UDC 582.669.2:581.143.6 https://doi.org/10.15407/biotech14.03.039

REGENERATION CAPACITY OF NARROW-LOCALIZED ENDEMIC SPECIES *Dianthus hypanicus* Andrz. *in vitro*

L. A. Koldar
L. L. Dzhus
M. V. Nebykov

Sofiyivka National Dendrological Park
of the National Academy of Sciences of Ukraine, Uman

E-mail: lyudmiladzhus88@gmail.com

Received 21.04.2021
Revised 18.05.2021
Accepted 30.06.2021

Development of effective technology of rare and endangered plant species mass reproduction *in vitro* is one of the urgent nowadays tasks. *Diantus hypanicus* Andrz. of the *Caryophyllaceae* Juss. family belongs to this group of plants. It is an endemic, narrow-localized species listed in the Red Book of Ukraine, the European Red List and the Annex to the Berne Convention. Prospects for the conservation of this species are associated with the study of its viability and require the development of effective reproduction methods.

Aim. The purpose of the experiment was to determine the effect of different growth regulators concentrations on the *D. hypanicus* explants regenerative capacity during *in vitro* propagation.

Methods. For the experiment the seed were used collected from plants of natural habitats and sown on a hormone-free nutrient medium according to the recipe of Murashige and Skoog. For 12–15 days, 4–6 cm long seedlings were obtained which were transferred to nutrient media modified with the content of growth-regulating substances: BAP 0.5–2.0 mg/l, IBA 0.01 mg/l and IAA 0.1 mg/l.

Results. Initiation of adventitious buds with subsequent shoot formation was observed for 12–16 days. Each variant plants differed in number, growth activity and intensity, morphometric parameters. After 35–40 days from the explant introduction, conglomerates with well-developed leafy shoots were formed. It was found that, when modifying nutrient media with growth regulators BAP, IBA, IAA of different concentrations, *D. hypanicus* explants were actively undergoing regeneration processes of different intensity.

Conclusions. It was proved that high ability to regenerate was the characteristic of explants cultured on a nutrient medium modified by the addition of BAP — 0.5 mg/l and IAA — 0.1 mg/l, where, on average, 18 ± 0.24 shoots of 2.42 ± 0.17 cm long were formed with a net reproduction of 19.06 ± 0.14. Shoots with 3.0–5.0 pairs of leaves that reached 3.5–6.0 cm were selected and transferred to nutrient media for rooting. Less developed shoots were planted on nutrient media for further reproduction. The next stage of the study is aimed at modifying nutrient media to achieve explants rhizogenesis and obtain a large number of plants necessary for the preservation of this endemic narrow-localized species in culture with subsequent repatriation to natural places of growth.

Key words: caryophyllaceae; protection; conservation; *in vitro* culture; growth regulators; morphogenesis; net reproduction.

Among the environmental measures related to the protection and conservation of rare and endangered plant species, special attention is paid to their introduction with subsequent reintroduction into natural habitats, which allows to harmoniously combine the conservation of plant diversity *in situ* and *ex situ*. Creating collections of these species in scientific and botanical institutions makes it possible to have a source for botanical, genetic, environmental and other experimental studies, as well as significantly expand the assortment of useful plants, in particular decorative [1].

One of the urgent tasks in the field of biodiversity conservation is the protection of rare and endangered species of the family *Caryophyllaceae* Juss. Plants in need of protection and conservation include the endemic, narrow-localized species *Dianthus hypanicus* Andrz. listed in the Red Book of Ukraine, the European Red List and the Annex to the Berne Convention (Red Book of Ukraine,

2009). The species has a world, national and regional sozological status, is protected in the National Nature Park "Bug Gard" and in a number of landscape reserves of the Mykolaiv region [2–8].

D. hypanicus is a perennial herbaceous 10–25 cm tall plant. It is propagated by seed and vegetatively. In mature generative age, due to the development of a large number of shoots (sometimes up to 120 pcs) or their intensive branching, plant forms pillow-like turf. Flowering rosetteless shoots reach 7–30 cm long. Inflorescences-monochasia often reduced to a single flower. Corolla color is from pale to dark pink. The fruit is a single-celled multi-seeded cylindrical 1.1–1.8 cm long and 0.2–0.3 cm in diameter capsule. Mature black-colored seed are thyroid, finely wrinkled on the surface. Flowering begins in June and lasts until the end of September. It enters the fruiting phase in August–October [9].

In terms of economic and commercial importance, *D. hypanicus* belongs to the ornamental plants, so their expansion in botanical gardens and parks is the basis for the popularization of this species among the general population. Under culture, *D. hypanicus* acts as a winter-hardy and drought-resistant species that grows in open sunny places, creating large beautiful curtains and blooms profusely during June–September.

The reasons for the decrease in the number of this species are the low competitiveness of seedlings, the requirement for optimal moisture supply, direct destruction or transformation of typical natural habitats due to hydraulic engineering, granite mining; cattle grazing; burning of grass in the surrounding areas, digging turf by local residents to use *D. hypanicus* as an ornamental plant [10, 11].

In recent decades, biotechnology methods, in particular microclonal propagation based on the totipotency of a plant cell, i.e. the ability of a plant to vegetative regeneration from somatic cells, have been successfully used to solve the problem of preserving the gene pool of rare and endangered plant species. The use of *in vitro* propagation methods allows the rapid reproduction of rare and endangered species in botanical gardens and arboretums [12, 13]. Therefore, the prospects for the *D. hypanicus* conservation require the development of effective reproduction methods, in particular in the *in vitro* culture.

Materials and Methods

Experimental studies of regenerative processes phytohormonal regulation in *D. hypanicus* explants were performed in the laboratory of microclonal plant propagation of the Sofiyivka National Dendrological Park of the National Academy of Sciences of Ukraine. Seed collected from plants of natural habitats of the National Natural Park "Bug Gard" (Mykolaiv region, Voznesensky district, near the village of Aktove, Mertvovod river valley) were used for research. Sterilization of *D. hypanicus* seed was performed according to the scheme: preliminary seed washing using "Biomoy" solution, and the main — sterilization with 75% aqueous solution of the commercial drug "Bilyzna" with exposures of 1.0; 1.5 and 2.0 min. Seed germination was performed on hormone-free agar media according to Murashige and Skoog (MS) [14]. The highest percentage (80%) of sterile and viable *D. hypanicus* explants was obtained by treating the seed with 75% aqueous solution of the drug "Bilyzna" with an exposure of 1 min [15].

The regenerative ability of explants obtained from seed germination was studied when adding exogenous phytohormones of auxin and cytokinin groups of different concentrations to the media: 6-benzyl-aminopurine (BAP), β-indolyl-butyric acid (IBA), β-indolyl-acetic acid (IAA). The reaction of nutrient medium (pH) was 5.7. Hormone-free nutrient medium was used as a control (Table 1).

The net reproduction (NR) of micro shoots was calculated by the formula: P = a/bc, where "a" is the number of formed shoots, "b" is the number of planted shoots; "c" is the number of passages [16]. The study was performed three times, in each of which there were used

Table 1. **Phytohormonal composition of modified nutrient media**

Nutrient medium variant	Growth regulators, mg/l		
	BAP	IBA	IAA
I (control)	–	–	–
II	0.5	–	–
III	0.5	0.01	–
IV	0.5	–	0.1
V	1.0	0.01	–
VI	1.0	–	0.1
VII	2.0	0.01	–
VIII	2.0	–	0.1

by 35 explants. The average number of regenerated shoots was calculated from the total number of studied explants.

Preparation of nutrient media, tools and materials, sterilization and cultivation of plant material were performed using the methods and recommendations of F. L. Kalinin, R. G. Butenko, V. A. Kunah, T. M. Cherevchenko [17–20]. Plants cultivation took place at a temperature of 24 ± 1 °C, light intensity of 3 000 lux and a 16-hour photoperiod. In the explants cultivating process, the morphogenesis analysis and plants regenerative capacity were carried out visually.

Results and Discussion

One method, which is based on the ability of isolated plant parts under favorable cultivation conditions to restore lost organs and thus restore whole plants, is the adventitious buds' direct regeneration directly by explant tissues. It should be noted that the ability to differentiate, morphogenesis (totipotency) and the whole plant formation depends on its species, genotype, specific tissue, cell type, and so on. That is, different genotypes within a species and different cell types of the same plant have different ability to regenerate.

A necessary condition for explants successful regeneration and morphogenesis in the *in vitro* culture is a nutrient medium containing a balanced composition of micro- and macroelements, carbohydrates, vitamins, amino acids, in particular growth regulators. Changing the relationship between cytokinins and auxins, which are part of the nutrient media, is the cellular basis and a powerful inducer of morphogenesis in explants and contributes to its successful passage. Cell division, differentiation and primary programming occur within three to five days and the direction of their further differentiation is controlled by exogenous inducers [21–25]. The main role is played by cytokinins, which induce the adventitious buds' development *de novo* from explant tissues [19]. Therefore, the shoots obtained as a result of *D. hypanicus* seed germination were transferred to the nutrient media modified by us. During 12–16 days in the basal part of the explant, meristem tissues were activated with the subsequent adventitious buds' initiation, which acquired a green color and increased in size. This served as the direct morphogenesis beginning in which additional shoots began to grow from the formed buds, which in some cases differed in growth activity (Fig. 1).

Fig. 1. **The initial stage of *D. hypanicus* explant regeneration**

Depending on the growth regulators content in the nutrient media and their concentrations, in different variants the shoots formation differed in number, morphometric parameters, growth intensity and vegetative mass increasing. During the second — third passage (35–40 days after introduction), dense conglomerates with well-developed shoots and dense foliage were formed (Fig. 2).

Evaluation of regeneration efficiency was performed during the third passage. The morphogenic potential of the studied genotypes' explants depended on the phytohormones of different concentrations presence in the nutrient media. The most

Fig. 2. ***D. hypanicus*** **regeneration on the 30th day of cultivation *in vitro***

effective data were obtained in variant IV on a medium with a content of BAP — 0.5 mg/l and β-IAA — 0.1 mg/l, where, on average, 18.24 ± 0.11 shoots of 2.42 ± 0.17 cm long were formed with a NR of 19.06 ± 0.14 (Fig. 3, Table 2).

Slightly lower morphometric values were in variant III (BAP content of 0.5 mg/l and IBA content of 0.01 mg/l) and variant V with the BAP content of 1.0 mg/l and IBA content of 0.01 mg/l, where NR was respectively 13.11 ± 0.17 and 12.17 ± 0.19 with the formed shoots number of 12.33 ± 0.13 and 11.16 ± 0.14, length of 2.19 ± 0.08 and 1.82 ± 0.09 cm. The manifestation of determination induced by growth regulators showed the readiness of the *D. hypanicus* plant system to transition and the regenerative meristem formation capable of laying growing points and differentiating the shoot rudiments.

At the 6-BAP content in the nutrient medium of 0.5 mg/l without the content of auxins, the NR decreased significantly and amounted to 3.12 ± 0.11. In the control variant, without the addition of growth regulators (var. I), weak single shoots were formed, in which leaves yellowing, turgor loss and growth cessation occurred within 12–15 days. With 6-BAP concentrations increasing to 2.0 mg/l with the 0.01 mg/l of β-IBA addition (var. VII), or 0.1 mg/l of β-IAA (var. VIII), the regenerative capacity of explants decreased, and morphometric parameters were respectively: the number of formed shoots of 4.28 ± 0.09 and 3.22 ± 0.18, length of 1.91 ± 0.13 and 1.87 ± 0.17, and NR was respectively 4.13 ± 0.09 and 3.15 ± 0.13.

The resulting shoots were sorted by size and divided into separate groups. Shoots reaching 3.5–6.0 cm were transferred to nutrient media for rooting. Less developed ones were planted on nutrient media for further reproduction.

The obtained results analysis shows that the intensity of regeneration processes by adventitious shoots explants and the net

Table 2. ***D. hypanicus*** **explants morphometric parameters depending on growth on modified nutrient media**

Variant	Growth regulators, mg/l			Average figures		Net reproduction
	BAP	IBA	IAA	formed shoots number, pcs.	shoots length, cm	
I	–	–	–	1.12 ± 0.09	0.81 ± 0.14	1.14 ± 0.15
II	0.5	–	–	3.09 ± 0.08	1.63 ± 0.13	3.12 ± 0.11
III	0.5	0.01	–	12.33 ± 0.13	2.19 ± 0.08	13.11 ± 0.17
IV	0.5	–	0.1	18.24 ± 0.11	2,.42 ± 0.17	19.06 ± 0.14
V	1.0	0.01	–	11.16 ± 0.14	1.82 ± 0.09	12.17 ± 0.19
VI	1.0	–	0.1	7.21 ± 0.12	1.66 ± 0.19	9.09 ± 0.12
VII	2.0	0.01	–	4.28 ± 0.09	1.91 ± 0.13	4.13 ± 0.09
VIII	2.0	–	0.1	3.22 ± 0.18	1.87 ± 0.17	3.15 ± 0.13

Fig. 3. **35-day** ***D. hypanicus*** **explants regenerated from one micro shoot**

reproduction increase are caused by the action of growth regulators different concentrations. The manifestation of the determination induced in this case showed the readiness of the *D. hypanicus* plant system for a certain competence, i.e. the plant cells ability to perceive inducing factors.

After two or three passages, plant groups with 2–18 shoots were formed in different study variants, which testified to the high ability of *D. hypanicus* explants to regenerate. The passage duration was 20–28 days and depended on the growth regulators content and their concentrations in the nutrient media, the explant development nature, cultivation conditions, and the reproduction rate. From the explants obtained in this manner, well-developed shoots were selected that were suitable for transfer to a nutrient medium to achieve rhizogenesis with subsequent adaptation to *ex vitro* conditions.

Conclusions

1. The nutrient media optimal composition for morphogenesis induction and shoots regeneration by *D. hypanicus* explants cultured *in vitro* due to the action of growth regulators different concentrations was selected.

2. It was found that for the successful passage of regeneration processes, with subsequent shoot formation, the most favorable was agar nutrient medium modified by the 6-BAP addition of 0.5 mg/l and β-IAA of 0.1 mg/l, where, on average, 18.24 ± 0.11 shoots were formed of 2.42 ± 0.17 cm long with a net reproduction of 19.06 ± 0.14.

The research was carried out as a personal initiative without any funding for this research.

The authors declare that they have no conflicts of interest.

REFERENCES

1. *Gogitashvili E. V., Muchaidze M. N.* Morphogenesis of oriental carnation (*Dianthus orientalis adams*). *Sci. J. "Science Rise: Biological Science"*. 2018, 4 (13), 42–48. https://doi.org/10.15587/2519-8025.2018.141405
2. *Bakanova V. V.* Floral and decorative perennials in open ground. *Kyiv: Nauk. dumka*. 1983, 156 p. (In Ukrainian).
3. Red Book of the RSFSR: plants. *Moskva: Rosahropromyzdat*. 1988, 590 p. (In Russian).
4. Red Data Book of the USSR: Rare and Endangered Species of Animals and Plants. V. 2. Main ed. collegium: *A. M. Borodin, A. G. Bannikov, V. Ye. Sokolov*. Ed. 2nd, rev. and add. *Lesn. prom-st*. 1984, 480 p. (In Russian).
5. Red Book of Ukraine. Flora. *Ya. P. Didukh. Kyiv: Hlobalkonsaltynh*. 2009, 912 p. (In Ukrainian).
6. European Red List of Globally Endangered Animals and Plants. *New York: UN*. 1992, 167 p.
7. Global Strategy for Plant Conservation. *BGCI: Rishmond, U. K*. 2002, 16 p.
8. *Derkach O. M. Directory of the most valuable territories and objects. Kyiv*. 2008, P. 50–51. (In Ukrainian).
9. *Didukh Ya. P., Fedoronchuk M. M., Burda R. I. Dianthus hypanicus* Andrz. Carnation lilac. *Ecoflora of Ukraine. V. III. Kyiv: Fitosotsiotsentr*. 2002, P. 426–427. https://doi.org/10.5281/zenodo.1494339
10. *Barmak I. M.* The state of model populations *Dianthus hypanicus* Andrz. (*Caryophyllaceae*) in the Kirovograd region. *Ukr. botan. zhurn.* 2006, 63 (5), 694–698. http://dspace.nbuv.gov.ua/handle/123456789/3571
11. *Sobko V. H., Haponenko M. B., Hnatyuk A. M., Derkach O. V., Minina Yu. V., Reshetyuk O. V.* Repatriation of phyto rarities as an active means of restoring populations and improving the biological state of the environment. The role of botanic gardens in the green construction of cities, resorts and recreational areas. *Mater. Inter. Science. Conf., May 20–24, 2002. Odesa: LATSTAR*. 2002, Part II, P. 138–141. (In Russian).
12. *Pushkarova N. O., Kalista M. S., Belokurova V. B., Kuchuk M. V.* Obtaining plant culture of endangered species *Crambe steveniana in vitro* and studying the effect of aseptic cultivation conditions on their biochemical composition. *Visn. Karazin Kharkiv National University. Biology Series*. 2016, V. 27, P. 155–162. (In Russian).
13. *Novikova T. I., Nabiyeva A. Yu., Poluboyarova T. V.* Conservation of rare and useful plants in the *in vitro* collection of the Central Siberian Botanical Garden. *Vesn. VOHyS*. 2008, 12 (4), 564–572. (In Russian).
14. *Murashige T., Skoog F.* A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Phusiol. Plant*. 1962, 15 (13), 473–497.
15. *Goncharuk L. L., Koldar L. A.* Features of *Dianthus hypanicus* Andrz. introduction in the *in vitro* culture. *Mater. int. sci. conf. "Dendrology, floriculture and garden and park construction" dedicated to the 200th anniversary of the Nikitskii Botanical Garden, Yalta, June 5–8*. 2012, V. 2, P. 95. (In Russian).

16. *Romadanova N. V., Seradzh N. A., Nurmanov M. M., Karasholakova L. N.* Wild apple *Malus sieversii* introduction to the *in vitro* culture. *Izdenister, netyzheler. Issledovaniya, rezultaty*. 2017, 3 (75), 103–110. (In Russian).
17. *Kalinin F. L., Sarnatskaya V. V., Polishchuk V. Ye.* Tissue culture methods in plant physiology and biochemistry. *Kyiv: Nauk. dumka*. 1980, 487 p. (In Ukrainian).
18. *Butenko R. H.* Induction of morphogenesis in plant tissue culture. *Hormonal regulation of plant ontogenesis. Moskva.* 1984, P. 94–97. (In Russian).
19. *Kunakh V. A.* Biotechnology of medicinal plants. Genetic and physiological-biochemical bases. *Kyiv*. 2005, 730 p. (In Ukrainian).
20. *Cherevchenko T. M., Kushnyr H. P.* Orchids in culture. *Kyiv: Nauk. dumka.* 1986, P. 77–88. (In Ukrainian).
21. *Kutas E. N.* Clonal micropropagation in the conservation of plant biodiversity. *Mater. inter. sci. conf., dedicated to the 75th anniversary of the establishment of the Central Botanical Garden of the National Academy of Sciences of Belarus "Theoretical and applied aspects of plant introduction as a promising direction in the development of science and the national economy." Minsk*. 2007, V. 1, P. 77–80. (In Russian).
22. *Melnychuk M. D., Novak T. V., Kunakh V. A.* Plant biotechnology. *Kyiv: Polihraf Konsaltynh.* 2003, 520 p. (In Ukrainian).
23. *Serzhuk O. P., Nebykov M. V., Sydoruk T. M. Hedysarum grandiflorum* Pall. reproduction features in the culture *in vitro*. *Mater. inter. sci. conf. of young botanists "Actual problems of botany, ecology and biotechnology" Kyiv: Fitosotsiotsentr*. 2006, P. 203–204. (In Ukrainian).
24. *Nebikov M. V., Honcharuk L. L. Dianthus hypanicus* Andrz. morphogenesis *in vitro*. Biodiversity conservation and plant introduction: *Mater. inter. sci. conf. to the 210th anniversary of the botanical garden of the Kharkiv national. University named after V. N. Karazin (8–11 spring 2014, Kharkiv). Kharkiv: FOP Tarasenko V. P.* 2014, P. 129–133. (In Russian).
25. *Timofeyeva O. A., Rumyantseva N. I.* Plant cell and tissue culture. *Visn. Karazin Kharkiv National University. Biology Series.* 2012, 91 p. (In Ukrainian).

РЕГЕНЕРАЦІЙНА ЗДАТНІСТЬ ВУЗЬКОЛОКАЛЬНОГО ЕНДЕМІЧНОГО ВИДУ *Dianthus hypanicus* Andrz. *in vitro*

Л. А. Колдар, Л. Л. Джус, М. В. Небиков

Національний дендрологічний парк «Софіївка» НАН України, Умань

E-mail: lyudmiladzhus88@gmail.com

Розроблення ефективної технології масового розмноження *in vitro* рідкісних і зникаючих видів рослин є одним із актуальних завдань сьогодення. До цієї групи рослин належить *Diantus hypanicus* Andrz. з родини *Caryophyllaceae* Juss. — ендемічний, вузьколокальний вид, занесений до Червоної книги України, Європейського червоного списку та до Додатку Бернської конвенції. Перспективи збереження цього виду пов'язані з вивченням його життєздатності та потребують розроблення ефективних методів розмноження.

Мета експерименту — визначити дію різних концентрацій регуляторів росту на регенераційну здатність експлантів *D. hypanicus* за розмноження *in vitro*.

Методи. Досліджували регенераційну здатність експлантів *D. hypanicus*, культивованих *in vitro*. Для проведення експерименту використовували насіння, зібране з рослин

РЕГЕНЕРАЦИОННАЯ СПОСОБНОСТЬ УЗКОЛОКАЛЬНОГО ЭНДЕМИЧЕСКОГО ВИДА *Dianthus hypanicus* Andrz. *in vitro*

Л. А. Колдар, Л. Л. Джус, М. В. Небыков

Национальный дендрологический парк «Софиевка» НАН Украины, Умань

E-mail: lyudmiladzhus88@gmail.com

Разработка эффективной технологии массового размножения *in vitro* редких и исчезающих видов растений является сегодня одной из актуальных задач. К этой группе растений относится вид *Diantus. hypanicus* Andrz. семейства *Caryophyllaceae Juss.* — эндемический, узколокальный вид, занесенный в Красную книгу Украины, Европейский красный список и в Приложение Бернской конвенции. Перспективы сохранения данного вида связаны с изучением его жизнеспособности и требуют разработки эффективных методов размножения.

Цель. Целью эксперимента является определение действия разных концентраций регуляторов роста на регенерационную способность эксплантов *D. hypanicus* при размножениии *in vitro*.

Методы. Исследовали регенерационную способность эксплантов *D. hypanicus*, культивируемых *in vitro*. Для проведения эксперимента

природних місцезростань, яке висівали на безгормональне живильне середовище за прописом Мурасіге–Скуга. Впродовж 12–15 діб одержували проростки завдовжки 4–6 см, які переносили на живильні середовища, модифіковані вмістом речовин, що регулюють ріст: БАП 0,5–2,0 мг/л, ІМК 0,01 мг/л та ІОК 0,1 мг/л.

Результати. Протягом 12–16 діб спостерігали ініціювання адвентивних бруньок з подальшим формуванням пагонів. Рослини кожного з варіантів відрізнялися кількістю, активністю та інтенсивністю росту, морфометричними показниками. Через 35–40 діб від уведення з одного експланта формувалися конгломерати з добре розвиненими облиственими пагонами. З'ясовано, що за модифікування живильних середовищ регуляторами росту БАП, ІМК, ІОК різних концентрацій у експлантів *D. hypanicus* активно відбувалися процеси регенерації різної інтенсивності.

Висновки. Доведено, що високою здатністю до регенерації характеризувалися експланти, культивовані на живильному середовищі, модифікованому додаванням БАП — 0,5 мг/л та ІОК — 0,1 мг/л, де в середньому було утворено 18±0,24 шт. пагонів завдовжки 2,42±0,17 см з коефіцієнтом розмноження 19,06±0,14. Пагони, які досягали 3,5–6,0 см з 3,0–5,0 парами листя, відбирали та переносили на живильні середовища для укорінення. Менш розвинені пасажували на живильні середовища для подальшого розмноження. Наступний етап дослідження спрямовано на модифікування живильних середовищ для досягнення експлантами ризогенезу та одержання великої кількості рослин, необхідних для збереження цього ендемічного вузьколокального виду в культурі з подальшою репатріацією в природні місця зростання.

Ключові слова: *Caryophyllaceae*; охорона; збереження; культура *in vitro*; регулятори росту; морфогенез; коефіцієнт розмноження.

использовали семена, которые высевали на безгормональную питательную среду по прописи Мурасиге–Скуга. В течение 12–15 дней получали проростки длиной 4–6 см, которые переносили на питательные среды, модифицированные содержанием регулирующих рост веществ: БАП 0,5–2,0 мг/л, ІМК 0,01 мг/л и ІОК 0,1 мг/л.

Результаты. В течение 12–16 дней наблюдали инициацию адвентивных почек с дальнейшим формированием побегов. Растения каждого из вариантов отличались количеством, активностью и интенсивностью роста, морфометрическими показателями. Через 35–40 дней после введения из одного экспланта формировались конгломераты с хорошо развитыми побегами. Установлено, что при модификации питательных сред добавлением регуляторов роста БАП, ІМК, ІОК разных концентраций у эксплантов *D. hypanicus* активно происходили процессы регенерации разной интенсивности.

Выводы. Доказано, что высокой способностью к регенерации характеризовались экспланты, культивированные на питательной среде, модифицированной добавлением БАП — 0,5 мг/л и ІОК — 0,1 мг/л, где в среднем из одного экспланта было получено 18±0,24 побегов длиной 2,42±0,17 см с коэффициентом размножения 19,06±0,14. Побеги, которые достигали 3,5–6,0 см с 3,0–5,0 парами листьев, отбирали и переносили на питательные среды для укоренения. Менее развитые пассажировали на питательные среды для дальнейшего размножения. Следующий этап исследований направлен на модификацию питательных сред для достижения эксплантами ризогенеза и получения большого количества растений, необходимых для сохранения данного эндемического узколокального вида в культуре с дальнейшей репатриацией в природные места произрастания.

Ключевые слова: *Caryophyllaceae*; охрана; сохранение; культура *in vitro*; регуляторы роста; морфогенез; коэффициент размножения.