Botanica SERBICA

(2022) 46 (2): 187-195

DOI: https://doi.org/10.2298/BOTSERB2202187C
journal homepage: botanicaserbica.bio.bg.ac.rs

*Original Scientific Paper*

# Micropropagation of rare bryo-halophyte *Hennediella heimii*

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**ABSTRACT:**
The rare moss species *Hennediella heimii* (Pottiaceae) was established in *in vitro* culture. Various treatments were tested to achieve axenical cultures. The most effective sterilising procedure was NaDCC treatment of sporophytes, keeping the spore viability and giving high disposal of xenic cohabiting organisms. The effects of plant growth regulators were studied regarding new shoot formation, i.e. bud formation on the protonemal filaments and protonemal patch size. Low concentrations of cytokinin and medium concentrations of auxin are shown to increase protonemal patch size and shoot production. Multiplication of *H. heimii* was observed to occur spontaneously on BCD medium type, but to achieve better and rapid biomass production and development it is suggested to grow it on a BCD medium enriched with auxin and cytokinin combined.

**Keywords:**
axenic culture, *in vitro*, plant growth regulators, mosses, multiplication, development

UDC: 561.32:631.53

Received: 02 March 2022
Revision accepted: 13 May 2022

## INTRODUCTION

Growing plants (including bryophytes) in controlled *in vitro* conditions has been recognised as a valuable method for the *ex situ* conservation of endangered and rare species, and their reintroduction into potential natural habitats (Sabovljević *et al.* 2003, 2012; Bijelović *et al.* 2004; Prence 2004; Rowntree 2006). Experimentation in controlled conditions is essential in understanding bryophyte development (Bopp 1977; Chaban *et al.* 1998; Duckett *et al.* 2004; Cove *et al.* 2006; Mallón *et al.* 2006; Clarke & Robinson 2008; Vujičić *et al.* 2009; Lobachevska *et al.* 2021), abiotic stress tolerance (Bopp & Werner 1993; Frank *et al.* 2005; Bogdanović *et al.* 2011; Ćosić *et al.* 2020a, b, 2021) or simply developmental biology and the biotechnology of bryophytes (Sabovljević & Sabovljević 2008, 2010; Decker & Reski 2020). Although the first ever *in vitro* culture of plants was established on bryophytes by Servettaz (1913), many difficulties have emerged in their *in vitro* culturing. The lack of cuticles and single cell layered phylloids are one of the reasons why bryophytes are sensitive to xenic organism disposal through the sterilisation process, making the establishment of *in vitro* cultures and growing bryophytes in axenic conditions a rather difficult and time-consuming process. In addition to the collection of rare and clean entities in proper developmental phases and storing bryophyte material prior to laboratory usage, microorganisms living on plants and the maintenance of genetic variation of species can also present a problem in the establishment of proper *in vitro* cultures (Duckett *et al.* 2004). However, bryophytes are excellent subjects for fundamental and applied studies including physiology, ecology, genetics, evolution and biotechnology, with many advantages compared to vascular plants (Sabovljević *et al.* 2003). The main disadvantages are the achievement of appropriate developmental stages and biomass yield of these slow growing plants, and problems in the extrapolation of known procedures having in mind that success in one species does not necessarily mean success

in another due to the lack of knowledge on species biology, especially that of rare and threatened taxa. On the other hand, the main advantages are haploid dominance (easier expression of traits) and non-demanding growth conditions (i.e. space and money saving).

In the last few decades, many investigations on plant growth regulators and their effects on bryophyte development in *in vitro* culture have been conducted, especially on the model moss organism *Physcomitrium patens* (Hedw.) Mitt. [syn. *Physcomitrella patens* (Hedw.) Bruch. & Schimp.] (e.g. Reski *et al.* 1991; Reski 1998; Decker *et al.* 2006; Prigge & Bezanilla 2010) and moss *Funaria hygrometrica* Hedw. (Bopp *et al.* 1978, 1991; Bopp & Jacob 1986). Most of the research was carried out with essential phytohormones, namely auxins and cytokinins, which are known to be the key regulators of growth and development in plants i.e. bryophytes (Cove & Ashton 1984). However, not much data can be found on plant growth regulators or their mode of action in various species of bryophytes although most are documented to be present and effective in studied representatives (Sabovljević *et al.* 2014a, b). Bryophyte morphogenesis is also documented in axenic conditions because of their simple structure, but not many species have been the subject of such studies. Recently, studies have focused on other bryophyte species (e.g. Bijelović & Sabovljević 2003; Sabovljević *et al.* 2003, 2006, 2012; Bijelović *et al.* 2004; Buczkowska *et al.* 2006; Chen *et al.* 2009; Vujičić *et al.* 2009; Awasthi *et al.* 2010, 2011, 2013). Still, we are far from obtaining a clear picture of the developmental mechanisms in such a diverse and phylogenetically distant plant group as bryophytes. Interestingly, rather few investigations are conducted on liverworts and hornworts. The responses of the tested bryophyte to auxins and cytokinins were rather specific, i.e. the same concentrations of hormone induced some changes in morphology in one species or group of bryophytes, whereas in others it had no effect or induced different changes (von Schwartzenberg 2009). According to the literature (summarised in Sabovljević *et al.* 2014b), auxin leads to the inhibition of protonema growth, the stimulation of rhizoid formation, the transformation of buds into filaments, callus induction and the suppression of phylloid development on stems (Sokal *et al.* 1997), but also the transition of chloronema to caulonema (Cove & Ashton 1984). On the other hand, cytokinins affect the formation of buds, their number and position along the caulonema, and protonemal cell divisions (Szweykowska *et al.* 1971). However, the interaction of those two plant regulators seems to be among the key regulators of developmental changes in mosses. It was previously reported that auxin could stimulate changes when applied in low concentrations, while high concentrations were rather inhibitory. Additionally, cytokinin affects a specific morphogenetic change in protonema development, but often through the interaction with auxin, which needs to be present in sufficiently high concentrations (Cove & Ashton 1984; Schumaker & Dietrich 1998).

The moss *Hennediella heimii* (Hedw.) R.H. Zander [syn. *Pottia heimii* (Hedw.) Hampe, *Desmatodon heimii* (Hedw.) Mitt.; Pottiaceae] is a circumpolar Boreo-temperate species (Hodgetts *et al.* 2019a). Its European range includes the Atlantic and Baltic coasts, from the Massif Central in France north to Iceland and Svalbard. It is present in central parts of Europe, but due to high habitat destruction (salty grassland type), its distribution there is scattered. Outside the European region the species occurs in northern Asia and a few localities are also known in central Asia, Japan, North America, south to New Mexico and Newfoundland, southern South America, Tasmania, New Zealand, the Subantarctic Islands and the Antarctic Peninsula (Blockeel *et al.* 2014).

In terms of its ecology this moss species can be regarded as a bryo-halophyte (Blockeel *et al.* 2014). It is the most characteristic bryophyte of salt-marshes, where it grows in grazed turf, on footpaths and on disturbed ground on upper marshes (Blockeel *et al.* 2014). It is also frequent in coastal habitats, including sandy or muddy ground between boulders on beaches, soil at the foot of cliffs and sea walls, the banks of dykes and tidal rivers, rock ledges and crevices, and short turf on cliff slopes and cliff tops (Hodgetts *et al.* 2019a). In central Europe it usually inhabits the edges of pools by salt springs, salt flats or salty grasslands. It has occasionally been recorded inland in ruderal, apparently non-saline habitats. The capsules are abundant, usually maturing from February to May, but recorded throughout the year.

The overall European population size seems to be large and therefore it is not red-listed for Europe (Hodgetts *et al.* 2019b), however, some local extinctions have been documented in Austria, Spain and the Lorraine region in Northeastern France (Mahévas *et al.* 2010; Garilleti & Albertos 2012; Hodgetts *et al.* 2019a). It is estimated to be Critically Endangered (IUCN: CR) in Slovakia, Endangered (IUCN: EN) in Hungary and Romania and Vulnerable (IUCN: VU) in Switzerland (Hodgetts & Lockhart 2020). It is red-listed as threatened in Austria, Germany and Poland, while in Ukraine it is rare. In Italy, Slovenia and Czechia, it is considered a Data Deficient species, while in Spain it is Extinct (Hodgetts & Lockhart 2020). In Serbia, this species is reported only once, more than a hundred years ago, from Southern Serbia in the district of Pčinjski (Pantović *et al.* 2021), in the area surrounding Vranje (Pavletić 1955).

The species is thus interesting for investigation not only from the developmental and eco-physiological perspective (not so many bryo-halophytes were previously described), but also as a subject of high conservation interest. The species expresses very interesting features regarding salt stress adaptation, including rapid ontogenesis, as well as biochemical and physiological changes (Ćosić *et al.* 2020a, b, 2021).

The aim of this research was to establish axenic *in vitro* cultures of *Hennediella heimii* and to define the conditions needed for the achievement of fully developed gametophytes and their easy micropropagation. An additional aim was to investigate the effect of essential growth regulators auxin and cytokinin on the development of this moss and biomass production.

## MATERIAL AND METHODS

**Plant material**. Bryo-halophyte *Hennediella heimii* originated from Hungary (accession Győr-Moson-Sopron area, Hungary; 11.05.2009 (N 47.673616°, E 16.831676°) leg. /det. B. Papp). Sporophytes of *H. heimii* dry material deposited in BEOU-Bryo (University of Belgrade Herbarium bryophyte collection) were used as the start material for the establishment of axenic *in vitro* cultures. Mature and fully developed sporophytes were separated and kept in Eppendorf tubes at +4°C before the sterilisation process, with the aim of preventing axenic cohabitant development after the cleaning process (e.g. fungal hyphae).

**Sterilisation of the plant material**. The first phase of the establishment of axenic *in vitro* cultures of *H. heimii* was the sterilisation of the herbarium material. The developed gametophytes and sporophytes – capsules and setae, were subject to the sterilisation process. Firstly, the sporophytes (enclosed capsule with spores) were gently separated, washed several times with distilled water and then immersed in solutions which serve as a disinfectant. Different concentrations of sodium hypochlorite (NaOCl) – 1%, 3%, 5%, 7%, 10%, and 13%, ethanol – 30%, 50%, and 70%, and sodium-dichloroisocyanurate (NaDCC) – 1%, 3%, and 5% were used as the sterilising solutions (Table 1). Combinations of 10% ethanol and different concentrations of NaOCl were also used. Additionally, the duration of the sterilisation process was also varied for 10% NaOCl and 3% NaDCC (60, 90 and 120 seconds) with the aim of testing whether the exposure time of the plant material to the sterilisation agents had any effect on the survival rate, whereas for the other sterilising solutions the duration of sterilisation was 90 seconds (Table 1). After the sterilisation process, the moss material was rinsed with sterile distilled water, the capsules were opened with sterile forceps and the spores released on solid basal BCD agar medium (containing $MgSO_4$, $KH_2PO_4$, $KNO_3$ and $FeSO_4$; see Sabovljević *et al.* (2009) for medium content and preparation details). The plant material was grown in sterile chambers at a constant temperature (18±2°C), humidity 60-70%, and a long day light regime (16 h light/8 h dark) for several weeks, and constantly checked for infections. When the axenic *in vitro* cultures were established, the plants were used for experiments.

**In vitro micropropagation of the plant material.** After the establishment of axenic cultures i.e. the achievement of axenic plantlets, solid agar BCD medium with the addition of different concentrations of auxin (indol-3-butyric acid, IBA) and cytokinin (6-benzylaminopurine, BAP) was used for the micropropagation of *H. heimii*. The effects of IBA and BAP on the plant explants were investigated, as well as their synergistic effect on morphogenesis. Various concentrations of both growth regulators were used: control (0), 0.03 µM, 0.1 µM, 0.3 µM, 1 µM, 3 µM and 10 µM. Approximately 40 plant explants (gametophores) of 5 mm in length were used per treatment to study the influence of phytohormones on morphogenesis. The experimental treatments were run in triplicate. The plants were grown in the same conditions as in the previous phase. After six weeks, morphogenesis parameters such as the index of multiplication (IM) and secondary protonemal patch diameter were measured. IM referred to the newly formed shoots from one initial plant explant. Changes in morphology were documented using a stereo microscope (Leica MZ75) after three and six weeks respectively.

**Table 1**. The influence of different treatments and sterilisation time on the survival of *Hennediella heimii* (spores) (NaOCl – sodium hypochlorite, NaDCC – sodium dichloroisocyanurate)

| Sterilization methods | Duration of sterilization (s) | Spore survival rate after sterilization (%) |
|---|---|---|
| 1% NaOCl | 90 | 0 |
| 3% NaOCl | 90 | 16 |
| 5% NaOCl | 90 | 19 |
| 7% NaOCl | 90 | 18 |
| 10% NaOCl | 60 | 9 |
| 10% NaOCl | 90 | 8 |
| 10% NaOCl | 120 | 8 |
| 13% NaOCl | 90 | 3 |
| 30% ethanol | 90 | 0 |
| 50% ethanol | 90 | 0 |
| 70% ethanol | 90 | 0 |
| 1% NaOCl + 10% ethanol | 90 | 0 |
| 3% NaOCl + 10% ethanol | 90 | 0 |
| 5% NaOCl + 10% ethanol | 90 | 0 |
| 7% NaOCl + 10% ethanol | 90 | 0 |
| 10% NaOCl + 10% ethanol | 90 | 0 |
| 13% NaOCl + 10% ethanol | 90 | 0 |
| 1% NaDCC | 90 | 18 |
| 3% NaDCC | 60 | 22 |
| 3% NaDCC | 90 | 27 |
| 3% NaDCC | 120 | 45 |
| 5% NaDCC | 90 | 41 |

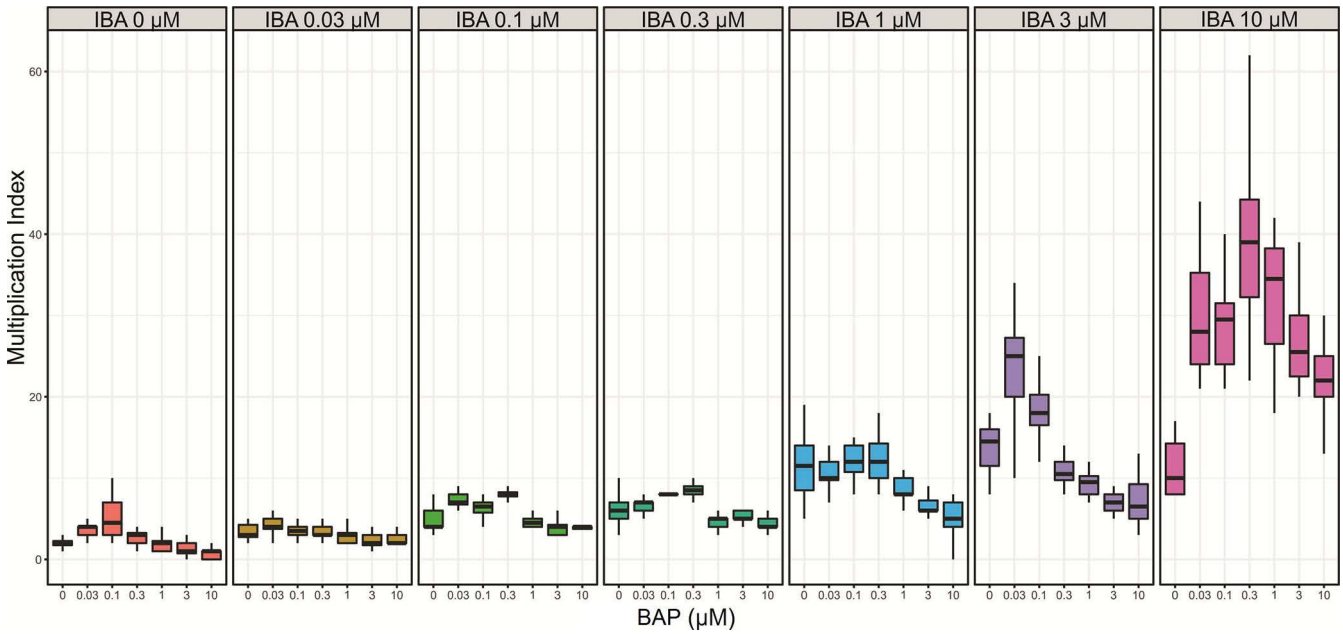


**Fig. 1**. The combined effects of auxin (indol-3-butyric acid, IBA) and cytokinin (6-benzylaminopurine, BAP) on the multiplication index of *Hennediella heimii*.

The obtained results were examined using exploratory data analysis. Factorial ANOVA, followed by Fisher's least significant difference test, were applied to check for differences in IM and protonema patch size in relation to the treatments. These analyses were performed using R 4.1.2 (R Core Team 2021).

## RESULTS

**Sterilisation test.** After applying different sterilisation treatments on the sporophyte material, the survival rate could be observed only for the spores, i.e. spore viability and survival within the capsules (Table 1). The gametophores tested previously were very delicate and did not survive the sterilisation process. On the other hand, the survival rate varied greatly for the spores depending on the duration of sterilisation and the chemical solution used. The greatest survival rate was measured for sporophytes sterilised with 3% NaDCC for 120 seconds (45% of vital axenic spores). However, when the sporophytes were treated with 5% NaDCC for 90 seconds, 41% survived. A lower survival rate was observed when NaOCl was used (0–19% survived spores), suggesting a high rate of spore damage although indirectly affected through the capsule walls. On the contrary, no sporophytes/spores survived the treatment with ethanol, nor the combined treatment with ethanol and NaOCl. According to the results obtained (Table 1), 3% NaDCC seemed to be the least harmful for the sterilisation of the sporophytes (i.e. spores) and the initiation of axenic *in vitro* cultures of *H. heimii*.

**In vitro micropropagation.** The greatest concentration of IBA (10 µM) mostly led to increased IM regardless of BAP additions in the medium (Fig. 1). High concentrations of IBA stimulated the growth of new buds and shoots on the plant explants. Nevertheless, plantlets grown on media with the addition of 0.03 µM IBA and 0.03 µM BAP combined, but also with the further addition of 0.1 µM IBA and 0.1 µM BAP, produced a significantly higher number of new shoots. Those results are evident in Fig. 2, particularly Figs. 2C & D, where the moss was grown on media with low concentrations of IBA and BAP. When compared to the plantlets grown on media with high concentrations of IBA (Figs. 2I & J), it was evident that low concentrations of growth regulators had a significant effect on the development of new buds and shoots.

Explants grown on the media containing only BAP did not form new shoots, suggesting its inhibitory effect on the formation of new shoots, since on the growth regulators of free media type some buds and shoots occurred. When the combination of low concentrations of IBA and BAP was added to the medium, the same pattern was observed, i.e. BAP exerted an inhibitory effect on the formation of new shoots. However, the greater the concentration of IBA in the medium, the more shoots were documented. Therefore, the optimal concentrations of auxin and cytokinin for bud formation in *H. heimii* could be regarded as a combination of 0.3 µM BAP and 10 µM IBA. In conclusion, IBA was necessary for shoot induction and applied in high concentrations it overcame the negative effect of exogenous BAP application.

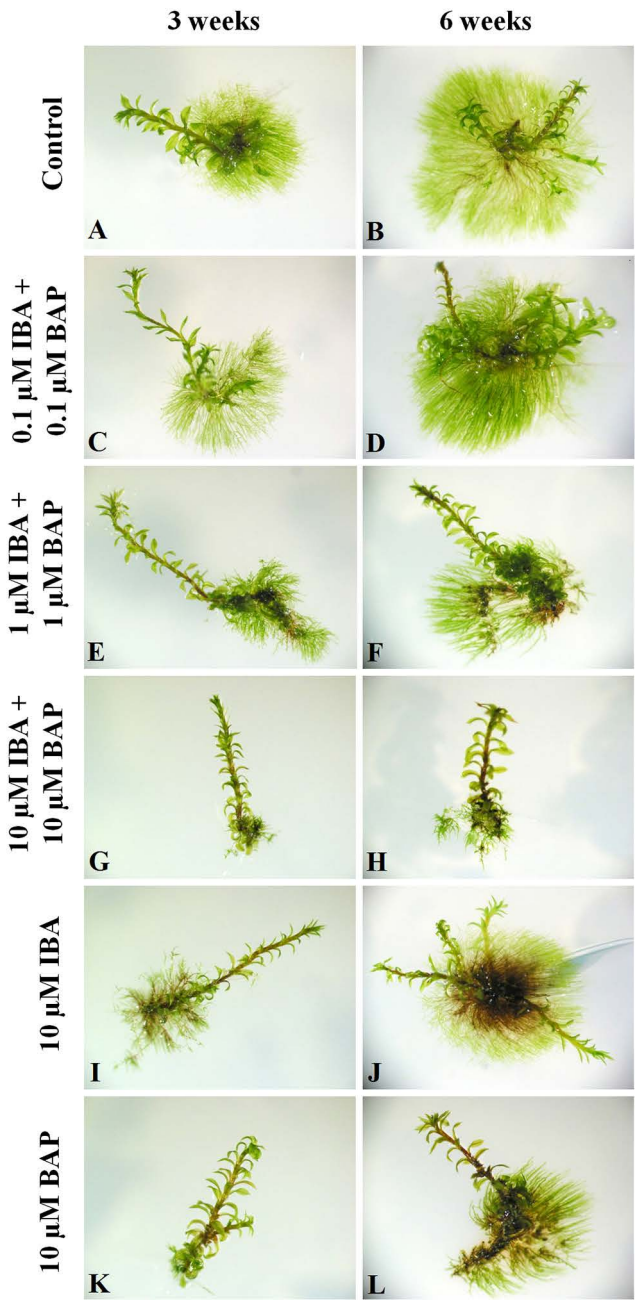


**Fig. 2**. The combined effects of auxin (indol-3-butyric acid, IBA) and cytokinin (6-benzylaminopurine, BAP) on *Hennediella heimii* development under different treatments.

In general, the diameters of the secondary protonema patches varied between 3 and 27 mm in the experiments. The smallest protonemal diameter was recorded in the plantlets grown on BCD media supplemented with 10 µM BAP (Fig. 3). High concentrations of BAP (3–10 µM) inhibited the growth of secondary protonema. When lower concentrations of BAP were used (0.3–1 µM BAP), the diameter of the secondary protonema patch decreased linearly compared to the control mosses. Low concentrations of IBA (0.03–1 µM) in combination with low concentrations of BAP positively affected the enlargement of the protonemal patch diameter. The largest protonemal patch diameter was detected in those plantlets grown on the medium supplemented both with 1 µM IBA and 1 µM BAP, (see Figs. 2E & F). Low concentrations of IBA as well as BAP (0.03 µM) induced the growth of secondary protonema. When IBA was added to the medium, the diameter of the protonemal patches increased compared to the control. However, the patch diameter was smaller than those documented in the plantlets grown on media supplemented with low concentrations of both IBA and BAP. In general, the combination of cytokinin and auxin in low concentrations greatly affected the formation of secondary protonema (Figs. 2 & 3). Massive protonemal development was observed in the control plants (Figs. 2A & B) and in the plantlets grown on the medium with low concentrations of IBA and BAP (0.1 µM and 1 µM) (Figs. 2C–F). Fewer protonema were observed when high concentrations of growth regulators were applied.

According to the results presented in Table 1, it can be concluded that the sterilisation of the plant material with ethanol proved to be lethal for both the gametophytes and sporophytes.

## DISCUSSION

Those results are in accordance with the sterilisation of the bryo-halophyte *Entosthodon hungaricus* (Boros) Loeske gametophytes (Sabovljević *et al.* 2012). As expected, the absence of cuticles and the ethanol applied leads to rapid chlorophyll extraction and the death of moss plants. The survival rate was null even with the combination of ethanol and NaOCl. The spores did not germinate after these treatments. The sterilisation of the plant material with NaOCl gave better results for *H. heimii,* similarly to *E. hungaricus,* where the survival of the spores with the sporophyte treatment was in the range of 33–90% (Sabovljević *et al.* 2012), suggesting no such harmful effects. In contrast to *E. hungaricus,* where the gametophores survived to some extent (2–11%) (Sabovljević *et al.* 2012), no *H. heimii* gametophyte survived the treatment with NaOCl. Based on data published to date, the most efficient sterilising agent for the initiation of *in vitro* cultures both from gametophytes and sporophytes is NaDCC (Sabovljević *et al.* 2003, 2012; Rowntree 2006). The application of 1% NaDCC for 3 minutes or 0.5% for 2 minutes has proved very effective in the initiation of *in vitro* cultures and the disposal of xenic organisms from bryophyte material.

Bryophytes are rather successful in autotrophic culture systems compared to vascular plants, making the addition of sucrose to the media unnecessary for their full growth and development (Lal 1984). In this research, BCD medium was used as the minimal medium in which bryophytes can be regenerated and multiplied, i.e. they

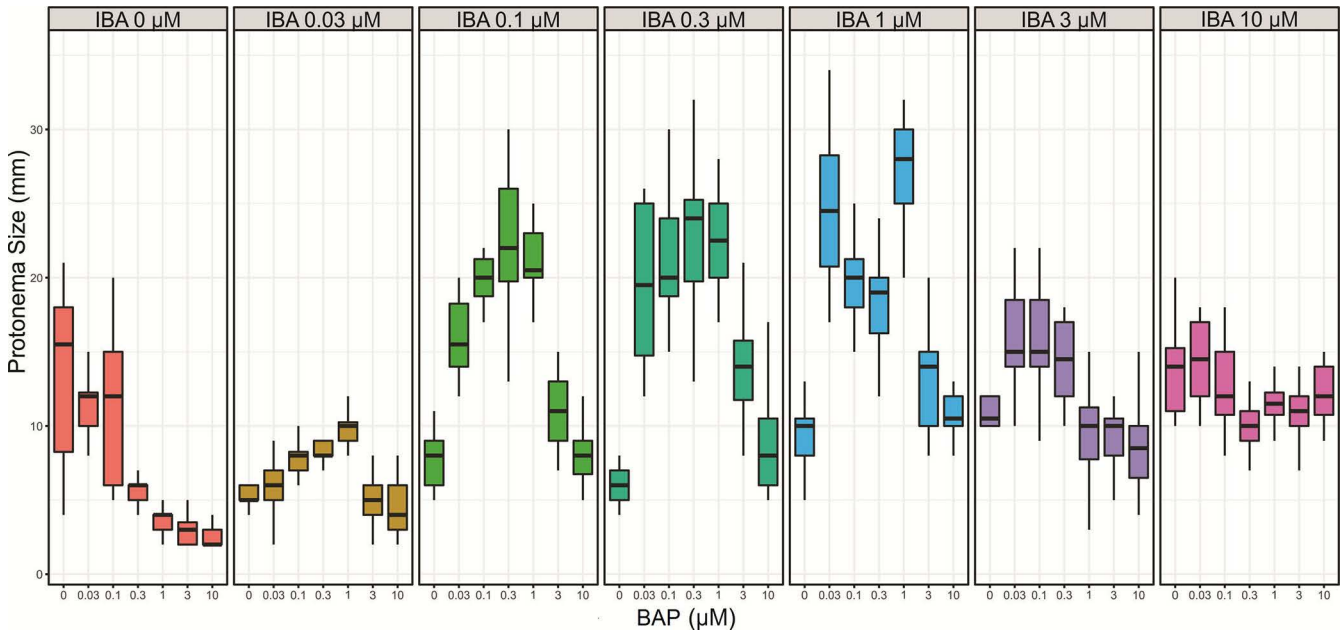


**Fig. 3**. The combined effects of auxin (indol-3-butyric acid, IBA) and cytokinin (6-benzylaminopurine, BAP) on the protonemal patch diameter of *Hennediella heimii*.

can normally grow. In general, the initiation of *in vitro* cultures from spores appears to be more successful than that from gametophores, due to the inner sterile conditions within the sporophyte capsules. However, cultures can be derived from gametophytes, but to significantly lesser extent (Sokal *et al*. 1997; Sabovljević *et al*. 2003; Nieto-Lugilde *et al*. 2018), and it is easier to start the axenic bryophyte cultures from spores originating from unopened capsules if no dormant spores or endophytes are present (e.g. Sabovljević *et al*. 2016). Temperature also influences the growth of protonema and new shoots. High temperatures such as 25°C seem to negatively affect the size and rate of bud formation (e.g. Bopp & Bathla 1990; Sabovljević *et al*. 2012). Thus, according to Sabovljević *et al*. (2012), BCD nutritive medium was the best among the tested mediums for massive propagation of *E. hungaricus*, on which gametophytes were spontaneously formed at a lower temperature (18°C) to that applied for vascular plants (25°C).

The sterilisation process of bryophytes appears to be *species*-specific (Sabovljević *et al*. 2003), and more alternative methods for culture initiations need to be found. Consequently, more detailed knowledge of the biology and ecology of certain species is key in understanding both its sterilisation process and plant culturing. Additionally, the state of the starting materials is crucial in selecting the axenic establishment procedure. Some of the main problems of axenically culturing bryophytes stem from the morpho-anatomy of the gametophyte and its sensitivity to chemicals, which complicates the disposal of microorganisms (Sabovljević *et al*. 2003, 2006, 2012; Bijelović *et al*. 2004).

Some bryophyte species are able to spontaneously form large numbers of new shoots such as *P. patens*, while others need a combination of different growth regulators applied exogenously, or other outer stimuli for the induction of buds and the growth of new shoots. Some previous studies have described the effects of auxin and cytokinins on moss morphogenesis, but less is known about their synergistic and/or antagonistic interactions (Sabovljević *et al*. 2014a, b). So far, tested bryophytes are known to respond to exogenous growth regulators and show similar response patterns to auxin and cytokinin (von Schwartzenberg 2009). However, there are exceptions and the optimal concentration for the initiation of certain developmental changes seems to be rather *species*-specific.

In some moss species, the protonema must achieve a critical size or critical age in order to initiate bud formation which is a rather *species*-specific feature (Chopra & Kumra 1988). In this research, *H. heimii* formed a large protonema in the control (no growth regulators added). The diameters of the protonemal patches increased when low concentrations of BAP and IBA were present in the medium, but decreased when high concentrations of both growth regulators were applied. Therefore, the optimal concentration of IBA for the protonemal growth of *H. heimii* is 1 µM, and the critically low concentration is 0.1 µM. The increased concentration of IBA resulted in a decrease in the protonemal patch diameter in *Bryum argenteum* Hedw. and *Atrichum undulatum* (Hedw.) P. Beauv. (Bijelović *et al*. 2004), which is in line with the results obtained in *H. heimii*. However, the protonemal patch diameter of *H. heimii* was significantly larger than

those in *B. argenteum* and *A. undulatum*. Moss *B. argenteum* formed new shoots when 0.1 and 1 μM IBA was added to the medium similarly to *H. heimii*, whereas IBA inhibited the formation of new shoots in *A. undulatum* (Bijelović *et al.* 2004). Nevertheless, low levels of auxins seem to be a key factor for caulonemal differentiation and bud formation from bud primordia, whereas high auxin concentrations are responsible for gametophyte induction (Cove & Ashton 1984), as also shown in this study. Bijelović *et al.* (2004) also demonstrated that protonemal patch diameter decreased with increasing concentrations of IBA, NAA (1-naphthaleneacetic acid) and BAP, thus suggesting that the size of the protonema depends on the addition of exogenous auxins (Chopra & Kumra 1988). Low concentrations of IBA did not affect the formation of many new shoots, while higher IBA concentrations (1 and 10 μM) led to a multiple increment in IM compared to the control plantlets. However, the control plantlets formed new shoots, suggesting *H. heimii* can spontaneously multiply on growth regulators of the free medium type, albeit somewhat more slowly.

On the other hand, cytokinins only affect bud formation on certain caulonemal cells (Ashton *et al.* 1979). Low concentrations of BAP (0.03 and 0.1 μM) had a positive impact on protonemal growth, although the diameter of the protonemal patch was similar to that in the control. However, when BAP was applied in combination with IBA, an increased in the protonemal patch diameter was observed indicating that although cytokinins are not the key factor for bud formation, they need to be present for some period to induce caulonemal differentiation and growth as well as the production of buds (Brandes 1973). Similar results were also obtained in other moss species (Bijelović *et al.* 2004).

Increased concentrations of BAP negatively affected the formation of new shoots in *H. heimii*, as well as in *B. argenteum* (Bijelović *et al.* 2004). On the other hand, low concentrations of BAP induced the formation of new shoots in *A. undulatum*, although the IM was still lower in *A. undulatum* than *B. argenteum* (Bijelović *et al.* 2004). Nevertheless, when IBA was present in high concentrations (1 or 10 μM), IM increased significantly. Moreover, low concentrations of cytokinins often lead to the development of normal gametophytes (Ashton *et al.* 1979) compared to very low or very high concentrations which lead to the formation of defective gametophytes (Chopra & Kumra 1988).

Although, this species is known to be a bryo-halophyte, the lack of sodium chloride did not affect its development, suggesting that it is a facultative halophyte or a species with very high salt tolerance.

Even though *H. heimii* formed new shoots and grew protonema on hormone-free medium, the addition of low concentrations of BAP and medium concentrations of IBA led to increased protonemal patch diameter and IM. Therefore, for the multiplication of *H. heimii,* it is better to use a combination of growth regulators in order to achieve a large amount of moss material for experiments, conservation purposes or biomass in a shorter time scale.

**Acknowledgements** – We acknowledge the support of the Ministry of Education, Science and Technological Development - Serbia (Grant No. 451-03-68/2022-14/200178).

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**REZIME**

Botanica SERBICA

## Mikropropagacija retke brio-halofite *Hennediella heimii*

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Retka vrsta mahovine *Hennediella heimii* (Pottiaceae) uspostavljena je u kulturi *in vitro*. Različiti tretmani su testirani da bi se dobila aksenična kultura. Najefektivniji sterilišući tretman bio je NaDCC-om na sporofite, jer su spore ostale vijabilne, a sa druge strane odstranio je sve strane organizme. Izučavan je i efekat regulatora rastenja biljaka na formiranje izdanaka odnosno formiranje pupoljaka, te uticaj na prečnik protoneme. Niska koncentracija BAP i srednja koncentracija IBA povećava dijametar protoneme i produkciju izdanaka. Iako se spontana multiplikacija odvija na BCD medijumu, bolje razviće i brza produkcija biomase odvija se za ovu vrstu na BCD medijumu obogaćenom kombinacijama fitohormona IBA i BAP.

**Ključne reči:** aksenična kultura, *in vitro*, regulatori rastenja kod biljaka, mahovine, multiplikacija, razviće