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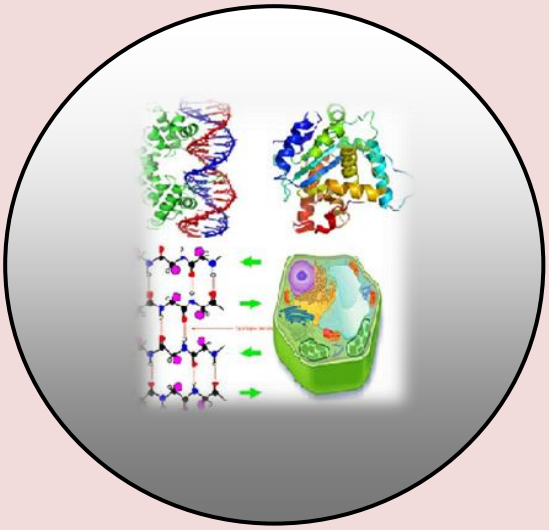

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RESEARCH PAPER

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Comparative Study on Variations in Composition of Murashige and Skoog medium effects on Propagation and Multiplication of *Asterella wallichiana* (Lehm. *et* Lindenb.)

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ABSTRACT

A study was conducted under laboratory condition to compare the effect of various status in composition of Murashige and Skoog (MS) medium on propagation and multiplication of a liverwort Asterella wallichiana viz. MS medium (1500 Lux), MS medium with 6 Benzylaminopurine (BAP, 1500 Lux), MS medium with 1Naphthalene acetic acid and Kinetin (NAA, KN, 1500 Lux), MS medium with Indole 3 acetic acid and kinetin (IAA, KN, 1500 Lux), MS medium with lux variation (IAA, KN, 6500-7000 Lux), MS medium (w/o agar, 1500 Lux), MS medium (with IAA, adenine sulfate, 2iP, 1500 Lux) and MS medium (with IAA, adenine sulfate, 2iP, without amino acids, 1500 Lux). MS medium (without supplement, 1500 Lux) was observed most effective in multiplication and propagation of Asterella wallichiana. Other medium was comparatively less promotary to propagation and multiplication of Asterella wallichiana. The least information is available on multiplications and propagation of Asterella wallichiana with various composition to select effective Murashige and Skoog medium. The study will be significant in in vitro propagation of Asterella wallichiana liverwort to their protection and helpful in the conservation of biodiversity of such types of liverworts.

Key words: Propagation, Multiplication, Liverwort, Murashige and Skoog medium.

INTRODUCTION

Asterella P. Beauv. has deeply incised cage like (beaked) pseudoperianth and the plants have characteristic fishy smell. This liverwort in the family aytoniaceae is reported approximately by 45-48 species worldwide constituting the second largest genus of order Marchantiales after *Riccia* L. (Long, 2006). In India 6 species of the genus have been wildly recognized (Long, 2006). In which *Asterella wallichiana* (Lehm. *et* Lindenb.) Pande *et al*. is widely distributed in India. The propagation of *A. wallichiana* in MS medium needs macro essential elements and growth regulator hormones for callus induction (Kumra, 1984). Many biologically active compounds and interesting chemical compounds have been isolated from different species of *Asterella*. The phytochemical steroids and triterpenoids have been reported from *Asterella angusta* (Siddiqui *et al*., 1993), *Asterella blumeana* (Neves *et al*., 1998) and *Asterella africana* (Figueiredo *et al*., 2006). *Asterella blumeana* has been observed to serve as effective tools in bioconversion reaction (Speicher *et al*., 2003). Antibacterial (Khanam *et al*., 2011) and antifungal activities have also been reported in *Asterella angusta* (Qu *et al*., 2007, Li *et al*., 2009). Dihydroptychantol A (DHA):

an antifungal active chemical obtained from *Asterella angusta* showed significant multidrug resistance (MDR) reverting activity in chemo-resistant cancer cells (Sun *et al*., 2009) and it was also tested for anti-cancer activities (Li *et al*., 2011). Also, *A. wallichiana* has potent antimicrobial activity against different bacterial strains (Sawant and Karadge, 2010). The work on callus induction in liverwort *A. wallichiana* using MS medium with variations in their compositions have been studied earlier (Kumra, 1984; Kumra and Chopra, 1987). Earlier, *in vitro* study on this plant by Awasthi *et al*. (2012) using spores as explants in inorganic medium (half strength Knop's macronutrients and Nitsch's trace elements with 10 ppm freshly prepared ferric citrate along with 1% sucrose) has been conducted. Many species of this genus are important and useful in one or other context and their conservation (*in situ* as well as *ex situ*) is very needful due to their localized population and in the similar way to protect the biodiversity of other liverworts, and to utilize their potential and for experimental studies. Least information is available on the suitable growth medium and appropriate chemical composition for their optimum multiplication and growth of liverworts, including *A. wallichiana*. Thus, *in vitro* studies on the multiplication and propagation are needed to get the plant tissue of required quantity without over harvesting of the plants from natural habitats, this study was conducted. The study may be helpful in conservation and utilization of *A. wallichiana* to their use for various purposes through their in vitro propagation.

MATERIALS AND METHODS

Fresh plants of *Asterella wallichiana* (Lehm. *et* Lindenb.) Pande *et al*. were collected from different localities of the Uttarakhand state i.e. New Tehri (Tehri garhwal), Nainital (Kumaun) in winters.

In the present study eight variations in nutritional composition of Murashige and Skoog medium (Murashige and Skoog, 1962) i.e. MS medium (1500 Lux), MS medium with 6 Benzylaminopurine (BAP, 1500 Lux), MS medium with 1Naphthalene acetic acid and Kinetin (NAA, KN, 1500 Lux), MS medium with Indole 3 acetic acid and kinetin (IAA, KN, 1500 Lux), MS medium with lux variation (IAA, KN, 6500-7000 Lux), MS medium (w/o agar, 1500 Lux), MS medium (with IAA, adenine sulfate, 2iP, 1500 Lux) and MS medium (with IAA, adenine sulfate, 2iP, without amino acids, 1500 Lux) were used, *In vitro* propagation was carried out with using apical portions of thalli as explants. Effects of different light intensities (1500 Lux & 6500-7000 Lux) on the growth were also studied in the taxon. In all above tried variations of MS medium only six variants were found suitable for tissue culture study for *Asterella wallichiana*. Two variants: MS medium (with IAA, adenine sulfate, 2iP, 1500 Lux) and MS medium (with IAA, adenine sulfate, 2iP, without amino acids, 1500 Lux) were found not suitable for the taxon in which the explants became dead at initial stages. Hence, they are not included here in the present study.

RESULTS AND DISCUSSION

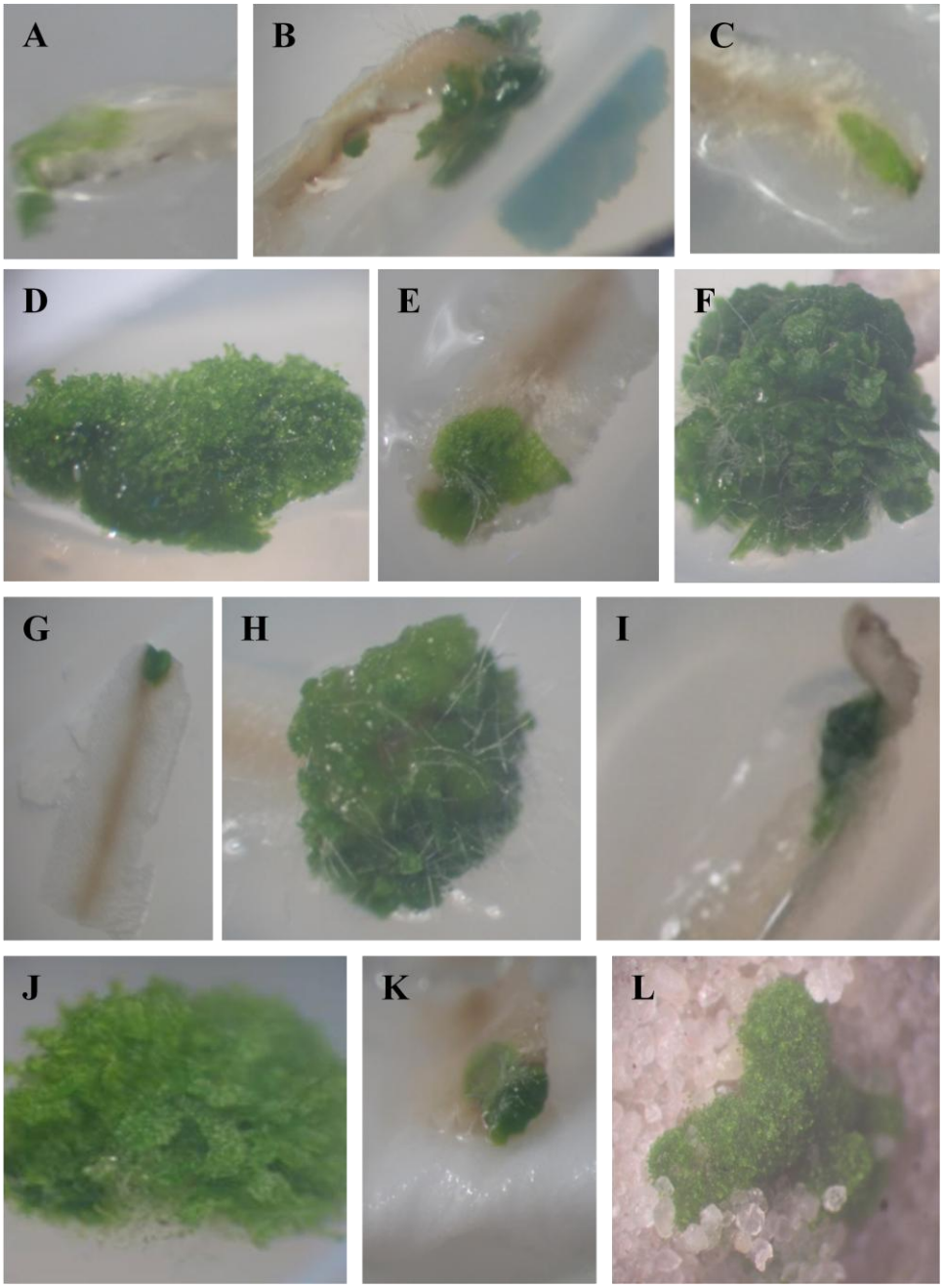
In all suitable MS medium (six variations) the callus was formed and differentiated into thalli except in MS medium (w/o agar using Paper-Bridge Technique). Calli were formed early in MS medium (1500 Lux) and MS medium (with BAP, 1500 Lux) in 14 days after inoculation (Plate: 1, figs.: A, C), while in MS medium (with NAA, KN, 1500 Lux) and MS medium (w/o agar, 1500 Lux) calli were formed late i.e. 40-45 days after inoculation (Plate: 1, figs.: E, K). Calli were formed within a month in MS medium (with IAA, KN, 1500 Lux), MS medium (with IAA, KN medium, 6500-7000 Lux) (Plate: 1, figs.: G, I). As far as percentage of callusing (number of explants having callus/ total number of explants × 100) is concerned, it was maximum in MS medium (1500 Lux) i.e. 100% and minimum in MS medium (with IAA, KN medium, 6500-7000 Lux) i.e. 40%. In MS medium (with BAP, 1500 Lux) 50% callusing, in MS medium (with NAA, KN, 1500 Lux) 90% callusing, MS medium (with IAA, KN, 1500 Lux) 70% callusing and MS medium (w/o agar, 1500 Lux) 50% callusing was observed. Calli mostly observed at dorsal surface of apical notch and sometimes on ventral dorsal surface rarely on median part of explants. Calli are green compact and globose in low light intensity but in high light intensity calli became pale due to late in differentiation process.

The differentiation of callus into thalli took minimum days in MS medium (1500 Lux) and MS medium (with IAA, KN, 1500 Lux) i.e. 50 days (Plate: 1, figs.: B, H) and maximum days in MS medium (with IAA, KN medium, 6500-7000 Lux) i.e. 100 days (Plate: 1, fig.: J).

In other variants, it took 80 days in MS medium (with NAA, KN, 1500 Lux) (Plate: 1, fig.: F) and 70 days in MS medium (with BAP, 1500 Lux) (Plate: 1, fig.: D). While in, MS medium (w/o agar, 1500 Lux) differentiation did not took place (Plate: 1, fig.: L). After thalli differentiation, rhizoids types (simple and tuberculate) of newly formed plants were also observed as they show variation in their numbers. It was observed that MS medium with BAP, only promote tuberculate rhizoid formation. Tissue culture experiments done in high Light intensity (6500-7000 Lux) shows delay in growth stages (i.e. callus formation, thalli differentiation and further new thalli formation) and thalli were also small in size. In *Asterella wallichiana* Murashige and Skoog medium (without any supplement and 1500 Lux) was found most favorable among in six suitable variants of MS medium i.e. MS (1500 Lux), MS (with BAP, 1500 Lux), MS (with NAA, KN, 1500 Lux), MS (with IAA, KN, 1500 Lux), MS (with IAA, KN, 6500-7000 Lux), MS (w/o agar, 1500 Lux). In *Asterella wallichiana* low light intensity (1500 Lux) was found most suitable in tissue culture experiments. In high light intensity (6500-7000 Lux) stunted growth was observed for long time period and thalli were smaller than normal size of plants.

Table 1. Different variants of Murashige and Skoog medium effects on the gametophyte of *Asterella wallichiana* (Lehm. *et* Lindenb.) Pande *et al.* in tissue culture study.

Different variants of MS medium	MS (1500 Lux)	MS + BAP (1500 Lux)	MS+ NAA+KN (1500 Lux)	MS+IAA +KN (1500 Lux)	MS+IAA+ KN (6500-7000 Lux)	MS w/o agar (1500 Lux)
Time taken for callus formation	14 days	14 days	45 days	25 days	30 days	40 days
Place of callus formation	At dorsal surface of apical notch and later on median part of explant	At dorsal & ventral surface of apical notch	At dorsal surface of apical notch	At dorsal surface of apex	At dorsal & ventral both surface of apical part	At Dorsal surface of apex and on ventral surface at median part of explant
Type of callus	Green, compact & globose	Green, compact	Dark green, globose and compact	Slight yellow-green and compact	Dark green and compact	Dark green, globose and compact
Percentage of callusing	100%	50%	90%	70%	40%	50%
Time taken for rhizoid formation	25 days	30 days	14 days	40 days	40 days	40 days
Percentage of both rhizoids	s=100%	t=100%	s= 99% t= 1%	s=75% t=25%	s=90% t=10%	s=50% t=50%
Time taken for calli to thalli differentiation	50 days	70 days	80 days	50 days	100 days	-



(PLATE: 1, Figs.: A-L)

A-L. *Asterella wallichiana (Lehm. et Lindenb.) Pande et al.*: Tissue culture in Murashige and Skoog Medium : A. Explant after two weeks of inoculation. B. Explant after fifty days of inoculation. Tissue culture in Murashige and Skoog Medium with BAP : C. Explant after two weeks of inoculation. D. Explant after seventy days of inoculation. Tissue culture in Murashige and Skoog Medium with NAA and Kinetin : E. Explant after forty five days of inoculation. F. Explant after eighty days of inoculation. Tissue culture in Murashige and Skoog Medium with IAA and Kinetin: G. Explant after twenty five days of inoculation. H. Explant after fifty days of inoculation. Tissue culture in Murashige and Skoog Medium with IAA & Kinetin with Lux variation: I. Explant after a month of inoculation. J. Explant after hundred days of inoculation. Tissue culture in Murashige and Skoog medium without agar using Paper-Bridge Technique: K. Explant after forty days of inoculation. L. Explant after a year of

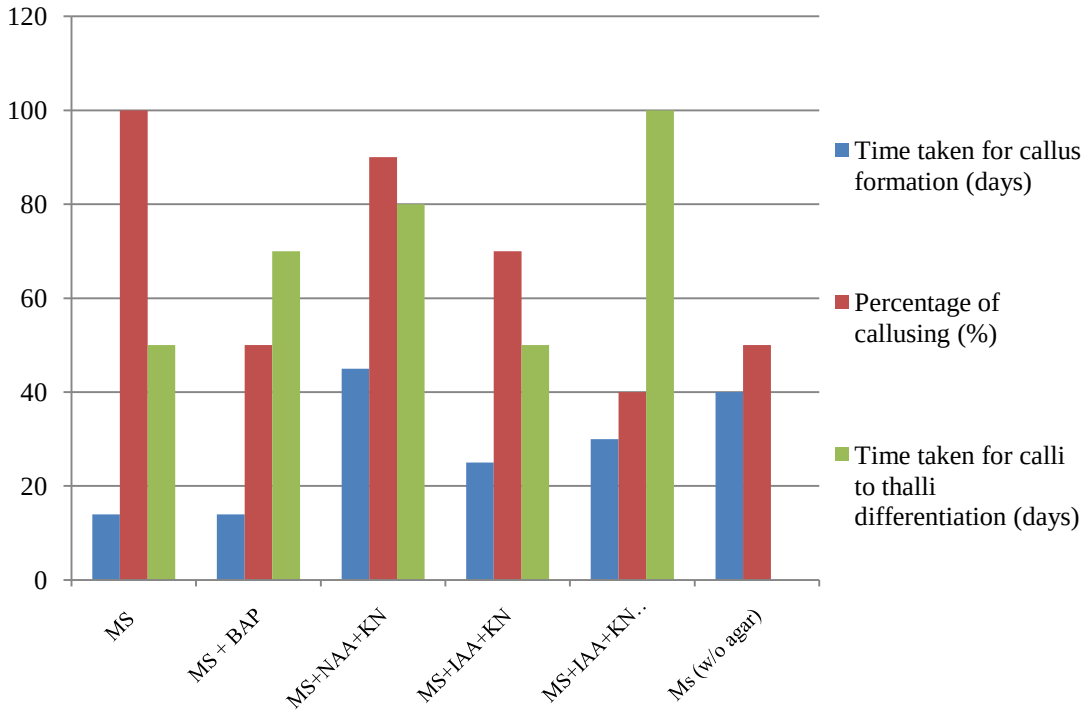


Figure 2. Comparison of different variants of MS medium used in *Asterella wallichaina* (Lehm. *et* Lindenb.) Pande *et al*.

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