Journal of
Plant Science & Research

Open Science Publications

Volume 12, Issue 1 - 2025

www.opensciencepublications.com

Agrobacterium Mediated Hairy Root Induction in *Lawsonia inermis* L.: A Step Forward for Secondary Metabolites Production

Research Article

Moharana A[1,2]*

[1]*Department of Botany, Ravenshaw University, Cuttack-753003, Odisha, India.*

[2]*ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack-753006, Odisha, India*

***Corresponding author:** Arpita Moharana, ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack-753006, Odisha, India. E-mail Id: arpiarpita22k@gmail.com

Article Information: **Submission:** 03/01/2025; **Accepted:** 05/02/2025; **Published:** 07/02/2025

Abstract

In this study, a protocol for hairy root production for bioactive compound enhancement of *Lawsonia inermis* through intervention of *Agrobacterium rhizogenes* was attempted. Two types of explants, leaf (both in vivo and in vitro) and internode (both in vivo and in vitro) were taken for hairy root transformation by two Agrobacterium rhizogenes strains (MTCC 532 and MTCC 2364). Among leaf and internode, better transformation observed in leaf than internode where as in vitro leaf was found most suitable explant for optimum transformation than in vivo leaf explant. Better transformation was achieved by MTCC 2364 than MTCC 532 in all leaf and internodal explants. Highest 83.3% hairy root induction was noticed in in-vitro leaves infected by MTCC 2364 for 40 minutes infection time with an O.D. value 0.6 and co-cultivation time 24-26 hours. Root emergence in in vitro leaf started within 15-17 days and highest c.a.9.2 roots were observed. The in vivo leaf was found to develop hairy roots within 20-23 days from the day of infection with 73.3% transformation efficiency infected by MTCC 2364 and developed c.a 8.4 roots.

Keywords: *Agrobacterium rhizogenes* strains (MTCC 532 and MTCC2364); *In vivo* leaf and internode; *In vitro* leaf and internode; Secondary metabolites

Introduction

Lawsonia inermis L. (syn. *Lawsonia alba* Lam. or *Lawsoniaruba* L.), commonly known as Henna or Mehendi [1,2] is a monotypic genus of family Lythraceae. India's ancient history discusses its many applications and significant importance in Ayurvedic or natural herbal remedies. Apart from cosmetic use, Mehendi leaves are used as a prophylactic against diarrhea, skin diseases, renal lithiase, and gastric problems. The flowers have also medicinal properties, used as refrigerant, soporific, febrifuge, cardio tonic, as an emmenagogue and applied against bruises. The seed powder is effective for dysentery, liver disorders, and associated problems. Besides the aerial plant part, the root plays an immensely significant role in the treatment of various diseases. Root has astringent properties and is used for sore eyes, bruises, and boils of children's heads. The root of this plant is helpful in the treatment of hysteria, gonorrhea, herpes, tumor, nervous disorders, and improves the liver and kidney [3-5]. Most importantly, in Odisha and in particular Koraput district and Kandhamal district, the root has also been used for the treatment of jaundice by common people, tribal people as well as local traditional healers [6,7]. They used root paste of *L. inermis* along with raw rice water for treatment of jaundice. Besides, pharmacologically the methanol extract of roots of *L. inermis* was most effective as an abortant [3] ethanolic root extract is effective as an antitumor, antiproliferative and has been used to improve the hepatic and renal function.[8,9] In addition, the presence of important secondary metabolites in the aerial part (*in vivo* and *in vitro*) as well as in root (*in vivo* and *in vitro*) has been proven by High Performance Thin Layer Chromatography (HPTLC; Moharana *et al*., 2018b) [10]. Due to the immense significant traditional and pharmacological properties of

ISSN: 2349-2805

L. inermis root, its popularization as herbal medicine and extraction of secondary metabolites for pharmacological uses is necessary. For pharmaceutical commercialization, large scale production of *L. inermis* root is a prerequisite.

In this context, *in vitro* root cultures in particular hairy root production could be the most appropriate alternative method. Biotechnological intervention through *Agrobacterium rhizogenes* can be used as a strategy for a sustainable industrial scale root production. *A. rhizogenes* (now known as Rhizobium rhizogenes), a soil-dwelling Gram-negative phytopathogenic bacteria when infect the plant caused "hairy root" disease. As a consequence, uncontrolled root growth at the site of injury and infection of the plant is found. The additional advantages of the production of secondary metabolites through *in vitro* hairy root culture includes fast growth, low doubling time, ease of maintenance of hairy roots, and genetic stability [11]. The biosynthetic ability of "hairy root culture" to produce secondary metabolites is often equal to or greater than mother plant. [12-14] Interestingly, hairy root has also been proven to synthesize those compounds which are known to accumulate in aerial part only. [15,16,14]

In short, *L. innermis* is a traditional medicinal plant of which root is a most important part attributing many pharmacological activities. Therefore, an increase in root biomass without uprooting the plant is a prerequisite for industrial-scale production of secondary metabolites particularly located in the root. So, in this part of work attempt has been taken to increase root biomass through hairy root cultures of *L. inermis* using *A. rhizogenes* for production and enhancement of secondary metabolites located in the root. Apart from that, factors influencing genetic transformation including explant source and type, culture matrix, bacterial strains, bacterial cell density, method of infection, and co-cultivation period were evaluated and standardized to maximize the efficiency of the transformation and hairy root production in *L. S.*

Material and Methods

Explant source (both *in vivo* and *in vitro*) for *Agrobacterium* transformation

For *Agrobacterium* infection experiment, two types of explants i.e., leaf and internode (mature and axenic) were taken. Young, fresh leaves and internodes were collected from a six years old *L. inermis* plant (Figure1A) present in campus of Ravenshaw University. The plant specimen which is used as the mature source (*in vivo*) of explants was identified and deposited in the herbarium of Botanical Survey, Odisha (voucher specimen number 2539/CBT). The mature leaves and internodal explants were kept under running tap water separately in a beaker with constant shaking of about 30 min for removal of external adherents like dust and dirt. Then, both types of explants were washed for 5 min with 1 % (v/v) aqueous solution of Teepol (Reckilt Benckiser Ltd., India), followed by five times rinsing with double distilled water. Then a 0.1 % (w/v) aqueous solution of mercuric chloride ($HgCl_2$, Hi-Media, India) was used for surface sterilization (3 min for leaves and 4 min for internodes), followed by five-six rinsing of sterile double distilled water [5]. After rinse, both leaves and internodal explants were ready for *Agrobacterium* infection.

For axenic (*in vitro*) internode and leaf, the *in vitro* regenerated plantlets maintained in our laboratory (Figure 1B) were used as a source of explant. The *in vitro* shoot culture was established by inoculation of

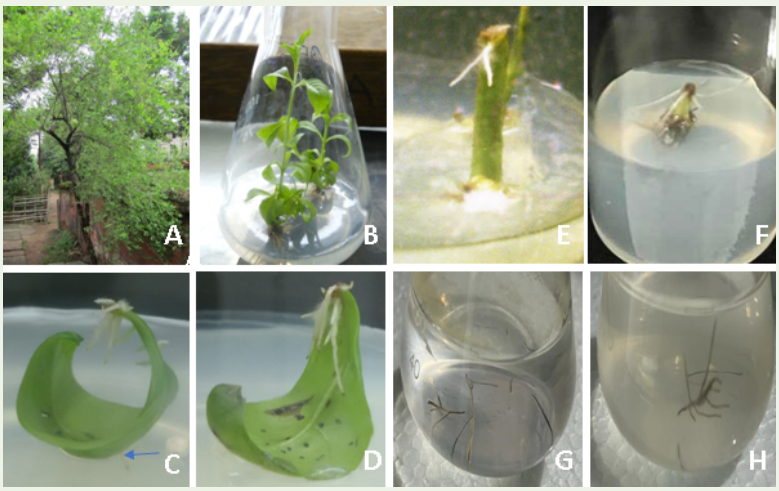

Figure 1: Agrobacterium transformation and hairy root development in L. inermis
A) Mature L. inermis plant as source of *in vivo* explant; B) Nodal explant mediated tissue culture regenerated plant as source of *in vitro* explant; C) Hairy root developed from *in vivo* leaf by MTCC 2364; D) Hairy root developed from *in vitro* leaf by MTCC 2364; E) Hairy root developed from *in vivo* internode by MTCC 2364; F) Hairy root developed from *in vitro* internode by MTCC 2364; G) Hairy root (excised from *in vivo* leaf) culture establishment in half MS liquid medium; H) Hairy root (excised from *in vitro* leaf) culture establishment in half MS liquid medium

Citation: Moharana A. *Agrobacterium* Mediated Hairy Root Induction in *Lawsonia inermis* L.: A Step Forward for Secondary Metabolites Production. J Plant Sci Res. 2025;12(1): 273

in vivo nodal explant on Murashige and Skoog, medium (MS, 1962) supplemented with BA (1.0mg/ml) and subsequent elongation on MS medium devoid of any plant growth regulators (PGRs) [5].

Bacterial strains and their maintenance

Two types of strains of *Agrobacterium rhizogenes*, MTCC 532 and MTCC 2364, were procured from IMTECH (Institute of Microbial Technology), Chandigarh, India and revived in nutrient broth (Hi-Media). For bacterial growth, the temperature was set at 26±1°C for 44 to 48 hours inside the incubator. The broth cultures were stored at 4 °C for the *Agrobacterium* transformation experiment. For explant infection, a 100µl aliquot of bacterial suspension was added into 50 ml of liquid medium in a 150-ml Erlenmeyer flask and incubated in an incubator shaker at 26±1°C. Prior to bacterial inoculation, the OD of both the bacterial suspensions of both the strains was adjusted to 0.6 (at 660 nm).

Agrobacterium transformation and hairy root induction in leaves (*in vivo* and *in vitro*) and internodes (*in vivo* and *in vitro*)

For the transformation and hairy root induction experiment, the*in vivo* internodal segments were excised (1.5-2.0cm) from both sides to eliminate the damaged tissue that were affected during the process of surface sterilization. The axenic internodes were excised (1.5-2.0cm) from *in vitro* regenerated shoots. Then both types of internodes were punctured on one tip side end, followed by the dipping in the bacterial suspension for 10-60 minutes to allow the infection. Likewise, leaves were excised from *in vitro* shoot culture, punctured around the midrib and dipped in the bacterial suspension for 10-60 minutes for *Agrobacterium* infection.

Both leaves (*in vivo* and *in vitro*) and internodes (*in vivo* and *in vitro*) were taken out of the bacterial suspension and rinsed with sterile distilled water, followed by drying with sterile tissue paper. Internodes were inoculated by the other side of the piercing and leaves were by their dorsal side up on solid half MS (agar 0.7 %, Hi-Media; pH 5.8±0.01). The co-cultivation time was optimized between 24-26 hours inside an incubator at 26±1°C. After co-cultivation, explants were transferred to fresh flask with solid half MS (agar 0.7 %, pH 5.8±0.01) and kept inside a dark culture room at 25±1°C for hairy root induction. One set of controls was maintained by the inoculation of both types of leaves and internode separately on solid half MS medium without exposure to bacterial suspension.

Establishment and mass enhancement of hairy root

After hairy root induction from both *in vivo* leaf and *in vitro* leaf, individual transformed roots (1.5–2.0 cm) were excised and transferred to a conical flask (Borosil, India) containing 100ml of liquid ½ MS medium without antibiotics incubated inside a dark culture room at 25±1°C. At one week interval, the hairy roots were transferred into a freshly prepared liquid ½ MS medium without antibiotics in the same conditions for root biomass enhancement.

Experimental design, statistical analysis and photography

For hairy root development, each treatment of the experiment, consisted of 5 replicates (culture flasks) and two explants/flask was

Table 1: Induction of hairy roots from *in vivo* and *in vitro* leaves infected by *A. rhizogenes* MTCC 2364 and MTCC 532 with different infection time.

Explant	Bacterial strain	Infection time (in minutes)	Days required for root emergence	Percentage of hairy root emergence	Number of hairy root/explant
In vivo leaf	MTCC 2364	10	20-23	Necrosis	Necrosis
		20		30.0^{hi}	3.2^{jk}
		30		63.3^{d}	7.1^{cd}
		40		**73.3 ^{c}**	**8.4 ^{b}**
		50		50.0^{f}	6.8^{de}
		60		Bacterial overgrowth	Bacterial overgrowth
	MTCC 532	10	25-28	Necrosis	Necrosis
		20		Necrosis	Necrosis
		30		26.6^{ij}	2.1^{l}
		40		33.3^{gh}	3.8^{gh}
		50		26.6^{ij}	3.4^{ij}
		60		Bacterial overgrowth	Bacterial overgrowth
In vitro leaf	MTCC 2364	10	15-17	Necrosis	Necrosis
		20		50.0^{f}	4.6^{ef}
		30		80.0^{ab}	7.7^{c}
		40		**83.3 ^{a}**	**9.2 ^{a}**
		50		53.3^{e}	6.8^{de}
		60		Bacterial overgrowth	Bacterial overgrowth
	MTCC 532	10	23-25	Necrosis	Necrosis
		20		26.6^{ij}	1.0^{m}
		30		33.3^{gh}	2.1^{l}
		40		36.6^{g}	4.2^{fg}
		50		26.6^{ij}	3.7^{hi}
		60		Bacterial overgrowth	Bacterial overgrowth

Data pooled from 02 explants per flask, 05 flasks per replication and the experiment repeated 03 times (2*5*3=30). Each value is the mean from 3 replications. Mean values marked by different letters are significantly different at $P < 0.05$ (DMRT).

Citation: Moharana A. *Agrobacterium* Mediated Hairy Root Induction in *Lawsonia inermis* L.: A Step Forward for Secondary Metabolites Production. J Plant Sci Res. 2025;12(1): 273

the experimental unit. Each experiment was repeated thrice at a 5 days interval. Mean value was taken from three biological replications. The percentage of explant showing hairy root induction, number of roots/explants, and root length were recorded after 45 days by visual observations. Mean values within column with different superscript alphabets are significantly different. Data were analyzed by analysis of variance (ANOVA) using Duncan's multiple range test ($p < 0.05$). Explants with hairy roots were photographed by a Canon DSLR P3000 camera and uploaded to the computer by inserting the memory card into computer.

Results

Agrobacterium transformation and hairy root induction in leaves (*in vivo* and *in vitro*):

For *Agrobacterium* transformation, both mature and axenic leaves, were taken into consideration. No hairy root development was observed in the control set of the experiment. Early rooting response and higher root regeneration frequency were observed in *in vitro* leaves than *in vivo* leaves (Table 1). The hairy root development in both types of leaves was observed mostly from the cut ends of the petiole and midrib. Root induction occurred in 15-17 days in *in vitro* leaf whereas, 20–23 days were required for rhizogenesis of *in vivo* leaf (Table 1) infected by MTCC 2364. The *in vitro* leaves resulted in the highest 8.5 (Figure 1C) hairy roots whereas, mature leaf developed the highest 7.2 (Figure 1D) hairy roots. Bacterial contamination was found more in *in vivo* explants, where as *in vitro* explants was devoid of such types of contamination.

Among the two MTCC 532 and MTCC 2364 *A. rhizogenes* strains used for hairy root formation, MTCC 2364 found much better in hairy root induction irrespective of explant types. The microbial strain MTCC 532, resulted in a lower percentage of root regeneration with few numbers of roots whereas, MTCC 2364 showed a higher transformation frequency in both *in vivo* (73.3) and *in vitro* (83.3) leaf. Apart from that, a comparatively higher number of days are required for rhizogenesis in the case of MTCC 532 than MTCC 2364 irrespective of explant types (Table 1).

Out of different infection time periods, 40 min. was found to be more effective in terms of the highest number of hairy root regeneration both in *in vivo* (8.4) and *in vitro* (9.2) leaves, whereas much lower infection time (10 mins.) did not show any response in any type of leaf explant rather the leaves eventually became brown and died after a few days of infection. Leaf explants infected for more time (60 min.) found excess bacterial growth around them with no hairy root (Table 1).

Table 2: Induction of hairy root from *in vivo* and *in vitro* internode infected by *A. rhizogenes* MTCC 2364 and MTCC 532 with different infection time.

Explant	Bacterial strain	Infection time (In minutes)	Days required for root emergence	Percentage of hairy root emergence	Number of hairy root/explant
In vivo internode	MTCC2364	10	25-30	Necrosis	Necrosis
		20		16.6^{f}	1.1^{klm}
		30		26.6^{e}	2.4^{fgh}
		40		**53.3** ^{b}	**3.1** ^{e}
		50		40.0^{cd}	2.6^{fg}
		60		Bacterial overgrowth	Bacterial overgrowth
	MTCC532	10	30-35	Necrosis	Necrosis
		20		Necrosis	Necrosis
		30		16.6^{f}	1.0^{klmn}
		40		40.0^{cd}	1.8^{hij}
		50		33.3^{d}	1.5^{ijkl}
		60		Bacterial overgrowth	Bacterial overgrowth
In vitro internode	MTCC2364	10	20-25	Necrosis	Necrosis
		20		40.0^{cd}	2.1^{ghi}
		30		43.3^{c}	4.5^{abc}
		40		**60.0** ^{a}	**4.9** ^{a}
		50		60.0^{a}	4.7^{ab}
		60		Bacterial overgrowth	Bacterial overgrowth
	MTCC532	10	25-30	Necrosis	Necrosis
		20		16.6^{f}	1.7^{hijk}
		30		26.6^{e}	2.8^{ef}
		40		33.3^{d}	3.6^{d}
		50		33.3^{d}	3.1^{e}
		60		Bacterial overgrowth	Bacterial overgrowth

Data pooled from 02 explants per flask, 05 flasks per replication and the experiment repeated 03 times (2*5*3=30). Each value is the mean from 3 replications. Mean values marked by different letters are significantly different at $P < 0.05$ (DMRT).

Citation: Moharana A. *Agrobacterium* Mediated Hairy Root Induction in *Lawsonia inermis* L.: A Step Forward for Secondary Metabolites Production. J Plant Sci Res. 2025;12(1): 273

The O.D. values for both the strains were optimized (data not shown) to 0.6 at 660 nm and co-cultivation time as 24-26 hours (data not shown) for optimum transformation in both types of leaves.

Agrobacterium transformation and hairy root induction in internodes (*in vivo* and *in vitro*)

Out of *in vivo* and *in vitro* internodes, higher transformation by *Agrobacterium* was observed in *in vitro* internodes than *in vivo* internodes. The *in vitro* internodal explant showed an early rooting response than the *in vivo* explant. The hairy root development in both types of internodes was observed mostly from the piercing cut end. Root induction occurred in 20-25 days on *in vitro* internodes whereas, 25–30 days were required for root emergence on internodes from mature explants (*in vivo*) (Table 2) infected by MTCC 2364. The *in vitro* and *in vivo* internodes infected by MTCC 532 showed root emergence in 25-30 and 30-35 respectively. The *in vitro* internodes resulted in the highest 3.1 (Figure 1E) hairy roots whereas, mature internodes developed the highest 4.8 (Figure.1F) hairy roots. Both types of internodal explants were found devoid of bacterial contamination. No hairy root development was observed in the control set of the experiment.

Among MTCC 532 and MTCC 2364 *A. rhizogenes* strains used for hairy root induction, MTCC 2364 found much better in hairy root development irrespective of explant types. The microbial strain MTCC 532, resulted in a lower percentage of root regeneration with a few numbers of roots whereas MTCC 2364 showed higher transformation frequency in both *in vivo* and *in vitro* internodes. Apart from that, a greater number of days are required for rhizogenesis in the case of MTCC 532 than MTCC 2364 irrespective of explant types (Table 2).

Out of different infection time periods, 40 min. was found to be more effective in terms of highest number of hairy root regenerations both in *in vivo* (3.1) and *in vitro* (4.9) internodes whereas much lower infection time (10 min.) did not show any response in any type of internodal explants rather, the internodes became brown and died after a few days of infection. The higher percentage of transformation frequency was also recorded with 40 min. of infection time in both *in vivo* (53.3 %) and *in vitro* (60 %) internodal explants. Internodal explants infected for more time (60 min.) found excess bacterial growth around them with no hairy root (Table 2).

The O.D. values for both the strains were optimized (data not shown) to 0.6 at 660 nm and co-cultivation time as 24-26 hours (data not shown) for optimum transformation in both types of internodes.

Establishment and mass enhancement of hairy root

Optimum hairy root induction was resulted by the infection of MTCC 2364 in leaf explant in terms of percentage of explant response (73.3 % in *in vivo* and 83.3 %*in vitro*) and number (8.4 in *in vivo* and 9.2 in *in vitro*) of hairy root regeneration as a result of transformation. So, the further experiment was carried out by taking hairy roots developed from both types of leaves. Actively growing individual roots (hairy roots; 1.5-2.0 cm) from transformed leaves, were excised and transferred to liquid ½MS medium. After 2 weeks, the roots in liquid medium showed a slight elongation as well as regeneration of secondary roots from the primary root (Figure 1 G),(Figure 1H)

Discussion

Out of the two strains, MTCC 532 and MTCC 2364 were selected for the hairy root induction experiment, the MTCC 2364 strain of *A. rhizogenes* was found more effective for hairy root induction in comparison to MTCC 532. These two strains were also previously used by Brijwal and Tamta, 2015[17] (*Berberis aristate*),Bathoju*et al.*, 2017[18] (*Chlorophytum borivilianum*) for hairy root induction. However, out of these two strains, MTCC 532 was reported by them as more effective in terms of percentage of hairy root induction, biomass, number, and length of hairy root than MTCC 2364, which is contrary to the result of this present study. At the same time, Vishwakarma *et al.*, 2017[14] and Mahakur*et al.*, 2024 [19] reported MTCC 2364 is more effective in hairy root formation than MTCC 532 in *Mucuna pruriens*and *Vitex negundo*respectively, which is similar to the result of the present work. On the contrary, Deore and Kide, 2015 [20] (*Chlorophytum* species) found MTCC 2364 as totally ineffective for hairy root development. Except for these two bacterial strains, Bakkali *et al.* [15] (1997) successfully developed hairy root from the *in vitro* leaf of *L. innermis*by the transformation of *Agrobacterium rhizogenes* NCIB 8196.

The types of explants influenced the hairy root production so, with a view to determie the suitable explant for optimum *Agrobacterium* transformation, different types of explants i.e., leaf (*in vivo* and *in vitro*) and internode (*in vivo* and *in vitro*) were used, out of which, both *in vivo* and *in vitro* leaf explants were more responsive than both types of internodal explants. Whereas, *in vitro* leaf showed better transformation percentage as well as more number root development than the *in vivo* leaf. Contrary to this result, internodal explant of *Clitoriaternatea* reported as better for *Agrobacterium* transformation than the leaf [21] (Swain *et al.*, 2012). Most of the researchers, including Thilip*et al.*, 2015[22] (*Withaniasomnifera*) and Jesudass*et al.*, 2020[23] (*Cucumis anguria*) suggested the use of *in vitro* or axenic explant for hairy root induction, which is similar to this part of the experiment. Preference of *in vitro* explants may be to avoid the crucial step of surface sterilization which is time consuming and has a chance of microbial contamination. But Swain *et al.*, 2012 [21] (*Clitoriaternatea*), Srinivasan *et al.*, 2023[23](*Aerva javanica*) also got success in regeneration of transformed roots from *in vivo* explants.

Agrobacterium cell density is a fundamental factor influencing genetic transformation system (Kumar *et al.*, 2006; Binka *et al.*, 2012; Shahabzadeh *et al.*, 2014; Asande *et al.*, 2020). [24-27] In this part of the experiment, the suitable O.D. value of the MTCC 2364 was found to 0.6 for the hairy root induction in all types of explants. Swain *et al.*, 2012[21] (*Clitoriaternatea*) suggested a 0.6 O.D. value as suitable for optimum hairy root production for *A. rhizogenes*A4T which corroborates the result of this part of the research work. But it is a fact that for perfect transformation the optical densities of *Agrobacterium* suspension cultures ranged from 0.1 to 1.0 depends on the genotype, *Agrobacterium* strain, and plant species (Asande*et al.*, 2020).[27] Higher *Agrobacterium* density can cause uncontrolled growth of bacterial cells thus limiting the explant survivility and subsequent reduction in transformation efficiency. Like-wise, lower cell density cannot induce transformation.

Citation: Moharana A. *Agrobacterium* Mediated Hairy Root Induction in *Lawsonia inermis* L.: A Step Forward for Secondary Metabolites Production. J Plant Sci Res. 2025;12(1): 273

The transfer of T-DNA to the plant genome from *Agrobacterium* during transformation process is time-dependent and therefore, transformation efficiency depends on both time of infection and co-cultivation duration (Markandan *et al*., 2015; Asande*et al*., 2020).[27,28]Apart from that, *A. rhizogenes* strains are different in their virulence, which leads to the different development rates of hairy root (Giri *et al*., 2001).[29]Variation in the infection durations had an influence on the transfer of T-DNA from *Agrobacterium* to plant cells of *L. inermis* from which, 40-min of infection time was recommended for successful transformation. Furthermore, the hairy root induction with 20- and 30-min infection periods resulted in significantly lower infection rates and number of roots. Due to inadequate bacterial infection duration, a shorter infection period like 10 min. was found ineffective. A higher rate of infectivity was not found when the infection duration crossed 40 min. Rather, 60 min infection time showed overgrowth of bacteria leading to explant contamination followed by necrosis. Therefore, 40 min was determined to be the optimal time for infection for *L. inermis*. Similar type of observation was reported by Srinivasan *et al*. (2023)[23]where, he suggested 20 min. as the optimum infection period for hairy root formation, but lower than 20 min. and higher than 20 min. are not preferable. Effect of infection time on transformation frequency using *A. rhizogenes* also proven to be dependent on plant species. Five minutes of infection to wounded explants was effective in inducing hairy roots in *Linum mucronatum* (Samadi *et al*., 2012)[30]and in *Agastache foeniculum* (Nouroz*iet al*., 2016),[31]whereas in *Silybum marianum* (Rahnama *et al*., 2008)[32]and in *Fagopyrum tataricum* (Thwe*et al*., 2016),[33-37]10 min of infection time was found effective for optimum transformation. Adding to that, 20 min. in *Artemisia annua* (Giri *et al*., 2001)[29]and one hour in *Berberis aristata*(Brijwal and Tamta, 2015)[17] were required for maximum hairy root formation.

Conclusion

To give a step forward for production and enhancement of root specific important phytochemicals, induction and establishment of hairy root in *L. inermis* by by*Agrobacterium* mediated transformation was carried out in which, *in vitro* leaf was found as the most suitable explant for hairy root induction by *A. rhizogenes* MTCC 2364 with O.D 0.6 and infection time 40 min. with co-cultivation time 24-26 hours. Factors impacting genetic transformation including explant source and type, culture matrix, bacterial strains, bacterial cell density, method of infection, and co-cultivation period were evaluated and standardised to maximize the efficiency of the transformation and hairy root production in *L. inermis*.

However, this part of the research work remains as a preliminary part of the work. Further research is under process i.e., enhancement of transformation efficiency, root number, and length. Molecular validation of transformed roots by PCR amplification, hairy root biomass enhancement, parameter optimization for production and enhancement of important secondary metabolites and phytochemical validation of secondary metabolites in both transformed root and roots from the mother plant will be accomplish. This part of the work might be a path forward for optimization of industrial scale production of roots biomass or bioreactor design aiming in the production of secondary metabolites, particularly accumulated in root, for drug manipulation to combat the antibiotic-resistance human pathogens, as *L. inermis* holds the status of a multipurpose medicinal plant.

Declaration of Conflicting Interests and Ethics: The author declares no conflict of interest

Funding support

The author declares that she has not taken any funding support to carry out this part of research work.

Authorship contribution statement

The Author designed, carried out the whole experiment, analyzed the data and wrote the entire manuscript

Acknowledgement

The author is grateful to Department of Botany, Ravenshaw University, Cuttack-753003 for providing necessary laboratory facilities. AM is highly obliged to Dr. Durga Prasad Barik and Dr. Soumendra Kumar Naik for their support, guidance, and providing the bacterial strains in carry out the experiment.

References

1. Sastri BN (1962) The wealth of India. Council of Scientific and Industrial Research, New Delhi.
2. Wagini NH, Soliman AS. Abbas MS, Hanafy YA Badawy EM (2014) Phytochemical analysis of Nigerian and Egyptian henna (*Lawsonia inermis* L.) leaves using TLC, FTIR and GCMS. Plant 2: 27-32.
3. Chaudhary GD, Goyal S, Poonia P (2010) *Lawsonia inermis* Linnaeus: a phytopharmacological review. International Journal of Pharmaceutical Sciences and Drug Research 2: 91-98.
4. Makhija I, Dhananjaya D, Kumar VS, Devkar R, Khamar D, et al. (2011) *Lawsonia inermis* – From traditional use to scientific assessment. African Journal of Pharmceutical Sciences and Pharmacy 2:145-165.
5. Moharana A, Das A, Subudhi E, Naik SK, Barik DP (2018a) Assessment of genetic fidelity using random amplified polymorphic DNA and inter simple sequence repeats markers of *Lawsonia inermis* L. plants regenerated by axillary shoot proliferation., Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 88:133-141.
6. Raut S, Raut S, Sen KS, Satpathy S, Pattnaik D (2012) An Ethnobotanical survey of medicinal plants in Semiliguda of Koraput District, Odisha, India. Botany Research International 5: 97-107.
7. Panigrahy J, Behera SK, Venugopal A, Leelaveni A (2016) Ethnomedicinal study of some medicinal plants from Kandhamal district, Odisha. International Journal of Herbal Medicine 4: 36-40.
8. Priya R, Ilavenil S, Kaleeswaran B, Srigopalram S, Ravikumar S (2011) Effect of *Lawsonia Inermis* on tumor expression induced by Dalton's lymphoma ascites in Swiss Albino Mice. Saudi Journal of Biological Sciences 18: 353-359.
9. Moutawallia A, Benkhouilia FZ, Doukkalib A, Benzeidb H, Zahidi A (2023) The biological and pharmacologic actions of *Lawsonia inermis* L. Phytomedicine Plus 3: 100468.
10. Moharana A, Barik DP, Naik SK, Rout KK (2018b) Comparative thin-layer chromatographic studies and development of a high-performance thin-layer chromatography method for the quantification of lawsone in natural and micropropagated plant parts of *Lawsonia inermis* L. Journal of Planar Chromatography 31: 155-162.
11. Pistelli L, Giovannini A, Ruffoni B, Bertoli A, Pistelli L (2010) Hairy root culture

Citation: Moharana A. *Agrobacterium* Mediated Hairy Root Induction in *Lawsonia inermis* L.: A Step Forward for Secondary Metabolites Production. J Plant Sci Res. 2025;12(1): 273

for secondary metabolites production. Electron Journal of Biotechnology 698:167-184.

12. Kim YJ, Wyslouzil BE, Weathers PJ (2002) Secondary metabolism of hairy root cultures in bioreactors. In vitro Cellular and Developmental Biology: Plant 38:1-10.

13. Yoon JY, Chung IM, Thiruvengadam M (2015) Evaluation of phenolic compounds, antioxidant and antimicrobial activities from transgenic hairy root cultures of gherkin (*Cucumis anguria* L.). South African Journal of Botany 100: 80-86.

14. Vishwakarma KS, Mohammed SI, Chaudhari AR, Salunkhe NS Maheswari VS (2017) Micropropagation and *Agrobacterium rhizogenes* mediated transformation studies in *Mucuna pruriens* (L.) DC. Indian Journal of Natural products Resources 8: 172-178.

15. Bakkali AT, Jaziri M, Foriers A, Heyden YV, Vanhaelen M, Hom`es J (1997) Lawsone accumulation in normal and transformed cultures of henna, *Lawsonia inermis.* Plant Cell Tissue and Organ Culture 51:83-87.

16. Liu YG, Shirano Y, Fukaki H, Tasaka M, Tabata S, Shibata D (1999) Complementation of plant mutants with large genomic DNA fragments by a transformation competent artificial chromosome vector accelerates positional cloning. Proceedings of National Academiof Sciences, 96: 6535-6540.

17. Brijwal L, Tamta S (2015) *Agrobacterium rhizogenes* mediated hairy root induction in endangered *Berberis aristata* DC. Springer plus 4: 443.

18. Bathoju G, Rao K, Giri A, (2017) Production of sapogenins (stigmasterol and hecogenin) from genetically transformed hairy root cultures of *Chlorophytum borivilianum* (Safed musli). Plant Cell Tissue and Organ Culture 131:369-376.

19. Mahakur, B, Moharana A, Madkami SK, Naik SK Barik DP (2024) Optimization of factors affecting *Agrobacterium*-mediated hairy root induction in *Vitex negundo* L. (Lamiaceae). International Journal of Secondary Metabolite 11: 244-254.

20. Deore SL, Kide A (2015) Induction and growth optimisation of hairy root culture of *Chlorophytum* species. BEMS Reports 1: 26-30.

21. Swain SS, Sahu L, Pal A, Barik DP, Pradhan C, Chand PK (2012) Hairy root cultures of butterfly pea (*Clitoriaternatea* L.): *Agrobacterium* 3 plant factors influencing transformation. World Journal Microbiology and Biotechnology 28: 729-739.

22. Thilip C, Raju CS, Varutharaju K, Aslam A, Shajahan A (2015) Improved *Agrobacterium rhizogenes*-mediated hairy root culture system of *Withania somnifera* (L.) Dunal using sonication and heat treatment. 3 Biotech 5: 949-956.

23. Srinivasan R, Kamalanathan D, Boobalan S, Saranyaa V, Mouliganesh S, et al. (2023) *Agrobacterium rhizogenes* influences aervine enhancement in hairy root culture of *Aerva javanica* (Burm.f.) Juss. ex Schult and in silico assessment of human breast cancer activity. Journal of Applied Biologyand Biotechnology 11: 148-158.

24. Kumar V, Sharma A, Prasad BCN, Gururaj HB, Ravishankar GA (2006) *Agrobacterium rhizogenes* mediated genetic transformation resulting in hairy root formation is enhanced by ultrasonication and acetosyringone treatment. Electronic Journal of Biotechology 9: 349-357.

25. Binka A, Orczyk W, Nadolska-Orczyk A (2012) The *Agrobacterium*-mediated transformation of common wheat (*Triticum aestivum* L.) and triticale (x *Triticaosecale*Wittmack): Role of the binary vector system and selection cassettes. Journal of Applied Genetics, 53: 1-8.

26. Shahabzadeh Z, Heidari B Hafez RF (2014) Induction of transgenic hairy roots in *Trigonella foenum-graceum* co-cultivated with *Agrobacterium rhizogenes* harboring a GFP gene. Journal of Crop Science and Biotechnology 16: 263-268.

27. Asande LK, Richard OO, Richard OO, Evans NN (2020) A simple and fast *Agrobacterium*-mediated transformation system for passion fruit KPF4 (*Passiflora edulis* f. *edulis* × *Passiflora edulis* f. *flavicarpa*). Plant Methods 16:141-153.

28. Markandan M, Subramanyam K, Ishwarya R, Elayaraja D, Ganapathi A (2015) Asssessment of factors influencing the tissue culture-independent *Agrobacterium*-mediated in planta genetic transformation of okra [*Abelmoschus esculentus* (L) Moench]. Plant Cell Tissue and Organ Cult 123: 309-320.

29. Giri A, Giri CC, Dhingra V, Narasu ML (2001) Enhanced podophyllotoxin production from *Agrobacterium rhizogenes* transformed cultures of *Podophyllum hexandrum*. Natural Product Letters 15: 229-35.

30. Samadi A, Carapetian J, Heidari R, Jafari M, Gorttapeh, A. H. (2012). Hairy root induction in *Linum mucronatum*ssp*mucronatum*, an anti-tumor lignans producing plant. *NotulaeBotanicae Horti Agrobotanici*, 40: 125-131.

31. Nourozi E, Hosseini B, Hassani A (2016) Influences of various factors on hairy root induction in *Agastache foeniculum* (Pursh) Kuntze. Acta Agriculturae Slovenica 107: 45-54.

32. Rahnama R, Hasanloo T, Shams MR, Sepehrifar R (2008) Silymarin production by hairy root culture of *Silybum mariamum* (L.) Gaertn. Iranian Journal of Biotechnology 6: 113-118.

33. Thwe A, Valan AM, Li X, Park CH, Kim SJ, et al. (2016) Effect of different *Agrobacterium rhizogenes* strainson hairy root induction and phenylpropanoid biosynthesis in Tartary buckwheat (*Fagopyrum tataricum* Gaertn). Frontiers in Microbiology 14: 318-328.

34. Al-Snafi AE (2019) A review on *Lawsonia inermis*: a potential medicinal plant. International Journal of Current Pharmaceutical Research 11: 1-13.

35. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

36. Muthiah JVL, Lakkakula S, Rajaiah J, Jayabalan S, Manikandan R (2016) Analysis of propagation of *Bacopa monnieri* (L.) from hairy roots, elicitation and Bacoside A contents of Ri transformed plants. World Journal of Microbiology and Biotechnology 32:131.

37. Srivastava S, Srivastava AK (2007). Hairy root culture for mass production of high value secondary metabolites, Critical Reviews in Biotechnology 27: 29-43.

Citation: Moharana A. *Agrobacterium* Mediated Hairy Root Induction in *Lawsonia inermis* L.: A Step Forward for Secondary Metabolites Production. J Plant Sci Res. 2025;12(1): 273