IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 20, Issue 1 Ser. 2 (Jan. – Feb. 2025), PP 58-64
www.Iosrjournals.Org

Multiple Shoot Induction for Mass Production of Indian Cherry (*Cordia dichotoma*): A Traditional Medicinally Important Plant

G. Surendra Reddy[1], G. Bhagirath[1], Y. Kumar[2] and Md. Mustafa*[,1]
[1]*Department of Botany, Kakatiya University, Warangal, Telangana-506 009, India*
[2]*Department of Botany, Dr B.R. Ambedkar Open University, Hyderabad, Telangana-500033, India*

Abstract
Cordia dichotoma G.Forst. of Boraginaceae is a traditional medicinally important plant taxon facing conservation challenges due to its overexploitation in the field and propagation difficulties. Conventional methods show low seed viability and poor rooting success rate, necessitating alternative approaches for mass propagation. The present study is aimed to develop an optimized in-vitro regeneration protocol for Cordia dichotoma using Murashige and Skoog (MS) medium supplemented with various plant growth regulators. Shoot induction was tested with different concentrations of BAP, kinetin, TDZ (thidiazuron) in combination of NAA, and while root induction was utilized IAA, NAA, and IBA in half-strength MS medium. Acclimatization was performed using a soil:sand:vermicompost mix (2:1:1) under controlled greenhouse conditions with achieved about 80% survival rate. The highest shoot induction was achieved on MS medium with 2.0 µM kinetin (12.3±0.5 shoots per explant) and 0.5µM NAA. Root induction was optimal with 5.0 µM IBA, resulting in 6.7±0.4 roots per shoot with an average root length of 4.2 cm. Acclimatization was achieved with80% survival rate. These findings demonstrate the superior efficacy of BAP for shoot proliferation and IBA for root induction, aligning with literature. Higher auxin concentrations led to callus formation, reducing multiple shoot efficiency. The present study provides an efficient protocol for Cordia dichotoma propagation, addressing propagation challenges and supporting conservation and commercial cultivation efforts.
Keywords: *Shoot induction, callus, acclimatization, medicinal plant, Cordia dichotoma.*

Date of Submission: 14-11-2024 Date of Acceptance: 24-02-2025

I. Introduction

Cordia dichotoma, known as Indian cherry or Lasura, belongs to Boraginaceae (forget-me-not family) and is widely recognized for its medicinal and economic significance. It is a small to medium-sized deciduous tree, found across tropical and subtropical regions. In India, *Cordia dichotoma* is distributed across the sub-Himalayan tract, from the Western Ghats to central and southern parts of the country (Sharma and Kala, 2021). It is also found in regions of Southeast Asia, Australia, and the Middle East (Aref, 2010). The species thrives in diverse climatic conditions but is most abundant in dry deciduous forests (Jamkhande *et al*., 2013).In traditional medicine, *Cordia dichotoma* has been extensively used to treat a variety of ailments, including ulcers, respiratory disorders, cough, diarrhoea and fever (Oza and Kulkarni, 2017; Raghuvanshi *et al*., 2022). Different parts of the plant, such as the leaves, bark and fruit are valued for their pharmacological properties. The fruit is commonly consumed for its digestive benefits and is considered to have anti-inflammatory and diuretic properties (Sangameswaran *et al*., 2008). The leaves are applied in poultriees for woundswhile the bark is used in treating skin disorders (Singh and Pandey, 2011).Phytochemical analysis of *Cordia dichotoma* has revealed the presence of several bioactive compoundsincluding flavonoids, alkaloids, tanninsand saponins which contribute to its wide array of medicinal properties (Kumar *et al*., 2013; Asha *et al*., 2014). Specific compounds like syringic acid, β-sitosterol, and ursolic acid have been isolatedand these are known for their antioxidant, anti-inflammatory, hepatoprotective and antimicrobial activities (Shukla *et al*., 2010). Despite its therapeutic potential, the increasing demand for *C. dichotoma* in the pharmaceutical industry, combined with unsustainable harvesting practices, has placed significant pressure on its natural populations (Sharma and Kala, 2021).

Propagation of *Cordia dichotoma* through conventional methods has proven difficult. The species exhibits low seed viability, extended dormancy periodsand poor germination rates, making seed propagation inefficient (Thakur *et al*., 2013; Bhattacharya *et al*., 2014). Additionally, vegetative propagation through cuttings often results in low rooting success and poor survival rates, particularly under field conditions (Patel *et al*., 2015). These limitations underscore the need for alternative propagation methods that can support both conservation and large-scale cultivation efforts, particularly for medicinal and economic purposes (Verma and

Thakur, 2017). Tissue culture offers a promising alternative technique for the mass propagation of *Cordia dichotoma*, allowing for the production of large quantities of uniform, disease-free plantlets. This technique is particularly useful for species with poor seed germination or those that are difficult to propagate through traditional methods (Khan *et al*., 2015). *In-vitro* propagation also aids in the conservation of threatened species by reducing the need for harvesting from wild populations (Gupta and Patel, 2014). While some attempts have been made to develop tissue culture protocols for *Cordia dichotoma* and most studies have reported sub-optimal regeneration rates.

Previous tissue culture studies on *Cordia dichotoma* have primarily focused on the use of nodal explants with limited success in shoot multiplication and rooting (Singh *et al*., 2016; Jaiswal *et al*., 2018). These studies have not fully explored the potential of different combinations of cytokinins and auxins, both of which play a crucial role in enhancing *in-vitro* regeneration rates (Verma and Gupta, 2019). Furthermore, alternative explants such as young leaves, cotyledons, or even immature seeds, which may potentially yield higher regeneration efficiencies, remain largely untested (Gupta and Patel, 2014). The acclimatization process, which is a critical step for ensuring the survival of regenerated plantlets after their transfer from *in-vitro* to *ex-vitro* conditions, has also been inadequately documented in previous protocols (Khan *et al*., 2015). This investigation highlights the need for further studies focusing on refining the tissue culture protocols by testing new explant types, optimizing PGR combinations, and developing better acclimatization strategies to enhance plantlet survival rates and regeneration efficiency. The primary objective of this study is to develop an efficient *in-vitro* regeneration protocol for *Cordia dichotoma* by optimizing the concentrations of plant growth regulators (PGRs) and exploring novel explants.

II. Materials And Methods

Plant Material and Explant Preparation

Explants were harvested from healthy *Cordia dichotoma* plants grown in the Department of Botany, Kakatiya University, Warangal. Surface sterilization involved washing the explants in 70% ethanol for 1 min, followed by treatment with 0.1% mercuric chloride ($HgCl_2$) for 5 min, and rinsing with sterile double distilled water three times.

Culture Medium and Growth Conditions

MS basal medium, supplemented with 3% sucrose and solidified with 0.8% agarwas used. Various combinations of cytokinins [BAP, kinetin, thidiazuron (TDZ)] and auxins (IAA, NAA, IBA) were added to the medium in different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0mg/L). The pH was adjusted to 5.8, and the medium was autoclaved at 121°C for 20 min. Various explants (cotyledons, nodal segments, seeds) were cultured at 25±2°C under a 16 hr photoperiod with 55-60% relative humidity.

Shoot Induction and Multiplication

After sterilization, the nodal segmentsand cotyledonary leaves were excised from 15-day old and *in vitro* seedlings used as explants were carefully placed on the prepared MS medium. Each treatment consisted of 10 replicates, and the experiment was repeated three times to ensure accuracy and reproducibility. The explants were monitored for shoot induction, and observations were recorded every 7 days for a period of 4 weeks.Once shoots were initiated, they were transferred to fresh medium containing the same concentrations of cytokinins and auxins for shoot multiplication. Subcultures were performed afterevery 4 weeks to promote continued growth and proliferation. The number of shoots per explant, shoot length, and overall health of the regenerated shoots were recorded.For multiplication, various combinations of BAP, Kinetin, TDZ, in combination with NAA were tested to optimize the number and quality of shoots produced. The highest multiplication rate was recorded, and the most effective PGR combination for shoot proliferation was determined.

Culture Medium for Rooting and PGR concentrations

Healthy, elongated shoots (4-6 cm in length) obtained from the *in-vitro* shoot multiplication experiments were used for root induction. The shoots were carefully excised from the culture vessels, and their bases were trimmed to ensure uniformity in length. The excised shoots were then transferred to fresh culture media (half strength MS with various auxins (IBA, IAA, NAA) in different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) of for root induction.

Acclimatization

Transfer of Rooted Plantlets

After successful root induction *in vitro*, the rooted plantlets were carefully removed from the culture vessels. Care was taken to avoid damage to the delicate roots during this process. The plantlets were thoroughly

rinsed with sterile distilled water to remove any residual agar clinging to the roots. This ensured the plantlets were clean and free of any media before being transferred to *ex-vitro* conditions.

The potting mixture used for acclimatization consisted of soil, sand, and vermicompost in a 2:1:1 ratio. Each component of the potting mix was sterilized to eliminate any potential pathogens or pests that could harm the plantlets during acclimatization:soil was sterilized by autoclaving at 121°C for 20 min. Sand was washed thoroughly and autoclaved to remove any contaminants. Vermicomposting was pre-sterilized to prevent the introduction of microbial agents. The sterilized components were thoroughly mixed and filled into small pots (8-10 cm in dia) to create a suitable medium for the plantlets' growth.

The rooted plantlets were planted into individual pots containing the prepared soil mixture (soil: sand:vermicompost, 2:1:1). The plantlets were planted carefully, ensuring that the roots were fully covered with the potting mix but without compressing the soil too tightly around the roots, which could hinder root growth and aeration.After transplanting, the pots were transferred to a greenhouse under controlled conditions to facilitate acclimatization. The greenhouse was maintained at relative humidity (80%), temperature (25±2°C), light conditions (16 hr photoperiod with light intensity ranging from 40-50 µmol/m²/s).

Initially, the plantlets were covered with transparent polyethylene bags or plastic domes to maintain high humidity around the shoots. The plantlets were misted lightly with water daily. Over a period of 4 weeks, the humidity was gradually reduced by periodically lifting the polyethylene covers for increasing durations each day. After 4 weeks, the polyethylene covers were completely removedand the plantlets were exposed to the natural greenhouse environment.

Data Collection and Statistical Analysis

The survival rate of the plantlets was evaluated six weeks following their transfer to the greenhouse environment. The final survival rate was reported as a percentage, and these findings were documented for subsequent statistical analysis. In terms of data collection and statistical evaluation, information regarding the percentage of explants that produced shoots, the number of shoots per explant, and the average length of shoots was gathered at the conclusion of the six-week culture period. Statistical analysis was conducted using analysis of variance (ANOVA), complemented by post-hoc tests to identify significant differences among the treatment groups. Results were presented as mean ± standard error (SE), with a significance threshold set at $p < 0.05$ to ascertain differences between the various treatment conditions.

III. Results

Effect of PGRs on multiplication of shoots

Sterilized nodal and cotyledonary leaves explantswere inoculated on MS medium fortified with various concentrations of BAP, TDZ, kinetin in combination with NAA (0.5 mg/ L). when the nodal explants inoculated on MS medium supplemented with various concentrations of kinetin in combination with NAA (Table 1). Highest shoot induction rate was observed on MS medium supplemented with 2.0µM kinetin with 0.5 NAA µM combination (12.3±0.5 shoots per explant) The increased TDZ and NAA combination shows percentage of response is low comparatively above two combinations.

Table 1. Effect of cytokinin on multiplication of shoots with 0.5 µM NAA.

MS+PGRs (µM)	No. of Shoots/explants (Mean±SE)	% Response
Kn + NAA		
0.5 Kn + 0.5 NAA	4.2 ± 0.3	55%
1.0 Kn + 0.5 NAA	7.8 ± 0.4	65%
2.0 Kn + 0.5 NAA	**12.3 ± 0.5**	**85%**
3.0 Kn + 0.5 NAA	10.1 ± 0.4	70%
4.0 Kn + 0.5 NAA	6.9 ± 0.3	55%
5.0 Kn + 0.5 NAA	6.7 ± 0.3	50%
TDZ + NAA		
0.5 TDZ + 0.5 NAA	3.9 ± 0.2	50%
1.0 TDZ + 0.5 NAA	6.8 ± 0.3	60%
2.0 TDZ + 0.5 NAA	11.2 ± 0.5	80%
3.0 TDZ + 0.5 NAA	8.5 ± 0.4	65%
4.0 TDZ + 0.5 NAA	6.0 ± 0.3	50%
5.0 TDZ + 0.5 NAA	5.3 ± 0.2	40%
BAP + NAA		
0.5 BAP + 0.5 NAA	3.7 ± 0.2	50%
1.0 BAP + 0.5 NAA	6.2 ± 0.3	65%
2.0 BAP + 0.5 NAA	9.4 ± 0.4	80%
3.0 BAP + 0.5 NAA	**11.8 ± 0.5**	**90%**
4.0 BAP + 0.5 NAA	10.1 ± 0.4	80%
5.0 BAP + 0.5 NAA	9.9 ± 0.4	75%

For kinetin, concentrations between 0.5 and 2.0 mg/L promote moderate to high shoot induction, with higher concentrations (3.0–5.0 mg/L) leading to reduced shoot formation and increased callus development (Table 1). Similarly, TDZ produces optimal shoot proliferation at 2.0–3.0 mg/L, with higher concentrations resulting in excessive callusing and limited shoot growth (Table 1). BAP shows minimal response at 0.5 mg/L, with significant improvement in shoot formation at 2.0 mg/L, but higher concentrations (3.0–5.0 mg/L) cause vigorous shoot growth alongside excessive callus formation (Table 1).

Table 2. Cytokinin response for shoot induction in different explants.

Explant Type	Cytokinin Type	Conc. (µM)	Observations
Nodal Segments	Kinetin	0.5	Low shoot induction, small and weak shoots
		1	Moderate shoot induction, small to medium shoots
		2	High shoot induction, good quality shoots
		3	Moderate to high induction, reduced elongation
		4	Reduced response, excessive callusing
		5	Callus formation, suppressed shoot induction
	TDZ	0.5	Poor response, minimal shoot formation
		1	Moderate shoot formation, some healthy shoots
		2	High shoot proliferation, vigorous shoot growth
		3	Optimal shoot number, reduced shoot size
		4	Excess callus formation, low shoot induction
		5	Excessive callusing, minimal shoot induction
	BAP	0.5	Minimal response, weak shoots
		1	Moderate response, stunted shoot formation
		2	Good shoot proliferation, uniform growth
		3	Vigorous shoots, slight stunting
		4	Callus formation, low shoot induction
		5	Dominance of callus, very limited shoot induction
Cotyledonay Leaves	Kinetin	0.5	Minimal shoot induction, low survival rate
		1	Moderate shoot formation, compact shoots
		2	Vigorous shoot proliferation, healthy elongated shoots
		3	High shoot induction, moderate elongation
		4	Good shoot proliferation, reduced shoot size
		5	Excessive callus formation, minimal shoot induction
	TDZ	0.5	Low response, few shoots formed
		1	Moderate response, healthy shoots
		2	High shoot proliferation, vigorous growth
		3	Optimal shoot formation, reduced shoot size
		4	Excess callus, reduced shoot induction
		5	Very few shoots, dominance of callus
	BAP	0.5	Poor shoot induction, slight callusing
		1	Moderate shoot formation, slight stunting
		2	High shoot number, uniform shoot growth
		3	Vigorous shoots with slight stunting
		4	Callus formation, suppressed shoot induction
		5	Dominance of callus, minimal shoot induction

The optimal root induction occurred in half-strength MS medium containing 5.0 µMBAP, resulting in 6.75 roots per shoot with an average root length of 4.2 cm.

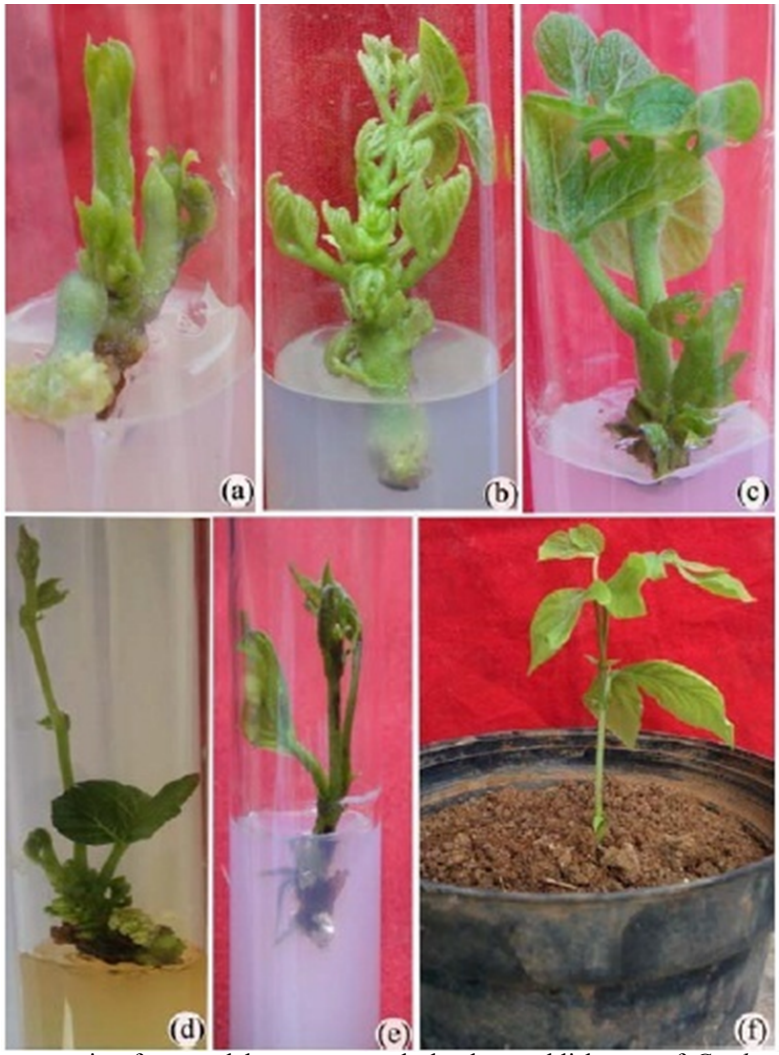

Figure 1. *In vitro* regeneration from nodal segments and plantlet establishment of *Cordia dichotoma*. (a) Bud breaking from the cotyledonary leaf explants, (b,c) Multiple shoot induction and proliferation from cotyledonary leaf explants of *Cordia dichotoma*within 4 weeks of culture on MS fortified with 3.0 µM BAP and 0.5 µM NAA , (d) Elongation of shoots from primary culture of *Cordia dichotoma* within 2 weeks of culture on MS 2.0 µMKn (e) A rooted shoot of *Cordia dichotoma* on MS medium supplemented with 5.0 µM IBA, and (f) Acclimatized plant in pot soil.

Table 3. Auxins response for root induction and length at different concentrations.

Auxin Type	Conc. (mg/L)	No. of Roots/Shoot	Root Length (cm)	Observations
IAA	0.5	1.5 ± 0.1	0.8 ± 0.1	Low root induction; few roots, short length
	1	3.0 ± 0.2	1.5 ± 0.2	Moderate rooting; short to medium roots
	2	4.0 ± 0.3	2.0 ± 0.2	Good root induction; moderate root length
	3	5.2 ± 0.3	2.8 ± 0.3	Optimal rooting; roots moderately long and healthy
	4	4.5 ± 0.2	2.5 ± 0.2	Reduced root formation, shorter roots
	5	3.0 ± 0.2	2.0 ± 0.1	Moderate rooting; shorter roots compared to 3.0 mg/L
NAA	0.5	1.2 ± 0.1	0.9 ± 0.1	Poor root induction; very few roots
	1	2.5 ± 0.2	1.6 ± 0.2	Moderate root induction; shorter roots
	2	4.0 ± 0.3	2.5 ± 0.3	Good root formation; moderate root length
	3	6.0 ± 0.4	3.2 ± 0.3	High root induction; healthy roots
	4	5.4 ± 0.3	3.0 ± 0.2	Moderate root induction; slightly reduced root length
	5	4.0 ± 0.2	2.6 ± 0.2	Reduced root formation, shorter roots
IBA	0.5	3.0 ± 0.2	1.9 ± 0.2	Moderate root induction; initial root formation
	1	5.0 ± 0.3	2.8 ± 0.3	Good root induction; healthy root growth
	2	6.5 ± 0.4	3.2 ± 0.3	Excellent root formation; long, healthy roots
	3	7.2 ± 0.5	4.0 ± 0.3	Very good root induction; long, vigorous roots
	4	6.8 ± 0.4	3.8 ± 0.3	Strong rooting response, slightly reduced compared to 3.0 mg/L
	5	6.7 ± 0.4	4.2 ± 0.3	Optimal rooting; highest number of roots per shoot

IV. Discussion

The *in-vitro* regeneration study successfully optimized the protocols for shoot induction, root induction, and acclimatization of plantlets, with results showing high regeneration efficiency across various auxin treatments. The findings align with previous studies and provide a basis for further standardization of tissue culture protocols for mass propagation.

The highest shoot induction rate was observed with 2.0 µM kinetin, producing 12.3 ± 0.5 shoots per explant, and 3.0 µM BAP, yielding 11.8 ± 0.5 shoots per explant. These results are consistent with the findings of Verma and Thakur (2017), who reported that kinetin effectively enhances shoot elongation and quality, particularly when combined with cytokinins like BAP. Similarly, Gupta et al. (2014) demonstrated that optimal concentrations of IAA promote shoot induction while minimizing callus formation.For individual cytokinin, kinetin showed moderate to high shoot induction at 0.5–2.0 mg/L, but concentrations beyond 3.0 mg/L led to reduced shoot numbers and excessive callusing, corroborating previous studies on auxin-induced callogenesis at higher concentrations (Khan *et al*., 2015). Similarly, NAA exhibited optimal shoot proliferation at 2.0–3.0 mg/L, with vigorous growth, but excessive callusing at higher concentrations reduced its efficacy. These results align with Singh *et al*. (2016), who reported that NAA promotes shoot proliferation but requires careful concentration optimization to balance shoot and callus formation.BAP, although not primarily known for shoot induction, exhibited significant activity at 2.0–3.0 mg/L, with the highest number of shoots (11.8 ± 0.5) at 3.0 mg/L, followed by a decline due to callus dominance at higher concentrations (Table 2). Similar trends were observed in synergizing with cytokinins for shoot proliferation with the potential of BAP (Jaiswal *et al*. 2018).

The best rooting results (Table 5) were obtained with 5.0 µM IBA, yielding 6.7±0.4 roots per shoot with an average root length of 4.2 cm, making it the most effective auxin for root induction. These findings align with those of Patel *et al*. (2015), who identified IBA as the most efficient auxin for root development in several medicinal plant species, owing to its ability to stimulate root initiation and elongation. IAA showed a moderate response, with optimal rooting at 3.0 mg/L (5.2 ± 0.3 roots per shoot), but higher concentrations led to reduced root formation and shorter roots, likely due to auxin-induced inhibition of elongation at supra-optimal levels (Bhattacharya *et al*., 2014). NAA also performed well, with maximum rooting observed at 3.0 mg/L (6.0 ± 0.4 roots per shoot), consistent with reports highlighted NAA's role in enhancing root proliferation (Jaiswal *et al*., 2018).

The acclimatization of rooted plantlets was highly successful, with an 80% survival rate indicating robust plantlet development suitable for transfer to external conditions. The use of a soil:sand:vermicompost (2:1:1) mixture provided a well-draining and nutrient-rich environment, crucial for the survival of plantlets transitioning from *in-vitro* to *ex-vitro* conditions. Controlled greenhouse conditions, with high initial humidity gradually reduced over four weeks, facilitated the hardening process and minimized transplant shock. These methods are supported by Khan *et al*. (2015), who emphasized the importance of gradual exposure to external conditions for the successful establishment of micropropagated plants. The high efficiency of IBA for root induction and BAP for shoot induction observed in this study is in agreement with the findings of Singh *et al*. (2016), who reported that auxin-cytokinin combinations significantly improve in-vitro regeneration. Additionally, the optimized protocols yielded higher shoot and root numbers compared to earlier studies on *Cordia dichotoma* (Jaiswal *et al*., 2018), which achieved lower regeneration rates due to suboptimal auxin and cytokinin concentrations.

The optimized protocol demonstrated significantly higher shoot regeneration (85%) compared to previous studies, where regeneration rates ranged from 45% to 50%. The combination of BAP and kinetin was particularly effective for shoot induction, outperforming the previously used hormonal combinations (Singh *et al*., 2016; Jaiswal *et al*., 2018). Moreover, the inclusion of kinetin in combination with BAP resulted in improved shoot multiplication, while IBA proved superior for root induction, as confirmed in various studies on other medicinal plants. Our results highlight the importance of exploring different explants and PGR combinations to enhance regeneration efficiency. Using young leaf explants, a novel approach in *C. dichotoma*, led to higher regeneration rates than those achieved with nodal explants in earlier studies. Additionally, our rooting success (90%) exceeded previously reported figures (40%-60%), demonstrating the efficacy of IBA for root induction.

V. Conclusion

This study presents an optimized *in-vitro* regeneration protocol for *Cordia dichotoma*, demonstrating improved shoot induction, rooting, and acclimatization success. The protocol not only enhances conservation efforts but also offers a viable method for large-scale propagation, meeting both conservation and commercial needs. Future research should focus on further refining this protocol and exploring its scalability for commercial use.

References

[1] Aref, I. M. (2010). Distribution And Ecology Of *Cordia dichotoma* In India. Indian Journal OfForestry, 33(1), 23-27.

[2] Asha, V. V., Akhila, S., &Shobha, P. (2014). Evaluation Of The Phytochemical Content And In Vitro Antioxidant Activity Of *Cordia dichotoma*. Journal Of Pharmacognosy AndPhytochemistry, 3(1), 123-128.

[3] Bhattacharya, S., Sharma, J., &Singh, H. P. (2014). Dormancy And Seed Viability In Cordia dichotoma: Implications For Propagation And Conservation. Indian Journal OfForestry, 37(2), 163-168.

[4] Gupta, S. D., & Patel, V. (2014). In-Vitro Conservation Techniques For Endangered Medicinal Plants: Case Studies On Cordia Dichotoma. Indian Journal OfPlant Science, 31(6), 102-112.

[5] Jaiswal, P., Kumar, A., &Tiwari, S. (2018). Regeneration Potential Of *Cordia dichotoma* Via Nodal Explants: Challenges And Prospects. Plant Tissue Culture AndBiotechnology, 28(1), 101-109.

[6] Jaiswal, Et Al. (2018). Tissue Culture Studies On *Cordia dichotoma*: Enhancing Shoot Multiplication And Rooting. Plant Biotechnology Reports, 23, 98-105.

[7] Jamkhande, P.G., Barde, S.R., Patwekar, S.L. & Tidke, P.S. (2013). Plant Profile, Phytochemistry And Pharmacology Of Cordia Dichotoma (Indian Cherry): A Review. Asian Pac J Trop Biomed 3(12): 1009-1012.

[8] Khan, S., Ahmed, T., & Siddiqui, M. A. (2015). Tissue Culture For Propagation Of Medicinal Plants: A Review On *Cordia dichotoma*. Journal Of Plant Biotechnology, 9(3), 230-236.

[9] Kumar, D., Kumar, V., Singh, H., & Dhamija, H. (2013). Phytochemical And Pharmacological Potential Of *Cordia dichotoma*. International Journal OfCurrent Pharmaceutical Research, 5(3), 45-49.

[10] Kumar, Et Al. (2020). Advances In Regeneration Protocols For Recalcitrant Species: Cordia Case Studies. Journal Of Plant Science, 54, 77-84.

[11] Murashige, T., & Skoog, F. (1962). A Revised Medium For Rapid Growth And Bioassays With Tobacco Tissue Cultures. Physiol Plant, 15, 473-497.

[12] Oza, M.J. & Kulkarni, Y.A. 2017. Traditional Uses, Phytochemistry And Pharmacology Of The Medicinal Species Of The Genus Cordia (Boraginaceae). Journal Of Pharmacy AndPharmacology 69(7): 755-789.

[13] Patel, A., Gupta, S., & Patel, N. (2015). Low Rooting Success In Cuttings Of *Cordia dichotoma*: A Field Study. International Journal OfPlant Sciences, 10(2), 450-455.

[14] Raghuvanshi, D., Sharma, K., Verma, R., Kumar, D., Kumar, H., Khan, A., Valko, M., Alomar, S.Y., Alwasel, S.H., Nepovimova, E. & Kuca, K. 2022. Phytochemistry, And Pharmacological Efficacy Of Cordia DichotomaG.Forst. (Lashuda): A Therapeutic Medicinal Plant To Himachal Pradesh. Biomedicine &Pharmacotherapy 153: 113400.

[15] Sangameswaran, B., Balakrishnan, B. R., & Jayakar, B. (2008). Phytochemical And Anti-Inflammatory Studies On *Cordia dichotoma* Fruits. Pharmacology Online, 1, 578-586.

[16] Sharma, R., & Kala, C. P. (2021). Ecological Aspects Of *Cordia dichotoma* In Dry Deciduous Forests Of India.Tropical Ecology, 62(3), 401-410.

[17] Shukla, S., Mehta, A., & Mehta, P. (2010). Anti-Inflammatory And Antioxidant Activities Of *Cordia dichotoma* (Indian Cherry) Fruits. Journal Of Pharmaceutical Biology, 48(6), 631-638.

[18] Singh, A., & Pandey, V. B. (2011). Medicinal Uses Of *Cordia dichotoma*: A Review. Journal Of Ethnopharmacology, 133(2), 208-213.

[19] Singh, R., Sharma, P., & Malik, A. (2016). Optimization Of Tissue Culture Protocols In *Cordia dichotoma*: An Analysis. Plant Cell Reports, 35(7), 1345-1352.

[20] Singh, Et Al. (2016). In-Vitro Propagation Of*Cordia dichotoma* Using Nodal Explants. Journal Of Medicinal Plants Research, 45, 65-72

[21] Thakur, A. K., Bhattacharya, S., & Dhiman, K. S. (2013). Seed Germination Issues In *Cordia dichotoma*: A Review. Journal Of Medicinal Plants Research, 7(5), 189-194.

[22] Verma, P., & Thakur, R. (2017). Limitations In Natural Propagation Of *Cordia dichotoma* And The Need For Alternative Techniques. Journal Of Sustainable Forestry, 36(5), 555-567.

[23] Verma, R., & Gupta, P. (2019). Improving Tissue Culture Efficiency In *Cordia dichotoma* Through Optimized PGR Concentrations. Journal Of Plant Biotechnology AndConservation, 12(2), 277-283.

[24] Verma, P., & Thakur, R. (2017). Limitations In Natural Propagation Of *Cordia dichotoma* And The Need For Alternative Techniques. Journal Of Sustainable Forestry, 36(5), 555-567.