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RESEARCH PAPER

In vitro propagation of the rare fern *Asplenium incisum* Thunb. via green globular bodies

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ABSTRACT

A promising direction for the conservation and propagation of ferns is the *in vitro* cultivation of ferns via green globular bodies (GGBs). *Asplenium incisum* Thunb. is a rare species of fern listed in the Red Data Book of several Far Eastern regions. The objective of the study was to estimate the efficiency of different media on GGB propagation and sporophyte regeneration of *A. incisum*. The Murashige and Skoog's medium with a half concentration of macro- and microelements (1/2MS), with 0.5 mg/L 6-benzylaminopurine (BAP) (variant 1) supplemented with MS full complement of vitamins, 2 % sucrose and 0.8 % agar, and adjusted to pH 5.8 was effective for the GGB propagation without formation of sporophyte. The medium 1/2MS without plant growth regulators (variant 2) supplemented with MS full complement of vitamins, 2 % sucrose and 0.8 % agar, and adjusted to pH 5.8 was effective for the GGB propagation with high sporophyte regeneration. The same media without ammonium nitrate and without vitamins had a negative effect on GGB production and sporophyte regeneration. The total number of initiated GGBs was 369 and 451 as a mean per explant after two months of *in vitro* culture for variant 1 and 2, respectively. The number of GGBs with developed sporophytes was 1 and 289, the sporophyte regeneration rate was 0.3 % and 64.1 %. BAP inhibited sporophyte regeneration but provoked the formation of new GGBs, so it is recommended to alternate variants 1 and 2 to obtain better results. Under *ex vitro* conditions, the sporophyte survival rate was 89 % in climatic room and 85 % in outdoors conditions in the first year. Sporophytes produced spores both in climatic room and outdoors conditions.

Keywords: *Asplenium incisum*, GGB, *in vitro* culture, fern, sporophyte

РЕЗЮМЕ

Шелихан Л.А. Размножение *in vitro* редкого папоротника *Asplenium incisum* Thunb. посредством зеленых глобулярных тел. Перспективным направлением в сохранении и размножении папоротников является культивирование папоротников *in vitro* посредством зеленых глобулярных тел (от англ. green globular bodies, GGBs). *Asplenium incisum* Thunb. – редкий вид папоротника, занесенный в Красные книги нескольких регионов Дальнего Востока. Целью работы была оценка влияния различных сред на размножение GGB и регенерацию спорофитов *A. incisum*. Питательная среда по Мурасиге и Скугу с половинной концентрацией макро- и микроэлементов (1/2 МС) с добавлением цитокинина (0,5 мг/л 6-бензиламинопурина, БАП) (вариант 1), с полным составом витаминов МС, с 2 % сахарозы, 0.8 % агара, при pH 5.8 была эффективна для размножения GGB без формирования спорофитов. Питательная среда 1/2МС без добавления регуляторов роста (вариант 2), с полным составом витаминов МС, 2 % сахарозы, 0.8 % агара, при pH 5.8 была эффективна для размножения GGB с высоким уровнем регенерации спорофитов. Те же среды без нитрата аммония и витаминов отрицательно влияли на продуцирование GGB и регенерацию спорофитов. Общее количество инициированных GGB составило 369 и 451 на эксплант после двух месяцев культивирования *in vitro* для варианта 1 и 2 соответственно. Количество GGB с развивающимися спорофитами составило 1 и 289; регенерация спорофитов составила 0.3 % и 64.1 %. БАП ингибировал регенерацию спорофитов, но провоцировал формирование новых GGB, поэтому для получения наилучших результатов рекомендуется чередовать варианты 1 и 2. В условиях *ex vitro* выживаемость спорофитов составляла 89 % в климатической комнате и 85 % в открытом грунте в первый год. Спорофиты продуцировали споры как в условиях климатической комнаты, так и в условиях открытого грунта.

Ключевые слова: *Asplenium incisum*, GGB, культура *in vitro*, папоротник, спорофит

Asplenium incisum Thunb. (Aspleniaceae) is a rare fern listed in the Red Data Books of several Far Eastern regions (Kozhevnikov 2008, Voronov 2008, Chernyagina 2018, Senchik 2020). In Russia it is only found in the Far East. It occurs in Kamchatka, central and southern parts of Sakhalin Island, southern Kuril Islands, Primorye and Khabarovsk Territories. In the Amur Region, it grows in the Zeya State Nature Reserve and the Khingan State Nature Reserve, but the number of populations has not yet been determined. Outside of Russia, it grows in China, Japan and Korea (Senchik 2020). The fern *A. incisum* has various medical properties such as detoxification ability (Jeong 2011) and the raw juice is used for medicinal purposes (Jeong 2011). *Asplenium incisum* has also been reported to

exhibit antibacterial activity against skin disease-causing bacteria, bronchodilator action and various pharmacological properties, including efficacy in the treatment of hepatitis, tinnitus and chronic manganese poisoning (Jeong 2011). According to Moon (2021), *A. incisum* has both anti-inflammatory and anti-osteoclastogenic activities, suggesting that it has great therapeutic potential in the control of periodontal disease. It is well known that the decline in the diversity of ferns in nature is caused by human activities and the destruction of their habitats (Kholia & Joshi 2010, Mehltreter 2010). It is therefore imperative to develop effective methods for their conservation and mass propagation.

In vitro cultivation of ferns is an efficient and rapid method of propagation (Shelikhan & Nekrasov 2018). Previously, we used spores as explants to introduce the rare *A. incisum* into *in vitro* culture (Shelikhan 2023b). In this study, we report the propagation of *A. incisum* via GGBs (green globular bodies) derived from juvenile sporophytes. Green globular bodies are vegetative shoots which are formed *in vitro* and have a high proliferation rate (Higuchi et al. 1987, Shelikhan 2023a). For further mass regeneration of sporophytes *in vitro*, such GGBs are promising meristem structures.

MATERIAL AND METHODS

Plant material

The object of this study was the fern *Asplenium incisum* (Aspleniaceae). Juvenile sporophytes obtained *in vitro* were used as explants for GGB production (Shelikhan 2023b). Young fronds and roots were removed from juvenile sporophytes with a scalpel. The remaining growth points (shoot bases) were placed on the following culture medium in flasks/jars (30 mL per 100 mL flask/jar): Murashige and Skoog's (1962) medium with a half concentration of macro- and microelements (1/2MS), with MS full complement of vitamins, plant growth regulators-free (PGRs-free), supplemented with 2 % (w/v) sucrose, 0.8 % (w/v) agar, and adjusted to pH 5.8 (Shelikhan 2024). Cultivation to obtain initial GGBs was performed at room temperature with a 16 h photoperiod (fluorescent daylight lamps, basic cool daylight, 36 W, 2500 Lm, Osram, Russia).

Propagation of GGBs

After one month of incubation, the resulting GGBs were used as explants for subculturing in flasks (100 mL containing 30 mL of one of the following media variants: A – 1/2MS PGRs-free; B – 1/2MS ammonium nitrate-free (-NH_4NO_3), vitamins-free (-vit.), PGRs-free; C – 1/2MS supplemented with the cytokinin 6-benzylaminopurine (BAP), 0.5 mg/L; D – 1/2MS (-NH_4NO_3; -vit.) containing BAP, 0.5 mg/L; E) 1/2MS supplemented with auxin indolylbutyric acid (IBA), 0.5 mg/L; F) 1/2MS (-NH_4NO_3; -vit.) containing IBA, 0.5 mg/L. All media were supplemented with 2 % (w/v) sucrose, 0.8 % (w/v) agar, and adjusted to pH 5.8. All cultures were incubated at room temperature (16 h photoperiod, cool daylight). The experiment was repeated three times. Explants were GGB fragments of 1–2 mm. For each variant of medium, 10 explants were used. After culturing for one and two months, GGBs and sporophytes (leafy shoots formed from GGBs) were separated from each other and counted under a Nikon SMZ645 stereomicroscope (Japan). After culturing GGBs and sporophytes (leafy shoots produced of GGB) were separated and counted under a stereomicroscope Nikon SMZ645 (Japan).

The following parameter was used for the estimation of effectiveness of a culture medium: the sporophyte regeneration rate (%) = (number of GGBs with developed sporophytes / total number of GGBs initiated) × 100 (Liao & Wu 2011).

Acclimatization of sporophytes

Two-month-old sporophytes were transferred from *in vitro* to *ex vitro* conditions (climatic room). Sporophytes were placed in plastic pots (one to two sporophytes per pot) with a pre-autoclaved mixture of peat and vermiculite (2:1, v/v). The pots with soil were placed in zip-lock bags and maintained at high humidity by spraying with water, as previously described for the experiments with *Polystychum craspedosorum* (Shelikhan 2024). Acclimatization was performed at room temperature with a 16 h photoperiod within six months. The experiment was carried out using fluorescent daylight lamps (basic cool daylight, 36 W, 2500 Lm, Osram, Russia). After another three months, ferns in soil pots were transferred outdoors for acclimatization to environmental conditions. Then, sporophytes were planted from the soil pots into a crevice in an artificial rock filled with soil at the Amur Branch of the Botanical Garden-Institute FEB RAS.

Morphological observations

Microscopic observations were carried out with the stereomicroscope Nikon SMZ 645 (Japan). Photographs of GGBs and sporophytes were taken with the cameras Sony Cyber-shot DSC-W630 (Japan) and Samsung Galaxy A32 (China).

RESULTS AND DISCUSSION

Propagation of GGBs

The initiation and proliferation of *A. incisum* GGBs was observed after 1–2 weeks of explant culturing *in vitro*. For other ferns, slightly later results have been described (Shelikhan 2023a). For example, *P. craspedosorum* started to produce GGBs after 2 to 3 weeks of culturing *in vitro* (Shelikhan 2024) and *Adiantum capillus-veneris* L. started to produce GGBs after 2 weeks (Li et al. 2015).

In all selected media, we observed that GGBs were increasing in size, becoming larger, looser and easier to separate. They formed groups of green globular bodies (Fig. 1:A, B). Similar results were obtained for *P. craspedosorum* (Shelikhan 2024). The color of the GGBs in *A. incisum* was light-green. In the studies by Thakur for *Matteuccia struthiopteris* (L.) Todaro and by Yu for *Cibotium barometz*, the green globular bodies varied from brown to dark-green and yellow-green, respectively (Thakur et al. 1998, Yu et al. 2017).

A single green globular body had two bipolar structures: a root meristem at the bottom and a shoot meristem at the top (Fig. 1:C). These processes are the same as those described for the GGBs of *P. craspedosorum* (Shelikhan 2024).

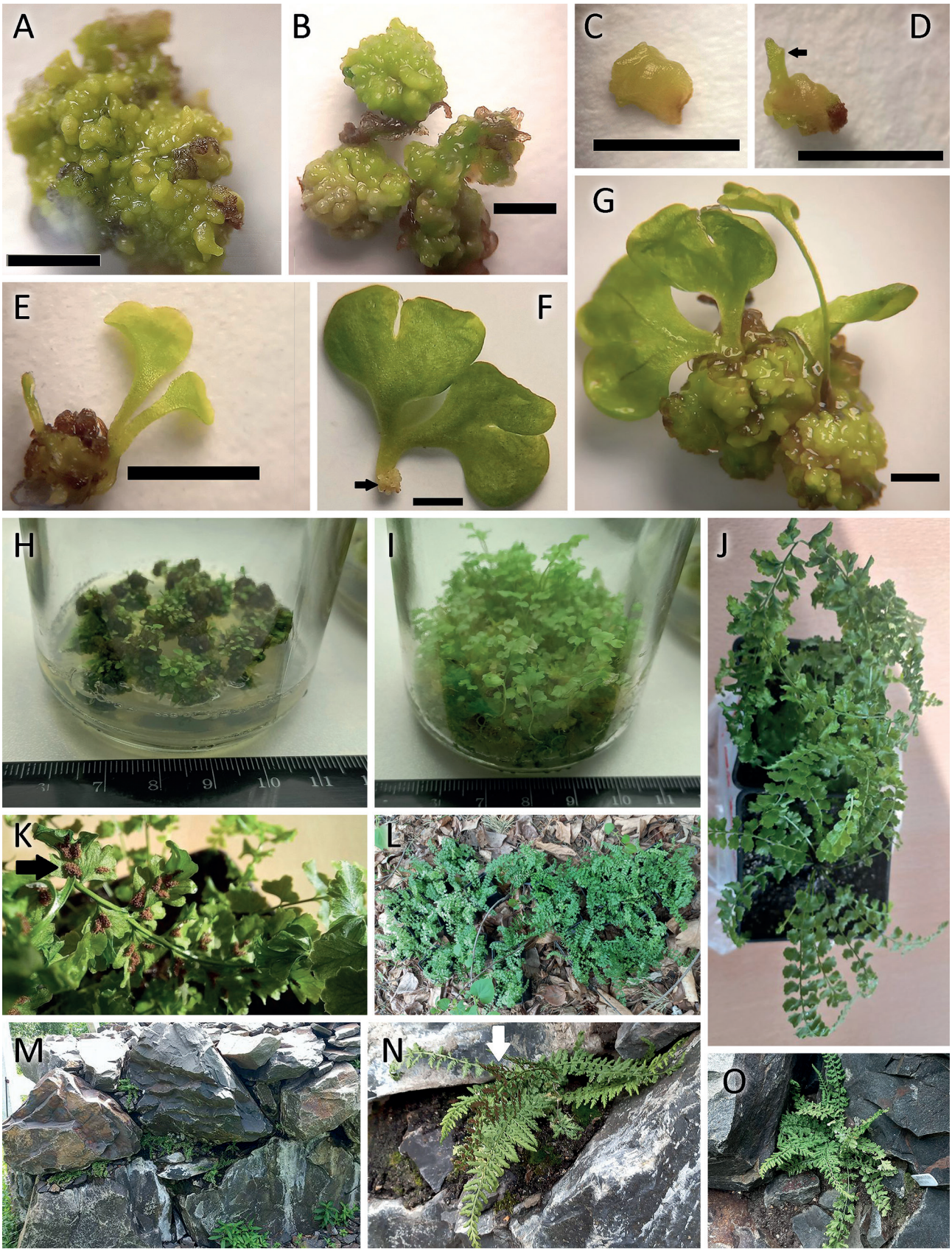

Figure 1 Propagation of *Asplenium incisum* Thunb. via green globular bodies (GGBs): A, B – groups of GGBs; C – a single GGB; D – differentiation of GGB. The arrow indicates the protrusion on the GGB; E, F, G, H – leafy shoots produced on GGBs. The arrow indicates GGB formation in the base of a leaf; I – regenerated sporophytes; J – sporophytes after 3 weeks of planting in the pots with soil; K – a sporophyte with sori. The arrow indicates the sori; L – acclimatization of sporophytes; M, N, O – sporophytes planted at the rocky site in the Amur Branch of the Botanical Garden-Institute FEB RAS; The arrow indicates the sori. Scale bars: 1 mm

These green globular bodies were easy to separate from each other. A single green globular body of *A. incisum* developed into a single sporophyte.

The proliferation and differentiation of *A. incisum* GGBs occurred simultaneously. During differentiation, protrusions (elongated tissue on the surface) developed on the GGBs, from which leafy shoots developed and then formed plantlets (Fig. 1:D, E, F, G). The single regenerated GGB and single regenerated leafy shoot were able to produce new GGBs (Fig. 1:F), these developmental stages are common to green globular bodies for different fern species (Shelikhan 2023a, 2024).

All of the *A. incisum* GGBs had a normal glass bead shape (Shelikhan 2023a). In our study, GGBs morphology was not changing during cultivation. This is in contrast to the results for *P. craspedosorum*, which showed deformation of the GGBs in a few cases (Shelikhan 2024). It is likely that deformations of the GGBs are species specific, as there have been few reports of such deformations (Yu et al. 2017, Shelikhan 2024). The more the sporophytes regenerated, the fewer GGBs were formed. A cluster of regenerated sporophytes developed from a cluster of GGBs (Fig. 1:H, I). Similarly observation is consistent with the studies of other ferns: *C. barometz* (Yu et al. 2017) and *P. craspedosorum* (Shelikhan 2024).

Effect of medium composition on GGB formation

GGB initiation was observed on all media. After one month of cultivation of explants, it was found that the highest number of GGBs (as a mean per explant) was 206 for medium C and 182 for medium A (Table 1). For *P. craspedosorum*, the total number of GGBs was approximately the same level on the same media, but BAP suppressed the initiation of GGBs in the case of *P. craspedosorum* (Shelikhan 2024). Also, in contrast to *P. craspedosorum*, there were no differences in color and size of the GGBs of *A. incisum* on the same different media. The average numbers of GGBs observed on media D, E, and F were 124, 150, and 122, respectively. The lowest number of GGBs was noted for medium B (102 GGBs) (Table 1). It is interesting to note that *P. craspedosorum* had the highest number of GGBs on medium B (Shelikhan 2024). In the case of *A. incisum*, ammonium nitrate and vitamins should be part of the medium for the development of GGBs. Thus, for *A. incisum*, 1/2 MS PGRs-free medium and the same medium with 0.5 mg/L BAP were the most productive for GGBs propagation after the first month of cultivation.

Following the cultivation of GGBs for two months (without passage), the production was gradually slowing down; however, the largest number of GGBs (as a mean per explant) was obtained on medium A (451) (Table 1). There were also positive dynamics of green globular body formation accounted for 369 and 337 GGBs on media C and E, respectively. The lowest number of GGBs was also detected on media B (185), D (177) and F (185) (Table 1). Medium C is recommended to maximize the production of GGBs without the formation of sporophytes. If the aim is to obtain GGBs and sporophytes separately, the lower total number of GGBs initiated is not a bad indicator. However, the media that can be used for mass production of GGBs are also variant A and variant E. It is believed that the need for exogenous growth regulators is dependent on the endogenous hormone levels of the plant (Shelikhan 2023a). This explains why media supplemented with BAP (medium C) and IBA (medium E) initiate less numbers of GGBs in *A. incisum*. In addition, the exclusion of ammonium nitrate and vitamins from the medium is a negative factor for the formation of GGBs by *A. incisum* compared to *P. craspedo-*

Table 1 Effect of medium composition on GGB formation and sporophyte regeneration in *Asplenium incisum* Thunb. after one and two months of *in vitro* culture

Medium	1/2MS PGRs-free	1/2MS (-NH_4NO_3; -vit.) PGRs-free	1/2MS 0.5 mg/L BAP	1/2MS (-NH_4NO_3; -vit.) 0.5 mg/L BAP	1/2MS 0.5 mg/L IBA	1/2MS (-NH_4NO_3; -vit.) 0.5 mg/L IBA
Medium Code	**A**	**B**	**C**	**D**	**E**	**F**
1 month						
Total number of GGBs initiated	182±6.2	102±6.2	206±5.8	124±5.5	150±5.2	122±5.6
Number of GGBs with developed sporophytes	8±0.9	1±0.6	1±0.7	0	1±0.8	1±0.6
Sporophyte regeneration rate (%)	4.4	1	0.5	-	0.7	0.8
2 months						
Total number of GGBs initiated	451±5.2	185±5.6	369±6.3	177±5.4	337±5.9	185±5.6
Number of GGBs with developed sporophytes	289±5.1	29±5.4	1±0.5	0	134±5.6	26±4.2
Sporophyte regeneration rate (%)	64.1	15.7	0.3	-	39.8	14.1

Notes: Results are expressed as a mean ± SD from three experiments, per initial explant

Abbreviations: 1/2MS – Murashige and Skoog's medium with a half concentration of macro- and microelements, a full complement of MS vitamins, 2 % (w/v) sucrose, 0.8 % (w/v) agar, and adjusted to pH 5.8; PGRs-free – plant growth regulators-free; -NH_4NO_3 – ammonium nitrate-free; -vit. – vitamins-free; BAP –6-benzylaminopurine; IBA –indolylbutyric acid; GGBs – green globular bodies.

sorum (Shelikhan 2024). Supplementation of the medium with ammonium nitrate is required for the high number of GGB initiation in *A. incisum*.

Sporophyte development

After one month of culturing *in vitro*, sporophyte development was low on all variants of the media except medium D on which no sporophytes were observed (Table 1). After two months of culturing *in vitro*, the number of GGBs with developed sporophytes (as a mean per explant) was higher on media A (289) and E (134); accordingly, the sporophyte regeneration rates were 64.1 % and 39.8 % for media A and E, respectively (Table 1). The number of GGBs with developed sporophytes and sporophyte regeneration rates were low on other media: B (29 and 15.7 %, respectively), C (1 and 0.3 %, respectively) and F (26 and 14.1 %, respectively). The regeneration of the sporophytes was strongly inhibited by BAP as found for media C and D, whereas GGBs proliferation was continuous in the presence of BAP (Table 1). Therefore, the best medium for the development of *A. incisum* sporophytes is medium A.

In the study on *C. barometz* (Yu et al. 2017), GGBs actively regenerated sporophytes on a medium without plant growth regulators and the sporophyte regeneration rate reached 68 %. It was reported that BAP inhibited sporophyte regeneration from GGBs of *Asplenium nidus* L. (Higuchi & Amaki 1989). The differentiation of GGBs was favored by the absence of BAP in the medium (Higuchi et al. 1987). In our study, we hardly observed any sporophyte regeneration on BAP-containing media. Therefore, a cytokinin should be added to the medium to inhibit sporophyte formation. For long-term maintenance or subculturing of pure GGB tissue without sporophyte regeneration, BAP may be the best choice (Liao & Wu 2011). The media without ammonium nitrate and vitamins had a negative effect on sporophyte development. These results differ from those obtained for *P. craspedosorum* which was able to produce GGBs and sporophytes on media without ammonium nitrate and vitamins (Shelikhan 2024). It appears that *P. craspedosorum* has an inherently lower nitrogen requirement than *A. incisum*. In many studies by other researchers, GGBs developed better into sporophytes when they were grown on media without plant growth regulators (Higuchi et al. 1987, Higuchi & Amaki 1989, Amaki & Higuchi 1992, Zhang & Su 2002, Shin & Lee 2009, Liao & Wu 2011, Yu et al. 2021). We recommend the alternation of media with BAP and PGRs-free medium for the best result in propagation of GGBs and sporophytes of *A. incisum*.

Sporophytes formed small roots after two months of cultivation in variants A and E. On media B, C and F, the sporophytes were very small and had no roots. However, sporophytes that had not yet rooted retained GGB root-like structures. On medium A and medium E, the sporophytes were equally dense. They had many roots and rhizoids. Depending on the species, it is known that sporophytes can spontaneously form roots *in vitro* (Liao & Wu 2011, Yu et al. 2017). The two-month-old sporophytes varied in size from 0.5 cm to 3.5 cm. Interestingly, in the study of *P. craspedosorum* (Shelikhan 2024), the size of two-month-old sporophytes did not exceed 1.5 cm. This is probably due to the biological characteristics of the species.

Acclimatization of sporophytes

For acclimatization, regenerated and rooted sporophytes of *A. incisum* were transferred to plastic pots with soil (Fig. 1:J). After 4 weeks of cultivation, sporophytes survival was high (89 %, n = 45). Similar results were observed for *P. bifurcatum* (88–95 %) and *P. craspedosorum* (85 %) (Yu et al. 2017, Shelikhan 2024). The plants that survived showed a normal phenotype. After 6 months in the plastic pots with soil, almost half of the sporophytes produced spores under room climate conditions (Fig. 1:K). Until now, there was no information about *A. incisum* being able to reproduce sexually in such a short period of time under room climate conditions. A total of 8–9 months elapsed between the production of the first sporophytes from GGBs and the first sporophytes producing spores. To the best of our knowledge, this is the first such report for this fern species. After 3 months, the ferns in plastic pots with soil were transferred outdoors for acclimatization to environmental conditions (Fig. 1:L). Following one week of the acclimatization, the plants of *A. incisum* were transferred from the pots with soil to the open ground consisted of a crevice in a rock filled with soil imitating natural conditions of the fern habitat (Fig. 1:M). Sporophytes survival in the rock with soil was 85 % (n = 40) in the first year outdoors. Same year (5 months later), with the beginning of the climatic autumn, we observed eight plants of A. insisum producing spores at the rocky site (Fig. 1:N, O). Thus, the obtained *A. incisum* sporophytes produced spores twice in the first year under different external conditions, demonstrating the efficiency of the above propagation procedure.

CONCLUSION

The *in vitro* production of *A. incisum* plants via GGBs presented in this study includes the initiation of GGBs from parts of juvenile sporophytes used as explants and the propagation of GGBs on different media. After 1–2 weeks of explant culture *in vitro*, the initiation and proliferation of *A. incisum* GGBs was observed. These processes occur slightly earlier than in *P. craspedosorum* (Shelikhan 2024) and *Adiantum capillus-veneris* L. (Li et al. 2015. Some morphological differences of *A. incisum* GGBs as compared to other fern species were also noted (GGB color and overall appearance, absence of deformations). We have found that the use of a medium with a half concentration of micro- and macrosalts, without added plant growth regulators and the same medium with added cytokinin 6-benzylaminopurine allows initiation of the high total number of GGBs. However, the cytokinin inhibits sporophyte formation. On the contrary, the absence of growth regulators in the medium causes the sporophytes to regenerate. Thus, we recommend the alternation of the media (BAP-containing and BAP-free) for the best results in propagation of GGBs and sporophytes of *A. incisum*. The procedure showed high sporophytes survival rates under *ex vitro* conditions (89 % in a climatic room and 85 % outdoors) in the first year of cultivation.

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LITERATURE CITED

Amaki, W. & H. Higuchi 1992. A possible propagation system of *Nephrolepis*, *Asplenium*, *Pteris*, *Adiantum* and *Rumohra* (Arachniodes) through tissue culture. *Acta Horticulturae* 300:237–244.

Chernyagina, O.A. (ed.) 2018. *Red data book of Kamchatka Territory. Vol. 2. Plants*. Kamchatpress, Petropavlovsk-Kamchatskii, 388 pp. (in Russian). [Красная книга Камчатского края / под ред. О.А. Чернягиной. 2018. Петропавловск-Камчатский: Камчатпресс. 388 с.].

Higuchi, H., W. Amaki & S. Suzuki 1987. *In vitro* propagation of *Nephrolepis cordifolia* Prsel. *Scientia Horticulturae* 32:105–113.

Higuchi, H. & W. Amaki 1989. Effects of 6-benzylaminopurine on the organogenesis of *Asplenium nidus* L. through *in vitro* propagation. *Scientia Horticulturae* 37:351–359.

Jeong, J.G. 2011. A herbalogical study on the plants of Aspleniaceae in Korea. *The Korea Journal of Herbology* 26:59–65.

Kholia, B.S. & R. Joshi 2010. International Year of Biodiversity – Adopting innovative measures for conservation. NeBIO. 1(2):55–61.

Kozhevnikov, A.E. (ed.) *2008*. *Red data book of the Primorsky Krai: Plants. Rare and vulnerable plant species*. AVK "Apel'sin", Vladivostok, 688 pp. (in Russian). [Красная книга Приморского края: Растения. Редкие и находящиеся под угрозой исчезновения виды растений и грибов / под ред. А.Е. Кожевникова. 2008. Владивосток: АВК "Апельсин". 688 с.].

Li, X., Y.H. Fang, J.D. Han, S.N. Bai & G.Y. Rao 2015. Isolation and characterization of a novel somatic embryogenesis receptor kinase gene expressed in the fern *Adiantum capillus-veneris* during shoot regeneration *in vitro*. *Plant Molecular Biology Reporter* 33(3):638–647.

Liao, Y.K. & Y.H. Wu 2011. *In vitro* propagation of *Platycerium bifurcatum* (Cav.) C. Chr. via green globular body initiation. *Botanical Studies* 52:455–463.

Mehltreter, K. 2010. Fern conservation. In: Fern ecology. 1st ed. (K. Mehltreter, L.R. Walker & J.M. Sharpe, eds), pp. 323–359, Cambridge University Press, Cambridge.

Moon, S.H., J.L. Son, S.J. Shin, S.H. Oh, S.H. Kim & J.M. Bae 2021. Inhibitory effect of *Asplenium incisum* on bacterial growth, inflammation, and osteoclastogenesis. *Medicina* 57(7):641.

Murashige, T. & F. Skoog 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15:473–497.

Senchik, A.V. (ed.) 2020. *Red data book of Amur Region: rare and endangered species of animals, plants and fungi. 2nd ed.* Blagoveshchensk, 502 pp. (in Russian). [Красная книга Амурской области: редкие и находящиеся под угрозой исчезновения виды животных, растений и грибов. 2е изд. / под ред. А.В. Сенчик. 2020. Благовещенск. 502 с.]

Shelikhan, L.A. 2023a. *In vitro* regeneration of fern via green globular bodies. *Russian Journal of Plant Physiology* 70(2):160–170.

Shelikhan, L.A. 2023b. *In vitro* cultivation of the rare fern *Asplenium incisum* Thunb. In: *Problems of plant introduction and conservation of biological resources: materials of the All-Russian scientific conference with international participation (21 November 2023)*, pp. 221–224, Publishing House Digital Polygraphy, Voronezh (in Russian). [Шелихан Л.А. 2023. Введение в культуру *in vitro* редкого папоротника *Asplenium incisum* Thunb. // Проблемы интродукции растений и сохранения биологических ресурсов. Материалы Всероссийской научной конференции с международным участием (21 ноября 2023 г.) Воронеж: Изд-во Цифровая полиграфия. С. 221–224.]

Shelikhan, L.A. 2024. *In vitro* propagation of the rare fern *Polystichum craspedosorum* (Maxim.) Diels. via green globular bodies. *Botanica Pacifica* 13(2):93–97.

Shelikhan, L.A. & E.V. Nekrasov 2018. Fern propagation via spore culture *in vitro* (a review). *Byulleten' Botanicheskogo sada-instituta DVO RAN* 20:23–42 (in Russian with English summary). [Шелихан Л.А., Некрасов Э.В. 2018. Размножение папоротников посредством спор в культуре *in vitro* (обзор литературы) // Бюллетень Ботанического сада-института ДВО РАН. Вып. 20. С. 23–42].

Shin, S.L. & C.H. Lee 2009. Medium composition affecting *in vitro* plant regeneration and acclimation of *Pteris cretica* 'Wilsonii'. *Korean Journal of Plant Resources* 22(5):394–402.

Thakur, R.C., Y. Hosoi, K. & Ishii 1998. Rapid *in vitro* propagation of *Matteuccia struthiopteris* (L.). Todaro – an edible fern. *Plant Cell Reports* 18:203–208.

Voronov, B.A. (ed.) 2008. *Red data book of the Khabarovsky krai: Rare and endangered plant and animal species*. Priamurskiye Vedomosti, Khabarovsk, 632 pp. (in Russian). [Красная книга Хабаровского края: редкие и находящиеся под угрозой исчезновения виды растений и животных / под. ред. Б.А. Воронова. 2008. Хабаровск: Издательский дом "Приамурские ведомости". 632 с.].

Yu, R., F. Li, G. Wang, J. Ruan, L. Wu, M. Wu, C. Yang & Q. Shan 2021. *In vitro* regeneration of the colorful fern *Pteris aspericaulis* var. *tricolor* via green globular bodies system. *In Vitro Cellular & Developmental Biology – Plant* 57(2):225–234.

Yu, R., G. Zhang, H. Li, H. Cao, X. Mo, M. Gui, X. Zhou, Y. Jiang, S. Li & J. Wang 2017. *In vitro* propagation of the endangered tree fern *Cibotium barometz* through formation of green globular bodies. *Plant Cell, Tissue and Organ Culture* 128:369–379.

Zhang, G. & W. Su 2002. *In vitro* rapid propagation from young leaf of *Sinephropteris delavayi* (Franch.) Mickel. J. *Journal of Yunnan University: Natural Sciences Edition*. 24(3):234–236.