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In-vitro propagation of three species of *Bryum* Hedw.: A comparative study

A. K. Asthana*, Vinay Sahu and Ankita Srivastava

CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India
E-mail: drakasthana@rediffmail.com*; sahuvinay8@gmail.com; s.ankita34@gmail.com
*Corresponding author

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ABSTRACT

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In-vitro propagation of three species of genus *Bryum*, viz. *B. argenteum* Hedw., *B. billardieri* Schwaegr. and *B. pallescens* Schleich ex Schwaegr. has been carried out to study their growth patterns and morphogenetic attributes. Plants of all the three species were freshly collected from Govind Wildlife Sanctuary (Uttarakhand). Axenic cultures of these three taxa have been established using spores as explants. Half-strength Knop's macronutrients were used for culture and found as most suitable for the growth of these plants under controlled laboratory condition. Uniform laboratory conditions were provided to the cultures of all the three *Bryum* taxa. Amongst them, *B. pallescens* and *B. argenteum* exhibited well grown plants as compared to *B. billardieri*. Observations were made on morphogenesis of protonema. A comparative account of variations in morphological attributes of culture grown plants and wild plants is provided.

Key-words: Axenic culture, *B. argenteum*, *B. pallescens*, *B. billardieri*, morphogenesis.

INTRODUCTION

Mosses contain numerous biologically active compounds (Mues 2000, Sabovljevic et al. 2001) but only a few species could be thoroughly studied for the isolation of important compounds due to their intermixed growth in nature and unavailability of sufficient pure population of one single species. This is the main reason that in spite of the presence of medicinally and pharmacologically interesting substances, these plants cannot be extracted sufficiently. The present study aims to develop such standardized culture method (in-vitro) that can be used to multiply the germplasm of required species under laboratory conditions so that bioprospection studies can be carried out without disturbing the diversity of plants in nature.

As far as studies on culture of Mosses are concerned, these were mainly carried out on spore germination and protonemal morphogenesis (Allsopp & Mitra 1956,1958, Nishida 1978, Nehira 1983), effect of various physical and chemical factors and growth substances on protonemal morphogenesis and bud induction (Mitra & Allsopp 1959, Mitra & Wareing 1959, Johri & Desai 1973, Chopra & Bhatla 1981) and reproductive biology of some mosses (Chopra & Rashid 1967, Chopra & Bhatla 1981, Nath et al. 2009). However, no substantial work has been done to multiply the living germplasm of these plants for bioprospection. Earlier, Sabovljevic et al. (2003) established axenic cultures of five moss species. As far as protocol for the in-vitro propagation and

Table1. Comparative account of protonemal morphogenesis and growth pattern in three species of genus *Bryum* Hedw. ± SD

Observations	*B. pallescens*	*B. billardieri*	*B. argenteum*
Spores Germination	3 days	3 days	3-4 days
Protonemal morphology (after 15 days of inoculation)	Spores germinated into protonema with longer filament composed of long cylindrical cells.	Spores develop into limited short dark brown protonema.	Spores develop normally, range between *B. pallescens* and *B. billardieri*.
Growth of culture (after 20 days of inoculation)	Protonema differentiated into green region mainly of chloronema and outer peripheral rarer network of longer caulonema, bud produced only on caulonema.	Less growth of protonema consisted of chiefly few chloronema and caulonema. Buds are few in numbers.	Protonemal growth uniform consisted of chiefly chloronema and only a few caulonema. No buds found.
Growth of culture (after 30 days of inoculation)	Protonema differentiated into leafy gametophores simultaneously. Large number of leafy gametophores developed.	Protonema differentiated into leafy gametophores simultaneously. Only few leafy gametophores developed	Protonema differentiated into leafy gametophores simultaneously. Large number of leafy gametophores developed.
Average length (in mm) of plants in 30 days	2.15 ± 0.53	0.6 ± 0.11	1.35 ± 0.34
Average length (in mm) of plants in 40 days	5.2 ± 0.94	1.25 ± 0.26	3.9 ± 0.74

multiplication of germplasm of Indian mosses is concerned, it has been provided by various workers (Awasthi et al. 2010a, b, c, Asthana & Sahu 2011, Sahu & Asthana 2013) to make available living plant material in bulk for further experimental studies.

The present study has been carried out on 3 species of *Bryum*, viz. *B. argenteum*, *B. pallescens* and *B. billardieri* belonging to family Bryaceae to observe their comparative growth rate and percentage growth in half-strength Knop's medium for conservation and propagation point of view. *Bryum billardieri* has the ability to tolerate different abiotic stress during its protonemal and gametophore stages including desiccation and salinity (Zavla et al. 2012). The aim of the present study is to observe the comparative morphogenesis of secondary protonema and gametophyte development of these three species.

MATERIAL AND METHODS

Plants having mature sporophytes of *Bryum argenteum*, *B. pallescens* and *B. billardieri* were collected from Govind Wildlife Sanctuary (Uttarakhand) in November 2012 and deposited in the Bryophyte Herbarium of National Botanical Research Institute, Lucknow (LWG). After drying, the plant material was kept in paper bags until the beginning of the experiment. Mature capsules were separated carefully from the plant. Mechanical impurity was removed after rinsing under the tap water. The mature undehisced capsules were disinfected in 4% sodium hypochlorite solution for 2 minutes and then washed repeatedly with sterilized distilled water. Half-strength Knop's solution solidified with 0.8% Agar used for inoculation of the spores. The pH of the media was adjusted to 5.8 before autoclaving it at 120°C for 15-20 minutes. The capsules were ruptured on a sterile glass slide and spores were spread on the media prepared with half-strength Knop's macronuturients. The cultures were maintained under controlled condition of light (2500-3000 lux, 16 hour/ 8 hour light/dark cycle) and temperature (22 ± 2°C).

Plate 1

Bryum argenteum: A. Germinating spores D. Protonemal stage, G. A young gametophore, J. In-vitro raised plant population, M: Plants after transferring on soil in pots.
Bryum billardieri: B. Germinating spores, E. Protonemal stage, H. Young gametophores, K. In-vitro raised plant population, N. Plants after transferring on soil in pots.
Bryum pallescens: C. Germinating spores, F. Protonemal stage, I. A young gametophore, L. In-vitro raised plant population, O. Plants after transferring on soil in pots.

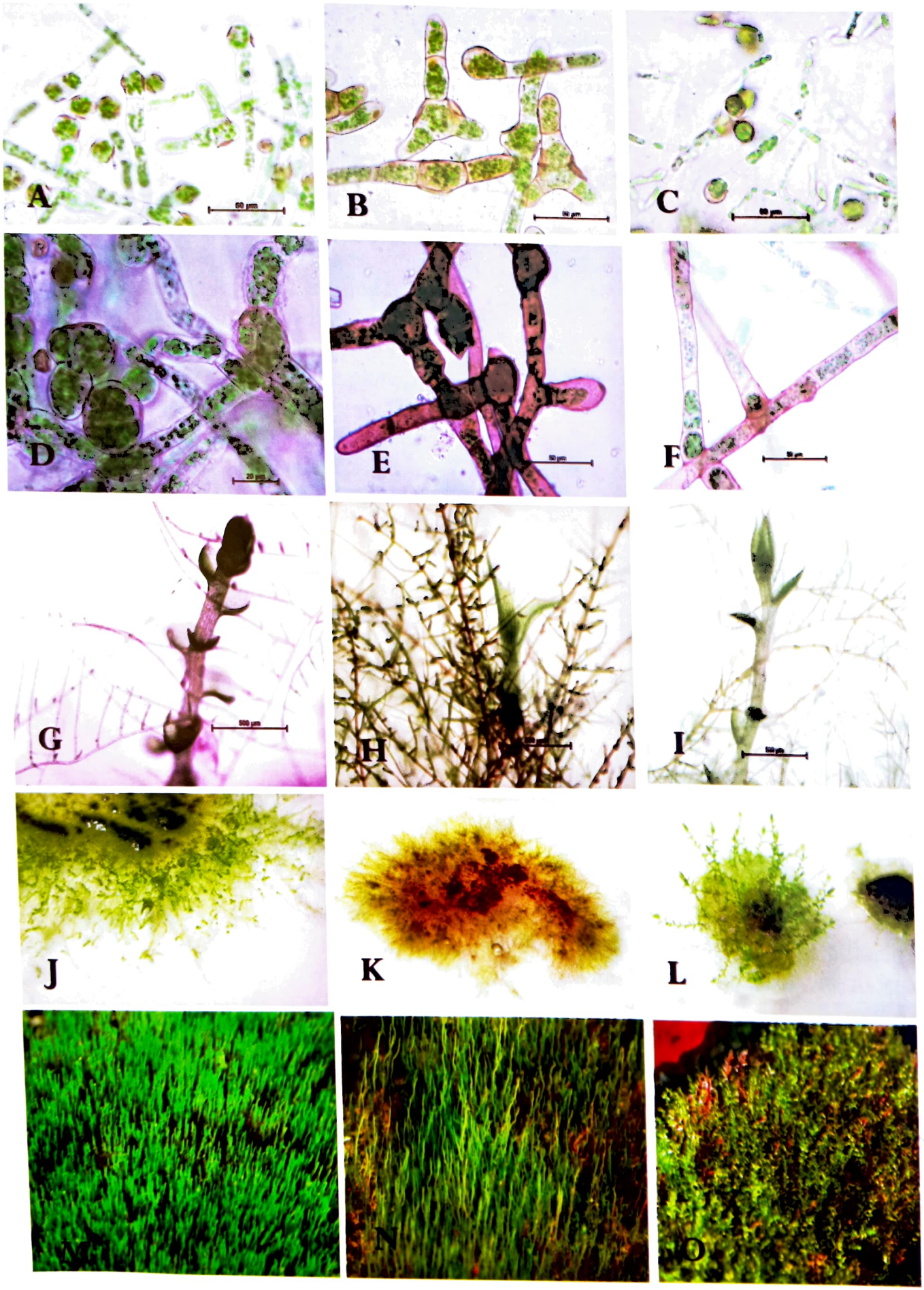
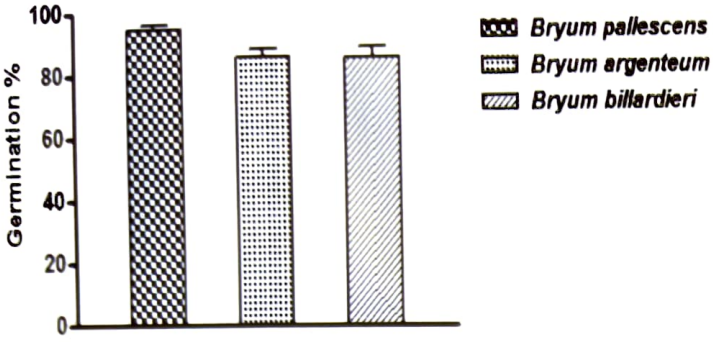
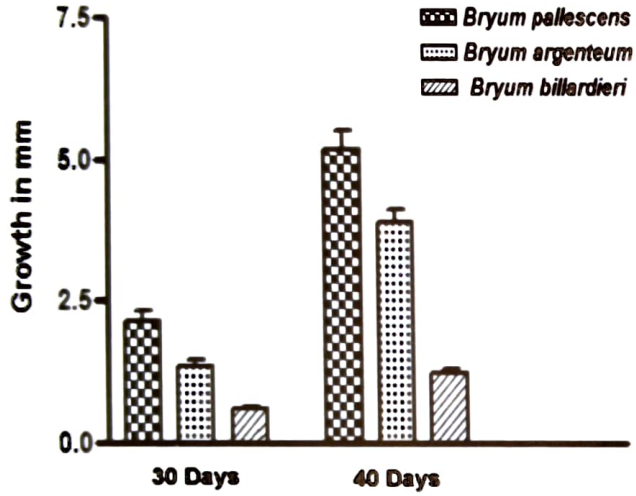


Plate 1



Text-figure 1. Germination percentage of spores of three species of *Bryum* after 3 days.



Text-figure 2. Growth of three species of *Bryum* in half-strength Knop's medium after 30 and 40 days. Bar Showing SD.

The well grown cultured plants were then transferred to the autoclaved soil after removing the agar from the plants for hardening and acclimatization under laboratory condition.

Specimens examined: India, western Himalaya, Uttarakhand, district Uttarkashi, Govind Wildlife Sanctuary, on way to Kedarkantha from Sankri (alt. ca 6547 ft), 5.11.2012, leg. Vinay Sahu 264884 (LWG), Halara Khadd (alt. ca 6250 ft), 7.11.2012, leg. Vinay Sahu, 264945 (LWG), Sankri (alt. ca 6467 ft), 7.11.2012, leg. Vinay Sahu, 264950 (LWG).

RESULTS AND DISCUSSION

The spores of *Bryum pallescens* and *B. argenteum* were green in colour, while the spores of *B. billardieri* were brown in colour. The spores germinated on the third day of inoculation. In case of *B. pallescens* and *B. argenteum* spores turned dark green, swelled and germ tube formed from endospore after rupturing exospores. The germination was monopolar as well as bipolar in all the above 3 species with no rhizoidal filaments. The germination percentage was highest in *B. pallescens* as compared to other two species (Text-figure 1). Although the plants were observed daily but the development and differentiation of chloronema and caulonema was observed after 20 days of germination of the spores. Later on after 30 days erect development of young gametophores took place differentiating the stem and leaves which were green in color in case of *B. argenteum* and *B. pallescens*, while brown in case of *B. billardieri* (Text-figure 2, Table 1). In erect gametophores no branching occurred. In the present study it has been observed that half-strength Knop's macronutrients are best suited for development of *B. pallescens* and *B. argenteum* followed by *B. billardieri*. The plants were transferred to pots filled with autoclaved soil for further growth and hardening. Morphogenetic observations have revealed that the arista in the leaves at top in case of *B. argenteum* was not hyaline as it is generally seen in the nature.

Earlier, some culture studies have also been carried out on some species of *Bryum* (Chopra & Bhatla 1981, Sarla 1987, Ashton & Raju 2001, Sabovljevic et al. 2005, 2010). Sabovljevic et al. (2002) used Murashige & Skoog mineral salts and vitamins (100 mg/l myo-inozitol and 30 gm/l sucrose supplement with 1 mg/l 2, 4 D and 2 mg/l kinetin) for in-vitro propagation of *B. argenteum* and *B. capillare* Hedw. In the case of *B. capillare* secondary protonema was developed in 10-40 days and after a month only caulonema was produced but no protonemal and caulonemal bud was formed, while in *B. argenteum* spores were germinated after 7 days and protonema formation takes place after 15 days and small leafy shoots appear after one month. In-vitro propagation of three species of *Bryum* (*B. coronatum* Schwaegr., *B. bicolor* and *B. capillare*) has also been done by Awasthi et al. (2010d). They found that only *B. coronatum* developed gametangia, while other two species produced rhizoidal gemmae. During present study, no micronutrients, vitamins, sugar and hormone were used for micropropagation of these three species.

Table 2. Showing comparative account of morpho-anatomical characters of In-vitro grown plants with wild plants of *Bryum* species

Plant name / Characters	*B. pallescens*		*B. billardieri*		*B. argenteum*	
	Nature	Culture	Nature	Culture	Nature	Culture
Plant	Stem erect with 2-3 subfloral innovation 10 – 20 mm	Stem erect without subfloral innovations about 0.5mm – 15mm	Robust, erect, branched by two or more subfloral innovations about 15 – 20 mm	Erect, not branched about 10-13mm	Stem erect, julaceous, glossy silvery white in colour about 0.5mm – 10 mm	Stem julaceous green in colour up to 30 mm
Leaf	Oblong-lanceolate margin revolute dentate at apex about 1.5 x 0.4mm	Oblong-lanceolate margin revolute dentate at apex about 0.7 x 0.2- 0.3 mm	ovate oblong spathulate, margin dentate-serrulate, recurved at tip about 4 x 1-2 mm	ovate, oblong spathulate margin dentate-serrulate, recurved at tip about 0.3x 0.2mm	Densely arranged, broadly ovate, margin flat 0.7x 0.4- 0.5mm	Densely arranged, broadly ovate margin flat 0.4 x 0.2mm
Leaf Apical cells (µm)	Hexagonal to rhomboidal, 56 x 7.5	Hexagonal to rhomboidal, 45 x 7.5 – 11.25	Rhomboidal, thin walled, 45 x 12 - 15	Rhomboidal thin walled, 30 - 45 x 9	Cells hyaline, narrow rhomboidal 45- 57 x 9	Cells hyaline, narrow rhomboidal 37.5 – 45 x 11.25
Middle cells (µm)	Longer than apical, 56 x 7.5 – 11	Longer than apical, 52 x 11	Shorter rhomboidal, 45 - 60 x 12	Shorter rhomboidal, 45- 51 x 12	Green, rectangular 45 - 60 x 6 - 9	Green, rectangular 37 x 11.25
Basal cells (µm)	Rectangular, 18 - 30 x 18 - 22	Rectangular, 30 x 15	Rectangular, 45 - 75 x 18	Rectangular, 15-30 x 12	Rectangular, 30 x 9	Rectangular, 30 x 15
Costa (mm)	excurrent, arista, 0.1	excurrent, arista, 0.04	excurrent, arista 0.3 – 0.4	excurrent, arista 0.009	Hyaline, excurrent, arista 0.1	Not hyaline, excurrent arista 0.1- 0.2

It was found that half-strength Knop's medium is best suited for the growth of these three species. A comparative study of these three species growing in culture and in nature has been done to assess the effect of controlled laboratory conditions on the growth of the plant (Plate 1). It was found that plants of *B. pallescens* and *B. billardieri* are smaller than plants growing in nature, while cultured plants of *B. argenteum* did not show the silver green colour and these were more robust as compared to plants in nature (Table 2).

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REFERENCES

Allsopp A. & Mitra G. C. 1956. The heterotrichous habit in the protonema of Bryales. Nature 178: 1063-1064.

Allsopp A. & Mitra, G. C. 1958. The morphology of protonema and bud formation in the Bryales. Annals of Botany 22: 95-115.

Ashton N. W. & Raju M. V. S. 2001. Development and germination of rhizoidal gemmae of *Bryum violaceum*. Cryptogamie Bryol 22: 3-11.

Asthana A. K. & Sahu V 2011. Growth responses of moss *Brachymenium capitulatum* (Mitt.) Par. in different culture media. Natl. Acad. Sci. Lett. 34(1-2): 1-4.

Awasthi V., Nath V. & Asthana A. K. 2010a. In-vitro study on micropropagation and reproductive behaviour of moss *Bryoerythrophyllum recurvirostrum* (Hedw.) Chen. Int. J. Plant Reproduct. Biol. 2: 31-37.

Awasthi V., Nath V. & Asthana A. K. 2010b. On the culture of a pleurocarpous moss *Entodon laetus* (Griff.) Jaeg. Natl. Acad. Sci. Lett. 33 (5, 6): 145-148.

Awasthi V., Nath V. & Asthana A. K. 2010c. In-vitro propagation of the endemic and threatened Indian liverwort: *Cryptomitrium himalayense* Kash. Curr. Sci. 98: 1440-1441.

Awasthi V., Nath V. & Asthana A. K. 2010d. Effect of Some Physical Factors on Reproductive behaviour of Selected Bryophytes. Intern. J. Plant Reprod. Biol. 2(2): 141-145.

Chopra R. N. & Bhatla S. C. 1981. Effect of physical factors on gametophyte induction, fertilization and sporophyte development in the moss *Bryum argenteum* grown in-vitro. New Phytol. 89: 439-447.

Chopra R. N. & Rashid A. 1967. Apogamy in *Funaria hygrometrica* Hedw. Bryololgist 70: 206-208.

Johri M. M. & Desai S. 1973. Auxin regulation of caulonema formation in moss protonema. Nature 245: 223-224.

Mitra G. C. & Allsopp A. 1959. The effect of sugar concentration on the development of protonema and bud formation in *Pohlia nutans* (Hedw.) Lindb. II. Phytomorphology 9: 55-63.

Mitra G. C. & Wareing P. F. 1959. The effect of light of various qualities on the development of the protonema and bud formation in *Pohlia nutans* (Hedw.) Lindb. Ann. Botany 9: 47-55.

Mues R. 2000. Chemical constituents and biochemistry. In: Bryophyte Biology. Shaw A. J. & Goffinet B. (Editors), Cambridge University Press, pp 150-181.

Nath V., Awasthi V. & Asthana A. K. 2009. In-vitro propagation and reproductive biology of moss *Funaria hygrometrica* Hedw. Intern. J. Plant Reproduct. Biol. 1: 103-108.

Nehira K. 1983. Spores germination, Protonema development and sporeling development. In: New Manual of Bryology, Vol. 1. R.M. Schuster, ed. The Hattori Botanical Laboratory, Nichinan, Miyazaki, Japan, pp. 343-385.

Nishida Y. 1978. Studies on sporeling types in mosses. J. Hattori Bot. Lab. 44: 371-454.

Sabovljevic M., Bijelovic A. & Dragicevic I. 2002. Effective and easy way of establishing in-vitro culture of mosses, *Bryum argenteum* Hedw. and *Bryum capillare* Hedw. (Bryaceae). Arch. Biol. Sci., Belgrade. 54 (1-2): 7-8.

Sabovljevic M., Bijelovic A. & Dragicevic I. 2003. In-vitro culture of mosses: *Aloina aloides* (K. F. Schultz) Kindb., *Brachythecium velutinum* (Hedw.) B. S. G., *Ceratodon purpureus* (Hedw.) Brid., *Eurhynchium praelongum* (Hedw.) B. S. G. and *Grimmia pulvinata* (Hedw.) Sm. Turkish J. Bot. 27: 441-446.

Sabovljevic M., Bijelovic A. & Grubisic D. 2001. Bryophytes as a potential source of medicinal compounds. Lekovite Sirovine 21: 17-29.

Sabovljevic A., Sabovljevic M., Grubisic D. & Konjevic R. 2005. The effect of sugars on development of two moss species (*Bryum argenteum* and *Atrichum undulatum*) during in-vitro culture. Belg. J. Bot. 138(1): 79-84.

Sabovljevic A., Sabovljevic M. & Vukojevic V. 2010. Effects of different cytokinins on chlorophyll retention in the moss *Bryum argenteum* (Bryaceae). Periodicum Biologorun 112(3): 301-305.

Sahu V. & Asthana A. K. 2013. An Observation on Growth Response of *Anomobryum filiforme* var. *concinnatum* (Spruce) Aman. (Bryaceae) in Different Culture Media. Natl. Acad. Sci. Lett. 36: 587-589.

Sarla 1987. Effect of auxin on in-vitro formation and behavior of gemmae in *Bryum capillare* Hedw. J. Hattori Bot. Lab. 62: 111-119.

Zavla A. M., Perez N. M., Becerra A. A. & Lopez M. A. V. 2012. Glucose inhibits the shoot bud formation in the moss *Bryum billardieri*. Cent. Eur. J. Biol. 7(4): 648-654.