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ADAPTATION OF *Gentiana lutea* L. PLANTS OBTAINED *in vitro* TO *ex vitro* AND *in situ* CONDITION

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The aim of the research was to develop the technology of introduction of the *Gentiana lutea* L. plants obtained by microclonal propagation into conditions *in situ*. Methods of cultivation of plant objects *in vitro* were used. There were chosen optimal conditions for rooting *G. lutea* shoots obtained through microclonal propagation *in vitro:* MS/2 medium with twice decreased concentration of NH_4NO_3without vitamins and sucrose supplemented with 3 g/l of mannite and 0.05 mg/l kinetin, and agar (4 g/l) in combination with perlite (16 g/l) used as a maintaining substrate; or the nutrient medium (MS/2 without vitamins and smaller concentration of NH_4NO_3) with gradual decrease of carbohydrates from 10 g/l to 2 g/l, and further rooting experimental shoots in tap water. Rooted plants were adapted to conditions *ex vitro* through planting them into flowerpots with soil and gradual changing hothouse regime for exposed one. The share of adapted to *in situ* conditions plants (21% after a year of planting) proves the used method to be resultative and promising. Thus, there was suggested the most efficient technology for revival of disturbed *G. lutea* populations that includes repatriation of rooted and adapted to *ex vitro* conditions plants obtained through microclonal propagation *in vitro*.

Key words: *Gentiana lutea* L., rooting *in vitro*, adaptation ex *vitro*, repatriation *in situ*.

Within a concept of preserving biodiversity of plant and animal world, the investigations aimed at finding ways to stabilize the number of disturbed populations and revival of destroyed natural populations of rare plant species are of current relevance. While protecting such plant species, it is necessary to apply not only passive preservation methods (inventory, including into local lists of rare plants and Red Books of various ranks, conserving on reserved territories), but use active methods (growing in botanical gardens, increasing number of rare species in natural conditions by seeding and planting of obtained *in vitro* cultures) too [1].

Rare plant species, requiring protection and revival, include medicinal *Gentiana lutea* L. The complexity of *G. lutea* seed restoration, excessive pastoral load and recreation have led to reducing areas and violated structures of this species population. *G. lutea* value for conventional and nontraditional medicine, determined by the pharmacological properties and potential need in raw material, has caused the necessity to estimate the species resources in Ukraine for creating fundamental principles of preservation and developing new approaches to its population revival.

The recent years being marked by the developed methods of *G. lutea* microclonal propagation in *vitro* [2, 3], the repatriation of propagated in *vitro* plants into natural conditions has become of great importance. The essential condition for successful repatriation is the use of individuals grown in most approximated to natural conditions, and therefore minimally changed genetically [4].

The objective of the investigation was to develop a scheme of reintroducing *G. lutea* plants developed by microclonal propagation into *in situ* conditions.

Materials and Methods

The object of the investigation was *G. lutea* — a perennial with indefinitely long life cycle (over 50 years) and lasting pre-generative period (5–10 years) [5]. The initial seed

material was gathered during expeditions in the Ukrainian Carpathians in mountainous populations on Petros Marmarosky, Pozhyzhevska, Sheshul-Pavlyk, Menchul Kvasy mountains and Lemska and Rogneska mountainous valleys.

For choice of rooting *G. lutea* shoots *in vitro* we took into consideration biological and ecological peculiarities of the species and the results of *G. lutea* introduction *in vitro* culture, obtained in the laboratory of ecology and biotechnology of Volodymyr Hnatiuk Ternopil National Pedagogical University. For rooting, we used *G. lutea* shoots obtained by microclonal propagation and then grown for 1.5–3 months until they became 15–20 mm high with 3–5 pairs of leaves. Each testing variant included three samplings with 8–10 shoots.

Fourteen variants of media were tested (tables 1, 2). In eight tested variants, the cultivated *in vitro* shoots were planted in Murashige and Skoog medium (1962) with half concentration of macro- and microsalts (MS/2) without sucrose and vitamins (table 1). There were experimented two MS modifications media: MS/2 (variants I, II, III, IV); and MS/2 with twice decreased concentration of NH_4NO_3 (variants V, VI, VII, VIII), as the decrease of macro- and micro-elements and nitrogen concentration are known to promote rooting wild strawberry, apple-tree and other plant cuttings [6–9]. In addition, the research of the component soil composition from natural *G. lutea* areas showed that the amount of both available and general nitrogen in them was comparatively low. Growth regulators are known to negatively influence adaptation of plants to *ex vitro* conditions [9], so kinetin (Kin) concentration in all media was reduced to 0.05 mg/l.

For each modification of nutrient medium, we used various maintaining substrates such as agar (8 g/l) (I, V); agar (4 g/l) and perlite (16 g/l) (IV, VIII); agar (4 g/l) and ground perlite (16 g/l) (III, VII); foam (II, VI); vermiculite (IX). We also attempted to enroot shoots in a sterilized soil from natural *G. lutea* habitats combined with peat and perlite in correlation 1:1:1 (X).

There was tested nutrient medium MS/2 without vitamins with twice decreased concentration of NH_4NO_3 which was added with 2 g/l sucrose (as the main CO_2 source) (XI); two variants with different concentrations of sucrose and mannite in correlation 1:1 (XII, XIII); a variant with mannite only (XIV) with its concentration 3 g/l (table 2). Agar (4 g/l) combined with perlite (16 g/l) was used as a maintaining substrate. Adding mannite was caused by its being osmotically active substance providing antioxidant protection and promoting plant survival in stress [10, 11]. Plants were cultivated for 120 days on the media mentioned above.

G. lutea was cultivated in jars with ventilatory covers. The plants, infected during relocation or not forming roots on nutrient media, were placed into settled tap water. Rooted specimens were planted into flowerpots with soil, having their roots previously washed with distilled water to relieve them from the medium remains.

To avoid dehydration of the plants and create the greenhouse effect the flowerpots were covered with glass. Every day the plants were sprayed and once a week they were watered with settled tap water. Air expositions were used to make them adapt to *ex vitro* conditions. In 1–1.5 months the flowerpots were definitely opened, watering was done in dependence on soil drying (1–2 times a week), and spraying was daily.

In early June (after 8–10 months of the experiment beginning) the adapted to *ex vitro* conditions and then grown for 3–4 months *G. lutea* were planted *in situ* conditions in the places of bare soil near adult gentian individuals.

General state of plants in *ex vitro* and *in situ* conditions was estimated by morphological values (plant height, number of shoots, pairs of leaves and number of internodes per plant). The obtained data were processed statistically [12].

Results and Discussion

The adaptation efficiency of the obtained *in vitro* plants to *ex vitro* conditions depends in the first place on their successful rooting, as the existence of well-developed root system provides better adaptation of plants to growth in soil and unstable conditions of unsterile environment (fluctuations of humidity, temperature, etc.). Thus, the research included three stages: rooting obtained by microclonal propagation plants *in vitro*, adaptation of rooted plants to *ex vitro* conditions, transfer of adapted to *ex vitro* plants to conditions *in situ*.

Rooting G. lutea sprouts obtained by microclonal propagation. The results of the research show that both the composition of nutrient medium and the type of maintaining substrate (table 1) influenced *G. lutea* rooting. *G. lutea* shoots planted in agarized media (8 g/l) (I, V) and in vermiculite (IX) necrotized on the

20–30th day of cultivation, and those placed in media with agar (4 g/l) in combination with ground perlite (16 g/l) (III, VII) — on 20–40th day. Evidently, the consistency of nutrient medium decelerates absorbing of nutrient substances by plant roots that leads to their death. This result is confirmed by scientific literature data, pointing to the fact that growth and development of roots *in vitro* are dependent on aeration of nutrient medium that, in its turn, is dependent on agar concentration. Rooting plant cuttings is decelerated; the development of secondary roots does not take place [7].

The shoots planted into the nutrient media with foam substrate and decreased concentration of agar (up to 4 g/l) in combination with perlite (16 g/l) survived and rooted in dependence on medium composition. The individuals cultivated in MS/2 without vitamins and sucrose (II, VI), died on the 20th (II) and 30th (VI) day. Twice decreased concentration of NH_4NO_3 in MS/2 medium (VI, VIII) was more efficient. As a result, the share of viable specimens on the 50th day of cultivation constituted 45% (VI) and 66% (VIII), and on the 120th day, it was 10 % and 15% respectively. Obviously, the composition of nutrient medium serves as a limiting factor in such a combination of nutrient medium and maintaining substrate.

Thus, one can assume that using MS/2 medium with twice decreased NH_4NO_3 concentration and maintaining substrates such as foam and agar (4 g/l) combined with perlite (16 g/l) positively influences rooting shoots. As the results of shoots survival on conditions of using foam and agar with perlite did not sufficiently differ, one can use both variants for further cultivation. However, referring to the objective of the research, we find the use of the second variant more efficient as semi-solid maintainig substrate will enable better adaptation of plant root system to growth conditions in soil. Other researchers prove positive effect of using agar combined with perlite as a maintaining substrate: plants of wild strawberries, apples, pears, ashes, lilac formed a well-developed root system on such a substrate [7].

On conditions of rooting *G. lutea* shoots in sterilized soil with adding peat and perlite in correlation 1:1:1 all the tested shoots died on 20–30th day. Evidently, soil sterilization

Table 1. **Rooting of *G. lutea* shoots *in vitro***

Days	Percentage of viable shoots, %								
	MS/2 medium without vitamins and sucrose supplemented with 0.05 mg/l Kin				MS/2 medium without vitamins and sucrose; with twice decreased concentration of NH_4NO_3, supplemented with 0.05 mg/l Kin				Vermiculite
	Maintaining substrate								
	Ag	F	Ag+gPr	Ag+Pr	Ag	F	Ag+gPr	Ag+Pr	
	I	II	III	IV	V	VI	VII	VIII	IX
10	51±3.8	84±3.5	42±2.4	100	49±3.2	100*	100*	100*	100
20	0	0	0	27±1.8	0	100*	18±1.4*	93±5.4*	43±3.2
30	0	0	0	0	0	100*	44±2.8*	93±5.4*	0
40	0	0	0	0	0	85±5.4*	0	71±4.7*	0
50	0	0	0	0	0	45±3.6*	0	66±4.2*	0
60	0	0	0	0	0	30±3.1*	0	48±3.9*	0
90	0	0	0	0	0	10±1.5*	0	43±3.3*	0
120	0	0	0	0	0	10±1.5*	0	15±1.8*	0

Notes: Ag, F, Ag + gPr, Ag + Pr — maintaining substrates;

Ag — agar (8 g/l), F — foam, Ag + gPr — agar (4 g/l) combined with ground perlite (16 g/l), Ag + Pr — agar (4 g/l) combined with perlite (16 g/l);

* — marked variants of the experiment in which the parameters of share of viable shoots during use of modified variants MS/2 media with twice decreased concentration of NH_4NO_3 reliable ($P \leq 0.05$) differ from the results on nutrient media MS/2 with full NH_4NO_3 concentration.

leads to death of mycorrhizal organisms that negatively affects growth and development of *G. lutea*, which is a mycorrhizal species [13].

An attempt to enroot *G. lutea* shoots on the medium suggested by Petrova and colleagues [14] and which was efficient for rooting and further adaptation of obtained *in vitro G. lutea* plants to *ex vitro* conditions, and then to *in situ* in Natural Park of Vitosha (Bulgaria) failed to have any positive results in our case. None of the cultivated *in vitro* shoots formed roots that is probably caused by genotype peculiarities and heterogeneity of natural habitats of plants.

The shoots cultivated on the medium without sucrose and additional supply of CO_2 died probably because of the complexity of adaptation to autotrophic type of nutrition, little photosynthetic activity and scarcity of oxygen. Therefore, for further cultivation, we used nutrient medium MS/2 without vitamins with twice decreased concentration of NH_4NO_3 and added carbohydrates (XI, XII, XIII, XIV) (Table 2).

The shoots cultivated on XI, XII and XIII variants of the medium were viable on the 30th day, but they did not form roots. That is why all the specimens for rooting were planted in tap water, covered with caps, which were gradually opened to adapt the plants to *ex vitro* conditions. On the 20th day after planting into water 38% of specimens demonstrated rhizogenesis, and on the 90th day 94% of the specimens formed roots. The *G. lutea* shoots grown on XIV variant of the medium also formed roots (Fig.1), the percentage of adaptation on the 120th day of cultivation was 97 (Table 2).

The analyzed results show that efficiency of rooting *G. lutea* shoots can be provided only by combination of optimal composition of nutrient medium and favourable for rooting maintaining substrate. The optimal medium among those tested proved to be MS/2 medium with twice decreased concentration of NH_4NO_3 without vitamins and sucrose supplemented with 3 g/l mannite and 0.05 mg/l Kin and agar (4 g/l) combined with perlite (16 g/l) used as a maintaining substrate. Gradual diminishing carbohydrates from 10 g/l to 2g/l in the nutrient medium (MS/2 without vitamins and twice decreased concentration of NH_4NO_3) with further rooting of the shoots in tap water proved equally efficient (Fig. 1).

Adaptation of the rooted plants to ex vitro conditions. Repatriation in disturbed populations of plants obtained through microclonal propagation *in vitro* first requires solving the problem of their adaptation

Table 2. **Rooting of *G. lutea* shoots**

<table>
<tr><td rowspan="3">Days</td><td colspan="4">Percentage of viable shoots, % / Percentage of rooted plants, %</td></tr>
<tr><td colspan="4">MS/2 medium with twice decreased concentration of NH_4NO_3 without vitamins, supplemented with 0.05 mg/l Kin, and agar (4 g/l) in combination with perlite (16 g/l) used as a maintaining substrate</td></tr>
<tr><td>2 g/l sucrose</td><td>2 g/l sucrose,
2 g/l mannite</td><td>1 g/l sucrose,
1 g/l mannite</td><td>3 g/l mannite</td></tr>
<tr><td></td><td>XI</td><td>XII</td><td>XIII</td><td>XIV</td></tr>
<tr><td>10</td><td>100/0</td><td>100/0</td><td>100/0</td><td>100/0</td></tr>
<tr><td>20</td><td>100/0</td><td>100/0</td><td>100/0</td><td>100/0</td></tr>
<tr><td rowspan="2">30</td><td>100/0</td><td>100/0</td><td>100/0</td><td rowspan="2">100/54±3.2</td></tr>
<tr><td colspan="3">Shoots planted into tap water</td></tr>
<tr><td>40</td><td colspan="3">100*/0</td><td>97±4.7*/97±4.7</td></tr>
<tr><td>50</td><td colspan="3">100*/38±2.8</td><td>97±4.7*/97±4.7</td></tr>
<tr><td>60</td><td colspan="3">94±5.3*/72±6.1</td><td>97±4.7*/97±4.7</td></tr>
<tr><td>90</td><td colspan="3">94±5.3*/94±5.3</td><td>97±4.7*/97±4.7</td></tr>
<tr><td>120</td><td colspan="3">94±5.3*/94±5.3</td><td>97±4.7*/97±4.7</td></tr>
</table>

Note: * — marked variants of the experiment in which the parameters of the share of viable shoots, on conditions of using modified variants of MS/2 media with twice decreased concentration of NH_4NO_3 supplemented with carbohydrates reliable ($P \leq 0.05$) differ from the results on the nutrient MS/2 medium with twice decreased concentration of NH_4NO_3 without carbohydrates (Table 1, VIII variant of nutrient medium).

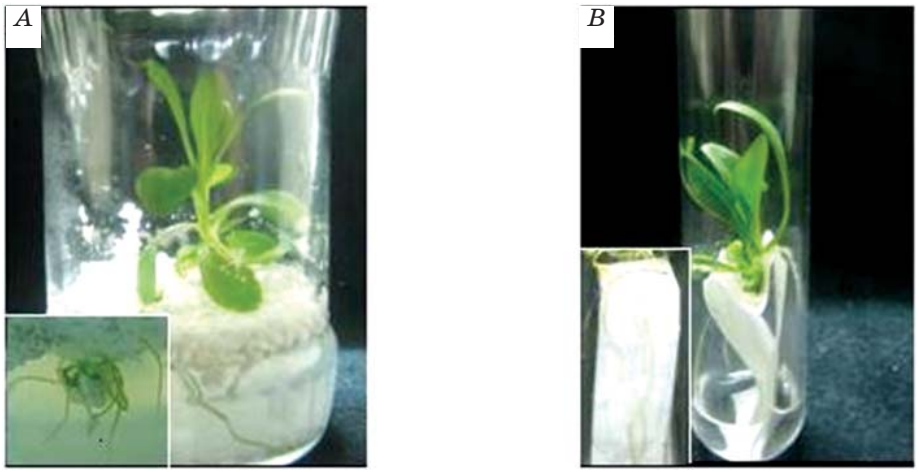

Fig. 1. **Rooting *G. lutea* shoots *in vitro*:**
A — rooting in the medium MS/2 with twice decreased concentration of NH_4NO_3 without vitamins and sucrose, supplemented with 3 g/l mannite and 0.05 mg/l Kin, the maintaining substrate is agar (4 g/l) combined with perlite (16 g/l); *B* — rooting in tap water

to *ex vitro* conditions. According to the research literature data [15–17], this process is labour intensive, as specific conditions of *in vitro* culture in many cases cause the formation of microshoots with defective physiological processes, morphological and anatomical structure. After transplantation from cultivation-vessels to *ex vitro* conditions such plants may be damaged because of altered cultivation conditions and therefore they require adaptation.

For adaptation *G. lutea* to *ex vitro* conditions the plants which formed roots in tap water and in the medium with twice decreased concentration of NH_4NO_3 and mannite were planted in soil (Fig. 2) To avoid drying of plants and their better adaptation to *ex vitro* conditions the flowerpots with planted specimens were covered by glass. Air expositions were done for 4–6 weeks, the duration and frequency of openings were gradually increased.

Percentage of adaptation for plants from various experimental variants somewhat differed (Fig. 3). Thus, on the 30th day the share of adaptation for plants which previously formed roots in water was higher compared to the plants cultivated in nutrient medium (XIV). However, the percentage of viable plants from both variants did not significantly differ hereafter and on the 150th day constituted 72.4% (rooted in tap water) and 70.8% (rooted in nutrient medium).

To estimate the condition of *G. lutea* individuals, we determined some morphometric parameters: height of plants (to clarify their adaptive capacity), number of pairs of leaves (leaves are the main organs of photosynthetic activity), and the number of internodes (to show adaptive changes of plants to *ex vitro* conditions, as internodes formation is the peculiarity of *G. lutea* plants cultivated *in vitro*).

It was shown that within the period from the 1st to 30th day the accretion of plants rooted

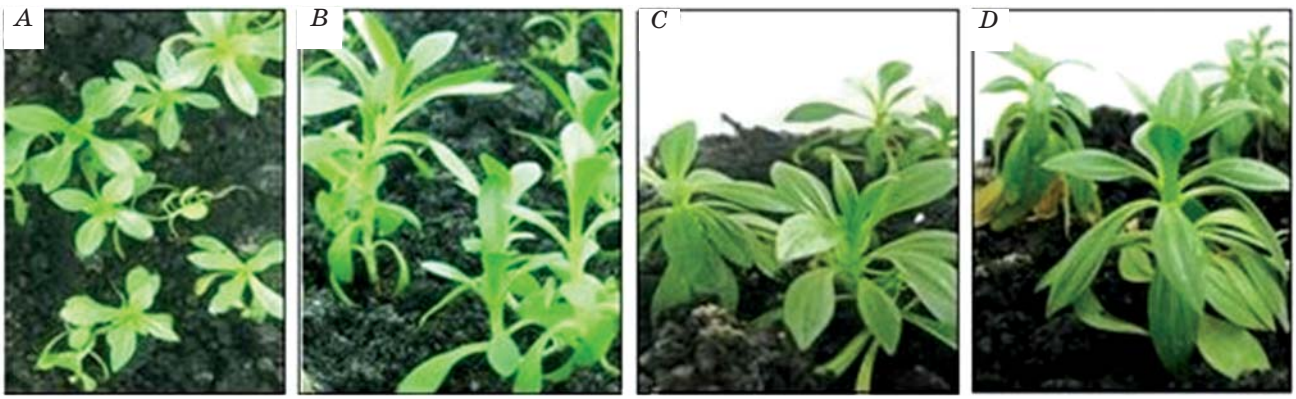

Fig. 2. ***G. lutea* planted in soil:**
vegetation duration 30 (*A*), 60 (*B*), 90 (*C*), 120 (*D*) days

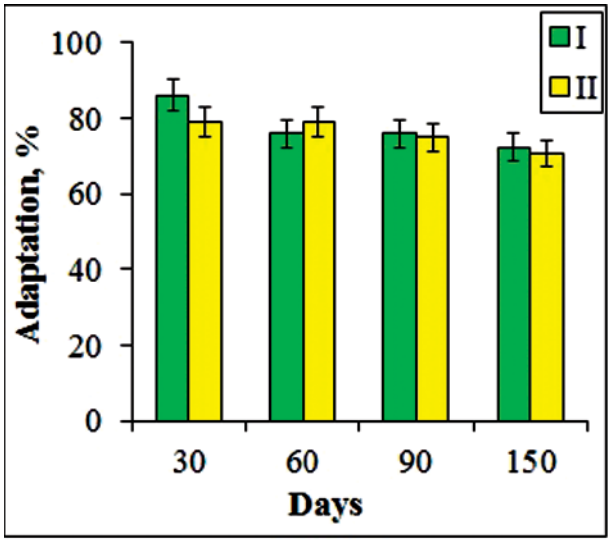

Fig. 3. **Adaptation in soil for plants rooted *in vitro*:**
I — percentage of adaptation for plants which formed roots in tap water;
II — percentage of adaptation for plants rooted in the nutrient medium MS/2 with twice decreased concentration of NH_4NO_3 without vitamins and sucrose, supplemented with 3 g/l mannite and 0.05 mg/l Kin, the maintaining substrate is agar (4 g/l) combined with perlite (16 g/l)

in the nutrient medium was 1.6 times higher compared with the plants which formed roots in water (Table 3). This phenomenon can be explained by the available reserve of nutrient substances stored in the organs of plants from the nutrient medium. From 30th to 90th day, the results were the opposite: accretion of the rooted in water plants was twice bigger that may testify to faster adaptation to *ex vitro* conditions. From 90th to 120th day the accretion of plant height was 1.3 times bigger for specimens rooted in the nutrient medium.

During growing in soil, the rooted in tap water plants formed on average 4–5 pairs of leaves and those rooted in the nutrient medium — 2–3 ones. That proves more intense photosynthetic activity, better viability and adaptation to *ex vitro* conditions. The number of internodes was the same in both variants. Besides, merome formation decreased in the process of cultivation and on 150th day it stopped (Table 3). Evidently, in *ex vitro* conditions *G. lutea* acquire morphological structure of intact plants, as the formation of radical rosette is representative for this species in nature [18].

Thus, the percentage of adaptation and accretion of plants rooted in tap water and in the nutrient medium did not practically differ. For rooting and further adaptation to *ex vitro* conditions, *G. lutea* can be planted in both water and medium MS/2 with twice decreased concentration of NH_4NO_3 without vitamins and sucrose, supplemented with 3 g/l mannite and 0.05 mg/l Kin. It is reasonable to use agar (4 g/l) combined with perlite (16 g/l) as a maintaining substrate.

Transplantation of adapted ex vitro plants into conditions in situ. At the beginning of June all viable and adapted to *ex vitro* conditions individuals were planted in natural conditions on Pozhyzhevska mountain (territory of the Carpathian National Natural Park) (Fig. 4). The area is located on the slope of northern-western exposition with steepness 20–40° 1450 m high above sea level.

According to literature data [19, 20], which were confirmed by our research, germination and taking to root of undergrowth for *Gentiana* species, including *G. lutea*, take place best in conditions of disturbed gramineous sodding. That is why the individuals were planted in places of bare soil to reduce the level of interspecies competition and provide optimal conditions for *G. lutea* adaptation and growth.

On the 3rd day of growth in natural conditions, we observed the loss of turgor in 15 ± 1.2% plants; on the 30th day the share of adapted plants constituted 97 ± 2.5%. The plants were viable, 2.5–7.5 cm high, with 6–16 pairs of leaves (Table 4). Evidently, their adaptation *in situ* was favoured by weather conditions of highland, as it rained almost every day in June–July, 2013. On the 60th day of growing the share of viable plants was 51 ± 1.8%. However, almost 40% of individuals died, having been eaten by small animals (rodents, lizards). Next year the share of viable plants equaled 21%.

General accretion of *G. lutea* height after 60 days of growing in natural conditions was insignificant (5.8 mm) that is probably caused not only by the complexity of adaptation, but biological peculiarities of the species too. Particularly, in the first years of ontogenesis *G. lutea* are known to grow slowly (2–5 cm/per year), forming 5–8 pairs of leaves [5]. The accretion of plants height from 30th to 60th day compared with the same value from 1st to 30th day was almost 3 times bigger that proves the successful adaptation. From 1st to 30th day the number of leaves was observed to increase, and from the 30th to 60th day — to decrease (Table 4). Probably the lack of rains in the first half of August resulted in drying out lower pairs of leaves.

Table 3. **Morphometric parameters of *G. lutea* planted in soil**

Morphometric parameters of plants rooted in tap water							
Parameters		at the time of planting	30th day	60th day	90th day	150th day	Total accretion
PH, mm	X± Sx	29±3.2	34±2.5	42±3.4	44±4.0	53±6.2	–
	Accretion	–	5±0.4*	8±0.6*	2±0.2*	9±0.6*	24±0.7
NPL, pieces	X± Sx	7.8±0.77	9.3±0.82	10.1±0.75	10.3±0.76	12.0±0.89	–
	Accretion	–	1.5±0.2*	0.8±0.1	0.2±0.1	1.7±0.2*	4.2±0.3
NI, pieces	X± Sx	3.1±0.35	4.2±0.33	4.3±0.33	4.5±0.43	4.5±0.37	–
	Accretion	–	1.1±0.1	0.1±0.04	0.2±0.05	0	1.4±0.2
Morphometric parameters of plants rooted in the nutrient medium[1]							
PH, mm	X± Sx	43±4.3	51±6.4	56±7.9	57±8.1	69±6.8	–
	Accretion	–	8±0.5	4±0.4	1±0.2	12±0.7	25±0.8
NPL, pieces	X± Sx	8.8±0.73	9.5±0.80	10.2±0.84	10.5±1.10	10.8±1.16	
	Accretion	–	0.7±0.1	0.7±0.1	0.3±0.05	0.3±0.05	2.0±0.2
NI, pieces	X± Sx	3.9±0.83	4.7±0.99	5.1±0.96	5.3±0.30	5.3±0.30	–
	Accretion	–	0.8±0.1	0.4±0.09	0.2±0.06	0	1.4±0.2

Notes: PH — plant height, NPL — number of pairs of leaves, NI — number of internodes; [1] — the medium MS/2 with twice decreased concentration of NH_4NO_3 without vitamins and sucrose, supplemented with 3 g/l mannite and 0.05 mg/l Kin, the maintaining substrate is agar (4 g/l) combined with perlite (16 g/l);
* — marked variants of the experiment in which the morphometric parameters of plants rooted in the nutrient medium MS/2 with twice decreased concentration of NH_4NO_3 without vitamins and sucrose, supplemented with 3 g/l mannite and 0.05 mg/l Kin reliable ($P \leq 0.05$) differ from the parameters of plants rooted in tap water.

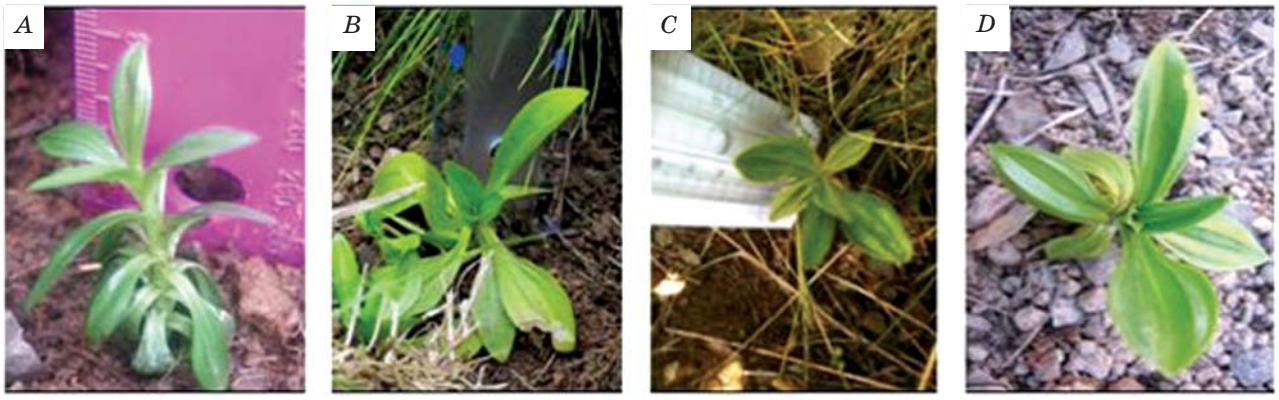

Fig. 4. ***G. lutea*** **planted in natural conditions:**
on 3rd (*A*), 30th (*B*), 60th (*C*) days of vegetation and a year after planting (*D*)

Within 60 days' period of *G. lutea* growing in natural conditions the length and width of lamina increased 7.8 and 2.9 times respectively. The formation of internodes stopped, and on the 60th day sporadic individuals had 1–2 internodes left, that proves that the plants acquired characteristic for the species habitus [18].

Thus, the conditions for rooting *G. lutea* shoots obtained by microclonal propagation have been chosen. It has been established that the biggest share of rooting shoots was provided by optimal combination of nutrient medium composition and supporting substrate. The medium MS/2 with twice decreased concentration of NH_4NO_3 without vitamins and sucrose, supplemented with 3 g/l mannite

Table 4. **Morphometric parameters of *G. lutea* planted *in situ* (Mt. Pozhyzhevska, 1450 m a.s.l.)**

Parameters		Time of measurement			Increase of morphometric parameters		
		1st day	30th day	60th day	1–30th day	30–60th day	60–90th day
Plant height, mm	X	41.4	42.9	47.2	1.5	4.3	5.8
	Sx	1.3	1.3	0.35			
	Sx′	1.84	1.63	1.70			
	Min	20.0	25.0	30.0			
	Max	70.0	75.0	75.0			
Number of internodes per plant, pieces	X	4.19	2.23	0.6	−2.67	−1.63	−4.30
	Sx	1.12	0.41	0.02			
	Sx′	1.47	0.58	0.13			
	Min	1.0	0	0			
	Max	10.0	4.0	1.0			
Number of pairs of leaves per plant, pieces	X	8.54	9.16	8.75	0.62	−0.41	0.21
	Sx	2.49	2.38	0.71			
	Sx′	2.69	2.64	2.97			
	Min	4.0	6.0	4.0			
	Max	13.0	16.0	14.0			
Length of lamina, mm	X	7.8	8.2	10.9	0.4	2.7	3.1
	Sx	0.08	0.1	0.13			
	Sx′	0.26	0.35	0.39			
	Min	5.0	5.0	7.0			
	Max	12.0	15.0	20.0			
Width of lamina, mm	X	3.6	4.7	6.8	1.1	2.1	3.2
	Sx	0.02	0.04	0.05			
	Sx′	0.09	0.1	0.17			
	Min	1.5	3.0	5.0			
	Max	4.5	7.0	10.0			

and 0.05 mg/l Kin and agar (4 g/l) combined with perlite (16 g/l) as a maintaining substrate proved to be the most efficient among all tested variants. Quite effective was also gradual diminishing of carbohydrates from 10 g/l to 2 g/l in the nutrient medium (MS/2 without vitamins and decreased concentration of NH_4NO_3) and further rooting of shoots in tap water.

There are chosen conditions for adaptation of rooted *in vitro* plants to conditions *ex vitro*. It has been found that plants rooted on both mentioned above variants can be used for this purpose. The obtained initial results of repatriation of *G. lutea* plants (21%) in natural habitats testify to efficiency of the suggested technology and rationality of its use for revival of disturbed gentian populations.

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АДАПТАЦІЯ ОДЕРЖАНИХ *in vitro* РОСЛИН *Gentiana lutea* L. ДО УМОВ *ex vitro* ТА *in situ*

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Метою роботи було розробити технологію перенесення одержаних шляхом мікроклонального розмноження рослин *Gentiana lutea* L. в умови *in situ*. Використовували методи культивування рослинних об'єктів *in vitro*. Підібрано оптимальні умови для вкорінення отриманих мікроклональним розмноженням пагонів *G. lutea in vitro:* живильне середовище МС/2 з половинним вмістом NH_4NO_3 без вітамінів та сахарози, доповнене 3 г/л маніту та 0,05 мг/л кінетину, з використанням як підтримувального субстрату агару (4 г/л) у поєднанні з перлітом (16 г/л) або поетапне зменшення в середовищі МС/2 без вітамінів та зі зменшеною концентрацією NH_4NO_3 концентрації вуглеводів з 10 г/л до 2 г/л із подальшим укоріненням цих пагонів у водопровідній воді. Укорінені рослини адаптовано до умов *ex vitro* шляхом висаджування їх у горщики з ґрунтом та поступовим переведенням тепличного режиму до відкритого. Частка адаптованих до умов *in situ* рослин — 21% через рік після висаджування — свідчить про перспективність розробленого способу культивування. Таким чином, запропоновано один з ефективних способів відновлення ушкоджених популяцій *G. lutea*, що включає репатріацію в них укорінених та адаптованих до умов *ex vitro* рослин, отриманих мікроклональним розмноженням *in vitro*.

Ключові слова: *Gentiana lutea* L., вкорінення *in vitro*, адаптація *ex vitro*, репатріація *in situ*.

АДАПТАЦИЯ ПОЛУЧЕННЫХ *in vitro* РАСТЕНИЙ *Gentiana lutea* L. К УСЛОВИЯМ *ex vitro* И *in situ*

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Цель работы — разработка технологии перенесения полученных путем микроклонального размножения растений *Gentiana lutea* L. в условия *in situ*. Использовали методы культивирования растительных объектов *in vitro*. Подобраны оптимальные условия укоренения полученных микроклональным размножением побегов *G. lutea in vitro*: питательная среда МС/2 с половинным содержанием NH_4NO_3 без витаминов и сахарозы, дополненная 3 г/л маннита и 0,05 мг/л кинетина, с использованием в качестве поддерживающего субстрата агара (4 г/л) в сочетании с перлитом (16 г/л) либо поэтапное уменьшение в среде МС/2 без витаминов и с уменьшенной концентрацией NH_4NO_3 концентрации углеводов с 10 г/л до 2 г/л с последующим укоренением этих побегов в водопроводной воде. Укоренившиеся растения адаптировали к условиям *ex vitro*, высаживая их в горшки с почвой, и постепенным переводом тепличного режима в открытый. Количество адаптированных к условиям *in situ* растений — 21% через год после высаживания свидетельствует о перспективности разработанного способа культивирования. Таким образом, предложен один из эффективных способов восстановления поврежденных популяций *G. lutea*, состоящий в репатриации в природные места произрастания укорененных и адаптированных к условиям *ex vitro* растений, полученных микроклональным размножением *in vitro*.

Ключевые слова: *Gentiana lutea* L., укоренение *in vitro*, адаптация *ex vitro*, репатриация *in situ*.