89, 241-246.
[10] Jakobsen, H., & Martens, H. (1994). Influence of temperature and aging of ovules and pollen on reproductive success in *Trifolium repens* L. *Annals of Botany*, 74, 493-501.
[11] Podolak, I., Koczurkiewicz, P., Michalik, M., Galanty, A., Zajdelm, P., & Janeczko, Z. (2013). A new cytotoxic triterpene saponin from *Lysimachia nummularia* L. *Carbohydrate Research*, 375, 1-20.
[12] Tsukanova, G.O., Andrienko, T.L., & Pryadko, O.I. (2002). Vegetation cover of the Dnipro islands within the city of Kyiv. *Ukrainian Botanical Journal*, 59(2), 135-140.
[13] Tsukanova, G.O. (2003). Sozological characteristics of the flora of the Dnipro islands and the adjacent part of the floodplain within the city of Kyiv. *Ukrainian Botanical Journal,* 60(4), 397-404.
[14] Stowe, L. (1979). Allelopathy and its influence on the distribution of plants in an Illinois old-field. *Journal of Ecology*, 67, 1065-1085.
[15] Svetla, D.Y., Sara, G., Ervin, F., Simcha, L.Y., & Moshe, A.F. (2003). Auxin type and timing of application determine the activation of the developmental program during *in vitro* organogenesis in apple. *Plant Science,* 165, 299-309.
[16] Richards, L.A., Dyer, L.A., Forister, M.L., Smilanich, A.M., Dodson, C.D., Leonard, M D., & Jeffrey, C.S. (2015). Phytochemical diversity drives diversity of tropical plant-insect communities. *Proceedings of the National Academy of Sciences*, 112, 10973-10978.
[17] Ruedenauer, F., Spaethe, J., & Leonhardt, S. (2016). Hungry for quality individual bumblebees forage flexibly to collect high-quality pollen. *Behavioral Ecology and Sociobiology*, 70, 1209-1217.
[18] Vanderplank, S., Rebman, J., & Ezcurra, E. (2017). Where to conserve? Plant biodiversity and endemism in mediterranean Mexico. *Biodiversity and Conservation*, 27, 109-122.
[19] Didukh, Y.P., & Didukh, Ya.P. (2011). *The ecological scales for the species of Ukrainian flora and their use in synphytoindication*. Kyiv: Phytosociocenter.
[20] Dogan, M. (2022). *In vitro* shoot regeneration of *Lysimachia nummularia* L. in solid and liquid culture medium. *CUPMAP*, 6, 875-881.
[21] Radchenko, V.H., Bilous, S.Yu., & Matiashuk, R.K. (2016). Peculiarities of obtaining aseptic culture of stem explants of *Lysimachia nummularia* L. in *in vitro* conditions. In *Actual problems of the forestry sector and horticulture* (p. 102). Kyiv: National University of Life and Environmental Sciences of Ukraine.
[22] Radchenko, V., Likhanov, A., & Matyashuk, R. (2017). Histochemical heterogeneity of secretory structures in the flowers of *Lysimachia nummularia* L. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, 1, 383-387.
[23] Ruedenaue, F., Leonhard, S., Lunau, K., & Spaethe, J. (2019). Bumblebees are able to perceive amino acids via chemotactile antennal stimulation. *Journal of Comparative Physiology*, 205, 321-331.
[24] Konvaliuk, I.I., Kravets, N.B., Drobyk, N.M., Melnyk, V.M., & Kunakh, V.A. (2010). Direct organogenesis *in vitro* of yellow tirlych (*Gentiana lutea* L.). *Biotekhnolohiia*, 3(5), 66-73.
[25] Murashige, T., & Scoog, F. (1962). Arevised medium for rapid, grow than bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15, 473-497.
[26] Driver, J.A., & Kuniyuki, J.H. (1984). *In vitro* propagatio of Paradox walnu troot stock. *HortScience*, 19, 507-509.
[27] Dogan, M., (2018). *In vitro* micropropagation from nodal explants of the medicinal plant *Lysimachia nummularia* L. *Journal of Agriculture and Nature*, 21(6), 875-881.
[28] Chornobrov, O., & Bilous, S. (2021). In vitro plant regeneration of Christmas cactus (Schlumbergera truncata (Haw.) Moran) by indirect morphogenesis. *Folia Forestalia Polonica, Series A – Forestry*, 63(1), 68-73.
[29] Mitrofanova, I.V. (2011). *Somatic embryogenesis and organogenesis as the basis of biotechnology for the production and preservation of perennial garden crops*. Kyiv: Agricultural Science.
[30] Butenko, R.G. (1975). *Experimental morphogenesis and differentiation in plant cell culture*. Moscow: Nauka.
[31] Hussain, S.A., Ahmad, N., & Anis, M. (2018). Synergetic effect of TDZ and BA on minimizing the post-exposure effects on axillary shoot proliferation and assessment of genetic fidelity in *Rauvolfia tetraphylla* (L.). *Rendiconti Lincei. Scienze Fisiche e Naturali*, 29(1), 109-115.

Морфогенез в культурі *in vitro* клітинних ліній рослин *Lysimachia nummularia* L. перспективних за вмістом біологічно активних речовин

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Анотація. *Lysimachia nummularia* L. – це рослини, що належать до родини *Primulaceae*, є особливо цінними як лікарська сировина, що використовується в народній медицині багатьох країн. Має чудову антибактеріальну та антиоксидантну здатність метаболітів. Саме тому мікроклональне розмноження *Lysimachia nummularia* L є актуальним питанням. Мета статті – розробити підходи до мікроклонального розмноження L. *nummularia*. Для мікроклонального розмноження L. *nummularia* використовували метод активації вже існуючих у рослині меристем ізольованих тканин і органів та індукція прямої регенерації безпосередньо тканинами експланту. Для добору високопродуктивних клітинних ліній представників роду *Lysimachia* калюсну культуру отримували шляхом непрямого морфогенезу із стеблових та листкових експлантів. З'ясовано, що формування тканин та органів L. *nummularia* у культурі *in vitro* залежало від складу живильного середовища й кількісного та якісного співвідношення регуляторів росту в ньому. Активну проліферацію мікропагонів L. *nummularia* в культурі *in vitro* відмічено на варіантах живильного середовища Мурасіге і Скуга та Драйвера Куньюки з додаванням 6-бензиламінопурину 4,0 мг·л$^{-1}$, індоліл масляної кислоти 0,03 мг·л$^{-1}$, гиберелової кислоти 0,1 мг·л$^{-1}$. Встановлено, що для мікроклонального розмноження, індукування й проліферації кореневої системи та отримання рослин-регенерантів L. *nummularia* найефективнішим є використання живильних середовищ за прописом Мурасіге і Скуга з додаванням тідіазурону 0,5 мг·л$^{-1}$ та 0,25 кінетину. Підібрано оптимальні умови для індукції калюсогенезу і отримання культури клітин і калюсних тканин L. *nummularia* та її пасажування в умовах *in vitro*. Показано, що оптимальним для накопичення біомаси калюсних тканин L. *nummularia* є модифіковане живильне середовище Мурасіге і Скуга, з 2,4-дихлорфеноцтовою кислотою 1,5 мг·л$^{-1}$ та індол-3-оцтовою кислотою 0,2 мг·л$^{-1}$, що забезпечували частоту калюсогенезу для першого й другого пасажу до 98,0 ± 0,2 %. Селективним шляхом виділено 5 клітинних ліній, які активно синтезують стильбеноїди та високопродуктивну клітинну лінію LN-EE 02/19, яка здатна синтезувати і накопичувати в калюсних тканинах до 10-12 мг/г мирицетрину. У результаті аналізу отримано клітинну лінію калюсної культури LN-EE 02/19, що дозволяє отримувати мирицетрин у кількостях до 10,0-12,0 мг·л$^{-1}$ сирої біомаси. Розроблений протокол може використовуватись як для рослин L. *nummularia* так і інших представників родини *Primulaceae*

Ключові слова: культура клітин, тканин та органів *in vitro*, живильне середовище, експлантат, мікроклональне розмноження, калюсогенез