

## CULTURE STUDIES AND *IN VITRO* PROPAGATION OF THE MOSS *BRYUM CORONATUM* SCHWAEGR

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*In vitro* propagation of *Bryum coronatum* has been done by using spores as explants. A full differentiation of protonema into well developed gametophytes along with development of gametangia (antheridia) has been achieved by inoculating a range of concentration of inorganic media supplemented with and without sucrose under laboratory conditions. *In vitro* raised plants were acclimatized and transferred to soil in order to their further proliferation.

**Key words;** *Bryum coronatum* Schwaegr., culture, propagation, spores.

*Bryum coronatum* Schwaegr., an acrocarpous moss of the family Bryaceae of the order Bryales is widely distributed in India. It is found in all the bryogeographical regions of the country viz. western Himalaya, eastern Himalaya, Gangetic plain, Rajasthan, central India, south India and Andaman and Nicobar islands (Lal 2005). During the assessment of bryophyte diversity in Pachmarhi Biosphere Reserve, this taxon has been collected from the rock crevices of Pandav caves, Pachmarhi at an altitude of ca 818.18 metre.

*Bryum coronatum* commonly found on rocks and on calcareous walls of old houses, exhibits distinctive feature like nodding or pendulous capsule, that distinguished it from other species of the genus. Although this taxon is widely distributed, but it is found in scattered patches in a few populations mixed with other species.

An attempt has been made to study the growth pattern of this species *in vitro* and multiplication of the species in order to facilitate the bulking up of the material in pure population in local conditions for further studies to explore the potential value of the species without overharvesting the species from its natural habitats.

Earlier some work on culture studies in reference to protonemal morphology and bud induction in some species of *Bryum* and other members of order Bryales have been carried out by Allsopp and Mitra (1958), Chopra and Rawat (1973, 1977), Chopra and Vashistha (1987). Chopra and Rawat (1977) achieved a little success to differentiate protonema of *Bryum coronatum* Schwaegr into some rudimentary leaves and gemmae like bodies. In the present study spores were germinated to produce chloronema and caulonema. Later on well developed gametophytes having sex organs (antheridia) were differentiated from the buds, produced on caulonema. Successful transfer of the cultured plants from culture media to soil has been carried out. Hence the described technique may prove useful for multiplication of this species in pure vegetative populations for the purpose mentioned earlier along with its conservation as *in vitro* germplasm resource.

### MATERIAL AND METHODS

#### Source of ex plants

Plants of *Bryum coronatum* having mature sporophytes were collected from the rocks and rock crevices of Pandav caves, Pachmarhi

(central India) in December, 2006 and specimens have been deposited in the Bryophyte Herbarium of National Botanical Research Institute, Lucknow (LWG). Before inoculating the media with explants (spores), mature capsules were dissected out from the plants and washed properly with running tap water followed by double distilled water (ddH<sub>2</sub>O). The capsules were surface sterilized by putting them into 1% sodium hypochlorite solution (NaOCl) for 8 to 10 minutes then washed with sterilized ddH<sub>2</sub>O twice.

**Specimen examined:** India, Madhya Pradesh, Pachmarhi, Pandav caves (alt. ca 818.18 m), 01.12.2006; Leg. V. Sahu and V. Awasthi, 227684 (LWG).

#### **Culture media and Laboratory conditions**

Culture medium used was Knop's macronutrient medium (Knop 1865) at full strength and half strength with and without 1% sucrose. Addition of Nitsch trace elements (as per description of Kaul *et al.* 1962) along with 10 ppm freshly prepared ferric citrate to the half strength Knop's medium was also tried. All the media were gelled with 0.8% agar (Bacto grade) and pH of the media was maintained at 5.8.

All the media and culture vessels (petridishes) were sterilized by autoclaving at 15 lb/sq inch for 15 minutes. Surface sterilized capsules of *Bryum coronatum* from Pachmarhi were ruptured in order to inoculate the following media with spores under Laminar Air Flow Cabinet.

I - Full strength Knop's macronutrients

II- Half strength Knop's macronutrients

III- Half strength Knop's macronutrients + 1% Sucrose

IV- Half Knop's + Nitsch trace elements + 10 ppm ferric citrate

V- Half Knop's + Nitsch trace elements + 10 ppm ferric citrate + 1% Sucrose.

After inoculation, cultures were maintained under controlled and aseptic conditions. Cultures were provided continuous illumination of 4000-5500 lux as well as alternate light of 16 hours and dark period of 8 hours with the help of a combination of fluorescent tubes. Temperature was maintained at  $21 \pm 2^{\circ}\text{C}$ .

#### **Acclimatization and Introduction**

When the gametophytes of *Bryum coronatum* became well developed and matured enough, their transfer from laboratory (culture medium) to soil was carried out in two successive steps. In first step the gametophytes were transferred to soil and kept in laboratory conditions. For this, the soil was autoclaved in order to remove all biological contaminants and saturated with sterilized mineral salts solution (Half Knop's macronutrients, Nitsch trace elements and 10 ppm ferric citrate). The cultured gametophytes with persistent protonema were transferred to the sterilized soil saturated with sterilized mineral salts solution filled in pots. The pots were kept in controlled laboratory conditions viz. alternate light of 4000-5500 lux for 10 hours and dark period of 14 hours. Mineral salts solution was sprinkled at two days interval. After six weeks the gametophytes were well established. Besides this a huge number of new gametophytes were also arisen from the protonema grew from persistent protonema attached with gametophytes. In second step the hardened and mature gametophytes were transferred with their substrata to the soil in Moss House, at Botanic Garden of N.B.R.I.,

Lucknow where humidity (60-100%) and optimum temperature ( $\pm 25^{\circ}\text{C}$ ) were maintained with the help of an advance fogger and sprinkler system.

### OBSERVATIONS

Spores of *Bryum coronatum* Schwaegr. turned green after 3 days of inoculation in continuous as well as in alternate light and dark conditions and after 4 days germinated to produce chloronema (Fig. 1A). The spores germination is both monopolar and bipolar. Half strength Knop's medium devoid of sucrose in continuous light was found best for spore germination as in this medium highest percentage of germination is noted. Sucrose inhibited the spore germination in continuous light, however in alternate light and dark conditions it favours the same. It has been observed that number of spores germinated increased with increase in days. Hence viability of all the spores are not same. Some spores germinate early in a particular condition while others late in same conditions.

In continuous dark period no spore germination was recorded in half strength Knop's medium. However, a very few spores (less than 1%) germinated in medium supplemented with sucrose. In such case the colour of protonema was brown instead of bright green, and development of chloroplast was very scarce. Spores germinated to produced branched chloronemata having abundant chloroplasts and transverse septa. After about 12-15 days of spores germination caulonemata were appeared, that can be differentiated from chloronemata as in former the number of chloroplasts was few and septa were oblique. Caulonemata produced several secondary chloronemal filaments (Fig. 1B,C). Hence about 1 month old culture contained only

protonemal stage in form of chloronema and caulonema. Later on buds were arisen on caulonema near the oblique septa. The number of protonemal buds produced was maximum in half strength Knop's + Nitsch trace elements with 10 ppm ferric citrate followed by the same medium supplemented with 1% sucrose. In about 45 days old culture young gametophores were differentiated from the protonemal buds (Fig. 1D) which developed into mature gametophytes in 30 days (Fig. 1E). At this stage antheridial clusters were appeared at the apex of male gametophytes (Fig. 1F,G). Amongst the media tried, half strength Knop's + Nitsch trace elements with 10 ppm ferric citrate possessed largest population of gametophytes, while in medium supplemented with sucrose, very few gametophytes were produced. Thus abundant growth of protonema and less number of buds were observed in medium supplemented with sucrose. Gametophytes were comparatively higher in medium devoid of sucrose than in that with sucrose. Protonemal bud induction was found dependent on the age of protonema and not on the size of its patch. When the protonema were transferred to fresh basal medium, buds induction and development of gametophyte were rapid irrespective of size of protonemal patch. When mature gametophytes were transferred to sterilized soil saturated with mineral salts solution they remained as such while protonemata attached with the gametophytes proliferate further and produce a large number of buds and ultimately gametophytes. Hence a large population of *B. coronatum* was developed in pot within one month of transferring the plants from culture media to soil.

### DISCUSSION

Excessively high concentration of macronu-

trients may cause poor growth of *Bryum coronatum*. Preference for a dilute culture solution has been noted in other bryophytes (Voth 1943). Light enhances the rate of spore germination while in presence of sucrose the number of spores germinated decreases in continuous light. Light stimulates the chloroplasts to photosynthesis in absence of

sucrose, when sucrose is present in medium, there is less need of photosynthesis, less activation of chloroplasts and less activation of vital activities through germination.

Addition of Nitsch trace elements along with ferric citrate fulfilled the need of micronutrients hence more number of protonemal buds and gametophytes were

**Table 1. Observations on *in vitro* growth of *B. coronatum* Schwaegr.**

• Ex plant used	Spores
• Medium optimum for spore germination	1/2 Knop's > Full Knop's > 1/2 Knop's + Nitsch trace elements > 1/2 Knop's + Nitsch trace elements + 1% Sucrose [1/2 K > K > 1/2 K + N > 1/2 K + N + S]
• Medium optimum for differentiation of protonemata in to gametophytes	1/2 K + N > 1/2 K > K > 1/2 K + N + S
• Time taken for germination of spores	3-4 days
• Time taken to induce bud formations when explants are spores	30 days
• Time taken to develop mature gametophytes with gametangia when explants are spores	60 -65 days
• Time taken to induce bud formation when explants are protonemata	18-20 days
• Time taken to develop mature gametophyte with gametangia when explants are protonemata	40-45 days
• Secondary inocula used for further multiplication	Protonema
• Time taken to develop a good population on soil after transferring of plants from medium to soil	30 days
• Medium suited best for multiplication and propagation of the species	1/2 strength Knop's + Nitsch trace elements + 10 ppm ferric citrate

produced in 1/2 Knop's + Nitsch trace elements medium.

When spores were used as explants they took more time to germinate and develop into gametophytes as compared to gametophytes developed from protonema used as explants, perhaps because of change in atmosphere and taking time to trigger regeneration, while in latter case protonema was adopted in the laboratory conditions and acquired fast regeneration ability. Besides this the age factor may also seem to contribute for the same.

The well development of rhizoids and leafy gametophytes with gametangia on basal medium devoid of any external support of growth regulators indicates the presence of endogenous auxin and kinetin like substances in *Bryum coronatum*.

Development of antheridia took place in culture media in continuous as well as in alternate light and dark conditions. Antheridial formation takes place earlier than archegonial formation. Gametangial induction was specifically inhibited with sucrose. It has been observed in other mosses viz. *Bryum argenteum* (Chopra and Bhatla 1981) and *Bartramidula bartramoides* (Rahbar 1981) where gametangial induction is specifically inhibited with concentrations of sucrose higher than one percent. Temperature is the critical factor for induction and maturation of gametophyte. Low temperature 18-20°C favours the gametangial induction while temperature above 22°C checks the gametangial induction. A similar response is shown by *Philonotis turneriana* which require a critical temperature of 18°C for gametangial induction (Kumra and Chopra 1983). In *Bryum coronatum* the unusual increase in gametophyte length, more number of leaves

and more vigorous growth of gametophytes in cultures in comparison to that in wild (natural habitat) seem to be taken place because of providing low temperature to plants which are habitual to moderate temperature  $\pm 25^{\circ}\text{C}$  of Pachmarhi. This also confirms the benevolence of low temperature of temperate or subtropical region for the growth of bryophytes. When the cultured gametophytes transferred to the sterilized soil saturated with mineral salts (1/2 Knop's + Nitsch trace elements along with ferric citrate) under laboratory conditions, the gametophyte further produces protonema, bud and then gametophytes only in 20-30 days due to 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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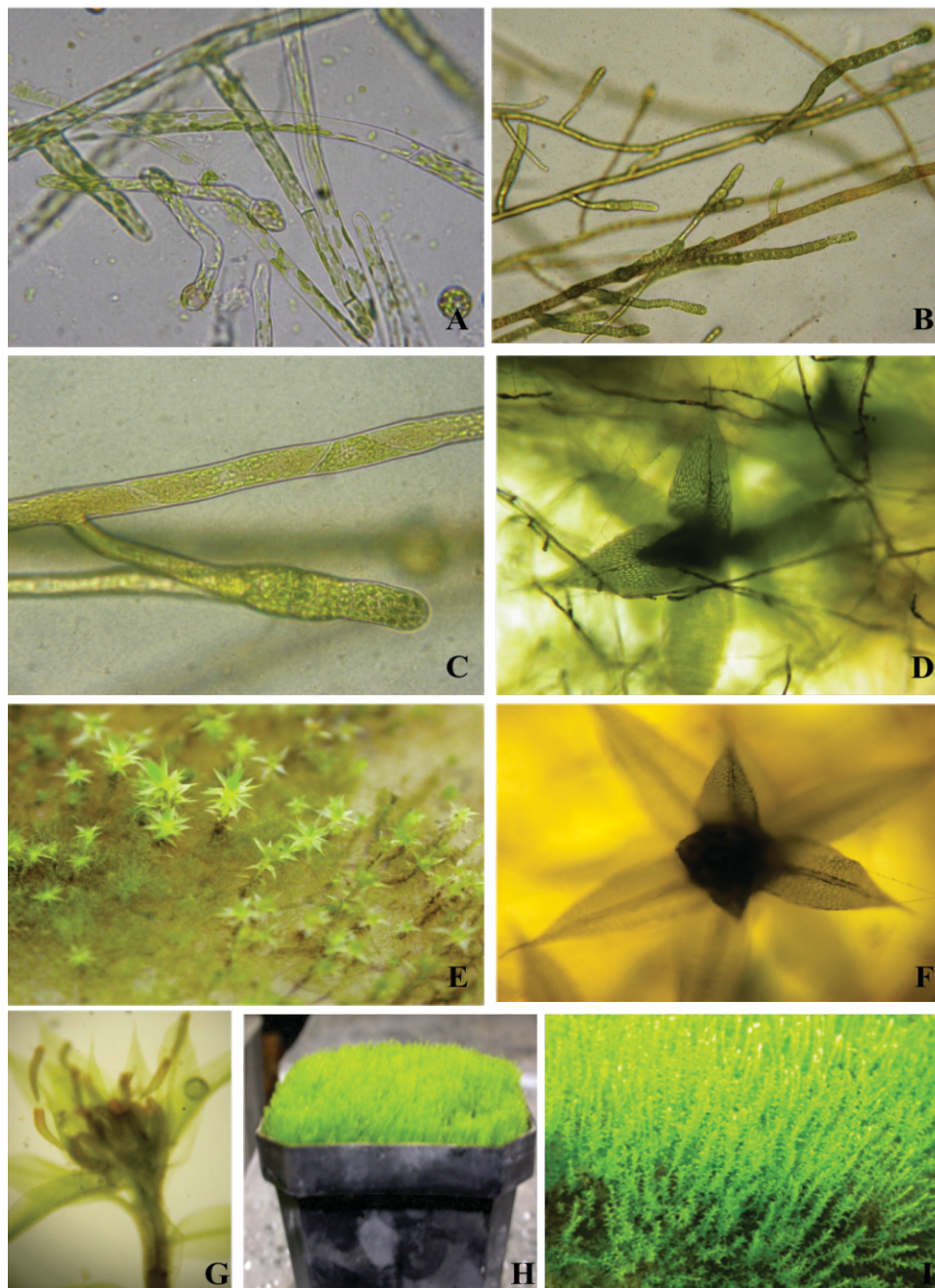
**Figure 1**

Figure -1. A-I. *In vitro* propagation of *Bryum coronatum* Schwaegr.

A. Germinating spores B. Growing protonema (after 15 days) C. Growing chloronema from caulonema (after 20 days) D. Emerging gametophores (after 40 days) E. Gametophyte population in culture (after 50 days) F. Antheridial cluster at apex of gametophyte (after 60 days) G. Gametophyte showing antheridia H, I. *In vitro* raised plants after 1 month of transfer on soil.

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