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Conservation of representatives of the *Drosera* L. genus using biotechnological methods

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Abstract. The paper examines the features of obtaining planting material of rare representatives of *Drosera spatulate* L. and *Drosera aliciae* L. using microclonal propagation for their preservation and subsequent cultivation in ex vitro conditions. The method of sterilisation of *D. spatulate* and *D. aliciae* explants was developed with 80-90% production of aseptic material. The influence of various sterilisation options on the development of microshoots was investigated. The two best solutions for sterilisation are: 0.1% $AgNO_3$ solution and 12.5% H_2O_2 solution. The features of organogenesis and regeneration of the whole organism from cultured tissues and organs of representatives of *Drosera* L. are investigated. The optimal nutrient medium was selected at the stage of introduction *D. spatulate* and *D. aliciae* into the in vitro culture (MS with added PVP 2 mg*l^{-1}). Optimal conditions for direct morphogenesis of *Drosera spatulate* L. and *Drosera aliciae* L. tissues were determined, which allowed mass production of improved, genetically homogeneous planting material. The effect of exogenous growth regulators at various stages of plant morphogenesis under in vitro conditions is shown. In vitro rhizogenesis conditions were improved. In particular, according to the results of the study, the highest coefficient of reproduction (1:8) and root system formation was obtained on a hormone-free medium with a halved content of macronutrients and a nutrient medium with the addition of 0.5 mg/l^{-1} IBA. Under such conditions, active morphogenesis and the formation of a developed root system were observed in all propagated plants. Based on the results of the conducted research, a method of microclonal reproduction was developed, which allowed for obtaining genetically stable, disease-free regenerative *D. spatulate* and *D. aliciae* plants with an optimally formed root system and vegetative mass. The resulting homogeneous planting material can be used in floriculture, creating terrariums, for pharmacological purposes, and for the purpose of introduction

Keywords: *Drosera* L., microclonal reproduction, morphogenesis

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Introduction

In Ukraine, due to the land reclamation works conducted in the last century, the territories of wetlands and swamps have substantially decreased. In addition, climate change also causes swamps to dry up, causing swamp plants to suffer. On the territory of wetland complexes, the area of localities of red book plant species has substantially decreased (Andrienko & Protopopova, 2010; Umanets & Moisienko, 2012; Chornobrov et al., 2019).

Biodiversity conservation is a major challenge in the face of rapid environmental changes. The problem of preserving extremely vulnerable stenotopic flora species is particularly acute. Exactly the species of the *Drosera* genus are indicators of climate change and are sensitive to fluctuations in the water level, etc. All members of the *Drosera* genus are endangered species, except for species of the *Drosera rotundifolia* genus, however, according to researchers (Rak & Kachula, 2017), they are listed in the new edition of the Red book of Ukraine in the category "vulnerable".

On the territory of Ukraine, there are three types of sundew: round-leaved (*Drosera rotundifolia* L.), English (*Drosera anglica* Huds.) and oblong-leaved sundew (*Drosera intermedia* Hayne) (Fig. 1).

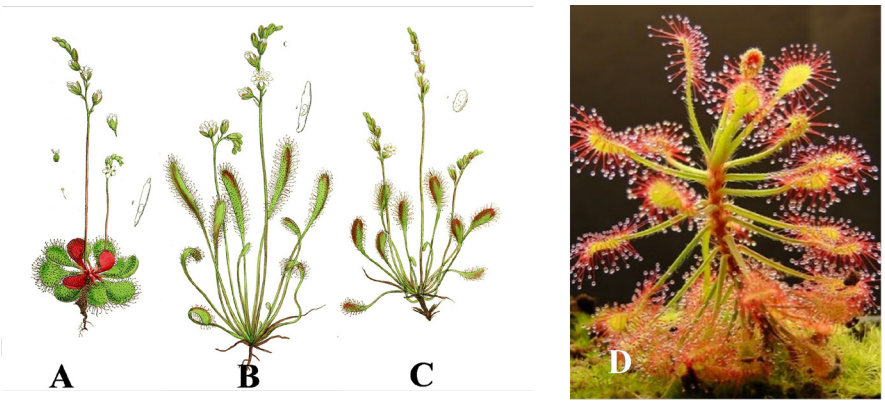

Figure 1. Representatives of *Drosera* L.: *a – Drosera rotundifolia* L.; b – *Drosera anglica* Huds.; c – *Drosera intermedia* Naupe; d – sundew appearence

D. rotundifolia is distributed in the arctic and temperate zones of Eurasia and North America (GBIF Secretariat, 2014). In Europe, it occurs almost all over the territory, but very unevenly. It is quite abundant in North and Atlantic Europe, occasionally in the middle and very rarely in the South (except for the South-East) (Melzig & Krenn, 2011).

In Ukraine quite recently *D. rotundifolia* was quite common in the forest zone and in the north of the forest-steppe, occasionally in the south of the forest-steppe and in the Carpathians, and very rarely in the north of the left-bank steppe (Andrienko, 2009; Chorna, 2006; Kiraly, 2009). However, according to recent observations, today there are a large number of locations of *D. rotundifolia* lost due to anthropogenic impact and drainage of swamps (Andrienko, 2009; Umanets, 2012).

The use of biotechnological methods for obtaining plant raw materials of *Drosera* L. is extremely relevant in both landscaping and pharmacology (Egan & map der Kooy, 2013), as they provide healthy, sterile plant material in large quantities, regardless of the season.

The optimal area for developing conservation strategies for endangered species is an integrated scientific approach that combines the use of biotechnological techniques for *in vitro* culture, rational collection management for efficient recovery of endangered populations and species (Chornobrov & Bilous, 2021), the use of molecular genetic techniques to document and maintain collections (Chung et al., 2013), assessment of the parameters of genetic diversity of natural and artificially created populations, replenishment of collections, and their exchange.

Using the *in vitro* culture method is an optimal modern solution that provides an opportunity of:

- restoration and reproduction of valuable, rare, and endangered species of flowering plants that are difficult to reproduce *in situ* and *ex situ*;
- creating a collection of genetically homogeneous plants *in vitro* culture;
- getting healthy planting material;
- mass production of valuable plant genotypes from the collections of botanical gardens.

The originality of this area is the creation of a genetic bank of samples of plant raw materials, which is an indispensable resource for characterising the conservation and sustainable use of biodiversity of the plant world, identifying the most productive genotypes of economic crops and crops with the increased adaptive potential to external environmental factors, quantifying the parameters of genetic diversity of natural and artificially created crops *ex situ* populations, and collections of individual taxa (Matthews, 1994; Chorna, 2006).

The purpose of the study – determination of the features of direct plant regeneration of *Drosera spatulate* L. and *Drosera aliciae* L. at different stages of microclonal reproduction.

Until now, such representatives of species of the *Drosera* genus were successfully cultivated *in vitro*: *D. binata* La Billardier, *D. natalensis* Diels (Anthony, 1992; Caniato et al., 1989), *D. capensis* L. (Curkovic & Berljak, 1996), *Drosera-regia* Stephens (Janssens, 1986), *D. rotundifolia* L. (Anthony, 1992; Bonnet et al., 1984; Kukulczanka & Czastka, 1987; Simola, 1978) and *D. spatulata* Labill. (Blehova et al., 1990, 1992). However, protocols for microclonal reproduction and composition of nutrient media (NM) have not yet been developed for *D. spatulata* (sundew), in particular, in Ukraine. Because of this, the most common method of propagation currently is the seed one (Fernández-Pascual, 2016), due to the ease of sterilisation to create primary crops.

Given the substantial demand for carnivorous plants (Baskin & Baskin, 2014) and the lack of developed methods of microclonal reproduction, the conservation and reproduction of rare and endangered species of this group of plants *ex situ*, as the creation of a genetic bank *in vitro* is becoming increasingly relevant.

Materials and Methods

The original explants were parts of the generative complex that are formed from buds restored in a single visible growth cycle, which contain a stem, leaves, buds, and a flower or inflorescence.

Methods and approaches generally accepted in biotechnology were followed, modifying them individually for each genotype to decontaminate the original explants (Chornobrov & Bilous, 2021).

Cultivation of meristem sites was conducted on modified Murashige and Skoog media (MS) (Murashige & Skoog, 1962) by adding phytohormones of various concentrations to their composition (Araújo et al., 2014; Baranyai & Joosten, 2016; Clapa et al., 2010).

In addition, activated carbon (1 $g^{*}l^{-1}$) was added to the medium (once), as a hydrocarbon food – sucrose (30 $g^{*}l^{-1}$), mesoinosite (100 $mg^{*}l^{-1}$), agar (0.7%), and two chelate forms of iron: ethylenediamine-di-2-hydroxy-phenylacetic acid (Fe-EDDHA) and ethylenediaminetetraacetic acid (Fe-EDTA), medium pH 5.6-5.7.

Explants were cultured at a temperature of 25±2°C, with a 16-hour photoperiod, for illumination with an intensity of 2000-3000 lx.

The main indicator of the effectiveness of the sterilising agent was the number of explants that developed normally.

Representatives of *Drosera spatulate* L. and *Drosera aliciae* L. were used as donor plants in the study, taken from container culture.

Results and Discussion

At the initial stage of introduction to *in vitro* culture Murashige and Skoog (MS) nutrient media with full or half-reduced mineral element content were used for regeneration.

During experimental work, a sterilising solution was selected, which was easily washed out of the tissues with distilled water to less harm the explant tissues. For this purpose, generally accepted methods were used to obtain sterile starting material. A solution of 70% ethyl alcohol (C_2H_5OH), 1.7 per cent and 1.25 per cent sodium hypochlorite NaClO solutions, hydrogen peroxide solutions (H_2O_2) of various concentrations – 12.5% and 25%, and 0.1% silver nitrate solution ($AgNO_3$). The reagents were used both separately and in combination with different exposures and washing modes to achieve this goal.

Each of the sterilising solutions and exposure time had a different effect on *Drosera* L. explants. Method of sterilisation using a 70% ethanol solution (30 seconds), then holding in a 1.25% NaClO solution with an exposure of 20 minutes and three times washing in sterile water did not damage tissues and did not inhibit plant development and ensured the sterility of explants by 60%. Notably, the sequence and exposure of the use of various sterilising reagents were determined empirically during operation, depending on the type of explant and the degree of its infection.

Sterilisation mode using 70% ethanol and holding for 30 seconds and in 0.1% $AgNO_3$ solution for 5-7-10 minutes and three times washing in sterile water for 10 minutes each time affected the plants in different ways. Sterilisation with a 10-minute exposure resulted in a complete loss of plant material, resulting in all plants being sterile but not viable.

In turn, the exposure is 7 minutes in 0.1% $AgNO_3$ solution allowed getting 50% of viable explants, and the exposure of 5 minutes was the most optimal. Under such conditions, the number of aseptic and regenerative plants was 80%.

In parallel with the two previous schemes, a 50% solution of hydrogen peroxide (H_2O_2) in two ratio options, 1:1 and 1:2, with different exposure times, was used to determine the most optimal method of sterilisation.

When using a 50% hydrogen peroxide solution in a ratio of 1:2, the yield of aseptic explants on the third day after administration was 80-90%. Therewith, washing the explants from the remains of the sterilising solution one time is enough. This method is the least toxic and effective for non-woody shoots.

Induction of organogenesis from *Drosera* L. meristem culture was conducted by culturing the obtained primary microshoots from aseptic explants obtained after their preliminary sterilisation.

On the sixth day of cultivation, induction of axillary buds was observed, which on day 10-14 had a size of 0.2-0.3 cm.

The yield of aseptic viable explants was 90-100%. After 2-3 days, active release of phenolic compounds was observed, which negatively affected the growth and development of explants. Polyvinylpyrrolidone (PVP) (1-2 $g*l^{-1}$) was added to the MS nutrient medium to avoid this, the adsorption properties of which contribute to the uniform distribution of nutrients in the medium and reduce the negative impact of secondary metabolic products.

The shoots were divided into segments and transferred to nutrient media containing different compositions and concentrations of phytohormones to induce morphogenesis (Table 1).

Table 1. Composition of components of the nutrient medium for induction of organogenesis

No.	NM components	Concentration by medium variants, $mg*l^{-1}$				
		1	2	3	4	5
1	Macronutrients of MS	100	100	30	100	100
2	Microelements of MS	1.0	1.0	1.0	1.0	1.0
3	vitamins of MS	1.0	1.0	1.0	1.0	1.0
4	Fe chelate	10	5	5	10	5
5	BAP	1.0	-	-	1.0	-
6	Glucocorticoids	0.1	-	-	-	-
7	Glycine	-	-	-	1.0	-
8	Kinetin	-	0.25	-	-	0.25
9	IAA	0.5	-	-	-	-
10	Mesoinosite, $g*l^{-1}$	0.1	0.1	0.1	0.1	0.1
11	Sucrose, $g*l^{-1}$	30	20	20	30	20
12	Agar, $g*l^{-1}$	6.7	6.7	6.7	6.7	6.7
13	PVP, $g*l^{-1}$	1	-	-	2	2
14	IBA	-	-	0.1	0.2	-
pH 5.6-5.7						

Induction of morphogenesis largely depended on the composition of NM and at the initial stages of reproduction took place mainly on basic MS media. Cultivation was conducted for 21 days (Fig. 2).

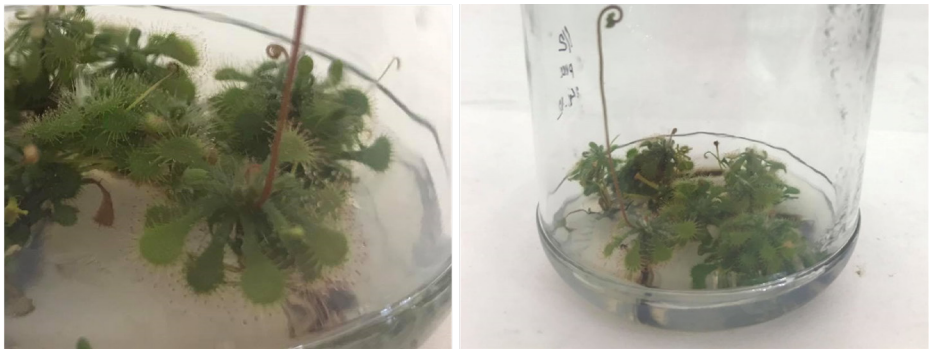

Figure 2. Regenerative plants *D. spatulate* on morphogenic medium on day 21 of cultivation

Analysing the data, it was concluded that morphogenesis of *D. spatulate* and *D. aliciae* most intensively passed on a medium with the addition of kinetin 0.25 mg*l^{-1} and PVP 2 g*l^{-1} explants had dimensions of 2.5-2.8 cm and typically rich green colour, formed roots of 2-3 pcs. on an explant, 3.5-4.9 cm long (Fig. 3). In turn, the release of phenols in the basal part and near the roots was observed on a medium of ½ MS without the addition of PVP. The accounting results are shown in Table 2.

Table 2. Features of explant development on different media variants (day 21 of cultivation)

No.	Composition of NM (PGR)	Hormone concentration, mg*l^{-1}	Number of explants, pcs.	Formation of shoots		Formation of roots	
				pcs.	cm	pcs.	cm
1	BAP Glucocorticoids IAA	1.0 0.1 0.5	2	2.7±0.9	0.5±1.3	-	-
2	Kinetin	0.25	2	3.3±0.56	2.3±1.1	2.5±4.3	1.6±3.2
3	IBA	0.1	2	2.8±3.6	0.62±0.8	3.8±3.1	1.3±2.4
4	IBA BAP	0.2 1.0	2	3.4±3.3	0.26±0.5	-	-
5	Kinetin AA	0.25 5.0	2	4.9±1.5	2.6±1.5	2.5±1.5	2.8±1.2

Note: AA– ascorbic acid; PGR – plant growth regulators: NM – nutrient medium

Figure 3. Regenerative plants *D. spatulate* and *D. aliciae* on morphogenic medium on day 21 of cultivation

When added to the medium BAP 1.0 mg*l^{-1}+ GC 0.5 mg*l^{-1} the attenuation of explants was determined(0.4-0.6 cm), which were characterised by the formation of weakened, deformed leaves that had a light colour. On a medium with the addition of 0.1 mg*l^{-1} IBA explants were also thin, weak – 0.4-0.7 cm, but simultaneously retained the ability to form roots (on one explant – up to 3-5 pcs.).

On NM supplemented with 0.2 mg*l^{-1} IBA + 1.0 mg*l^{-1} BAP did not observe a substantial difference in the size of explants, and the roots were formed much smaller in size. The most optimal environment was the use of 0.25 mg*l^{-1} kinetin and the addition of PVP 2 g*l^{-1}, and hormone-free NM MS with a halved composition of macronutrients. Active growth and induction of rhizogenesis were observed in this medium.

On 21-28 days of cultivation, formed regenerative plants were obtained, the total size of which reached 8 cm with a well-formed root system. The resulting regenerative plants were used for further mass multiplication *in vitro*.

After the successful production of sterile viable regenerative plants *D. spatulate* and *D. aliciae*, the components of the nutrient medium for induction of rhizogenesis *in vitro* of *Drosera* L. were selected. For this purpose, sterile plants were divided into separate microparticles and planted on NM, different in composition: MS+IAA (0.5 mg*l^{-1}); MS+IBA 0.5 mg*l^{-1}+ IAA 0.5 mg*l^{-1}; MS+IBA 4 mg*l^{-1}+ 0.5 mg*l^{-1} BAP; h/f MS and ½ MS (Table 3).

Table 3. Influence of plant growth regulators on morphogenesis of *D. spatulate* and *D. aliciae*

Medium	Viable microshoots		Coefficient reproduction	Presence of a root system
	pcs.	%		
Hormone-free MS	10	100	9	available
½ MS	10	100	8	available
MS + BAP 0.5 mg*l^{-1}	2	20	8	absent
MS + BAP 1.0 mg*l^{-1}	3	30	3	absent
MS + BAP 1.5 mg*l^{-1}	1	10	4	absent
MS + kinetin 1 mg*l^{-1}	3	25	4	absent
MS + kinetin 2 mg*l^{-1}	3	30	3	absent

Table 3. Continued

Medium	Viable microshoots		Coefficient reproduction	Presence of a root system
	pcs.	%		
MS + kinetin 3 mg*l^{-1}	8	80	4	absent
MS + IBA + 0.5 mg*l^{-1}	10	100	5	absent

As can be seen from the table, the addition of low concentrations of cytokinins BAP and kinetin to NM had a positive effect on the growth of regenerative plants and contributed to the formation of the root system in 50% of plants. The addition of auxin IBA at a concentration of 0.5 mg*l^{-1} also had a positive effect on the induction of explant tissue rhizogenesis. Within two weeks, explants formed a developed root system and doubled their growth. As a result of research, the highest coefficient of reproduction (1:8) and root system formation was obtained on a hormone-free medium with a halved content of macronutrients and on NM with the addition of 0.5 mg*l^{-1} IBA. All plants showed active morphogenesis and formed roots (Fig. 4).

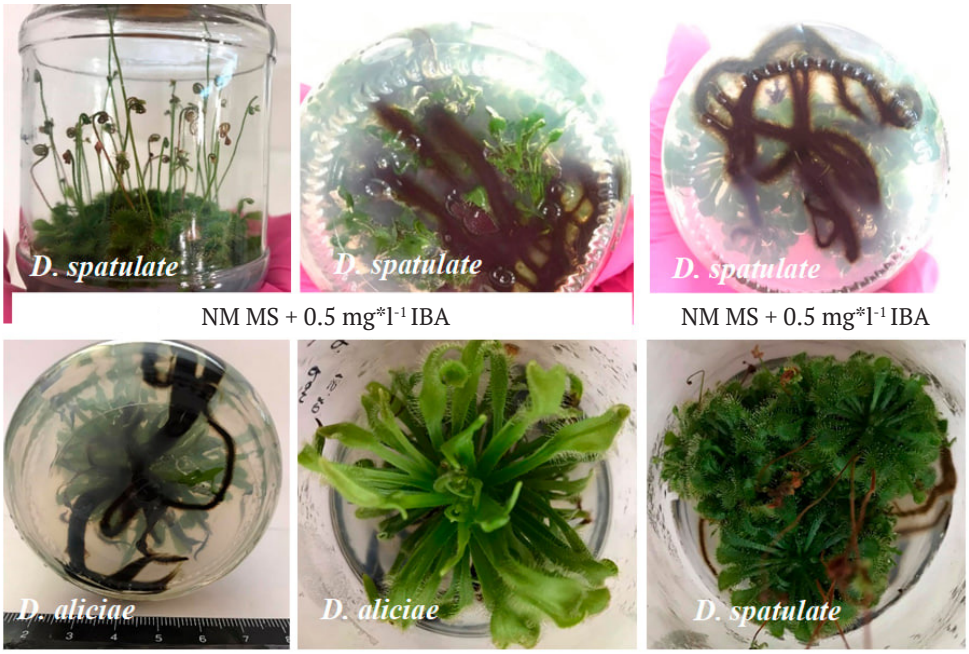

Figure 4. Formation of a developed root system and induction of microshoot growth on NM ½ MS

Summarising the results obtained, the most effective can be considered NM ½ MS, which can be used both for plant cultivation and root formation, with the only exception being forced induction. If rapid root formation to transfer plants to the soil is necessary, it is best to use NM with the addition of 0.5 mg*l^{-1} IBA.

Conclusions

Based on the results of the conducted research, a method of microclonal reproduction was developed, which allowed for obtaining genetically stable, disease-free regenerative plants *D. spatulate* and *D. aliciae* with an optimally formed root system and vegetative mass. The resulting

homogeneous planting material can be used in floriculture, creating terrariums, for pharmacological purposes, and for the purpose of introduction. The method of explant sterilisation of *D. spatulate* and *D. aliciae* with 80-90% production of the aseptic culture of plant tissues was developed. The influence of various sterilisation options on the development of microshoots was investigated. It was identified that sterilisation using a 0.1% $AgNO_3$ (5 min) solution is the most effective and three times washing in dH_2O, and sterilisation with explants kept for 30 seconds in a solution of 70% ethanol and 12.5% solution H_2O_2 with a single wash 10 min in dH_2O. It was experimentally established that NM MS with the addition of 2 $g*l^{-1}$ PVP is optimal at the stage of introduction of *D. spatulate* and *D. aliciae* in *vitro*. Microshoot regeneration of *D. spatulate* and *D. aliciae* were investigated depending on the type of explant and the composition of the NM. Morphogenesis was most effective on NM with the addition of 0.25 $mg*l^{-1}$ kinetin and on h/f MS. Such cultivation conditions provided 100% regeneration of regenerative plants with a reproduction rate of 1:8. After investigating the effect of cytokinins on microclonal reproduction of *D. spatulate* and *D. aliciae,* it was identified that the development and induction of multiple shoot formation *in vitro* works best in a h/f MS environment. It is necessary to add NM 0.5 $mg*l^{-1}$ IBA to induce the formation of the root system.

References

[1] Andrienko, T., & Protopopova, V. (2010). *Insectivorous plants of Ukraine*. Kyiv: Alterpress [in Ukrainian].

[2] Anthony, J. L. (1992). In vitro propagation of Drosera spp. *HortScience,* 27, 850.

[3] Araújo, P. V., Porto, M., Silva, A., Almeida, J. D., Lourenço, J., Schwarzer, U., Silveira, P., & Peixoto, M. (2014). *Drosera rotundifolia* L. – mapa de distribuição (Drosera rotundifolia L. – distribution map). Flora-On: Flora de Portugal Interactiva, Sociedade Portuguesa de Botânica. Available at http://www.flora-on.pt/wDrosera.

[4] Baranyai, B., & Joosten, H. (2016). Biology, ecology, use, conservation and cultivation of round-leaved sundew (Drosera rotundifolia L.): a review. *Mires and Peat*, *18*, 1–28. Available at http://www.mires-and-peat.net/.

[5] Baskin, C. C., & Baskin, J. M. (2014). *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, London.

[6] Blehova, A., Erdelsky, K., & Bobak, M. Cultivation of organ and callus culture of Drosera spatulata Labill. in in vitro conditions. Acta Facultatis Rerum Naturalium Universitas Comenianae *Physiol. Plant*, *28*, 93–102.

[7] Chorna, H. (2006). *Flora of reservoirs and swamps of the Forest-Steppe of Ukraine. Vascular plants*. Kyiv: Phytosocial center [in Ukrainian].

[8] Chornobrov, O. Yu., & Bilous, S. Yu. (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. https://doi.org/10.2478/ffp-2021-0007

[9] Chornobrov, O., Bilous, S., Chornobrov, O., & Manko, M. (2019). Peculiarities of morphogenesis of the endangered species of willow (Salix spp.) in vitro. *BIOLOGIJA*, *65* (1), 48–55.

[10] Chung, M. Y., Lopez-Pujol, J. & Chung, M. G. (2013). Population history of the two carnivorous plants Drosera peltata var. nipponica and Drosera rotundifolia (Droseraceae) in Korea. *American Journal of Botany, 100* (11), 2231–2239. http://dx.doi.org/10.3732

[11] Clapa, D., Alexandru, F. & Pacurar, I. (2010). In vitro multiplication, conservation and ex vitro acclimation of Drosera rotundifolia. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, UASVM Horticulture, 66* (1)/2009, 34–39.

[12] Curkovic Perica, M., & Berljak, J. (1996). In Vitro Growth and Regeneration of Drosera spatulata Labill. on Various Media. *HortScience*, *31* (6). https://doi.org/10.21273/HORTSCI.31.6.1033

[13] Egan, P. A., & Van der Kooy, F. (2013). Phytochemistry of the carnivorous sundew genus *Drosera* (*Droseraceae*) – Future perspectives and ethnopharmacological relevance. *Chemistry & Biodiversity, 10* (10), 1774–1790. http://dx.doi.org/10.1002

[14] Fernández-Pascual, E. (2016). Comparative seed germination traits in bog and fen mire wetlands. *Aquatic Botany, 130*, 21–26. http://dx.doi.org/10.1016/j.aquabot.2016.01.001

[15] GBIF Secretariat. (2014). *Drosera rotundifolia* L. species in GBIF backbone taxonomy. Available at http://www.gbif.org/species/3191030.

[16] Janssens, J. (1986). In vitro propagation of Sundew Drosera-regia Stephens. Meded Fac Landbouwwet. *Rijksuniv. Gent, 51*, 61–66.

[17] Konvaliuk, I., Kravets, N., & Drobik, N. (2010). Direct organogenesis of in vitro yellow gentian (Gentiana Lutea L.). *Journal of Biotechnology*, *3* (5), 66–73.
[18] Kiraly, G. (Ed.). (2009). *Új magyar füvészkönyv. Magyarország hajtásos növényei*. Határozókulcsok. – Jósvafı: ANP Igazgatóság.
[19] Kukulczanka, K. & Czastka, B. (1987). Propagation of Drosera L. in vitro. *Wiad Bot*., *31*, 61–64.
[20] Matthews, R. F. (1994). Drosera rotundifolia. Fire Effects Information System, US Department of Agriculture Forest Service, Rocky Mountain Research Station, *Fort Collins*. Available at http://www.fs.fed.us/database/feis/plants/forb/drorot/ all.html.
[21] Melzig, M. F., Pertz, H. H., & Krenn, L. (2011). Anti-inflammatory and spasmolytic activity of extracts from Droserae Herba. *Phytomedicine, 8*, 225–229. http://dx.doi.org/10.1078/0944-7113-00031
[22] Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant, 15*, 473–497.
[23] Rak, O., & Kachula, I. (2017). Cancer OO NATURA 2000 network as an innovative system of protection of rare species and settlements in Ukraine. *Proceedings of the scientific-practical seminar (Kyiv, February 15, 2017) / Series: "Conservation Biology in Ukraine" 1*, 131–132.
[24] Simola, L. K. (1978). Dipeptides as nitrogen sources for Drosera rotundifolia in aseptic culture. *Physiol. Plant*, *44*, 315–318.
[25] Umanets, O., & Moisienko, I. (2012). The most southern find of Drosera rotundifolia in Ukraine. *Black Sea Botanical Journal*, *8* (3), 342–346.

Збереження представників роду *Drosera* L. з використанням біотехнологічних методів

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Анотація. У роботі проведено дослідження особливостей отримання садивного матеріалу рідкісних представників *Drosera spatulate* L. та *Drosera aliciae* L. з використанням мікроклонального розмноження з метою їх збереження й подальшого культивування в умовах *ex vitro*. Відпрацьовано методику стерилізації експлантатів *D. spatulate* та *D. aliciae* з 80-90 % отриманням асептичного матеріалу. Вивчено вплив різних варіантів стерилізації на розвиток мікропагонів. Найкращими розчинами для стерилізації визначено два: 0,1-відсотковий розчин $AgNO_3$ та 12,5-відсотковий розчин H_2O_2. Досліджено особливості органогенезу та регенерації цілого організму з культивованих тканин та органів представників *Drosera* L. Підібрано оптимальне живильне середовище на етапі введення у культуру in vitro *D. spatulate* та *D. aliciae* (МС із додаванням PVP 2 мг·л$^{-1}$). Визначено оптимальні умови прямого морфогенезу тканин *Drosera spatulate* L. та *Drosera aliciae* L., що дало змогу масового отримання оздоровленого, генетично однорідного садивного матеріалу. Показано дію екзогенних регуляторів росту на різних етапах морфогенезу рослин в умовах *in vitro*. Удосконалено умови ризогенезу in vitro. Зокрема за результатами досліджень найвищий коефіцієнт розмноження (1:8) та формування кореневої системи було отримано на безгормональному середовищі з удвічі зменшеним вмістом макроелементів і живильному середовищі з додаванням 0,5 мг·л$^{-1}$ ІМК. За таких умов у всіх розмножуваних рослин спостерігали активний морфогенез і формування розвиненої кореневої системи. За результатами проведених досліджень було розроблено методику мікроклонального розмноження, яка дала можливість отримати генетично-стабільні, вільні від хвороб рослини-регенеранти *D. spatulate* та *D. aliciae* з оптимально сформованою кореневою системою та вегетативною масою. Отриманий однорідний садивний матеріал можна використовувати у квітникарстві, створенні тераріумів, для фармакологічних цілей та з метою інтродукції

Ключові слова: *Drosera* L., мікроклональне розмноження, морфогенез