An *in vitro* study on organogenesis of *Pteris vittata* L.

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ABSTRACT

The present study emphasized on *in vitro* organogenesis and large scale multiplication protocol for a rare fern *Pteris vittata* L. The young croziers were harvested and sterilized with 0.05% (w/v) $HgCl_2$ for two minutes and rinsed thrice in sterile distilled water. The sterilized young croziers were cut into 1.0 cm length and cultured on different media augmented with various concentrations and combinations of plant growth regulators. Murashige and Skoog's (MS) medium added with 2,4-D 1.5 mg/l induced maximum percentage of callus formation. Maximum number (55 ± 1.3) and frequency (70.4 ± 0.72) of shoot formation were observed in MS medium augmented with BAP 1.0 mg/l and IAA 0.5 mg/l. Half strength MS medium augmented with IBA 1.0 mg / l induced maximum number (8.3 ± 0.63) of roots formation with high frequency (80.8 ± 0.33), followed by IAA 1.0 mg/l. The highest percentage of aposporous gametophyte formation was observed on MS medium added with BAP 1.0 mg/l. The micropropagated plants showed maximum percentage (90.6 ± 1.42) establishments during hardening and 52.0 ± 0.9 percentage establishments in the field condition.

Key words: *Pteris vittata*, *in vitro*, organogenesis, callus, crozier.

INTRODUCTION

In the Western Ghats, the South of Palghat Gap alone constitutes about one - third of the ferns flora of India. It contains more than about 700 species of ferns. Most of them occur in streams and stream banks in evergreen forests and while some occur on exposed roadsides and clearing (Manickam and Irudayaraj, 1992). The Western Ghats contains forty four threatened ferns, which should be conserved in priority basis (Manickam, 1995). *Pteris vittata* is one of the rare species in Tamil Nadu as reported from Coimbatore hills, Kothayar Hills, Palni hills (Beddome, 1864 ; Manickam, 1986). Sukumaran *et al* (2009)and Sacred grove forests of Kanyakumari -District, Tamil Nadu. Recently, Ghanthi Kumar *et al* (2012) reported in urban area of palayamkottai, Tirunelveli-District, Tamilnadu, particularly in open sewage-water canal. Generally, it has high Argenic tolerance and good accumulate abilities (Ma et al., 2001; Chen et al., 2002).

Tissue culture has been acting as a promising technique to obtain genetically pure elite populations under *in vitro* conditions.

The *in vitro* propagation has been proving as a pivotal tool for enormous production of medicinal as well as rare plant species, products thereof and germplasm conservation (Hassan and Khatun, 2010). The conservation through micropropagation, which allows making numerous clones of rare plants by exploring the totipotency of suitable tissues isolated from the mother plant (Kanungo and Sahoo, 2011). Hence, *in vitro* propagation methods offer powerful tools for mass- multiplication of threatened plant species (Murch *et al.*, 2000; Roja *et al.*, 1991). With this background, the present study has focused on the *in vitro* organogenesis and large scale multiplication protocol for the rare fern *Pteris vittata* L. using crozier as explants.

MATERIALS AND METHODS

The IUCN least concern fern Pteris vittata L. was collected from kodaikanal hills, near kodaikanal Botanical Garden, Perumalmalai, Dindugul -District, Tamilnadu. The croziers used as the source of explants. The explants were cut into 1.5 cm and washed in running tab water for 10 minutes and sterilized with 0.05% (w/v) $HgCl_2$ for 2 minutes which rinsed thrice in sterile distilled water. The sterilized young croziers were again trimmed into 1.0 cm length and cultured on different media (Knudson C medium, Knop's medium and MS's medium) augmented with different combinations and concentrations of plant growth regulators (0.5 mg/l - 2.5 mg/l of BAP, Kin, IBA and IAA and 2, 4-D). The cultures kept warm at 25+2° C under cool fluorescent light (2000 lux 14 hr photoperiod). The *in vitro* raised sporophytes were sub - cultured on to root inducing half strength of MS medium with different concentrations of IBA and IAA. For Hardening, the *in vitro* raised plantlets were removed from culture, washed thoroughly with tap water planted in small polycups filled with sterile garden soil (3:1), covered by imperforated polybags, and hardened for four weeks in a mist chamber before transfer to field.

RESULTS AND DISCUSSIONS

The young crozier segments were used as explants, the surface sterilized using 0.05% (w/v) HgCl2 for two minutes and cultured on MS medium (Murashige & Skoog,1962) added with various concentration of 2, 4- D. Maximum percentage (70.5 ± 0.61) of callus formation was observed in 1.5 mg/l of 2, 4- D (Table 1).

The *in vitro* derived calli were sub-cultured in MS medium added with BAP (0.5-2.5 mg/l) and Kin (0.5-2.5 mg/l) individually or in combination with IAA (0.5-2.5 mg/l). After 6 weeks, the saprophytic plants were formed on the surface of the callus in MS medium augmented with BAP 1.0 mg/l and IAA 0.5 mg/l. Maximum number (55 ± 1.3) and frequency (70.4 ± 0.72) of shoot formation was observed in MS medium augmented with BAP 1.0 mg/l and IAA 0.5 mg/l (Table 2).

Table 1: Influence of PGR on *in vitro* callus induction from young crozier explants of *Pteris vittata*

MS - medium + PGR		% of callus formation ± S. D.
2, 4- D	0.5	-
	1.0	67.6 ± 0.23
	1.5	70.5 ± 0.61
	2.0	52.4 ± 0.33
	2.5	-
BAP	0.5	-
	1.0	-
	1.5	42.3± 0.13
	2.0	56.4 ± 1.41
	2.5	53.1 ± 0.23
IAA	0.5	-
	1.0	-
	1.5	-
	2.0	58.1 ± 0.11
	2.5	63.2 ± 0.53

Table 2: Influence of PGR on *in vitro* shoots induction from *in vitro* derived callus of *P.vittata*

MS - medium + PGR		% of Gametophytes	% of Saprophytes	Mean no. of Shoots
BAP	0.5	-	-	-
	1.0	50.4 ± 0.21	70.5 ± 0.61	12.5 ± 0.51
	1.5	62.1 ± 0.02	52.4 ± 0.33	11.4 ± 0.23
	2.0	58.0 ± 0.41	57.6 ± 0.23	10.2 ± 0.13
	2.5	-	-	-
Kin	0.5	-	-	-
	1.0	-	-	-
	1.5	42.3± 0.13	56.4 ± 1.41	16.4 ± 0.01
	2.0	56.4 ± 1.41	53.1 ± 0.23	23.1 ± 0.03
	2.5	-	-	-
BAP+IAA	0.5+0.2	62.5± 0.23	66.1 ± 0.21	36.4 ± 1.41
	1.0+0.5	88.2 ± 1.21	70.4 ± 0.72	55.0 ± 1.30
	1.5+0.8	57.4 ± 0.55	58.5 ± 0.01	18.1 ± 0.21
	2.0+1.0	-	-	-
	2.5+1.2	-	-	-

(Data represented 6 weeks of *in vitro* plant)

For root formation, the *in vitro* derived Saprophytic plant ie shoots were cultured in half strength of MS medium added with different concentrations of IBA and IAA. Maximum number (8.3 ± 0.63) of roots induction observed on half strength of MS medium added with 1.0 mg /l of IBA with high frequency (80.8 ± 0.33) of root formation (Table 3) followed by IAA 1.0 mg/l(Plate 1). Many researcher have successfully developed protocol for fern segments such as rhizome, crozier, front etc., through tissue culture (Sara,2001; Vallinayagam, 2003). Similar work was done by Padhya (1987). According to Cheema and Kaur (1986) reported *in vitro* regeneration of some aquatic ferns. According to Kshirsagar and Mehra (1978) finding of callus induction from rhizome segments, Cheema (1983) obtained callus from fertile leaf. Many research works have been conducted wherein morphological traits are employed for studying and monitoring the variations arising in the tissue cultured plants (Iriondo and Perez, 1990; Lakhanpaul et al, 1991; Dhawan, 1993; Oh et al, 1995 and Nair, 2000). All the plants raised through tissue culture were morphologically uniform and showed uniform growth.

Table 3. Influence of PGR on root formation on the *in vitro* raised shootlets of of *P.vittata*

½ MS - medium + PGR		% of Root formation ± S.D.	Mean no. of roots
IBA	0.5	-	-
	1.0	**80.8 ± 0.33**	**8.3 ± 0.63**
	1.5	65.7 ± 0.72	3.2 ± 0.63
	2.0	-	-
	2.5	-	-
IAA	0.5	-	-
	1.0	72.3 ± 1.21	6.3 ± 0.13
	1.5	53.3± 0.13	3.4 ± 0.21
	2.0	-	-
	2.5	-	-

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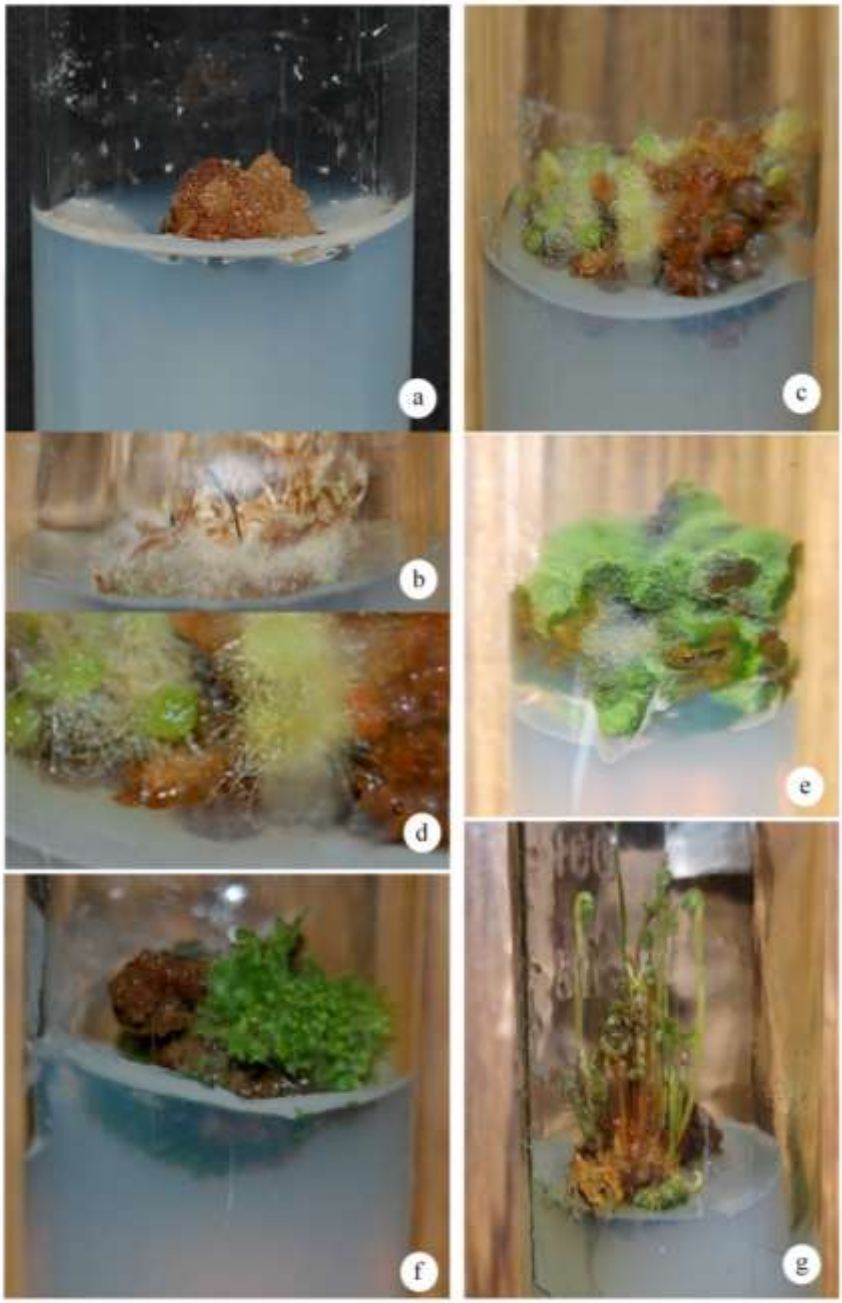
Plate : 1

In vitro organogenesis formation in *Pteris vittata* L.



a) Calli formation from the crozier ;
b) Aposporous gametophyte formation from the crozier derived calli;
c) Glands with aposporous gametophyte;
d) Close up view of aposporous gametophyte origin from the crozier derived calli;
e) Gametophyte formation occurred on the crozier derived calli;
f) Gametophyte formation on the crozier derived calli;
g) Sporophyte and rootlets formation from the crozier derived calli