# Morphogenetic Studies and *In vitro* Propagation of Two Mosses: *Philonotis thwaitesii* Mitt. and *Brachythecium plumosum* (Hedw.) B.S.G.

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ABSTRACT: Well differentiated gametophytes of *Philonotis thwaitesii* Mitt., an acrocarpous moss and *Brachythecium plumosum* (Hedw.) B.S.G., a pleurocarpous moss have been raised *in vitro* by inoculating their spores into a range of concentrations of inorganic media. Half-strength Knop's macronutrients + Nitsch's trace elements with 10 ppm ferric citrate was found the most suitable for growth and multiplication of these species in continuous light of 4,000-5,500 lux and at 21 ± 2ºC temperature. Protonemal buds developed on caulonema in *P. thwaitesii* while on chloronema in *B. plumosum.* Sucrose stimulated caulonemal differentiation, abundant rhizoid production and a few but much robust gametophyte development. Emergence of hyaline rhizoids like filaments has been observed in *P. thwaitesii* upon contact of gametophores with solid substratum while true rhizoids all along the length of gametophytes of *B. plumosum* emerges upon placing them horizontally on culture medium. Variable growth forms (weft-like branched as well as erect unbranched habit) have been observed in both species in culture conditions. Sub-culturing of *P. thwaitesii* gave rise to new population via passing through chloronemal and caulonemal stage while *B. plumosum* directly regenerated into new gametophytes by-passing protonemal stage. *In vitro* raised plants were acclimatized and transferred to soil in order to their further proliferation.

KEY WORDS: Acrocarpous moss, growth forms, *in vitro* propagation, pleurocarpous moss, spores.

## INTRODUCTION

*In vitro* cultivation is not only promising for the use of bryophytes in various aspects of cellular, developmental and morphogenetic studies but also an essential tool in supplying stable research materials for phytochemists as well as botanists. Culture studies in different bryophyte taxa have been carried out in the past regarding to nutritional requirement (Basile, 1975), spore germination, sporeling and regeneration studies (Inoue, 1960; Fulford, 1975; Renzaglia, 1978), controlled differentiation (Allsopp, 1957), while particularly on mosses, the studies were centered on spore germination and protonemal morphogenesis (Allsopp and Mitra, 1956; Nishida, 1978; Duckett et al., 1998), induction of buds in various mosses (Allsopp and Mitra, 1958), effect of various physical and chemical factors and growth substances on protonemal morphogenesis and bud induction (Mitra and Wareing, 1959; Mitra and Allsopp, 1959; Brandes and Kende, 1968; Johri and Desai, 1973; Chopra and Bhatla, 1981; Bopp, 1983) and reproductive biology of some mosses (Chopra and Rashid, 1967; Chopra and Bhatla, 1981; Nath et al., 2009; Awasthi et al., 2010). But so far, only a little work has been done in cultivating bryophytes in order to make them available in bulk amount for their potential use or further bioprospection studies inspite of knowing the fact that mosses are potentially rich in many biologically active substances (McCleary et al., 1960; Sakai et al., 1988; Sabovljevic et al., 2001). In this context, Decker and Reski (2007, 2008) have established moss bioreactors for improved bio-pharmaceuticals. Recently, Sabovljevic et al. (2009) initiated a work in this regard and developed protocol to isolate secondary metabolites from axenic cultures of bryophytes.

Pleurocarpous mosses have not been attended in culture of bryophytes inspite of their sound economic importance in horticulture industry. They are of wide use in horticulture and frequently used as mulching material in maintaining freshness of fruits, vegetables, bouquet etc. because of their high water holding capacity and permeability to air (Ishikawa, 1974). In the alpine highlands of the north west Himalayas, Indians frequently use the some common pleurocarpous moss species i.e. *Brachythecium salebrosum, Macrothamnium submacrocarpum, Actinothuidium hookeri, Hypnum cupressiforme, Floribundaria floribunda, Neckera crenulata* etc. in wrapping apples and plums and also making bedding, mattress, cushions, pillows by stuffing mosses into coarse linen sacks by spreading them on the muddy floor because of their properties of insect repellence and rot resistance (Pant and Tewari, 1989). Medicinal value of these mosses has also been come into knowledge as possessing an antimicrobial activity (McCleary and Walkington 1966;

Gupta and Singh, 1971). Species of *Hypnum*, *Plagiothecium*, *Entodon* and *Brachythecium* possess antibacterial, antifungal and anti-tinnitus activities (Wolters, 1964; Dayal and Mishra, 1966; Banerjee and Sen, 1979). These mosses are found in specific habitat and majority of them are epiphytic. Hence culturing the pleurocarpous mosses may be a good strategy to augment the scope of their use without overharvesting the valuable biodiversity from its natural habitat.

Till now, only a few preliminary works have been carried out on culture of these plants. Nishida (1978) studied the sporeling types among various mosses including some pleurocarpous moss taxa, while Sabovljevic et al. (2003) established the axenic cultures of *Brachythecium velutimum* (Hedw.) B.S.G. and *Eurhynchium praelongum* (Hedw.) B.S.G. only up to protonemal stage and bud formation. Recently, Awasthi et al. (2010a) established axenic culture of well-developed branched gametophyte of *Entodon laetus* (Griff.) Jaeg. In the present contribution, axenic cultures of two moss taxa viz. *Philonotis thwaitesii* Mitt., an acrocarpous moss belonging to family Bartramiaceae and *Brachythecium plumosum* (Hedw.) B.S.G., a pleurocarpous moss belonging to family Brachytheciaceae were established by inoculating their spores into agar media and the comparative morphogenetic studies have been carried out on these two species. The well-differentiated cultured plants were acclimatized and subsequently transferred on soil where they further propagated. Hence the described technique may be useful as a model for establishing axenic cultures of not only potential moss taxa having economic or medicinal value but also of rare, endangered and threatened moss taxa. The first phase should be multiplication and propagation in pure vegetative population that can be harvested in other bioprospection studies followed by reintroduction of the cultures of species to native and potentially suitable habitats. Recently, *in vitro* propagation of an endemic and threatened Indian liverwort *Cryptomitrium himalayense* Kash. has also been carried out (Awasthi et al., 2010b) that has paved the way for restoration of such an important taxon and other rare taxa.

## MATERIALS AND METHODS

Plants of *P. thwaitesii* and *B. plumosum* having mature sporophytes were collected from Mussoorie and Mukteshwar (western Himalaya) respectively. Voucher specimens have been deposited in Bryophyte Herbarium of National Botanical Research Institute, Lucknow (LWG), India. Specimens examined and used in this study were *Philonotis thwaitesii* Mitt.: India, western Himalaya, Mussoorie (alt. ca 2,142 m), on wet rock, 29.10.2009, *V. Sahu, 251454* (LWG), and *Brachythecium plumosum* (Hedw.) B.S.G.: India, western Himalaya, Mukteshwar, 5 km from Ramgarh (alt. ca 2,181 m), on wet rock, 04.11.2008, *A.K. Asthana & V. Sahu, 248977* (LWG).

For procuring explants (spores), healthy, mature and undehisced capsules were detached from the herbarium specimen and washed properly with running tap water followed by double distilled water. Now these washed capsules were put into 1% sodium hypochlorite solution for 6 to 8 minutes for their surface sterilization and subsequently washed with sterilized double distilled water twice.

Spores of the two species were inoculated into eight type of culture media: media containing full-strength Knop's macronutrients (Knop, 1865), half-strength Knop's macronutrients, one-fourth strength Knop's macronutrients, full-strength Knop's + 2% sucrose, half-strength Knop's + 1% sucrose, one-fourth strength Knop's + 0.5% sucrose, half-strength Knop's + Nitsch's trace elements + 10 ppm ferric citrate (Kaul et al., 1962) and half-strength Knop's + Nitsch's trace elements + 10 ppm ferric citrate + 1% sucrose, by dissecting surface sterilized capsules under aseptic conditions in a Laminar Air Flow Cabinet. All media and glasswares were sterilized by autoclaving at 15 lb/sq. in. for 15 minutes. After inoculation, three replicates of each medium were maintained under controlled and aseptic conditions, cultures were provided continuous illumination of 4,000–5,500 lux as well as alternate light and dark period of 16 hours and 8 hours respectively with the help of a combination of fluorescent tubes. Temperature was maintained at 21 ± 2ºC.

When the gametophytes of both species were well differentiated and matured enough, their acclimatization and introduction to soil have been carried out as per description of Nath et al. (2009).

## RESULTS

Spores of *P. thwaitesii* Mitt. turned bright green after 3 – 4 days of inoculation in continuous illumination as well as in alternate light and dark conditions. After 5 – 6 days, they germinated to give rise one or two germ tubes that formed through the clefts of the exospore and developed into chloronemata which consist of long cylindrical cells with abundant rounded chloroplasts. Half-strength Knop's macro- nutrients medium devoid of sucrose in continuous light was found the best for spore germination as in this medium almost all the spores germinated within 5 – 6 days. In continuous dark, no spore germination occurred. Chloronemal branches abundantly occurred on a main filament of chloronema from early stages of development (Fig. 1A).

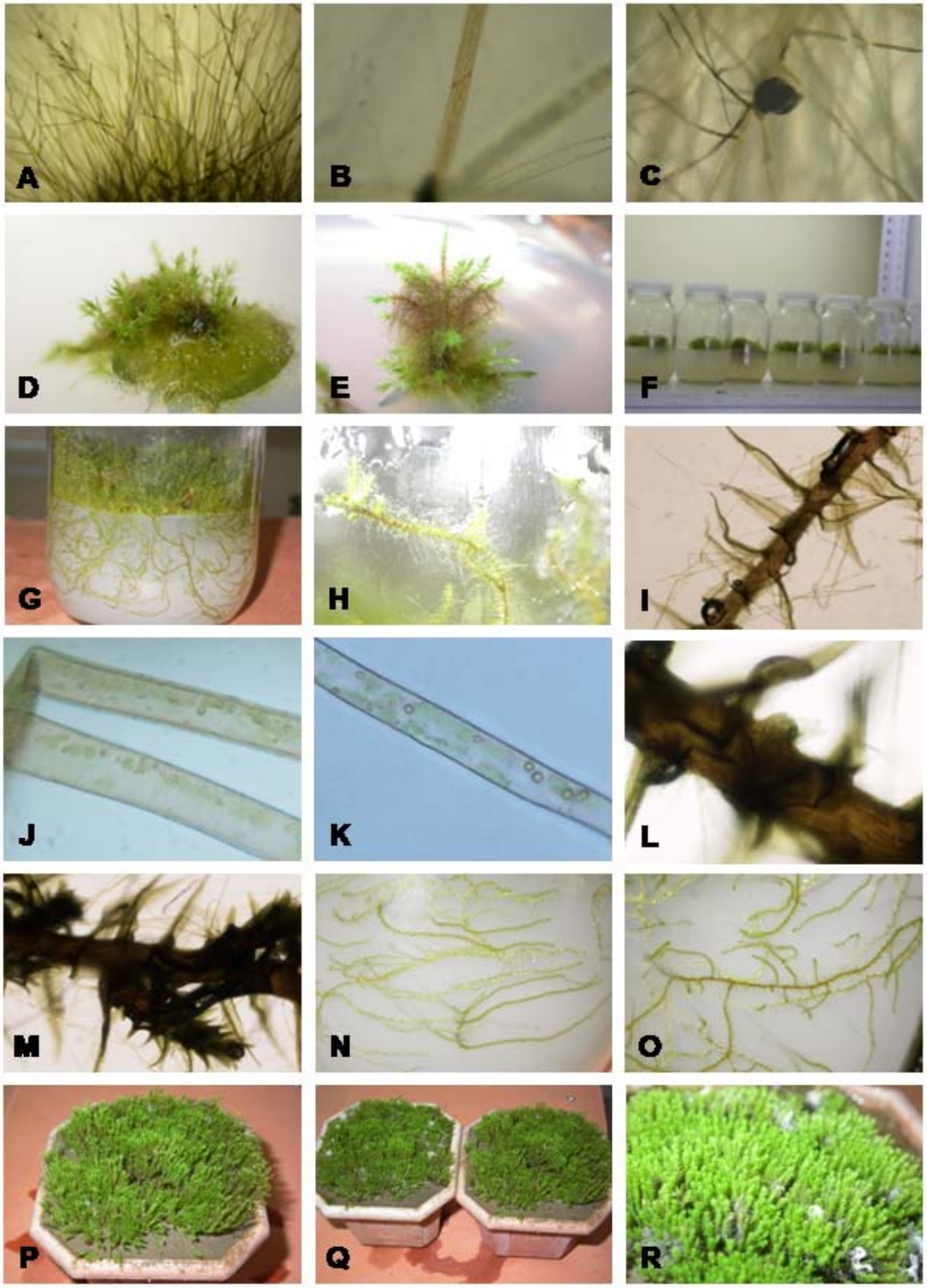


**Fig. 1. Micropropagation of *Philonotis thwaitesii* Mitt. A: Early stage of protonemal development. B: Differentiation of caulonema. C: Protonemal buds on caulonema. D: Growing young gametophores. E: Profuse growth of rhizoids in medium supplemented with 1% sucrose. F, G: Micropropagation of the gametophytes. H: Emergence of hyaline rhizoids like filaments upon contact with glass surface. I, J, K: Enlarged view of rhizoids like filaments showing presence of chloroplasts and oil globules like inclusions. L, M: Development of branch initial and lateral branch at the region of rhizoidal growth. N, O: Growth of gametophytes inside the medium showing abnormal increase in length as well as development of many later branches. P, Q, R: *In vitro* raised plants transferred on soil in pots.**

Many chloronemal branches also grew upward showing a positive phototropism but the remainder grew horizontally. After ca 20 – 25 days of spore germination, the terminal end of chloronema became hyaline that subsequently differentiated into caulonema (Fig. 1B). Caulonema can be differentiated from chloronema. The filament of caulonema is broad, brown with oblique septa and few spindle-shaped chloroplasts while the filaments of chloronema are narrower, bright green with plenty of rounded chloroplasts and transverse septa. Caulonema tended to grow downward inside the medium hence showed a negative phototropism. Caulonema further produced several secondary chloronemal filaments as its side branches; hence ca 35-day-old culture contained only heterotrichous protonemal stage in a form of chloronema and caulonema. In medium supplemented with 1% sucrose, the caulonemal differentiation occurred early (within 12 – 15 days of germination) and the number of caulonemal filaments was also larger than in medium without sucrose.

Protonemal buds were arisen after 35 – 40 days of inoculation on the caulonema (Fig. 1C), just near the oblique septa of caulonemata. The maximum number of protonemal buds was observed in half-strength Knop's macronutrients + Nitsch's trace elements with 10 ppm ferric citrate. In 60-day-old culture, young gametophores were differentiated from buds (Fig. 1D) in a form of erect main axis around which leaves were spirally arranged. Induction of protonemal buds was found dependent on a critical size and not on the age of protonema. When the protonema or gametophores were transferred to fresh basal media, bud induction and gametophyte development occurred only when the growth of protonemal patch reached up to a critical size (ca 3 cm in diameter), irrespective of the age of explants (protonema or intact gametophores). Among all media tried, half-strength Knop's + Nitsch's trace elements possessed largest population of gametophytes, while in media supplemented with sucrose, abundant growth of rhizoids (Fig. 1E) and fewer in number but more robust gametophytes were observed. In half-strength Knop's macronutrients devoid of any trace elements and sucrose, the raised gametophytes were thin, delicate and their leaves were yellowish, small, narrower and distantly arranged. Hence half-strength Knop's macronutrients + Nitsch's trace elements with 10 ppm ferric citrate devoid of sucrose proved optimal basal medium for the micropropagation of this species in which a well developed population of erect gametophores developed in ca 80 – 90 days of spore germination (Figs. 1F, G).

In those gametophytes that were in contact with the side glass surface of the culture bottle, many hyaline rhizoids like filaments emerged from the main axis (Figs. 1H, I). These filaments were usually aseptate and possessed certain green plastids like structure and few oil globules like inclusions (Figs. 1J, K), hence differ from normal rhizoids. Gametophores bearing such filaments also tended to branch near these filaments or near solid surface (Figs. 1L, M) while remaining gametophores in middle of the population were either unbranched or branched sparingly at terminal end. Furthermore, the gametophytes differentiated from buds on downwardly-grown caulonema, beneath the surface of the culture medium grew abnormally to increase their length. To develop many side branches on the main axis of such gametophytes gave rise to the appearance of well-branched gametophytes (Figs. 1N, O), resembling the habit of many pleurocarpous mosses.

When the leafy gametophores placed on fresh optimal basal medium vertically, several secondary protonemal filaments were proliferated from the basal rhizoidal area that subsequently developed into population of gametophytes passing through chloronemal and caulonemal stages. Similar growth pattern occurred on soil when the cultured plants were transferred to soil containing minerals (half-strength Knop's macronutrients + Nitsch's trace elements with 10 ppm ferric citrate). Hence by regular sub-culturing of these gametophytes as well as protonema, a large enough population was developed in about 120 days on pots (Figs. 1P, Q, R).

Spores of *B. plumosum* (Hedw.) B.S.G. (Fig. 2A) turned bright green after 4 – 5 days of inoculation in continuous illumination as well as in alternate light and dark conditions and after 6 – 7 days they germinated to produce branched chloronema with abundant rounded chloroplasts and vertical septa. Spore germination is usually monopolar or occasionally bipolar. Half-strength Knop's macronutrients medium devoid of sucrose in continuous light was found the best for spore germination. No spore germination was occurred in absolute dark. After ca 20 days of spore germination the distal end of chloronema started to become hyaline on account of disappearance of chloroplasts but only a few chloronemal filaments were differentiated into conspicuous caulonema (brown coloured filaments having a few spindle shaped chloroplasts and oblique septa). One-month-old culture contained mainly green chloronema and a few caulonema or hyaline chloronema (Fig. 2B). After ca 35 – 40 days of inoculation, protonemal buds were differentiated from the main chloronemal filaments or basal cells of the chloronemal branches and distributed throughout the protonemal patch in a scattered manner (Fig. 2C) in contrary to the previous acrocarpous moss species where they were developed into only on caulonema and

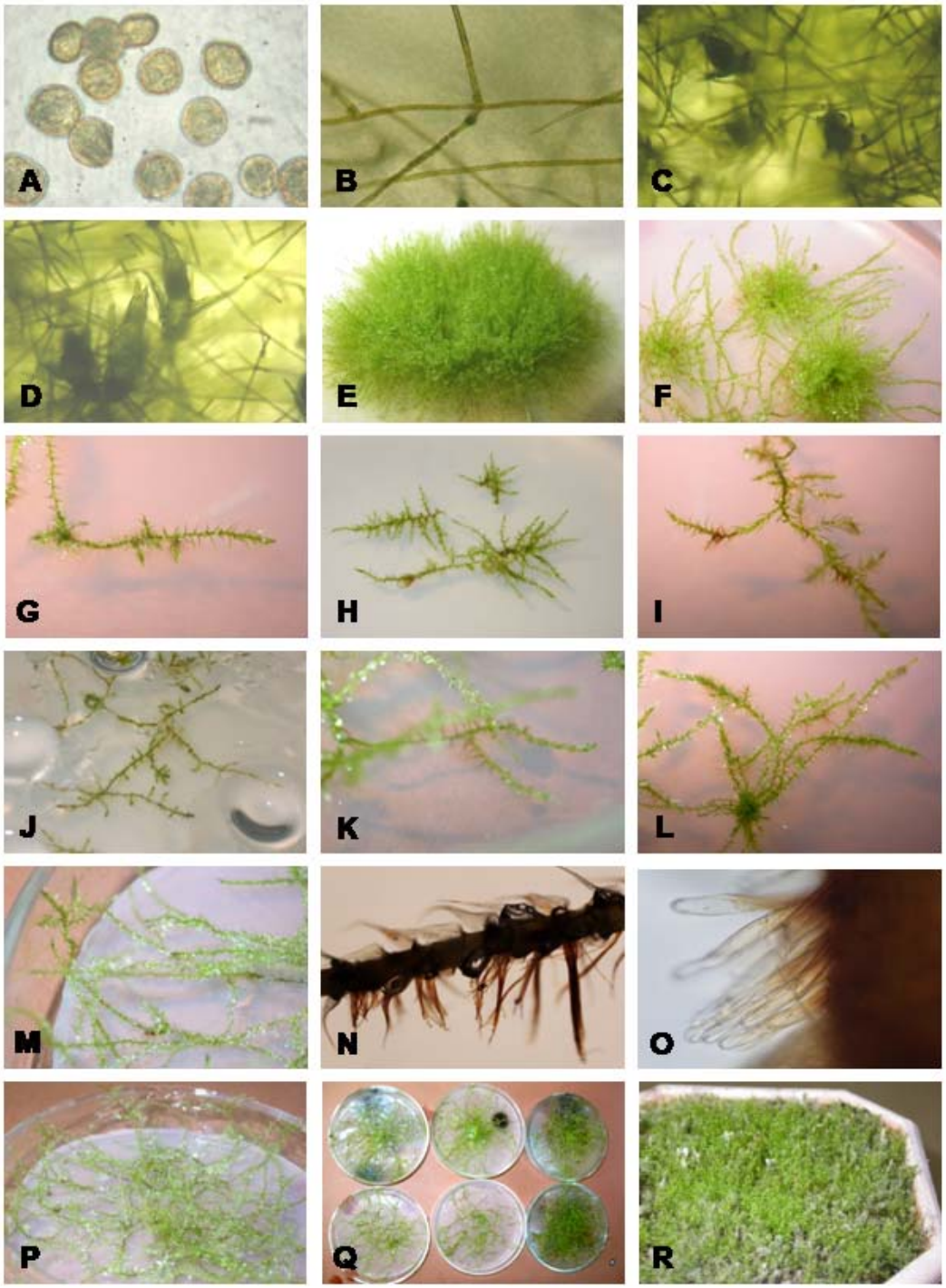


**Fig. 2. Micropropagation of *Brachythecium plumosum* (Hedw.) B.S.G. A: Spores. B: Protonemal stage (green coloured chloronema and brown coloured caulonema). C,D: Protonemal buds giving rise to young gametophores. E: *In vitro* raised population of erect unbranched gametophytes. F: Creeping growth of gametophytes at the margin of protonemal patch. G, H: Development of lateral branches and rhizoids on main axis of gametophytes. I: Well branched creeping gametophyte showing emergence of rhizoid at places of contact with medium. J: Enhanced number of branches in high humidity. K: Development of rhizoids all along the length of gametophytes. L: Sub culturing of protonema resulted in direct regeneration of gametophyte. M: Branching and uplifting tendency of gametophyte near the glass surface of margin wall of petridish. N: Enlarged view of rhizoids growing all along the length of axis. O: Emergence of rhizoid upon contact with solid substratum (enlarged view). P, Q: *In vitro* raised gametophytes. R: Development of weft forming population upon transferring of *in vitro* raised plants on soil in pots.**

in centripetal manner (in perfect ring). Protonemal buds induction was found dependent on the age rather than a critical size of protonema. Protonemal buds subsequently developed into young gametophores (Fig. 2D) in form of erect main axis with spirally-arranged leaves. Half-strength Knop's macronutrients + Nitsch's trace elements + 10 ppm ferric citrate possessed largest population while in medium with 1% sucrose, abundant growth of protonema and rhizoids, but less number of buds and gametophores were observed. In ca 70-80-day-old culture, a well developed population of erect gametophores developed (Fig. 2E).

In erect gametophores, as such, no branching occurred although a few superficial branch initials developed on main axis; they never develop into a branch. However, when the gametophores on the margin of protonemal patch came in contact with medium by chance, the rhizoids were developed at the point of contact and a creeping growth started (Fig. 2F). Similarly, when the erect gametophores were placed horizontally on fresh medium, no regeneration in form of protonema occurred, instead rhizoids all along the length of axis (Fig. 2K) and many branch initials were developed (Fig. 2G) near the area of rhizoid emergence, hence they proliferated in length as well as in lateral/erect branches. Thus, in ca 120 days, well-differentiated branched gametophytes were developed (Figs. 2H, I), that can be differentiated into two parts i.e. prostrate main axis and lateral/erect secondary branches. At higher moisture level, an enhanced number of branches have been observed (Fig. 2J). Rhizoids emerged from the main axis were morphologically similar to caulonema being brown in colour and with oblique septa; however no spindle shaped plastids could be observed. In a medium supplemented with sucrose, proliferation of rhizoids as well as branches was pronounced and resulted in development of much robust gametophytes. Upon sub-culturing of protonema or gametophores, a very restricted growth of protonema occurred, instead the buds in protonema or fragments of gametophores sub-cultured, gave rise into creeping and branched gametophyte directly without passing through the chloronemal and caulonemal stages (Figs. 2L, M). Hence, by regular sub-culturing the axenic cultures of well-branched gametophytes were established in ca 120 days (Figs. 2P, Q).

In aerial part of the gametophytes that came in contact with glass surface, the rhizoids developed from the main axis but they differed from those of *P. thwaitesii* because of brownish colour, oblique septa and lack of any plastid-like structures or any inclusion (Figs. 2N, O).

Upon transferring the cultured gametophytes whether branched or unbranched into the soil saturated with minerals (half-strength Knop's macronutrients + Nitsch's trace elements with 10 ppm ferric citrate), no further profuse growth of protonema occurred, in contrast to *P. thwaitesii*, instead the gametophytes or fragments of gametophytes directly regenerated into well-branched gametophytes. Hence in about one month, the soil surface was covered with weft-formed population of *B. plumosum* (Fig. 2R). A comparative account of protonemal morphogenesis and growth pattern of *P. thwaitesii* Mitt. and *B. plumosum* (Hedw.) B.S.G. is provided in Table 1.

## DISCUSSION

Excessively high concentration of macronutrients may cause poor growth of these moss species. Preference of dilute culture solution has been noted in other bryophytes (Voth, 1943; Awasthi et al., 2010, 2010b). Furthermore presence of zinc and copper has been demonstrated as inhibitor of germination of gemmae in *Marchantia polymorpha* and spores of *Funaria hygrometrica* (Coombes and Lepp, 1974). This may be the reason that a half-strength Knop's macronutrient medium devoid of any trace element and sucrose was found the most suitable for spore germination in both the species. Differentiation of caulonema from chloronema as well as rhizoid formation was found to be stimulated by sucrose. Since these morphogenetic events are facilitated under the effect of auxin (Bopp, 1983), auxin-like substances are produced or activated in the presence of sucrose in *P. thwaitesii*. Protonemal buds were formed spontaneously in *P. thwaitesii* as soon as a critical size was reached in a quite similar fashion as in *Funaria* (Bopp and Brandes, 1964). In medium supplemented with sucrose, the growth of protonema was much pronounced and bud formation was scarce, while in medium devoid of sucrose, the number of buds and gametophores was large. Cytokinin induces bud formation in mosses (Bopp, 1968; Valadon and Mummery, 1971). It is probable that an endogenous cytokinin synthesized in spontaneously formed caulonemal cells. These act as target cells for the bud induction, in medium devoid of sucrose. The cytokinin production in the culture is light dependent (Hartman, 1973); therefore maximum number of buds and gametophores were developed in continuous light.

The rhizoid like hyaline filaments regenerated from stem upon contact with solid substrata like glass surface or medium surface. Such stimulation of rhizoids has also been observed in *Calliergon stramineum* (Duckett,

**Table 1. Comparison of protonemal morphogenesis and growth pattern between *Philonotis thwaitesii* Mitt. and *Brachythecium plumosum* (Hedw.) B.S.G.**

| Observations | *Philonotis thwaitesii* Mitt. | *Brachythecium plumosum* (Hedw.) B.S.G. |
|---|---|---|
| Time taken in germination of spores | 5-6 days | 6-7 days |
| Protonemal morphology (after 15 days of inoculation) | Germinated spores gave rise to extensive growth of protonema with longer filaments composed of long cylindrical cells. | Germinated spores gave rise limited growth of protonema having short cylindrical cells. |
| Protonemal morphology (after 30 days of inoculation) | Protonema clearly differentiated into central green denser region mainly of chloronema and outer peripheral rarer network of longer caulonema. | Uniform growth of protonema consisted of chiefly chloronema and only a few caulonema. |
| Position and time taken in development of protonemal buds | Buds produced only on caulonema and in perfect rings in a protonemal patch after ca 35-40 days of inoculation. | Buds abundantly produced from entire protonema in scattered manner after ca 35-40 days of inoculation. |
| Growth of culture (after 55 days of inoculation) | Growth of protonema as well as leafy gametophores occurred simultaneously. | Growth of leafy gametophores dominated over the growth of protonema. |
| Time taken in development of population of erect leafy gametophores from spores | ca 85-95 days | ca 70-80 days |
| Sub-culturing | Sub-culturing of gametophyte gave another population only after passing through chloronema and caulonema stages. | Sub-culturing of gametophyte or its fragments produced well-branched leafy gametophytes without passing through any protonemal stage. |
| Rhizoids formation | Rhizoids usually branched and produced only at the basal part of gametophyte all around the axis. | Rhizoids usually unbranched and abundantly produced all along the length of gametophyte and its branches and in only one plane. |

1994). In contrast to the main rhizoid axes with their oblique septa and brown-coloured cell walls, the filaments regenerated from stem have non-pigmented wall, several chloroplasts and few oil globules like inclusion. Such formation of rhizoids like filaments is intimately associated with the straggling habit of this species and forms a remarkable parallel in miniature to organ of attachment in climbing vascular plants. This view also strengthened by the fact that, the gametophores inside the culture medium and also in contact with solid substratum gave rise to branched gametophores, resemble the straggling and climbing habit of pleurocarpous mosses.

In culture of *B. plumosum*, the protonemal development is of *Bryum* type as categorized by Nishida (1978), but differentiation of a few caulonemal filaments also occurred, while Nishida (1978) reported absence of caulonemal filament in *B. plumosum* in his study on protonemal morphogenesis in mosses. Although caulonemal differentiation occurred, formation of buds occurred only on chloronema in *B. plumosum.* Protonemal bud induction depends on the age of protonema rather than its size as upon sub-culturing of protonema with buds, the new buds developed again without waiting for further growth of protonema up to a critical size. Similar observation was found in another pleurocarpous moss *Entodon laetus* (Griff.) Jaeg. when it growing epiphytically (Awasthi et al., 2010a). Once protonemal buds developed they grew rapidly into leafy gametophores. These features i.e. restricted growth of protonema, only a few or inconspicuous caulonema and rapid growth of leafy gametophyte shows affinities with the development pattern of epiphytic mosses (Nishida, 1978). Since the investigated species was collected from wet rock, it may be possible that the habitat affected the protonemal morphogenesis and the characteristic of caulonema development in terrestrial mosses also occurred.

Development of gametophyte of *B. plumosum* also follows the growth pattern of *Entodon laetus* in which no branching occurred in erect gametophores (Awasthi et al., 2010a). Branching occurred only in gametophores on the margin of protonemal patch when came in contact in medium by chance or upon placing erect gametophores horizontally on medium. In such condition emergence of rhizoid all along the length of axis or particularly at point of contact with medium preceded emergence of new branch initials that grew further into branches. Lack of regeneration of protonema upon sub-culturing of intact gametophyte or fragment of gametophyte also revealed affinity with the developmental pattern of epiphytically growing pleurocarpous moss *E. laetus* (Awasthi et al., 2010a). Restricted growth of protonema and bypassing the

caulonemal stage as a pre-requisite for bud induction in contrary to many other terrestrial acrocarpous mosses reveal the high regenerative capacity of pleurocarpous mosses. The plant or plant's fragments are capable to develop in mature gametophyte on a suitable nutrient providing substratum without passing through chloronema and caulonema.

## CONCLUSION

Acrocarpous mosses are characterized by erect or ascending shoot system that are either unbranched or sparingly branched while pleurocarpous are generally characterized by creeping shoot system with extensive lateral branching. In present work, *Philonotis thwaitesii* (an acrocarpous moss) revealed erect as well as creeping and branched habit (when grew inside the medium), while *Brachythecium plumosum* (a pleurocarpous moss) revealed branched habit only upon contact of entire main axis with nutrient medium otherwise unbranched. It suggested that the growth forms are in part, at least, influenced by the microenvironment especially moisture conditions at the time of maximum growth and sometimes can be altered by a change in environmental condition. As in above case, obviously higher moisture level inside the medium than above the medium resulted in many branches and profuse growth.

In nature, there is an example of *Thamnobryum alopecurum* that normally possesses dendroid gametophores, but produces creeping weft-like forms under conditions of increased humidity and shade (Meusel, 1935; Birse, 1957). Another example is of *Hylocomium splendens*, a normally weft-forming moss produces an essentially turf form in alpine and arctic region (Schofield, 1972, 1981). Such variation in growth forms may assist in interpreting the bryophyte community as related to the environment.

When the cultured gametophytes were transferred to the sterilized soil saturated with minerals under laboratory conditions, the adhering protonema of *P. thwaitesii* further proliferated to produce buds and then gametophores only in 20 – 25 days while plants of *B. plumosum* proliferated in weft-form and sufficient enough population of well-branched plants in 30 days. This rapid and vigorous growth of gametophytes occurred on account of availability of a more suitable and natural substratum in addition to the required favourable conditions viz. low temperature, availability of minerals, humidity and light.

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# 兩種苔類植物的形態發生研究及組織培養繁殖：*Philonotis thwaitesii* Mitt. 和 *Brachythecium plumosum* (Hedw.) B.S.G.

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摘要：本文提出一種頂生蒴苔 *Philonotis thwaitesii* Mitt. 和另一種側生蒴苔 *Brachythecium plumosum* (Hedw.). B.S.G. 此二種苔類植物的組織培養繁殖方式；這兩種苔類能以其孢子接種到一定濃度範圍的無機培養基中，誘導產生發育良好的配子體。實驗發現在添加有 Nitsch's trace elements 以及 10 ppm ferric citrate 之 1/2 強度 Knop's macronutrients 培養基中，以 21 ± 2°C 溫度及 4000 – 5500 lux 連續光下培養，最適合這二個物種的生長與複製。*P. thwaitesii* 之原絲體的芽可發育自軸絲體；而 *B. plumosum* 之原絲體的芽則發育自綠絲體。蔗糖能刺激軸絲體的分化且使豐富的假根產生，卻造成少數但較強韌的配子體發育。培養 *P. thwaitesii* 時，當其配子枝與固體基質接觸後，則發現有透明假根狀的絲狀體出現；然而培養 *B. plumosum* 時，其真正假根的出現則在其被水平放置於培養基後，假根沿著配子體長軸生長出來。在不同培養條件下，這兩個物種均能觀察到多變的生長形態（緯線狀的分枝和直立不分枝的習性）。*P. thwaitesii* 透過綠絲體和軸絲體的繼代培養，可以促使新的群體發生；而 *B. plumosum* 則可直接藉由原絲體再生新的配子體。此兩種物種以組織栽培所培育的植物體可以被馴化，並移植到土壤中更進一步的繁殖。

關鍵詞：頂生蒴苔、生長形式、組織培養繁殖、側生蒴苔、孢子。