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An Observation on Morphogenetic Response of *Marchantia polymorpha* subsp. *ruderalis* Bischl. & Boissel.-Dub. in Different Culture Media

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Abstract

The growth pattern of *Marchantia polymorpha* subsp. *ruderalis*, using gemmae as explant, in different culture media in combination with auxin and cytokinins has been studied. The plant growth was maximum in ½ KNOP's followed by KNOP's, Hoagland and Murashige & Skoog media. The normal growth of gemmae was inhibited at higher concentrations of hormone (1 mg L^{-1} Kinetin + 1 mg L^{-1} Auxin, 1.5 mg L^{-1} Kinetin + 1.5 mg L^{-1} Auxin, 0.5 mg L^{-1} 2,4D) and callus like tissue was produced, while at lower concentrations (0.1 mg L^{-1} Kinetin+0.1 mg L^{-1} Auxin) gemmae developed normally and rhizoids were profusely produced on the surface of young thalli. Cultured thalli were transferred to the soil in pots and introduced in moss house.

1. Introduction

Marchantia L. belongs to family Marchantiaceae and is cosmopolitan in distribution. It possesses antimicrobial, anti-hepatic, antipyretic and diuretic properties. As far as its diversity is concerned, recently, Singh and Singh (2013) recognized 10 valid species and infraspecific taxa of the genus in India. Asakawa (1988) reported the isolation of compounds Marchantin A, B and C from *Marchantia polymorpha*, which were cytotoxic to KB cells. In China species of *Marchantia* are used as traditional medicinal herbs for the treatment of hepatitis and are mixed with vegetable oil to treat bites, boils, burns, cuts, eczema and wounds (Ando, 1983; Saroya, 2011). *Marchantia polymorpha* L. is also known to treat fractures, cuts, poisonous snake bites, burns, scalds, and open wounds (Qu *et al*., 2007; Mewari and Kumar, 2008). According to Shrisat (2008) tribal community of Melghat area, Maharashtra, India is using *Marchantia polymorpha* against inflammation and skin disease. Earlier some research work on other species of *Marchantia* have been carried out by Kaul *et al.* (1961, 1962), Kaul and Kaul (1974), Nath *et al.* (2008), Awasthi *et al.* (2011) and Sahu *et al.* (2010) with respect to nutritional requirements, response against heavy metals and effect of auxins and antiauxins. The genus contains terpenoids, flavonoids, steroids, and bis (bibenzyls) (Asakawa, 1995, Niranjan *et al.,* 2014). In spite of its high potential value it is quite difficult to obtain the sufficient amount of pure population of fresh plants in nature. *In vitro* propagation of *M. polymorpha* will facilitate the bulk production of this species for studies on its chemical and pharmaceutical aspects and further isolation of novel chemical compounds. In view of this, present study has been carried out on a plant population growing in western Himalaya.

2. Materials and Methods

The gemmae of *M. polymorpha* subsp. *ruderalis* were obtained from Govind Wildlife Sanctuary, Uttarkashi, Uttarakhand. Voucher specimens have been deposited in Bryophyte Herbarium of National Botanical Research Institute, Lucknow (LWG), India. Specimens examined: India, western Himalaya, Uttarakhand, Uttarkashi, on way to Kedarkantha (ca 3124 m), on soil, 6.10.2013, leg., K. K. Rawat, 254868A (LWG).

The gemmae were surface sterilized with 0.1% sodium hypochlorite solution for 2 minute and washed

Table 1: *In vitro* propagation of *M. polymorpha* subsp*. ruderalis* in different Culture media.

S. N.	Growth substances	3 days		15 days		45 days	
		A.L. (mm)	A.W. (mm)	A.L. (mm)	A.W. (mm)	A.L. (mm)	A.W. (mm)
1.	MS	0.86±0.12	0.6±0.10	4.65±0.47	3.4±0.69	8.8±1.98	4.8±0.78
2.	1/2 KNOP's	2.3±0.44	1.34±0.42	9.2±1.09	4±1.41	15±2.62	7.9±0.73
3.	KNOP's	1.01±0.144	0.66±0.10	3.8±0.47	2.15±0.69	11.2±0.47	7.2±0.699
4.	Hoagland	1.21±0.27	0.812±0.15	4.9±0.56	2.85±0.74	10±1.41	5.6±0.72

MS=Murashige and Skoog Media, A.L= average length, A.W= average width

repeatedly with sterile double distilled water. The gemmae were inoculated in Half strength KNOP's macronutrient, KNOP's, Hoagland and Murashige & 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°C-23°C) and relative humidity of 50-60% and provided with illumination of 2400–2500 lux as well as alternate light and dark period of 16h and 8 h respectively with the help of a combination of fluorescent tubes.

In order to observe the influence of different mineral nutrients and hormone concentrations on the morphogenesis of this species, the following combination was used. All the hormone concentrations were prepared with 1/2 KNOP's macronutrients medium.

(a) Murashige and Skoog media (MS)

(b) Half strength KNOP's macronutrient

(c) Full strength KNOP's

(d) Hoagland no. 2 basal salt mixture

(e) 1/2 KNOP's macronutrients +0.1 mg L^{-1}Kinetin+0.1 mg L^{-1}Auxin

(f) 1/2 KNOP'S macronutrients +1 mg L^{-1}Kinetin + 1 mg L^{-1}Auxin

(g) 1/2 KNOP's macronutrients +1.5mg L^{-1} Kinetin + 1.5 mg L^{-1}Auxin

(h) 1/2 KNOP's macronutrients +0.5 mg L^{-1}2,4D

3. Results and Discussion

3.1. Responses of Gemmae to several growth substances

The growth of gemmae was observed on 3rd day. In all the media gemmae were bright green in colour and started to elongate towards the axis of two opposite notches. Two thalli, one from each notch, were developed followed by emergence of rhizoids from the ventral surface of the gemmae; in some cases rhizoids developed on the dorsal surface as well. In all the media germination of gemmae took place. It has been observed that gemmae growth were maximum in the ½ KNOP's followed by Hoagland, KNOP's and MS on 3rd day. On 15th Day, in MS and Hoagland media dark green thallus developed with only smooth-walled rhizoids, while in the case of the Half strength KNOP's and KNOP's macronutrient media light green, delicate, thalli developed. Thalli growth was best in the case of Half strength KNOP's. On 45th Day in the case of Half strength KNOP's maximum growth was observed followed by KNOP's and Hoagland (Table 1). In all the media simple and tuberculate rhizoids and light green scales developed on the ventral surface of the thallus, while 1-3 gemma cups developed on the dorsal surface of the each thallus in Half strength KNOP's medium as compared to only one gemma cup on those in Hoagland and MS media. It was found that gemma cups were not developed in Full strength KNOP's media. The population of the species has been successfully grown in Half strength KNOP's macronutrient. The plants were transferred to the soil in pots for hardening after 45 days (Fig. 1). In the present experiment biomass production and moisture content of plant after 30 and

Table 2: Plants biomass produced in pots and moisture content.

Days	Fresh wt. (A.V.)	Dry wt. (A.V.)	Moisture content (%)
30 days	6.471±0.131	0.3488±0.05	94.59
45 days	9.12475±0.22	0.45015±0.01	94.14

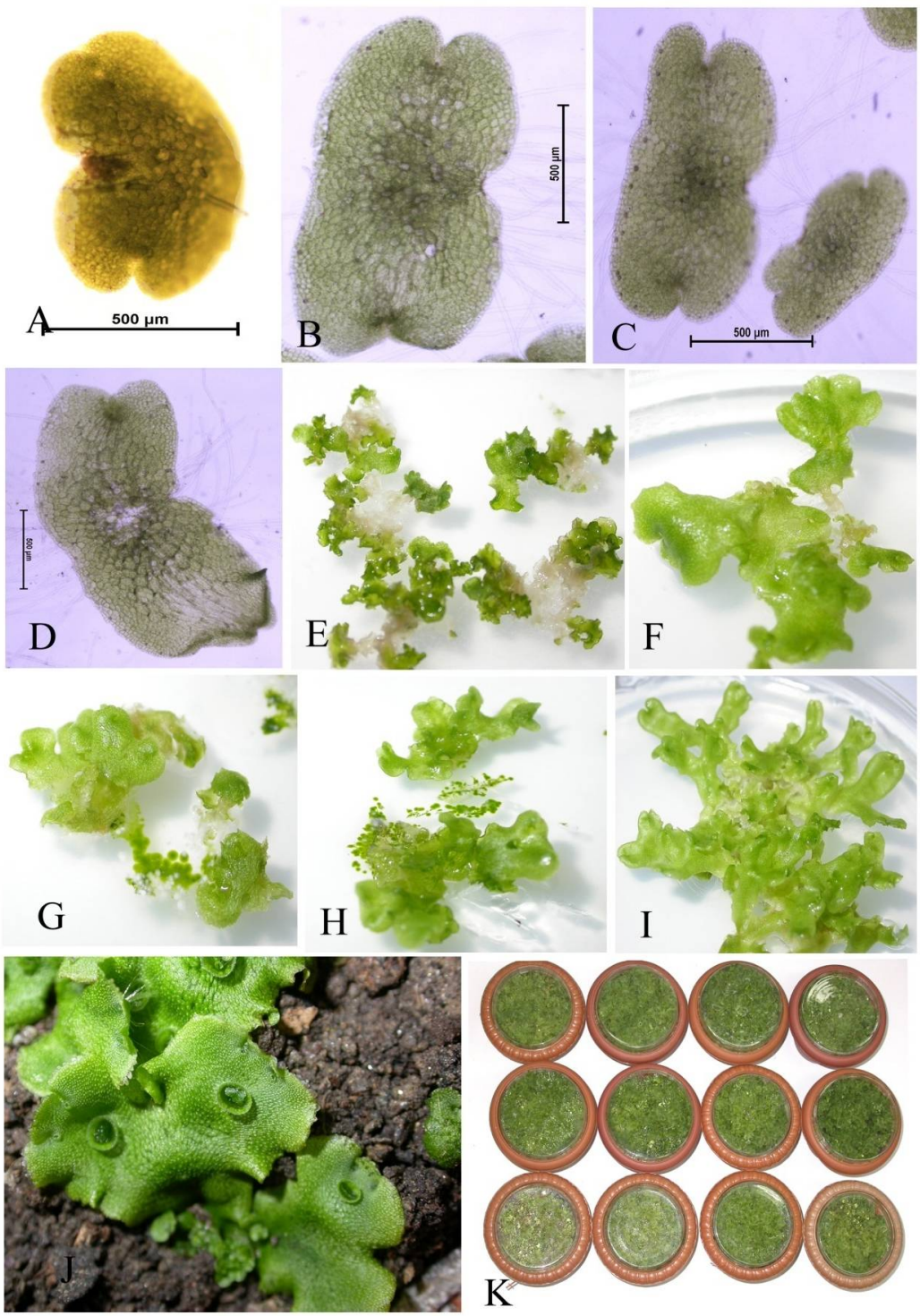

Fig. 1: *In vitro* growth and multiplication of *M. polymorpha* subsp. *ruderalis*. Growth of gemmae in different media after 3 days (A-D) A: MS media, B: Half strength KNOP's media, C: KNOP's media, D: Hoagland; Thalli growth in different media after 15 days (E-H) E: MS media, F: Half strength KNOP's media, G: KNOP's media, H: Hoagland, I: Dense population of thalli in Half strength KNOP's media, J-K Thalli after transferring on soil in pots.

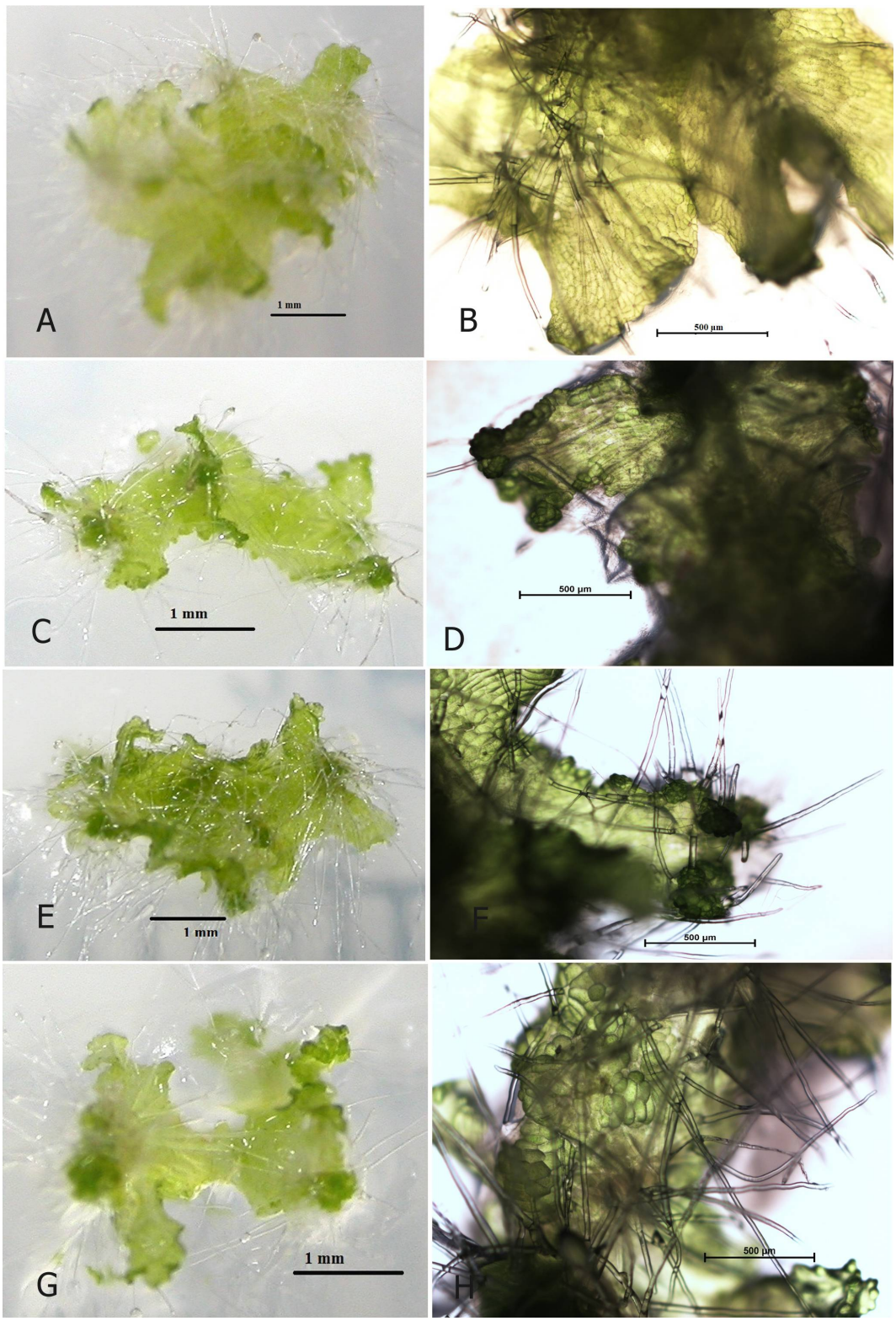

Fig. 2: *In vitro* growth and multiplication of *M. polymorpha* subsp. *ruderalis* in different concentration of hormones after 15 days. A, B: Differentiating thalli in 0.1 mg L^{-1} Kinetin+0.1 mg L^{-1} Auxin, C, D: 1 mg L^{-1} Kinetin + 1 mg L^{-1} Auxin, E, F 1.5 mg L^{-1} Kinetin + 1.5 mg L^{-1} Auxin, G, H: 0.5 mg L^{-1} 2,4D.

Table 3: Responses of the *M. polymorpha* subsp. *ruderalis* to different concentrations of Growth substances.

S. N.	Growth substances and their concentrations	3 Days		15 Days	
		A.L. (mm)	A.W. (mm)	A.L. (mm)	A.W. (mm)
1.	1/2 KNOP's	2.3±0.44	1.34±0.42	9.2±1.09	4±1.41
2.	0.1 mg L^{-1} Kinetin + 0.1 mg L^{-1} Auxin	1.5±0.37	0.88±0.08	2.9±0.22	1.7±0.27
3.	1 mg L^{-1} Kinetin + 1 mg L^{-1} Auxin	2±0.141	1.06±0.16	3.4±0.894	1.8±0.27
4.	1.5 mg L^{-1} Kinetin + 1.5 mg L^{-1} Auxin	1.96±0.08	1.1±0.1	3.4±0.54	1.7±0.27
5.	0.5 mg L^{-1} 2,4D	2.3±0.27	1.34±0.13	3.6±0.54	1.9±0.22

45 days of transfer into pots were also calculated (Table 2). This may further lead to bulk propagation of species for conservation and large biomass production of this species for applied studies.

3.2. Responses of Gemmae to different concentration of Auxins and cytokinins

At lower concentration (0.1 mg L^{-1} Kinetin + 0.1 mg L^{-1} Auxin) gemmae developed normally but rhizoid formation was induced not only on ventral surface but also on dorsal surface of thalli. At higher concentrations of auxins and cytokinins (1 mg L^{-1} Kinetin + 1 mg L^{-1} Auxin, 1.5 mg L^{-1} Kinetin + 1.5 mg L^{-1} Auxin, and 0.5 mg L^{-1} 2,4D) globular or irregular masses of callus-like tissue produced at the distal end of the thallus which did not differentiate. The callus tissue was usually green in colour. In the present experiment it was found that higher concentrations of auxins and cytokinins in combination with half strength Knop's media resulted in callus induction, while the maximum growth was observed in Half strength KNOP's medium (Table 3, Fig. 2).

It is evident from the observations mentioned above that thalli of *M. polymorpha* subsp. *ruderalis* responded to stimulatory and inhibitory effects of higher and lower concentrations of auxins and cytokinins in a similar way like root and shoot buds of higher plants. According to Bünning (1952, 1956), polarity is one of the factors which is involved in the development of a specific form. Fitting (1936) has also discussed about the polarity in *Marchantia* and *Lunularia*. It is the basis of all kinds of differentiation by direct effects of physical or chemical factor. The suppression of polarity will prevent normal differentiation but may allow cells to divide and re-divide. On the basis of this hypothesis it may be assumed that the action of these growth substances on young thalli could be due to their direct effect on the internal gradient of the dividing protoplasts.

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