Original Article

In vitro MICROPROPAGATION OF RARE AND ENDANGERED MOSS *Entosthodon hungaricus* (Funariaceae)

MICROPROPAGAÇÃO In Vitro DO MUSGO RARO Entosthodon hungaricus *(Funariaceae), AMEAÇADO DE EXTINÇÃO*

Marko S. SABOVLJEVIĆ[1]; Beáta PAPP[2]; Aneta SABOVLJEVIĆ[1]; Milorad VUJIČIĆ[1]; Erzsébet SZURDOKI[2]; José Gabriel SEGARRA-MORAGUES[3]

1. Institute of Botany and Garden, Faculty of Biology, University of Belgrade, Belgrade, Serbia. marko@bio.bg.ac.rs; 2. Botanical Department, Hungarian Natural History Museum, Budapest, Hungary; 3. Centro de Investigaciones sobre Desertificación-CIDE, Valencia, Spain

ABSTRACT: The moss *Entosthodon hungaricus* (Boros) Loeske is an European endemic species typical of dry and saline soils extending from the Iberian Peninsula to Aral-Caspian steppes, similarly to some other xerothermic bryophytes. However, the distribution range is fragmented and localities are quite scattered and the species is considered as rare and vulnerable because of its ephemeral characteristics and specialized ecology. With the aim to develop an active protection plan for this species, the *ex situ* conservation requirements of *E. hungaricus* were developed. The axenic culture in *in vitro* conditions were established, and the optimal growth parameters were adjusted to achieve fully developed gametophytes ready to be reintroduced to its native range and other potentially native areas, where this species was once reported but has not been collected in recent times, suggesting its local extinction (i.e. some areas in Vojvodina, N. Serbia). Starting materials were derived from recent herbarium specimens and from fresh materials collected from Hungarian populations. Several means for sterilization of stating material and growing nutritive media were assayed in different regimes of light and temperature. Here we describe the conditions to achieve full plant development and for its micropropagation. Such materials are adequate for *ex situ* conservation purposes and for experimental introductions in native and potentially native areas. The first axenical culture of *E. hungaricus* was successfully established, and the first *in vitro* micropropagation of this rare and endangered species was achieved. Our study contributes to the conservation biology as well as for the potential use of this moss species in biotechnological research.

KEYWORDS: *Entosthodon hungaricus*. Moss. E*x situ.* Micropropagation.

INTRODUCTION

In vitro culture has been recognized as an important tool in bryophyte *ex situ* conservation and reintroduction trials (PRENCE 2004; ROWNTREE; RAMSAY 2005; ROWNTREE 2006; SARASAN et al. 2006; REED et al. 2011). This is also a key technique for developmental studies (DUCKETT et al. 2004; VICTORIA et al. 2011). *In vitro* cultures of bryophytes also give opportunities to increase our knowledge of certain aspects of the biology of the species (MARTÍNEZ; PRICE 2011). Besides, *in vitro* cultivation of bryophytes is needed for biotechnological research (SABOVLJEVIC; SABOVLJEVIC 2008; 2010; SABOVLJEVIC et al. 2006a; 2010; 2011) and a variety of experimentations in controlled conditions, for example, effects of day length on photosyntetic pigments and antioxydative metabolism of in vitro cultured moss *Atrichum undulatum* (CVETIC et al., 2009), effects of gypsiferous or salt substrata on bryophytes (BOGDANOVIC et al., 2009; 2011), bioactivities of bryophyte extracts, chlorophyll retention in bryophytes or developmental studies (SABOVLJEVIC et al. 2010a; b; c). In addition, axenic cultures are needed for the development of species-specific markers, as those procedures are sensitive to DNA from other organisms living associated to bryophytes, and for obtaining critical amounts of biomass for the analysis or production of specific chemical compounds.

The moss *Entosthodon hungaricus* (Boros) Loeske (syn. *Funaria hungarica* Boros) was described from Hungary (BOROS, 1924). It was long considered as an European endemic species with scattered distribution until later it was found in other countries out of Europe in Israel, Kazakhstan, Kirgizia and because of its synonymization with *E. maroccanum* (Meyl.) Hebr. & Lo Giudice, also in Morocco (PISARENKO et al., 2001 and the references therein).

Prior to its synonymization with *E. maroccanum*, it was considered to be a typical species of the central European steppes, with and Aral-Caspian distribution. In Europe it has quite a strange range extending from the Mediterranean to Central Europe, similarly to some other species often considered as xerothermic (POCS et al., 2004). It has been reported from Austria, Greece, Germany, Hungary, Romania, Serbia, Slovakia,

Received: 09/10/11
Accepted: 05/02/12

Spain and Ukraine (PISARENKO et al. 2001 and references therein). Nonetheless, despite its widespread European distribution, populations are scattered and the range highly discontinuous and fragmented. Altogether to its distribution range characteristics, its ephemeral habit and specialized ecology and habitat type on gypsum and saline substrates have contributed to its consideration under different categories of menace. Thus, it has been considered as rare and threatened in Europe (ECCB, 1995). Additionally, it has been included in the red lists from several European countries (PAPP, 2008; SOLTES et al., 2002; SERGIO et al., 2006; SAUER, 2005; AHRENS, 2005; SABOVLJEVIC et al., 2004).

The aim of this paper was to establish for the first time axenical culture in *in vitro* conditions for the rare and endangered moss *E. hungaricus* and, to search for the conditions for full plant development and for its *ex situ* micropropagation. Such knowledge is essential for designing *ex situ* conservation biology schedules and for future introduction and reintroduction as well as for evaluating the biotechnological potential of this moss species. The effects of culture methods, media and exogenous conditions on completely developed *n* generation (fully developed gametophytes) of this moss were studied aiming at assessing whether this species can be propagated *in vitro* and which are the optimal conditions for its culture under artificial conditions. Our data will contribute future conservation biology and biotechnological experiments.

MATERIAL AND METHODS

Plant materials:

E. hungaricus were collected at (1.) Hungary, Bács-Kiskun County, Kisrét at Szabadszállás, saline-alkali area, 46°51'46,5" N, 19°12'14,6" E, 100 m, 2010.04.01 and (2.) Hungary, Bács-Kiskun County, Szappanszék at Fülöpháza, saline-alkali area, 46°53'11,1" N, 19°25'35,3" E 115 m, 2010.04.01. The voucher specimens is deposited in the bryophyte collections of BP and BEOU.

Fully developed plants were collected in plastic boxes, and kept at + 4°C prior to sterilization for 24 h. Both, gametophyte without developed sporophytes (green leafy shoots) and sporophytes (capsules with setas) were separated, washed in rinsed water tree times under cheese cloths and finally washed in distillated water. With an aim to produce genetically diverse axenic cultures of *E. hungaricus*, the way of establishing whole plants from the variety of spores has been studied. The problems in establishing moss culture have been elaborated elsewhere (ROWNTREE et al., 2011).

The sterilization process was tested through the addition of increasing concentrations of sodium hypochlorite (NaOCl) 1, 3, 5, 7, 10 or 13% or ethanol 10, 30, 50 and 70% or sodium dichloroisocyanurate (NaDCC) 1, 3, 5% and a combination of NaOCl at the already described concentrations and 10% Ethanol (Table 1). Sterilization time was kept constant in all cases for 90 seconds in the first round of trials. Additionally, longer (120 s) and shorter (60 s) exposure time of materials to sterilization agents were tested at the concentrations that showed significantly higher survival rates and absence of contamination at 90 s. Spore germination was assayed from young and fresh mature capsules. Spore germination rates were evaluated on a variety of conditions, including nutritive media (MS; MS/2; BCD w/w sucrose, SABOVLJEVIC et al. (2009) for further details), light (long day/short day photoperiod) and temperature (18°C/25°C). Once the spore had germinated, the influence of the different media and culture conditions applied on primary protonema were estimated in views of achieving the highest percentage of bud formation and gametophore development (index of multiplication). The developmental rates of secondary protonema from detached gametophores were estimated for the purpose of massive micropropagation. Index of multiplication is considered as number of newly developed shoots, while the secondary protonema development is measured by the diameter increase within certain time frame.

RESULTS AND DISSCUSION

The gametophytes of *E. hungaricus* are very delicate (Figure 1A) and do not survive even very short-time surface sterilization with diluted ethanol followed by water washing (Table 1). This is due to the absence of cuticles in mosses and the one cell-layered leaves of *E. hungaricus*. Similarly, when NaOCl was combined with ethanol, survival rates after surface sterilization were negligible (Table 1). The ethanol produces a quick degradation of chlorophyll and thus, is lethal for the gametophores of *E. hungaricus*. Similar negative results regarding survival rates were also obtained after the sterilization of sporophytes with ethanol (Table 1). The sterilization with NaOCl at various concentration rendered better results at least for sporophytes (survival rates of spores ranged from 33%-90% depending on the concentration of NaOCl) and occasionally for gametophytes

(survival rates 2%-11%). The best results of NaOCl sporophyte sterilization and spore survival, maximum survival rate and absence of contamination, were achieved at NaOCl concentration of 3% for 90 seconds followed by a double rinse of sporophytes with distilled water (Table 1). In this case, survival rate of spores contained in capsules reached up to 90%.

Table 1. Influences of disinfector and disinfection time on explants of *E. Hungaricus (*NaOCl – Sodium hypochlorite; NaDCC – Sodium dichloroisocyanurate)

Sterilization methods	Duration (s)	Results (Survival rate, %)	
		Gamethophyte	Sporophyte
1% NaOCl	90	0 (100% dead or contaminated)	0 (alive but contaminated)
3% NaOCl	90	11 (89% dead or contaminated)	90
5% NaOCl	90	7	70
7% NaOCl	90	2	63
10% NaOCl	90	0 (100% lethal)	48
13% NaOCl	90	0 (100% lethal)	33
10% ethanol	90	0 (100% lethal)	0 (effective but no spore gerimantion)
30% ethanol	90	0 (100% lethal)	0 (effective but no spore gerimantion)
50% ethanol	90	0 (100% lethal)	0 (effective but no spore gerimantion)
70% ethanol	90	0 (100% lethal)	0 (effective but no spore gerimantion)
1% NaOCl +10% ethanol (50:50, v/v)	90	0 (100% lethal)	0 (effective but no spore gerimantion)
3% NaOCl +10% ethanol (50:50, v/v)	90	0 (100% lethal)	0 (effective but no spore gerimantion)
5% NaOCl +10% ethanol (50:50, v/v)	90	0 (100% lethal)	0 (effective but no spore gerimantion)
7% NaOCl +10% ethanol (50:50, v/v)	90	0 (100% lethal)	0 (effective but no spore gerimantion)
10% NaOCl +10% ethanol (50:50, v/v)	90	0 (100% lethal)	0 (effective but no spore gerimantion)
13% NaOCl +10% ethanol (50:50, v/v)	90	0 (100% lethal)	0 (effective but no spore gerimantion)
1% NaDCC	90	48	69
3% NaDCC	90	66	80
5% NaDCC	90	37	66
10% NaOCl	60	8	75
10% NaOCl	120	2	45
3% NaDCC	60	55	72
3% NaDCC	120	30	66

Spore germination started two days after being transferred to nutritive media. Spores germination rates where high in all nutritive media, ranging from 57% to 72% depending if the starting material was derived from herbarium vouchers or from fresh materials. Nonetheless higher percentages were obtained in those media that did not included sugar in their composition. Primary protonema started to develop five days after the spore inoculation. Higher bud production was achieved from primary protonema developed from fresh materials irrespective of the photoperiod applied to cultures. High temperature (25ºC) did not preclude bud formation in primary protonema, however their growth rate was slower and they reached overall smaller sizes. BCD nutritive medium free from growth regulators and sugar emerged as the best one among the tested nutritive media for massive propagation and subculturing. In this medium the gametophores spontaneously

produced "colonies" when grown in cooler (18ºC), irrespectively of the longer or shorter photoperiod (Figure 1B).

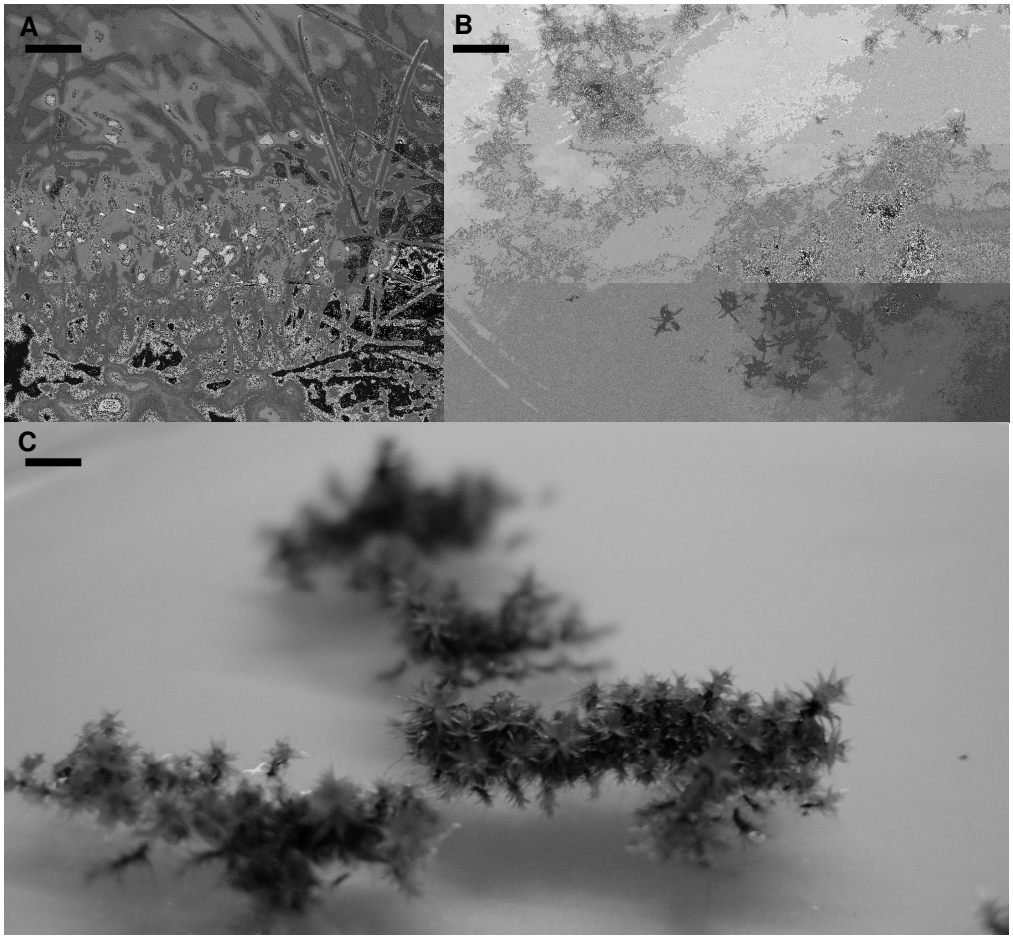

Figure 1. *Entosthodon hungaricus*. A. Fully developed plants with sporophytes in the wild. B. Gametophores developed on primary protonema developed after spore germination. C. Gametophores developed on secondary protonema. (Bars = 10 mm)

Secondary protonema started to develop a week after the inoculation of appropriate growing media with leafy shoots (sterile gametophores). As reported by Lian et al. (2010) for *Bryum argenteum* Hedw., and by Decker and Reski (2004) for *Physcomitrella patens* (Hedw.) Bruch & Schimp., a model moss species for physiological studies and closely related to *Entosthodon* Schwägr., the fragmentation of primary protonema retarded bud induction. Similarly, nutritive media enriched with sucrose also prevented or retarded the induction of buds compared to the nutritive media in which sucrose had not been not added. Protonema developed larger in diameter on both MS and BCD nutritive media enriched with sucrose (15 g L^{-1} or 30 g L^{-1}) while on nutritive media that did not contain sugar (MS/2) the diameters were significantly smaller (Figure 2).

After the subculturing of primary protonema to new media, faster growth rates were observed with MS nutritive medium compared to BCD one. However, bud induction was faster on BCD than on MS. After the subculturing of gametophores, secondary protonema developed discs with larger diameters in those nutritive media supplemented with sucrose. Contrastingly, a poorer protonema development was obtained with BCD. In this medium, secondary protonema hardly spread further around the inoculated gametophore (Figure 2). Nonetheless, small secondary protonema cultured on BCD quickly developed a large number of buds that rapidly grew into fully developed gametophytes and soon formed moss turfs. These gametophores rather than protonema can be readily used as starting explants for further micropropagation since they take only 45 to 60 days to develop into moss turfs (Figure 1C). This result is confirmed by the index of multiplication (Figure 3). The gametophore development on primary protonema developed after spore germination

(Figure 3, upper right), and a cushion of gametophores well developed on secondary protonema.

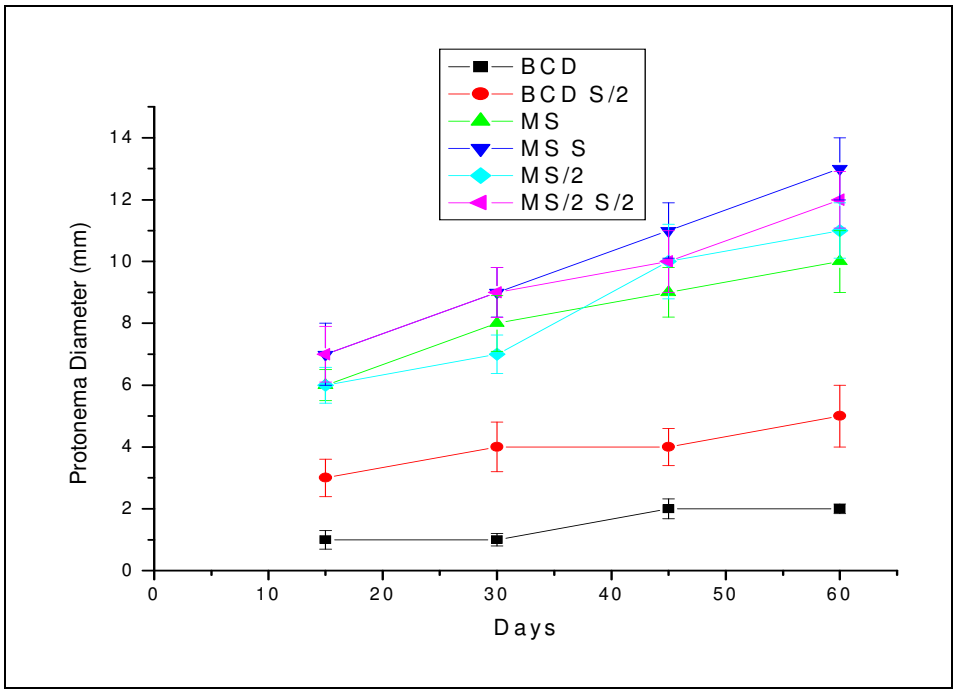

Figure 2. Secondary protonema diameter after 60-day culture of *Entosthodon hungaricus* using different nutritive media.(Means ± standard deviation, n= 30)

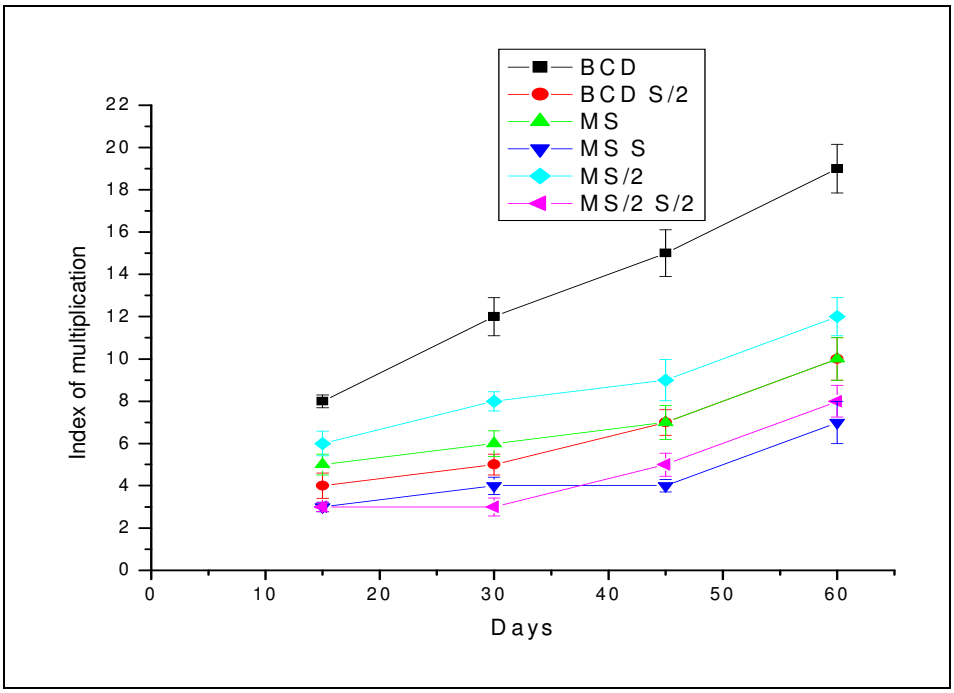

Figure 3. Index of multiplication after 60-day culture of *Entosthodon hungaricus* using different nutritive media. (Means ± standard deviation, n = 30)

Different species of bryophytes show dissimilar responses to surface sterilization methods and require of alternative culture conditions (SABOVLJEVIC et al., 2003). The insight into biology of species, growth conditions in wild, life cycle and history, morpho-anatomical features and

the cohabitation with a variety of other organisms is acknowledged during collection, preparation and adequate sterilization treatments of such divers group like bryophytes (VUJICIC et al., 2009; 2011; 2012). Delicate vegetative and reproductive structures of bryophytes represent some challenge for establishing axenic cultures while disposing or killing xenic organisms (CHEN et al. 2009; BIJELOVIC, 2003; SABOVLJEVIC 2003; SABOVLJEVIC et al., 2006b, 2012). Thus, the application of an adequate methodology for successfully obtaining axenic cultures of bryophytes requires of a large knowledge on bryophyte biology.

Besides, the development of different bryophyte species is affected differently by exogenous chemical and physical conditions. Even, within-species variation has been observed on cultures established with the same axenical conditions, suggesting the better adaptation of particular genotypes to *in vitro* culture conditions. Also, congeneric species the different genera of a family may respond differently to axenic conditions. Experiments testing the performance of gametophores to a variety of exogenously added growth regulators and culture conditions are currently under way. These are aimed at achieving the sexual maturity of gametophores and at sporophyte development, as well as achieving *ex situ* conservation of this interesting and rare moss species.

ACKNOWLEDGEMENTS

The Serbian Ministry of Science is acknowledged for the support grants 173024 and 173030, and the Serbian-Hungarian Bilateral cooperation entitled "*Ex situ* conservation of rare and endangered bryophytes in Hungary, Serbia and Europe". JGS-M was supported by a Spanish Ministry of Science and Innovation "Ramón y Cajal" postdoctoral contract.

RESUMO: O musgo *Entosthodon hungaricus* (Boros) Loeske é uma espécie endêmica Européia típica de solos secos e salinos que se estendem da Península Ibérica até as estepes Aral-Cáspias, similar a outras briófitas de clima seco. Entretanto, a distribuição é bastante dispersa e fragmentada e a espécie é considerada muito rara e vulnerável devido às suas características efêmeras e ecologia especializada. Com o intuito de desenvolver um plano de proteção a essa espécie, foram elaborados os requisitos de preservação *ex situ das E. hungaricus.* As condições para a cultura axênica *in vitro* foram estabelecidas e os parâmetros ideais de crescimento foram atingidos para conseguir gametófitos completamente desenvolvidos, prontos para serem reintroduzidos em suas áreas nativas e em outras áreas potencialmente nativas, onde essa espécie já foi relatada. Porém, não houve coleta da mesma nos últimos anos, o que sugere uma extinção local (por exemplo, algumas áreas em Voivodina, Norte da Sérvia). Os materiais iniciais foram derivados de espécies recentes de herbários e de materiais frescos coletados de populações Húngaras. Várias formas de assepsia do material inicial e dos meios de crescimento nutritivo foram ensaiadas em diferentes regimes de luz e temperatura. No trabalho descrevemos as condições para obter desenvolvimento completo da planta e sua micropropagação. Os materiais são adequados para os fins de conservação *ex situ* e para as introduções experimentais em áreas nativas e/ou potencialmente nativas. O estudo contribui para a conservação biológica bem como para o potencial uso dessa espécie de musgo em pesquisas biotecnológicas.

PALAVRAS-CHAVE: *Entosthodon hungaricus*. Musgo. E*x situ*. Micropropagação.

REFERENCES

BIJELOVIC, A.; SABOVLJEVIC, M. Callus induction and plant regeneration in the moss *Aloina aloides* (Schultz.) Kindb. (*Pottiaceae, Bryopsida*). **Archives of Biological Sciences,** Belgrade, v. 55 n. 3-4: p. 77-80, 2003.

BOGDANOVIC, M.; SABOVLJEVIC, M.; SABOVLJEVIC, A.; GRUBISIC, D. The influence of gypsiferous substrata on bryophyte growth: are there obligatory gypsophilous bryophytes? **Botanica Serbica,** Belgrade, v. 33, n. 1, p. 75-82, 2009.

BOGDANOVIC, M.; ILIC, M.; ZIVKOVIC, S.; SABOVLJEVIC, A.; GRUBISIC, D.; SABOVLJEVIC, M. Comparative study on the effects of NaCl on selected moss and fern representatives. **Australian Journal of Botany**, Collingwood, v. 59, n. 8, p. 734-740, 2011.

BOROS, A. *Funaria hungarica,* nov. spec. **Magyar Botanikai Lapok,** Budepest, v. 23, p. 73-35, 1924.

CHEN, Y.Y.; LOU, Y.X.; GUO, S.L.; CAO, T. Successful tissue culture of the medicinal moss *Rhodobryum giganteum* and factors influencing proliferation of its protonemata. **Annales Botanici Fennici**, Helsinki, v. 46, p. 516-524, 2009.

CVETIC, T.; SABOVLJEVIC, A.; BOGDANOVIC PRISTOV, J.; SABOVLJEVIC, M. Effects of day length on photosyntetic pigments and antioxydative metabolism of in vitro cultured moss *Atrichum undulatum* (Hedw.) P. Beauv. (Bryophyta). **Botanica Serbica,** Belgrade, v. 33 n.1, p. 83-88, 2009.

DECKER, E.L.; RESKI R. The moss bioreactor. **Current Opinion in Plant Biology,** London, v. 7, p. 166-170, 2004.

DUCKETT, J.G.; BRUCH, J.; FLETCHER, P.W.; MATCHAM, H.W.; READ, D.J.; RUSSEL, A.J.; PRESSEL, S. *In vitro* cultivation of bryophytes : a review of practicalities, problems, progress and promise. **Journal of Bryology,** Cambridge, v. 26, p. 3-20, 2004.

ECCB Red Data Book of European Bryophytes. Trondheim, p. 291, 1995.

GRIMS, F. Rote Liste gefährdeter Laubmoose (Musci) Österreich. In: NIKLFELD, H. (ed.) Rote Listen gefährdeter Pflanzen Österreich. Grüne Reiche Bundesministerium Gesundheit Umweltschutz v. 5, p. 138-151, 1986.

LIANG, S.F.; SUN, Y.; ZHU, R.L. *In vitro* micropropagation of *Bryum argenteum* Hedw. **Cryptogamie, Bryologie,** Paris, v. 31, n. 3, p. 233-239, 2010.

MARTÍNEZ, K.; PRICE, M. Brood cells in the rare, epiphytic moss *Tayloria rudolphiana* (Garov.) Bruch et Schimp. (Splachnaceae). **Cryptogamie, Bryologie,** Paris, v. 32, n.1, p. 3-12. 2011.

PAPP, B. Selection of Important Bryophyte Areas in Hungary. **Folia Cryptogamica Estonica,** Tallin, v**.** 44, p. 101-111, 2008.

PISARENKO, O.Y.; IGNATOVA, E.A.; IGNATOV, M.S. *Entosthodon hungaricus* (Boros) Loeske (Funariaceae, Musci) in Altaisky territory, South Siberia. **Arctoa**, Moskow, v. 10, p. 97-102, 2001.

POCS, T.; SABOVLJEVIC, M.; PUCHE, F.; SEGARRA-MORAGUES, J.G.; GIMENO, C.; KÜRSCHNER, H. *Crossidium laxefilamentosum* Frey and Kürschner (*Pottiaceae*), new to Europe and to North Africa. Studies on the cryptogamic vegetation on loess clifs, VII.. **Journal of Bryology**, Cambridge, v. 26, p. 113-124, 2004.

PRENCE, V.C. Ex situ conservation methods for bryophytes and pterydophytes. In: GUERRANT, E.O. Jr.; HAVENS, K.; MUNDER, M. (eds.), Ex situ plant conservation. Suporting species survival in the wild.Washington, USA, Island Press. p. 206-227, 2004.

REED, B.M.; SARASAN, V.; KANE, M.; BUNN, E.; PRENCE, V.C. Biodiversity conservation and conservation biotechnology tools. **In vitro cellular and developmental biology –Plant,** Oxfordshire, v. 47, n. 1, p. 1-4, 2011.

ROWNTREE, J.K. Development of novel methods for the initiation of in vitro bryophytes culture for conservation. **Plant cell tissue and organ culture,** Dordrecht, v. 87, p. 191-201, 2006.

ROWNTREE, J.K.; RAMSAY. M.M. Ex situ conservation of bryophytes: progress and potential of a pilot project. **Boletin de la sociedad espanola de briologia**, Madrid, v. 26-27, p. 17-22, 2005.

ROWNTREE, J.K.; PRESSEL, S.; RAMSAY, M.M.; SABOVLJEVIC, A.; SABOVLJEVIC, M. In vitro conservation of European bryophytes. **In vitro cellular and developmental biology –Plant,** Oxfordshire, v. 47, n. 1, p. 55-64, 2011.

SABOVLJEVIC, M.; BIJELOVIC, A.; DRAGICEVIC, I. *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 Journal of Botany,** Ankara, v. 27, p. 441 – 446, 2003.

SABOVLJEVIC, M.; CVETIC, T.; STEVANOVIC, V. Bryophyte Red List of Serbia and Montenegro. **Biodiversity and Conservation,** Dordrecht, v. 13, p. 1781-1790, 2004.

SABOVLJEVIC, A.; SOKOVIC, M.; SABOVLJEVIC, M.; GRUBISIC, D. Antimicrobial activity of *Bryum argenteum.* **Fitoterapia,** Amsterdam, v. 77 n. 2, p. 144-145, 2006a.

SABOVLJEVIC, A.; CVETIC, T.; SABOVLJEVIC, M. The establishment and development of the Catherine's moss *Atrichum undulatum* (Hedw.) P.Beauv. (Polytrichaceae) in *in vitro* conditions. **Archives of Biological Sciences,** Belgrade, v. 58, n. 2, p. 87-93, 2006b.

SABOVLJEVIC, A.; SABOVLJEVIC, M. Bryophytes, a source of bioactive and new compounds. In: GOVIL, J. N. (ed.). Phytopharmacology and Therapeutic Values IV, the Series "Recent Progress in Medicinal Plants". Studium Press, Houston, Texas, USA. p. 9-25, 2008.

SABOVLJEVIC, A.; SABOVLJEVIC, M.; JOCKOVIC, N. In vitro Culture and Secondary Metabolite Isolation in Bryophytes In: MOHAN, J. S.; SAXENA, P. K. (eds.) Protocols for In Vitro Cultures and Secondary Metabolite Analysis of Aromatic and Medicinal Plants. Methods in Molecular Biology Humana Press, p. 117-128, 2009.

SABOVLJEVIC, M.; SABOVLJEVIC, A. Potentials of bryophytes for biotechnological use In: GUPTA, V. K. (ed.). Comprehensive Bioactive natural products V.1 - Potential & Challenges Edited by: Studium Press LC, p. 211-233, 2010.

SABOVLJEVIC, A.; SOKOVIC, M.; GLAMOCLIJA, J.; CIRIC, A.; VUJICIC, M.; PEJIN, B.; SABOVLJEVIC, M.. Comparison of extract bio-activities of *in situ* and *in vitro* grown selected bryophyte species. **African Journal of Microbiology Research,** Nairobi, v. 4 n. 9, p. 808-812, 2010a.

SABOVLJEVIC, A.; SABOVLJEVIC, M.; GRUBISIC, D. Gibberellin influence on the morphogenesis of the moss *Bryum argenteum* Hedw. in *in vitro* conditions. **Archive of Biological Sciences,** Belgrade, v. 62, n.2, p. 373-380, 2010b.

SABOVLJEVIC, A.; SABOVLJEVIC, M.; VUKOJEVIC, V. Effects of different cytokinins on chlorophyll retention in the moss *Bryum argenteum* (Bryaceae). **Periodicum Biologorum,** Zagreb, v. 112, n. 3, p. 301-305, 2010c.

SABOVLJEVIC, A.; SOKOVIC, M.; GLAMOCLIJA, J.; CIRIC, A.; VUJICIC, M.; PEJIN, B.; SABOVLJEVIC, M. Bio-activities of extracts from some axenically farmed and naturally grown bryophytes. **Journal of Medicinal Plants Research,** Nairobi, v. 5, n. 4, p. 565-571, 2011.

SABOVLJEVIC, A.; VUJICIC, M.; SKORIC, M.; SABOVLJEVIC, M. Axenically culturing the bryophytes: establishment and propagation of the pleurocarpous moss *Thamnobryum alopecurum* Nieuwland ex Gangulee (Bryophyta, Neckeraceae) in in vitro conditions. **Pakistan Journal of Botany**, Karachi, v. 44, n. 1, p. 339-344, 2012.

SARASAN, V.; CROPPS, R.; RAMSAY, M.M.; ATHERTON, C.; MCMICHEN, M.; PRENDER-GAST, G.; ROWNTREE J.K. Conservation in vitro of threatened plants – progress in past decades. **In vitro cellular and developmental biology – Plant,** Oxfordshire, v. 42, p. 206-214, 2006.

SAUER, M.; AHRENS, M. Rote Liste und Artenverzeichnis der Moose Baden-Württembergs. Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg, p. 142, 2005.

SERGIO, C.; BURGUÉS, M.; CROS, R.M.; CASAS, C.; GARCÍA, C. The 2006 red list and an updated checklist of the Iberian Peninsula (Portugal, Spain and Andorra). **Lindbergia,** Lund, v. 31, p. 109-125, 2006.

SOLTES, R.; KUBINSKA, A.; JANOVICOVA, K. Extinction risk to the bryophytes in Slovakia, reasons and evaluation. **Portugaliae Acta Biologica,** Lisbon, v. 20, p. 57-63, 2002.

VICTORIA, F.C.; OLIVEIRA, A.C.; PETERS, J.A. Establishment of the moss *Polytrichum juniperinum* Hedw. under axenic conditions. **Bioscience Journal,** Uberlandia, v. 27, n. 4, p. 673-676, 2011.

VUJICIC, M.; SABOVLJEVIC, A.; SABOVLJEVIC, M. Axenically culturing the bryophytes: a case study of the moss *Dicranum scoparium* Hedw. (Dicranaceae, Bryophyta). **Botanica Serbica,** Belgrade, v.33, n. 2, p. 137-140, 2009.

VUJICIC, M.; SABOVLJEVIC, A.; SABOVLJEVIC, M. Axenically culturing the bryophytes: establishment and propagation of the moss *Hypnum cupressiforme* Hedw. (Bryophyta, Hypnaceae) in *in vitro* conditions. **Botanica Serbica,** Belgrade, v. 35, n. 1, p 71-77, 2011.

VUJICIC, M.; SABOVLJEVIC, A.; SINZAR-SEKULIC, J.; SKORIC, M.; SABOVLJEVIC, M. *In vitro* development of the rare and endangered moss *Molendoa hornschuchiana* (Hook.) Lindb. ex Limpr. (Pottiaceae, Bryophyta). **HortScience**, Alexandria, v. 47, n. 1, p. 84-87, 2012.