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Calli, cell and plantlet suspension cultures of bryophytes

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ABSTRACT

FELIX, H. (1994). Calli, cell and plantlet suspension cultures of bryophytes. *Candollea* 49: 141-158. In English, English and French abstract.

Calli, cell cultures or cultures of whole plantlets have been established of a range of bryophytes. The cultures have favourable biochemical and physiological characteristics, making them a system of high potential for morphological and physiological studies as well as for the in vitro production of valuable bryophyte-derived metabolites. To date relatively few papers have been published on cultures of bryophytes, the use of these cultures for production of metabolites is even more rare. This review summarizes the publications on calli, cell cultures or cultures of whole plantlets of bryophytes.

RÉSUMÉ

FELIX, H. (1994). Calli, cultures de cellules et de plantes entières des bryophytes. *Candollea* 49: 141-158. En anglais, résumés anglais et français.

Des calli, des cultures de cellules ou des plantes entières ont été établis pour de nombreuses bryophytes. Les cultures ont des caractères biologiques et physiologiques favorables pour des études morphologiques et physiologiques et pour la production de substances de haute valeur. Jusqu'à maintenant il n'y a pas beaucoup de publications sur la culture des bryophytes. L'utilisation des cultures pour la production des métabolites est encore plus rare. Cet exposé rassemble les publications sur les calli et les cultures de cellules et de plantes entières.

KEY-WORDS: Bryophytes — Mosses — Cell culture — Secondary metabolites — Protonemata culture — Plantlet culture — Photoautotropic growth.

1. Introduction

Plant cell culture has been proposed as an alternative supply of plant mass and phytochemicals to the normal plantation system. For bryophytes there are no extended plantation systems, thus the need for bryophyte cell cultures is even more obvious. Such systems can provide a product of constant quality and quantity, free from constraints of season, climate or disease considerations. No process, however, will be adapted unless it can compete on cost, or confer some real advantages over existing sources. Bryophyte cell cultures can act as a supply of completely novel substances opening new market areas.

In this review it will be shown, that progress in bryophyte cell culture did not go as fast as with cell culture techniques of higher plants, although even there only a few commercial applications are known. There is, however, an interesting fact with bryophyte cell cultures: Many research groups cultivate whole plantlets or protonemata in culture instead of single cells or cell aggregates. This is not possible with higher plants.

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Bryophyte calli have been discovered a long time ago (VON WETTSTEIN, 1953; MEYER, 1953). Bryophyte cell cultures were mainly used to answer morphological and physiological questions. A research group in Saarbrücken (Germany; ZINSMEISTER & al., 1991) uses bryophyte culture techniques to get enough plant material to isolate interesting secondary metabolites, which can be screened in medical and agrochemical tests.

Several authors summarized the use of calli and suspension cultures. HUNECK (1986) stated that relatively few papers have been published on the biochemistry and chemistry of cell cultures of bryophytes. CHOPRA & KUMRA (1988) discussed the study of callus formation and its differentiation as an additional tool to investigate life cycles.

The present review lists the bryophytes cultivated as calli, cell suspension or plantlet suspension; it does not contain extended information on axenic cultures of whole bryophytes on solid media, which can be found elsewhere (LAL, 1984; SARGENT, 1988; McQUEEN, 1991). Additionally information is summarized about the investigations carried out with these cultures. The aim of this review is to encourage further research in this branch of biology and bio-organic chemistry.

2. Establishment of cell cultures

As shown in Table 1 (row A) bryophyte cell cultures originate from different sources: a) from spores, b) from gametophytes, c) from gametophytic buds and gemmae, d) from sporogons, e) from protoplasts. There is indication that the origin of the callus plays a role: Different types of callus tissues appear to originate from different tissues of thallus (TRIPATHY, 1978a, 1978b). They differ in their external and internal morphology, pattern of growth, potentiality for differentiation and physiological requirements.

In a first step bryophyte parts need to be sterilized. The majority of species are surface sterilized using the washing machine technique (BASILE, 1972; BASILE & BASILE, 1988) on either shoot tips or unopened capsules. Clorox (9%) for 1 minute works best for most species, but the time and concentration have to be modified for delicate and tough specimens. Spore suspensions from surface sterilized capsules are spread on agar-plates, and spores from untreated capsules are spread by the top technique described by DOYLE (1967). A few species, e.g. *Treubia* and *Cryptothallus,* have proven to be totally recalcitrant to surface sterilization, probably due to obligate, symbiotic fungi and fungal and bacterial (especially blue-green algae) contaminants, which are able to destroy bryophyte cell cultures.

LAL (1984) and SARGENT (1988) list a whole number of species cultured under sterile conditions together with recommended growth conditions. SIMON (1988) emphasizes particularly the sterile cultivation of *Sphagnum* species, which can be a source for calli or suspension cultures. KATOH (1988) describes in a general way possibilities to get calli and suspension cultures. Table 1 summarizes literature on culture of bryophytes in general (row a) and literature on effects of different factors on growth of calli or suspension cultures (rows c, d, e, f, g, h). Plant hormones and other compounds like chelated iron, vitamines, light, temperature, humidity, sugars, pH, bacteria all influence growth. Plant hormones (BOPP, 1983; COVE & ASHTON, 1984) and sugars (KATOH, 1988; JONES, 1978) seem to play a major role. According to the literature (Table 1, row c) hormones strongly influence bryophytes as well as higher plants. UV light can substitute for growth regulators (KUMRA & CHOPRA, 1982). The effect of sugars (Table 1, row f) was tentatively explained by JONES (1978) who found soluble carbohydrates in inorganic culture solutions containing aseptically cultivated *Sphagnum* shoots. He concluded that to grow normally in liquid culture, *Sphagnum* requires sugars to compensate for those carbohydrates lost by leakage. Activated charcoal can inhibit calussing of protonema (RAHBAR, 1982), indicating adsorption of an essential compound.

Not all bryophytes are cultured in the form of undifferentiated tissue (calli, suspension), many authors use axenic suspension cultures of whole plantlets, protonemata or chloronemata (Table 1, row q). These cultures have special requirements. Repeatedly researchers mentioned that inorganic media are insufficient to allow normal growth and development of *Sphagnum* in axenic cultures (SIMOLA, 1969; HINTIKKA, 1972; JONES, 1978; BAKER & BOATMAN, 1985; KAJITA

& al., 1987). In non-agitated batch cultures the protonema development, bud formation, and growth of shoots are poor without any addition of an organic carbon source. SIMOLA (1969, 1975, 1979) studied the effect of several sugars, amino acids and dipeptides on the growth of *Sphagnum* in axenic cultures. Ca. 200-230 mg dry weight were produced in 100 days when one 10 mm shoot tip was used as inoculum per flask containing nutrient medium with 1% sucrose as a carbon source (SIMOLA, 1977; RUDOLPH & al., 1988).

Several authors developped fermenters for the cultivation of bryophytes (Table 1, row B; BOYD & al., 1988; RUDOLPH & al., 1988; JACUBOWSKI & RUDOLPH, 1989; OHTA & TAKATA, 1990; ZINSMEISTER & al., 1991). RUDOLPH & al. (1988) describe a continuous feed fermenter for whole plantlets. Multicellular organisms form large aggregates, which are difficult to deal with in fermenters, for clumping soon takes place, together with layering. It is difficult to achieve adequate agitation, especially turbulence, without damaging the branched leafy moss shoots. Therefore common types of stirrers are not appropriate. An airlift fermenter (rising current of air) is specially suited to agitate delicate moss shoots. The airlift system described by BOYD & al. (1988) provides a supply of protonemal tissue for analysis, and allows time courses to be followed, for example after the addition of a plant hormone. *Physcomitrella patens* will develop from the protonemal stage in this system to give small plantlets, consisting of a protonemal ball with gametophores, but such development is delayed compared with growth on solid media (20-24 days instead of 10-14 days). If gametophytic tissue is required, the fermenter will need some alterations, as clogging of the outflow will be a problem. Alternatively, if longer production runs of protonemal tissue are required, it may be possible to manipulate the culture medium in order to extend the protonemal phase. To keep the culture axenic proved to be most difficult, even though there was no organic carbon source in the medium. Only by a rigorous test of all possible contamination sources was this problem eliminated. For continuous culture, a representative outflow is essential, in order that the specific growth rate, calculated from the dilution rate and the steady state dry weight, is accurate.

3. Effect of physical parameters on growth

As can be expected the environmental conditions play a major role in the growth of bryophytes in cultures. The influence of light including UV light (Table 1, row d) seems to be especially important. Bryophytes grow better in culture, if light is not completely absent. Photoautotrophic growth of bryophytes in culture is easier achieved than with higher plants (see chapter on photoautotrophic growth), most probably this is due to a higher chlorophyll content. Other physical parameters like temperature, humidity and pH (Table 1, rows e and g) were also investigated.

4. Photoautotrophic growth

The majority of cell cultures of higher plants exhibit a low rate of photosynthetic growth, probably because the chlorophyll content of the cells is low. Callus cells from bryophytes contain a high concentration of chlorophyll (ALLSOPP, 1957; WARD, 1960a & 1960b), but few attempts have been made to establish photoautotrophic suspension cultures of bryophyte cells (Table 1, row k). The cells of liverworts grow rapidly in the light in liquid culture media containing glucose, they contain a high amount of chlorophyll. In contrast to most cultured cells from seed plants, liverwort cells apparently do not grow in the dark even when glucose is present. A cell line isolated from *Marchantia polymorpha* was capable of photoautotrophic growth under air enriched with 1% CO_2 (KATOH, 1983b), *Sphagnum imbricatum* could also be grown under elevated CO_2 concentration (KAJITA & al., 1987). When chlorophyllous cells of *Marchantia paleacea* that had been grown heterotrophically in the dark were transferred to phototrophic conditions, these cells started to grow photoautotrophically without a lag phase (NGUMI & al., 1990), may-be the chlorophyll content is less controlled by light.

Table 1. — Bryophytes listed alphabetically together with a brief discription of investigations carried out.

Bryophytes	*A*	*B*	*Investigations carried out*																			*References*
			a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	
Anoectangium thomsonii	2	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	SAXENA & RASHID, 1982
Anthoceros punctatus	2	c,s	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	TAKAMI & al., 1988a
A. punctatus	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
Asterella angusta	2	c	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	RASHID, 1971
A. wallichiana.....	3	c	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	KUMRA, 1984; KUMRA & CHOPRA, 1987
A. wallichiana.....	2	c	.	.	.	x	x	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	CHOPRA & KUMRA, 1986
Athalamia pusilla.....	2	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MEHRA & PENTAL, 1976
Atrichum sp.....	1	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MITSUBISHI-GAS-CHEM, 1988
Atrichum undulatum.....	2	s	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1987
A. undulatum	2	c	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	WARD, 1960b
A. undulatum	2	c	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976
Barbula unguiculata	2	s	.	.	.	x	.	x	.	.	.	.	.	.	.	.	x	.	.	.	.	.	TAKIO & al., 1986
B. unguiculata.....	2	s	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	TAKIO, 1987
B. unguiculata.....	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	TAKAMI & al., 1988b; TAKIO & al., 1990
B. unguiculata.....	2	s	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	TAKIO, 1989
B. unguiculata.....	2	s	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	TAKIO & NAGATA, 1990
Bartramidula bartramioides..	2	s	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	RAHBAR & CHOPRA, 1982
Blasia pusilla.....	2	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ALLSOPP & ILAHI, 1970b
Bryum argenteum.....	2	s	.	x	.	x	x	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	CHOPRA & BHATLA, 1981
B. coronatum	2,4	c	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	KUMRA & CHOPRA, 1982
Calypogeia azurea	1	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990
C. azurea	2,3	s	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	KATOH & TAKEDA, 1990
C. granulata	2	s	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	.	TAKEDA & KATOH, 1981 & 1983a & 1983b
C. granulata	2,3	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	KATOH & TAKEDA, 1990
C. muelleriana	2,3	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	KATOH & TAKEDA, 1990
C. peruviana	2,3	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	KATOH & TAKEDA, 1990
C. tosana	2	c	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	ABE & OHTA, 1983
C. tosana	2,3	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	KATOH & TAKEDA, 1990
Cephaloziella sp.	2	c	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MEYER, 1953
Climacium americanum	1	c	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976
Conocephalum conicum	3	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990
Dawsonia sp.....	1	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MITSUBISHI-GAS-CHEM., 1988
Dicranella schreberi.....	2	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ULYCHNA, 1971
Entodon seductrix	2	c	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976
Eurhynchium striatum	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	x	.	.	.	.	HANSEN & ROSSI, 1991
Exormotheca sp......	1	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MITSUBISHI-GAS-CHEM., 1988
Fossombronia sp.....	1	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MITSUBISHI-GAS-CHEM., 1988
Fossombronia pusilla.....	2	c	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ALLSOPP, 1957
F. pusilla	1	c	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ILAHI, 1972
F. pusilla	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	SAUERWEIN & BECKER, 1990
F. pusilla	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990

Table 1 (cont.)

Bryophytes	*A*	*B*	*Investigations carried out*																			*References*
			a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	
Funaria hygrometrica	2	c	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	VON WETTSTEIN, 1953
F. hygrometrica.....	2	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BÜNNING & VON WETTSTEIN, 1953
F. hygrometrica.....	2	s	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	SZWEYKOWSKA & al., 1968; JOHRI, 1974 & 1975; HANDA & JOHRI, 1976 & 1979
F. hygrometrica.....	5	c	.	x	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	RASHID & CHOPRA, 1969
F. hygrometrica.....	2	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1975
F. hygrometrica.....	2	s,f	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	NAEF, 1975
F. hygrometrica.....	2	c	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976; SPIESS & al., 1977a
F. hygrometrica.....	2	s	.	.	x	.	.	.	.	.	x	.	.	.	.	.	.	x	.	.	.	.	HANDA & JOHRI, 1977
F. hygrometrica.....	2	s	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	SUSSMANN & KENDE, 1977
F. hygrometrica.....	2	s	x	.	.	.	.	.	.	.	x	.	.	.	.	.	.	x	.	.	.	.	SHARMA & al., 1979
F. hygrometrica.....	2	s	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	RESKI & ABEL, 1985
F. hygrometrica.....	2	s	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	x	.	.	.	.	JOHRI & HENRIQUES, 1990
F. hygrometrica.....	2	s	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	x	.	.	.	.	PADIDAM & JOHRI, 1991; PADIDAM & al., 1991
Heterophyllium haldaneanum	2	c	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976
Heteroscyphus bescherellei...	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
Hyophila involuta	2	c	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	RAHBAR & CHOPRA, 1980; RAHBAR, 1982; MEHRA, 1984
Jungermannia infusca	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
J. subulata.....	4	s	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	OHTA & al., 1981
J. subulata.....	2	s	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	OHTA & HIROSE, 1982
J. subulata.....	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	ABE & OHTA, 1983
J. subulata.....	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	ONO & OKAMOTO, 1984
Leptobryum pyriforme	2	s	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	x	.	.	.	.	EULER & al., 1988
Lophocolea heterophylla.....	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	ABE & OHTA, 1983
Makinoa crispata.....	1	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ILAHI & ALLSOPP, 1969 & 1970
Mannia androgyna.....	2	c	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	KAPUR & CHOPRA, 1991
M. dichotoma.....	2	c	x	x	.	x	x	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	KAPUR & CHOPRA, 1989; CHOPRA & KAPUR, 1989
Marchantia nepalensis.....	1	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	KAUL & al., 1961 & 1962
M. nepalensis	1	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	TRIPATHI, 1978a & 1978b
M. nepalensis	4	s	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	ONO & al., 1979
M. nepalensis	2	s	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	KATOH & al., 1979
M. paleacea var. *diptera*	2	s	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	NGUMI & al., 1990
M. paleacea var. *diptera*	2	s	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	x	.	.	.	TAKIO, 1987 & 1990
M. paleacea var. *diptera*	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	TAKIO & al., 1988
M. paleacea var. *diptera*	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
M. palmata	3	c	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	DHINGRA-BABBAR & CHOPRA, 1985, 1986a & 1986b
M. palmata	3	c	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	CHOPRA & DHINGRA-BABBAR, 1986
M. polymorpha	4	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	SCHNEIDER & al., 1967

Table 1 (cont.)

Bryophytes	*A*	*B*	*Investigations carried out*																			*References*
			a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	
Marchantia polymorpha	4	c	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO, 1973
M. polymorpha	4	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO, 1974, 1976a & 1976b
M. polymorpha	4	s	x	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	OHTA & al., 1977
M. polymorpha	4	s	.	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	ONO & al., 1979
M. polymorpha	2	s	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	KATOH, 1983a & 1983b; KATOH & al., 1979 & 1980
M. polymorpha	4	s	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	OHYAMA & al., 1982 & 1983; TANAKA & al., 1984
M. polymorpha	2	s	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	KONNO & al., 1983; TAKIO, 1987; TAKIO & HINO, 1988
M. polymorpha	4	c	.	.	x	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	SUGAWARA, 1983
M. polymorpha	2	s	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	ABE & OHTA, 1983 & 1984; OHTA & al., 1983 & 1984
M. polymorpha	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	ONO & OKAMOTO, 1984
M. polymorpha	4	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	x	.	.	.	.	IMOTO & OHTA, 1985
M. polymorpha	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	KONNO & al., 1987; SATO & KATO, 1988
M. polymorpha	2	s	.	.	.	.	.	.	.	.	.	x	.	.	x	.	.	.	.	.	.	.	OHTA & TAKATA, 1990
M. polymorpha	4	c	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	HAMADA & al., 1991
Moerckia flotowiana	1	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ALLSOPP & ILAHI, 1969; ILAHI & ALLSOPP, 1969 & 1970
Noteroclada confluens	2	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ALLSOPP & ILAHI, 1970a & 1970b
N. confluens	1	c	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ILAHI, 1972
Pellia endiviifolia	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
P. epiphylla	2	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ILAHI & ALLSOPP, 1969 & 1970
P. epiphylla	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990
P. neesiana	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
Petalophyllum ralfsii	2	c	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ILAHI, 1972
Physcomitrella patens	2	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	BEUTELMANN & al., 1978
P. patens	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	GRIMSLEY & al., 1977; WANG & al., 1980; 1981a & 1981b
P. patens	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	GRIMSLEY & WITHERS, 1983
P. patens	2	s	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	RESKI & ABEL, 1985
P. patens	2	s	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	ABEL & al., 1989
P. patens	2	s,f	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	BOYD & al., 1988
Physcomitrium coorgense	3	c	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	LAL, 1961
P. pyriforme	5	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BAUER, 1957 & 1961
P. pyriforme	2	c	.	x	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MENON & LAL, 1972
P. pyriforme	2	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MENON & LAL, 1974
P. pyriforme	2	c	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	.	MENON & HARTMANN, 1988a & 1988b
P. pyriforme × *F. hygrometrica*	5	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BAUER, 1961 & 1963

Table 1 (cont.)

Bryophytes	*A*	*B*	*Investigations carried out*																		*References*
			a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	
Physcomitrium pyriforme × *F. hygrometrica*	5	c	.	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	BAUER, 1966; HARTMANN 1971; BEUTELMANN, 1973; BEUTELMANN & BAUER, 1977
P. pyriforme × *F. hygrometrica*	5	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	HARTMANN, 1972
P. pyriforme × *F. hygrometrica*	5	c	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	HARTMANN, 1973
P. pyriforme × *F. hygrometrica*	5	c	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	HARTMANN, 1974; HARTMANN & KILBINGER, 1974
Plagiochila arctica	3	s	.	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	LAW & al., 1985
P. arctica	3	s	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BASILE, 1979
P. ovarifolia	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
Plagiomnium vesicatum	3	s	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	.	.	MAEDA, 1979
Polytrichum commune	2	c	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	WARD, 1960a & 1960b
P. commune	1	c	x	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	WARD & FREDERICK, 1967
P. commune	2	c	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976
P. commune	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
P. formosum	2	s	.	x	x	.	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	NAEF & MISSET, 1973
P. juniperinum	6	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	GAY, 1976
Pottia intermedia	2	c	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	RIPETSKIJ, 1978 & 1979
Pylaisiella selwynii	2	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS & al., 1973
P. selwynii	2	c	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976; SPIESS & al., 1977a, 1977b & 1981
P. selwynii	2	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1979
Reboulia hemisphaerica	2	c	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ALLSOPP, 1957
R. hemisphaerica	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
R. hemisphaerica	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	TAKIO & al., 1990
R. hemisphaerica	1	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990
R. hemisphaerica	1	c,f	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	MARAIS & BECKER, 1990
Rhytidiadelphus squarrosus	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	x	.	.	.	.	HANSEN & ROSSI, 1991a, 1992b
Riccardia multifida	1	c	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ALLSOPP & ILAHI, 1969; ILAHI, 1972
R. multifida	1	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ILAHI & ALLSOPP, 1969
R. pinguis	1	c	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ILAHI & ALLSOPP, 1969 & 1970; ILAHI, 1972
R. pinguis	1	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ALLSOPP & ILAHI, 1969; ILAHI & ALLSOPP 1970
Riccia crystallina	1	c	.	x	x	x	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	SOOD, 1974; CHOPRA & SOOD, 1973
R. fluitans	3	s	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	LISA, 1971
R. frostii	3	c	.	x	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	VASHISTHA & CHOPRA, 1989
Ricciocarpos natans	2	c,s	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ONO & al., 1988
R. natans	1	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	WURZEL & BECKER, 1990; WURZEL & al., 1990
R. natans	1	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990

Table 1 (cont.)

Bryophytes	*A*	*B*	*Investigations carried out*																		*References*
			a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	
Scapania nemorea	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990
S. nemorea	4	c	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BASILE, 1965
Sphaerocarpos donnellii	3	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	MACHLIS & DOYLE, 1962; MACHLIS, 1962
S. donnellii	3	s	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	MILLER & MACHLIS, 1968
S. donnellii	3	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	x	GRUSAK & al., 1980
S. texanus	3	s	x	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MONTAGUE & TAYLOR, 1971
S. texanus	3	s	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	STAHLY & al., 1977
Sphagnum cuspidatum	2	s	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BAKER & BOATMAN, 1985
S. cuspidatum	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	RUDOLPH & al., 1988
S. fallax	2	s	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	HINTIKKA, 1972
S. fallax	2	f	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	x	.	.	.	.	JACUBOWSKI & RUDOLPH, 1989
S. fallax	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	RUDOLPH & al., 1988
S. fimbriatum	2	s	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	SIMOLA, 1977
S. imbricatum	2	s	.	.	x	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	KAJITA & al., 1987
S. imbricatum	2	s	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	TAKIO, 1987
S. imbricatum	2	s	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	TAKAMI & al., 1990
S. magellanicum	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	RUDOLPH & al., 1988
S. magellanicum	2	f	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	x	.	.	.	.	JACUBOWSKI & RUDOLPH, 1989
S. majus	2	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	RUDOLPH & al., 1988
Symphyogyna brongniartii	2	s	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	x	.	.	.	.	SPOERLE & al., 1990
Thuidium delicatum	2	c	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	SPIESS, 1976
Trichocolea tomentella	1	s	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	BECKER, 1990

Areas of research:
- a establishment of cultures
- b differentiation, morphogenetic studies
- c effects of plant hormones and other compounds
- d effects of light (UV light included) on growth
- e effects of temperature or humidity on growth
- f effects of sugars on growth
- g effect of pH on growth
- h effects of *Agrobacterium tumefaciens* and other bacteria
- i enzymology
- k photoautotrophic growth
- l chloroplast DNA
- m excretion of compounds
- n production of primary and secondary metabolites
- o culture of protoplasts
- p photosynthetic activity
- q whole plants in culture (incl. protonemata, chloronemata)
- r mutants
- s preservation of cultures
- t oxygen metabolism
- u cell wall biosynthesis

A = Origin of cultures:
- 1 callus of undefined origin
- 2 from spores
- 3 from whole gametophytes, surface sterilized
- 4 gametophytic buds, gemmae
- 5 sporogons
- 6 from protoplasts

B = Type of culture:
- c callus
- s suspension culture
- f fermenter

Bryophyte	Type of culture	Doubling time (days)	References
Barbula unguiculata	photoautotrophic growth, suspension culture	4,5	TAKAMI & al., 1988b
Physcomitrella patens	protonemata in airlift fermenter	1,1	BOYD & al., 1988
P. patens	protonemata in spherical aeration vessel	4,4	WANG & al., 1981a
Marchantia polymorpha ...	suspension culture, photoautotrophic growth	1,1	KATOH, 1983b
M. polymorpha.....	jar-fermenter, photoautotrophic growth	2	OHTA & TAKATA, 1990
Pellia endiviifolia	suspension culture	7,4	ONO & al., 1988
Ricciocarpos natans.....	axenic culture of whole bryophytes	4,1	WURZEL & BECKER, 1990
Sphagnum sp.....	cell suspension culture in the light	3,3	KAJITA & al., 1987
Sphagnum fallax	fermenter, whole plants	5	RUDOLPH & al., 1988
Marchantia paleacea	cell suspension culture, photoautotrophic growth	4,7	NGUMI & al., 1990

Table 2. — Growth rates of bryophyte cultures.

A high cell density makes it quite difficult to irradiate cells in large-scale culture such as a jar-fermenter. If illumination is provided from outside of the vessel, only the cells near the surface of side walls will receive sufficient intensity of light. In order to cultivate liverwort cells in a jar-fermenter under illumination, a new culture method was developped in which light is introduced directly into the culture vessel by using optical fibres (OHTA & TAKATA, 1990).

5. Growth rates in suspension cultures and fermenters

In general microbial cells have rapid growth rates with doubling times measured in hours, whereas in plant cell suspension cultures this is measured in days. One of the fastest growing cultures has been tobacco with doubling times of 18 hours, but a more typical time would be 2-3 days (NOGUCHI & al., 1977). Comparing these values with doubling times of bryophyte cultures (Table 2), the growth rates of bryophytes are very similar. It is, however, remarkable, that without special optimization some bryophytes grow as fast as the particular tobacco strain. Over all, similar problems will be observed in a bioreactor with higher plants and bryophytes, a run of a plant suspension can be from one to four weeks which means a much greater difficulty in maintaining sterility than with bacteria, because slow growth of plant cells means that almost any contamination will outgrow the culture.

6. Storage of cell cultures

Continuous vegetative propagation in sterile culture is used as a storage method for bryophytes, since many do not produce spores due to self-sterility or the presence of a developmental block in the gametophyte. Usually it is possible to cope with subculturing demands. However, the number of stock strains can increase beyond this point and, more importantly, there is evidence of strains undergoing changes with time in culture. The risk of mutations accumulating in culture and risks of loss of irreplaceable strains by microbial contaminations, which can be very difficult to eradicate, have led GRIMSLEY & WITHERS (1983) to develop a stable storage by cryopreservation in liquid nitrogen.

7. Bryophytes used

In Table 1 bryophytes are listed alphabetically together with a brief discription of the investigations carried out and the kind of culture used (callus, suspension culture, culture of whole plants, fermenters). It is evident, as in many areas of biology, that there are favorite organisms, e.g. *Marchantia polymorpha*, *Physcomitrium pyriforme*, *Sphagnum* spp. Compared to the countless number of investigations carried out using cell cultures of higher plants, the use of bryophyte cultures is more limited. Not even all bryophyte families were considered. The reason for this neglect may be, that there are no crops among bryophytes, bryophytes are also not used as a model for higher plants. The interest may increase a little bit, if there is a need for sufficient plant material to isolate secondary metabolites (ZINSMEISTER & al., 1991).

8. Investigations carried out

Cell cultures of hepatics and mosses are used for the same purposes as cultures of higher plants, namely to get reproducible and comparable results. Essentially the same investigations are carried out: studies of morphogenesis, differentiation, effects of different factors on growth, physiology, biochemical studies, enzymes, photosynthesis, oxygen metabolism, cell wall biosynthesis, genetics, production of primary and secondary metabolites.

8.1. Differentiation, morphogenetic studies

Bryophytes have proven to be a good material to study morphogenesis (Table 1, row b). Calli can readily be induced from different parts of the gametophyte and from the sporophyte, and it can be made to differentiate into gametophytes or sporophytes, respectively, by modifying the cultural conditions. This is possible by variation of single elements or by changing the relative concentration of elements in the different solutions (Table 1, rows c and f). Therefore it must be shown for each bryophyte material which of the nutritional solutions may provide the optimal results in vitro. The use of protoplasts as starting material for morphological studies has also been described (Table 1, row o). Agrobacteria, the causal agents of the crown gall, cane gall, and hairy root diseases in higher plants, as well as other bacteria, accelerate normal development and induce abnormal changes in bryophytes also. Pathogenic strains are typically most effective in bringing about these developmental changes, which require physical contact between the bacteria and the moss. SPIESS & al. (1981) conclude that many bacteria closely associated with mosses in nature are capable of influencing moss development in the protonemal stage and thus could play a role in the normal initiation of gametophore development. While some of these strains may prove to be agrobacteria, the tests employed were insufficient to establish such an identification. Besides studying the effect of bacteria the influence of fungi on bryophyte development also deserves further attention (FELIX, 1988).

8.2. Biochemical studies

Compared to the biochemical knowledge about higher plants, much less is known about bryophytes. Bryophyte cell cultures are an ideal source for tissue, because reproducible and comparable results can be obtained. A few enzymological investigations were carried out (Table 1, row i), whereby enzymes were extracted from cell cultures. Chloroplast DNA was examined in bryophytes (Table 1, row l), photosynthetic activity (Table 1, row p), oxygen metabolism (Table 1, row t) and cell wall biosynthesis (Table 1, row u) were followed. A detailed description of the reports is not given here, because the experiments must be seen in other contexts than just the use of cell cultures.

8.3. Genetic studies

COVE & ASHTON (1984) and KNIGHT & al. (1988) describe in detail the use of mutants to study the role of hormones in moss development and other processes, in these reviews it was not indicated, whether also cell cultures were mutagenized. BEUTELMANN & al. (1978) treated spores with N-methyl-N'-nitro-N-nitrosoguanidine and established cell cultures from them. TAKIO (1990) selected mutants directly from cell cultures after incubation of cells in chlorate.

8.4. Production of primary and secondary metabolites

Between 25% and 30% of prescribed pharmaceuticals are extracted from plant sources. Plants are also used as sources of food colourings and flavourings, and also in the production of agrochemicals. These products supply a market worth billions of dollars per annum. For compounds of plant origin the source is usually assumed to be a higher plant. The chemical analysis of bryophytes was neglected for a long time, the main reason for this fact may be the difficulty to collect sufficient amounts of bryophytes to isolate the pure compound. The easier it was to characterize minute amounts of isolated compounds the more secondary metabolites could be investigated. Nevertheless only about 5% of all known bryophyte species were investigated for their chemical constituents.

An interesting feature of bryophytes is that they are relatively free from attack by parasitic microorganisms (FELIX, 1988), e.g. herbarium specimen need no special treatment like those of higher plants. The cause of this may be anatomical, immunological or may be due to the presence of antimicrobial activity or other factors. In recent publications on disease resistance of plants (KUC, 1990) and on the role of secondary metabolites in chemical defence mechanisms (HARBORNE, 1990) bryophtes were not mentioned at all, obviously this phenomenon was not studied in bryophytes. In this context it would also be interesting to study phytoalexins as part of induced defence reactions in bryophytes as it is done in higher plants (BARZ & al., 1990). It is not known whether phytoalexins exist in bryophytes at all, some hints can be found in several publications (ZINSMEISTER & MUES, 1990). Besides antibiotic activity of bryophytes (BANERJEE & SEN, 1979; ASAKAWA, 1990) other biological effects of compounds from bryophytes were observed: allergenic effects, cytotoxic and cytostatic effects, cardiotonic activity, carcinogenic activity, antiinflammatory activity, vasopressin-antagonistic activity, release of hyperoxide, piscicidal, molluscicidal and feed repellent effects, growth regulation in higher plants, enzyme inhibition (HUNECK, 1983; ZINSMEISTER & MUES, 1990; ZINSMEISTER & al., 1991; ASAKAWA, 1990). A relatively large screening program for antitumor agents was carried out (SPJUT & al., 1986). In this report the problem of collecting enough material was mentioned: samples weighing 0.5 — 2 kg (dried) were collected for each of 208 species and varieties. In vitro cultures offer the possibility to obtain sufficient material (BECKER, 1990; KATOH & TAKEDA, 1990; OHTA & TAKATA, 1990; SAUERWEIN & BECKER, 1990). This technique is used for intensive screening programs at the University of Saarbrücken, Germany (BECKER, 1990; ZINSMEISTER & al., 1991).

Mainly liverwort cultures were screened for secondary metabolites. SAUERWEIN & BECKER (1990) and WURZEL & BECKER (1990) found that *Fossombronia pusilla* and *Ricciocarpos natans*, respectively, produced the same pattern of secondary metabolites as field collected material (perrottianal, santonin, 7 terpenes with antimicrobial activity). The content of the terpenoid decreases on nitrogen and phosphorous-deficient media. MORAIS & BECKER (1990) found that differentiated cultures on agar produced sesquiterpenes in tenfold increased amount compared to undifferentiated suspension cultures. TAKEDA & KATO (1981, 1983a, 1983b) found that *Calypogeia granulata* had a higher yield in essential oils in suspension cultured cells than for freely growing, intact plants. The hypothesis of bryophytes producing secondary metabolites independent of their developmental stage is controversial (H. BECKER, personal communication). ABE & OHTA (1983, 1984) and OHTA & al. (1983, 1984) investigated prelunularic acid and lunularic acid in various liverworts. Lunularic acid may be a secondary metabolite or an endogenous bryophyte growth regulator, it may control drought resistance. The accumulation of lunularic acid is inversely correlated with the amount of phosphate in the medium. The age-dependent accumulation of

lunularic acid can be explained as the result of depletion of phosphate in the medium. Some compounds are excreted into the medium (Table 1, row m). Excretion does not seem to be dependent on the size of the molecules.

HAMADA & al. (1991) describe a green cell suspension culture of the liverwort *Marchantia polymorpha* converting testosterone to 6β-hydroxytestosterone and epitestosterone to androst-4-ene-3,17-dione.

Commercial interest in secondary metabolites of bryophytes from cell or whole plant culture origin is still rare. Lack of material for extraction and following difficulties with structure analysis may have been the reason. The chemical company BASF together with ZINSMEISTER & al. (1991) developped an extended screening program. MITSUBISHI-GAS-CHEM. (1988) may also have a commercial interest in secondary metabolites.

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