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In vitro micropropagation of Cheilanthes farinosa (Forssk.) Kaulf. Pteridaceae

Kavitha R.
jesuskavi96@gmail.com
PSG College of Arts and Science, Coimbatore, Tamil Nadu

Prabakaran R.
raju.prabakaran@gmail.com
PSG College of Arts and Science, Coimbatore, Tamil Nadu

ABSTRACT

Cheilanthes farinosa is a pteridophytic plant belongs to the family Pteridaceae is a rare plant of rhizome up to 1 cm. in diam. Sori minute, 1 mm. in diam, closely set along the margins of the pinna segments. The plant roots are used to treat eczema and stomachache; fronds are used to treat menstrual disorders. Rhizomes and roots are used to treat gout, rheumatism and used as general tonics. It is used in the treatment of inflammatory, skin disorders and rheumatism etc., so the Cheilanthes farinosa plant was chosen for conservation through micropropagation. The sporangia are used as explant. MS medium with 3.5mgL-1 of BAP and GA3 shows the high growth rate and 2.4mgL-1 of IBA and KIN shows the slow growth rate. After 1 month of inoculation, the gametophytic body was developed. And it was subculture to MS medium containing various concentrations of BAP and GA3.Key words: Conservation, fronds, gametophyte, pteridaceae, sporangia, and subculture.

Keywords— *Conservation, Fronds, Gametophyte, Pteridaceae, Sporangia, Subculture.*

1. INTRODUCTION

Micropropagation is the art of growing plants using plant tissue culture techniques. It refers to multiplication of plant cells, tissues, organs using solid, semisolid or liquid medium under *invitro* conditions. It is used to avoid the diseases in crops, and it helps in early flowering and fruiting. Through this method we can produce mass multiplication of cells, each cell have the capacity to develop individual plant.

Cheilanthes farinosa was taken in Kurunthamalai hills, which is a small town in the district of Coimbatore, this is 24 km from the city of Coimbatore and only 4 km from Karamadai. *Cheilanthes farinosa* has Erect, with tufted fronds and with dark-brown subulate entire rhizome-scales up to 7 mm. Lamina up to 39 x 18 cm. oblong, acute in outline, 2–3-pinnatifid, dark-green above, the lower surface covered with pale-yellow to whitish powder. Sori discrete, appearing continuous at maturity; indusia separate to continuous, entire to lacerate-ciliate. In India extract of washed rhizome freed from scales is useful to the unconscious patients suffering from epilepsy.

2. DISTRIBUTION OF THE PLANT

Kenya Elgeyo District Cherangani Hills, Arror Valley, 26 Aug. 1969, Mabberley & McCall 240!KENYA Fort Hall District Thika, Blue Posts Hotel, 12 Aug. 1967, Faden 67/583!;Kenya Londiani District Tinderet Forest Reserve, Camp 2, 19 June 1949, Maas Geesteranus 5042!Tanzania Mbulu District Ngorongoro crater rim near where main road meets it, 2 Mar. 1964, Verdcourt 4025!;Tanzania Lushoto District W Usambaras, near Magamba, Kosti, 21 Sept. 1981, Mtui & Sigara 66!;Tanzania Mbeya District Poroto Mts, Kikondo Camp, 19 Jan. 1961, Richards 13959!Uganda Karamoja District Mt Debasien, Zebiel, 9 Jan. 1937, A.S. Thomas 2219!;Uganda Kigezi District Kirwa, Dec. 1938, Chandler & Hancock 2536!;UGANDA Mbale District Bugishu, Butandiga, Dec. 1935, Eggeling 2430!

3. MATERIALS AND METHODS

3.1 Source of plant materials

The plant material was collected in Fronds with mature sori from Kurunthamalai hill Coimbatore, Tamil Nadu, India. Spore with fronds were collected and used for various experiments for developing the protocol for explant preparation and regeneration.

3.2 In vitro studies

The present investigations on *in vitro* studies of *Cheilanthes farinosa* was carried out in the tissue culture laboratory of the Department of Botany, PSG College of Arts & Science, Coimbatore, India. *In vitro* techniques like spore germination, direct regeneration and *in vitro* gametophytic development were attempted.

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3.3 *In vitro* Protonema Initiation

Sterilized Fronds with mature sori were inoculated on water agar media, Murashige and Skoog's (MS) basal salts in ½ strength and half strength MS media containing BAP 1.0mg to 2.0mg and NAA 0.1mg to 0.3mg concentrations and full MS medium containing IBA 1.0mg to 3.0mg and KIN 1.0mg to 3.0mg were added respectively for protonema initiation. The cultures were incubated in 22 ± 2°C under 16/8 hour (light and dark) condition period to observe rate of germination.

3.4 Culture media employed and their composition

MS (Murashige and Skoog's, 1962) basal medium in two different strength; full strength (MS) and half strength (MS) were employed in the present study. The composition of the medium is given below

Table 1: Chemical composition of Murashige and Skoog's (1962) medium

S no.	Component	mg/l
	Major salts	
1	NH_4NO_3	1650
2	KNO_3	1900
3	$CaCl_2.2H_2O$	440
4	$MgSO_4.7H_2O$	370
5	KH_2PO_4	170
	Minor salts	
6	$MnSO_4.4H_2O$	16.8
7	$ZnSO_4.7H_2O$	8.6
8	H_3BO_3	6.2
	Micro salts	
9	$Na_2MoO_4.2H_2O$	0.251
10	$COCl_2.6H_2O$	0.025
11	$CuSO_4.5H_2O$	0.025
12	KI	0.830
	Iron	
13	$FeSO_4.7H_2O$	27.86
14	Di sodium EDTA	37.26
	Vitamins	
15	Nicotinic acid	0.5
16	Thiamine HCl	1.0
17	Pyridoxine HCl	0.5
	Amino Acids	
18	Glycine	2.0
19	Meso Inositol	100.0
20	Sucrose	30g
21	Agar	8g
	pH	5.8

3.5 Culture conditions

All the cultures were maintained in the culture room at a temperature of 25±2°C and relative humidity of 65-70%. The cultures were kept under white light at intensity of 3000 Lux provided from white fluorescent lamps (Philips, India) with 12 hours photoperiodic duration.

3.6 Explants selection and mode of sterilization

The explants Fronds with mature sori harvested from the field grown plants were treated with detergents (teepol and vim gel), fungicides (bavistin-methyl-3-benzimidizole carbomate solution and Carbendazim), antibiotics (amphicilin) and surface sterilants namely 70% alcohol and mercuric chloride ($HgCl_2$) with various concentrations at different time for sterilization. The surface sterilized explants were trimmed gently with the help of sterile surgical blade (Lisyter No: 10) and aseptically inoculated on to pre-cooled autoclaved medium.

3.7 Protonema Initiation and gametophytic Formation

Fronds with mature sori explant from field grown plants were used as primary explants. The explants were cultured on MS medium supplemented with various concentrations of growth regulators (3.5mgL^{-1} of BAP and GA3, 2.4mgL^{-1} of IBA and KIN). Twenty five explants were used for each culture. The percent of explants responding for protonema induction, gametophytic formation, nature of spore and number of days taken for spore induction were recorded after 40 days. In the subsequent subcultures, the callus and other parts obtained *in vitro* cultures were harvested and used as explants. Sub culturing was carried out at regular interval of 15-20 days.

3.8 Gametophytic initiation and multiplication

Fronds with mature sori explants from field grown plants were used as primary explants. Fronds with mature sori explants were cultured on MS medium supplemented with various concentrations of growth regulators (BAP, IBA and NAA). Fifty explants were used for each culture. Each experiment was repeated twice. The per cent of explant responding for protonema development and gametophytic initiation, spore number per explant and total number of spore per explant per sub culture were recorded after 60 days. In

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the subsequent sub cultures, the thallus obtained *in vitro* produced gametophytic body were harvested and used as explants to culture on the same medium. Sub-culturing was carried out at the regular interval of 15-20 days.

4. RESULTS

Cheilanthes farinosa is a Pteridophytic plant with creeping rhizome and ovate to lanceolate scales. Sori minute, 1 mm in diameter, closely set along the margins of the pinna segments; indusium small, semi-transparent, variously lacerate. The plant is a lithophyte and lives on rocks. Roots are used to treat eczema and stomachache; fronds are used to treat menstrual disorders. Rhizomes and roots are used to treat gout, rheumatism and used as general tonics. It is used in the treatment of inflammation, skin disorders and rheumatism. It has anti-nociceptive properties. *Cheilanthes farinosa* is also used to treat mental disorders in India. The present study was to evaluate *in vitro* spore germination and direct regeneration of *Cheilanthes farinosa*.

4.1 *In vitro* spore germination

The percentage of *in vitro* seed germination of *Cheilanthes farinosa* in different media conditions are shown in table 1. Significant differences in all parameters like percentage of germination, no of protonema and gametophye development were observed and recorded on 60th day of germination. It was observed that full strength MS with 3.5 mg/L of IBA & GA3 showed the highest growth percentage (83.34±2.36) of spore germination and protonema initiation with (333.7±3.74) number of protonema, average number of gametophyte from protonema was (290.56±2.87) and their percentage of gametophyte development was (98.48±0.65) observed in Plate1. Followed by 2.5 mg/L of IBA & KIN showed (25.16±0.22) percentage of spore germination and protonema initiation with (123.7±0.85) number of protonema, average number of gametophyte from protonema were (97.4±0.80) and their percentage of gametophyte development was (77.1±0.74) observed in Plate2.

4.2 Direct gametophyte development

Direct gametophyte formation through sub-culturing of *Cheilanthes farinosa (*plate1. E, F), using various growth regulators such as BAP & GA_3 at different concentrations are summarized in table 2. It was observed that full strength MS with 1.0 mg/L of BAP & GA_3 showed the highest growth percentage of (36.18±1.57) number of gametophyte plantlets subcultured with (65.04±1.83) number of gametophyte were developed after subculture and their percentage of gametophyte development was (76.45±1.74). Followed by 2.0 mg/L of BAP & GA3 showed the highest growth percentage of (36.17±0.45) number of gametophyte plantlets subcultured with (37.57±0.65) number of gametophyte were developed after subculture and their percentage of gametophyte development was (10.09±0.20).

Table 2: In vitro Spore Germination and Gametophyte Regeneration of *Cheilanthes farinosa* (Forsk.) Kaulf.

S.no	Types of media	Concentrations of growth regulator					Spore germination and protonema initiation %	Number of protonema	Average number of gametophyte from protonema	Percentae of gametophyte development
		BAP	NAA	IBA	KIN	GA_3				
1	½ MS Medium	1.0	0.1	-	-	-	-	-	-	-
		1.5	0.2	-	-	-	-	-	-	-
		2.0	0.3	-	-	-	-	-	-	-
2	HOgland No 1 Solution	1	-	-	-	-	-	-	-	-
		0.5	0.1	-	-	-	-	-	-	-
		1	0.2	-	-	-	-	-	-	-
		1.5	0.3	-	-	-	-	-	-	-
		2.0	0.4	-	-	-	-	-	-	-
3	Full MS Medium	-	-	1.0	1.0	1.0	-	-	-	-
		-	-	1.5	1.5	1.5	-	-	-	-
		-	-	2.0	2.0	2.0	-	-	-	-
		-	-	2.5	2.5	2.5	-	-	-	-
		-	-	3.0	3.0	3.0	-	-	-	-
		-	-	**3.5**	**3.5**	**3.5**	**83.34±2.36**	**333.7±3.74**	**290.56±2.87**	**98.48±0.65**
		-	-	2.0	2.0	-	-	-	-	-
		-	-	**2.5**	**2.5**	-	**25.16±0.22**	**123.7±0.85**	**97.4±0.80**	**77.1±0.74**
		-	-	3.0	3.0	-	-	-	-	-
		-	-	3.5	3.5	-	-	-	-	-

Table 3: Effect of various concentrations of BAP and GA_3 on subculture of *Cheilanthes farinosa* (Forsk.) Kaulf.

S.no	Type of media	Concentration of growth regulators		Number of gametophyte plantlets subcultured	Number of gametophyte developed after subculture	Percentage of gametophyte development
		BAP	GA_3			
1	Full M.S Medium	0.2	0.2	-	-	-
		0.3	0.3	-	-	-
		0.5	0.5	-	-	-
		1.0	1.0	36.18±1.57	65.04±1.83	76.45±1.74
		2.0	2.0	36.17±0.45	37.57±0.65	10.09±0.20
		3.0	3.0	-	-	-

PLATE 1

In vitro **Spore Germination and Gametophyte Regeneration of** ***Cheilanthes farinosa*** **In the concentration of (3.5 mg/L of IBA & GA_3)**

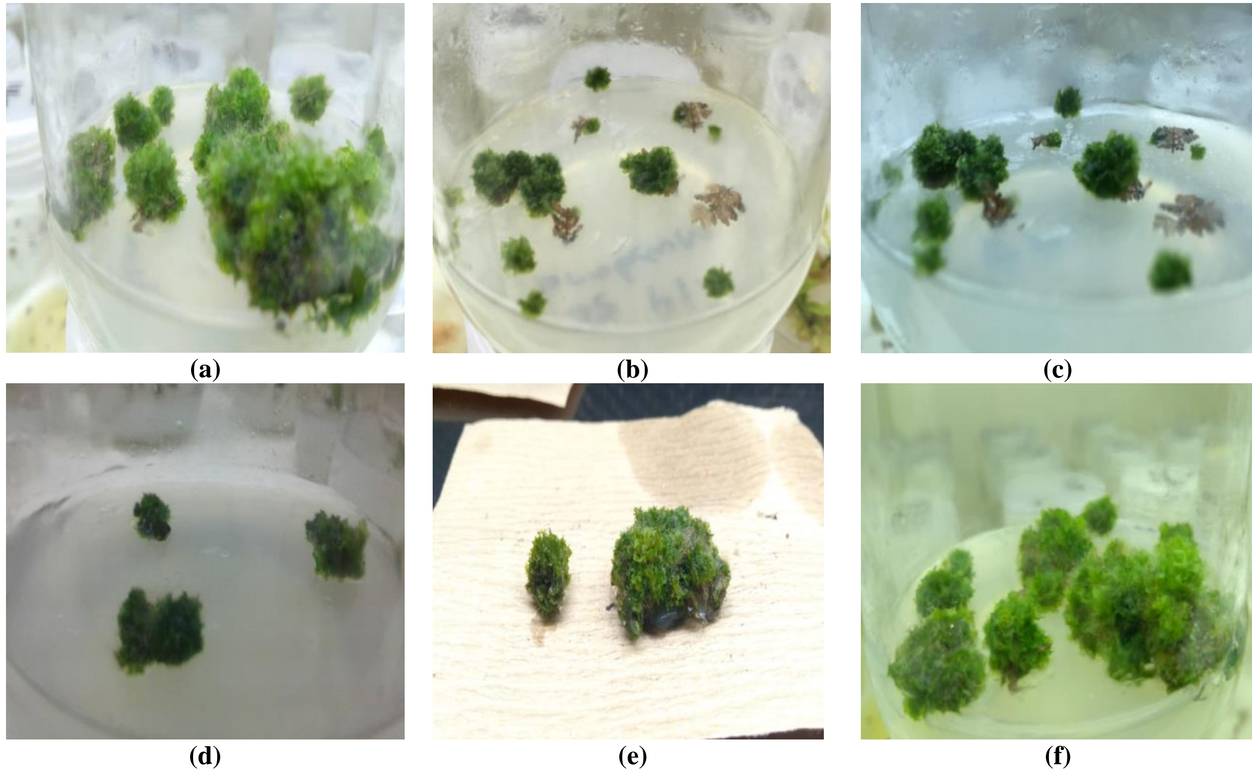

Fig. 1: (a) Spore germination and Protonema initiation, (b) Protonema development after 30 days, (c) Gametophyte initiation after 15 days, (d) Gametophyte development after 15 days, (e) During sub-culture of gametophyte after 60 days, (f) Sub-cultured gametophyte plantlets.

PLATE 2

In vitro **Spore Germination of** ***Cheilanthes farinosa*** **In the concentration of (2.5 mg/L of IBA & KIN)**

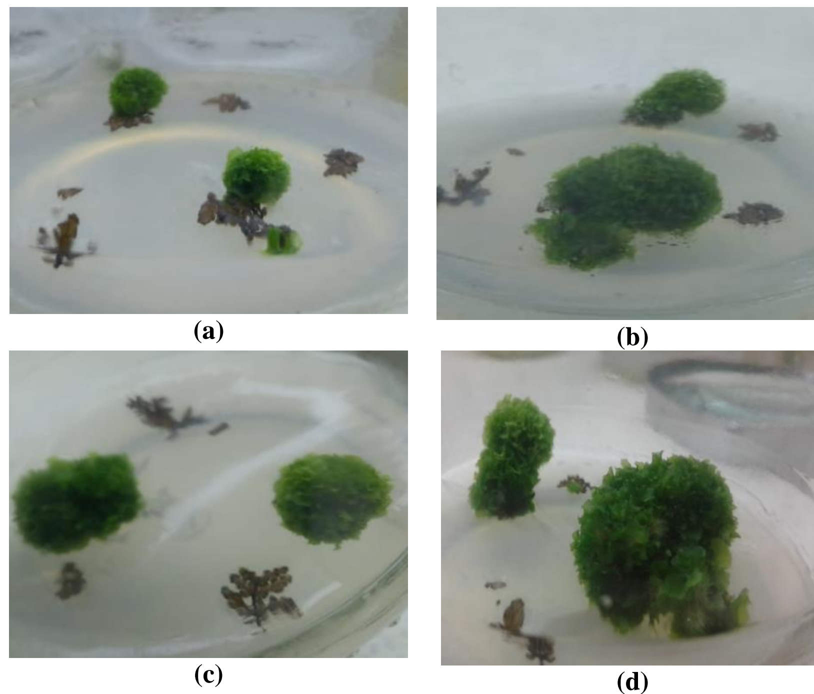

Fig. 2 (a) Spore germination and Protonema initiation, (b) Protonema development after 30 days, (c) Gametophyte initiation after 15 days, (d) Gametophytic development after 30 days.

PLATE 3

Sub-cultured Gametophyte plantlets in the combination of BAP and GA3

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Fig. 3: Sub-cultured Gametophyte plantlets in the combination of BAP and GA3

5. DISCUSSION

In present study, *in vitro* spore germination percentage of *Cheilanthes farinosa* were determined by culturing spores with fronds on full strength MS medium supplemented with 3.5 mg/L of IBA & GA_3 showed the highest growth percentage. Similarly, a combination of full strength MS medium with 2,4-D (2.26μM) and BAP (2.22μM) was found to be ideal for profuse callusing (80%) against other combinations in *Pteris vittata.* (Shukla and Khare, 2012)

In present study, number of protonema and gametophye development were observed and recorded on 60th day of germination. Similarly, gametophyte development was observed within 8 weeks of *in vitro* culture in *Cibotium schiedei, Cyathea brownii, Cyathea dealbata, Cyathea leichhardtiana, Cyathea robertsiana, Cyathea schanschin, Cythea smithii.* Katarzyna Goller, Jan Jaroslaw Rybczynski, (2007).

In current study, the Protonema development was observed after 30 days and Gametophyte initiation and development took place within a span of 30 days, by continuous sub-culture of gametophyte. On the contrary, Successful spore culture establishment in Asplenium nidus was reported recently (Khan *et al.,* 2008). The callus and dermal hair were developed within 60-70 days. After 70-100 days, the callus were elongated and bulged. In 100-120 days of culture maintenance, embryo development started, which resulted in the formation of sporophytic leaf.

6. CONCLUTION

The present study demonstrated a simple and efficient method for high frequency of *in vitro* spore germination, direct regeneration of *C. farinosa*. It is used in the treatment of inflammation, skin disorders and rheumatism. The process of *in vitro* plant regeneration supports conservation of plant species from indiscriminate exploitation from its natural resources, ultimately enabling to keep pace with commercial needs.

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