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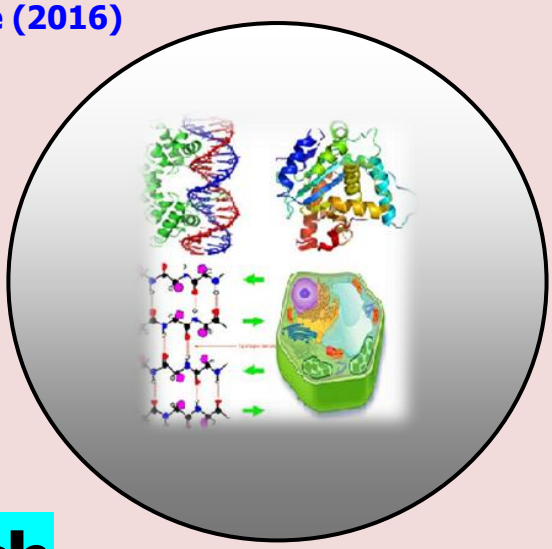

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RESEARCH PAPER

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A Study on *in vitro* Propagation of *Plagiochasma appendiculatum* Lehm. *et* Lindenb. A Medicinally Important Liverwort

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ABSTRACT

Establishment of axenic culture of a medicinally important liverwort Plagiochasma appendiculatum has been carried out by inoculation of spores and gametophytes using different nutrient's media via different methods under controlled conditions to determine optimal conditions for the growth of this plant which can be utilize for the production of bioactive compounds used in various medicinal purposes.
Key words: Liverwort, Nutrient Media and Bioactive Compounds.

INTRODUCTION

A large number of bryophytes are known for their medicinal values (Kumar *et al*., 2000; Singh *et al*., 2006). The bryophyte *Plagiochasma appendiculatum* (a thalloid liverwort) is potentially very important for its bioactive compounds and medicinal importance, this plant has been found to act as biogeoindicator associated with Iron-haematite and Calc-tufa $CaCO_3$ detected in Kumaon Himalaya region (Pant and Tewari, 1998). Variations in some metabolites and nitrate reductase activity have been studied in polluted urban environment (Shankar *et al.*, 2000). The species has also been found useful in the biotest for water quality assessment, especially for heavy metals by using thallus deterioration as a simple biomonitoring parameter (Ghate and Chaphekar 2000; Saxena and Saxena 2000; Nath *et al.*, 2011). This plant also used as ethnic medicine by Gaddi Tribes of Kangra Valley, Himachal Pradesh for the cure of burns, boils and blisters of skin have been reported earlier (Kumar *et al.*, 2000; Singh *et al.*, 2011). Sesquiterpenoids (Joshi *et al.*, 2001), potent antimicrobial, wound healing and anti-oxidant activities (Singh *et al.*, 2006) and antifungal as well as antibacterial activities of *P. appendiculatum* have been observed (Deora and Jain 2008; Bodade *et al*., 2008; Dey and De 2011). Least informations are available on conditions and nutrients level for optimal growth of *P. appendiculatum* to obtain the maximum medicinal value. In present study attempt has been made to grow this plant *in vitro* through various techniques of plant tissue culture so that its year round availability enables us to extract bioactive compounds according to need.

MATERIALS AND METHODS

Fresh plants of *Plagiochasma appendiculatum* Lehm. *et* Lindenb. were collected from different localities of the Uttarakhand state i.e. Dharchula (Pithoragarh), Mussoorie (on the way of company garden), Joshimath (Chamoli) and Nainital in different time periods for regeneration, spore germination and tissue culture study. For regeneration study of different portions of collected *Plagiochasma* plant (Apical, median, midrib and

lateral) were observed up to 30 days in different concentrations (10, 20, 30, 40 and 50%) of Knop's solution (22±2^{o}C temperature and 6500-7000 Lux in continuous light condition). For spore germination preliminary experiments in MS (Murashige and Skoog) medium and different concentrations of Knop's solution were carried out. 20% Knop's solution was found suitable for spore germination and experiment was carried out further (22±2^{o}C temperature and 6500-7000 Lux in continuous light condition). Four nutrient media: MS medium (Murashige and Skoog, 1962), NN (Nitsch and Nitsch, 1969) medium, Knop's medium (Basile and Basile 1988) and Knudson medium (Basile and Basile 1988) were used with or without supplementation of growth hormones for tissue culture in the plant *Plagiochasma appendiculatum*. Total six variations i.e. MS (2000-2500 Lux), MS (with BAP, 2000-2500 Lux), MS (with NAA, KN, 2000-2500 Lux), MS (with IAA, KN, 2000-2500 Lux), MS (with IAA, KN, 6500-7000 Lux), MS (w/o agar, 2000-2500 Lux) in MS medium and three variations i.e. NN (2000-2500 Lux), NN (NAA, KN, 2000-2500 Lux), NN (w/o agar, 2000-2500 Lux) in NN medium were tried initially but success was not achieved in each variants. Best results were obtained in three variations of MS medium (MS (2000-2500 Lux), MS (with BAP, 2000-2500 Lux) and MS (with IAA, KN, 6500-7000 Lux)) and NN medium (NAA, KN, 2000-2500 Lux).

RESULTS

Regeneration

In regeneration experiments of *Plagiochasma*, no regenerants were observed in the apical portions of explants due to inhibitory influence of apical cell. Regenerants were observed in median, midrib and lateral portions of explants from the cut end which was nearer to the growing point. The regenerants always arose from the midrib tissue. Maximum growth was observed in half Knop's solution in all the portions (apical, median, midrib and lateral) of explants in comparison to other concentrations (40, 30, 20 and 10%) of Knop's solution and distilled water (Figure1).

Number of Regenerants

The number of regenerants did not increase with the increase in concentration of Knop's solution. They were found maximum at 20% of Knop's solution (Figure 2). This showed that the number of regenerants did not depend upon a single factor (concentration of nutrient medium).

Spore germination

For spore germination, liquid medium (Knop's solution) with low nutrients supply (20% Knop's solution) was found best in all the tried media (MS medium with agar, 10, 20, 30, 40 and 50% Knop's solution).

High percentage of germination of spores in least time was achieved in 20% Knop's solution in *Plagiochasma appendiculatum* (Figure 3; Plate: 1, figures: A, B). While in other medium, percentage of germination was very low and took long period of time. Further, growth of developing thalli originated from spores was found best in half Knop's solution in spite of 20% Knop's solution (Plate: 1, figure: C).

Formation of Calli

In three suitable variants of MS medium (MS medium (2000-2500 Lux), MS medium (with BAP, 2000-2500 Lux) and MS medium (with IAA, KN, 6500-7000 Lux)) calli were formed and differentiated into thalli (Plate: 1, figures: D-L). The callus formation took place within a month in all variants (Plate: 1, figures: D, G and J). Calli were green, compact and globose in all MS variants except in MS medium (with BAP, 2000-2500 Lux) where calli were green but fragile. Calli were generally formed at apical notch but they were also observed at median part of explant (as in MS medium (with IAA, KN, 6500-7000 Lux)). Percentage of callusing (number of explants having callus/ total number of explants) × 100) was maximum in MS medium (with IAA, KN, 6500-7000 Lux) (70%). In MS medium (2000-2500 Lux) and MS medium (with BAP, 2000-2500 Lux) percentage of callusing were equal (20%).

Formation of rhizoids

Rhizoids formed after thallus formation in suitable concentration of nutrient medium. In MS medium (2000-2500 Lux) rhizoids were simple (s) type (100%) only and were present on ventral surface of new thalli. Rhizoids formation took place in 105 days in MS medium (2000-2500 Lux). In MS medium (with BAP, 2000-2500 Lux) rhizoids were only tuberculate (t) type (100%) and were developed on callus before its differentiation into thalli. Rhizoids formation took 50 days in MS medium (with BAP, 2000-2500 Lux).

In MS medium (with IAA, KN, 6500-7000 Lux) rhizoids formation took maximum days i.e. 160 days. They were present on ventral surface of new thalli and were mostly of simple (s= 92%), whereas tuberculate (t = 8%) type.

Thalli formation

Differentiation of callus into thalli, took two months in MS medium (2000-2500 Lux) while 105 days in MS medium (with BAP, 2000-2500 Lux) and three months (maximum days) in MS medium (with IAA, KN, 6500-7000 Lux) (Figure 4).

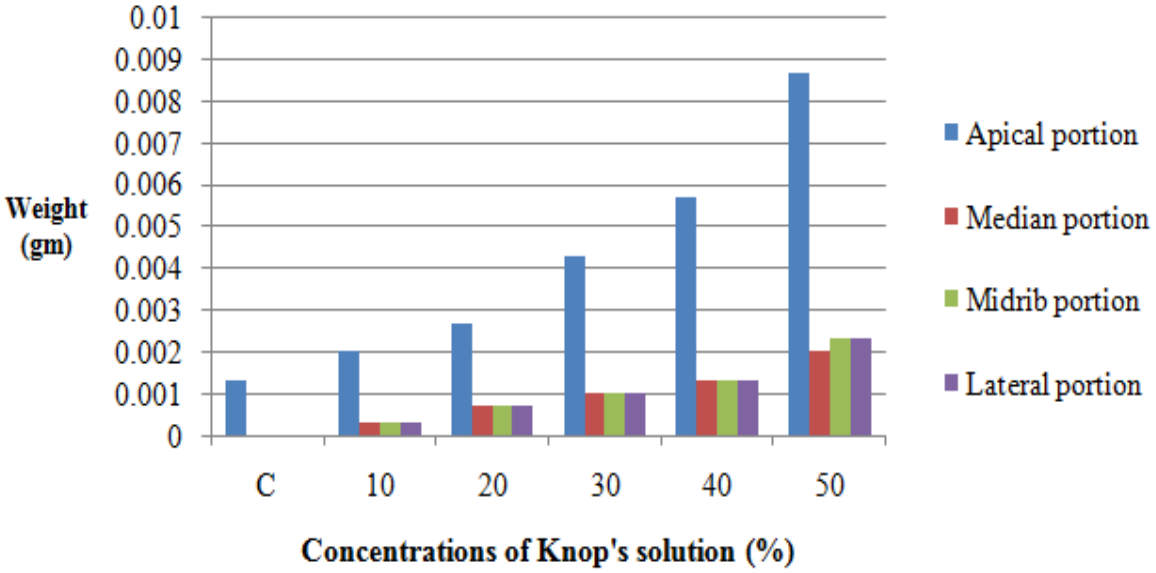


Figure 1. Effect of different concentrations of Knop's solution on growth of various portions of gametophyte of *Plagiochasma appendiculatum* Lehm. *et* Lindenb.

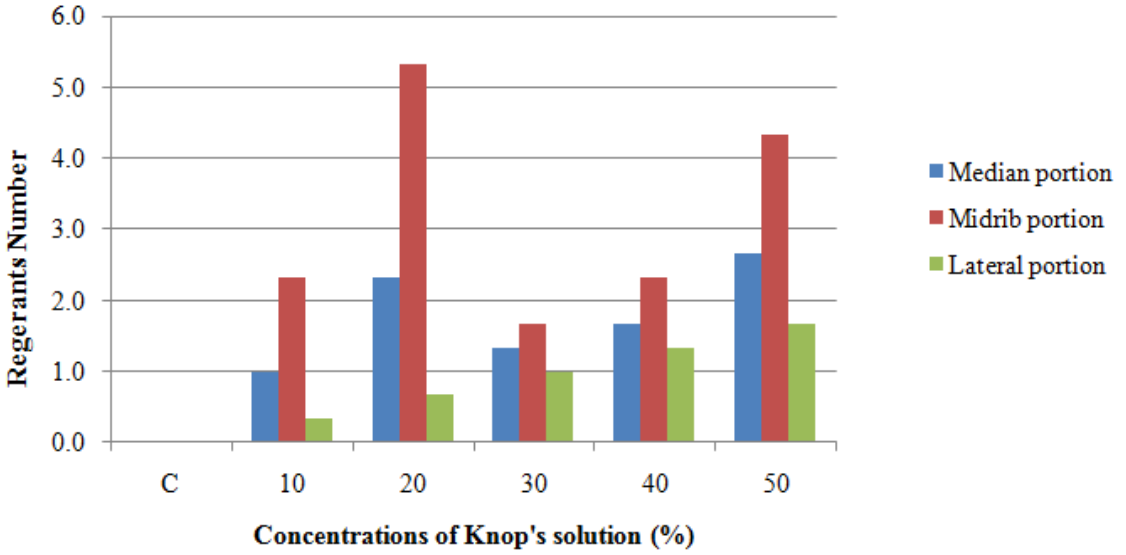


Figure 2. Effect of different concentrations of Knop's solution on number of regenerants in various portions of gametophyte of *Plagiochasma appendiculatum* Lehm. *et* Lindenb.

Other mediums

In Nitsch medium (NN), three variants were tried for tissue culture study i.e. NN medium (2000-2500 Lux), NN medium (with NAA, KN, 2000-2500 Lux) and NN medium (w/o agar, 2000-2500 Lux). In all tried variants, only NN medium (with NAA, KN, 2000-2500 Lux) was found suitable for tissue culture study. NN medium (with NAA, KN, 2000-2500 Lux) supported apical growth which led to thalli formation. Time taken for apical growth in the medium was two weeks (Plate: 1, figure: M). Apical growth was compact and dark green. Percentage of apical growth formation was 60%. Rhizoids were formed within two weeks and were mostly of simple (s) type (s= 99%, t=1%). Thalli formation took place in 40 days (Figures: N and O). Knop's medium and Knudsen's medium were found not suitable for this taxon (under 2000-2500 Lux) at initial stage.

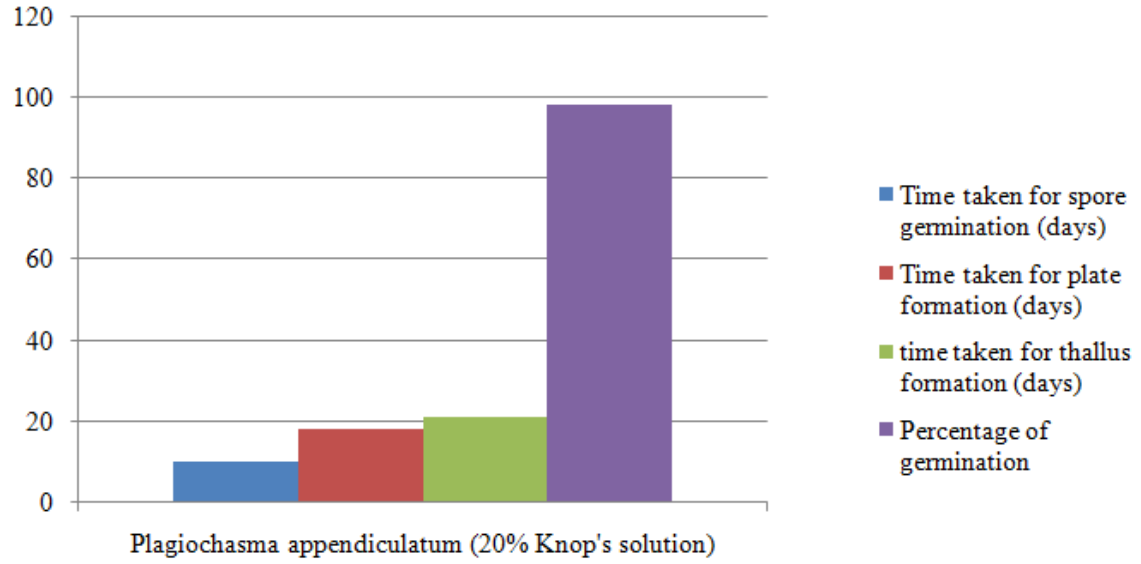


Figure 3. Spore germination in *Plagiochasma appendiculatum*.

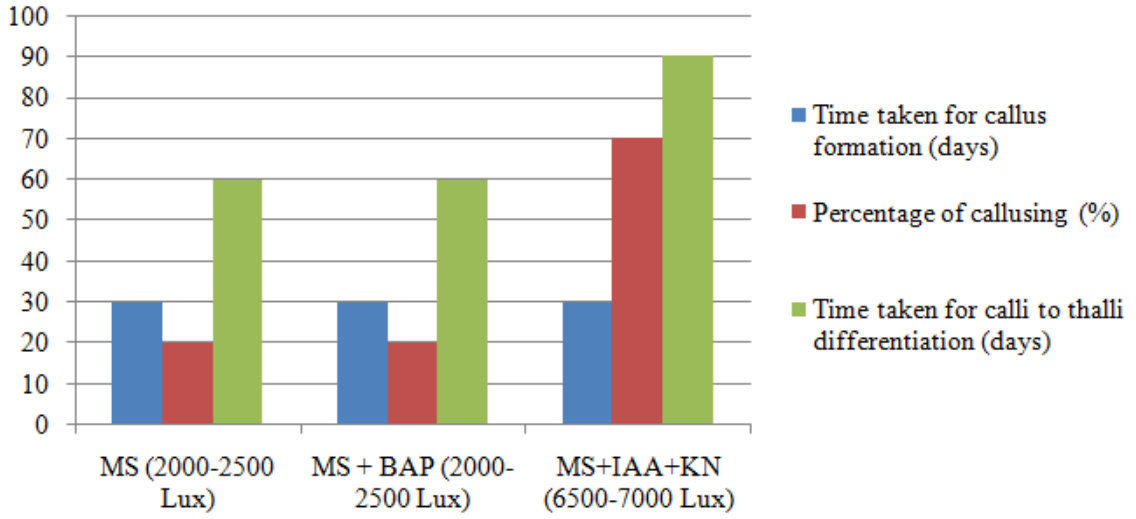
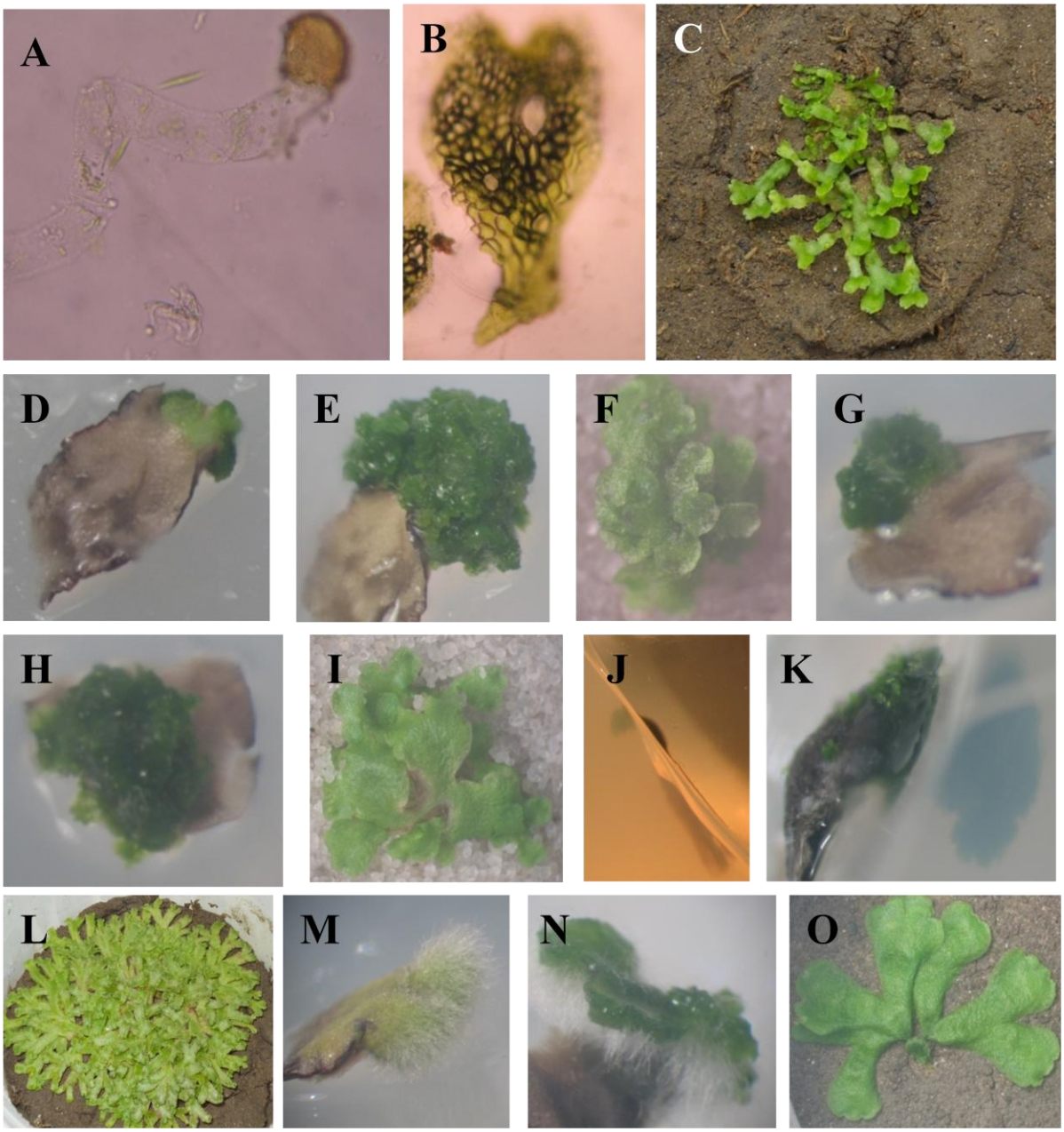


Figure 4. Comparison of different variants of MS medium used in *Plagiochasma appendiculaum* Lehm. *et* Lindenb.

Plagiochasma



(Plate 1. Figures: A-O)

A-O. *Plagiochasma appendiculatum* Lehm. *et* Lindenb.: A. Germinating spore in 20% Knop's solution. B. Developing thalli after three weeks in 20% Knop's solution. C. Thalli after four months of inoculation in 50% Knop's solution. D. Explant in MS medium after a month of inoculation. E. Explant in MS medium after three and half month of inoculation. F. Explant in MS medium after five months of inoculation. G. Explant in MS with BAP medium after a month of inoculation. H. Explant in MS with BAP medium after three and a half month of inoculation. I. Explant in MS with BAP medium after seven months of inoculation. J. Explant in MS medium with IAA & Kinetin (Lux variation) after a month of inoculation. K. Explant in MS medium with IAA & Kinetin (Lux variation) after two months of inoculation. L. Explant in MS medium with IAA & Kinetin (Lux variation) after four hundred days of inoculation. M. Explant in Nitsch medium with NAA & Kinetin after two weeks of inoculation. N. Explant in Nitsch medium with NAA & Kinetin after forty days of inoculation. O. Explant in Nitsch medium with NAA & Kinetin after five months of inoculation

DISCUSSION

Since, it is always been difficult to collect uncontaminated single species of liverwort in wild, hence the in vitro propagation of such an important plant will be helpful for the continuous supply of bioactive compounds of medicinal uses. In the present *in vitro s*tudy for propagation and multiplication of *Plagiochasma appendiculatum* were tried to propagate through regeneration, spore germination and tissue culture. It has been clearly observed that population in mass is difficult to obtain through regeneration as regenerants were found to grow very slow and it takes time as observed earlier also by some workers (Awasthi *et al*., 2012). Spore germination method is best for propagation and multiplication of plants in terms of time and mass but availability of mature and viable spores impose limitation to propagate the population through spore germination. Tissue culture method has been found most suitable way for the propagation and multiplication of plants as availability of vegetative plants is many times more than the availability of fertile plants. The propagation of vegetative parts for study the medicinal value of bryophytes including *Plagiochasma* has been observed. In *Plagiochasma appendiculatum* high light intensity (6500-7000 Lux) was found best in tissue culture experiments for its differentiation into thalli from callus and for further growth of thalli. Medium light intensity (2000-2500 Lux) inhibits its proper differentiation from callus and took long period.)

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