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In vitro Propagation of Saprophytic Moss *Splachnum sphaericum* Hedw.

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Abstract

In vitro propagation of *Splachnum sphaericum* Hedw. has been carried out to study its growth pattern and morphogenetic attributes. Plants of this species were freshly collected from Se La Pass, Tawang, Arunachal Pradesh. Axenic culture of the species has been established using spores as explants. Half Knop's macronutrients, Half Knop's + Vitamins, Hoagland and Murashige & Skoog media were used for culture. Half Knop's macronutrients medium was found as most suitable for the growth of plants under controlled laboratory condition and after 50 days a well grown dense population of gametophores has been achieved. Observations made on morphogenesis of protonema and gametophyte development in different media are provided.

Introduction

Iwatsuki and Steere (1975) reported *Splachnum sphaericum* from East Nepal (Topke Gola, altitude 3600 m, growing on dung). It was also known from North America, Greenland, Europe, Siberia, and China (Smith, 2004; Ignatov *et al*., 2006). This species was reported for the first time in India from Arunachal Pradesh in eastern Himalaya (Ellis *et al*., 2016). The species was found growing on Yak dung, thus showing its habitat specificity. The present study has been carried out on *S. sphaericum* to observe its comparative growth rate and percentage growth in different media (Hoagland; Half Knop's macronutrients; Half Knop's + Vitamins; Murashige and Skoog media) from conservation and propagation point of view. The objective of present study is to observe the comparative morphogenesis of protonema and gametophyte development in different medium. Present study is important from the conservation point of view of the taxon which is known only from Arunachal Pradesh in Indian bryoflora.

Materials and Methods

The plants of *S. sphaericum* bearing sporophytes were collected from Se La Pass, Tawang, Arunachal Pradesh in the month of June, 2015 (Fig. 1). After drying, the plant material was kept in brown paper bags. The voucher specimen has been deposited in Bryophyte Herbarium of National Botanical Research Institute, Lucknow, India (LWG). Mature capsules were separated carefully from the plants and surface sterilized with 2% sodium hypochlorite solution for 2 minutes and washed repeatedly with sterile double distilled water. The spores were inoculated in Half strength Knop's macronutrients, Half Knop's + Vitamins, Hoagland, and Murashige and Skoog media. The pH of the media was maintained at 5.8 before autoclaving. The media was autoclaved at 15 psi for 15 minutes. The experiment was carried out in laboratory under controlled temperature (20-23°C) and relative humidity of 50-60% and provided with illumination of 2400–2500 lux, alternate light and dark period of 16 and 8 hours respectively with the help of a combination of fluorescent tubes. The observations were made on daily basis after the inoculation.

Specimens examined

India, eastern Himalaya, Arunachal Pradesh, Tawang, Se La Pass, 27°30'47.1" N, 91°51'31.6" E, 4137 m, on Yak dung, 17 June 2015, K. K. Rawat 300254 (LWG).

Fig. 1: Plants of *Splachnum sphaericum* Hedw. in nature

Table 1: Comparative account of protonemal morphogenesis and growth pattern of *Splachnum sphaericum* in different media

Number of days	Media used			
	HK	H	1/2 K+ V	MS
3 days	Germ tube developed, green in colour, mostly mono-polar, few bipolar, germination 82% ± 6.7	Germ tube developed, green in colour, bi- and mono- polar both, germination 74 %± 8.3	Germ tube developed, green in colour, mostly mono- polar, germination 88% ±4.2	Germ tube developed, green in colour, mostly mono-polar, germination 80%±2.2
15 days	Protonema differentiated into green region mainly of chloronema and longer filaments, caulonema	Protonema differentiated into green region mainly of chloronema and longer filaments, caulonema	Protonema differentiated into green region mainly of chloronema and longer filaments, caulonema	Protonema with longer filaments, mainly of chloronema developed
30 days	Many leafy gametophores produced from caulonema, average length 1.5-1.8 mm	Few leafy gametophores produced, average length less than 1 mm	Many leafy gametophores produced from caulonema, average length 1.0-1.5 mm	No leafy gametophores produced
40 days	Erect leafy gametophores developed, average length 2-3 mm	Erect leafy gametophores developed, average length 1.8- 2.5 mm	Erect leafy gametophores developed, average length 2-3 mm	Erect leafy gametophores Not Developed
50 days	Dense Population of leafy gametophores developed, average length of plants 4-6 mm	Population not so dense, average length of plants 3-4 mm	Dense Population of leafy gametophores developed, average length of plants 4-6 mm	Erect leafy gametophores Not Developed

Media: HK- Half Knop's; H- Hoagland; 1/2 K +V - Half Knop's + Vitamins; MS - Murashige and Skoog media

Results and Discussion

The spores of *S. sphaericum* at the time of inoculation were green in colour. The spores germinated on the third day of inoculation in all the media. The germ tube formed from endospore after rupturing exospores. The germination percentage was highest in Half Knop's + Vitamins followed by Hoagland, Half Knop's, and Murashige and Skoog media. The germination was usually monopolar in Half Knop's, Half Knop's + Vitamins and Murashige and Skoog media while bi- and monopolar both in Hoagland. The development and differentiation of chloronema and caulonema was observed after 15 days of the germination of spores. In media containing Hoagland, Half Knop's + Vitamins and Half Knop's macronutrients protonema differentiated into green region mainly of chloronema and longer filaments caulonema, however, in Murashige and Skoog medium chloronema were comparatively more longer. After 30 days, development of young gametophores from caulonema took place in Hoagland; Half Knop's + Vitamins and Half Knop's macronutrients (Table 1).

The best growth was observed in half Knop's macronutrients media, while Murashige and Skoog media leafy gametophores were not produced. On 40th day, development of erect leafy gametophores were observed differentiating into the stem and leaves in all the media, except in Murashige and Skoog medium. On 50th day, dense populations of leafy gametophores were observed in Half Knop's + Vitamins and Half Knop's macronutrients (Fig. 2). No leafy gametophores were developed in Murashige and Skoog medium. Cytokinin induces bud formation in mosses (Valadon and Mummery, 1971). Probably it may be the reason that media containing excessive nutrient or trace elements restrict the formation of Cytokinin, that is the reason, no erect plants developed in Murashige and Skoog medium. Awasthi *et al*. (2010) also found that excessively high concentration of macronutrients may cause poor growth of moss species and that may be the reason that half-strength Knop's macronutrient medium, devoid of any trace element and sucrose, was the most suitable for rapid growth of *S. sphaericum*. Gonzalez *et al*. (2006) studied *in vitro* propagation of *S. ampullaceum* Mitt. in ten different mineral media with different sucrose, Nitrogen and B5 Vitamins contents and found that in sucrose and high ammonium media, there was very little effect on protonema diameter and number of buds, while in low ammonium media number of buds reduced, and the media with B5 vitamins improved the long term culture of this species.

The plants were transferred to pots after 50 days and average length of the plants were 3-4 mm bearing 8-14 leaves, for further growth and hardening. In the present study it was found that plant growth was best in Half Knop's

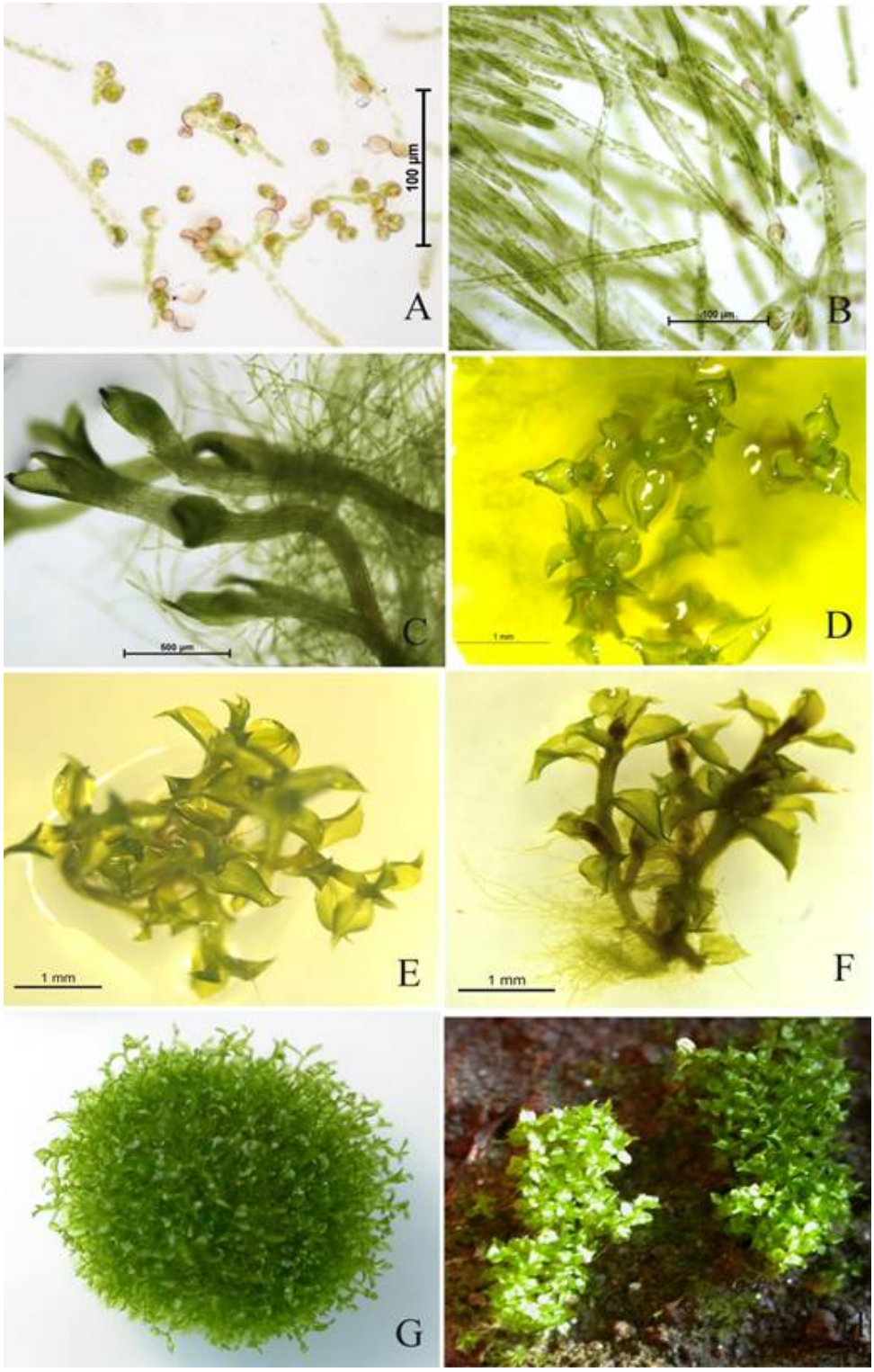

Fig. 2: *In vitro* growth and multiplication of *Splachnum sphaericum.* A: Germinating spores; B: Protonema stage; C: Young gametophores produced from caulonema after 30 days; D-F: Development of erect leafy gametophores; G: Dense population of erect plants; H: Plants after transferring on soil in pots

+ Vitamins and Half Knop's macronutrients medium. The study showed that media containing excessive nutrient or trace elements restricted the growth of *S. sphaericum*.

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