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Influence of auxin and rooting substrate on adventitious rooting in *Chukrasia tabularis* mini-cuttings: Implications for clonal propagation

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Abstract
Chukrasia tabularis, a secondary timber with potential for plantation forestry and genetic conservation, exhibits rapid growth and variable seed output. Yet, shoot borer susceptibility affects stem shape, while short seed viability and heterozygosity management are vital for quality. The innovative technique of mini-clonal propagation holds significant importance in tree improvement initiatives, especially for efficiently propagating superior genetic variants of the tree species on a larger scale. As *Chukrasia tabularis* is widely utilized for timber, effective methods for clone propagation become crucial. This newfound knowledge aids propagators, geneticists, and tree enhancement experts in optimizing propagation approaches, reducing costs, and improving root formation success. In this study the effects of varying concentrations of IBA (Indole-3-butyric acid), IAA (Indole-3-acetic acid), and NAA (Naphthaleneacetic Acid) powders (500 ppm, 1000 ppm, 1500 ppm, and 2000 ppm), alongside untreated cuttings as controls were investigated. Stem cuttings of 10-15 cm were immersed in these growth hormones before planting in 4 different rooting media (cocapith: perlite mixture, cocapith: perlite+ biochar, sand: soil and decomposed cocapith: perlite). The study discovered that treating *Chukrasia tabularis* stem cuttings with IBA at 1000 ppm before planting in the cocapith:perlite mixture significantly enhanced root development (74%) and survival rates (99%).

Keywords: *Chukrasia tabularis,* mini clonal, IBA, IAA, NAA

Introduction
Chukrasia tabularis, native to Southeast Asia and also known as Chittagong wood or Indian mahogany, is a deciduous tree prized for its durable, termite-resistant wood (Dorthe Jøker, 2000) [11]. This medium-sized tree can reach heights of 40 meters, featuring a wide crown, pinnate leaves with 5–11 leaflets, and a straight trunk. Fragrant clusters of yellowish-white flowers are followed by small winged seeds. Propagation through stem cuttings is effective; mature, disease-free stems with 2-3 nodes and lengths of 15-20 cm are used (Kalinganire and Pinyopusarerk, 2000) [12].

Stem-cutting propagation offers advantages like preserving genetic traits and rapid clone multiplication. It aids the successful cultivation of this valuable timber species when properly managed. In today's global market, forestry plantations for industrial purposes must meet customer demands, achieve cost-effectiveness, ensure sustainability, and minimize environmental impact. Raw material's impact on overall performance should be considered for enhanced competitiveness (Parthiban and Fernandaz 2017) [20]. Tree breeding programs are pivotal in achieving rapid genetic gains. Effective vegetative propagation methods, like cloning systems, are crucial. Such methods rapidly translate genetic gains into industrial benefits. Inter-specific hybridization and clonal forestry are efficient strategies. They yield high-quality tree clones with desirable traits, ensuring environmentally friendly products that meet customer demands, enhancing market competitiveness (Assis and T.F., 2000) [1].

Vegetative propagation maintains genetic superiority and boosts production for economically significant tree species (Tewari, 1994) [24]. Standardizing species-level vegetative propagation methods is essential for mass multiplying planting stock. Agro-forestry and social forestry satisfied 80% of wood-based industries' raw material needs (Kulkarni, 2008). Forest-based industries rely on vegetative propagation for productivity, quality, pest resistance, and coppicing ability (Campinhos and Ikemori, 1983) [5]. Eucalyptus vegetative propagation was standardized industrially using mature plants (Boulay *et al.*, 1987) [3].

The study aimed to explore the impact of various growth hormones, concentrations, and rooting media on *Chukrasia tabularis* mini cutting root induction.

Materials and Methods

The study was conducted at Forest College and Research Institute in Mettupalayam, Tamil Nadu, India (11°9 N latitude and 77°56 E longitude). The region experiences hot summers and cold winters in a semi-arid tropical environment. The annual average rainfall in the area is 895 mm. The average highest and lowest temperatures in the area are approximately 30 °C and 20 °C, respectively.

The stem cuttings were collected in different months from February to August and a low-cost polytunnel was utilized for the rooting investigation. The experiment followed a Completely Randomized Block design with three replications.

Standardization of protocol for stem cuttings

Stem cuttings (10-15 cm) from the mother tree were immersed in 2% Bavistin and treated with IBA, IAA, and NAA at various concentrations. Control cuttings were untreated. Planted in different root trainers with cocapith: perlite, cocapith: perlite + biochar, Sand: soil, and decomposed cocapith. Softwood and semi-hardwood cuttings were placed in a polytunnel, 70-80% humidity, 25-35 °C. Rooting parameters were assessed at 6 and 12 weeks.

Statistical analysis

ANOVA highlighted significant interactions (@0.5 significance) between auxins, concentrations, root count, length, and survival.

Results and Discussion

After 45 days of treatment, the study found IBA positively influenced *Chukrasia tabularis* stem cutting root initiation and growth. After 12 weeks, analysis showed significant effects of IBA, IAA, and NAA plant hormones, varied concentrations, and different growth mediums cocapith perlite (3:1), cocapith perlite+biochar (3:1 +10gm/kg), sand: soil (1:3), and decomposed cocapith on rooting parameters.

Effect of auxin treatment in cocapith: perlite rooting media

In this study, the effects of three auxins - IBA, IAA, and NAA - were investigated on root growth using a cocapith: perlite-based rooting medium. Particularly, IBA exhibited the most favorable outcomes (Table 1). Mini-cuttings in a sand-based medium treated with 1000 ppm IBA showed superior root formation (74%), 10.67 roots, 10.33 cm length, and 99% survival. A 1500 ppm IBA treatment resulted in lower root formation (28.50%), 3.33 roots, 10.33 cm length, and 50% survival. For IAA, 1000 ppm yielded 18.50% root formation, 2.67 roots, 5.67 cm length, and 20% survival. The 1500 ppm IAA treatment had 3.70% root formation, 1.33 roots, 5.66 cm length, and 10% survival. Regarding NAA, 1500 ppm led to 4.74% root formation, 1.33 roots, 1.17 cm length, and 3% survival. The 1000 ppm NAA treatment resulted in 2.59% root formation, 1.67 roots, 1.50 cm length, and 3% survival.

Table 1: Optimization of growth hormone concentration for clonal propagation of *Chukrasia tabularis*

Sl. No.	Treatments		Rooting %	Root number per cutting	Root length per cutting (cm)	Survival %
1	IBA	1000	74.00	10.67	10.33	99.00
		1500	28.50	3.33	10.33	50.00
		2000	9.85	0.67	0.83	10.00
2	IAA	1000	18.50	2.67	5.67	20.00
		1500	3.70	1.33	5.66	10.00
		2000	1.85	1.00	0.84	5.00
	NAA	500	1.70	1.00	5.17	5.00
		1000	2.59	1.67	1.50	3.00
		1500	4.74	1.33	1.17	3.00
4	Control		0.00	0.00	0.00	0.00
	Grand mean		14.543	2.367	2.94	20.5
	S.E(d)		0.608	0.098	0.119	1.676
	C.D (0.05)		1.278	0.205	0.251	3.520

The impact of auxin treatment within a cocapith: perlite + biochar rooting medium

IBA exhibited the most favorable outcomes among the tested auxins (IBA, IAA, NAA), as detailed in Table 2. Particularly, the 1000 ppm IBA treatment displayed superior results: 70% root formation, 9.83 roots, 7.67cm length, and 95% survival. The 1500 ppm IBA treatment resulted in 17.55% root formation, 4.67 roots, 3.00cm length, and 45% survival.

Regarding IAA, the most effective results were observed with the 1000 ppm treatment: 17.55% root formation, 5.33 roots, 2.67cm length, and 20% survival. The 1500 ppm IAA treatment showed 1.96% root formation, 1.17 roots, 0.68cm length, and 6% survival.

For NAA, the 1000 ppm treatment yielded best results in 3.9% root formation, 1.5 roots, 1.66cm length, and 3% survival. The 500 ppm NAA treatment exhibited 1.95% root formation, 0.83 roots, 0.68cm length, and 4.5% survival.

Table 2: Optimization of growth hormone concentration for clonal propagation of *Chukrasia tabularis* (Cocapith: perlite + biochar) rooting media

Sl. No.	Treatments		Rooting %	Root number per cutting	Root length per cutting	Survival %
1	IBA	1000	70.2	9.83	7.67	95.00
		1500	17.55	4.67	3.00	45.00
		2000	1.95	1.17	1.00	7.00
2	IAA	1000	17.55	5.33	2.67	20.00
		1500	1.96	1.17	0.68	6.00
		2000	1.94	1.00	0.67	3.00
	NAA	500	1.95	0.83	0.68	4.50
		1000	3.9	1.50	1.66	3.00
		1500	0.78	0.33	0.33	2.00
4	Control		0.00	0.00	0.00	0.00
	Grand mean		11.78	2.37	2.58	18.55
	S.E(d)		1.23	0.147	0.147	0.424
	C.D (0.05)		2.58	0.309	0.309	0.891

The impact of auxin treatment within sand: soil rooting medium

IBA, IAA, and NAA were evaluated as auxins, with IBA yielding the most advantageous results, as shown in Table 3. Significantly, the 1000 ppm IBA treatment displayed superior outcomes: 15% root formation, 5.11 roots, 6.21cm length, and 70% survival. The subsequent effective treatment involved 1500 ppm IBA, resulting in 3% root formation, 4.7 root count, 4.27cm length, and 43% survival.

In the case of IAA treatment, the best results were seen with 1000 ppm IAA, showing 4% root formation, 3.38 roots, 1.51cm length, and 20% survival. The 1500 ppm IAA treatment demonstrated 1.5% root formation, 2.19 roots, 0.7 cm length, and 6% survival.

For NAA treatment, optimal outcomes were attained with 500 ppm NAA, leading to 1.40% root formation, 1.67 roots, 0.91cm length, and 10% survival. Subsequently, the 1000 ppm NAA treatment displayed 1.00% root formation, 1.59 roots, 1.01 cm length, and 5% survival.

Consistent with prior findings, the 1000 ppm IBA concentration yielded superior root formation, count, and length.

Table 3: Optimization of growth hormone concentration for clonal propagation of *Chukrasia tabularis* (sand: soil) rooting media

Sl. No.	Treatments		Rooting %	Root number per cutting	Root length per cutting	Survival %
1	IBA	1000	15.00	5.11	6.21	70.00
		1500	3.00	4.70	4.24	43.00
		2000	0.30	2.03	2.81	3.00
2	IAA	1000	4.00	3.38	1.51	20.00
		1500	1.50	2.19	0.70	6.00
		2000	0.50	0.98	0.11	2.00
	NAA	500	1.40	1.67	0.91	10.00
		1000	1.00	1.59	1.01	5.00
		1500	0.20	0.78	0.30	3.00
4	CONTROL		0.00	0.00	0.00	0.00
	Grand mean		2.99	2.24	1.78	16.20
	S.E(d)		0.12	0.09	0.05	0.682
	C.D (0.05)		0.25	0.19	0.09	1.432

Effect of auxin treatment in cocapith: perlite rooting media

Auxins IBA, IAA, and NAA were assessed, with IBA yielding the best results (Table 4). The 1000 ppm IBA treatment displayed excellence 8% root formation, 4.78 roots, 4.24cm root length, and 64% survival. The 1500 ppm IBA treatment exhibited 1% root formation, 3.03 roots, 1.81cm root length, and 40% survival.

For IAA, 1000 ppm IAA showed the highest effectiveness: 2% root formation, 3.20 roots, 0.56cm root length, and 20% survival. The 1500 ppm IAA treatment had 1.02% root formation, 2.19 roots, 0.12cm root length, and 6% survival.

In the case of NAA, the 500 ppm NAA treatment achieved 1% root formation, 1.67 roots, 0.29cm root length, and 8% survival, while the 1000 ppm NAA treatment resulted in 0.63% root formation, 1.46 roots, 0.09cm root length, and 4.5% survival.

Table 4: Optimization of growth hormone concentration for clonal propagation of *Chukrasia tabularis* (decomposed Cocapith: perlite) rooting media

Sl. No.	Treatments		Rooting %	Root number per cutting	Root length per cutting	Survival %
1	IBA	1000	8.00	4.78	4.24	64.00
		1500	1.00	3.03	1.81	40.00
		2000	0.30	1.54	0.49	13.00
2	IAA	1000	2.00	3.20	0.56	20.00
		1500	1.02	2.19	0.12	6.00
		2000	0.35	0.98	0.11	3.00
	NAA	500	1.00	1.67	0.29	8.00
		1000	0.63	1.46	0.09	4.50
		1500	0.29	0.40	0.03	3.30
4	CONTROL		0.00	0.00	0.00	0.00
	Grand mean		1.46	1.93	0.77	16.18
	S.E(d)		0.11	0.10	0.07	1.45
	C.D (0.05)		0.23	0.20	0.15	3.04

The interaction between auxin types and concentrations significantly affected root formation, root count, root length and survival. 1000 ppm IBA consistently yielded better results in terms of root formation, root count, and root length, as seen in the earlier results.

The study revealed that among the different hormonal concentrations tested, treatment with 1000 ppm of IBA (Indole-3-butyric acid) in cocapith: perlite rooting media proved to be the most effective and beneficial for simulating higher rooting in stem cuttings of *Chukrasia tabularis*. The application of exogenous IBA (Indole-3-butyric acid), IAA (Indole-3-acetic acid) and NAA (Naphthaleneacetic Acid) might have stimulated carbohydrate metabolism, leading to the release of energy within the stem cuttings. This increased energy availability could have played a crucial role in supporting the initiation and development of adventitious root primordia in the rooting zone of the cuttings.

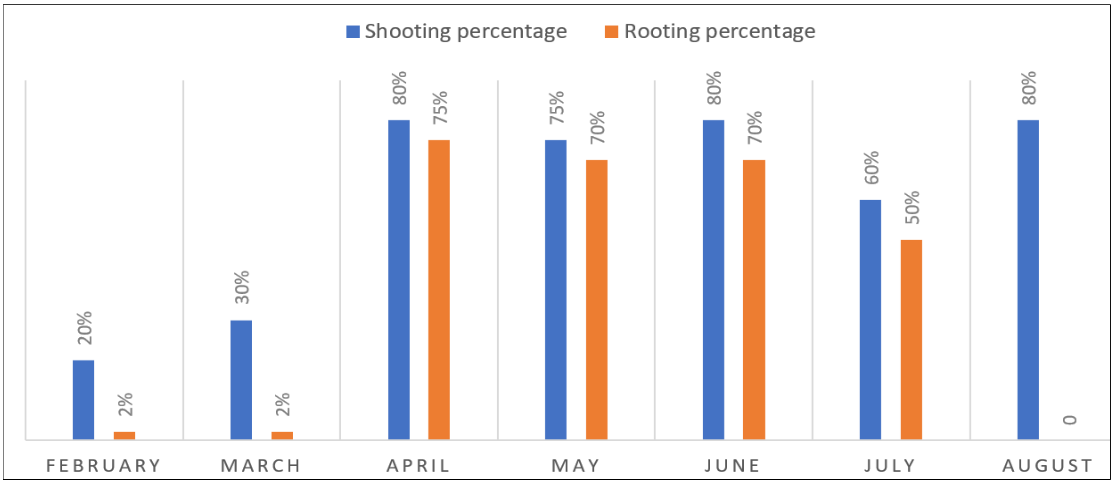

Fig 1: Data collected on a monthly basis from the cuttings.

This indicates that plantings conducted in spring and rainy seasons exhibited more favorable outcomes compared to cuttings performed in late winter (Figure 1). Rooting of stem cuttings is influenced by various factors such as cutting season, stem diameter, source tree age, propagation medium, and specific auxin type and concentration (Hartmann *et al.*, 1997) [8]. For Eucalyptus, mini-cuttings are recommended in autumn and winter (De Lima *et al.*, 2022) [6]. Burin *et al.* (2020) [4] found higher mini-cutting numbers during summer for *Cabralea canjerana.*

From the present study, we found that external application of rooting hormone has shown rooting among all IBA treatments of 1000 ppm for promoting profuse rooting in the *Chukrasia tabularis* stem cuttings compared to other treatments applied. A study involving E. grandis × E. urophylla clones demonstrated optimal survival rates when treated with 2000 ppm IBA, aligning with findings from *Eucalyptus pellita* cuttings. Kiruba *et al.* (2023) [14] highlighted the significance of root formation, protein synthesis, and peroxidase activity. Interestingly, a concentration of 4000 ppm IBA effectively initiated roots and impacted various parameters. Research with *Casuarina equisetifolia, Gmelina arborea,* and *Ceiba pendanadra* documented the advantages of externally applied hormones. Similar results were observed with *Pongamia pinnata* using IBA treatment (Kumaran *et al.*, 2010) [16]. Successful stem-cutting rooting hinges on factors like season, stem traits, mother tree age, propagation medium, and auxin specifics (Karapatzak *et al.*, 2022; Gehlot *et al.*, 2014; Schwambach *et al.*, 2008) [13, 7, 23]. In the macro propagation of *Lannea coromandelica* (Kanna *et al.*, 2017) [20], varying growth regulators and media significantly affected sprouting percentages. Immersion in 3000 ppm IBA achieved a remarkable 63.49% sprouting in softwood cuttings.

An ideal rooting medium should offer adequate moisture, and nutrients for root growth, and prevent cut-end desiccation. Various media were assessed, with cocapith: perlite showing superior results over other options. Gehlot *et al.* (2014) [7] emphasized optimal rooting media and growth regulators for mini-cutting vegetative propagation. Sand surpassed vermiculite and soil as a rooting medium, benefiting *Azadirachta indica*. Kanna *et al.* (2017) [20] noted higher sprouting with a soil-sand mix and coir pith medium for *Lannea coromandelica.*

The present study reported, that the cuttings after planting in different rooting media were placed in a mist chamber, low-cost polytunnels and then finally moved out to the greenhouse, this showed much better results than in plantings in open conditions (0%). According to Carvalho *et al.* (2021) [27], *Plathymenia reticulata* mini-cuttings achieved a 67% survival rate after a month of intermittent misting in the rooting area. In subsequent greenhouse evaluations, the rate increased to 70%, highlighting controlled environmental conditions' positive impact (Wedling; Xavier, 2005) [25].

In the present study, softwood and semi-hardwood were collected and showed good results, among the two semi-hardwood have shown better results. Earlier research (Samantaray, Maiti 2010) [28] highlighted consistent preservation of genetic traits in micro-propagated plants from shoot tips and axillary buds, attributed to meristem culture's resilience against genetic alterations.

Conclusion

The study underscores the effectiveness of using 10-15 cm *Chukrasia tabularis* cuttings treated with 1000 ppm IBA and planted in a cocapith: perlite mix (3:1) for robust root growth and cutting viability. This has implications for propagation, genetics, and tree improvement practices, streamlining clone generation for various applications.

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