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Combination of PGRs for Rapid and Enhanced Micropropagation of *Tinospora Cordifolia*

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Abstract:- *Tinospora cordifolia* is a priority medicinal plant with significant therapeutic value, particularly in Indian medicine. However, for quick multiplication, this plant requires a regeneration technique. *T. cordifolia* nodal explants were used to design a successful regeneration technique.When nodal segments were cultivated on MS media enriched with BAP and 2-iP, a high frequency of multiple shoot development was produced. On this medium and hormonal concentration, the maximum mean number of shoots per nodal explant, the longest shoot length, and an 86 percent response were attained. *Tinospora cordifolia* (Willd) Miers has a successful *in vitro* micropropagation strategy based on shoot tip explants. Young and mature shoot tip (YST and MST) explants from two different-aged plant sources (15 days and 3 years) were treated simultaneously in the micropropagation methodology to develop and evaluate their effectiveness.6-benzyladenine (BA) and kinetin (KN) performed better in bud break and shoot growth than the other cytokinins and synergetic treatments, at both individual and combined dosages.Multiple shoot induction and development were improved when the auxin indole-3-acetic acid (IAA) was added to the appropriate cytokinin doses of BA and KN.Additional gibberellic acid and an antioxidant (ascorbic acid) supplementation improved shoot bud induction, shot number, and shoot length considerably.Also, this review article gives an idea of application of *Tinosporacordifolia* and how to enhanced micropropagation by the combination of various Plant Growth Regulators and its concentration.

Fig. 1: Tinospora Cordifolia

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I. INTRODUCTION

According to the World Health Organization, traditional medicines utilizing plant extracts or their active ingredients are used by 80% of the world's population. India's mega-biodiversity and knowledge of rich old traditional medical systems (Ayurveda) give a good foundation for the use of a large number of plants in general healthcare and relief of common diseases (Pandey MM *et al*., 2008).

Medicinal plants have been utilized as medicines and cures for humans in all civilizations from prehistoric times. The extraction and development of various medicines and chemotherapeutics from these plants, as well as some traditionally used herbal treatments, has led to a growing dependence on medicinal plants in industrialized nations. In the meantime, medicinal plant stocks in poor nations are diminishing and in risk of extinction as a result of rising trade demands for lower-cost healthcare items, such as more targeted medications and biopharmaceuticals (Debnath *et al*., 2006).

Natural medications have been made from medicinal plants. This technique can be traced back to primordial times. Plants have been used in medicine in a variety of ways, including crude extracts for therapeutic purposes due to the presence of natural chemical constituents such as berberine, morphine, psilocin, vincristine (Balandrin MF *et al*., 1985), and natural compounds for the synthesis of drugs such as tubocurarine, colchicine, nicotine, quinine, and others. Many latest medicines, such as digitalis, vinblastine, aspirin, quinine, and paracetamol, were developed from natural substances found in medicinal plants such as foxglove (*Digitalis purpurea*), madagascar periwinkle (*Vinca rosea*), willow bark (*Salix spp.*), and quinine bark (*Cinchona officinalis*) (Briskin DP. 2000).

Due to urbanization, deforestation, and destruction of whole plants to get plant extract for the creation of medications by pharmaceutical corporations, medicinal plant species are becoming increasingly threatened (Mohammed SM *et al*., 2012). As a result, medicinal flora is rapidly disappearing from its natural environment.

The aseptic cultivation of explants of tissues and organs in closed tubes with prescribed culture medium and under regulated environmental conditions is known as micropropagation. Micropropagation is the most economically efficient and practically oriented plant biotechnology at the moment, resulting in the fast creation of a large number of clonal plants of various plant species that are virus- and pathogen-free in many circumstances. Furthermore, micropropagation is currently the key technical link in the production of transgenic and other somatically engineered plants. The capacity to regenerate complete plants from cells, tissues, or organs into which "foreign" DNA has been introduced and expressed is critical to the efficient production of transgenic plants. Furthermore, new modalities in molecular biology, as well as micropropagation and other tissue-culture techniques, can allow for rapid testing of new genotypes or field selections of plants.

Micropropagation or tissue culture technique may provide a consistent and dependable supply of medicines, and it can also be used to develop plant cells on a big scale and extract essential metabolites (Hussain *et al*., 2012). Micropropagation (also known as *in vitro* propagation) is the most frequent name for clonal, true-to-type plant multiplication using a range of tissue, cell, and organ culture techniques. Other applications of plant tissue culture, such as axenic or aseptic culture and plant tissue culture, are not always strictly propagation.

Exogenously applied chemical substances called plant growth regulators (PGRs) control stem elongation by suppressing gibberellin production or releasing ethylene. PGRs have been and continue to be primarily utilized to shorten straw and thereby boost lodging resistance in current, high-input cereal management. In addition to stem elongation, there is evidence that PGRs have the capacity to alter grain yield formation and plant stand structure. Often, these changes occur as a result of changes that are comparable to those caused by daylength.

Tinospora cordifolia is a significant substance in Indian medicine and has been used in medicine from the dawn of time. Fevers, diabetes, dyspepsia, jaundice, urinary difficulties, skin illnesses, chronic diarrhea, and dysentery are all treated with this well-known Indian bitter. It's also been suggested as a therapy for heart disease, leprosy, and helminthiasis.

The starch extracted from the stem is very nutritious and digestive, and it's used to treat a variety of ailments (Kirti Sinha *et al*., 2004). *Tinospora cordifolia* is known by different name in various different languages in India viz, garo in Gujarati, Amritavalli in Sanskrit, Guduchi in Marathi, Guluchi in Oriya, gurcha in Hindi, Tippa-teega in Telugu, Shindilakodi in Tamil, Amruthu, Chittamruthu in Malayalam, Amrutha balli in Kannada, Rasakinda in Sinhala (LB Gaur *et al*., 2014).

II. TINOSPORA CORDIFOLIA

Alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic chemicals, and polysaccharides are among the substances found in *Tinospora cordifolia.*

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Fig. 2: Different Parts of *Tinospora Cordifolia*

A. Growth Constraints

For cultivation, *T. cordifolia* prefers medium black or red soil. Giloy may be cultivated in a wide range of soils, from sandy to clay loam, with success. However, in order for it to flourish, the soil must be well-drained, wet, and rich in organic matter.

Seeds and vegetative cuttings can be used to propagate *T.cordifolia.* However, both approaches are unsuitable for large-scale production and have issues with standard propagation methods. The primary issues connected with clonal propagation are low seed viability, poor seed set, and seed germination.Vegetative cuttings are also unsuitable because of their low production and reliance on weather conditions for continued growth. Plant tissue culture techniques may be acceptable approaches for large-scale production in less time and area, given the growth limits.

B. Nutritional and Elemental Analysis

Fiber (15.9%), ample protein (4.5% -11.2%), appropriate carbohydrates (61.66%), and low fat (3.1%) are all common in *T. cordifolia*, as with high potassium (0.845%), chromium (0.006%), iron (0.28%), and calcium (0.28%). It has a nutritional value of 292.54 calories per 100 g (M.I. Khan *et al*., 2011). The elemental makeup of deseeded *T. cordifolia* fruit was reported to be iron, copper, zinc, magnesium potassium, and sodium. The biological activities of many illnesses are linked to the lack or abundance of certain trace elements (B. Kavya *et al*., 2015).

C. Threats to T. cordifolia

Because of its extensive therapeutic benefits, this plant has been overexploited by pharmaceutical firms and those seeking traditional treatments, resulting in a severe shortage of this plant to fulfil current demand. *T. cordifolia* has been classified among 29 highly priority medicinal plants of India's agro-climatic zone 8 (Rajasthan, Uttar Pradesh, and Madhya Pradesh), as determined by the National Medicinal Plant Board in New Delhi. NMPB, New Delhi, India, has also categorized this plant among 178 medicinal plant species in high volume trade. As a result, this plant has been chosen for a review article to educate the public and scientific community about its morphology, growth constraints, a variety of chemical compounds, medicinal properties, pharmaceutical products, research work done to date in various aspects, various research projects sanctioned by various funding agencies, and so on.

D. Application of T. cordifolia

Tinospora cordifolia is used to treat a variety of ailments. For its general tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic, and anti-diabetic characteristics, the plant is widely utilised in Ayurvedic medicine. The herb is also used in Ayurvedic 'Rasayanas' to strengthen the immune system and immunity to illnesses. This plant's root has been shown to have anti-stress, anti-leprotic, and antimalarial properties (Nadkarni KM *et al*., 1982). In Ayurvedic literature, the plant is cited as a component of various chemical medicines that are used to treat general debility, dyspepsia, fevers, and urinary disorders. This climber has also been credited with antiviral effects against Ranikhet sickness in poultry. Tinospora stems are bitter, stomachic, and diuretic (Nayampalli SS *et al*., 1988), stimulating bile secretion, causing constipation, as well as quenching thirst, burning sensations, and vomiting. They also enrich the blood and heal jaundice. The mature stem extract is beneficial for skin problems (Aiyer KN *et al*., 1983; Raghunathan K *et al*., 1982). *T. cordifolia* root and stem are used as an antidote to snakebite and scorpion sting in conjunction with other medications (Kirtikar KR *et al*., 1982). Gout has been treated using a decoction of the leaves, and young leaves have been used as a liniment for erysipelas. Root is a strong emetic and is used to treat visceral blockage; its watery extract is also used to treat leprosy. Fruit that has been pulverised is used as a tonic, as well as for jaundice and rheumatism. This herb has been linked to the treatment of diabetes (StanelyMainzen Prince P *et al*., 1999; Stanely P *et al*., 2000; StanelyMainzen Prince P *et al*., 2001). In humans, *Tinospora cordifolia* has been used to treat throat cancer (Chauhan K., 1995). It has also been found that the plant species is effective in the treatment of allergic rhinitis (Badar VA *et al*., 2005).

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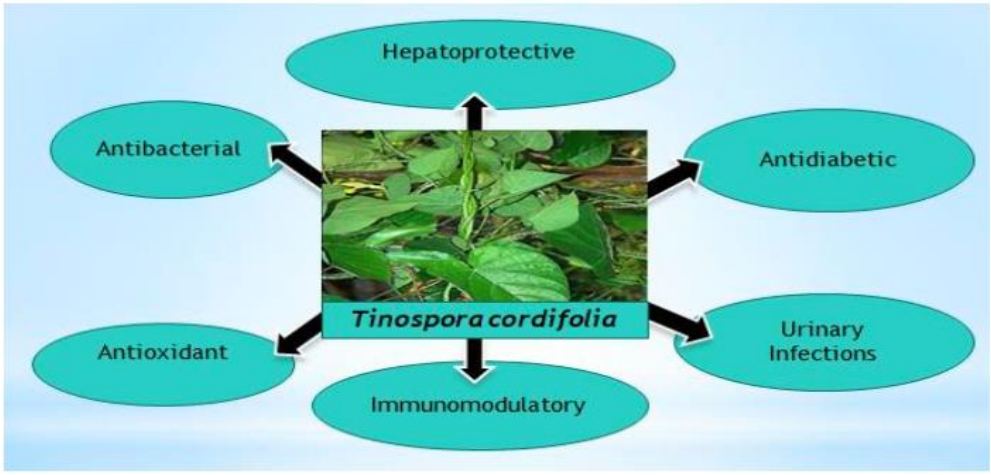

Fig. 3: Application of *Tinospora Cordifolia*

Tinospora cordifolia has also been shown to be effective in the treatment of a variety of bacterial infections. The plant, in particular, has antibacterial properties against a variety of disease-causing pathogens. A disc diffusion analysis 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 supports*Tinosporacordifolia's* usage in traditional medicine to treat a variety of infectious disorders (Jeyachandran R *et al*., 2003).

III. MICROPROPAGATION

Plants contain a wide range of therapeutic properties (Shrestha S*et al*., 2020).

All micropropagation techniques share the same fundamental characteristics of plant regeneration *in vitro*. Due to the unique totipotency of plant cells, micropropagation relies on the regeneration of new plants from small explants of various sources, and includes induced and enhanced cell division, formation of a callus tissue, further proliferation of the callus, and/or multistep differentiation and regeneration of events that lead to organogenesis (organ formation, i.e., roots and/or shoots) or somatic embryogenesis (seed-like embryos are formed from somatic cells).

Excision of an explant from a source plant, as well as exposure to culture medium components and changing environmental circumstances, are all part of micropropagation. Wounding reactions, as well as a number of physiological, hormonal, metabolic, and molecular responses, are triggered (which are shown in the box on the upper left-hand side). Dedifferentiation of certain explant tissues and increased cell division are the results of these processes (right-hand side boxes). Callus proliferation (left-hand side boxes) and/or redifferentiation are the outcomes of enhanced mitotic activity. When callus is sub cultured onto fresh growth media on a regular basis, mitotic activity continues and callus proliferates. Furthermore, after dedifferentiation, a new morphogenetic pattern is generated under the direction of cytoskeleton and cell cycle genes, which is followed by redifferentiation processes. This leads to the formation of new shoot and/or root meristems (organogenesis), as well as somatic embryogenesis. Dedifferentiation and redifferentiation are not required for micropropagation via improved growth of axillary branches.

Tinospora cordifolia is quickly disappearing from its native environment, despite its vast therapeutic benefits in traditional and modern health systems. Biotechnological options for rapid diffusion, scaling up secondary metabolites, and conserving precious, rare, and fragile medicinal plants should also be applied, even if the traditional strategy is insufficient to offset depletion (Sinha A*et al*., 2015). Because of the regeneration, induction, and micropropagation of calluses, the culture of plant tissue in the current sample was quite effective. Micropropagation *in vitro* is one of the greatest alternatives for quick clonal mass multiplication for a nice, healthy, high-yielding plant with minimal illness (Poudel K*et al*., 2018). Other technological approaches for creating organisms, such as genetically altered organisms and effective metabolite *in vitro* development, need cell culture (Mangal M*et al*., 2012). Tissue culture has been used to effectively reproduce the plant as an attractive alternative. It thrives in practically every type of soil and under a variety of environmental conditions. When the neem tree is properly trained, it will demonstrate a higher level of medicinal nobility. This can also be enhanced by sowing seeds during the monsoon, although seedlings develop much slower than cuttings. Seed viability, on the other hand, is extremely low, and seedlings are a major difficulty in large-scale clonal replication. The plant is hardy and may be harvested throughout the tropics and subtropics, but mostly in dry and wet environments. It can't handle a lot of rain or if there's a lot of water on the ground (Kattupalli S*et al*., 2019). The finding, replication, and survival of this species' sensitive genotypes may be aided by biotechnological technologies. Furthermore, biotechnological tools and techniques may be used to replicate and enhance acceptable genotypes, and improved micropropagation can be used for industrial processing of secondary plant metabolites.

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A. Objectives of Micropropagation and Related Applications

- Large-scale clonal propagation in situations where traditional vegetative propagation is impossible or impractical; when stock plant material is scarce; or where the traditional vegetative propagation coefficient (rate) is very low.
- Initial fast clonal multiplication of novel variations (using progeny from sexual breeding and uncommon wild species)
- Embryo rescue is the process of germination of seeds and embryos *in vitro* to salvage sexual progeny that would otherwise be nonviable.
- Pathogen-free propagation material was recovered.
- Crop enhancement with *in vitro* gene banks.
- Somatic cell genetics (haploid production from *in vitro* grown pollen, somatic protoplast fusion, or *in vitro* selection of somaclonal variation) breeding.
- The first transgenic (genetically modified) plants were created.

Micropropagation is a particularly efficient approach when it is combined with pathogen-free plant recovery and/or breeding programs, such as for the quick creation of sexual hybrids, the recovery of transgenic plants, and/or as a stage in somatic breeding.

B. Advantagesand Disadvantages of Micropropagation

Micropropagation has considerable quality, quantity, and cost benefits over traditional vegetative propagation for many species. However, there are drawbacks to micropropagation, as well as considerable gaps in our understanding of how and why plant tissue culture works. If the full potential of plant biotechnologies is to be realized, several factors must be considered. In commercial micropropagation, the hunt for new procedures and more favorable results continues.

a) Advantages
- Production of a Large Number of Clonal Propagules in a Relatively Short Time Span Using Conventional Techniques on the Same Plant.
- Production of disease-free plant material that can be free of viral, bacterial, and fungal contamination (Cassells AC., 1998; Cassells AC., 1997; Cassells AC. *et al*., 1998; Cassells AC., 1988).
- Production of a Large Stock of Clonal Propagation Material that is True to Type.
- The ability to safely ship large amounts of plant material in a timely, efficient, and cost-effective manner.
- The ability to quickly and in large quantities bring new technologies or newly bred plants and selections to market.

b) Disadvantages
- "Handicaps for Large-Scale Commercial Application of Micropropagation," Pierik wrote in 1988 (Kunert KJ *et al*., 2002).
- Internal infections, vitrification, and toxic exudates; increased ethylene and CO_2 levels; frequent mutations; lack of fundamental understanding of organogenesis or embryogenesis; special difficulties with woody species; internal infections; vitrification; and toxic exudates; Physical development variables (light, temperature, humidity, and the gas phase) are ignored; losses occur during the transition from *in vitro* to acclimation. a lack of actual automation and high labour expenses Many recently discovered procedures are uneconomical; "Commercial production is frequently under-controlled." Commercial micropropagationists are still dealing with the same or comparable challenges in the twenty-first century, over 20 years later, but from a position of greater awareness and appreciation for the complexity of marketed plant tissue culture.
- Contamination (Cassells AC., 1988)
- Somaclonal Variation at Unacceptably High Levels (Sasson A., 1993)
- Plant Stress
- High Production Costs

C. Micropropagation of Tinospora cordifolia in various Stages

- Shoot induction of *Tinospora cordifolia* nodal explants using shoot induction media containing silver nitrate, with the addition of KIN.
- After 20 days, shoot induction with KIN in M.S. media.
- The expanding initiation culture is sub-cultured again, this time with leaves.
- Elongation culture with KIN supplementation to create a single big leaf.
- Initial stage of multiplication in BA shoot culture media.
- On shoot induction medium, nodal explants produce several shoots.
- After 4 weeks, shoot multiplication in M.S. culture medium was allowed with BA.
- Shoot elongation with BA and KIN on M.S. medium.
- After 12 and 28 days, elongated shoots were supplemented with BA and KIN respectively.
- Elongated shoots were supplemented with BA and KIN after 12 and 28 days, respectively.
- *In vitro* rooting with IBA on half strength M.S. medium.
- Plantlets in pots that have been acclimated.
- Plantlets that have been acclimated in pots.
- *Tinospora cordifolia* plants in a container, five months old tissue cultivated.

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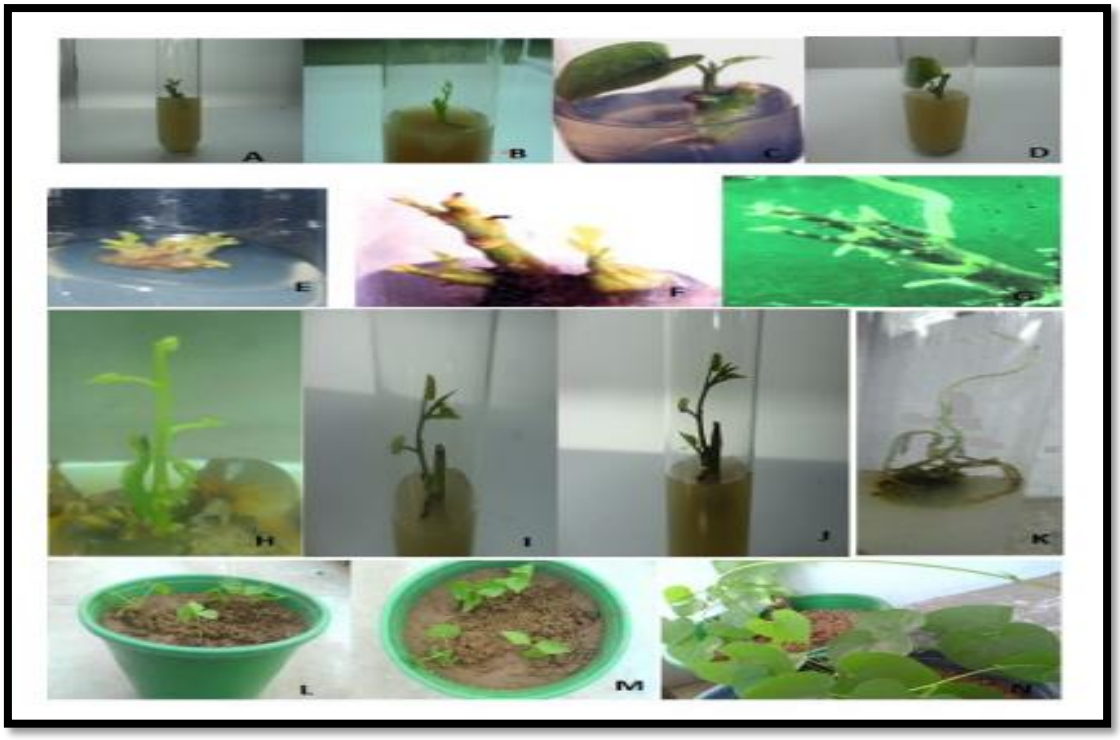

Fig. 4: Micropropagation stages of *T. Cordifolia*

IV. PLANT GROWTH REGULATORS (PGRS)

Plant growth regulators (also known as plant hormones or growth regulators) are substances that are used to control the growth of a plant or a plant component. Hormones are plant-produced chemicals that regulate typical plant activities such as root growth, fruit set and drop, growth, and other developmental processes.

Any substance or mixture of substances intended to accelerate or retard the rate of growth or maturation, or to otherwise alter the behavior, of ornamental or crop plants or their products, through physiological action; but does not include plant nutrients, trace elements, nutritional chemicals, plant inoculants, or soil amendments.

Plant growth regulators were first used in agricultural production in the United States in the 1930s. The first discovery and application were with acetylene and ethylene, which improved pineapple blossom output. As a result, their use has increased dramatically, and they are now important components of agricultural commodity production. Despite the fact that they are not real plant growth regulators, several herbicides and insecticides have a growth-regulating impact on plants.

A. Different PGRs and its Combination

Auxins regulate processes such as cell growth, cell wall acidification, cell division initiation, and meristem structure, resulting in either disorganized tissue (callus) or specified organs (usually roots), and they stimulate vascular differentiation. Auxins appear to be important actors in sustaining apical dominance, abscission, root development, tropistic curvatures, leaf senescence, and fruit ripening in organized tissue.

Cytokinin have two primary features that make them effective in culture: cell division stimulation (frequently in combination with auxins) and release dormancy of lateral buds (Binns, AN., 1994).

For routine usage, stock solutions of several PGRs such as cytokinin and auxins were produced and kept at 40°C. To proliferate axillary buds, different concentrations of several cytokinin, such as BA, KN, 2-iP, and TDZ, were utilised alone or in combination.*In vitro* rooting was achieved by elongating the started micro shoots in varying strengths of MS salts combined with variable concentrations of auxin, namely IAA and IBA.

Utilizing mature *in vivo* nodal explants of *T. cordifolia*, *in vitro* regeneration was effectively performed using a direct tissue culture approach. Only three cytokinins (BA, 2-iP and TDZ) showed a favourable response when tested alone at varying concentrations. Kn, on the other hand, elicited no reaction when administered alone. On MS medium combined with BA, the largest number of shoots length was obtained throughout the study. As the concentration of BA declined/increased beyond certain thresholds, the number of shoots and shot length reduced (Sivakumar V *et al*., 2014; Khanapurkar RS *et al*., 2012). In tylophora indica, Kn was responsible for optimum *in vitro* shoot development.

B. Effect of Cytokinin on Shoot Production

Within 15 days of inoculation on MS medium containing cytokinins, the infected shoot tips and bases developed shoot primordia ranging in quantity and length at all doses. The most shoots/explant were generated in basal MS medium with 2.0 mg/L BA, whereas KN was less effective. Individually varying the concentrations of BA and KN had no effect on the number of shoots or the duration of the

shoots. KN was mixed with BA in equal quantities at varied concentrations, assuming that it was significant in inducing shoot elongation. The number of shoots generated, as well as their length, showed a little improvement. At any concentration, there was no callus development on any of the explants. After 15–20 days of culture, multiple shoot development occurred.

C. Effect of Auxins on Shoot Production

To improve shoot production, the optimal concentration of cytokinins (BA 2.0 mg/L + KN 1.0 mg/L) was paired with various auxins (IAA and NAA). Within 15–20 days of culture, YST (8.2 shoots/explant) and MST (6.0 shoots/explant) on MS medium enriched with BA (2.0 mg/L) + KN (1.0 mg/L) + IAA (0.5 mg/L) showed a considerable increase in shoot number.

D. Effect of Additives on Shoot Production

AdS in the culture media greatly decreased shoot induction and the quantity of shoots, but GA_3 dramatically improved shoot formation and development. On the YST and MST, GA_3 in combination with BA (2.0 mg/L) + KN (1.0 mg/L) + IAA (0.5 mg/L) generated the maximum response of 92.1% and 91.3%, respectively. A shoots/explant with a good length were detected.

E. Effect of Antioxidants on Shoot Production

Various antioxidants (PVP, ascorbic acid, and charcoal) were utilized at varied doses to regulate phenolic exudation from cut ends, which significantly reduced the number and quality of shoots. PVP, followed by ascorbic acid, significantly decreased phenolic exudation among the treatments. On medium containing of AA, the greatest proportion of YST explants reacted with the greatest number of shoots per explant. Charcoal generated a good response, but increasing the dosage had a significant impact on the quantity of shoots. PVP and charcoal were the least effective, causing shoot induction and multiple shoot development to be inhibited, respectively.

V. CONCLUSION

T. cordifolia is a traditional medicinal plant in South Asia that has a wide range of biological properties and is mostly utilised as a therapeutic medication. Despite its amazing therapeutic potential, *T. cordifolia* is quickly disappearing from its native environment. As a result, it's critical to choose, identify, and conserve planting material using biotechnological improvement.Many hormone-like growth regulators have already been found, but despite the large list of their effects and advances in explaining certain signal transduction pathways, we still have a long way to go before we completely comprehend the complicated interplay between plant hormones. *In vivo* and *in vitro*, growth and development processes are too complicated, with interdependent physiological stages (competence, induction, determination, initiation, expression) that have diverse needs. As tissues travel through periods of changing sensitivity to certain hormones, a single phytohormone can play the role of inducer/stimulator or inhibitor several times throughout time.Nevertheless, for cells in culture, a variety of growth active substances (biochemical and/or morphogenic) are available to regulate their growth and differentiation. This enables the performance of systematic empirical investigations, particularly with recalcitrant tissues that do not react to traditional phytohormones. Although auxins and cytokinins will continue to be the most often employed plant hormones in tissue culture, it is obvious that many additional growth active chemicals may be used to improve growth and differentiation *in vitro*. Many of these possible phytohormones have not yet been studied *in vitro*, as stated in this article.

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