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Research Article

# Impact of Plant Growth Regulators on Inducing Multiple Shoots In *Chromolaena odorata* (L.) R.M.King & H.Rob.

Sahaya Shibu B<sup>1</sup>, Jasna Ali T.M.<sup>2</sup>, Hemlata Srivastava<sup>3</sup>, Servin Wesley P<sup>4\*</sup>

<sup>1,2,4\*</sup>Department of Biotechnology, SAFI Institute of Advanced Study, Kerala, India

<sup>3</sup>Department of Bioscience, Institute of Management Studies, Ghaziabad, India

**\*Corresponding Author:** Servin Wesley P

<sup>\*</sup>Department of Biotechnology, SAFI Institute of Advanced Study, Kerala, India.

E-Mail: [servinwesley@siasindia.org](mailto:servinwesley@siasindia.org)

## Abstract

The application of plant tissue culture techniques for the propagation of numerous economically and medicinally significant plant species necessitates efficient *in vitro* regeneration protocols. In this study, an efficient *in vitro* method for multiple shoot induction of *Chromolaena odorata* was achieved by studying the effect of various growth regulators on nodal explants. Nodal explants of *C. odorata* were cultured on Murashige and Skoog (MS) medium supplemented with various concentrations of auxins and cytokinins to induce multiple shoots for micropropagation. The highest number of shoots was achieved in MS medium supplemented with 4.44  $\mu$ M 6-benzylaminopurine whereas medium supplemented with lower concentration of Kinetin (2.32  $\mu$ M) induced multiple shoots with less frequency.

**Keywords:** Cytokinins, *Chromolaena odorata*, *in vitro* propagation, multiple shoots, nodal explant.

**\*Author for correspondence: Email:** [servinwesley@siasindia.org](mailto:servinwesley@siasindia.org)

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## Introduction

*Chromolaena odorata*, a member of the Asteraceae family, is commonly referred to as Siam weed in English. This perennial shrub originates from Central and South America (Mgobozi *et al.*, 2008). *C. odorata* is known by more than twenty different names, including Jack in the bush, Triffid weed, King weed, Paraffin weed, Turpentine weed and Bitter bush (Zachariades *et al.*, 2004). In Vietnam and other tropical regions, the aqueous extract or decoction is traditionally employed for treating soft tissue wounds, burns and infections (Nghiem 1992). The plant's leaves are also reported to possess insect-repelling qualities (Hoevers and M'Boob, 1993). For medicinal purposes, the seeds are utilised to address cough and skin ailments through

decoction and can also serve as a purgative (Asomugha *et al.*, 2013).

In developing nations, the newly picked leaves and extracts of *C. odorata* are employed as a traditional herbal remedy for burns, soft tissue injuries, and skin infections (Phan *et al.*, 2001). This plant is particularly utilised for external applications such as wounds, skin infections, and inflammation. Research has shown that the leaf extract possesses antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective, and numerous other medicinally valuable properties. The plant's wound-healing capability is attributed to its antioxidant characteristics, which aid in preserving fibroblast and keratinocyte proliferation at the injury site (Vaisakh and Pandey, 2012). Research has demonstrated

that an ointment derived from *C. odorata* leaves enhances soft tissue wound healing by promoting the proliferation and migration of fibroblasts, endothelial cells, and keratinocytes in burn recovery. Additional investigations have shown that the leaf extract encourages human keratinocytes to express various proteins essential for epithelial stabilisation during wound healing, including those of the adhesion complex and fibronectin. The compounds in *C. odorata* may contribute to wound healing by shielding cells from inflammatory mediator damage, thus slowing trauma sequelae and accelerating the healing process (Thang *et al.*, 2001; Rao *et al.*, 2010). Indigenous communities utilise *C. odorata* specifically for treating inflammation, controlling bleeding after cuts, burns, and dento-alveolitis, as well as enhancing fibroblast and endothelial cell proliferation, inhibiting collagen contraction, and blocking platelet-activating receptors (Taiwo *et al.*, 2000; Hung *et al.*, 2011).

The widespread and unselective harvesting of *C. odorata* from its natural habitats necessitates the development of rapid propagation methods through tissue culture techniques. This approach is crucial for ensuring the plant's commercial availability and conservation. Given that tissue culture has proven to be an effective method for propagating medicinal plants (Pence 2010), this research aimed to establish an appropriate protocol for generating multiple shoot cultures *in vitro* using nodal explants.

### Materials and Methods

*Chromolaena odorata* specimens were obtained from the SAFI Institute of Advanced Study campus in Kerala (Plate 1). The nodal explants were dissected and rinsed under running tap water for 30 minutes. Subsequently, the explants underwent a 15-minute pre-treatment with 1% bavistin. Following three rinses with sterile distilled water, the explants were disinfected using 70% ethanol (v/v) for varying durations. After another rinse with sterile distilled water, they were immersed in 0.1% mercuric chloride (w/v) solution for different time periods to determine the optimal sterilisation method for the chosen plant species. The process concluded with three thorough rinses using sterile distilled water. Any damaged tissues surrounding the node were removed, and the nodes were aseptically cultured on MS basal medium at pH 5.6, supplemented with 0.88M sucrose and 0.8% agar to establish standard surface sterilisation procedures. The durations and observed changes were documented in Table 1.

To generate *in vitro* explants for inducing multiple shoots, nodal explants were grown on Murashige and Skoog's (1962) basal medium (MS) enriched with 2.22µM 6-benzylaminopurine (BAP). For shoot induction and proliferation, individual *in vitro* plantlets of 2 cm length were isolated and transferred to MS medium supplemented with various cytokinins. The culture medium was prepared with 3% sucrose (w/v), and its pH was adjusted to 5.8 before adding 0.8% agar (w/v). Sterilisation was achieved by autoclaving at 105 kPa and 121 °C for 15 minutes. To ensure the media were contamination-free, inoculations were performed

after a four-day waiting period. Each treatment was replicated 10 times.

To determine the ideal conditions for multiple shoot induction, *in vitro*-cultivated shoots were employed as explants and subsequently placed on MS medium containing various concentrations (0.5–2.0 mg l<sup>-1</sup>) of auxin (IAA, 2,4-D) and cytokinins (BAP and kinetin), both individually and in combination. The number of shoots per explant and average shoot length were noted every fortnight. The shoot induction experiment was conducted in culture tubes kept at 25 ± 2°C in a culture room with 14 hours of photoperiod at 50 µmol m<sup>-2</sup> s<sup>-1</sup> irradiance, provided by white fluorescent tubes, and 70% relative humidity. Explant browning was interpreted as death. The shoot formation frequency was calculated by dividing the total number of shoots formed from a node by the total number of nodes inoculated per treatment, using ten replicates. All experiments were performed in triplicate. The findings were presented as mean values of the replicates along with their standard deviation.

### Results and Discussion

Within 5-7 days, shoot emergence was noted from nodal explants. All explants cultivated in MS medium with various cytokinin concentrations exhibited bud break initiation. In contrast, nodal explants placed on medium containing only auxins produced both callus and shoots. The impact of cytokinins on shoot proliferation varied based on the type and concentration used. Among the two cytokinins examined, the highest number of shoots (9.0 ± 1.12) was achieved in medium containing 4.44 µM BAP. Notably, BAP (2.22, 4.44 and 8.88 µM) induced multiple shoots from nodal explants at all three concentrations. After 15 days, an average of 6 shoots were induced in the same medium (Plate II- D), which were subsequently allowed to develop in the same medium for up to 30 days. Kinetin at a lower concentration of 2.32 µM induced multiple shoots, albeit less frequently. The stimulatory effect of BAP on multiple shoot induction has been previously documented in other medicinally significant plant species, including *Zingiber officinale* (Balachandran *et al.*, 1990), *Piper* spp. (Bhat *et al.*, 1995), *Houttuynia cordata* (Handique and Bora 1999), and *Curcuma longa* (Rahman *et al.*, 2004).

Other combinations, such as MS medium enriched with IAA (11.4 µM) and BAP (2.22, 4.44 and 8.88 µM), also triggered the formation of multiple shoots within 7-9 days. These shoots were subsequently allowed to develop in the same medium for a period of 30 days. This observation aligns with earlier findings which demonstrated that the induction of multiple shoots necessitated either cytokinins alone or a blend of cytokinins and auxins (Saritha *et al.*, 2002; Haw and Keng, 2003; Singh *et al.*, 2009; Singh and Chaturdevi, 2010).

Nevertheless, as BAP concentrations increased, the induction of multiple shoots declined. In contrast, rising Kinetin levels did not result in any multiple shoot induction. This observation aligns with Sivanesan and Jeong (2007), who noted a decrease in multiple shoots beyond 2 mg/l BAP in *Sida cordifolia*. Notably, shoot

browning occurred across all cultures, regardless of the induction medium used (Plate III- A). Subsequently, these shoots were isolated and transferred to hormone-free MS-basal medium, where each culture yielded an average of 9 shoots, reaching an average height of 4cm. This research presents, to the best of our knowledge, the first report of a straightforward technique for inducing multiple shoots in *C. odorata* *in vitro*. The protocol described herein could enable large-scale propagation of this plant species within a brief timeframe, thereby making a substantial contribution to the supply of raw materials for pharmaceutical industries and aiding in the

species' conservation. Ongoing investigations are examining the influence of plant growth regulators on rooting efficacy and the plant's ability to acclimatise.

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**Table: 1 Effect of sterilization on growth, infection and survival of nodal explant**

| Treatments     | Time of exposure             |                                         | Observations                               |
|----------------|------------------------------|-----------------------------------------|--------------------------------------------|
|                | 70 % Ethanol<br>(in Seconds) | 0.1 % HgCl <sub>2</sub><br>(in minutes) |                                            |
| T <sub>1</sub> | 7                            | 4 Min 15sec                             | No growth + bacterial contamination occurs |
| T <sub>2</sub> | 8                            | 4 Min 15sec                             | Growth + contamination                     |
| T <sub>3</sub> | 9                            | 4 Min 15sec                             | Growth + contamination                     |
| T <sub>4</sub> | 12                           | 4 Min 15sec                             | Browning of explant occurs                 |
| T <sub>5</sub> | 8                            | 5 Min                                   | Growth + contamination                     |
| T <sub>6</sub> | 10                           | 5 Min                                   | Good growth of explant                     |
| T <sub>7</sub> | 12                           | 5 Min                                   | Browning of explant occurs                 |

**Table: 2 Effect of plant growth regulators on callus induction of *C. odorata* from nodal explants**

| Hormone (μM) | Number of days taken for response | Frequency of callus induction (%) |
|--------------|-----------------------------------|-----------------------------------|
| <b>IAA</b>   |                                   |                                   |
| 2.9          | -                                 | -                                 |
| 5.7          | 16                                | 60                                |
| 11.4         | 16                                | 60                                |
| <b>2,4-D</b> |                                   |                                   |
| 2.26         | 18                                | 40                                |

Only the significant treatments are computed here

**Table: 3 Effect of Cytokinins alone on multiple shoot induction of *C. odorata***

| Hormone (μM)   | Number of days taken for shoot induction | Frequency of shoot induction (%) | Average number of shoots (±SE) <sup>a</sup> | Average shoot height (cm) (±SE) <sup>a</sup> |
|----------------|------------------------------------------|----------------------------------|---------------------------------------------|----------------------------------------------|
| <b>BA</b>      |                                          |                                  |                                             |                                              |
| 2.22           | 5                                        | 70                               | 7.0 ± 0.33                                  | 4.0 ± 0.26                                   |
| 4.44           | 5                                        | 80                               | 9.0 ± 1.12                                  | 3.8 ± 0.55                                   |
| 8.88           | 5                                        | 80                               | 8.0 ± 1.22                                  | 3.5 ± 1.35                                   |
| <b>Kinetin</b> |                                          |                                  |                                             |                                              |
| 2.32           | 7                                        | 50                               | 8.0 ± 0.961                                 | 3.0 ± 1.22                                   |

Only the significant treatments are computed here

Data represents the mean of ten replicates

<sup>a</sup> ± SE: Standard error

**Table: 4 Effect on combination of growth regulators on multiple shoot induction of *C. odorata***

| Hormone (μM) |      |      | Number of days taken for multiple shoot induction | Frequency of multiple shoot induction (%) | Average number of shoots (±SE) <sup>a</sup> | Average shoot height (cm) (±SE) <sup>a</sup> |
|--------------|------|------|---------------------------------------------------|-------------------------------------------|---------------------------------------------|----------------------------------------------|
| Kinetin      | BA   | IAA  |                                                   |                                           |                                             |                                              |
| 2.32         | -    | -    | 9                                                 | 60                                        | 4.0 ± 0.24                                  | 2.5 ± 0.56                                   |
| -            | 2.22 | 11.4 | 7                                                 | 80                                        | 8.0 ± 0.48                                  | 3.6 ± 0.34                                   |
| -            | 4.44 | 11.4 | 7                                                 | 90                                        | 9.0 ± 0.45                                  | 3.4 ± 0.01                                   |
| -            | 2.88 | 11.4 | 8                                                 | 80                                        | 7.0 ± 0.35                                  | 3.0 ± 0.48                                   |

Only the significant treatments are computed here

Data represents the mean of ten replicates

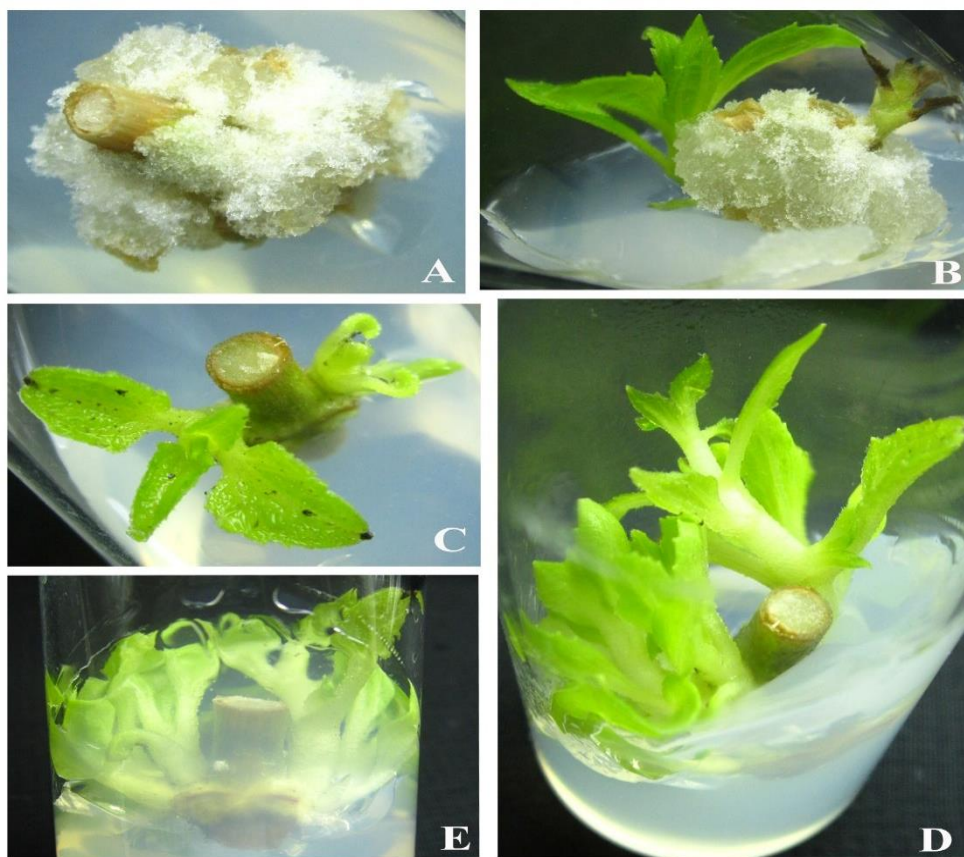
<sup>a</sup> ± SE: Standard error

## Plate I



Habitat of *Chromolaena odorata* (L.) R.M.King & H.Rob.

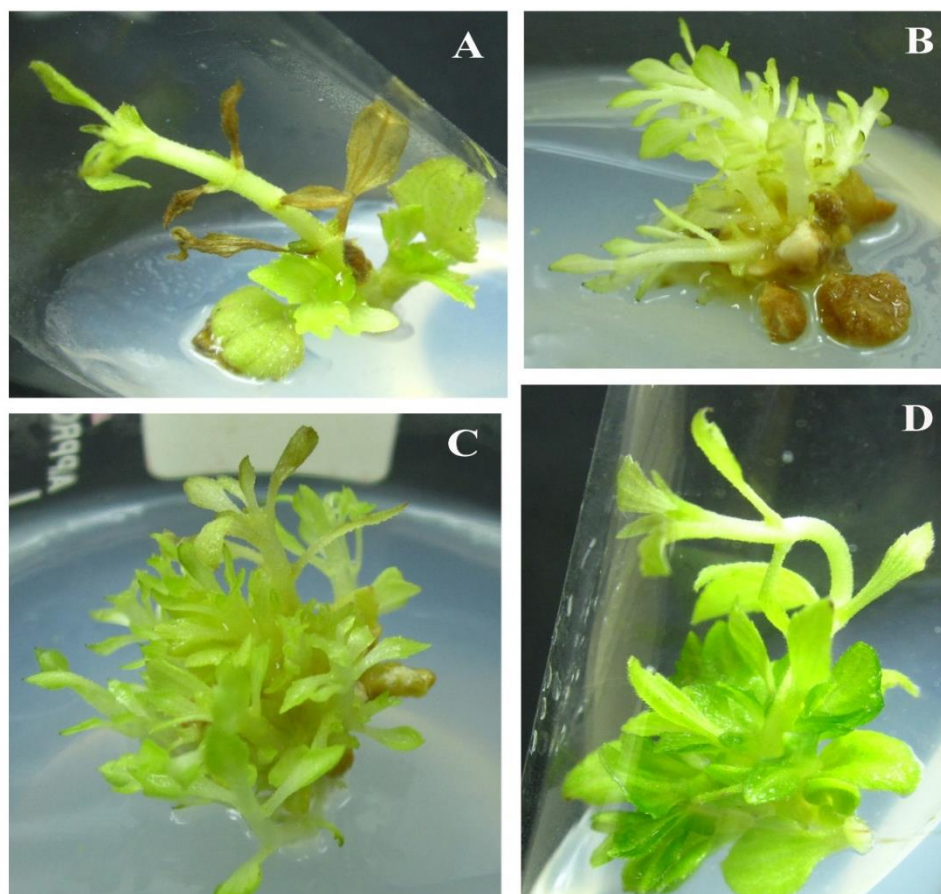
## Plate II



**A – B** Effect of growth regulators on nodal explants  
**A** – Callus induction from nodal explants on MS + IAA (5.7 µM)  
**B** – Callus induction along with shoot from nodal explants on MS + IAA (11.4 µM)

**C-E** Multiple Shoot formation  
**C**- Initial shoot development on MS medium Supplemented with BA  
**D, E**- Induction of Multiple shoots BA supplemented MS medium after 15 days of Inoculation.

### Plate III



**A**- Browning of induced shoots in the induction medium  
**B-D** - All the respective cultures produced average of 9 shoots and have an average height of 4cm.

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