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Original Article

Clonal propagation of *Adiantum capillus* – veneris

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Abstract

Adiantum cap illus - veneris is an important medicinal fern in South India. In the present study, establishment of protocol for its mass propagation of *A. cap illus* - veneris was initiated using spores as an explant. Explants were cultured on MS basal medium and were incubated in the dark at 22±2°C. After 12 weeks germination of spores, calli and prothalli were obtained. After germination, cultures were transferred on to fresh medium every 4-week. Prothalli were sub-cultured on MS medium supplemented with BAP (0.01-2 mg^{-1}/L), 2,4-D (0.1-1.5 mg^{-1}/L) and combination with BAP and 2,4-D where they multiplied successfully. Callus induction and protonema formation was achieved in higher percentage in case of MS medium supplemented with 1.5+2.0 μM^{-1}/L 2,4-D+BAP and 0.75+1.50 μM^{-1}/L 2,4-D+IBA and 4% glucose. They were sub-cultured into phytohormone-free MS medium with 4% glucose, finely cultured ferns were transferred to poly cups and maintained for 15 days in green house condition.

Key words: Maiden hair fern, *Adiantum capillus* - veneris, spores, calli

Abbreviations: MS- Murashige and Skoog Medium; BAP – Benzylaminopurine; 2,4-D; 2,4-Dichlorophenoxy acetic acid, PGRs – Plant Growth Regulators

Introduction

Mass propagation of plants through *in vitro* culture is one of the best and most successful option of commercial application of plant tissue culture technology. The first successes in the field of the intensive multiplication of plants through *in vitro* techniques are cited around 1970, the fern *Nephrolepis exal tata bost oniensis* being the first plant micropropagated *in vitro* with a commercial purpose (Cachita-Cosma and Dorina,1987). Recently, there has been much progress in this technology for some medicinal plants (Bertrand *et al* ., 1999, Fernandez *et al*.,1999). Tissue culture propagation and its importance in conservation of genetic resources and clonal improvement have been described by many workers (Barz *et al*., 1977; Datta and Datta,1985;Kukreja *et al* ., 1989; Jusekutty *et al*., 1993).

Ferns, a lower group of plants having a rich source of medicines which are used as decoction or infusion to ease labor pains. These plant extracts used as depurative, to reduce fever and as a hair wash (Burkhill,1935).

The genus *Adiantum* (L.) belongs to the family Adiantaceae, which consists of 150 to 200 species worldwide distributed in North America, United States, South Dakota, British Columbia, Canada and India (Fernald, and Lyndon,1950; Hickman,1993; Paris and Cathy,1993; Lellinger and David,1985; Gleason and Cronquist,1991;Victor *et al*.,2003). Whole plants of *A. cap illus*-veneris are used as tonic for cough, throat infection, visual tumours and menstrual problems (Guhabaskhi *et a l*.,1999). Active constituents include 21-OH-adiantone, isoquercitrin, kaemferol, letuol, terpenoids, 3α-4α-poxyfilicane, flavones tannic acid, gallic acid and essential oils were reported to be responsible for the potent medicinal values of this fern (Irudayaraj and Patric Raja,1998; May,1978). Victor *et al* ., (2003) reported the antimicrobial activity of leaves and pinnae oils. In the present study a rapid protocol for *in vitro* mass production of *A. ca pillus* - veneris through high-frequency calli from spore explants followed by successful establishment of regenerated fern was achieved.

Materials and Methods

Fern material

Fresh plant (fern) material was collected from Aluthakanni River, Tenkasi, Tirunelveli District, Tamil Nadu, South India.

Spore collection

Spores were collected from *A. capillus*- veneris fronds and dried on filter paper in an oven at 30ºC for three days. The spores were separated from sporangia by filtering through tissue paper, and were stored in glass jars under refrigeration at 7 ± 1 ºC.

Spores sterilization

Spores of *A. ca pillus*- veneris were sterilized in 2% sodium hypochlorite for 10 minutes, filtered through sterile filter paper by vacuum, washed several times with sterile distilled water and dried in a laminar flow hood for 30 min (De Brum and Randi, 2002).

In-vitro culture medium

The culture medium used in the present study was Murashige and Skoog (1962) basal medium with 3% (w/v) sucrose and 0.8% (w/v) agar. The medium was further augmented with different concentrations of BAP (0.01–2.0µM), 2, 4-D (0.01 – 2.0µM) separately and in combination in the concentration range of 0.01–2.0µM (Table 1). Spores of *A. capillus* - veneris were aseptically inoculated in MS basal medium on 100ml sterile bottle. Spore germination in different stages like protonema stage, gametophyte, young sporophytes formation was observed at a temperature of 20±5^{o}C and at a light intensity between 3-15 µMolm^{-2}s^{-1}. Morphogenetic response was examined at the given cultured condition. Germinated aseptic cultures were transferred into fresh media in six weeks interval. Further, the *in vitro* developed shoots were transferred into rooting medium supplemented with 2,4-D 0.01-1.0µM/L in combination with 0.01-2.0µM/L IBA. After root development, regenerated plantlets were transferred to pots for hardening.

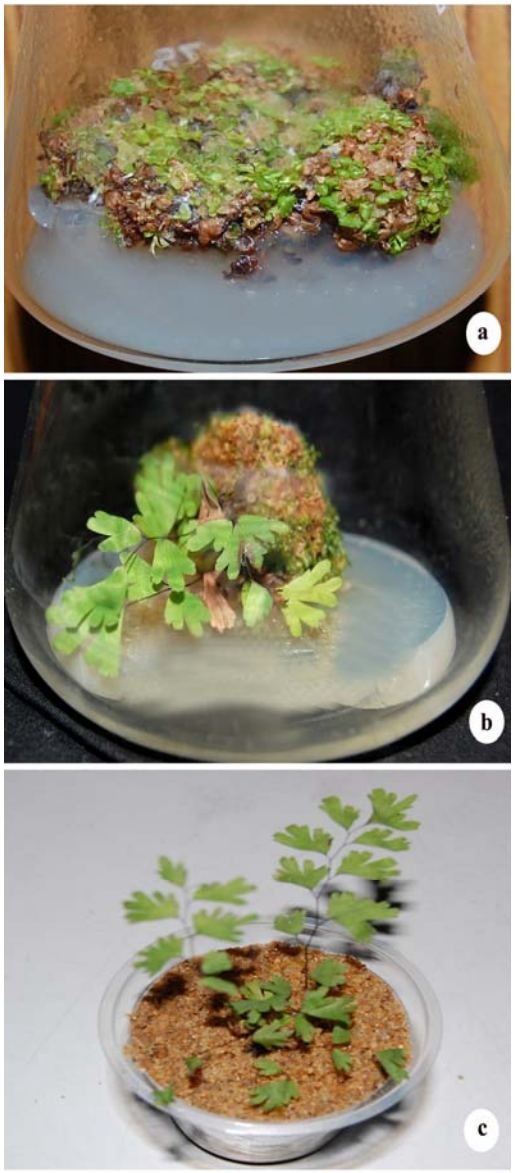
Results and Discussion

The protocol for *in vitro* culture of *A. capillus* - veneris, is summarized in Fig.1. The germination of spore started within 3-5 days. Spores have been used as the explant source for successful high frequency regeneration of plants.

Although the regeneration of plants from spores is quite difficult *in vitro* , optimization of every step from initiation to acclimatization makes it more feasible to produce plants from spores (Banks,1999). Due to the phenomenon of vegetative reproduction of the gametophyte, frequently noticed *in vitro*, a high frequency of regeneration has been obtained, a fact that is also reported by Fernandez *et al*.,(1999). The excellent capacity of *in vitro* multiplication of the gametophyte can however influence the number of the sporophytes formed, as was noticed in *Asplenium*, *Dryopteris*, *Osmunda*, etc., as the absence of the sporophyte presupposes a nutritional competition between the two generations. This presupposition is supported by the fact that, when the multiplication of the gametophyte is blocked in *Osmunda*, the production of sporophytes increases (Fernandez *et al*., 1999).

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 (Fig.1). Table 1 shows the responses obtained when the spores of *A. ca pillus* - veneris were cultured on MS medium supplemented with different concentrations of 2,4-D/ BAP in combination. Medium containing 1.5 and 2.0 µM/L BAP showed the best frequency of calli production rates and also BAP (2.0µM/L) in combination with 2,4-D (1.5µM/L) and 0.75+1.50µM/L 2,4-D+IBA. This observation authenticate that plants need both auxins and cytokinins which should be supplied *in vitro* and the ratio between auxin and cytokinin seems to be very important for multiplication (Bertrand *et al* ., 1999, Fernandez *et al*., 1999).

There are many reports in which application of growth regulators were highlighted towards either enhanced or suppressed plant growth which directly influence the rate of multiplication (Fernandez and Revilla, 2003). The *in vitro* regeneration of Pteridophyta is currently used for the mass multiplication of the ornamental and medicinal species, and also for the endangered ones, with a view to preserving them *ex situ*.



While, in the spore culture, only one prothallus is, as a rule, obtained from a single spore, in the case of the green sporangia culture, from a single explant a colony is obtained, formed numerous prothalli, on account of the fact that, in various manners, the secondary gametophytes are formed (Soare,2008). The experiments of growth with isolated prothalli and pairs of prothalli have shown that, on average, 56% of the isolated prothalli are successful in forming the sporophyte and the percentage is much larger in the case of the pairs of prothalli, which indicates the fact that a crossed fecundation, between the gametes on different prothalli, is produced in *Asplenium trichomanes* (Suter *et al*., 2000). In the present study it was possible to obtain a higher multiplication of prothalli and further propagation, hardening, maintenance, hardening and field transfer is underway. This approach could be a vital one for the *ex situ* conservation of this medicinally important fern species and similar this may be extended for such similar species too.

Fig 1 (a). Prothalli growth on MS medium
(b). Subcultureed on calli forming prothallus with young sporophytes
(c). Hardening of *A. capillus* -veneris

Table -1. Frequency of callus induction, protonema regeneration and root induction of *A. capillus* -veneris in different concentrations of PGRs

PGRs	Concentration (µM)	No. of Explants	No. of calli (%)	No. of protonema	Frequency of callus induction
	0.01	93	0	93	0.0
	0.02	94	0	94	0.0
	0.05	91	0	91	0.0
	1.00	92	0	92	0.0
2,4-D	1.50	93	0	93	0.0
	2.00	91	0	91	0.0

PGRs	Concentration (µM)	No. of Explants	No. of calli (%)	No. of protonema	Frequency of shoot induction
BAP	0.01	92	75	21	81.52
	0.02	95	79	16	83.16
	0.05	94	76	15	80.85
	1.00	95	81	11	85.26
	1.50	91	84	8	92.31
	2.00	92	87	6	94.67
2,4-D+ BAP	0.01 + 0.01	91	79	16	86.81
	0.02 + 0.02	95	81	13	85.26
	0.05 + 0.05	93	82	9	88.17
	1.00 + 1.00	92	85	8	92.39
	1.50 + 1.50	94	89	5	94.68
	1.50 + 2.00	95	90	4	94.74

PGRs	Concentration (µM)	No. of Explants	No. of new Shoots	No. of Roots	Frequency of root induction %
2,4-D + IBA	0.01 + 0.01	91	10	3	30
	0.02 + 0.02	95	10	3	30
	0.03 + 0.05	93	10	5	50
	0.05 + 1.00	92	10	7	70
	0.75 + 1.50	94	10	8	80
	1.00 + 2.00	95	10	7	70

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