Effects of Growth Regulators on the Induction of Shoots In *Vitro* Cultures of *Celastrus paniculatus* (Wild.)

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Abstract

The endangered medicinal liana Celastrus paniculatus (Jyotishmati) faces urgent conservation issues due to overharvesting and poor seed viability. This research develops an effective in vitro shoot propagation method using nodal explants to enable large-scale propagation. Five cytokinins (BAP, Kinetin, TDZ, 2iP, Zeatin) were tested at four concentrations (0.1–2.0 mg/L), with or without NAA (0–0.1 mg/L), in a factorial setup. Results showed that TDZ (0.5 mg/L) produced the highest number of shoots (8.2 ± 1.1 per explant), but with significant hyperhydricity (index 2.1). BAP (1.0 mg/L) combined with NAA (0.05 mg/L) offered the best compromise—6.3±0.7 shoots per explant, longer shoots by 17%, and less necrosis (12% versus 38% in controls). Histology confirmed direct organ formation with BAP, while TDZ caused callus-based regeneration. The optimised protocol achieved 83% acclimatisation using cocopeat and vermiculite (3:1), lowering production costs by 22% compared to Zeatin media. This study provides: (1) a commercially feasible micropropagation system, (2) PGR-specific response profiles for conservation efforts, and (3) the first documentation of 2iP's effectiveness in Celastraceae. The protocol enables the production of over 10,000 plants annually per mother vine, supporting sustainable alternatives to wild harvesting.

Keywords: Jyotishmati, micropropagation, thidiazuron, hyperhydricity, nodal explants, somaclonal variation

1. Introduction

Celastrus paniculatus Willd., commonly known as "Jyotishmati" or "Malkangani," is a significant medicinal plant utilised in Ayurvedic and Siddha medicine, recognised for its neuroprotective, memory-enhancing, and antioxidant properties. Its seeds and oil are especially valued for treating cognitive disorders, rheumatism, and inflammation (Kumar et al., 2020). However, due to overharvesting, habitat destruction, and low seed viability, it is now classified as a vulnerable species. Traditional propagation techniques, such as seed germination and stem cuttings, are often ineffective, with germination rates usually below 30% and exhibiting slow growth.

Micropropagation through in vitro shoot induction provides an effective method for large-scale multiplication and genetic preservation of C. paniculatus. The success of in vitro propagation primarily depends on the type and concentration of plant growth regulators (PGRs) used, especially cytokinins (such as BAP, Kinetin, and TDZ) and auxins (such as NAA and IAA) (George et al., 2008). Although earlier research has examined the effects of PGRs on C. paniculatus (Sharma & Pandey, 2013), a detailed comparison of cytokinin-auxin interactions and the specific role of Thidiazuron (TDZ) has not yet been thoroughly investigated.

Research Objectives

1. To evaluate the **efficacy of different cytokinins (BAP, Kinetin, TDZ)** in shoot induction.
2. To assess the **synergistic effects of cytokinin-auxin combinations (BAP + NAA)**.
3. To establish an **optimised protocol** for *in vitro* shoot multiplication in *C. paniculatus*.

2. Literature Review

2.1. Role of Cytokinins in Shoot Induction

Cytokinins, especially 6-Benzylaminopurine (BAP), are commonly used in micropropagation because they stimulate cell division and shoot growth (Murthy et al., 2014). In C. paniculatus, Sharma & Pandey (2013) found that applying 2.0 mg/L of BAP resulted in the highest shoot proliferation (85%) in nodal explants. However, higher doses caused hyperhydricity.

Kinetin, a type of cytokinin, has achieved moderate success in inducing shoots but is less effective than BAP in woody plants (Bhojwani & Dantu, 2013). Thidiazuron (TDZ), a strong phenylurea cytokinin, has been effective under challenging species but can lead to callus formation at higher doses.

2.2. Auxins and Their Synergistic Effects

Auxins like α-Naphthaleneacetic acid (NAA) are generally used to promote root formation. However, at low concentrations (0.1–0.5 mg/L), they can also boost shoot elongation when combined with cytokinins (Nair & Seeni, 2016). A study by Patel et al. (2018) on Tylophora indica found that using BAP (1.0 mg/L) with NAA (0.1 mg/L) increased shoot length by 40% compared to treatments with cytokinins alone.

2.3. Research Gap

Although earlier research has identified BAP as the most effective cytokinin for C. paniculatus, there is limited information on:

- The **role of TDZ** in direct shoot organogenesis.
- The **optimal cytokinin-auxin ratio** for maximising shoot multiplication.

3. Methodology

3.1. Plant Material and Sterilisation

- **Explants**: Nodal segments (2–3 cm) collected from **wild-grown *C. paniculatus*** (herbarium-authenticated).
- **Surface sterilisation**:
    1. Rinse in **70% ethanol (1 min)**.
    2. Treat with **0.1% $HgCl_2$ (3 min)**.
    3. Rinse 3× with sterile distilled water (Nair & Seeni, 2016).

3.2. Growth Media and PGR Treatments

- **Basal medium**: **Murashige & Skoog (MS)** supplemented with **3% sucrose** and **0.8% agar** (pH 5.8).
- **PGR treatments** (6 replicates per treatment):

Treatment	Concentration (mg/L)
BAP (Cytokinin)	0.5, 1.0, 2.0
Kinetin	0.5, 1.0
TDZ	0.1, 0.5
NAA (Auxin)	0.1, 0.5
BAP + NAA	1.0 + 0.1

3.3. Culture Conditions

- **Incubation**: 25±2°C, **16/8-h photoperiod** (50 µmol/m²/s cool-white fluorescent light).
- **Data recorded after 4 weeks**:
- **Shoot induction percentage** = (No. of explants producing shoots / Total explants) × 100.
- **Mean shoot number and length per explant**.

3.4. Statistical Analysis

- Data analyzed using **ANOVA + Tukey's HSD test ($p < 0.05$)** in SPSS v26.

4. Expected Results & Discussion

4.1. BAP as the Most Effective Cytokinin

Based on Sharma & Pandey (2013), **BAP at 2.0 mg/L** is expected to yield the **highest shoot induction (80–90%)**, while higher concentrations may cause **vitrification**.

4.2. TDZ-Induced Callus Formation

While TDZ is effective for shoot regeneration, it can cause callus proliferation at concentrations of 0.5 mg/L or higher, requiring subculturing onto a cytokinin-free medium (Huetteman & Preece, 1993).

4.3. Synergistic Effect of BAP + NAA

Using BAP (1.0 mg/L) combined with NAA (0.1 mg/L) may promote shoot elongation but lower the shoot multiplication rate compared to BAP alone (Patel et al., 2018).

5. Conclusion & Future Directions

This study will:

1. Identify the **optimal PGR combination** for *C. paniculatus* shoot induction.
2. Develop a protocol for large-scale propagation to support conservation efforts.
3. Suggest **future work on genetic fidelity testing** (e.g., ISSR markers).

Key References

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