

ESTABLISHMENT AND DEVELOPMENT OF THE CATHERINE'S MOSS *ATRICHUM UNDULATUM* (HEDW.) P. BEAUV. (POLYTRICHACEAE) IN *IN VITRO* CONDITIONS

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Abstract - The effect of sucrose and mineral salts on morphogenesis of the Catherine's moss (*Atrichum undulatum*) in *in vitro* culture was tested. *In vitro* culture of this species was established from disinfected spores on Murashige and Skoog (MS) medium. Apical shoots of gametophytes were used to investigate the influence of sucrose and mineral salts on protonemal and gametophyte growth and multiplication. Paper also treats morpho-anatomical characteristics of plants grown in nature and plants derived from *in vitro* culture.

Key words: Brzophytes, morphogenesis, Catherine's moss, growth, multiplication

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INTRODUCTION

The Catherine's moss [*Atrichum undulatum* (Hedw.) P. Beauv.] is among the largest European terrestrial moss species. It is widespread across Europe, and due to its size is widely used in moss biology research (e.g., Bequerel, 1906; Gemmell, 1953; Word, 1960; Sitte, 1963; Wolters, 1964; Brown and Lemmon, 1987; Ono *et al.*, 1987; Lindemann *et al.*, 1989; Miles and Longton, 1990; Stoneburner *et al.*, 1992; Rütten and Santarius, 1992; Santorius, 1993; Imura, 1994; Meyer and Santorius, 1998; Becket *et al.*, 2000; Ligro-ne *et al.*, 2002; Gang *et al.*, 2003; Bijelović *et al.*, 2004; Lin *et al.*, 2005; Sabovljević *et al.*, 2005). However, cultivation of this species remains problematic, and it is therefore not easy to maintain controlled growth conditions while investigating biology of the Catherine's moss. Axenic culturing of bryophytes seems to be so complicated that many investigators gave up the attempt. However, due to possible interaction with other organisms in non axenic conditions, sterile culturing is necessary for certain experimental procedures.

Moreover, bryophytes as models for plant biology investigations have great advantages, over vascular plants: (1) relatively simple structure compared to other

higher plants, (2) haploid gametophyte of the dominant vegetative phase, and (3) lower chromosome numbers (Gang *et al.*, 2003). Cells of bryophytes, especially in suspension culture, have been noted as ideal materials for morphogenetic, genetic, physiological, biochemical, and molecular studies (Ono *et al.*, 1988).

According to Felix (1994), 31 liverworts, 18 mosses, and one hornwort have been used as experimental objects in the sterile culture of bryophytes. Some new species have lately been added to the list of mosses (Sabovljević *et al.*, 2002; Sabovljević *et al.*, 2003; Bijelović and Sabovljević, 2003). Systems of suspension culture have been established from 27 liverworts, 16 mosses, and one hornwort (Gang *et al.*, 2003). However, progress in bryophyte tissue culture has not gone as fast as in culture of the cells of other higher plants, and the number of cases achieved still does not satisfy sufficiently the demands of various research fields (Felix, 1994). Many data are still controversial, and numerous species react in different ways or express different pathways of development under the same conditions (Bijelović *et al.*, 2004; Sabovljević *et al.*, 2005). Also, very few data can be found on comparison of bryophyte development comparison *in vitro* and *in vivo*.

MATERIAL AND METHODS

Plant material was collected on roadside soil during investigation of Mt. Avala (Sabovljević and Cvetić, 2003). Voucher specimens are deposited in the BEOU herbarium of Belgrade University.

The collected material of *A. undulatum* was in the sporophyte phase, but with intact opercula. Cultures were initiated from almost mature spores, from unopened capsules that were taken for sterilization. After collection, the sporophytes were separated carefully from the gametophytes, placed in glasses, covered with cheese cloth, and rinsed with tap water for 30 minutes. Sporophytes were then disinfected for 7 minutes with a 13% solution of sodium hypochlorite. Finally, the sporophytes were rinsed three times in sterile deionized water.

As a basal medium for establishment of *in vitro* culture, we used Murashige and Skoog (1962) (MS) medium containing Murashige and Skoog mineral salts and vitamins, 100 mg/l inositol, 0.70% (w/v) agar (Torklak purified, Belgrade), and 3% sucrose. In order to observe the influence of sucrose and/or mineral salts on the morphogenesis of this species, the following medium compositions were used:

- 1) MS₁: 1/10 strength of MS mineral salts;
- 2) MS₂: 1/10 strength of MS mineral salts, 1.5% sucrose;
- 3) MS₃: 1/10 strength of MS mineral salts, 3% sucrose;
- 4) MS₄: half strength of MS mineral salts;
- 5) MS₅: half strength of MS mineral salts, 1.5% sucrose;
- 6) MS₆: half strength of MS mineral salts, 3% sucrose;
- 7) MS₇: MS mineral salts;
- 8) MS₈: MS mineral salts, 1.5% sucrose;
- 9) MS₉: MS mineral salts, 3% sucrose (basal medium).

The pH of the media was adjusted to 5.8 before autoclaving at 114°C for 25 minutes.

Cultures were kept at 25 ± 2°C, and light (16/8 hours

of light to darkness) was supplied by cool-white fluorescent tubes at a photon fluency rate of 47 μmol/m²s.

Cultures were subcultured for a period of 4-6 weeks.

For analysis of MS mineral salts and sucrose influence, 10 mm long apical segments were transferred to nutrient media (MS₁-MS₉). For each medium composition, approximately 40 transplants of *A. undulatum* were cultivated in four Petridishes. The influence of medium composition was quantified by measuring elongation of initial gametophyte explants and the index of multiplication.

Descriptive statistics and parametric statistical testing (ANOVA) were performed using Microcal ORIGIN 6.1 software.

Morphological characteristics of *in vivo* and *in vitro* grown plants were compared.

RESULTS

Surface sterilization of sporophytes was effective, and a high percentage of spores germinated on basal MS medium (supplemented with 3% sucrose). Spore germination of *A. undulatum* occurred one month after establishing *in vitro* culture. Protonema developed 15 days after spore germination, and bud formation occurred two months after spore germination. The number of developed buds was not high, and they remained for a long time in the bud phase without growing to fully developed gametophytes. Four weeks after bud development, buds started to grow to fully developed gametophytes.

To study the influence of sucrose and MS mineral salts on morphogenesis of *A. undulatum*, gametophyte shoots were used in the following experiments. For each medium composition (MS₁ – MS₉), approximately 40 transplants of *A. undulatum* were cultivated in four Petridishes (Fig.2).

According to our results, that higher sucrose concentrations (MS₃, MS₆, and MS₈) tended to have a positive effect on shoot elongation, but these differences were not statistically significant at the 0.05 level (Fig. 1A).

The media MS₇ and MS₉ were effective as well, although plants remain significantly smaller than the native counterparts (Fig. 3).

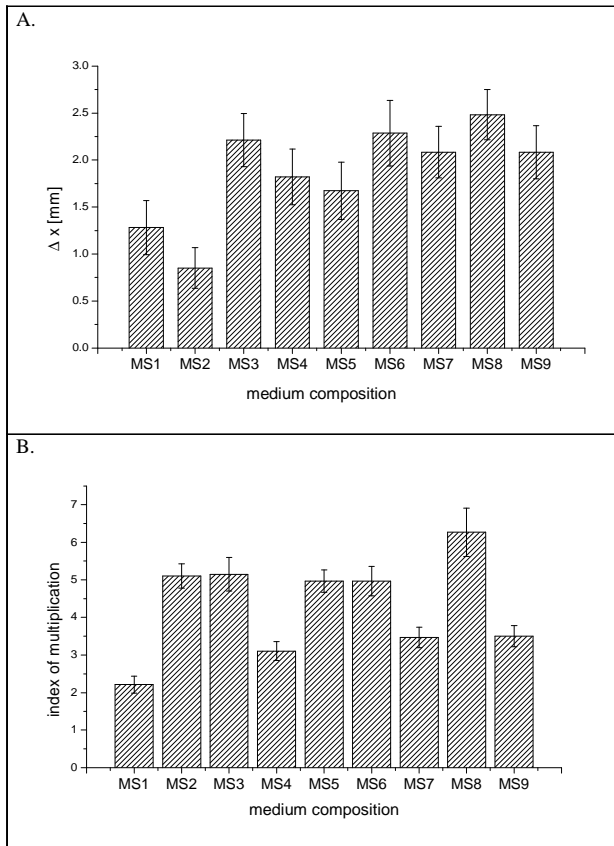


Fig. 1. Effect of different mineral salts and sucrose content of agarose nutrient media on elongation (A) and multiplication (B) of *Atrichum undulatum* shoot explants. Δx presents growth of gametophytes after 6 weeks.

Analyses of the index of multiplication (Fig. 1B) showed a statistically significant difference between plants grown on media with no sucrose and media supplied with 1.5% sucrose. The presence of sucrose promoted multiplication in any mineral composition ($MS_1 < MS_2$, $MS_4 < MS_5$, $MS_7 < MS_8$). High sucrose concentration combined with dilute mineral solution (MS_3 , MS_6) had the same positive effect on multiplication as moderate sucrose concentration, but when combined with high mineral concentration, high sucrose had no effect at all (MS_7/MS_9).

Nevertheless, our results suggest that both elongation and multiplication of *A. undulatum* shoots are in most cases stimulated by sucrose presence in the medium, even if the latter did not contain mineral salts (MS_3 and MS_6). According to our results (Fig. 1), 3% sucrose was more effective than 1.5% sucrose, except in MS_9 medium.

The highest index of multiplication of plants developed on MS_8 medium (Fig. 1B) indicates that gametophytes of *A. undulatum* could develop and even multiply better on media supplemented with a lower amount of sucrose (1.5%), while in this case a higher concentration of sucrose (3%) promoted protonema development. Similarly, it was shown that *Bryum argenteum* also multiplies much better on a medium supplemented with 1.5% sucrose compared to media with higher sucrose concentrations (S a b o v l j e v i ć *et al.*, 2005).

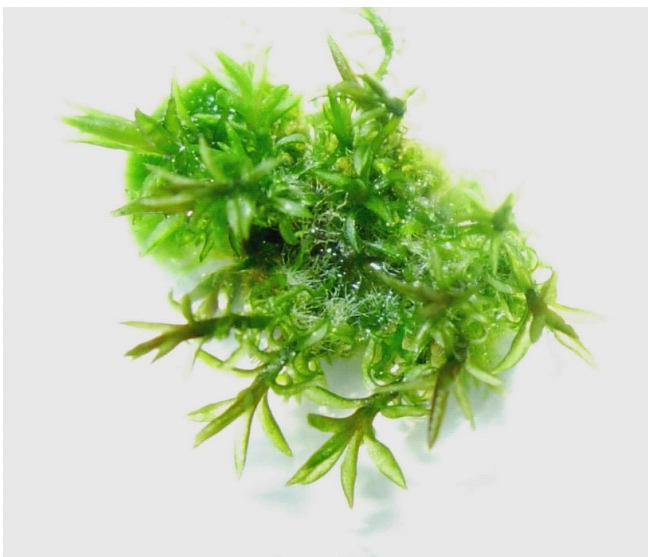


Fig. 2. *Atrichum undulatum* grown in *in vitro* culture on MS medium supplemented with 1.5% sucrose and full mineral salts.



Fig. 3. *Atrichum undulatum* grown in *in vitro* culture on MS medium without sucrose and with full mineral salts. Plants remain small.

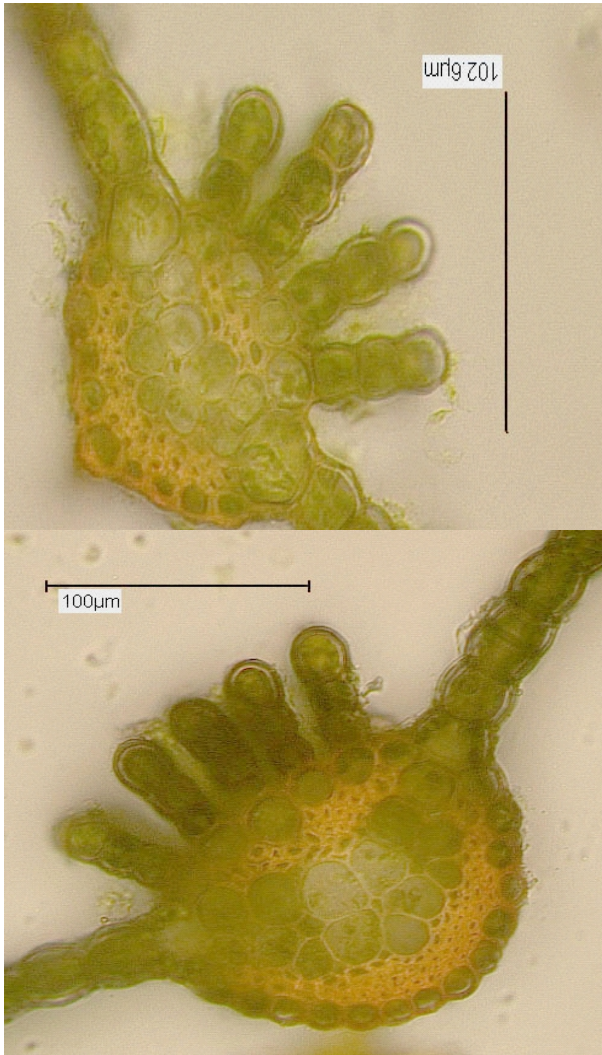


Fig. 4. Cross section of the phylloid of *Atrichum undulatum* grown in nature, with fully developed filaments.

According to the scarce data published to date, sugars have a positive effect on protonemal growth and development of mosses under *in vitro* conditions (Chopra and Kumra, 1988). Also, literature data indicate that sugar has a positive effect on bud induction and shoot formation in *in vitro* culture of some moss species (Mitra and Allsopp, 1959).

Data on mineral nutrition of bryophytes are lacking. It is assumed that bryophytes have macronutrient and micronutrient requirements closely similar to those of vascular plants (Bates, 2000; Zechmeister *et al.*, 2002).



Fig. 5. Cross section of the phylloid of *Atrichum undulatum* grown in nature, juvenile leaf with incompletely developed filaments

However, an additional problem in consideration of bryophyte nutrient needs is the specific nature of mineral uptake and transport of mineral elements in bryophyte tissue (Brown and Bates, 1990), which are significantly altered when minerals present are bound in an organic complex (Vukojić *et al.*, 2004).

Atrichum undulatum grown on a medium supplemented with sugars developed until the juvenile phase and remained in this stage with further subculturing (Sabovljević *et al.*, 2005), like plants on a medium supplemented with phytohormones (Bijelović *et al.*, 2004). On a medium that was supplemented just with mineral salts (without sugars), plants in axenic culture developed into fully developed plant, but never reached the size of native counterparts. There were no significant morphological differences between plants from nature and from *in vitro* culture other than size differences. Phylloids grown in *in vitro* culture formed fully developed lamellae on costa, similar to plants grown in nature. However, plants developed in axenic culture under any growth conditions have tenderer constitution of phylloids and lamellae (Figs. 4, 5, 6A, and 6B).

CONCLUSION

Our attempt to achieve initiation and propagation of both protonema and gametophytes of *A. undulatum* was

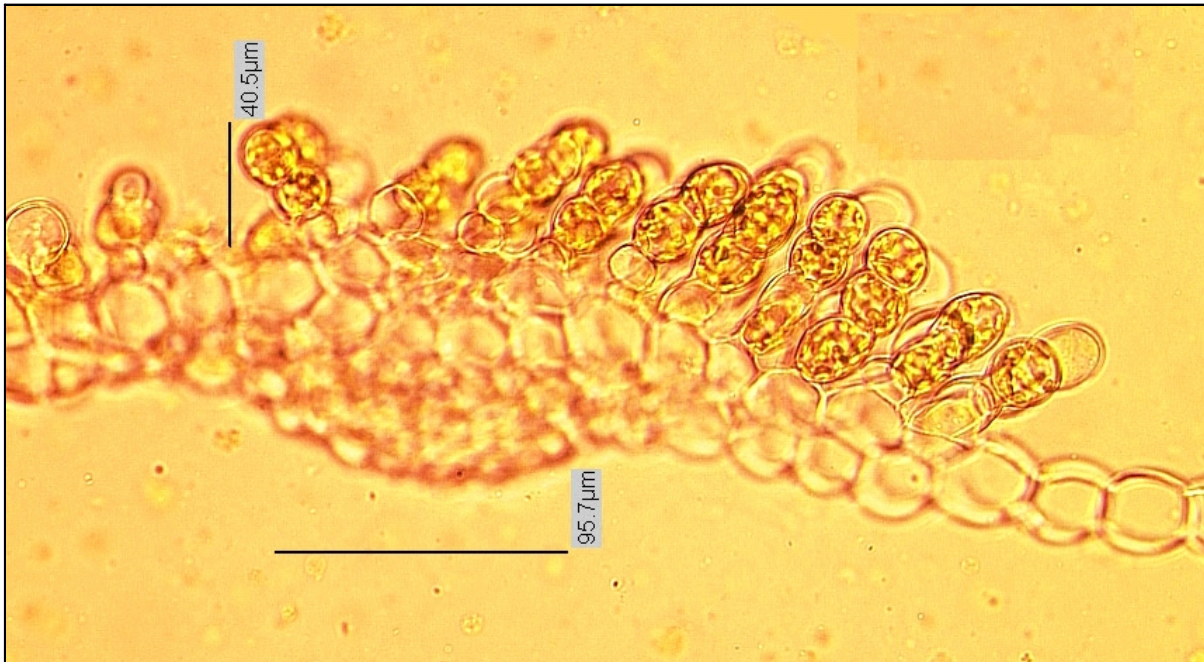


Fig. 6A. Cross section of the phyloid of *Atrichum undulatum* grown in *in vitro* conditions, with fully developed but tender filaments.



Fig. 6B. Cross section of the phyloid of *Atrichum undulatum* grown in *in vitro* conditions, with fully developed but tender filaments.

successful. The optimal medium for gametophyte development contained MS salts and 1.5% sucrose. A higher sucrose concentration (3%) inhibited full plant development (plants remain in juvenile stage) and promoted protonemal development.

Further experiments should be performed in order to elucidate effects of mineral composition and carbon availability on the development of different moss species, since results so far are sparse and controversial.

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УСПОСТАВЉАЊЕ *IN VITRO* КУЛТУРЕ И РАЗВИЋЕ МАХОВИНЕ *ATRICHUM UNDULATUM* (HEDW.) P. BEAUV. (POLYTRICHACEAE)

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Испитиван је утицај сахарозе и минералних соли на морфогенезу маховине *Atrichum undulatum* у *in vitro* култури. Култура *in vitro* маховине *Atrichum undulatum* успостављена је од стерилних спора на медијуму Murashige – Skoog. Вршни изданци гаметофита су коришћени у истраживању утицаја сахарозе и минералних соли на

раст протонеме и гаметофита, те на мултипликацију.

У раду су дати упоредни подаци о морфоанатомским карактеристикама биљака одраслих у природи и биљака одраслих у контролисаним условима *in vitro* културе.