

Original Research

In Vitro rapid multiplication of *Pergularia daemia* - A medicinal plant

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ABSTRACT:

A protocol for *in vitro* multiple shoot development and plantlet formation using leaf explant was prescribed for *Pergularia daemia* (Forssk.) Chiov. medicinally important plant species. Young apical leaf explant was used for callus induction on MS medium containing TDZ and NAA at 1.5 and 0.2 mg^l⁻¹ respectively showed maximum callus induction (77%). The amount of callus responded for shoot formation (75%) was obtained in the MS medium containing IBA (1.5 mg^l⁻¹) and TDZ (0.3mg^l⁻¹). The elongated shoots were rooted on half strength medium supplemented with IBA (1.5 mg^l⁻¹) and Kn (0.3 mg^l⁻¹) for shoots rooted. Regenerated plantlet were successfully acclimatized and hardened off inside the culture and then transferred to green house with better survival rate.

Keywords:

Pergularia daemia, MS medium, TDZ, Multiple shooting, Acclimatization.

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INTRODUCTION

In the last few decades medicinal plants form the backbone of traditional medicine with intense pharmacological properties. The use of herbs as medicinal plants due to the presence of various chemical substances is ever increasing. With the increase concern about its medicinal value, large-scale exploitation from natural resources had lead to the marked depletion from natural stock (Aravind *et al.*, 2013). To overcome this problem, medicinal plants can be raised through a technique called plant tissue culture. It is a technique of growing plant cells, tissues and organs in an artificially prepared nutrient medium under aseptic condition (Kumar, 2003).

Pergularia daemia (Forssk.) Chiov. belonging to the family Asclepiadaceae, having lot of medicinal properties. The whole plant is used as an anti-helminthic, laxative, antipyretic and expectorant, also used to treat infertile diarrhea and malarial intermittent fevers (Kirtikar and Basu, 1999; Nadkarani, 1976; Anonymous, 1995). Extract of this plant is taken orally for gastric ulcers, uterine and menstrual complaints. The leaves are useful in leprosy and haemorrhoids. The fresh pulped leaves are applied as a poultice to relieve carbuncles. Leaf juice is used as an amenorrhea, catarrhal infections and dysmenorrheal, infantile diarrhea and also used to reduce body pain. In addition dried leaves rused as an antirheumatic, asthma, amenorrhea, dysmenorrheal, bronchitis, whooping cough, heals cuts and wounds and finally to facilitate parturition. Latex of this plant is used for toothache (Hebbar *et al.*, 2004). The stem bark of this plant is a good remedy for cold (Dokosi, 1998) and fever (Bruce, 2000) and also used as antipyretic and appetizer latex of the plant is used for boils and sores. Dried roots are used as an abortifacient, emetic, bronchitis and used for cough, asthma and constipation, while the fresh roots used as an abortifacient and used to treat gonorrhoea. The root is used to treat mental disorders, anemia, leprosy and piles.

To date, there has been no report on *in vitro* regeneration of *P. daemia*. Herein, we described the optimization of culture conditions and plant growth regulators required for callus initiation, shoot regeneration and rooting of plantlets from immature leaflets of *P. daemia*.

MATERIALS AND METHODS

Explant sterilization

Leaf segments from young and healthy branches of *P. daemia* were used as explants. They were collected from pot cultured individuals maintained in a mist chamber. The collected immature leaves were washed in running tap water twice and then treated with few drops of tween-20 solution to remove the superficial dust particles. They were then surface sterilized with 5% antibiotics (ampicillin and rifampicin) to eliminate bacterial contamination followed by three rinses in sterile double distilled water. To eliminate fungal contamination the explants were treated with bavistin (fungicide) and rinsed with double distilled water 2-3 times. Furthermore, they were treated with freshly prepared 0.1% (w/v) HgCl₂ solution (Merk, India) for 10 minutes followed by 4-5 rinses with sterilized double distilled water.

Media and culture condition

Leaf explants were inoculated on MS medium (Murashige and Skoog, 1962) containing 3 % sucrose solidified with 1 % agar (Tissue culture grade, Himedia, India) and the pH of the medium was adjusted to 5.6 to 5.8 with 0.1N sodium hydroxide or 0.1N hydrochloric acid prior to the addition of agar. Approximately, 30 ml of medium was dispensed in culture bottles and autoclaved at 15 lbs pressure for 15 min. The cultures were maintained under white fluorescent lamp having 2000 lux light intensity. The incubation temperature was 25±2°C and the relative humidity was 55±5% with 16h light and 8hrs dark period in every 24h cycle.

Table 1. Effect of growth regulators on callus induction from leaf explants of the species, *Pergularia daemia*

S. No	Growth regulators (mg/l)				Days required for callus formation after inoculation	Callus formation (%)
	BAP	TDZ	NAA	IAA		
1	0.0	0.5	0.2	0.0	23	59.31 ^g ± 0.82
2	0.0	1.0	0.2	0.0	23	68.22 ^h ± 1.63
3	0.0	1.5	0.2	0.0	28	77.34 ^j ± 0.82
4	0.0	2.0	0.2	0.0	19	74.44 ⁱ ± 1.63
5	0.0	2.5	0.2	0.0	26	69.00 ⁱ ± 1.63
6	0.0	3.0	0.2	0.0	28	60.18 ⁱ ± 0.82
7	0.0	0.3	0.0	0.0	18	15.56 ^a ± 1.63
8	0.0	0.6	0.0	0.0	19	23.76 ^b ± 0.82
9	0.0	0.9	0.0	0.0	21	35.97 ^c ± 1.63
10	0.0	1.2	0.0	0.0	17	51.46 ^f ± 1.63
11	0.0	1.5	0.0	0.0	23	69.00 ^h ± 0.82
12	0.5	0.0	0.	0.0	18	25.21 ^b ± 1.63
13	1.0	0.0	0.2	0.0	20	41.38 ^d ± 1.63
14	1.5	0.0	0.3	0.0	22	58.45 ^g ± 1.63
15	2.0	0.0	0.4	0.0	24	69.64 ^h ± 0.82
16	2.5	0.0	0.5	0.0	28	72.32 ⁱ ± 1.63
17	3.0	0.0	0.6	0.0	27	71.17 ⁱ ± 1.63
18	0.0	0.3	0.0	0.2	15	15.89 ^a ± 0.82
19	0.0	0.6	0.0	0.4	17	23.43 ^b ± 0.82
20	0.0	0.9	0.0	0.6	18	41.00 ^d ± 0.82
21	0.0	1.2	0.0	0.8	20	46.00 ^c ± 1.63
22	0.0	1.5	0.0	1.0	19	47.00 ^c ± 1.63

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Callus induction medium

The explants were transferred to culture bottles containing 25ml MS medium supplemented with different concentrations and combinations of BAP and NAA for callus induction.

Shoot induction medium

MS medium containing different concentrations and combinations of IBA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and TDZ at 0.3 mg/l was used for shooting attributes.

Rooting of elongated shoots and acclimatization

The proliferated shoots (5-6cm long) were excised carefully and transferred onto the MS medium supplemented with IBA, IAA and Kn for root formation. The rooting attributes (rooting %, number of roots/shoot and root length) were determined. After proper root formation, the rooted plantlets were carefully removed

from the medium and washed with 1% bavistin to remove fungal contamination. Then they were transferred to hardening medium containing various combinations of hardening mixtures in different proportions and maintained in greenhouse condition to know the survivability rate.

Statistical analysis

Triplicates was maintained for all experiments and the observed data were statistically processed and means were compared according to Duncan's Multiple Range Test (P<0.05).

RESULTS AND DISCUSSION

Calli formation was observed in leaf explants after 28 days. The best response of callus (77%) was observed in the MS medium supplemented with cytokinin TDZ (1.5 mg l⁻¹) and auxin, NAA (0.2 mg l⁻¹)

Table 2. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of leaf derived callus of the species, *Pergularia daemia*

S. No	Growth regulators (mg/l)				Culture response (%)	No. of shoots/callus	Shoot length (cm)
	IBA	TDZ	Kn	BAP			
1	0.5	0.0	0.0	0.3	0.5	0.0	0.0
2	1.0	0.0	0.0	0.3	1.0	0.0	0.0
3	1.5	0.0	0.0	0.3	1.5	0.0	0.0
4	2.0	0.0	0.0	0.3	2.0	0.0	0.0
5	2.5	0.0	0.0	0.3	2.5	0.0	0.0
6	3.0	0.0	0.0	0.3	3.0	0.0	0.0
7	0.5	0.3	0.0	0.0	0.5	0.3	0.0
8	1.0	0.3	0.0	0.0	1.0	0.3	0.0
9	1.5	0.3	0.0	0.0	1.5	0.3	0.0
10	2.0	0.3	0.0	0.0	2.0	0.3	0.0
11	2.5	0.3	0.0	0.0	2.5	0.3	0.0
12	3.0	0.3	0.0	0.0	3.0	0.3	0.0
13	0.5	0.0	0.2	0.0	0.5	0.0	0.2
14	1.0	0.0	0.2	0.0	1.0	0.0	0.2
15	1.5	0.0	0.2	0.0	1.5	0.0	0.2
16	2.0	0.0	0.2	0.0	2.0	0.0	0.2
17	2.5	0.0	0.2	0.0	2.5	0.0	0.2
18	3.0	0.0	0.2	0.0	3.0	0.0	0.2
19	0.5	0.0	0.0	0.0	0.5	0.0	0.0
20	1.0	0.0	0.0	0.0	1.0	0.0	0.0
21	1.5	0.0	0.0	0.0	1.5	0.0	0.0
22	2.0	0.0	0.0	0.0	2.0	0.0	0.0
23	2.5	0.0	0.0	0.0	2.5	0.0	0.0
24	3.0	0.0	0.0	0.0	3.0	0.0	0.0

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT

(Table 1). The result is supported by Thambiraj and Paulsamy (2012). Further studies were carried out for shoot regeneration capacity of the callus. Shoots were initiated from the callus obtained leaf explants. The best result of shooting (75%) was observed on the MS medium fortified with IBA (1.5 mg l⁻¹) and TDZ (0.3 mg l⁻¹). The maximum number of multiple shoots 10.38 shoots/callus and shoot length (6.2 cm) were produced in the same concentrations and combinations of growth regulators (Table 2). Basalma *et al.* (2008) already reported that the combination of IBA and TDZ was effective for shoot formation in the plant species like *Carthamus tinctorius*. Similar kind of observation on the requirement of cytokinin (TDZ) for better callusing were reported in *Adhatoda vasica* species (Mandal and

Laxminarayana, 2014).

Induction of rooting is an important step for *in vitro* plant propagation. Excised shoots were inoculated on MS medium with IBA and Kn for proper root development. The rooting responses were summarized in Table 3. Maximum rooting (72%), number of roots (9.76 roots/ shoot) and root length (6.0 cm) was observed on the MS medium supplemented with IBA and Kn at 1.5 and 0.3 mg l⁻¹ respectively (Table 3). These findings are in agreement with those reported by Sujatha and Reddy, 1998; Ahn *et al.*, 2007; Alam *et al.*, 2010; Ramanathan *et al.*, 2011.

After the development of roots, the plantlets were taken out from the culture bottles and washed with sterilized distilled water to remove adhering agar

Table 3. Effect of different concentrations of growth regulators on root number, rooting percentage and root length after the subculturing of leaf callus derived *in vitro* produced shoots of the species, *Pergularia daemia*

S. No	Growth regulators (mg/l)			Shoots rooted (%)	No. of roots/shoot	Root length cm)
	IBA	IAA	Kn			
1	0.5	0.0	0.0	40.36 ^b ± 0.82	4.35 ^{abc} ± 0.82	3.5 ^{a-d} ± 0.41
2	1.0	0.0	0.0	48.79 ^d ± 1.63	4.76 ^{abc} ± 0.41	2.8 ^{ab} ± 0.65
3	1.5	0.0	0.0	52.59 ^c ± 0.82	5.48 ^{bcd} ± 1.63	3.0 ^{abc} ± 0.82
4	2.0	0.0	0.0	57.43 ^g ± 0.41	4.25 ^{abc} ± 0.82	4.8 ^{c-f} ± 0.65
5	2.5	0.0	0.0	60.90 ^h ± 1.63	6.46 ^{cde} ± 0.82	5.4 ^{ef} ± 0.33
6	3.0	0.0	0.0	48.00 ^d ± 0.82	5.56 ^{bcd} ± 1.63	4.0 ^{b-e} ± 0.82
7	0.5	0.0	0.1	54.75 ^c ± 0.65	4.26 ^{abc} ± 0.41	3.0 ^{abc} ± 0.82
8	1.0	0.0	0.2	65.47 ^e ± 1.63	5.38 ^{bcd} ± 0.82	3.0 ^{abc} ± 1.63
9	1.5	0.0	0.3	72.38 ⁱ ± 0.82	9.76 ^{cde} ± 0.82	6.0 ^a ± 0.82
10	2.0	0.0	0.4	69.19 ^j ± 1.63	8.27 ^c ± 1.63	5.5 ^f ± 0.82
11	2.5	0.0	0.5	67.46 ⁱ ± 0.82	8.65 ^{de} ± 0.41	5.0 ^{def} ± 0.41
12	3.0	0.0	0.6	56.57 ^g ± 0.41	5.98 ^a ± 0.82	4.0 ^{b-e} ± 0.82
13	0.0	0.3	0.0	35.86 ^a ± 1.63	2.43 ^{ab} ± 1.63	1.5 ^{abc} ± 1.63
14	0.0	0.3	0.0	41.45 ^b ± 0.82	3.23 ^{ab} ± 0.82	4.2 ^{b-f} ± 0.16
15	0.0	0.3	0.0	48.34 ^d ± 1.63	4.49 ^{abc} ± 1.63	3.1 ^{abc} ± 0.82
16	0.0	0.3	0.0	49.64 ^d ± 0.82	5.75 ^{bcd} ± 0.41	3.4 ^{a-d} ± 0.33
17	0.0	0.3	0.0	52.28 ^e ± 1.63	6.00 ^{cde} ± 1.63	2.5 ^{ab} ± 0.41
18	0.0	0.3	0.0	54.17 ^{ef} ± 0.82	4.37 ^{abc} ± 0.82	3.2 ^{a-d} ± 0.16

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT

medium, so that the chance of contamination could be stopped. Then these juvenile plantlets were transferred to the hardening medium containing garden soil, sand and vermicompost (1:1:1 ratio by volume) where the leaf callus derived plantlets survivability rate was higher 71% (Table 4). Admixture of all these three components may offer conducive environment by providing proper nutrients, adequate aeration and required minerals respectively to the plantlets.

From the above study, it is concluded that multiple shoot and root cultures of *Pergularia daemia* were established from leaf explants on MS medium supplemented with combination of hormones. This protocol has potential for large-scale micropropagation

Table 4. Effect of different composition of hardening medium on survivability rate of leaf callus derived *in vitro* rooted plantlets of the species, *Pergularia daemia*

S. No	Hardening medium composition (V/V)	No. of plantlets under hardening	No. of plantlets survived	Survivability (%)
1	Red soil + sand (1:1)	50	22	38 ^a ± 1.63
2	Garden soil + sand + vermicompost (1:1:1)	50	39	71 ^c ± 0.82
3	Decomposed coir waste + perlite + compost (1:1:1)	50	34	67 ^d ± 0.41
4	Vermicompost + soil (1:1)	50	30	63 ^c ± 1.22
5	Red soil + sand + vermicompost (1:1:1)	50	28	56 ^b ± 0.82

Means in column followed by different letter (s) are significant to each other at 5% level according to DMRT.

and application in molecular plant breeding research programs.

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