In vitro morphogenetic studies on *Tinospora cordifolia* (Willd.) Miers

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ABSTRACT

Tinospora cordifolia (also known as *Tinospora sinensis* (Lour.) Merr.) belongs to the Menispermaceae family and is native to Myanmar, Sri Lanka, and China. In recent research, an effort was made to generate plantlets in vitro using MS medium. This process involved two distinct steps. Firstly, explants were cultured to initiate callus formation through indirect organogenesis. The findings of this initial step are detailed in the paper. The second step aimed to induce morphogenesis and generate plantlets from the callus. However, the callus has shown no response thus far, delaying the production of plantlets.

Key words: *Tinospora cordifolia*, MS media, *in - vitro* morphogenetic studies, callus

Introduction:

T. cordifolia (synonym: *Tinospora sinensis (Lour.) Merr.)* is commonly referred to as Guduchi/Amrita, with Latin names *Tinospora cordifolia (Wild) Hook. f. & Thomson*, English Tinospora Gulancha/Indian Tinospora, and Hindi Giloya. It belongs to the Menispermaceae family and can be observed in Myanmar, Sri Lanka, and China [1]. It grows well in the tropical climate, frequently reaches high heights, and scales the trunks of big trees [2]. The roots and mature stem of *T. cordifolia* are essential in the preparation of Cheriyarasnadikasayam, Dhanvantharamtailam, and Amrtaristam in Ayurveda and tribal medicine. The ideal growing season for this plant is from spring to summer. Due to their invaluable contribution to the health and wellbeing of human cultures, medicinal plants are known as "green gold." Most developing nations employ traditional medicine and medicinal plants as a normative foundation for the maintenance of good health, as has been well documented. In India, using medicinal plants in conjunction with traditional knowledge is a very common medical practice. All segments of the Indian population use medicinal plants extensively, and it has been estimated that over 7500 plant species are utilized by various ethnic groups. Pharmaceutical corporations and indigenous people have overused this plant for traditional treatments. It is a plant known as Rasayana and is considered to be of major medicinal significance in the Indian system. The contemporary medical system has also acknowledged its efficacy. Although the stem is permitted for use in medicine, the entire plant is utilized medicinally. This is because the stems contain more alkaloid than the leaves do [3]. It is used to treat conditions like diabetes, fever, jaundice, respiratory problems, neurological problems, and rheumatism as well as to support the operation of numerous organs. Alkaloids, diterpenoids, lactose, glycosides, steroids, and sesquiterpenoids are only a few of the many groups of bioactive substances. The anti-cancer properties of *Tinospora cordifolia* are primarily observed in animal models.

According to the World Health Organization, 80% of the global population mostly uses traditional medicines that involve plant extracts or their active ingredients. The mega-biodiversity of India and its knowledge of the rich, ancient traditional medical systems (Ayurveda, Siddha, Unani, Amchi, and local health traditions) provide a solid foundation for the use of many plants in general healthcare and the treatment of common human maladies [2]. The plant is used in ayurvedic "Rasayanas" to strengthen the body's defenses against infection and the immune system. The anti-stress, anti-leprotic, and anti-malarial properties of this plant's root are well known [4].

Drug manufacturing companies in India obtain nearly 90% of the raw materials for medicinal plants from natural forests [3]. The severe scarcity of wild medicinal plants, including *T. cordifolia*, is caused by the rising demand for natural medicinal raw materials. The National Medicinal Plant Board, New Delhi, has listed *T. cordifolia* as one of the highly prioritized medicinal plants in the agro-climatic zones of Rajasthan, Uttar Pradesh, and Madhya Pradesh in India due to its high demand [5]. Concern over various aspects of medicinal plants has grown in recent years, and more attention is being paid to a wide range of research topics, from metabolomics to in vitro propagation. The species is currently endangered in India's natural habitats. The National Medicinal Plant Board (NMPB) of India recently gave this species priority for mass reproduction. The commercial level cultivation of this species is promoted in this context by biotechnological methods [6]. This plant's poor seed viability, low germination rate, and high susceptibility to infections make it difficult to propagate by stem cuttings and conventional means. As an alternative, plantlets can be produced in large numbers with true to type and healthy stocks using in vitropropagation techniques that use direct and callus-mediated shoot induction [5].Utilizing nodes, young leaves and shoot tips - in the current research, the method of propagating *T. cordifolia in vitro* is discussed with results obtained.

Materials and methods:

Fig 1: *Tinospora cordifolia*

For the current study, mature and healthy plants of *Tinospora cordifolia*were collected from Doddanekkundi, Bengaluru. Shoot meristems, nodes and young leaves were taken as explants for the *in vitro* cultivation of plant.

Fig 2: Different explants of *Tinospora cordifolia* (Willd) Miersa.Young leaves b. Nodes c. Shoot

The explants (Fig 2) nodes, young leaves, and shoot meristems were first rinsed under the tap water multiple times to get rid of dirt particles for surface sterilization. After that, the explants in the culture bottles were cleaned for ten minutes using a 5% teepol solution, which served as a detergent. The explants were then placed inside autoclaved distilled water until the next sterilization process, after being repeatedly cleaned with tap water to get rid of any detergent residue. After washing, the explants were placed in a laminar air flow (LAF) cabinet to go through additional chemical sterilization. Following 15 minutes of washing in 0.2% mercuric chloride ($HgCl_2$), the explants were rinsed again in double-distilled water. The explants were then finally sterilized by washing them with hydrogen peroxide (H_2O_2) for 10 seconds. The explants were subsequently washed three times in double distilled water for about two minutes each to eliminate residues of mercuric chloride and hydrogen peroxide, both of which are hazardous to plants.

The sterile plants were then transferred to a sterile plate, and the explants were removed with a sterile blade. Young leaves, nodes, and shoot meristems were among the explants. The instruments used in this study were sterilized in a hot air oven for 1 to 2 hours at 200℃. The explants were removed and inoculated within the LAF unit in the inoculation chamber. Explants were inoculated into culture bottles that included sterilized growth media with sterilized forceps. Murashige and Skoog growth media with varying hormonal concentrations were utilized in the current research. prior to autoclaving, the pH was kept at 6, and the media was transferred into the culture bottles, capped, wrapped up and autoclaved at 121℃ at 15 psi pressure for a duration of twenty minutes. Afterwards, the media was allowed to solidify. M.S (Murashige and Skoog) media 1 (Set 1), media 2 (Set 2), media 3 (Set 3), and media 4 (Set 4) were prepared with varying hormonal concentrations. On the basis of literature review, different hormone concentrations were taken into account. Explants were inoculated in 09 test tubes of each media type. Following explant inoculation, the culture bottles were closed with caps and sealed using cling wrap to prevent

contamination before being moved to the culture room, which was set to 23°C and was provided with 2000 lux of light. The observations were made on a weekly basis, and any variations were noted.

Table 1: Hormonal concentrations of different sets of MS media prepared

Media	Conc. Of 2,4 - D	Conc. Of NAA	Conc. Of BAP	Observation
MS media Set 1	6 mg/L	-	3 mg/L	Callus growth was observed in leaf explant after 4 weeks
MS media Set 2	-	6 mg/L	3 mg/L	Callus growth was observed in nodal explants and shoot meristem after 1 week
MS media Set 3	3 mg/L	3 mg/L	4 mg/L	Callus was observed in a nodal explant and shoot meristems after 1 week
MS media Set 4	3 mg/L	6 mg/L	3 mg/L	Callus was observed in aleaf explant and shoot meristems after 2 weeks

Table 1. Hormonal concentrations of different sets of MS media prepared

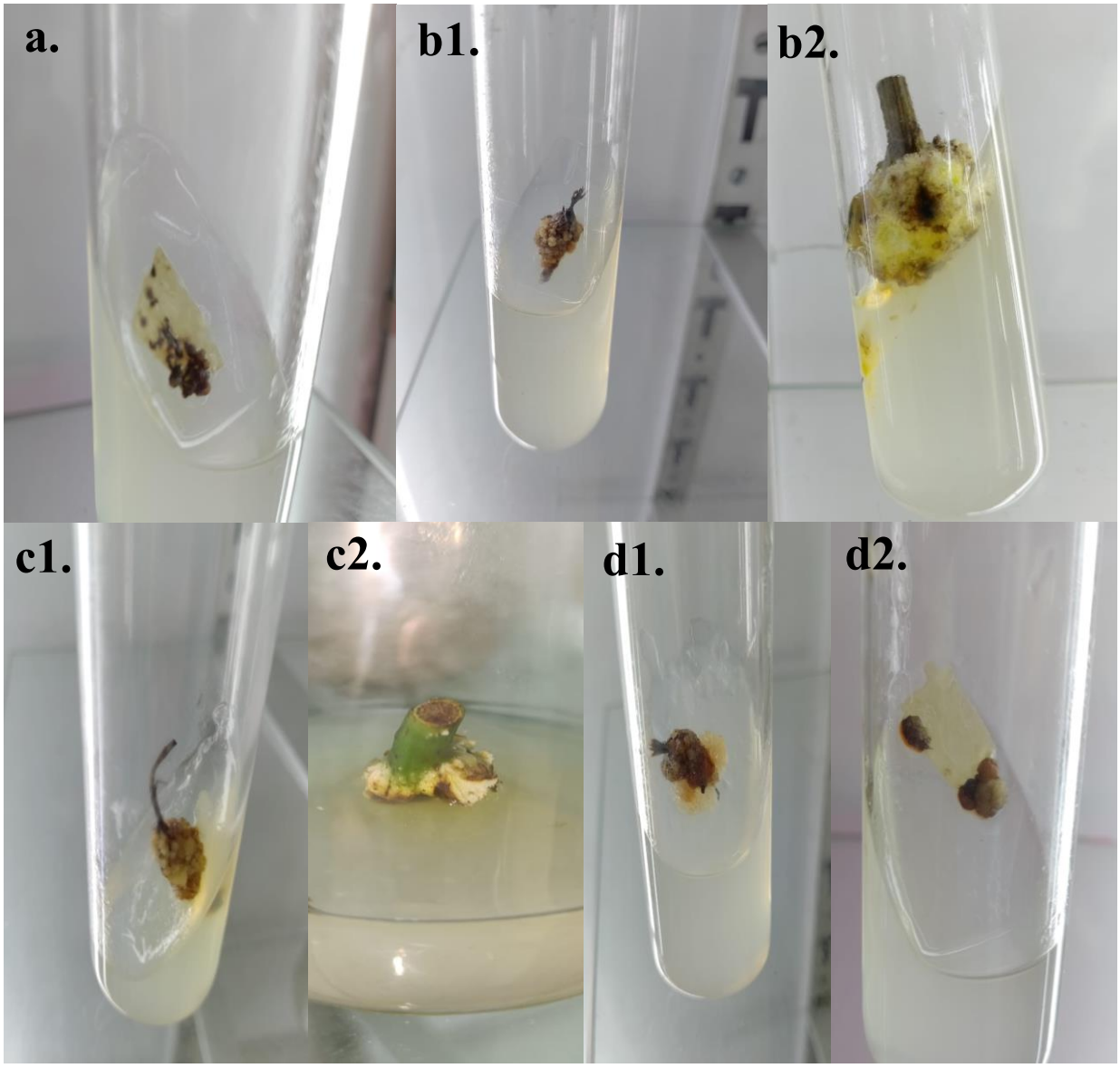

Fig 5: a. Callus growth on leaf explant in MS media set 1, b1. & b2. Callus growth on shoot meristem and nodal explant in MS media set 2, c1.& c2. Callus growth on shoot meristem and nodal explant in MS media set 3, d1. & d2. Callus growth on shoot meristem and leaf explant in MS media set 4.

Media	Total number of test tubes inoculated	Total number of test tubeswhich showed callus growth	Growth percentage
MS media Set 1	Leaf -3 Meristem- 3 Node -3 Total no - 09	Leaf- 1	11.11%
MS media Set 2	Leaf -3 Meristem- 3 Node -3 Total no – 09	Meristem 3 Node- 2	55.55%
MS media Set 3	Leaf -3 Meristem- 3 Node -3 Total no - 09	Meristem- 2 Node -1	33.33%
MS media Set 4	Leaf -3 Meristem- 3 Node -3 Total no - 09	Leaf -1 Meristem -4	55.55%

Table 2: Growth % of different sets of MS media prepared

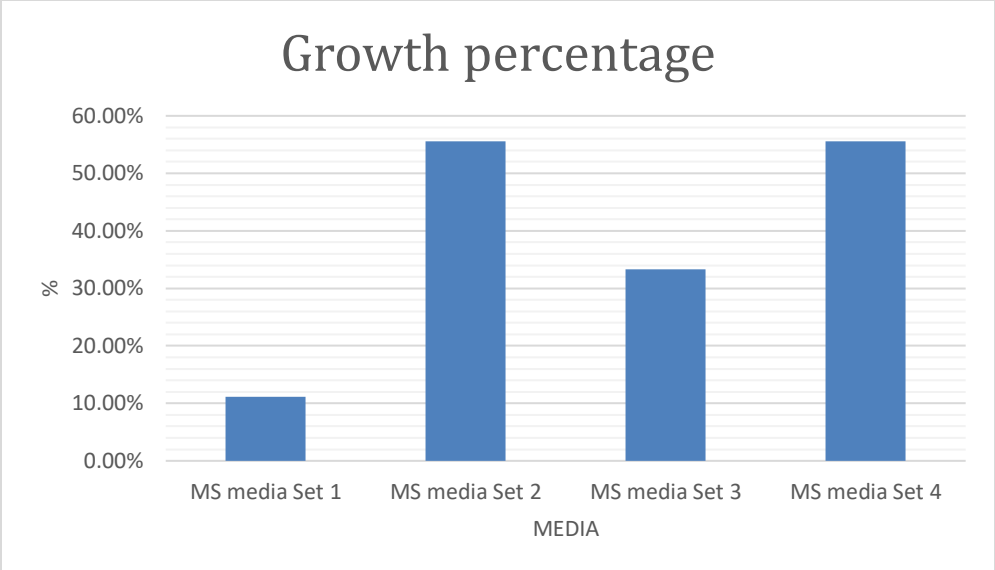

Fig 4: Graph showing the growth % of different MS media sets

After 1-2 weeks of primary culture, explants showed callus growth on different culture media. Callus formation was seen in nodal, shoot meristem and leaf explants, when inoculated on the Media containing the different combinations of 2,4– Dichloro phenoxy acetic acid ,Benzyl amino purine and Naphthalene acetic acid (Table 1).In MS media set 1, 6mg/L of 2,4 – Dichloro phenoxy acetic acid and 3mg/L of Benzyl amino purine was used for media preparation and 9 test tubes were inoculated withleaf, node and shoot meristem explants of 3 each.The Callus growth was observed only in one of the leaf explants and the growth percentage was 11.11%. In MS Media set, 2- 6mg/L of Naphthaleneacetic acid and 3 mg/L of Benzyl amino purine was used for media preparation and 9 test tubes were inoculated with leaf, node andshoot meristem explantsof 3 each. The Callus growth was observed only in 2 nodal explants and 3 shoot meristems. The growth percentage was 55.55%.In MS media Set 3, 3mg/L of 2,4 – Dichlorophenoxy acetic acid, 3mg/L of Naphthaleneacetic acid and 4 mg/L of Benzyl amino purine was used for media preparation and 9 test tubes were inoculated with leaf, node and shoot meristem explants of 3 each.The callus growth was observed in 1of the nodal explants and 2 shoot meristems. The growth percentage was 33.33%.In MS media Set 4, 3mg/L of 2,4 – Dichlorophenoxy acetic acid, 6mg/L of Naphthalene acetic acid , 3mg/L of Benzyl amino purine was used for media preparation and 9 test tubes were inoculated with leaf, node and shoot meristems explants of 3 each. The callus growth was observed in 1 of the leaf explants and 4 shoot meristems. The growth percentage was 55.55%.

Discussion:

The current study reports, effective mass propagation protocols for *T. cordifolia* through indirect organogenesis from leaves, shoot meristems and nodal explants. Callus induction was readily obtained from all the sets of MS media prepared. Media with NAA and BAP and media with 2,4 – D, NAA and BAP showed good results with the formation of callus after 1 – 2 weeks of inoculation. Whereas, media with 2,4 – D and BAP did not show good results as the growth percentage was very less when compared to other media compositions. It was also observed that the Shoot regeneration efficiency of the callus was dependent on explant type, age of explant, regeneration medium and PGRs used. *Tinospora cordifolia* has been proven to be effective in the treatment of a number of microbial infections. Furthermore, the plant exhibits significant antimicrobial properties against a variety of pathogenic microbes. A disc diffusion examination of the antibacterial activity of *Tinospora cordifolia* aqueous, ethanol, and chloroform extracts against a variety of gram positive and gram negative bacteria indicated substantial antibacterial activity. This discovery validates the use of *Tinospora cordifolia* in traditional medicine to treat a variety of infectious disorders [7].*Tinospora cordifolia's* methanol extracts have been proven to be effective against microbial infections. Plant extracts inhibited the growth of a variety of bacteria, including *E. coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Shigella flexneri, Salmonella paratyphi, Salmonella typhimurium, Pseudomonas aeruginosa, Enterobacter aerogene*, and *Serratia marcesenses.* [8,9]. *Tinospora* extract was shown in mouse models to aid in bacterial clearance and to increase neutrophil phagocytic and intracellular bactericidal capacities. [10]. *Tinospora* stem extract produced by soxhlet extraction and purified using several chromatographic methods in a mixed solvent system demonstrated antibacterial activity against *Staphylococcus albus, Escherichia coli* and *Staphylococcus aureus*. [11]. *Tinospora cordifolia* grown on *Azadirachta indica* tree hydro-alcoholic extract revealed significant antimicrobial properties resembling *Azadirachta indica. Klebsiella pneumonia,Staphylococcus aureus, Aspergillus niger,Escherichia coli, Pseudomonas sp, Aspergillus fumigates, Pencilliumsp and mucor sp* were all examined for antibacterial activity. [12]. *Tinospora cordifolia* is also known to have antibacterial efficacy against urinary tract microorganisms [13].In the most recent research, silver nanoparticles produced from the stem of *Tinospora cordifolia* were tested for antibacterial efficacy against multidrug-resistant strains of Pseudomonas aeruginosa recovered from burn victims. *Tinospora cordifolia's* silver nanoparticles have very excellent antibacterial qualities, making them an excellent source of antibiotics [14].

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