



## Preliminary hairy root induction in aerial plant parts of *Paederia foetida* using *Agrobacterium rhizogenes*

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### Abstract

Plant diseases are mainly caused by plant pathogens, some of which have the ability to modify the plants genetically. *Agrobacterium rhizogenes* is such bacteria which has the ability to genetically modify the plant. *Paederia foetida* L. is one of the important medicinal plants of tremendous medicinal importance. This study aims to extract essential secondary metabolites from the roots of *Paederia foetida* for the design and manufacture of drugs without the plant being uprooted. Thus helps in the conservation of the plant species. We used the previously established plant tissue culture protocol as a pre-requisite for the development of hairy roots in *Paederia foetida* L. in this paper. Via the research aid of two *Agrobacterium rhizogenes*, MTCC 2364 and MTCC 532 wild type strains. *In vivo* and *in vitro* leaves and internodes were used as explants and to infection techniques viz, injecting and dipping for 15, 30 & 45 minutes were implemented. Highest (53.3 %) result was recorded in *in vivo* internodal explant with 30 min dipping but 15 min and 45 mins dipping showed less response (20.0%). Hairy roots induction in medicinal plants can provide the supplement to the pharmaceutical companies without disturbing or uprooting the plants, which will lead to the conservation of medicinal plants.

**Keywords:** *Agrobacterium rhizogenes*, *Paederia foetida*, secondary metabolites MTCC 2364, MTCC 532

### Introduction

In all religions and cultures, plants have been used since ancient times as an everlasting source of food and medicine. Medicinal plants are reported to be an essential component of biodiversity and the environment. Techniques of tissue culture are used not only for the propagation but also for the protection of medicinal plants [1]. Medicinal plants are known to be a life-saving medicine resource for the bulk of the world's population. India has a rich heritage of traditional medicine systems, such as Ayurveda, Sidha, Unani and Folk Medicines, focused primarily on botanical formulation [2]. Among these systems Ayurveda and Unani are most developed and widely practiced in India [3].

*Paederia foetida* L., known simply as skunk vine or Gandha prasarini (Hindi) relates to the family Rubiaceae. It is an extensive smelling perennial climber. It is native to eastern and southern Asia [4]. The foul smell of the plant is primarily due to the presence of sulphur compounds mainly dimethyl sulphides within the leaves [5]. *Paederia foetida* is well known for its use in Ayurvedic medicines of India [6]. It is also reported to be used in the treatment of gout, diarrhea, dysentery and piles [7]. As an emetic, and even for the treatment of piles and inflammation, the roots and barks are used [8]. *Agrobacterium rhizogenes* is a gram-negative, rod-shaped soil bacterium that produces hairy root diseases in plants. It usually occurs singly or in pairs [7]. The bacteria are known for its ability to induce proliferative, multi-branched adventitious roots at the site of infection known as hairy root syndrome [9]. *Agrobacterium rhizogenes* has been mainly used for the production of important secondary metabolites for medicinal purposes and genetic engineering [10]. The ability to move its Ri plasmid to a host plant is another importance of this bacterial species in the

environment, and the plasmid will alter the plant's genome to express genes that support its survival under extreme conditions and produce opine compounds that are used as an essential source of nitrogen [11]. The mechanism by which *Agrobacterium rhizogenes* infects the wounded plant cells is the attraction to the wound site of the bacterial cells due to the synthesis of phenolic compounds such as acetosyringone, which by auto phosphorylation triggers the virulence genes of bacterial Ri plasmid. If the bacteria reach the site of the wound, they infect the plant by incorporating their pathogenic Ri plasmid into the genome of the plant, resulting in hairy root disease development. The process of genetic transformation depends upon a large sized plasmid 200-800 kilo-bases in the bacterium called pRi [12, 13].

### Materials and Methods

#### Study area

The research was carried out from January-June, 2020 at Ravenshaw University, Cuttack, Odisha.

#### Collection of Plant Materials

The nodes of a healthy plant of *Paederia foetida* L. were collected from Department of Botany, Ravenshaw University, Cuttack, Odisha (Fig. 1 A).

#### *In vivo* and *in vitro* Explants Preparation

The nodes of a healthy plant of *Paederia foetida* L. from Department of Botany, Ravenshaw University, Cuttack, Odisha, India was used as an explant for the *in vitro* shoot regeneration whereas internode and leaf explants were used for *Agrobacterium rhizogenes* infection. The node, internode and leaf explants were firstly washed under running tap water for about 30 minutes followed by 10

minutes treatment with 2% (v/v) aqueous solution of Teepol (4ml Teepol with 200ml water) and rinsed 5 times with distilled water. Then the explants were treated with 2% (w/v) aqueous solution of Bavistein for 5 minutes (2gm with 100ml distilled water) and rinsed 5 times with distilled water. Then the explants were surface sterilised with 0.1% (w/v) aqueous solution of mercury chloride (HgCl<sub>2</sub>) for 5 minutes followed by 5 rinses with sterile double distilled water. The sterilized nodal explant was then inoculated in the MS + BAP 2.0 mg/l media for *in vitro* shoot multiplication while inter node and leaf were used for *Agrobacterium* infection for hairy root induction. The cultures were kept in the culture room with required light and temperature shoot multiplication and *Agrobacterium* infected explants in dark. Following this, *in vitro* shoots were obtained from the *Paederia foetida* which becomes the source for *in vitro* leaf and internode for agro infection.

### Explant Types for *Agrobacterium* infection”

The leaves and internodes from the *in vivo* and *in vitro* shoots of *Paederia foetida* L. was used as explants. Rather the *in vitro* explants were collected from previous cultures maintain in the laboratory.

### *Agrobacterium rhizogenes* Strains”

For infecting the plant material, two wild type strains of *Agrobacterium rhizogenes* (MTCC 2364 and MTCC 532) obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India, were used. For growth and maintenance of the bacterial strains, nutrient broth (NB) media was used (Fig. 1 B & C).

### Infection and Co-cultivation of explants with Different Strains”

The surface sterilized leaf and internode explants were placed on glass petri dishes containing bacterial strain. Using a surgical scalpel, the leaf discs (5x5 mm) and the internode (1-1.5 mm) were intentionally wounded and the explants were submerged in it. In another method, the bacterial strain was injected into the leaf explants. Incubation time was given for 30, 45 and 60 minutes. The explants was removed from bacterial strains and dipped in antibiotic solution i.e. Cefotaxime for 5 minutes. Then the explants were washed with distilled water (Fig. 1 E & F).

### Plant Tissue Culture Medium”

MS basal medium with 3% sucrose (w/v) as the carbon source and gelled with 0.7% agar (w/v) was used as the medium. Also, the pH was maintained at 5.8 the basal medium was then autoclaved at 121 C for 17 minutes. After waiting for another 20 minutes, the flasks of tissue culture medium were kept in the culture room under required conditions to obtain the solidified MS medium.

### Inoculation of Explants in MS Medium”

The disinfected internode and leaf explants were cut into a particular size (1.0-1.5 cm) with the help of sterilized surgical blades and forceps, then the trimmed explant with the help of forceps was inoculated in the flask of MS media with utmost care. The flask was closed with the help of cotton plug. After inoculation in the MS media, the explant was kept in the culture room with required conditions for growth of the hairy root. Data pooled from 15 explants (05 flasks and 03 explants per flasks).

## Results and Discussion

Four categories of explants were taken for the induction of hairy roots. These were *in vitro* leaf & internode and *in vivo* leaf & internode which were used as explants for hairy root production in *Paederia foetida* L. Two strains of *Agrobacterium rhizogenes* were used for the production of hairy roots. These two strains were MTCC 532 and MTCC 2364. The most used methods of infection are by directly injecting the bacterial strain into explant and the other one is dipping the explants in bacterial strain. The explants were dipped into both the strains MTCC 532 and MTCC 2364 for different time intervals of 15 minutes, 30 minutes and 45 minutes (Fig 1 D).

Out of the four types of explants, *in vivo* internode explant showed swellings at the infected region in less time period using the dipping 30 mins method for MTCC 532 strain, whereas the other three explants were not responded or less responded during this period (Table 1, Fig. 1 E & F). Highest (53.3 %) result was recorded in *in vivo* internodal explant with 30 min dipping but 15 min and 45 mins dipping showed less response (20.0%). Comparing both *in vivo* and *in vitro* leaf explants, *in vivo* leaf showed response to agro infection but the others do not. When comparing the explant types i.e. leaf and internode, internode found more responsive than leaf. Experiment under progress due to COVID-19 pandemic all results could not be recorded.

In the existing work by (Brijwal *et al.*, 2015), the strain MTCC 532 and MTCC 2364 of *Agrobacterium rhizogenes* had been used for hairy root induction in endangered *Berberis aristata* DC. In hairy root induction and *in vitro* grown callus (61.11 ± 1.60 % transformation frequency), strain MTCC 532 was found to be more efficient than strain MTCC 2364, and *in vitro* grown callus (61.11 ± 1.60 % transformation frequency) was found to be ideal for leaves (42.59 ± 0.92 % transformation frequency) and nodal segments (34.25 ± 0.92 % transformation frequency) of *in vitro* grown micro shoots<sup>[14]</sup>. (Sajjalaguddam RR *et al.*, 2016) had also used MTCC 532 and MTCC 2364 strain of *Agrobacterium rhizogenes* for hairy root induction in *Abrus precatorius*<sup>[15]</sup>. In terms of transformation effectiveness and number of hairy roots, MTCC 532 proved better than MTCC 2364 out of the two strains. (Bhagat *et al.*, 2019) had used two strains of *Agrobacterium rhizogenes* such as MTCC 532 and MTCC 2364 for hairy root induction in *Rauwolfia Serpentina*. Higher transformation efficiency was established using MTCC 532 (31%) and MTCC 2364 (24%) with 30 minutes infection time. Transformation efficiency was further enhanced to 55% with 125 cm acetosyringone. MTCC 532 was proven a better strain over MTCC 2364 in all the tested factors<sup>[16]</sup>.

In the existing works by (Thilip *et al.*, 2017) had used R1000, MTCC 2364 and MTCC 532 strains of *Agrobacterium rhizogenes* for hairy root induction in *Withania Somnifera* (L.) Donal<sup>[17]</sup>. The Strain R1000 showed the highest transformation rates (50.6%) MTCC 2364 was efficient than MTCC 532 (18.6%). (Chowdhury, 2015) had used MTCC 2364 MTCC 532 and ATCC 15834 strains of *Agrobacterium rhizogenes* for hairy root induction in *Eclipta alba* (L.) Hassle. The highest transformation efficiency of 87.5±0.77 % in shoot tips, 83.33±0.66 % in nodes and 45.83±0.21 % in leaves was shown by the Strain MTCC 2364. For ATCC 15834, the highest transformation efficiency of 83.33±0.64 % in shoot tips, 75±0.53 % in nodes and 33.33±0.21 % in leaves, the highest

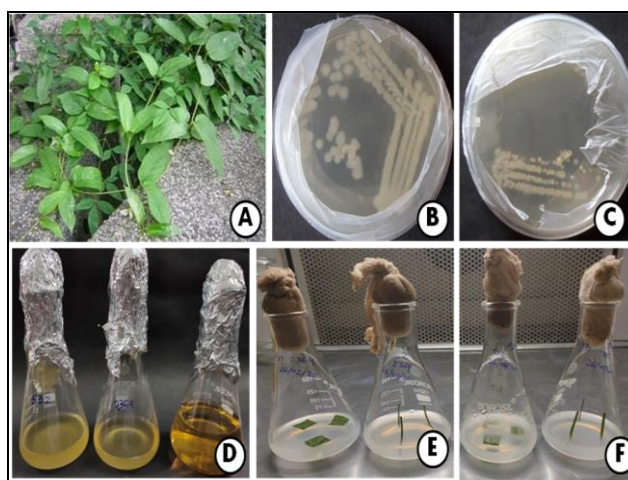
transformation frequency of  $79.16 \pm 0.61$  % in leaves, was achieved for MTCC 532 strain. MTCC 2364 was found to

be the most productive for both the frequency of transformation and the numbers of hairy roots/explants<sup>[18]</sup>.

**Table 1:** *A. Rhizogenes* mediated hairy root production in *Paederia foetida*

Agrobacterium strains, Techniques and time (min.) of infection			Percentage of hairy root production			
			Leaf		Internode	
			In vivo	In vitro	In vivo	In vitro
MTCC 532	Injecting		NR	NR	NR	NR
	Dipping	15 mins	NR	NR	R	NR
		30 mins	R	NR	R +	NR
		45 mins	NR	NR	R	NR
MTCC 2364	Injecting		NR	NR	NR	NR
	Dipping	15 mins	NR	NR	NR	NR
		30 mins	NR	NR	NR	NR
		45 mins	NR	NR	NR	NR
		45 mins	NR	NR	NR	NR

Data pooled from 15 explants (05 flasks and 03 explants per flasks). NR- No response, R- Explant responded, R+- Swelling at the infected region.



**Fig 1:** (A) *Paederia foetida* plant @ Ravenshaw university (B) Streaking of *Agrobacterium* strain MTCC 532 (C) Streaking of *Agrobacterium* strain MTCC 2364 (D) Working broth cultures of *Agrobacterium* strain MTCC 532 and MTCC 2364 (E) Inoculation of infected leaf and internode explants by MTCC 532 (F) Inoculation of infected leaf and internode explants by MTCC 2364

## Conclusions

Less result was recorded due to COVID-19 pandemic situations but the experiment was set nicely to develop the hairy root in *Paederia foetida* as discussed by the other workers in different medicinal plants. Hairy root induction in medicinal plants can provide the supplement to pharmaceutical companies without disturbing the plants or uprooting the plants. This leads to the conservation of the medicinal plant.

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