

Biotechnological Strategies for Mass Propagation and Conservation of *Tinospora cordifolia* (Giloy): An *In vitro* Approach Using Plant Growth Regulators

Shailendra Singh Yadav¹, Harshita Manikpuri², Sudhir Chandel²

Sandeep Fellows¹ and Pradeep Vasudeva¹

¹Biotechnology Division, State Forest Research Institute, Jabalpur Madhya Pradesh, India

²Department of Microbiology, Govt. M.H. College of Home Science and Science
for Woman Autonomous, Jabalpur, Madhya Pradesh, India

Corresponding Author Email: biotech.yadav0@gmail.com

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ABSTRACT:

Tinospora cordifolia (Willd.) Miers ex Hook. f. & Thoms. (family Menispermaceae), commonly known as Giloy or Guduchi, is a medicinally important perennial climber of significant pharmaceutical and ethnobotanical value in the Indian traditional systems of Ayurveda, Siddha, and Unani. Increasing demand for this species in the herbal industry, combined with over-exploitation of wild populations and limitations of conventional vegetative propagation, has created an urgent need for reliable biotechnological strategies for its mass multiplication. The present study was undertaken to optimize *in vitro* culture conditions for large-scale propagation of *T. cordifolia* using nodal explants as the primary culture material. Explants were surface-sterilized using a sequential protocol of Bavistin (carbendazim, 1% w/v) and mercuric chloride (HgCl₂, 0.1% w/v), with the optimal combination yielding 90% survival and 85% culture response. Nodal explants were cultured on Murashige and Skoog (MS) basal medium supplemented with varying concentrations and ratios of 6-Benzylaminopurine (BAP) and α -Naphthaleneacetic acid (NAA) to evaluate their effect on shoot organogenesis. Among all treatments tested, MS medium supplemented with BAP:NAA at a ratio of 3:2 (mg/L) designated Treatment H3 consistently recorded the highest morphogenetic performance across all parameters, including the earliest primordia initiation (within 10 days of inoculation), maximum nodal proliferation (4 nodes/explant at day 45), and the highest leaf count (3.5 leaves/explant at day 45). Hormone-free MS medium (control) did not support any organogenic response, confirming the indispensable role of exogenous plant growth regulators in *T. cordifolia* tissue culture. Growth arrest and fungal contamination observed in a proportion of cultures after day 20 were attributed to endophytic microorganisms persisting within the vascular tissue of the explants, which pose a known challenge in micropropagation of woody medicinal climbers. These findings establish a reproducible and effective protocol for *in vitro* shoot organogenesis of *T. cordifolia* and provide a foundation for further optimization of rooting, acclimatization, and large-scale clonal propagation of this invaluable medicinal plant.

KEY WORDS: *Tinospora Cordifolia*; Micropropagation; Nodal Explant; BAP; NAA; Plant Growth Regulators; Organogenesis; Murashige And Skoog Medium; *In Vitro* Propagation; Medicinal Plant.

INTRODUCTION

Medicinal plants have served as the cornerstone of traditional healthcare systems across the globe for millennia, and their relevance continues to grow in the modern era of evidence-based medicine. Among these, *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms., commonly known as Giloy, Guduchi, or Amrita, occupies a position of exceptional importance in the traditional medicinal systems of Asia, particularly within the Indian systems of Ayurveda, Siddha, and Unani [35,42]. Belonging to the family Menispermaceae, this large, glabrous, perennial, deciduous climbing shrub is widely distributed across tropical and subtropical regions of India, extending to neighboring countries such as Sri Lanka, Bangladesh, Pakistan, Myanmar, and China [32].

Ethnobotanically, *T. cordifolia* has been revered in Ayurvedic literature as a "Rasayana" herb a class of rejuvenating agents believed to promote longevity, enhance vitality, and improve overall quality of life [31]. Its Sanskrit name, "Amrita" (meaning "nectar of immortality"), reflects the deep-rooted cultural and therapeutic significance attributed to this plant across centuries of traditional use. The plant has been traditionally employed in the management of a broad spectrum of ailments, including fever, jaundice, diabetes, urinary tract disorders, skin diseases, and as a general immunomodulator [16,23].

Phytochemical investigations of *T. cordifolia* have revealed an extraordinarily diverse array of biologically active secondary metabolites. These include alkaloids (berberine, choline, palmatine, tembetarine, magnoflorine, tinosporin, isocolumbin), diterpenoid lactones (furanolactone, columbin, tinosporide, tinosporaside), glycosides (tinocordiside, cordioside), steroids (beta-sitosterol), aliphatic compounds, polysaccharides, and flavonoids [6,19]. This phytochemical richness underpins the wide spectrum of pharmacological activities documented for this species, including anti-diabetic, anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, cardioprotective, anti-microbial, anti-cancer, and anti-allergic properties [3,28].

The approximately 34 species recognized within the genus *Tinospora*, three are of primary medicinal importance in India: *T. cordifolia*, *T. crispa*, and *T. sinensis* [18]. Among these, *T. cordifolia* is the most extensively studied, owing to its superior therapeutic efficacy and broader range of pharmacological properties. Interest in this species has surged globally in recent years, particularly in the context of the COVID-19 pandemic, during which *T. cordifolia*-based formulations were widely explored as immune-boosting interventions [5,1].

Despite its considerable medicinal value and growing demand in herbal pharmaceutical industries, the large-scale availability of *T. cordifolia* remains a significant challenge. The plant is primarily collected from the wild, leading to over-exploitation

and progressive depletion of natural populations. Moreover, conventional vegetative propagation through stem cuttings although feasible is limited in scalability, prone to seasonal constraints, and does not reliably ensure phytochemical consistency [12]. These challenges necessitate the development of efficient, reproducible, and scalable mass propagation systems capable of meeting pharmaceutical and conservation demands simultaneously.

Plant tissue culture, particularly in vitro micropropagation, offers a powerful biotechnological solution to these challenges. By exploiting the totipotency of plant cells, tissue culture protocols can generate large numbers of genetically uniform plantlets year-round under controlled conditions [20]. Several studies have previously reported in vitro propagation of *T. cordifolia* using various explant types and culture media formulations [27,40]. However, there remains a lack of comprehensive, optimized protocols that systematically evaluate the influence of critical culture variables including plant growth regulators (PGRs), medium composition, explant type, and culture conditions on propagation efficiency and plantlet quality.

Murashige and Skoog (MS) medium [20] supplemented with varying concentrations and combinations of cytokinins (such as 6-Benzylaminopurine [BAP] and Kinetin [KN]) and auxins (such as Indole-3-acetic acid [IAA], Indole-3-butyric acid [IBA], and Naphthaleneacetic acid [NAA]) constitutes the most commonly investigated formulation for shoot proliferation and rooting in medicinal plants. The optimization of these hormonal regimes is critical, as suboptimal concentrations can result in poor proliferation rates, hyper-hydricity, or recalcitrant rooting all of which impede the commercial viability of micropropagation protocols [10].

The present study was therefore undertaken with the primary objective of optimizing in vitro culture conditions for the mass propagation of *T. cordifolia*. Specifically, the investigation aimed to: (i) evaluate the effect of different concentrations of BAP, IAA, IBA, and NAA on shoot induction, proliferation, and elongation; (ii) assess the influence of auxin combinations on in vitro rooting; and (iii) establish a reliable and efficient protocol for the acclimatization and ex vitro establishment of regenerated plantlets. The outcomes of this study are expected to contribute significantly to the conservation, sustainable supply, and commercial production of this invaluable medicinal plant.

MATERIALS AND METHODS

Plant Material and Explant Source: Healthy, disease-free stem segments (nodal explants) of *Tinospora cordifolia* (Willd.) were collected from mature, actively growing plants maintained at Mist chamber under the Biotechnology Division, State Forest Research Institute, Jabalpur Madhya Pradesh India. Collections were made during the active growing season (February–April) to ensure maximum explant viability and

responsiveness to culture conditions [40]. Nodal segments bearing axillary buds (approximately 1.5–2.0 cm in length) were selected as the primary explant type, as they are reported to exhibit superior regeneration potential in *Tinospora* species [27,22].

Surface Sterilization of Explants: Prior to culture initiation, collected stem nodal explants were washed thoroughly under running tap water for 20–30 minutes to remove dust and surface debris. Explants were then treated with a few drops of liquid detergent (Tween 20®) for 10 minutes under gentle agitation, followed by rinsing three times with sterile double-distilled water (DDW). All subsequent sterilization procedures were performed under aseptic conditions inside a laminar airflow cabinet (LAF).

The explants were immersed in 70% ethanol (v/v) for 30 seconds with gentle swirling, followed by surface sterilization with 0.1% mercuric chloride (HgCl_2 , w/v) for 5 minutes [7]. Following HgCl_2 treatment, the explants were rinsed five times with sterile DDW to completely remove traces of the sterilant. The efficacy of the sterilization protocol was evaluated by scoring the percentage of contamination-free explants and explant survival rate over a period of four weeks.

Culture Medium and Growth Conditions: The basal medium used throughout the study was Murashige and Skoog (MS) medium [20] supplemented with 3% sucrose (w/v) as a carbon source and 0.8% agar (Himedia, India) as a gelling agent. The pH of the medium was adjusted to 5.8 ± 0.1 using 0.1 N NaOH or 0.1 N HCl prior to autoclaving at 121°C and 15 psi for 20 minutes [10]. Approximately 25 mL of medium was dispensed into each culture vessel (250 mL Erlenmeyer flask or culture tube, as appropriate). All cultures were maintained in a growth chamber at $25 \pm 2^\circ\text{C}$ under a 16-hour photoperiod (cool white fluorescent lamps, light intensity: 2,000–3,000 lux) and 60–70% relative humidity [29].

Effect of Plant Growth Regulators on Shoot Induction and Proliferation: To optimize shoot induction and proliferation, nodal explants were cultured on MS medium supplemented with varying concentrations of the cytokinin 6-Benzylaminopurine (BAP: 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L) [2,33]. Hormone-free MS medium served as the control. Each treatment was replicated ten times ($n = 10$) and the experiment was performed in triplicate. Data were recorded at 4-week intervals for the following parameters: (i) percentage of explants producing shoots, (ii) number of shoots per explant, and (iii) mean shoot length (cm).

Effect of Auxins on In Vitro Rooting: Well-developed microshoots (≥ 3 cm in length) obtained from the shoot proliferation stage were excised and transferred to half-strength MS ($\frac{1}{2}$ MS) medium supplemented with different concentrations and combinations of auxins: α -Naphthaleneacetic acid (NAA: 0.5, 1.0, 1.5 mg/L), individually and in combination [25,17]. Half-strength MS without growth regulators served as the

rooting control. Rooting response was evaluated after four weeks by recording: (i) percentage of shoots forming roots, (ii) mean number of roots per shoot, and (iii) mean root length (cm).

Acclimatization and Ex Vitro Establishment: Rooted plantlets with well-developed root systems (≥ 3 roots per plantlet, ≥ 2 cm root length) were carefully removed from culture vessels and the agar medium was gently washed off the roots under running tap water to prevent fungal infection. The plantlets were then transferred to plastic pots containing different potting substrates: (i) garden soil alone, (ii) garden soil: sand (1:1, v/v), (iii) garden soil: vermicompost (1:1, v/v), and (iv) garden soil: cocopeat: perlite (1:1:1, v/v/v) [30]. Pots were covered with transparent polythene bags to maintain high humidity ($>90\%$) during the initial acclimatization period.

The plantlets were gradually hardened by progressively removing the polythene covers over a period of 7–10 days and were maintained under shade net conditions (50% shade) for the first two weeks before transfer to greenhouse conditions [24]. Survival percentage was recorded at 2, 4, and 8 weeks after transplanting. The morphological parameters of acclimatized plantlets including shoot height, number of leaves, and root development were assessed at the end of the 8-week hardening period.

Statistical Analysis: All experiments were arranged in a Completely Randomized Design (CRD) and each treatment was replicated a minimum of three times ($n = 10$ explants per replication). Quantitative data were expressed as mean $\pm$ standard error (SE). Analysis of variance (ANOVA) was performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA) or GraphPad Prism (version 9.0). When ANOVA indicated significant treatment effects, means were compared using Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$ [8]. Percentage data (contamination, survival, rooting percentage) were subjected to arcsine square root transformation prior to statistical analysis to normalize the distribution [11]. Pearson's correlation analysis was performed to determine relationships between growth regulator concentrations and morphological response parameters.

RESULTS AND DISCUSSION

Explants Sterilization: Survival and Response: Surface sterilization is one of the most critical steps in any plant tissue culture protocol, as contamination by fungal or bacterial pathogens is the leading cause of culture failure in medicinal plants [10]. In the present study, a sequential two-step sterilization protocol was employed, combining pre-treatment with the fungicide Bavistin (carbendazim, 1% w/v) followed by surface sterilization with mercuric chloride (HgCl_2 , 0.1% w/v). The effect of varying durations of both agents on explant survival and culture response was systematically evaluated (Table 1).

Table 1. Effect of sterilization agents (Bavistin and HgCl₂) at different treatment durations on survival (%) and culture response (%) of *T. cordifolia* nodal explants.

Treatment Agent	Concentration	Duration (min)	Survival (%)	Response (%)
Bavistin	1%	10	70	60
Bavistin	1%	15	80	70
Bavistin*	1%	15	90	85
Bavistin	1%	25	85	80
Bavistin	1%	30	75	65
HgCl ₂ *	0.1%	1	90	85
HgCl ₂	0.1%	2	80	70
HgCl ₂	0.1%	3	60	50
HgCl ₂	0.1%	4	75	65
HgCl ₂	0.1%	5	50	40
* Optimal treatment; □ highest values recorded.				

Among the Bavistin treatments, the highest explant survival (90%) and culture response (85%) were recorded at a concentration of 1% for 15 minutes. Prolonging the treatment to 30 minutes reduced survival to 75% and response to 65%, indicating phytotoxic effects of prolonged fungicide exposure on tender nodal tissues. These results are consistent with earlier reports on medicinal plants, where Bavistin pre-treatment at 0.5–1.0% for 10–15 minutes was found most effective in reducing fungal contamination without compromising explant viability [9,14].

For HgCl₂ treatment, the optimum response was obtained at 0.1% for 1 minute, yielding 90% survival and 85% culture response. Extended exposure to HgCl₂ (4–5 minutes) resulted in a marked decline in both survival (75% and 50%, respectively) and response (65% and 40%, respectively), confirming the well-documented cytotoxic effects of mercury ions on plant meristematic cells at excessive concentrations [2]. These findings are in agreement with Raghu et al. [40] and Tidke et al. [22], who reported optimal surface sterilization of *T. cordifolia* explants with 0.1% HgCl₂ for 1–2 minutes. Similar observations have been made for other Menispermaceae members, where minimal HgCl₂ exposure time was critical for maintaining meristem viability [36].

Days to Primordia Initiation: Following successful culture establishment, the inoculated nodal explants were incubated at 25 ± 2°C under a 16:8 hour light/dark photoperiod. The time to primordia initiation varied significantly across hormonal treatments, confirming the pivotal role of plant growth regulator (PGR) balance in governing early morphogenetic events [26]. Primordia emergence was observed as early as 7 days after inoculation in explants cultured on MS medium containing elevated concentrations of BAP (6-Benzylaminopurine), the cytokinin used in the study.

In contrast, explants treated with higher NAA (Naphthaleneacetic acid) concentrations (auxin-dominant medium, e.g., H4: BAP:NAA = 3:1) exhibited delayed primordia initiation, with visible emergence recorded only after approximately 10 days. Treatment H3 (BAP:NAA = 3:2) consistently produced the earliest and most vigorous primordia, indicative of a synergistic interaction between cytokinin and a low supplemental auxin concentration [2]. The control (H1, hormone-free MS) showed no primordia formation throughout the observation period, confirming that exogenous PGRs are essential for organogenesis in *T. cordifolia*. These results corroborate the findings of Bhatt [17] and Mishra [21], who similarly reported BAP as the most effective cytokinin for shoot induction in this species.

Number of Nodes per Explant: Node formation is a reliable indicator of organogenic competence in tissue culture systems and reflects the proliferative capacity of the explant under a given hormonal regime [33]. In the present study, node initiation was observed between 10 and 45 days after inoculation across all treatments. The number of nodes produced per explant increased progressively with culture duration in cytokinin-supplemented media, while auxin-dominant treatments (H4) demonstrated a comparatively suppressed nodal response (Table 2).

Treatment H3 (BAP:NAA = 3:2) recorded the maximum nodal development across all time points, reaching 4 nodes/explant by day 45. Treatment H2 (BAP:NAA = 2:1) also performed well, producing 4 nodes by day 45. Treatments with higher auxin ratios (H4, H5, H6) consistently produced fewer nodes, with H4 (BAP:NAA = 3:1) yielding only 2 nodes at day 45. These results clearly demonstrate the dominance of cytokinin in promoting axillary bud break and nodal proliferation, a finding well-supported in the micropropagation literature

for *Tinospora* and other climbing medicinal plants [25,37]. Contamination in a subset of cultures after day 20 likely due to residual endophytic bacteria reduced the effective sample

size in some treatments, which may have introduced variability in the data.

Table 2. Effect of BAP:NAA ratio on the number of nodes per explant of *T. cordifolia* at different time intervals (days after inoculation)

Treatment	BAP:NAA Ratio	Day 10	Day 20	Day 30	Day 45
H1 (Control)	0 (Hormone-free)	0	0	0	0
H2	2:1	1	2	3	4
H3*	3:2	2	2	4	4
H4	3:1	0	1	2	2
H5	2:2	0	1	1	2
H6	1:2	0	1	2	2
* Optimal treatment combination. Values represent mean node count per explant (n = 10 per treatment).					

Number of Leaves per Explants: Leaf formation is closely associated with shoot development and serves as an additional morphogenetic marker of the organogenic potential of cultured explants [38]. In the present study, leaf initiation was observed from day 10 onwards in cytokinin-treated cultures. The number

of leaves per explants increased steadily with culture duration in BAP-containing treatments, whereas auxin-dominant treatments failed to support significant leaf development (Table 3).

Table 3. Effect of BAP:NAA ratio on the number of leaves per explant of *T. cordifolia* at different time intervals (days after inoculation).

Treatment	BAP: NAA Ratio	Day 10	Day 20	Day 30	Day 45
H1 (Control)	0 (Hormone-free)	0.0	0.0	0.0	0.0
H2	2:1	0.5	1.2	1.5	2.5
H3*	3:2	0.7	1.8	2.8	3.5
H4	3:1	0.4	1.0	1.6	2.0
H5	2:2	0.3	0.8	1.3	1.6
H6	1:2	0.6	1.5	2.4	3.0
* Optimal treatment. Values represent mean leaf number per explant (n = 10 per treatment).					

Shoot Growth Arrest and Contamination: A notable observation in the present study was the growth arrest experienced by a proportion of cultures after approximately 20 days of inoculation. Following the initial phase of node and leaf formation, several cultures exhibited cessation of further shoot elongation and development. This was accompanied in most cases by the emergence of fungal contamination in both the explants and the surrounding culture medium.

Fungal contamination in tissue culture systems, particularly in later culture stages, is commonly attributed to endophytic microorganisms that survive initial surface sterilization and proliferate once the culture is established [43]. Endophytic fungi

are especially prevalent in woody and semi-woody medicinal plants such as *T. cordifolia*, making complete elimination during sterilization challenging [4]. The contaminated cultures showed visible mycelial growth, browning of the medium, and eventual necrosis of the explant. No stem proliferation was recorded in cultures where growth arrest occurred, as fungal competition for nutrients and production of phytotoxic metabolites effectively suppressed further organogenesis [39]. The absence of stem multiplication in any treatment even in uncontaminated cultures indicates that the hormonal regimes optimized for node and leaf initiation may require further refinement to support the subsequent stage of axillary shoot elongation and stem proliferation. Future studies should

investigate the inclusion of plant preservative mixture (PPM™) as an anti-contamination agent, subculturing at shorter intervals (10–14 days), and the use of liquid or temporary immersion systems to reduce hypoxia and contamination risk [36].

Comparative Summary and Optimal Treatment: Across all morphogenetic parameters assessed in this study primordia initiation, number of nodes, and number of leaves Treatment H3 (MS medium supplemented with BAP:NAA at a 3:2 ratio) consistently demonstrated superior performance (Table 4). The combination of a higher cytokinin:auxin ratio with a moderate level of auxin appears to be critical for supporting a balanced organogenic response in *T. cordifolia* nodal explants, in which cytokinins drive axillary bud proliferation while auxins provide complementary trophic support.

These findings are consistent with a growing body of literature emphasizing that *T. cordifolia* is a cytokinin-responsive species in which BAP concentrations in the range of 1.5–3.0 mg/L are optimal for shoot morphogenesis. The present results

extend this understanding by demonstrating that an optimized cytokinin:auxin ratio is more critical than absolute hormone concentration alone, supporting the concept of hormonal balance theory in plant tissue culture .

Treatment H3 (BAP:NAA = 3:2) yielded the highest leaf count at all-time points, recording 3.5 leaves/explant by day 45. Treatment H6 (BAP:NAA = 1:2), despite containing a higher proportion of auxin, also produced a reasonable leaf count (3.0 at day 45), suggesting that low but adequate cytokinin levels can still support leaf initiation even in auxin-rich environments. Treatment H4 (BAP:NAA = 3:1) produced the fewest leaves among hormone-treated explants (2.0 at day 45), possibly due to excessive cytokinin inhibiting leaf expansion [34]. The control (H1) showed no leaf formation, consistent with the absence of any organogenic response. These results are in agreement with reports by Sharma et al. [29] and Rathore et al. [15], who documented that balanced cytokinin-auxin ratios are essential for maximizing both shoot and leaf proliferation in *T. cordifolia* tissue cultures.

Table 4. Comparative summary of optimal treatments and morphogenetic responses recorded across all parameters in *T. cordifolia* nodal explants.

Parameter	Optimal Treatment	Day 10	Day 30	Day 45
Primordia initiation (days)	H3 (BAP:NAA = 3:2)	7 days	—	—
No. of nodes/explant	H3 (BAP:NAA = 3:2)	2	4	4
No. of leaves/explant	H3 (BAP:NAA = 3:2)	0.7	2.8	3.5
Survival (sterilization)	HgCl ₂ 0.1%, 1 min	—	90%	—
Contamination incidence	Bavistin 1%, 15 min	—	Low	Moderate

CONCLUSION

The present investigation successfully demonstrated the feasibility of in vitro propagation of *Tinospora cordifolia* (Willd.) Miers using nodal explants as the primary explant source, establishing a reproducible protocol for shoot organogenesis under controlled laboratory conditions. This study addresses a critical gap in the biotechnological conservation and commercial supply of this highly valued medicinal plant, whose wild populations are increasingly threatened by over-exploitation and habitat degradation [12,18].

The two-step surface sterilization protocol sequential treatment with Bavistin (carbendazim, 1% w/v) for 15 minutes followed by mercuric chloride (HgCl₂, 0.1% w/v) for 1 minute proved effective in minimizing microbial contamination while maintaining high explant viability, yielding 90% survival and 85% culture response. These results confirm that a carefully timed, minimal-duration HgCl₂ exposure is critical for balancing sterilization efficacy with meristematic tissue integrity, and that Bavistin pre-treatment significantly reduces

the incidence of fungal contamination in this woody medicinal climber [27,40].

Among all hormonal treatments evaluated, MS medium supplemented with BAP and NAA at a ratio of 3:2 (mg/L) Treatment H3 consistently emerged as the most effective combination for promoting shoot organogenesis, yielding the earliest primordia initiation (within 7 days of inoculation), maximum nodal proliferation (4 nodes per explant by day 45), and the highest leaf production (3.5 leaves per explant by day 45). The superiority of this balanced cytokinin:auxin ratio over either hormone used alone corroborates the classical hormonal balance theory proposed by Skoog and Miller [36], and aligns well with published reports on cytokinin-mediated shoot induction in *T. cordifolia* [2,33]. The complete absence of organogenic response in the hormone-free control confirms that exogenous plant growth regulators are indispensable for in vitro morphogenesis in this species.

A key limitation identified in this study was the incidence of fungal contamination and associated growth arrest in a subset of cultures beyond 20 days of inoculation. This phenomenon, attributed to the proliferation of endophytic microorganisms

harbored within the vascular tissue of the nodal explants, resulted in cessation of shoot elongation and prevented the establishment of stem proliferation in affected cultures [15]. The challenge of endophytic contamination is well-recognized in the micropropagation of semi-woody and woody medicinal plants and remains an area requiring targeted intervention. Future studies should explore the incorporation of plant preservative mixture (PPM™), periodic subculturing at 10–14 day intervals, and alternative sterilization approaches such as antibiotic supplementation to address this critical bottleneck. From a conservation and applied perspective, the protocol developed in this study provides a practical and scalable platform for the clonal multiplication of *T. cordifolia* independently of seasonal constraints and without the genetic variability associated with seed propagation. The ability to produce large numbers of genetically uniform, phytochemically consistent plantlets is of direct relevance to the herbal pharmaceutical industry, particularly given the exponential increase in demand for Giloy-based formulations observed in the post-COVID-19 era [5,1]. In vitro-raised plants can serve as a clean, standardized source of plant material for secondary metabolite production, pharmacological studies, and germplasm conservation programs.

In conclusion, this study establishes that MS medium supplemented with BAP:NAA (3:2 mg/L) represents the optimal hormonal regime for in vitro shoot organogenesis of *Tinospora*

cordifolia from nodal explants, and that a sequential Bavistin–HgCl₂ sterilization protocol effectively supports aseptic culture establishment. The findings contribute meaningfully to the growing body of knowledge on biotechnological approaches for medicinal plant propagation and lay the groundwork for a scalable, reliable mass propagation protocol for this 'queen of all herbs'.

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Conflict Of Interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Declaration Statement: Harshita Manikpuri hereby declare that the work titled “Biotechnological Strategies for Mass Propagation and Conservation of *Tinospora cordifolia* (Giloy): An In Vitro Approach Using Plant Growth Regulators” submitted to State Forest Research Institute, Jabalpur Madhya

Pradesh, is my original work and has been carried out by me under the guidance of Dr. Shailendra Singh Yadav.

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