RESEARCH PAPER

Micropropagation Studies on a Traditionally Important Medicinal Plant – *Pergularia daemia* Forssk. (Chiov.)

Sujil Victor J.[1], **Ben C. P.**[2*]
[1](Reg. No.: 23213162261008), Research Scholar, Department of Botany and Research Centre, Scott Christian College (Autonomous), Nagercoil – 629 003 (Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627 012, Tamil Nadu)
[2]Assistant Professor, Department of Botany and Research Centre, Scott Christian College (Autonomous), Nagercoil – 629 003
***Corresponding Author:** Ben C. P., Assistant Professor, Department of Botany and Research Centre, Scott Christian College (Autonomous), Nagercoil – 629 003
Email: ben.christal@gmail.com
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ABSTRACT
Tissue culture forms a vital technique in conserving medicinal plants by facilitating rapid, mass and clonal propagation year round irrespective of the unfavourable climatic conditions, utilizing a minimal plant material. Also the synthesis of medicinally important phytochemicals in plants can be improved using this technique. Deriving tissue culture protocol supports biodiversity by reducing pressure on wild populations and provides a sustainable method for producing bioactive compounds. Here, an efficient micropropagation protocol was developed for the mass propagation of *Pergularia daemia*, a traditionally important medicinal plant.
Explants for the study were obtained from species in their natural habitat. These explants viz., shoot tips, axillary buds, and leaf discs were cultured on Murashige and Skoog's medium (MS) fortified with individual as well as combinations of various plant growth regulators like benzyl amino purine (BAP), indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), kinetin, and 2,4-dichlorophenoxyacetic acid (2,4-D) in varied concentrations. Shoot tip explants exhibited 90% response rate with BAP (2.0 mg/L). Additionally, axillary bud explants produced multiple shoots in a combination of BAP (1 mg/L) and IAA (0.1 mg/L), achieving a 90% growth rate within five weeks. Inoculated leaf disc explants transformed into callus with 100% efficiency with 2,4-D (2.0 mg/L). *In vitro* shoots derived from the shoot tip and axillary bud explants were sub-cultured to medium fortified with NAA and IBA for root establishment. Where, IBA (2.0 mg/L) exhibited effective rhizogenesis.
This study concludes with deriving a successful tissue culture protocol for *Pergularia daemia* with a means of conservation and sustainable utilization.
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Introduction

Although many allopathic medicines have been discovered globally, humans still rely heavily on the medicinal plants for curing ailments. Nature remains the best source of medicinal raw materials. However, overexploitation, rapid urbanization, climate change, and habitat destruction have led to the decline of naturally occurring medicinal plants. To combat this issue, plant tissue culture protocols have been developed with the aim of conserving and sustainably utilizing the medicinal plant species. Micropropagation has proven to be a reliable method for conserving such valuable plants.

Pergularia daemia is a perennial twining herb belonging to the family Asclepiadaceae. This plant is commonly known as the 'Hairknot Plant' in english and in tamil as 'Seenghal Kodi'. It is widely distributed in tropical and subtropical regions of the world. In India, it is found at the altitudes of 1000 m in the Himalayas and 900 m in Southern parts (Dahanukar *et al*., 2000). The stem is pentagonal, pale yellow, smooth, hollow, 2–4 mm thick, and slightly bitter (Bhusari et al., 2018). This thin, hispid, foul-smelling perennial climber has opposite, membranous leaves measuring 3–9 cm in length and width. Leaves are broadly oblong to orbicular or deeply cordate, with a sharp or short-acuminate apex and hairy undersides. Petioles are 2–9 cm long. Flowers are greenish-yellow or dull white with purple tints, found in axillary, drooping clusters. Fruits (follicles) are lanceolate and covered in soft spines, measuring around 5 cm. Seeds are pubescent and broadly ovate. In Central India, flowering occurs from August to January, with fruit maturation between October and February. Stems often die back in February and regrow during the rainy season (Parrotta, 2001).

P. daemia encompasses a wide array of phytochemicals which includes alkaloids,

triterpenoids, flavonoids, saponins, steroids, tannins, and coumarins (Rajan & Averal, 2016). These compounds possess anti-helmintic, antipyretic, antimalarial, antibacterial, anti-inflammatory, antidiarrheal, and analgesic properties (Nithyatharani & Kavitha, 2018). Ethanolic extracts of aerial parts of *P. daemia* exhibits hepatoprotective activity (Kumar & Mishra, 2008). The leaf decoction of the plant is used to treat inflammation with 100% fidelity level (Priyan & Muneeswaran, 2024). The latex obtained from the plant is used to treat venereal diseases, arthritis, muscle aches, asthma, rheumatism, snake bites, and as fish poisoning (Van Damme et al., 1922), while the milky sap is used to relieve eye irritation (Irvine et al., 1952). This study investigates the regeneration potential of shoot tip, axillary bud and leaf disc explants on MS medium fortified with various plant growth regulators.

Materials and Methods

Fresh, disease-free shoots and branches of *P. daemia* were collected from Scott Christian College campus which served as the source of explants. In the laboratory, young and healthy explants viz., shoot tips, axillary buds, and leaf discs were selected. These selected explants were washed under running water to remove all surface impurities. This was followed by treatment with surfactant (Teepol) for 10–20 minutes, followed by sterilization with 70% ethanol for 3 seconds and 0.5% sodium hypochlorite for 3 - 5 minutes. Immediately after surface sterilization, the explants were rinsed seven times with sterile double-distilled water inside the laminar air flow chamber.

Explants (0.5 - 1 cm) were inoculated aseptically onto Murashige and Skoog (1962) medium containing 3% sucrose fortified with different plant growth regulators (PGRs). The pH of the medium was adjusted to 5.8, and was solidified using 0.8% agar. The culture medium was autoclaved at 121°C for 15 minutes. Cultures were maintained at 24 ± 2°C under cool white fluorescent light (4000 lux, 16 hours/day) and 60–65% relative humidity. All experiments were repeated thrice.

Shoot tips and axillary buds were cultured on MS medium with cytokinins (BAP) and auxins (IAA, NAA, Kinetin, and 2,4-D) for direct shoot proliferation as well as for callus induction. Regenerated shoot buds were transferred to rooting media supplemented with NAA and IBA. Leaf discs were cultured on medium supplemented with 2,4-D and BAP for callus, shoot, and root induction. Rooted plantlets were washed to remove agar and transferred to a potting mix (sand : cowdung : red soil) in the ratio of 1:1:2 and sprayed weekly with quarter-strength liquid MS medium. Regenerated plantlets were acclimatized in greenhouse conditions and were introduced to the field.

Results and Discussion

Response of Shoot tip Explant

Shoot tip explants cultured on MS medium supplemented with BAP, IAA, BAP + NAA and BAP + IAA (1 mg/L) showed significant shoot proliferation. The highest response (90%) was recorded with BAP at 2.0 mg/L, with a maximum shoot length of 1.9 ± 0.20 cm (Figure 4: a-d). These results are in consistent with previous studies on *Dendrobium primulinum* (Pant & Thapa, 2012) and *Ficus carica* (Ling *et al*., 2018). In contrast, IAA alone (1.0 mg/L) produced only 30% response rate, with a shoot length of 1.0 ± 0.10 cm. This indicates the role of cytokinins like BAP in inducing apical shoot growth (Table -1).

Table 1. Effects of BAP, NAA and IAA and their combinations on shoot production from shoot tip explants of *P. daemia* after five weeks of inoculation.

PGR's and concentration (mg/l)	% of Shooting Response	Mean no of days required for shoot initiation ±S.D.	Mean shoot number/Explant±S.D.	Mean length of shoots (cm) ±S.D.
Control	-	-	-	-
BAP				
2.0	90	7.0±0.60	05±0.87	1.9±0.20
1.5	80	7.0±0.70	04±0.66	1.5±0.20
1.0	50	9.0±0.70	01±0.50	1.2±0.40
IAA				
1.5	50	8.0±0.20	03±0.50	1.4±0.10
1.0	30	10.0±0.40	01±0.50	1.0±0.10
0.5	-	-	-	-
BAP+NAA				
2.0, 0.1	50	9.0±0.79	04±0.66	1.7±0.60
1.0, 0.1	60	7.0±0.32	01±0.62	1.3±0.40
0.1, 1.0	40	5.0±0.46	01±0.50	0.7±0.10
BAP+IAA				
2.0, 0.1	50	11.0±0.87	03±0.46	3.0±0.30
1.0, 0.1	50	8.0±0.54	01±0.54	2.0±0.10
0.1, 1.0	-	-	-	-

Note : Each experiment was performed with 3 replicates and was repeated twice.

Response of Axillary Bud explant

Axillary buds taken from the first or second nodes showed strong multiple shoot induction on MS medium supplemented with BAP (1 mg/L) and IAA (0.1 mg/L). After five weeks, a 90% response rate was recorded, with shoot length of 7.2 ± 0.18 cm (Figure 4: e, f). Similar findings were reported in *Caralluma edulis* (Kaur et al., 1992), *Caralluma lasiantha* (Aruna et al., 2012), *Ceropegia bulbosa* (Jogdand et al., 2016), and *Holostemma annulare* (Sudha et al., 1998). However, IAA when supplied individually (1.0 mg/L) exhibited a minimal growth rate (2.0 ± 0.1 cm) with 20% response rate (Table -2).

Table 2. Effects of BAP, NAA and IAA and their combinations on shoot production from axillary bud explants of *P. daemia* after five weeks of inoculation.

PGR's and Concentration (mg/l)	% of Shooting Response	Mean no of days required for shoot initiation ±S.D.	Mean shoot number/Explant ±S.D.	Mean length of shoots(cm) ±S.D.
Control	-	-	-	-
BAP				
2.0	50	6.2±0.50	3.0±0.03	4.0±0.80
1.5	60	7.5±0.22	4.1±1.05	4.0±0.68
1.0	30	3.3±0.62	3.1±0.04	4.0±0.50
IAA				
1.5	20	11.0±1.42	3.0±0.02	2.0±0.1
1.0	-	-	-	-
0.5	-	-	-	-
BAP+NAA				
2.0, 0.1	70	7.0±0.45	3.0±0.05	4.5±0.58
1.0, 0.1	90	7.2±0.22	6.0±0.07	7.2±0.18
0.1, 1.0	50	3.0±0.82	3.0±0.14	3.6±0.11
BAP+IAA				
2.0, 0.1	40	5.7±0.80	3.1±1.05	3.0±0.18
1.0, 0.1	45	4.1±0.12	2.4±0.10	2.5±0.35
0.1, 1.0	20	6.0±0.61	2.0±0.15	0.7±0.19

Note : Each experiment was performed with 3 replicates and was repeated twice.

Response of Leaf Disc Explant

Leaf discs cultured on MS medium with 2.0 mg/L 2,4-D proliferated in to callus within 7-9 days with 100% response rate (). The proliferated callus further induced shoot formation (4.66 ± 0.31) and root production which confirmed 2,4-D's effectiveness in caulogenesis and rhizogenesis (Figure 4: g-i; Table - 3). This result aligns with studies reported in *Allium sativum* (Haider *et al.,* 2015), *Datura stramonium* (Amiri *et al.,* 2011), and *Origanum vulgare* (Kumari & Saradhi, 1992).

When stem explants were cultured on MS medium supplied with 1.5 mg/L BAP, the entire explant was proliferated in to callus with 75% response rate in 10-14 days. The multiplied callus tissues have undergone caulogenesis (7.83 ± 0.18) and rhizogenesis (70%). Callus induction was less efficient with stem explant when compared with that of leaf disc (Table - 3). Corroborative results were also reported with the stem and leaf disc explants of *Calotropis gigantea* (Talitha *et al*., 2022).

Table 3. Effects of different concentrations of 2,4-D and BAP on callus production, caulogenesis and rhizogenesis from leaf disc and stem explants of *P. daemia* after five weeks of inoculation.

PGR's (mg/L)	Callusing (%)	Days required for callusing	Mean no. of shoots from callus (X ±SE)	% of rooted plants
Leaf Disc				
2,4-D				
2.0	100	7- 9	4.66 ± 0.31	90
1.5	90	7- 9	3.30 ± 0.07	70
1.0	80	7- 9	3.50 ± 0.22	60
Stem				
BAP				
2.0	70	10 - 14	7.83 ± 0.18	70
1.5	75	10 - 14	7.33 ± 0.11	70
1.0	70	10 - 14	6.14 ± 0.22	70

All experiments were repeated thrice. Data are r epresented as mean ± standard error

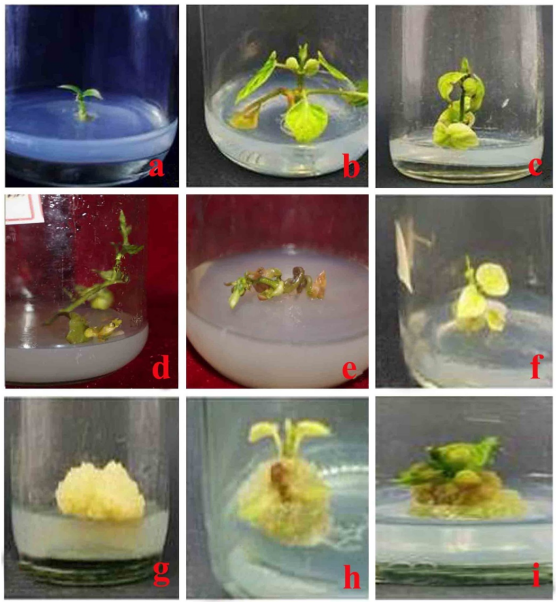


Fig 1: Micropropagation of *P. daemia* (a – i). a – d : Shoot proliferation from shoot tip explants. **e & f :** Shoot proliferation from axillary bud explants**.g :** Callus initiation from leaf disc explants**. h & i :** Caulogenesis and rhizogenesis from the callus.

Effect of *in vitro* Rhizogenesis

Rooting was most successful on MS medium with IBA (1.0 mg/L), showing 80% response with 6.83 ± 0.12 root number and 4.10 ± 0.12 cm root length (Table - 4). Similar results were reported in *Dendrobium primulinum* (Pant & Thapa, 2012) and *Chrysanthemum morifolium* (Waseem *et al.,* 2009), where IBA proved to be more effective than NAA. *Curculigo latifolia* also showed similar rooting success with IBA (Babaei *et al*., 2014).

Table 4. Effects of different concentrations of IBA and NAA on root production from *in vitro* derived micro-shoots of *P. daemia* after five weeks of inoculation.

PGR's (mg/L)	Rooting response (%)	Mean root number/Explant (X ±SE)	Mean root length (cm)
IBA			
2.0	70	5.33 ± 0.31	3.60 ± 0.17
1.0	80	6.83 ± 0.12	4.10 ± 0.12
	50	4.16 ± 0.15	

0.5			4.90 ± 0.18
NAA 2.0 1.0 0.5	 60 40 30	 4.33 ± 0.03 3.50 ± 0.07 3.16 ± 0.30	 2.40 ± 0.16 3.16 ± 0.13 3.20 ± 0.20

All experiments were repeated thrice. Data are r epresented as mean ± standard error

Conclusion

Pergularia daemia is renowned for its significant medicinal properties, which has led to its extensive exploitation. Continued overharvesting, coupled with threats such as urbanization, habitat destruction, disease, and climate change, poses a serious risk to its survival, potentially leading to declinity. In this context, the present study aimed to develop an efficient regeneration protocol through tissue culture technique to support the conservation and mass propagation of *P. daemia*. Direct organogenesis from shoot tip explants demonstrated a high regeneration potential when cultured on media supplemented with BAP (2.0 mg/L), facilitating effective shoot induction. Furthermore, axillary bud explants responded positively to a combination of BAP and IAA (1 mg/L & 0.1 mg/L), resulting in multiple shoot formation. For indirect morphogenesis, leaf disc explants cultured on media containing 2,4-D (2.0 mg/L) showed significant callus induction and regeneration response. Root induction (rhizogenesis) was successfully achieved using IBA (1.0 mg/L) supplementation. The findings of this study have established a reliable and rapid *in vitro* propagation protocol for *P. daemia*, offering a viable approach for its large-scale propagation and conservation.

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