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Research Article

IN VITRO CLONAL PROPAGATION FROM LEAF EXPLANTS OF *PERGULARIA DEMIA* (FORSSK.) CHIOV.

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ABSTRACT

The development of rapid *in vitro* propagation by multiple shoots induction of *Pergularia daemia* an important medicinal plant belonging to Asclepiadaceae family. Multiple shoot were induced *in vitro* from the leaf segments on Murashige and Skoog medium (MS) containing 6-benzylamino purine (BAP) alone or in combination with naphthaleacetice acid (NAA). Maximum number of shoots (7.3 shoots per explants) was observes on the medium containing BAP 0.5 mg/l in combination with NAA (1.0 mg/l). Regenerated shoots were rooted on half strength MS supplemented with 0.5 mg/l IBA. The rooted *in vitro* raised plantlets were acclimatized in growth chamber for 4 weeks and successfully transferred to mist chamber with high survival rate. The regeneration protocol developed in this study to provide a basis for germplasm conservation and for further investigation of medicinally active constituents of the elite medicinal plants.

Keywords: Acclimatization, Callus, Leaf segments, *In vitro* propagation.

INTRODUCTION

Pergularia daemia (Forssk.) Chiov. is a slender foul smelling perennial milky twining herb with hispid stems belongs to the family Asclepiadaceae. It is distributed throughout the hotter parts of India up to 900m. The plant is acrid, amenorrhoea, anthelminitc, antipyretic, asthma, astringent, cough, dysmenorrhoea, emetic, emmenagogue, expectorant, inflammations, intermittent fevers, laxative, leucoderma, metropathy, strangury, thermogenic, urethrorrhoea, vitiated conditions of vata and kapha[1].

The leaves are bitter, thermogenic, anthelminitc and depurative. The fruit are aerid, thermogenic and digestive and are useful in vitiated conditions of kapha and dyspepsia. The plant extract is useful in anti-inflammatory activity[2], antidiabetice activity[3], uterine and menstrual disorders and in facilitating parturition. The plant has highly medicinal values to rectify various types of disorders such as kapha, helminthiasis, haemorrhoids and leprosy[1]. Phytochemically the plant has been investigated for cardenolides, alkaloids, triterpenes, and saponins[4] and also contains various triterpenes and steroidal compounds[5]. There is no standard protocol for *in vitro* propagation of the *Pergularia daemia*. The main aim of the present investigation is to standardized protocol for induction of adventitious buds and root from leaf explants on *in vitro* condition for conservation of germplasm.

MATERIALS AND METHOD

Plant material

Pergularia daemia plants were collected from Southern parts of Pudukottai district, Tamil Nadu, India and planted in college botanical garden. The plant specimen was confirmed by Botanical Survey of India, Coimbatore, TamilNadu, India.

Explants and surface sterilization

Small tender twigs were collected from 5-6 months old grown plants, cut into 0.5-1.0 cm leaf segments from tender twigs used as explants for the induction of multiple shoots.

Explants were washed thoroughly under running tap water for 15 min then washed with 2% (v/v) Tween 20 (mild detergent) solution for 10 minutes, and then surface sterilized by submersion in 70% ethanol for 60 seconds followed by rinsing in $HgCl_2$ (0.1%) for 2 minutes and washed thrice with sterile distilled water.

Culture medium and conditions for plant regeneration

Under laminar flow cabinet explants were inoculated aseptically on Murashige and Skoog medium[6] supplemented with various concentration of 6-benzylamino purine (BAP) alone or in combinations with naphthalene acetic acid (NAA) and kinetin (Table.1). All media were adjusted to pH 5.8 and 0.8% agar and 30 gl^{-1} sucrose was added. About 15 ml of the medium were dispersed in each culture bottle and sealed with plastic cover before autoclaving at 121°C for 15 minutes under pressure of 15 Psi. The media were left cool as slant in the culture room until use. All cultures were maintained for 16 hr light at 1200 lux using fluorescent lamps and 25 ± 2°C. Results were observed at regular intervals and data were collected from three independent experiments and calculated the average and standard error (SE).

Effects of basal medium strength on multiple shoot induction

In evaluation on the abilities of different basal media to support the shoot culture establishment of full and half strength of MS medium supplemented with 0.5 mg/l BAP in combination with 1.0 mg/l NAA.

Effect and concentration of auxin for *in vitro* rooting

For root induction, *in vitro* developed shoots were excised and cultured into half strength MS medium supplemented with different concentrations (0.5-1.5 mg/l) of IBA, IAA and NAA separately.

Acclimatization and mist chamber transfer

The rooted plantlets (9-10 cm length) were taken out from the culture tubes and washed to remove adhered agar and traces of medium. Plantlets were then transferred to 8.0 cm diameter plastic pot containing sterilized soil and sand mixture in the ratio of 3:1. These plants were maintained inside the growth chamber for two weeks and irrigated gently once a day. After wards the plants were grown under mist chamber conditions.

RESULTS AND DISCUSSION

In order to establish an efficient *in vitro* micropropagation system for *Pergularia daemia* from leaf explants were incubated on MS solid medium supplemented with varying levels of either BAP/Kin alone and in combination with NAA respectively. Leaf segments cultured on MS basal medium without growth regulators did not show any response. However on MS basal medium supplemented with various concentration of cytokinin alone or in combination with auxin swelled in their size after 1-2 weeks of culture and differentiated auxiliary shoots in another 2 weeks (Table 1 & Fig.1 a, b).

The combination of BAP (0.5 mg/l) and NAA (1.0mg/l) were positively affected the multiplication rate of the *Pergularia daemia* compared with BAP alone successful results of shoot multiplication from leaf segments explants cultured on MS medium were obtained. The multiplication at the rate of 95% explants produced shoot with an average of 7.3 explants (Table 1) of *Pergularia daemia* compared with BAP and Kinetin alone and kinetin combination with NAA (Fig.1 c).

The regenerated shoots were excised and placed on the half strength MS medium supplemented with different concentrations 0.5-1.5 mg/l of NAA, IAA and IBA alone for rooting (Table 2). IBA is clearly more effective in promoting root induction than NAA and IAA. The optimum rooting efficiency for shoots (95%) as well as the best root number per shoot were obtained on MS media supplemented with 0.5 mg/l and 1.0 mg/l IAA respectively (Fig.1 d). Plantlets on MS medium fortified with high concentration of auxin 1.5 mg/l grew slowly, turned yellowish thicker and shorter with callus forming capacity at the basal cut end.

The hardening process of *Pergularia daemia* was carried out by transferring 9-10 cm length rooted plantlets to 8.0 cm diameter plastic pot containing mixture of sterilized soil and sand 3:1 ratio. Hardening of potted plants for 2 weeks in a growth chamber (Fig.1 e). The survival percentage of the plantlets was 90% after transplantation to soil and sand mixture. Plants transferred to the net house have established well in the soil and appeared to the morphologically uniform and were successfully adapted to mist chamber conditions.

Development of efficient and reproducible regeneration protocol from cells or tissues holds tremendous potential for the production of high quality plant based medicines [7]. The plant regeneration from leaf segments is considered to be one of the most promising ways for multiplying a selected variety have of its type showing the same agronomic characteristics. It is evident from the results that *Pergularia daemia* can be easily clonally mass prospected *in vitro* using leaf segments as explants. Although, *in vitro* micropropagation of *Pergularia daemia* has not been reported before but micro propagation of various plant species including many medicinal plants has been reported. Plant regeneration from shoot and leaf meristems has yielded encouring results in medicinal plants like *Solanum nigrum*, *Cunila galioides* and *Rauwolfia micrantha* [8, 9, 10].

Our results showed that BAP in combination with NAA were more effective for shoot multiplication than Kinetin alone or in combination with NAA. The effects of auxins and cytokinins on shoot multiplication and *in vitro* rooting of various medicinal plants have been reported[11].

BAP alone with NAA induces a high rate of shoot proliferation of *Hemidesmus indicus, Gymnema sylvestre* and also shown that the number of shoots per explants depends on concentration of the growth regulators[12,13]. In this investigation proved that the MS medium supplemented with BAP 0.5mg/l and NAA 1.0 mg/l for maximum shoot induction, and IBA 0.5 mg/l for root induction in this species.

Table 1: Effects of different concentration of plant regulators on *in vitro* shoot proliferation from leaf segments of *Pergularia daemia* after 4 weeks of culture.

Plant growth regulators (mg/l)			No. of shoots explants (mean ±SE)	Regeneration culture (%)
BAP	**NAA**	**KIN**		
0.5			3.3±0.83	64.0
1.0			2.0±0.33	52.0
2.0			3.0±0.58	39.0
4.0			3.0±1.00	42.0
0.5	0.5		1.7±0.67	56.0
1.0	0.5		1.3±0.33	61.0
2.0	0.5		3.1±0.76	67.0
4.0	0.5		3.2±0.31	53.0
0.5	1.0		7.3±0.88	95.0
1.0	1.0		5.6±0.22	79.0
2.0	1.0		4.8±0.11	82.0
4.0	1.0		3.9±0.10	65.0
		0.5	3.0±0.58	69.0
		1.0	2.7±0.88	55.0
		2.0	2.3±0.33	52.0
		4.0	5.0±0.28	65.0
	0.5	0.5	4.3±0.22	52.0
	0.5	1.0	3.4±0.11	59.0
	0.5	2.0	2.8±0.14	48.0
	0.5	4.0	2.3±0.33	49.0
	1.0	0.5	3.3±0.22	57.0
	1.0	1.0	3.2±0.88	42.0
	1.0	2.0	4.0±0.33	61.0
	1.0	4.0	2.9±0.31	63.0

Table 2: Effects of auxins for rooting of *in vitro* derived shoots of *Pergularia daemia* after 4 weeks of culture.

Auxin (mg/l)	Number of shoots cultured	Number of roots per shoot (mean±SE)	Rooting (%)
NAA			
0.5	30	4.8±0.10	49.0
1.0	30	6.1±0.12	45.0
1.5	30	2.3±0.33	58.0
2.0	30	3.0±0.58	63.0
IBA			
0.5	30	9.38±0.82	92.0
1.0	30	3.9±0.63	71.0
1.5	30	5.5±0.97	69.0
2.0	30	2.8±0.11	46.0
IAA			

0.5	30	5.23±0.11	43.0
1.0	30	6.8±0.27	66.0
1.5	30	5.6±1.30	55.0
2.0	30	3.5±1.63	69.0

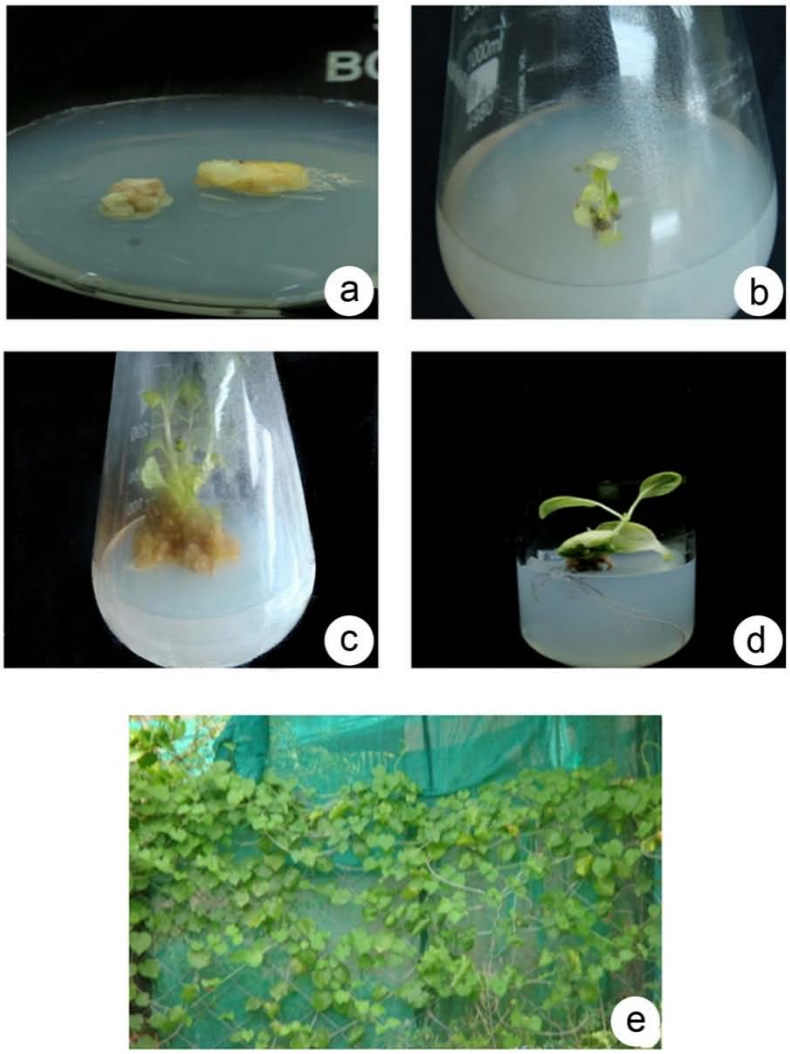

Fig.1 *In vitro* clonal propagation of *Pergularia daemia* from leaf explants
a. Callus proliferation from the leaf explants of *Pergularia daemia.*
b. Shoot initiation from leaf explants of *Pergularia daemia* (Direct regeneration).
c. Multiple shoot formation from callus of leaf explants on MS medium with 0.5mg/l BAP and NAA 1.0 mg/l (In direct regeneration).
d. Rooting stage of *Pergularia daemia* on half strength MS medium supplemented with 0.5mg/l IBA.
e. Regenerated plants growing in net house.

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