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Reviving, In Vitro Differentiation, Development, and Micropropagation of the Rare and Endangered Moss *Bruchia vogesiaca* (Bruchiaceae)

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Abstract. **This study provides the results of the developmental biology of the highly rare and endangered moss species *Bruchia vogesiaca* (recorded in less than 30 localities in the Northern Hemisphere, mainly western, central, and southwestern Europe). The aim of the study was to achieve the fully developed gametophyte and to propagate it for the purpose of conservation, reintroduction, and introduction to potential habitats free from xenic contamination. These gametophytes will be used for the study of genetics and genomics of this species. The micropropagation of *B. vogesiaca* was successfully applied on BCD medium supplemented with 0.1 μM BA and on BCD supplemented with 0.3 μM IBA and 0.3 μM BA for numerous gametophore production. The highest production of secondary protonema was achieved on MS/2 S/2 medium enriched with 0.1 or 0.3 μM IBA and 0.3 μM BA. Rather successfully applied micropropagation of this threatened moss species enables better knowledge of its biology and is of great value for its conservation biology and developmental research. Chemical names used: indole-3-butyric acid (IBA), N_6-benzyladenine (BA), Murashige and Skoog medium (MS).**

Culturing plant tissues and organs under axenic conditions was first established and profitably used in bryophytes (mosses, liverworts, and hornworts), especially mosses (Lal, 1984; Servettaz, 1913). Since then, comparatively little work has been carried out using bryophytes. Just a few mosses have been used to investigate the plant growth regulators influence on their development in vitro [e.g., *Funaria hygrometrica* Hedw. and *Physcomitrella patens* (Hedw.) Bruch & Schimp., both from Funariales (Bijelović et al., 2004)]. Von Schwartzenberg (2009) reported bryophytes as interesting model systems in plant growth regulation research not only because they have simple organ structure, but also because they respond to many plant growth regulators. Not all tested bryophyte species react the same way to exogenously applied stimuli (i.e., growth regulators; Sabovljević et al., 2003; Vujičić et al., 2012). Mosses provide excellent and very convenient material for in vitro culture and they are very good experimental models for studies on basic molecular, cytological, and developmental plant biology (Duckett et al., 2004; Gonzales et al., 2006). However, axenic cultures of bryophytes are rather hard to establish and maintain (Sabovljević et al., 2003), especially those with unknown biology (Rowntree et al., 2011).

The moss *Bruchia vogesiaca* Schwägr. (Bruchiaceae) is recorded in the Northern Hemisphere (western Europe, North America, and China) in less than 30 localities (Weddeling et al., 2005). The distribution is rather fragmented and concentrated in the hilly belt of central, western, and southwestern Europe (like in southwest France; up to the alpine zone in more Atlantic situations, like in Portugal). Out of Europe, it has been collected few times in the United States: New York and New Hampshire (Rushing, 1986) and only once in China: Fujian (Cao and Gao, 1988) and none of these has been recently confirmed. *B. vogesiaca* is extremely rare in Europe. It is listed in national red lists and red data books as well as in the European level as an endangered species (European Committee for Conservation of Bryophytes, 1995) and it is reported in the annex II of the European Habitat directive. In Germany and Austria, it is considered as extinct (Grims and Köckinger, 1999; Ludwig et al., 1996), as vulnerable in Iberian Peninsula (Sérgio et al., 2006), and as endangered in France (Deperiers, 2000). Recently, it was newly recorded in England (Holyoak, 2007). Also, natural hybrids of female *Bruchia vogesiaca* × male *Trematodon ambiguus* (Hedw.) Hornschuch, another species of the bruchiaceae, were recorded in France (Frahm and Ho, 2010).

Bruchia vogesiaca inhabits the open wet edges of oligo-mesotrophic fens and moors as a shuttle species. Although the plants produce some sporophytes, the spores are rather too large for long-distance dispersal and probably water-dependent for spreading. Hugonnot et al. (2011) reported the difference in reproductive effort, i.e., less sporophyte production in some French populations, as a consequence of habitat destruction and pollution. All these are reasons for its high threat and rarity. Sites with such ecology become rarer everyday and active measures for protection are needed as proposed by Hugonnot et al. (2011) and Weddeling et al. (2005).

Bryophytes are often overlooked in conservation initiatives as diminutive, although some ex situ bryophyte collections exist (Rowntree, 2006; Rowntree et al., 2011). This is rather the result of their lower economical interest as well as the difficulty of establishing and maintaining stable in vitro cultures (Duckett et al., 2004; Sabovljević et al., 2003). Hence, protonemata can remain in early stages of development if specific exogenous physical and/or chemical factors are not involved. Such an inducing factor is not always easily to define, and if known, it is not easy to adjust the intensity and duration of its application (e.g., Bijelović et al., 2004; Sabovljević et al., 2005).

The aim of this study was to establish the in vitro culture of *B. vogesiaca* to examine its development under in vitro conditions, the

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