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Towards *ex situ* conservation of the moss *Campyliadelphus elodes* (Amblistegiaceae, Bryophyta)

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ABSTRACT:
The rare and threatened moss *Campyliadelphus elodes* (Amblysetgiaceae), whose population is in decline in Europe, was the subject of studies investigating its biological characteristics so as to establish a captive culture and an *ex situ* population. Herbarium material from Hungary was used to establish propagation from the vegetative parts under axenic laboratory conditions. Growth optimisation was tested by selecting the most suitable growth medium and evaluating the effects of plant growth regulators on rapid propagation and multiplication. The KNOP medium proved to be the most effective for the rapid propagation of the species when grown at a temperature of 18 ± 2°C, with 60–70% humidity, and a long-day photoperiod (16 h light/8 h dark cycle). The tested concentrations of auxin and cytokinin did not significantly improve the development and multiplication of *C. elodes*. Subsequently, the laboratory-cultivated material was used to establish an *ex situ* population at the Belgrade Botanical Garden, thus contributing to the species' sustainable preservation.

Keywords: bryophyte, protection, conservation physiology, threatened species, propagation, *in vitro*

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INTRODUCTION

The pleurocarpous moss *Campyliadelphus elodes* (Lindb.) Kanda (syn. *Campylium elodes* (Lindb.) Kindb.; *Kandaea elodes* (Lindb.) J. Kučera & Hedenäs, Amblystegiaceae) is classified as a Near Threatened (NT) species in Europe (Hodgetts *et al.* 2019a). Its overall population across Europe is declining. In various regions, particularly Central Europe, the species has been observed to be diminishing, and it is not commonly found throughout much of its range (Hodgetts *et al.* 2019b). This species is a perennial inhabitant of fens and other wetland habitats which are threatened by rapid climate change and severe hydrological fluctuations. These habitat types are often associated with numerous other threatened moss and liverwort species.

The species is dioecious and therefore sexual reproduction is rarely documented as inferred by the absence of sporophytes. Although vegetative propagation by patch separation is its main method of dispersal, no specific propa-

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gules or spread-vectors have been documented to date. The current threats to this species include habitat changes such as succession, eutrophication, alteration of the water regime, and drought, as well as land use and management. At a national and regional level, the species is red-listed in Finland, Norway, Great Britain, Ireland, Austria, Belgium, the Czech Republic, Germany, the Netherlands, Poland, Slovakia, Switzerland, Albania, Hungary, Romania and Lithuania (Hodgetts *et al.* 2019b). In Serbia, it has only been recorded once and it is red-listed as vulnerable (IUCN: VU; Sabovljević *et al.* 2024), with a high probability of extinction due to habitat destruction. Therefore, the species requires a physiological approach to conservation and active protection, at least in some parts of its European range.

Since the data on the biology of *C. elodes* remains limited, investigating the effects of different types of growth media and plant growth regulators on its morphogenesis is needed. This will provide valuable insights into the growth and development of this rare and threatened species under axenic conditions, i.e. providing the data for its *ex situ* conservation and propagation in captivity. To deepen our understanding of the nutrient requirements and *in vitro* propagation techniques for this species, the initial phase of this study focused on assessing the effects of three distinct types of growth media (namely BCD, KNOP, and MS/2), which are also used for the cultivation of other bryophyte species.

In this study, we investigated the following key issues: (1) What is the optimal positioning of the explants and the most suitable type of growth medium for the micropropagation of the species under axenic conditions? (2) How do exogenously applied plant growth regulators (PGRs) affect the morphogenesis of the species, and can the positioning of the explants influence their effects? (3) Can leafy gametophores be successfully utilised for acclimation and propagation under controlled, non-sterile conditions, as well as in outdoor environments, to prepare them for reintroduction into natural habitats?

MATERIAL AND METHODS

Plant material and in vitro culture establishment. The plant material for this study was collected on April 24, 2017, in Veszprém County, Hungary, from wetland habitat type *Caricetum elatae* Koch, 1926, at Gyepükaján (N 47°02’30.2”, E 17°17’13.9”, 160 m); leg./det. Beáta Papp. The sample is deposited in the Hungarian Natural History Museum in Budapest (voucher number BP192617).

Surface sterilisation of the gametophytes was conducted using various concentrations of sterilisation agents, including ethanol, sodium hypochlorite (NaOCl), and sodium dichloroisocyanurate (NaDCC) for different durations, as previously described (e.g. Jadranin *et al.* 2023; Ćosić *et al.* 2025). Since previously tested solutions either caused lethal effects on both gametophytes and spores or failed to eliminate contamination by xenic organisms, we applied a 3% NaDCC solution, which showed the greatest effectiveness in sterilising the moss gametophore material when applied for 5 minutes.

Experimental design. After more than two years of cultivating axenic (contaminant-free) cultures of *C. elodes* on minimal KNOP medium (Knop 1865) under standardised conditions [temperature of 18 ± 2°C, 60–70% humidity, and a long-day photoperiod (16 h light/8 h dark cycle)], cohabitant-free explants were propagated for the experiments. Decapitated explants, each measuring 1 cm in length, were subjected to different media types with or without the addition of plant growth regulators. Removing the tip of the plantlets, i.e. decapitation, serves to eliminate the majority of dividing cells in the apex, thereby ensuring that each explant has an equal opportunity of undergoing

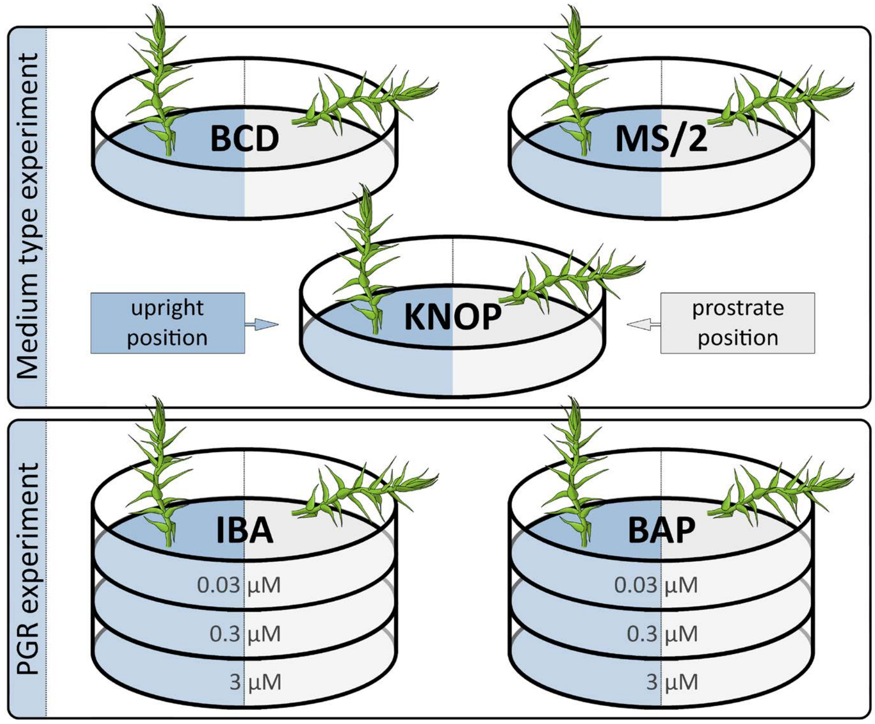

Fig. 1. A graphical illustration of the experimental design. The "Medium type experiment" involved three distinct solid media (KNOP, MS/2 and BCD), with the explants placed in two positions, either upright or prostrate. The "PGR experiment" consisted of various setups, where different concentrations (0.03, 0.3 and 3 μM) of IBA and BAP were added to the KNOP medium, with the explants positioned either upright or prostrate.

the dedifferentiation process and producing new shoots. Namely, gametophore initials undergo three rounds of cell division to generate a tetrahedral-shaped apical cell, which subsequently cleaves spirally to produce a leafy shoot.

Such explants were subjected to two different experimental setups (shown in Fig. 1). In the "Medium type experiment" the plantlets were exposed to three different growth media types to determine the optimal media composition for *in vitro* growth and development. Individual explants were placed on solid KNOP medium, half-strength Murashige & Skoog medium (Murashige & Skoog 1962), and BCD medium (Sabovljević *et al.* 2009). Additionally, the explants were positioned both upright and prostrate on the media to investigate the influence of position on the morphogenesis of the investigated species, as well as the nutrient transport through the plantlets.

In the "PGR experiment" the plantlets were grown on media containing different concentrations of plant growth regulators (PGRs), specifically auxins and cytokinins, to examine their effects on the growth and development of the target moss. Different concentrations (0.03, 0.3, and 3 μM) of IBA (indole-3-butyric acid) and BAP (6-benzylaminopurine) were added to the KNOP medium, i.e. exogenously applied on the moss explants which were placed in both upright and prostrate positions. The KNOP medium was selected for further experiments because the gametophores grew well and formed numerous new shoots without secondary protonema as confirmed in previously conducted experiments aimed at the selection of the best medium type.

The pH of the media in all the experimental setups was adjusted to 5.8 prior to sterilisation at 121°C for 30 minutes. All the treatments used in this study consisted of four individual explants in five separate Petri dishes, i.e. a total of 20 gametophores per treatment. The explants were cultured under axenic conditions in sterile Petri dishes at a temperature of 18 ± 2°C and ca. 60–70% humidity. They were exposed to a long-day photoperiod (16 h light/8 h dark cycle) with fluorescent tubes (Tesla Pančevo) providing a light intensity i.e. PPFD (Photosynthetic Photon Flux Density) of 50 μmol m^{-2} s^{-1}.

After 4 weeks of experimentation, the morphogenetic changes were observed and measured, including the formation of new shoots on the initial explant (i.e. index of multiplication), and the survival rate. The diameter of the secondary protonemal patches was not measured due to visibility i.e. non-vigorous development. The morphological parameters were measured and photographed using a Leica MZ stereomicroscope (Leica MZ 7.5, Bi-Optic Inc., Santa Clara, CA, USA).

Acclimation of *in vitro* propagated plant material. The moss material, grown under laboratory-controlled axenic conditions, was acclimated in two stages. Initially, it was moved to xenic conditions with the application of rainwater, following the controlled parameters outlined for axenic cultivation (temperature of 18 ± 2°C, 80% humidity, and a long-day photoperiod of 16 hours of light and 8 hours of darkness). The plantlets were placed on filter paper soaked in non-sterilised rainwater collected within the Botanical Garden Jevremovac at the Faculty of Biology, University of Belgrade. These plants were then allowed to adapt and coexist with naturally occurring xenic organisms, i.e. to ensure interaction with airborne and waterborne inhabitants without negative i.e. lethal consequences.

In the second stage, the moss plants were placed in semi-shaded, inclined plastic basins (at an angle of 30°) in the autumn (November 10, 2021). The plants were positioned at the edge of the water in the transition area of the inclined basin and covered with an inert plastic net to fix them onto the moist filter paper. The basins were maintained free of leaf litter and other external contaminants.

Statistical analysis. The statistical analysis was performed using the R programming language (v. 4.3.2) (R Core Team 2023). Initial data exploration included the Shapiro-Wilk normality test and Levene's test for homogeneity of variance, both of which indicated violations of normality and homoscedasticity across the groups. Consequently, a nonparametric factorial ANOVA was conducted employing the Align Rank Transform (ART) procedure (Elkin *et al.* 2021; Wobbrock *et al.* 2011) from the "ARTool" R package (Kay *et al.* 2021). The factorial models were constructed with the "art" function and the significance of the main effects and interactions was evaluated using the "anova" function. Post-hoc contrast tests were conducted using the "art.con" function from the same R package.

RESULTS AND DISCUSSION

The multiplication index (IM) of *C. elodes* ($p < 0.001$) was significantly influenced by the main effects of explant position (EP) and medium type (MT). However, no significant interaction between these main effects was observed. Among the tested conditions, the prostrate-positioned plants grown on KNOP minimal medium exhibited the highest IM values, followed by the prostrate-positioned explants on BCD and MS/2 media, respectively (Fig. 2). Across all media types, the prostrate-positioned plants consistently showed higher IM values compared to the upright-positioned plants ($p < 0.05$). Although no statistically significant differences in IM were observed between the prostrate- and upright-positioned plants grown on BCD or KNOP media, a higher number of newly developed shoots was recorded for those plants grown on KNOP medium (Fig. 2).

Moreover, the plants developed normally, producing numerous new shoots from the primary explants grown both on BCD and KNOP media in both positions (Fig. 3A, B, D, and E). In contrast, those grown on MS/2 exhibited less vigorous growth (Fig. 3C and F). Overall, since the highest index of mul-

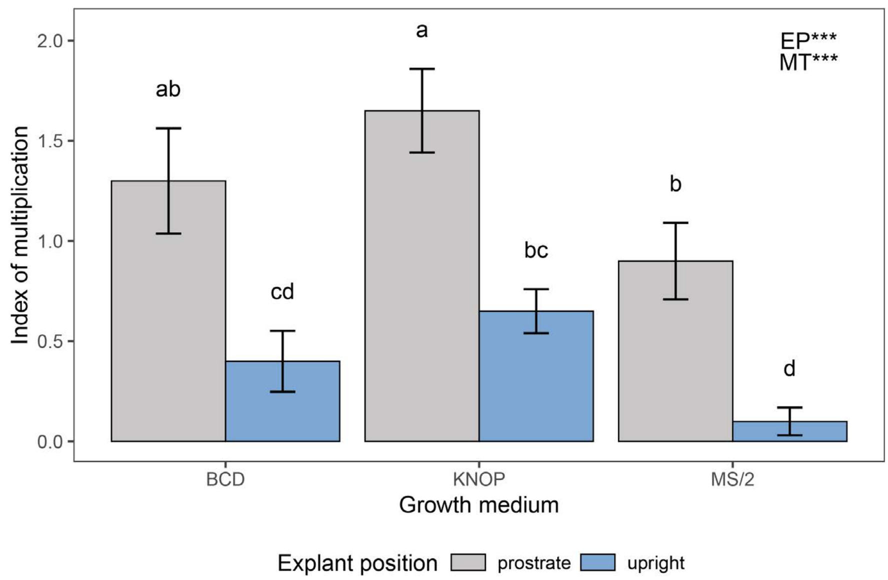

Fig. 2. The effects of explant positioning and different growth media types on the index of multiplication of *Campyliadelphus elodes*. The data are presented as the mean ± standard error. Different letters above the bars indicate statistically significant differences $p < 0.05$) among the experimental groups. The significance of the main effects of explant position (EP) and media type (MT) after factorial analysis is denoted by symbols (***$p < 0.001$).

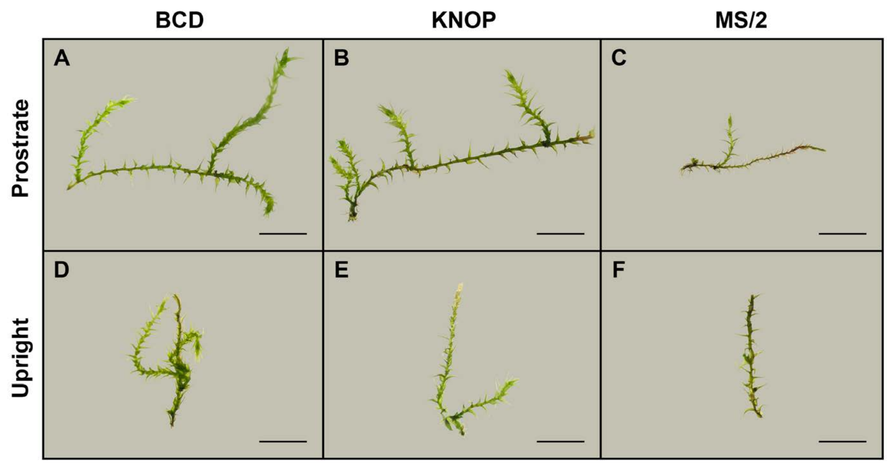

Fig. 3. The appearance of *Campyliadelphus elodes* explants grown on BCD, KNOP, and MS/2 media. Scale bars = 2 mm.

tiplication was recorded for the plants grown on KNOP medium, it could be considered optimal for the rapid biomass production of *C. elodes*.

In the mosses grown on IBA enriched medium, the main effects of both explant positioning and the interaction between explant position and IBA concentration in the medium significantly influenced the IM values in *C. elodes* ($p < 0.001$ and $p < 0.05$, respectively) (Fig. 4A). No significant differences in the IM values were observed among the tested IBA concentrations present in the medium (control, 0.03, 0.3, and 3 μM IBA) with either the prostrate or upright-positioned moss explants. However, a significant difference in IM was observed in all the experimental groups between the prostrate and upright-positioned plants ($p < 0.05$), with the prostrate-positioned plants exhibiting substantially higher IM values (Fig. 4A).

In those mosses grown on media enriched with BAP, the IM values were significantly influenced by explant positioning, BAP concentration, and their interaction ($p < 0.001$) (Fig. 4B). The plants grown on KNOP medium without exogenously added BAP (control group) exhibited the highest IM values

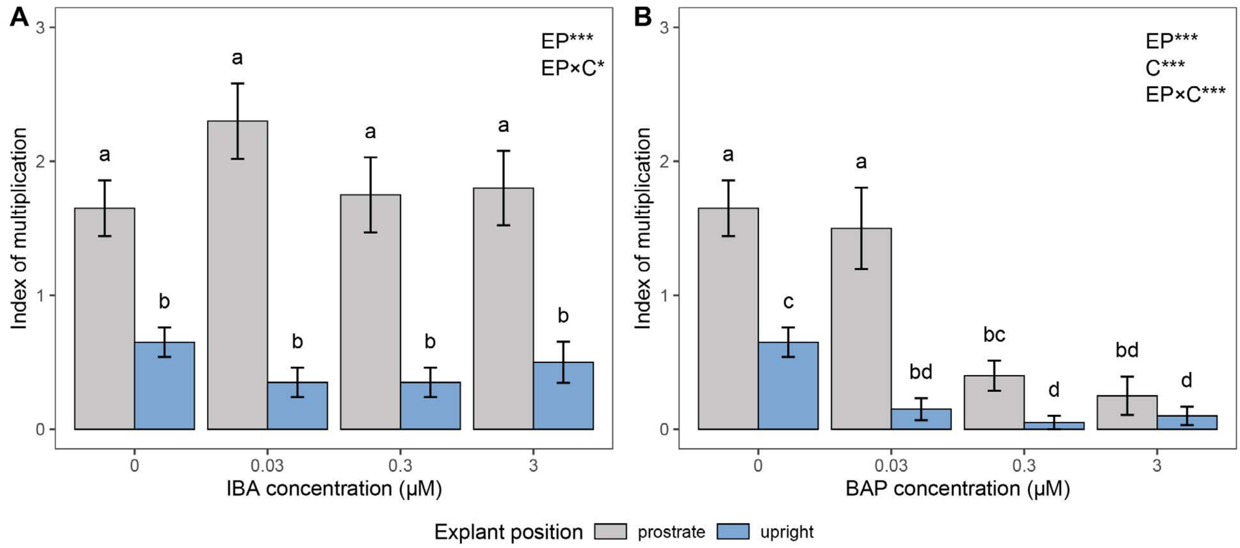

Fig. 4. The effects of explant positioning and varying IBA (A) and BAP (B) concentrations on the index of multiplication of *Campyliadelphus elodes*. The data are presented as the mean ± standard error. Different letters above the bars denote statistically significant differences ($p < 0.05$) among the experimental groups. The significance of the main effects of explant position (EP), plant growth regulators concentrations (C), and their interaction (EP×C) after factorial analysis is denoted by symbols (***$p < 0.001$, *$p < 0.05$).

(number of newly developed shoots) in both explant positions, with the IM values decreasing with increasing BAP concentrations (Fig. 4B). In the prostrate-positioned plants, higher BAP concentrations (0.3 and 3 μM) significantly decreased the IM values compared to the control group ($p < 0.05$) (Fig. 4B). On the other hand, for the upright-positioned explants, all the applied BAP concentrations significantly inhibited new shoot development, leading to a decrease in the recorded IM values compared to the control group ($p < 0.05$) (Fig. 4B).

The appearance of the plants further confirmed the results presented in Fig. 4. Namely, the addition of IBA did not significantly affect the development of new shoots, especially in the prostrate-positioned plants (Fig. 5 A–C) compared to the control group (Fig. 3B). Similar morphological traits were recorded for the upright-positioned plants which generally had lower IM values (Fig. 5 D and F) than the upright-positioned control plants (Fig. 3E). This suggests that exogenous IBA did not efficiently promote the massive propagation of *C. elodes*.

On the other hand, the application of BAP alone significantly decreased the IM values (Fig. 4B), particularly in prostrate-positioned plants subjected to higher BAP concentrations (Fig. 5H and I). A similar trend was also documented in the plants grown in the upright-position in the presence of BAP (Fig. 5J–L). Overall, the addition of BAP seems not to be favourable for the production of new shoots in *C. elodes,* although small buds were visible on the primary explants (Fig. 5H, K, and L).

In late autumn (10 November 2021), the moss plants, which had been developed under *in vitro* conditions and subsequently acclimatised, were placed outdoors in the Botanical Garden Jevremovac (Fig. 6). By the following spring 2022, the plants remained green and moist and had also begun to develop and spread significantly. Over the course of a year, spanning both the winter and summer seasons, the plants were completely submerged on rainy days but were never allowed to dry out completely during hot summer days. Under these conditions, they successfully covered an entire 4 dm^2 basin area (Fig. 6C).

The obtained results clearly show that the highest index of multiplication (IM) was observed for both the upright and prostrate positioned plantlets

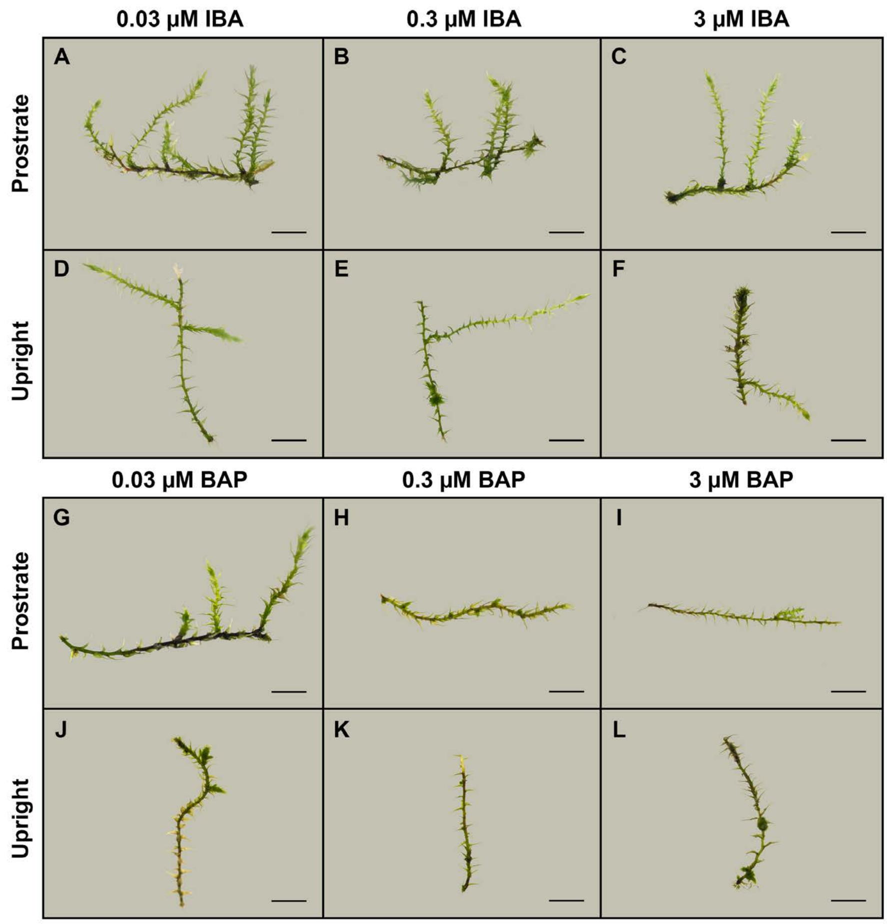

Fig. 5. The appearance of *Campyliadelphus elodes* explants grown on KNOP medium supplemented with different IBA (A-F) and BAP (G-L) concentrations. The control group plants are shown in Figures 3B and 3E. Scale bars = 2 mm.

on the KNOP medium, with the BCD medium also proving to be suitable for the propagation of *C. elodes* (Figs. 2, 3A and B). These findings are not unexpected considering that the KNOP medium is the most widely used for the cultivation of numerous bryophyte species such as mosses *Physcomitrella patens* (Hedw.) Bruch & Schimp., *Physcomitrium pyriforme* Hedw., *Atrichum undulatum* (Hedw.) P. Beauv., *Plagiomnium undulatum* (Hedw.) T.J.Kop., *Aulacomnium androgynum* (Hedwig) Schwagrichen, *Rhynchostegium murale* W. P. Schimper, *Brachythecium rutabulum* (Hedw.) Schimp., *Thuidium* sp., and liverwort *Marchantia polymorpha* L., for example (Beike *et al.* 2010), as well as for some other bryophyte representatives i.e. *Sphagnum* sp. (Såstad *et al.* 1998; Beike *et al.* 2014; Natcheva & Cronberg 2023). Similarly, a study on *Drepanocladus lycopodioides* (Brid.) Warnst., a pleurocarpous species from the Amblystegiaceae family, which inhabits environments similar to *C. elodes*, found the KNOP medium to be the most suitable for *in vitro* propagation (Jadranin *et al.* 2024). In addition to the KNOP medium, the BCD medium also showed strong potential for the cultivation of *C. elodes* when prostrate-positioned explants were considered. Previous studies have shown that the BCD medium is favourable for the development of various bryophyte species, such as *Molendoa hornschuchiana* (Hook.) Lindb. ex Limpr. (Vujičić *et al.* 2012), *Bruchia vogesiaca* Nestl. ex Schwägr. (Sabovljević *et al.* 2012), bryo-halophyte *Hennediella heimii* (Hedw.) R.H. Zander (Ćosić *et al.* 2022), *Vesicularia montagnei* (Bel.) Fleisch. (Hu *et al.* 2023), as well as *Amblystegium serpens* Schimp. (Cvetić *et al.* 2005). As with BCD, the prostrate-positioned

Fig. 6. *Campyliadelphus elodes* grown in *ex situ* conditions in semi-shaded, inclined plastic basins with rainwater within the Botanical Garden Jevremovac, Faculty of Biology, University of Belgrade, Serbia. A) Plants began to develop and grow over the plastic net, five months after outside inoculation (early spring, 10 March 2022); B) Spring to summer growth of plants (10 June 2022); C) Appearance of plants grown for a year when they fully covered the space of plastic basins (10 November 2022).

explants also developed well on the MS/2 medium, but to a significantly lesser extent compared to the other two tested growth media (Figs. 2, 3C and 3F). Although it is not frequently used, the MS/2 medium has proven to be effective for the growth of some moss species such as *Pterigoneurum sibiricum* Otnyukova (Jadranin *et al.* 2023), *Rhodobryum giganteum* (Schwägr.) Paris (Chen *et al.* 2009), and liverwort *Lunularia cruciata* (L.) Dumort. ex Lindb. (Mukhia *et al.* 2019).

There is no universal nutrient media composition for bryophyte cultivation as confirmed by studies on multiple species. Bryophytes are highly specific to their microhabitats and are therefore likely to differ in their nutrient requirements (apart from the basic ones) for rapid development and propagation. In addition, the three main lineages of bryophytes are rather phylogenetically distant, so it is not surprising that they display varying responses to different media supplements and formulations. The investigated species *C. elodes* thrives in moist, swampy areas and fens rich in nitrogen and calcium, especially those that are periodically flooded. All the tested media in this study were found to align with the specific nutritional needs of the species, albeit with different impacts. The KNOP medium contains only $Ca(NO_3)_2 \times 4H_2O$ as a nitrogen source, while the BCD medium provides nitrogen in higher concentrations in the form of KNO_3 (Sabovljević *et al.* 2009). Thus, both the KNOP and BCD media most likely meet the nutrient requirements of *C. elodes*, as expected. However, due to differences in nitrogen sources and nitrate concentrations, the KNOP medium is assumed to offer a more balanced nitrate ratio relative to the other macroelements in its composition and/or accessibility to plantlets, thereby favouring the multiplication of this species. On the other hand, the MS/2 medium consists of two different nitrogen sources, namely NH_4NO_3 and KNO_3, which may have an adverse effect on the formation of new shoots in the investigated species.

When observing the positioning of the explant within a single media type, it becomes clear that the position of the plant significantly affects the uptake of nutrients from the medium (Fig. 2). The rostrate-positioned explants grown on selected types of media produced a higher number of newly formed shoots, suggesting that this position is particularly favourable for the production and new development of *C. elodes* gametophores (Figs. 2, 3A–C). Additionally, prostrate-positioned explants have a longitudinal side immersed in the medium, which improves the whole-body nutrient uptake from the substrate. In contrast, another study carried out on pleurocarpous moss *D. lycopodioides* showed that upright-positioned explants produced a higher number of new shoots (Jadranin *et al.* 2024). In the present study, it can be concluded that higher biomass production, i.e. higher IM values occurs when the plant surface is in close contact with the medium, allowing a larger surface area to contribute to nutrient uptake. Although further detailed studies are required, these results provide a solid basis for future research on the propagation of the selected species.

The second phase of this study, i.e. the effects of selected plant growth regulators on *C. elodes* moss development and morphogenesis, did not show the expected results bearing in mind that cytokinins and auxins play important and interdependent roles in several steps of gametophytic development in bryophytes (Chopra & Sarla 1986). Mosses are known to synthesize cytokinins and auxins in specific forms, primarily the *cis*-zeatin (*cis*Z) type in nature, the isopentenyl adenine (iP) type under controlled conditions, and free indole-3-acetic acid (IAA) (Drábková *et al.* 2015). Nevertheless, a deeper understanding of how growth regulators influence developmental changes in bryophytes is still required. The addition of IBA to the medium showed no significant differences compared to the control treatment (PGR-free medium) (Fig. 4A). Furthermore, exogenous IBA did not affect the morphological changes of the tested plantlets (Fig. 5A–F). Therefore, it can be concluded that the tested IBA concentrations were mostly ineffective in promoting the propagation of *C. elodes*. Moreover, the addition of IBA was found to reduce the formation of new buds in previous studies on moss species *D. lycopodioides* (Jadranin *et al.* 2024), *Entosthodon pulchellus* (H. Philib.) Brugués (Ćosić *et al.* 2025), and *P. sibiricum* (Jadranin *et al.* 2023). This effect is likely due to the fact that the application of auxins can sometimes disrupt the endogenous hormone balance, thereby inhibiting bud formation in mosses, particularly at higher concentrations (Sarla & Chopra 1987). On the other hand, interesting results were obtained regarding the positioning of *C. elodes* explants. The prostrate-positioned explants developed significantly more newly formed shoots compared to those positioned upright (Fig. 4A). This finding supports our hypothesis that the prostrate position facilitates the efficient uptake of nutrients and hormones from the medium.

Similar to IBA, the lowest BAP concentration (0.03 μM) in the prostrate-positioned explants showed no difference compared to the control treatment (Fig. 4B). On the other hand, when the BAP concentration was increased, both the prostrate- and upright-positioned plants exhibited significant inhibition of new bud formation. However, this result was expected, as low concentrations of cytokinins often promote the development of normal gametophytes (Ashton *et al.* 1979) compared to very high concentrations which lead to the formation of defective gametophytes (Chopra & Kumra 1988). In addition, cytokinins mainly affect bud formation on the caulonemal cells when the protonema is formed (Ashton *et al.* 1979). Similar results have been reported in studies on *D. lycopodioides* – also from the family Amblystegiaceae (Jadranin *et al.* 2024), as well as in acrocarpous mosses such as *H. heimii* (Ćosić *et al.* 2022), *Bryum argenteum* Hedw., *A. undulatum* (Bijelović *et al.* 2004), and *Plagiomnium cuspidatum* Hedw (Nyman & Cutter 1981). In contrast, some studies have shown that exogenously applied BAP leads to an increase in the number of newly formed buds, as observed in *P. patens* (Ashton *et al.* 1979). The supplementation with IBA and BAP in our study exerted no positive effect on the biomass production of *C. elodes*. Nonetheless, it has been shown that certain concentrations of exogenously added PGRs may have a positive effect on the development of some mosses (Sarla & Chopra 1987). Therefore, further experiments investigating a wider range of PGR concentrations as well as different types of PGRs applied individually or in combination would be valuable to determine the optimal concentrations for the mass propagation of certain species. In this study, the addition of PGRs had no positive effect on promoting the development and propagation of *C. elodes* since the highest number of newly formed buds was recorded in PGR-free conditions, i.e. the control plants (Fig. 4A and B). The spontaneous development of buds on KNOP minimal medium has also been recorded for other bryophyte species, such as *P. patens* (von Schwartzenberg 2009), *E. pulchellus* (Ćosić *et al.* 2025), and *D. lycopodioides* (Jadranin *et al.* 2024). Nevertheless, investigating the impact of PGRs on morphogenesis is not only valuable for large-scale propagation, but also for the induction of sex organs, which are rarely obtained in axenic conditions. Further research is needed on a wider range of PGRs across diverse species to assess their interactions with other key growth conditions. Additionally, molecular sex markers would serve to determine whether the *ex situ* vegetative plants are male or female specimens, an area of research urgently needed for both wild and captive population conservation.

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COMPREHENSIVE PLANT BIOLOGY

REZIME

Ex situ konzervacija mahovine *Campyliadelphus elodes* (Amblistegiaceae, Bryophyta)

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Retka i ugrožena mahovina *Campyliadelphus elodes* (Amblysetgiaceae), čije su populacije u opadanju u Evropi, bila je predmet studija koje su istraživale njene biološke karakteristike kako bi se uspostavila kultura u kontrolisanim uslovima i populacija *ex situ*. Za propagaciju iz vegetativnih delova u akseničnim laboratorijskim uslovima korišćen je herbarski materijal iz Mađarske. Optimizacija rasta je testirana odabirom najpogodnijeg medijuma za rast i procenom efekata regulatora rastenja biljaka na brzu propagaciju i multiplikaciju. Podloga KNOP se pokazala kao najefikasnija za brzo razmnožavanje vrste kada se uzgaja na temperaturi od 18 ± 2°C, vlažnosti od 60–70% i dugodnevnom svetlosnom ciklusu (16 h svetlost/8 h mrak). Ispitane koncentracije auksina i citokinina nisu značajno poboljšale razvoj i umnožavanje *C. elodes.* Laboratorijski kultivisan material je potom korišćen za uspostavljanje *ex situ* populacije u Botaničkoj bašti u Beogradu, čime se doprinelo održivom očuvanju vrste.

Ključne reči: briofite, zaštita, konzervaciona fiziologija, ugrožene vrste, propagacija, *in vitro*