

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/287390630>

In vitro propagation and conservation of rare species *Fritillaria meleagris* L. from floral explants

Article in *Contemporary Problems of Ecology* · November 2015

DOI: 10.1134/S1995425515060128

CITATIONS

0

READS

223

3 authors:



[Dinara Muraseva](#)

Central Siberian Botanical Garden

5 PUBLICATIONS 1 CITATION

[SEE PROFILE](#)



[T. I. Novikova](#)

18 PUBLICATIONS 6 CITATIONS

[SEE PROFILE](#)



[Anna Erst](#)

Central Siberian botanical garden (CSBG)

30 PUBLICATIONS 8 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



ISSR identification of genetic stability the regenerated plants of *Hedysarum theinum* [View project](#)



Production of phytoecdysteroids by hairy root cultures of some species of the genus *Silene* L. [View project](#)

All content following this page was uploaded by [Anna Erst](#) on 23 December 2015.

The user has requested enhancement of the downloaded file.

In Vitro Propagation and Conservation of Rare Species *Fritillaria meleagris* L. from Floral Explants

D. S. Muraseva, T. I. Novikova, and A. A. Erst

Central Siberian Botanical Garden, Siberian Branch, Russian Academy of Sciences,
ul. Zolotodolinskaya 101, Novosibirsk, 630090 Russia

e-mail: dsmuraseva@csbg.nsc.ru

Received June 5, 2015; in final form, June 15, 2015

Abstract—The peculiarities of the in vitro regeneration of the rare species *Fritillaria meleagris* L. from floral explants have been studied for the first time. Upon the establishment of in vitro culture and during the multiplication stage, the most effective is the B5 nutrient medium supplemented with 0.44 μ M BAP, 3.22 μ M NAA, and 2.28 μ M IAA. During the multiplication stage, the regeneration rate reaches 80% and the number of bulblets per explant is 3.9 ± 0.3 . It is established that the morphogenesis of de novo formed structures depends on the mineral composition of the medium: the use of mineral-based B₅ leads to adventitious shoot formation (gemma genesis), while the use of BDS causes morphogenic callus formation and the gemmorrhizogenesis on its surface. The stimulating effect of low temperatures (+7°C) on bulblet development and adaptation to ex vitro conditions is noted.

Keywords: *Fritillaria meleagris* L., in vitro regeneration, floral explants, morpho-histological analysis, adventitious shoot formation, biodiversity conservation

DOI: [10.1134/S1995425515060128](https://doi.org/10.1134/S1995425515060128)

INTRODUCTION

The problems of plant diversity conservation arising from ecosystem destabilization caused by environmental pollution, anthropogenic load, an increase in the number of invasive species, and climate change require the search and development of new strategies. One effective approach to successfully preserving rare species under ex situ conditions is the use of biotechnologies (Engelmann, 2011). The processes of in vitro regeneration are the basis not only for the development of multiplication technologies, but also for basic research on plant morphogenetic development. One fundamentally important property of morphogenesis is the versatility of its ways under both in vivo and in vitro conditions; it makes it possible to explore the potential of plant cells and modulate the processes of development in the organs and tissues culture (Batygina, 2010).

Snake's head fritillary—*Fritillaria meleagris* L. (Fam. Liliaceae) is a rare species with a disjunctive habitat. It is spread in Western Siberia and the European part of Russia, as well as in Eastern Europe, Kazakhstan, and the Mediterranean; it is listed in the Red Data Book of the Russian Federation (2008), with a status of 3c. Natural populations of this ephemeral suffer from its bulbs being gathered and dug up due to the attractive color of its flowers and early blooming. The low rate of natural reproduction of *F. meleagris* is also one of the causes of the vulnerability of natural populations of this species. A number of studies were

devoted to the conservation and propagation of snake's head fritillary, which proved the effectiveness of alternative biotechnological approaches instead of traditional breeding methods (Vechernina, 2004; Nikolic et al., 2008; Vetchinkina, 2010; Subotic et al., 2010; Petric et al., 2011, 2013; Jevremovic et al., 2010).

In vitro plant regeneration, particularly that of monocots, is often challenging (Laslo et al., 2011). Somatic cells of this class of higher plants are differentiated very early and then lose their mitotic activity and morphogenetic potential. Embryos or organs with meristematic zones are used most often for in vitro multiplication. However, the seeds of species of the genus *Fritillaria* have a deep morphophysiological type of dormancy that makes it difficult to use them as primary explants (Nikolaeva et al., 1999; Vetchinkina et al., 2012).

Other types of explants are used for in vitro culture initiation of fritillaries: bulbous scale, for example, for *F. verticillata* (Vechernina, 2004), *F. meleagris* (Laslo, 2011), and *F. thunbergii* (Paek, Murthy, 2002); leaf base for *F. meleagris* (Subotic et al., 2010); petals for *F. imperialis* (Mohammadi-Dehcheshmeh et al., 2007, 2008), and bulblets for *F. unibracteata* (Gao et al., 1999).

The use of floral organs as primary explants ensures the preservation of the mother plant, which is especially important when working with rare species; it makes it possible to avoid a high level of contamination that is typical for explants from the underground organs, and it is an alternative to the bulb scales that make up the bulb

of the donor plant, especially in the case of their limited number (1–3 scales) (Ziv and Lilien-Kipnis, 2000; Mohammadi-Dehcheshmeh et al., 2008).

It was found that the in vitro culture regeneration processes of the genus *Fritillaria* can occur in two ways: through somatic embryogenesis or gemmogenesis; the de novo formation of the structures may occur either directly or indirectly through a phase of callus formation (Paek and Murthy, 2002; Vechernina, 2004). According to the literature, somatic embryogenesis, which often has an intermediate stage of callus formation, is most typical for *F. meleagris* (Petric et al., 2010; Subotić et al., 2010). Since the ultimate aim of these works is to preserve the germplasm of rare and endangered species and to maintain it in a stable condition, an important point in the development of micropropagation techniques is to study the ways of in vitro morphogenesis.

The aim of this research is to study the morphogenesis of a rare species *F. meleagris* in the in vitro culture of petals as the basis for the development of highly efficient and stable systems of reproduction and conservation of the species.

MATERIALS AND METHODS

Closed flower buds of *F. meleagris* (totally, 37 buds) served as the starting material for its introduction to in vitro culture; they were taken in 2013–2015 from the plants growing at the introduction site of the Laboratory of Ornamental Plants of the Central Siberian Botanical Garden, Siberian Branch, Russian Academy of Sciences (Novosibirsk).

Preparation of an Aseptic Culture

Surface sterilization was performed by immersion into 20% aqueous solution of Domestos for 20 min, then the buds were washed three times with sterile distilled water. Sterile petals were separated from the receptacle and used as primary explants. The culture medium developed for *F. imperialis* was used at the stage of the introduction to in vitro culture (Mohammadi-Dehcheshmeh et al., 2008): mineral foundation by the prescription of Gamborg and Eveleigh (B_5) (Gamborg and Eveleigh, 1968) and growth regulators $0.44 \mu\text{M}$ 6-benzylaminopurine (BAP) in combination with $3.22 \mu\text{M}$ α -naphthaleneacetic acid (NAA) and $2.28 \mu\text{M}$ indole-3-acetic acid (IAA). The hormone free B_5 medium was used as a control.

In Vitro Propagation

Conglomerates of regenerated microbulblets were transferred to media for further propagation. Modified B_5 and Dunstan and Short (BDS) (Dunstan and Short, 1977) nutrient media supplemented with BAP, NAA, and indole-3-acetic acid (IAA) at concentrations of 0.2 – $10.0 \mu\text{M}$ were used at the stage of propagation. Plant material was cultivated under conditions

of 16 h illumination/ 8 h darkness at $23 \pm 2^\circ\text{C}$ by standard methods (Kalinin et al., 1980). Subculture period was 35 – 40 days.

Morphohistological Analysis

A Stereo Discovery v. 12 stereo microscope (Carl Zeiss, Germany) was used for morphological studies. A detailed histological study of the processes of morphogenesis was carried out on permanent microscopic preparations prepared according to Pausheva (1988). The plant material was fixed in FAA–ethanol: formaldehyde: glacial acetic acid (100 : 7 : 7). Washing and further storage was performed in 70% ethanol. Thin sections were stained with hematoxylin according to Ehrlich with aniline blue tint. The histological analysis of plant development was carried out using an Axioskop-40 light microscope (Carl Zeiss, Germany) equipped with AxioCam MRc5 digital camera using AxioVision v. 4.8 software for receiving, processing, and image analysis.

Rooting and Adaptation to Ex Vitro Conditions

At the stage of rooting, the microbulblets were planted on B_5 medium with a twice-reduced content of macro- and microsalts supplemented with $5 \mu\text{M}$ NAA. All nutrient media for rooting contained particulate activated carbon (0.5 mg/L). To induce rhizogenesis, microplants were placed under low positive temperature in a light thermostat (Rumed, Germany) at 7°C for 1.5 – 2 months. Indicators of growth and development were recorded at the end of this period: the diameter of the bulb and the number of bulb scales and the number and the length of roots. $1/2 B_5$ nutrient medium and $23 \pm 2^\circ\text{C}$ served as a control.

To adapt the regenerated plants to ex vitro conditions, they were planted in containers filled with a mixture of shredded coconut fiber and sand (3 : 1) closed with a film and transferred to the greenhouse.

Statistical Analysis of the Results

All experiments were carried out twice. The statistical analysis package Microsoft Excel was used to process the results. The diagrams show the average arithmetic values and confidence intervals. This paper discusses the differences significant at the 95% significance level.

RESULTS AND DISCUSSION

When petals are used as primary explants, in addition to providing a high degree of sterility, it is necessary to reduce the damaging effect of the sterilizing agent. We developed the surface sterilization mode for flower buds using a 20-min exposure to a 20% solution of Domestos; it proved to be effective, and explant sterility reached 91–98%. The viability of primary explants was 76%.

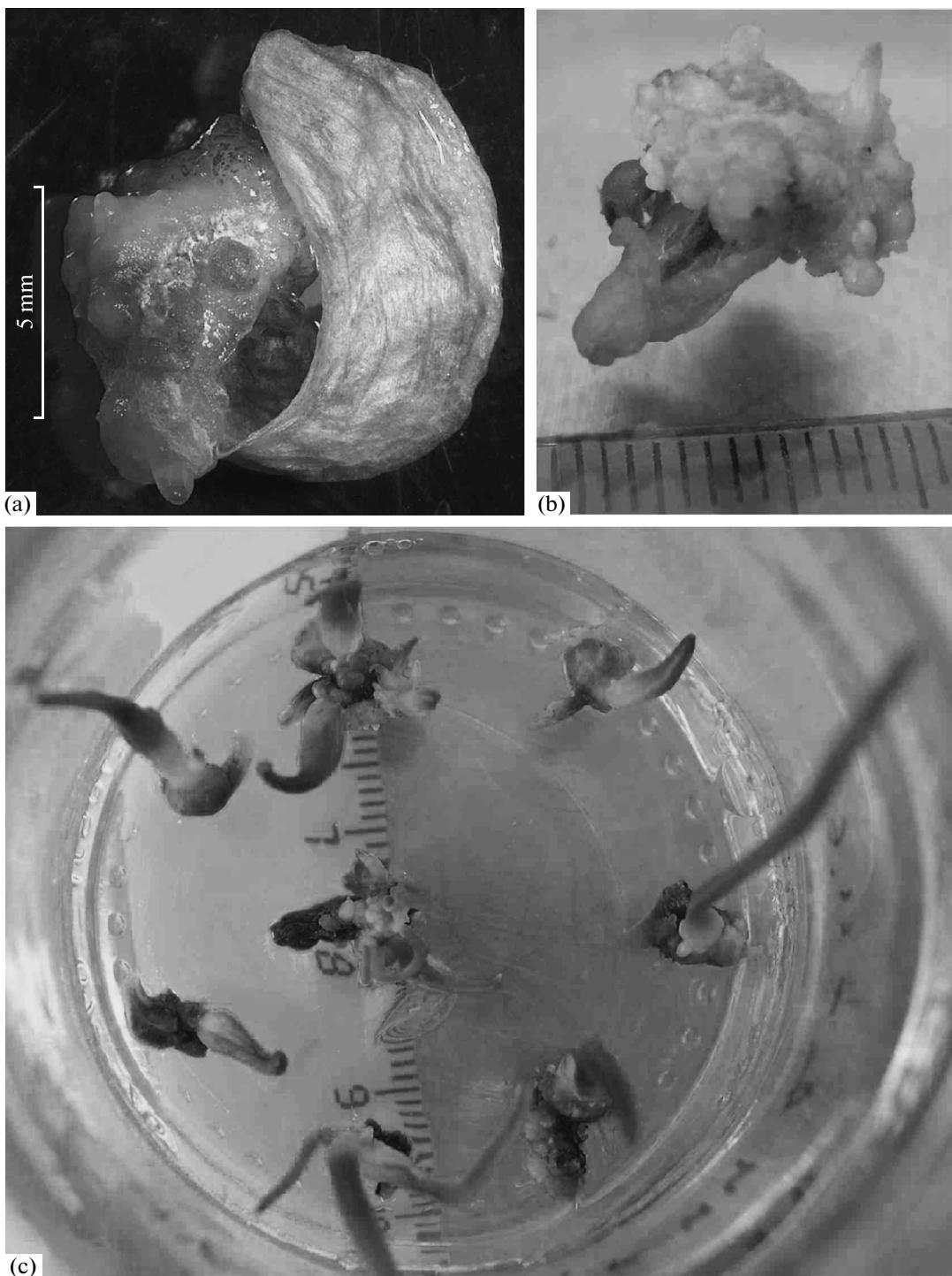


Fig. 1. *F. meleagris* introduction to an in vitro culture using petals as primary explants and B₅ nutrient medium supplemented with 0.44 μ M BAP + 3.22 μ M NAA + 2.28 μ M IAA: (a) the overgrowth of a leaf base, formation of protuberances on 25th day; (b) the development of microbuds, 34th day; and (c) de novo shoot formation after 65 days of culturing.

Shoot regeneration did not occur in the control hormone free medium; leaf tissue was subjected to necrosis. Using B₅ nutrient medium supplemented with 0.44 μ M BAP combined with 3.22 μ M NAA and 2.28 μ M IAA contributed to the activation of morphogenic processes in the tissues of the petals. The appear-

ance of the first protuberances on the surface of the explant in the area of the petal attachment to the receptacle was noted 23–25 days after the introduction to tissue culture and occurred without the formation of callus, but with the overgrowth of the leaf base tissue (Fig. 1). The regeneration reached 56% in this

medium; 4.4 ± 0.5 bulbs were formed, on the average, per one explant. The effectiveness of this nutrient medium was observed earlier by Mohammadi-Dehcheshmeh et al. (2008) using *F. imperialis* floral explants.

Microbulblets that formed at the stage of initiation to in vitro culture were separated from the primary explant and cultivated on various media (B_5 and BDS) in order to optimize the stage of multiplication using different concentrations and combinations of auxins and cytokinins (Fig. 2). The frequency of microshoot regeneration ranged from 60 to 81% in all tested media (table). Assessing the impact of growth regulators on the adventitious shoot formation of *F. meleagris*, it was found that the greatest number of shoots was formed on B_5 medium supplemented with BAP 0.44 μ M + NAA 3.22 μ M + IAA 2.28 μ M. This combination of B_5 mineral base and growth regulators with a predominance of auxins promoted the formation of 3.9 ± 0.3 adventitious microbulblets per explant; the frequency of regeneration was 80%. It is seen from the diagram (Fig. 2) that the use of only auxins or cytokinins at the stage of multiplication decreased the de novo formation of microbulblets. Similar results were obtained culturing *Lilium davidii* var. unicolor (Xu et al., 2009) and *Allium chinense* (Yan et al., 2009). In general, the combination of cytokinins and auxins is the most effective to induce the shoot formation in geophytes (Kim et al., 2005). In many studies, the researchers used high concentrations of growth regulators (22–89 μ M) (De Bruyn et al., 1992; Slabbert et al., 1993), which often facilitated the emergence of somaclonal variation.

When the concentration of BAP increased to 10 μ M, an inhibition of shoot formation (to 1.9 shoots per explant) and reduced frequency of regeneration (60%) are noted; this is accompanied by an increase in time required for microbulblets regeneration. The regeneration of adventitious shoots on control hormone free media was also found, which can be explained by the accumulation of exogenous growth regulators contained in nutrient media at the stage of initiation to in vitro culture in the tissue of an explant (table).

The influence of the mineral composition of the nutrient medium on the efficiency of *F. meleagris* in vitro multiplication was established. Thus, the use of B_5 medium promoted more active regeneration that did not depend on growth regulators. The use of two mineral bases allowed us to reveal the different ways of morphogenesis in tissue culture: the cultivation of microbulblets derived from the petals on B_5 medium led to an active shoot formation, while tissue overgrowth of the microbulblet bulb scale and active callus formation (72–87%) were noted on the BDS medium, wherein embryolike structures were formed on the callus surface. We showed that the use of different combinations of growth regulators effected only the activity of the regeneration process and did not affect the way

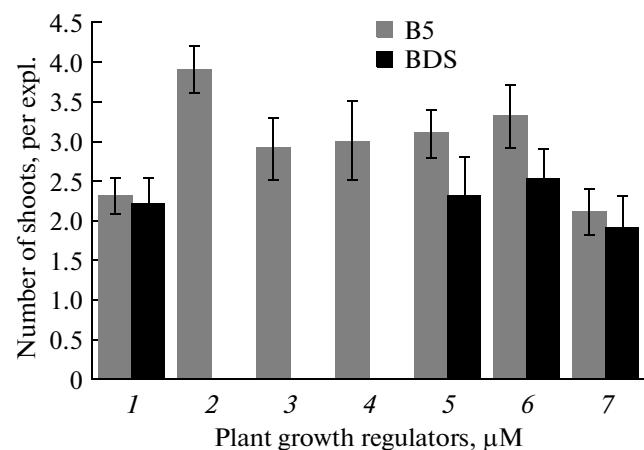


Fig. 2. Effect of the components of the nutrient medium on the in vitro regeneration of *F. meleagris* microbulblets. (1) Without growth regulators; (2) BAP 0.44 + NAA 3.22 + IAA 2.28; (3) NAA 0.3 + IAA 0.2; (4) NAA 3.2 + IAA 2.3; (5) BAP 0.5; (6) BAP 5 + NAA 2; (7) BAP 10 + NAA 2.

of morphogenesis (Fig. 2). Such an effect of a medium's mineral base on the in vitro morphogenesis is considered by Ramag and Williams (2002).

To identify the exact way of morphogenesis in the in vitro culture of *F. meleagris*, we performed a morphohistological analysis of de novo structures.

As was mentioned previously, when the microbulblets were cultured (multiplication stage) on B_5 nutrient medium, the formation of microbuds was noted in the bulb base during the passaging to fresh nutrient medium. Morphohistological analysis revealed that there is a direct adventitious shoot formation (gemmogenesis) without an intermediate stage of callus formation (Fig. 3).

The cultivation of microbulblets on BDS nutrient medium supplemented with 5 μ M BAP and 2 μ M NAA resulted in the formation of dense morphogenic callus. The formation of embryolike structures of elongated shape was observed on the surface of callus mass dur-

Frequency of adventitious shoot formation in *F. meleagris* in vitro culture at the stage of multiplication

Growth regulators, μ M	Regeneration, %	
	B_5	BDS
Control	71	30
BAP 0.44 + NAA 3.22 + IAA 2.28	80	—
NAA 0.3 + IAA 0.2	45	—
NAA 3.2 + IAA 2.3	47	—
BAP 0.5	81	58
BAP 5 + NAA 2	77	64
BAP 10 + NAA 2	73	60

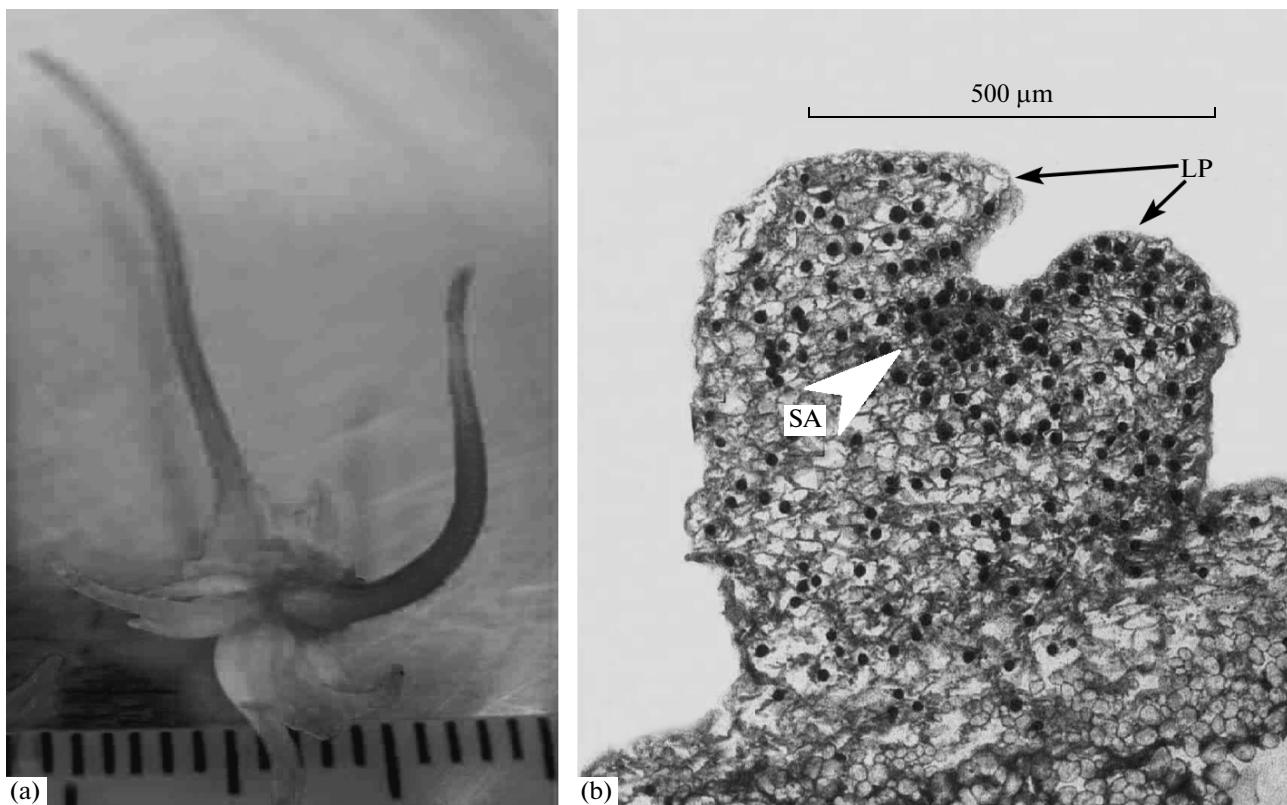


Fig. 3. Formation of *F. meleagris* adventitious microbulbets at the stage of multiplication on B₅ nutrient medium supplemented with 0.44 μM BAP + 3.22 μM NAA + 2.28 μM IAA: (a) a conglomerate of adventitious microbulbets at the end of the passage; (b) developing microbulbets, shoot apex (SA) and leaf primordia (LP) are visible.

ing its growth (Fig. 4). No differentiation of bulb scales occurred at the early stages of development. The asynchronous development of de novo structures should be noted: both initiated protuberances and well-developed microshoots were being observed simultaneously.

A histological examination of embryolike structures revealed the presence of both shoot and root apexes, as well as the presence of elongated procambium cells in the parenchyma of the buds (Figs. 5a, 5b). Further proliferation of these cells led to the appearance of strands of primary conduction system, connecting the leaf primordia and root apex, the formation of which was observed in the 35th–42nd day of culturing (Fig. 5c). The apical meristem of a microshoot had a tunica-corpus type of organization with clearly visible protoderm (Fig. 5d). Presence of well-developed root apexes in microshoots at the late stages of morphogenesis indicates formation of adventitious roots (Figs. 5c, 5e). At the same time formation two root apexes may be explained by the development of adventitious roots, which is typical of all bulbous geophytes.

The study of the structure of emerging structures suggests that the initiation of meristems, giving rise to microshoots, occurs not on the surface of the callus, but in deeper cell layers. Despite of the presence of shoot and root apexes, we did not reveal the processes

of somatic embryogenesis in callus tissue of *F. meleagris* due to the absence of bipolarity at the early stages of in vitro morphogenesis. Consequently, the regeneration processes in callus of *F. meleagris* occur through gemmorrhizogenesis.

The question on the origin of de novo structures and the initiation factors of regeneration processes was discussed in many papers. Shimada and Otani (1997) obtained *F. camtschatcensis* adventitious bulbs by direct organogenesis; this type of morphogenesis in in vitro culture was also shown for *F. thunbergii* (Paek and Murthy, 2002) and *F. roylei* (Joshi et al., 2007) from the tissues of bulbous scales, while for *F. imperialis* both indirect somatic embryogenesis and direct adventitious shoot formation from the tissue of tepals are possible (Mohammadi-Dehcheshmeh et al., 2007, 2008). Indirect somatic embryogenesis was obtained for *F. meleagris* using mature zygotic embryos as primary explants and then from the scales of formed microbulbets (Petric et al., 2011).

Geophytes, developing both in vivo and in vitro conditions, are characterized by the presence of a dormancy period, which delays germination and the further development of bulbs when they are transferred to ex vitro conditions (Paek and Murthy, 2002; Nikolić et al., 2008). Several authors obtained results pointing

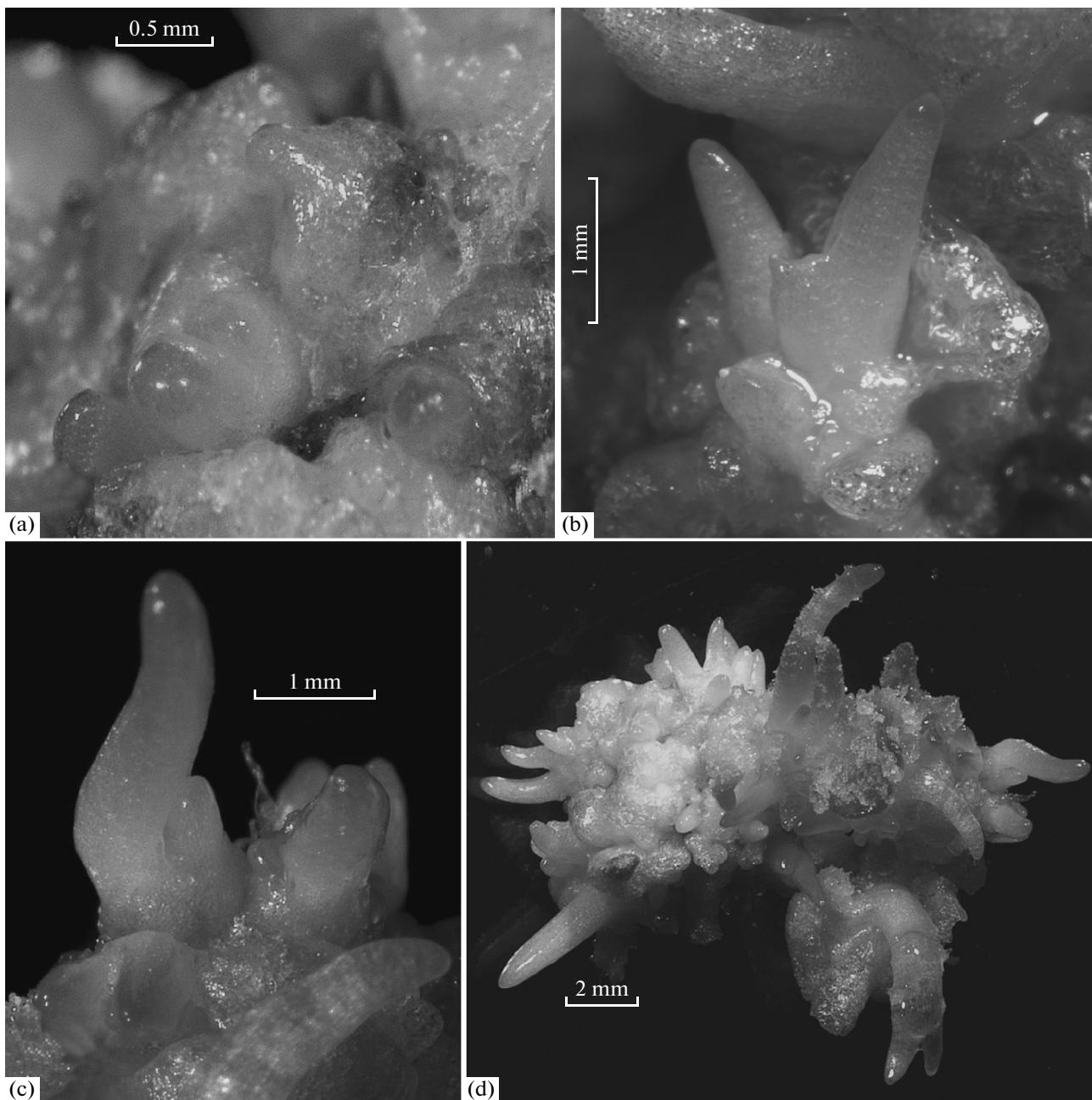


Fig. 4. Development of *F. meleagris* embryolike structures on BDS nutrient medium, supplemented with 5 μ M BAP and 2 μ M NAA: (a) protuberances on the surface of morphogenic callus, (b) developing embryolike structures, 25th day, (c) microshoot at 32nd day of culturing, and (d) the conglomerate of microshoots at the surface of callus.

to the stimulating effect of low temperatures on root formation and dormancy overcoming of bulbs developing in vitro (Yae et al., 2001; Erst et al., 2014), including those for *F. meleagris* (Jevremović et al., 2010).

To overcome the dormancy of microbulblets, to stimulate their growth and root development, we used the method of cold stratification with the maintenance of microbulblets at a low positive temperature (7°C). As a result, we obtained well-rooted *F. meleagris*

microplants; rooting rate reached 98%. However, the comparison of the growth of bulbs and in vitro rooting at different temperatures (at 23 \pm 2°C and at 7°C) showed no significant differences: microbulblets consisted on average of 3.1 \pm 0.4 scales; their diameter ranged from 4 to 5.5 mm. At the end of 6–7 weeks, regardless of the temperature, the bulbs had 3–4 roots with a length of 18–23 mm.

At the same time, we noted that cultivation at low temperatures promoted more rapid germination and

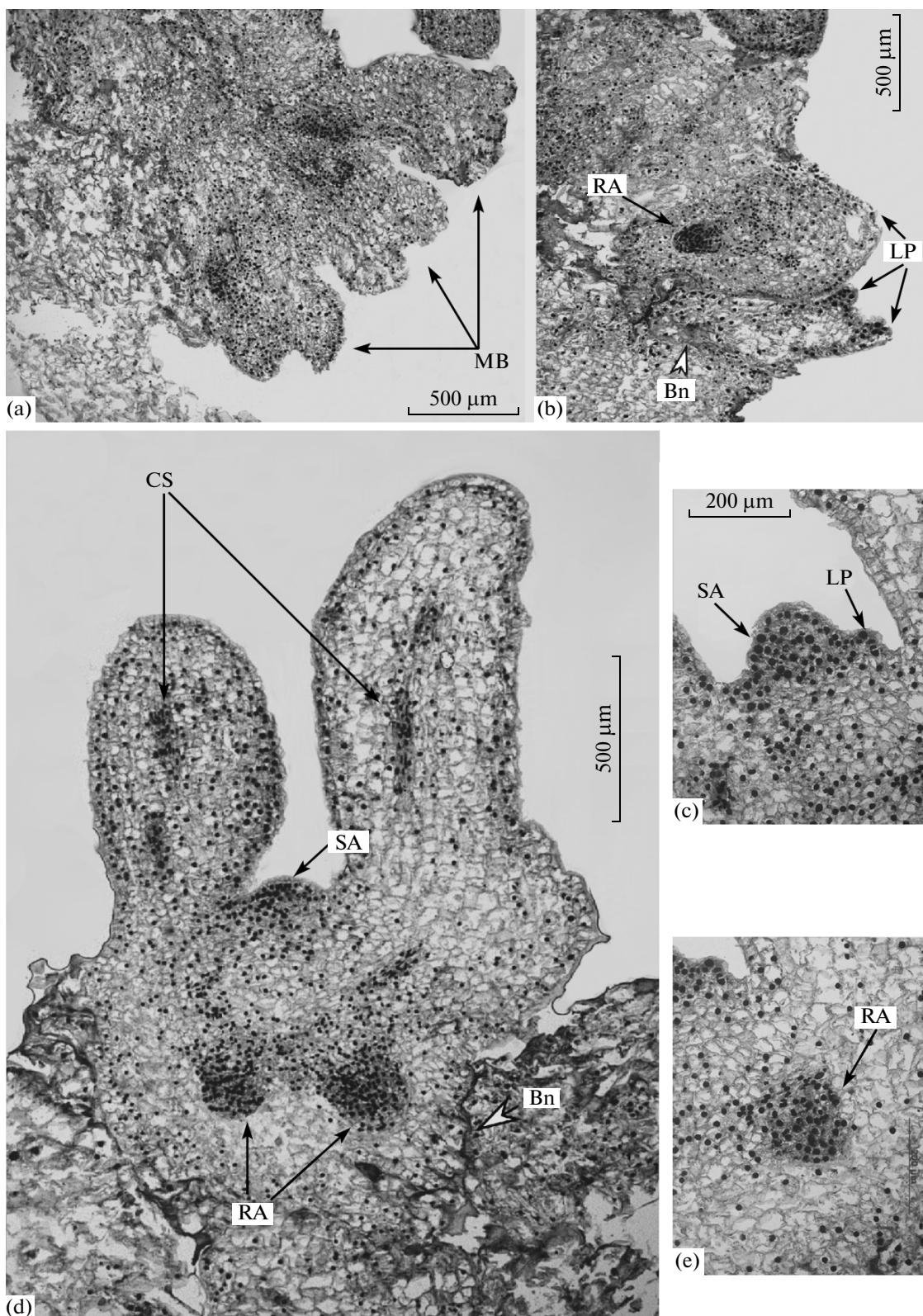


Fig. 5. Histological analysis of *F. meleagris* indirect gemmorrhizogenesis in the in vitro culture on BDS nutrient medium, supplemented with 5 μ M BAP and 2 μ M NAA: (a) development of microbuds on the surface of morphogenic callus, 30th day of culturing; (b) microshoots with well-developed root apex and leaf primordia; (c) formed microshoot with developed shoot and root apexes, the boundary between the callus tissues and shoot, and the primary conduction system clearly visible, 40th day of culturing; (d) formation of the shoot apex, tunica-corpus type of organization, and initiation of another leaf primordium are visible; and (e) initiation of the root apex. (SA) Shoot apex, (RA) root apex, (Bn) the boundary between the callus tissues and embryo, (LP) leaf primordium, (CS) vessels of primary conducting system, and (MB) microbuds.

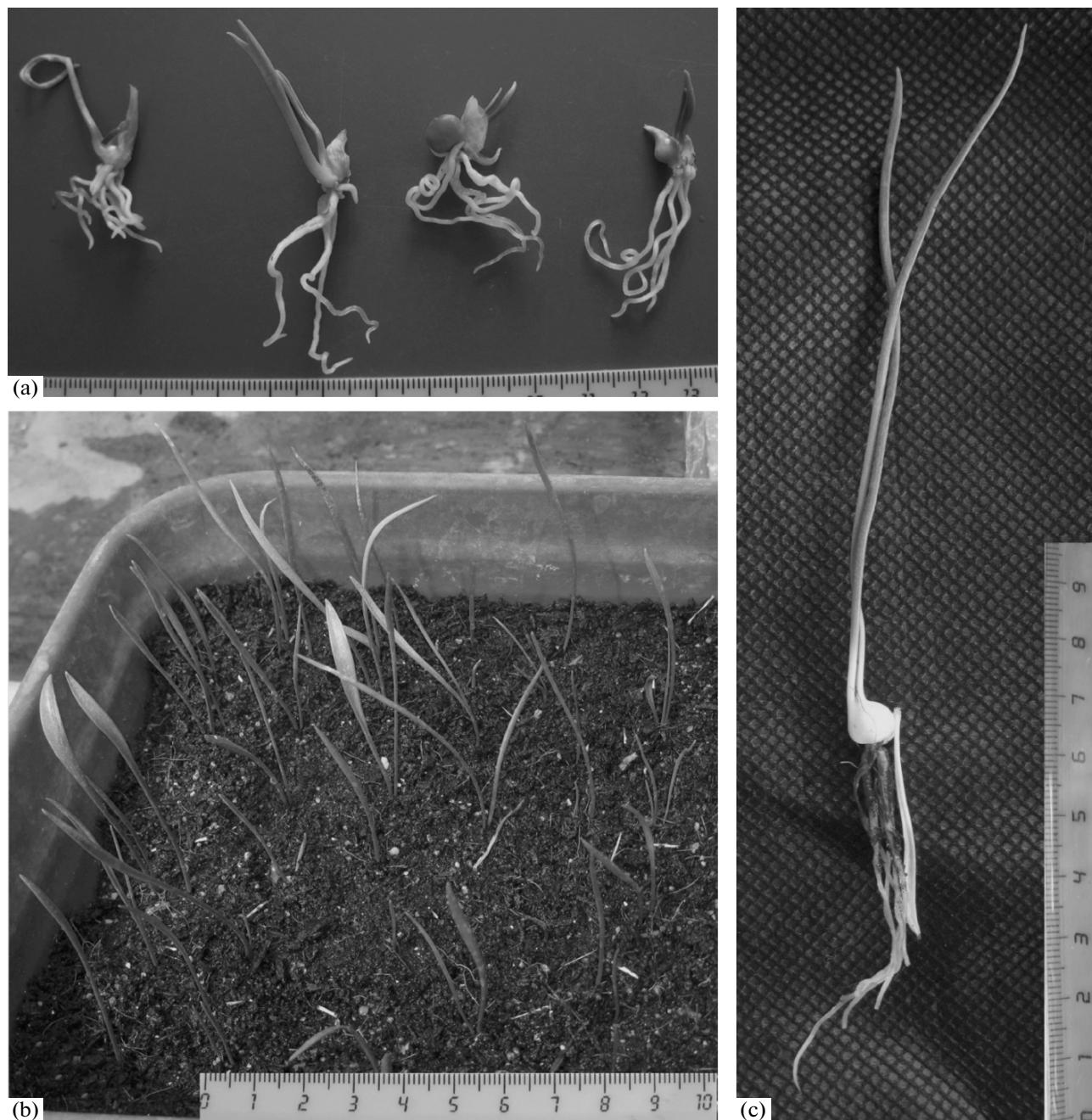


Fig. 6. Rooting and adaptation of *F. meleagris* microbulblets: (a) rooted microbulbs after 6 weeks at 7°C, B₅ culture medium supplemented with 5 µM NAA; (b) sprouting bulbs in a mixture of crushed coconut fiber and sand (3 : 1), greenhouse; (c) the bulb of *F. meleagris* after 5 months of cultivation in the open field.

the development of *F. meleagris* bulbs during their subsequent transferring to ex vitro conditions. Thus, the germination of the first leaf formed in ex vitro conditions from the microbulblets preconditioned at the temperature of 7°C was noted on 51th–56th day, whereas in the absence of low-temperature treatment the germination was observed only after 89–94 days. It should be noted that bulbs germinate synchronously in both cases.

Using a mixture of crushed coconut fiber and sand (3 : 1), it was possible to obtain a 70% level of adaptation to ex vitro conditions. The growth of regenerants under greenhouse conditions contributed to the development of 2–3 assimilating leaves with a well-developed leaf blade of linear form (Fig. 6). Further cultivation of *F. meleagris* plants was carried out in the open field. Termination of the growing season was noted 2–2.5 months after the germination of the first leaf.

CONCLUSIONS

In this study, petals are used as an explant for the introduction to in vitro culture of a rare species *F. meleagris* for the first time. This type of explants is a promising alternative to the previously used bulb scales, because it makes it possible to save the mother plants and overcome problems with contamination. The use of media of different compositions at the stage of multiplication (B₅ and BDS) revealed the influence of a mineral base on both the activity of regeneration and in vitro morphogenesis. It was established that the regeneration processes are implemented more actively on B₅ medium, regardless of the growth regulators. Thus, the formation of de novo structures on this medium occurs through the direct gemmogenesis, whereas in BDS medium it occurs through gemmohizogenesis with the stage of callus formation. The adaptation conditions were optimized and the stimulating effect of low temperatures on the germination and development of *F. meleagris* bulbs was shown at their subsequent transfer to ex vitro conditions.

The results of our research that revealed the different ways of snake's head fritillary morphogenetic in vitro development from the floral organs are of some interest in both the development of multiplication technologies for a rare species and understanding the regeneration processes in the culture of isolated organs and tissues of monocots.

ACKNOWLEDGMENTS

The research was funded by OPTEK and UMNIK grants.

REFERENCES

- Batygina, T.B., Kruglova, N.N., Gorbunova, V.Yu., et al., *Ot mikrospory – k sortu* (From Microspore to the Cultivar), Moscow: Nauka, 2010.
- De Bruyn, M.H., Ferreira, D.I., Slabbert, M.M., and Pretorius, J., In vitro propagation of *Amaryllis belladonna*, *Plant Cell, Tissue Organ Cult.*, 1992, no. 31, pp. 179–184.
- Dunstan, D.J. and Short, K.C., Improved growth of tissue cultures of the onion *Allium cepa*, *Physiol. Plant*, 1977, vol. 41, no. 1, pp. 70–72.
- Engelmann, F., Use of biotechnologies for the conservation of plant biodiversity, *In Vitro Cell. Dev. Biol.: Plant*, 2011, vol. 47, pp. 5–16.
- Erst, A.A., Erst, A.S., Shaulo, D.N., and Kul'khanova, D.S., Preservation and reproduction in vitro of rare species of genus *Fritillaria* (Liliaceae), *Rastit. Mir Aziat. Ross.*, 2014, no. 1(13), pp. 64–70.
- Gamborg, O.L. and Eveleigh, D.E., Culture methods and detection of glucanases in cultures of wheat and barley, *Can. J. Biochem.*, 1968, vol. 46, no. 5, pp. 417–421.
- Gao, S.L., Zhu, D.N., Cai, Z.H., Jiang, Y., and Xu, R., Organ culture of a precious Chinese medicinal plant – *Fritillaria unibracteata*, *Plant Cell, Tissue Organ Cult.*, 1999, no. 59, pp. 197–201.
- Jevremović, S., Petrić, M., Živković, S., Trifunović, M., and Subotić, A., Superoxide dismutase activity and isoenzyme profiles in bulbs of snake's head fritillary in response to cold treatment, *Arch. Biol. Sci.*, 2010, no. 62, no. 3, pp. 553–558.
- Jiménez, V.M., Involvement of plant hormones and plant growth regulators on in vitro somatic embryogenesis, *Plant Growth Regul.*, 2005, no. 47, pp. 91–110.
- Joshi, S.K., Dhar, U., and Andola, H.C., In vitro bulblet regeneration and evaluation of *Fritillaria roylei* Hook. a high value medicinal herb of the Himalaya, *Acta Hort.*, 2007, no. 756, pp. 75–84.
- Kalinin, F.L., Sarnatskaya, V.V., and Polishchuk, V.E., *Metody kul'tury tkanei v fiziologii i biokhimiï rastenii* (The Methods of Tissue Culture in Physiology and Biochemistry of the Plants), Kyiv: Naukova Dumka, 1980.
- Kim, M.S., Jeon, J.H., Youm, J.W., Kim, J.H., Lee, B.C., Kang, W.J., Kim, H.S., and Joung, H., Efficient plantlet regeneration via callus formation from leaf segment of *Lilium* oriental hybrid "Casa Blanca", *J. Plant. Biotech.*, 2005, no. 7, pp. 129–134.
- Krasnaya kniga Rossiiskoi Federatsii: rasteniya i griby* (The Red Data Book of Russian Federation: Plants and Fungi), Moscow: KMK, 2008.
- Laslo, V., Zăpărțan, M., and Agud, E., In vitro conservation of certain endangered and rare species of Romanian spontaneous flora, *An. Univ. Oradea, Fasc. Prot. Med.*, 2011, vol. 16, pp. 252–261.
- Mohammadi-Dehcheshmeh, M., Khalighi, A., Naderi, R., Ebrahimi, E., and Sardari, M., Indirect somatic embryogenesis from petal explant of endangered wild population of *Fritillaria imperialis*, *Pak. J. Biol. Sci.*, 2007, vol. 10, no. 11, pp. 1875–1879.
- Mohammadi-Dehcheshmeh, M., Khalighi, A., Naderi, R., Sardari, M., and Ebrahimi, E., Petal: a reliable explant for direct bulblet regeneration of endangered wild populations of *Fritillaria imperialis* L., *Acta Physiol.*, 2008, no. 30, pp. 395–399.
- Nikolaeva, M.G., Lyanguzova, I.V., and Pozdova, L.M., *Biologiya semyan* (Biology of Seeds), St. Petersburg: Nauchno-Issled. Inst. Khim., S.-Peterb. Gos. Univ., 1999.
- Nikolić, M., Mišić, D., Maksimović, V., Jevremović, S., Trifunović, M., and Subotić, A., Effect of low temperature on rooting rate and carbohydrate content of *Fritillaria meleagris* bulbs formed in culture in vitro, *Arch. Biol. Sci.*, 2008, vol. 60, no. 1, pp. 5–6.
- Otani, M. and Shimada, T., Micropagation of *Fritillaria camschatcensis* (L.) KerGawl. "Kuroyuri," *Bull. Res. Inst. Agric. Sci., Ishikawa Agric. Coll.*, 1997, vol. 5, pp. 39–44.
- Paek, K.Y. and Murthy, H.N., High frequency of bulblet regeneration from bulb scale sections of *Fritillaria thunbergii*, *Plant Cell, Tissue Organ Cult.*, 2002, no. 68, pp. 247–252.
- Pausheva, Z.P., *Praktikum po tsitologii rastenii* (Practical Manual on the Plant Cytology), Moscow: Agropromizdat, 1988.
- Petrić, M., Subotić, A., Jevremović, S., and Trifunović, M., Somatic embryogenesis and bulblet regeneration in snakehead fritillary (*Fritillaria meleagris* L.), *Afr. J. Biotech.*, 2011, vol. 72, no. 10, pp. 16181–16188.

- Ramage, C.M. and Williams, R.R., Mineral nutrition and plant morphogenesis, *In Vitro Cell. Dev. Biol.: Plant*, 2002, no. 38, pp. 116–124.
- Slabbert, M.M., De Bruyn, M.H., Ferreira, D.I., and Pretorius, J., Regeneration of bulblets from twin scales of *Crinum macowanii* in vitro, *Plant Cell, Tissue Organ Cult.*, 1993, no. 33, pp. 133–141.
- Subotić, A., Trifunović, M., Jevremović, S., and Petrić, M., Morpho-histological study of direct somatic embryogenesis in endangered species *Fritillaria meleagris*, *Biol. Plant*, 2010, vol. 54, no. 3, pp. 592–596.
- Vechernina, N.A., *Metody biotekhnologii v selektsii, razmnozhenii i sokhranenii genofonda rastenii: monografiya* (The Methods of Biotechnology in Selection, Reproduction, and Preservation of the Gene Pool of the Plants: Monograph), Barnaul: Altaisk. Gos. Univ., 2004.
- Vetchinkina, E.M., Biological features of cultivation in vitro of seeds and embryos of rare plant species, *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Moscow, 2010.
- Vetchinkina, E.M., Shirnina, I.V., Shirnin, S.Yu., and Molkanova, O.I., Preservation of rare plant species in genetic collections in vitro, *Vestn. Balt. Fed. Univ. im. I. Kanta*, 2012, no. 7, pp. 109–118.
- Xu, L.F., Ma, F.W., and Liang, D., Plant regeneration from in vitro cultured leaves of Lanzhou lily (*Lilium davidii* var. *unicolor*), *Sci. Hortic.*, 2009, no. 119, pp. 458–461.
- Yae, B.W., Han, B.H., and Goo, D.H., Dormancy breaking and in vitro growth of in vitro bulblets in *Lilium* oriental hybrid “Casablanca,” *J. Kor. Soc. Hortic. Sci.*, 2001, no. 42, pp. 99–102.
- Yan, M.-M., Xu, C., Kim, C.-H., Um, Y.-C., Bah, A.A., and Guo, D.-P., Effects of explant type, culture media and growth regulators on callus induction and plant regeneration of Chinese jiaotou (*Allium chinense*), *Sci. Hortic.*, 2009, vol. 123, no. 1, pp. 124–128.
- Ziv, M. and Lilien-Kipnis, H., Bud regeneration from inflorescence explants for rapid propagation of geophytes in vitro, *Plant Cell Rep.*, 2000, no. 19, pp. 845–850.

Translated by M. Shulskaya