PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.11153

eISSN 2348-1900

RESEARCH ARTICLE

In vitro rooting and *ex vitro* acclimatization protocol for *Cordyline terminalis* (L.) Kunth

Sweta Subhashree Jena[1*], Lilymoony Tripathy[2], Hafeef Roshan K T[3], Vishal Patel[4], Jeegarkumar Panchal[5], Madhurjit Singh Rathore[6], Lalit Upadhyay[7], Yandava Swarupa[8], Pratyush Jena[9*] & Kumaresan Marappan[10]

[1]Department of Floriculture & Landscaping, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India
[2]AICRP on Floriculture, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India
[3]Kerala Agricultural University, ICAR-Krishi Vigyan Kendra, Kottayam 686 563, Kerala, India
[4]Shri Vaishav Institute of Agriculture, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore 452 001, Madhya Pradesh, India
[5]Governors State University, University Park, Illinois 60484, USA
[6]Biotechnology Centre, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, 466 001, Madhya Pradesh, India
[7]Krishi Vigyan Kendra, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Reasi 182 301, Jammu & Kashmir, India
[8]Indian Institute of Millet Research, Hyderabad 500 030, Telangana, India
[9]College of Forestry, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India
[10]Vels Institute of Science, Technology and Advanced Studies, Chennai 600 117, Tamil Nadu, India

*Correspondence email - jenaswetasubhashree@gmail.com; jenapratyush17@gmail.com

Received: 06 August 2025; Accepted: 02 November 2025; Available online: Version 1.0: 07 January 2026

Cite this article: Sweta SJ, Lilymoony T, Hafeef RKT, Vishal P, Jeegarkumar P, Madhurjit SR, Lalit U, Yandava S, Pratyush J, Kumaresan M. *In vitro* rooting and *ex vitro* acclimatization protocol for *Cordyline terminalis* (L.) Kunth. Plant Science Today (Early Access). https://doi.org/10.14719/pst.11153

Abstract

An efficient *in vitro* rooting and *ex vitro* acclimatization protocol was developed for *Cordyline terminalis* (L.) Kunth, commonly known as the Ti plant, an economically important ornamental foliage species. The study was conducted at the Biotechnology-cum-Tissue Culture Centre, All India Coordinated Research Project (AICRP) on Floriculture and Landscaping, Odisha University of Agriculture and Technology, Bhubaneswar. Nodal segments were cultured on Murashige and Skoog (MS) medium supplemented with various concentrations and combinations of 6-benzylaminopurine (BAP), α-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) for shoot initiation and organogenesis. Among the combinations tested, BAP (1.0 mg L^{-1}) and NAA (0.5 mg L^{-1}) induced the highest shoot proliferation and morphogenetic response. For rooting, both *in vitro* and *ex vitro* approaches were evaluated. *In vitro* rooting was achieved on plant growth regulator (PGR)-free half-strength MS medium, while *ex vitro* rooting was optimized using quick dips in indole-3-butyric acid (IBA) at 25 ppm, which yielded the highest rooting percentage (96.6 %), root number (3.6) and root length (3.90 cm) in potting mixture. Hardening was performed under open conditions, plastic cover and net house environments using various substrates. The highest survival (40 %) was recorded in the net house using a vermiculite + sand substrate, which although moderate, represented the most effective survival outcome under tropical field conditions. Additionally, nutritional standardization trials revealed that soil drenching with nitrogen-phosphorus-potassium (NPK) (19:19:19) at 0.75 g $plant^{-1}$ significantly improved plant height and leaf number, while foliar application enhanced leaf length and breadth. This comprehensive protocol demonstrates the potential for mass propagation of *C. terminalis* with high rooting efficiency and improved survival rate. The findings provide a practical, reproducible and cost-effective strategy for sustainable micropropagation and commercial-scale cultivation of this valuable ornamental species.

Keywords: *Cordyline terminalis*; *ex vitro* acclimatization; hardening; IBA quick dip; *in vitro* rooting; micropropagation

Introduction

Tissue culture has gained momentum in the production of large-scale ornamental foliage plants for commercial purposes due to its rapid propagation, increased survival percentage, disease-free and uniform propagules and greater exotic market demands for cultivars of *Cordyline*, *Dracaena*, *Aglaonema*, *Alpinia*, etc. Bhubaneswar has a tropical savanna climate with eastern coastal plains and > 50 % laterite soil, having full potential for growing these vibrant exotic foliage plants on a large scale, which plays a significant role in the introduction of new varieties in the country (1). Micropropagation is an economic means of multiplying desirable plant species when time, space and personnel are limited.

Cordyline terminalis (L.) Kunth, commonly known as the Ti plant or Red Dracaena, is a valuable ornamental species cultivated for its vibrant foliage, adaptability and cultural significance in tropical landscapes (2). Belonging to the family Asparagaceae, it holds prominent commercial value in interior landscaping, floral arrangements and potted plant markets due to its broad leaf architecture and striking coloration (3). However, its conventional propagation through stem cuttings is slow,

disease-prone and inefficient in generating uniform planting material (4, 5). Moreover, conventional propagation methods produce only four daughter plants from a single mother plant and in some new varieties, propagation through rhizomes is not feasible (6). Therefore, micropropagation through *in vitro* techniques is essential to accelerate propagation rates and reduce the dependence on mother plants (7).

In vitro micropropagation has emerged as a reliable tool for large-scale production of genetically uniform and disease-free planting stock in ornamental species (8). Among the stages of micropropagation, root induction and acclimatization are particularly critical, as successful rooting and the subsequent transition from *in vitro* to *ex vitro* environments are often associated with high mortality due to physiological stress and poor root system development (9). Efficient rooting-whether *in vitro* or *ex vitro* is strongly influenced by endogenous and exogenous hormonal cues, primarily auxins such as indole-3-butyric acid (IBA), which have been widely reported to promote root initiation across species (10).

In *Cordyline* species, few studies have reported complete micropropagation protocols, with most emphasizing shoot induction and providing limited information on rooting responses (11, 12). Although *C. fruticosa* has been propagated through nodal explants and rhizomatous cultures, the rooting stage in *C. terminalis* has not been extensively optimized (13). Moreover, the integration of *ex vitro* rooting during the primary hardening stage has gained attention for its cost-effectiveness and enhanced plantlet survival, as it eliminates the need for prolonged *in vitro* culture and reduces contamination risks (14). Acclimatization is another indispensable phase that determines the success of micropropagated plantlets under greenhouse or nursery conditions. Transitioning plantlets from high humidity, sugar-enriched *in vitro* media to heterotrophic soil conditions requires physiological and metabolic adjustments, including root hair formation, lignification and photosynthetic competence (15, 16). A well-developed root system, established either *in vitro* or *ex vitro*, is vital for this process.

Given the economic and ecological relevance of *C. terminalis* and the limited reports on its complete regeneration protocol, the present study aimed to optimize *in vitro* rooting using different hormonal combinations, evaluate *ex vitro* rooting efficiency under variable substrate conditions and assess acclimatization and hardening efficiency for standardized nursery performance. This integrated and cost-effective approach is novel, as it combines *in vitro* and *ex vitro* rooting, acclimatization and nutrient supplementation into a single, scalable micropropagation system suitable for commercial nurseries and ornamental foliage production.

Materials and Methods

Plant material and explant preparation

Nodal segments, shoot tips and leaf bits of *C. terminalis*, measuring approximately 1.5 cm-2.0 cm in length, were collected from healthy, disease-free stock plants maintained in a greenhouse at Biotechnology-cum-Tissue Culture Centre in Baramunda under Floriculture and Landscaping Department, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. Explants were collected during the winter season (November-January) of 2022-2023, when contamination levels were minimal and physiological activity was optimal. Explants were thoroughly washed under running tap water for 15 min, followed by immersion in a solution of 2 % (v/v) tween-20 for 10 min with constant agitation. After multiple rinses with sterile distilled water, explants were surface sterilized using 0.1 % (w/v) mercuric chloride ($HgCl_2$) for 4-10 min under aseptic conditions in a laminar airflow cabinet, followed by three rinses with sterile distilled water.

Culture medium and conditions

Murashige and Skoog (MS) medium was used as the basal medium throughout the experiments. The medium was supplemented with various concentrations of plant growth regulators (PGRs) for different stages. For shoot multiplication, BAP (0.5-3.0 mg L^{-1}) in combination with NAA (0.5 mg L^{-1}) and 2,4-D (0.5-2.0 mg L^{-1}). For *in vitro* rooting, half- or full-strength MS without or with plant hormones such as BAP, NAA and 2,4-D.

The media were solidified with 0.8 % (w/v) agar and adjusted to pH 5.8 before autoclaving at 121 °C and 15 psi for 20 min. Cultures were incubated at 25 ± 2 °C with a 16 hr photoperiod (40 µmol m^{-2} s^{-1} light intensity from cool white, fluorescent lamps) and 60 %-70 % relative humidity.

In vitro root induction

Studies on *in vitro* rooting were carried from the elongated shoots (stage 2) in MS basal medium. Shoots (3 cm-4 cm in height) derived from multiplication media were excised and transferred to rooting media containing different plant bio regulators (PBRs) concentrations (BAP, NAA and 2,4-D), as well as hormone-free MS and half-strength MS medium for comparative analysis. Cultures were maintained under the same photoperiod and temperature conditions for 4-6 weeks and data on root initiation, number of roots and root length were recorded.

Ex vitro rooting and substrate standardization

For *ex vitro* rooting, regenerated shoots (5 cm-7 cm) were directly treated with IBA solutions (10, 15, 20 and 25 ppm) by quick dip for 5 min and transplanted into polybags containing peat and potting mixture of cocopeat: vermicompost (1:1 w/w). The pots were maintained under greenhouse conditions (30 ± 2 °C, 70 %-80 % RH) with intermittent misting. After 30 and 60 days, rooting percentage, root number and root length were evaluated. Survival percentage was also recorded at the end of the hardening phase.

Acclimatization and hardening

For *in vitro* rooted plants, sterile water was added to the culture vessels after removing the cotton plugs and kept as such for 5 to 10 min. Rooted plantlets were taken with the forceps, washed gently to remove media and transferred to plastic crates filled with sterile potting mixture mainly Cocopeat. It was kept in low poly tunnels for 2 weeks and then transferred to potting mixture (peat + sand + vermicompost, 1:1:1). Initially, the plants were kept in a mist chamber under high humidity (90 %-95 %) for 10 days, gradually shifted to greenhouse conditions and watered regularly. Morphological parameters like shoot height, leaf number and survival percentage were recorded after 60 days. The tissue culture plantlets were treated with 0.2 % carbendazim (50 % WP) solution for 30 min before planting out. After planting out, the plantlets were drenched with 0.2 % carbendazim solution.

Standardization of hardening treatments

The *in vitro* plantlets of *C. terminalis* were subjected to various post-transplanting hardening techniques to evaluate survival under different environmental and substrate conditions. Treatments included:

- Open condition (mud pots) with media such as fine sand, coconut fibre, peat moss, vermiculite and mixtures of peat/ sand or vermiculite/sand.
- Plastic cover (polythene bags with holes) for 2 weeks.
- Net house conditions using the same substrates.
- Pre-planting treatment with 0.2 % carbendazim for 30 min.
- Rooting media incorporated with triadimefon (1 mg/L) in select cases.
- Post-planting treatments included foliar spray or soil drenching with triadimefon (20 mg L^{-1}).
- Plantlets were watered at 2-day intervals and monitored for survival (%) at 2, 4, 6 and 8 weeks post-transplanting.

Field trial and evaluation

Effect of nutrient solution

The tissue culture plantlets were supplied with a solution of 19:19:19 NPK complex. The treatments included soil drenching on alternate days, with the quantity applied per plant per week (g $plant^{-1}$ $week^{-1}$) as follows: control (0.00), 0.50, 0.75 and 1.00. For foliar application, treatments were applied daily, with the quantity applied per plant per week (g $plant^{-1}$ $week^{-1}$) as follwos: control (0.00), 0.25, 0.50 and 0.75. Observations were recorded on plant height, leaf number, leaf length and leaf breadth.

The complete micropropagation protocol followed for *C. terminalis* is illustrated in Fig. 1. It highlights the sequential stages from culture establishment to *in vitro* and *ex vitro* rooting, indirect organogenesis pathways and subsequent acclimatization and nutritional standardization.

Statistical Analysis

All experiments were conducted in a completely randomized design (CRD) with three replications. Each replication included 10 explants. Data were subjected to analysis of variance (ANOVA) using MS Excel and mean comparisons were made using Duncan's multiple range test (DMRT) at $p \leq 0.05$. Percentage data were arcsine-transformed prior to analysis to meet statistical assumptions.

Results and Discussion

In vitro morphogenetic responses

Nodal explants of *C. terminalis* cultured on MS medium with different concentrations of BAP (0.5–3.0 mg L^{-1}) in combination with NAA (0.5 mg L^{-1}) or 2,4-D (0.5–2.0 mg L^{-1}) exhibited varied morphogenic responses (Table 1). The highest frequency of multiple shoot bud formation (++++) was observed with 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, which also promoted intense basal callusing and rhizogenesis. This indicates that BAP, a cytokinin, in optimal concentration stimulates axillary meristem development, a pattern similarly observed in *C. terminalis* and other monocots (3). These findings highlight the

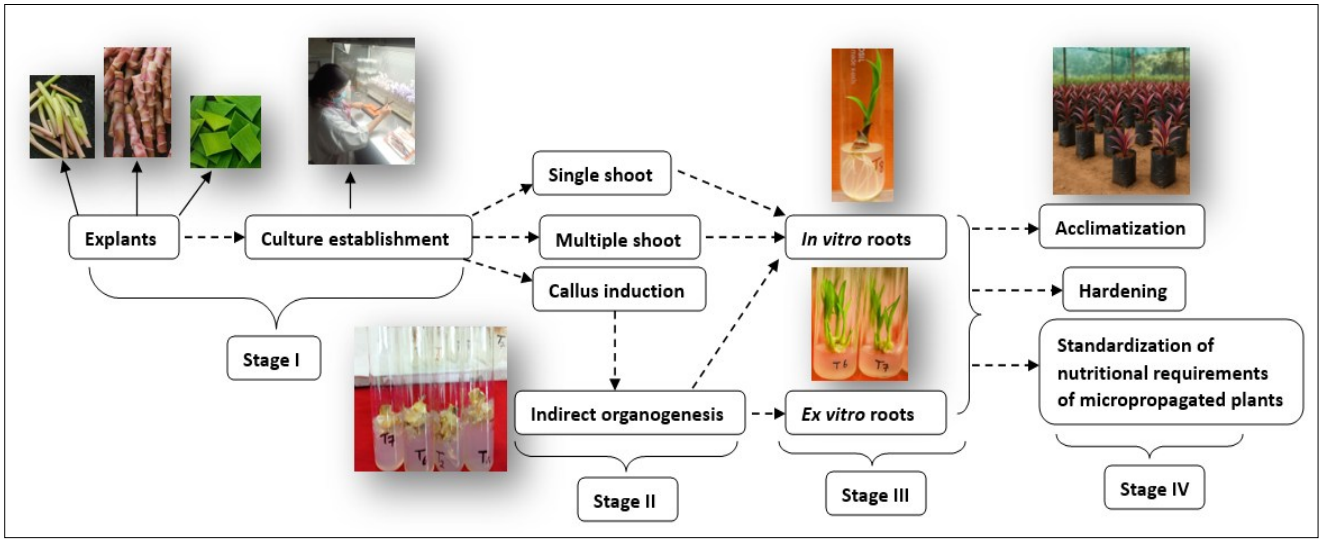

Fig. 1. Schematic representation of the micropropagation protocol in *Cordyline terminalis.*

Table 1. Effect of BAP, NAA and 2,4-D combinations on *in vitro* morphogenetic responses of *Cordyline terminalis* nodal explants

Culture period: 4 weeks					Basal medium: MS
Treatments			Nature of response		
BAP (mg/L)	NAA (mg/L)	2, 4-D (mg/L)	Multiple axillary buds	Callus growth	Others
0.5	0.5	0.0	++	+++	Callusing of base and rhizogenesis
1.0	0.5	0.0	++++	++++	Callusing of base and rhizogenesis
1.5	0.5	0.0	+	+++	Callusing of base and rhizogenesis
2.0	0.5	0.0	+++	+++	Callusing of base
2.5	0.5	0.0	-	+	Callusing of base
3.0	0.5	0.0	+	+++	Callusing of base
0.0	0.0	0.5	+	++	Callusing of base
0.0	0.0	1.0	+	++	Callusing of base
0.0	0.0	1.5	+	+++	Callusing of base
0.0	0.0	2.0	+	+++	Callusing of base
Half MS Basal medium			+++	-	Direct organogenesis and rhizogenesis
Full MS Basal medium			+++	-	Direct organogenesis and rhizogenesis

Note: nodal explants of *Cordyline terminalis* were cultured on MS medium with different BAP, NAA and 2,4-D combinations for 4 weeks. '+' indicates number of shoot buds: + (≤ 3), ++ (4-10), +++ (11-20), ++++ (≥ 21), - no response. Final treatment used half-strength MS.

synergistic effect of BAP and NAA in promoting shoot bud formation, consistent with previous reports in *Cordyline* species, which revealed that axillary bud development took place after 3 weeks and was highest in media containing 0.5-1.0 mg L^{-1} of BAP (17). At lower BAP concentration (0.5 mg L^{-1}), moderate shoot proliferation (++), callusing (+++) and rhizogenesis were recorded. However, increasing BAP beyond 2.5 mg L^{-1} resulted in reduced shoot initiation and excessive basal callusing, likely due to hormonal imbalance or inhibitory effects at supra-optimal levels (18). The auxin 2,4-D, in isolation, failed to induce organized shoots, supporting its known role in promoting dedifferentiation and callus formation rather than direct shoot organogenesis (5).

Interestingly, early rhizogenesis (root-like structures) was observed in media with low PGR (Plant growth regulators) levels such as BAP 0.5-1.5 mg L^{-1} + NAA 0.5 mg L^{-1} and also in hormone-free MS and ½ MS media, indicating that root induction with direct organogenesis (shooting and rooting) can occur without exogenous PGRs under favourable basal conditions. This is likely due to reduced osmotic stress and a favourable endogenous auxin-cytokinin balance. These results also align with earlier studies where low auxin -cytokinin ratios or PGR-free conditions favoured rooting over shoot proliferation in monocotyledonous ornamentals (19, 20).

In vitro rooting influenced by $HgCl_2$ sterilization

Surface sterilization with 0.1 % $HgCl_2$ for 10 min (T_8) resulted in the most efficient *in vitro* rooting, producing an average of 5.33 ± 0.06 roots per explant, root length of 7.67 ± 0.18 cm and early root emergence at 47.33 ± 0.14 days (Table 2). These roots were long, creamy white and exhibited healthy root hairs-traits favourable for *ex vitro* transfer (Fig. 2). Such enhanced rooting following appropriate sterilization is attributed to reduced microbial load and improved tissue receptivity.

In vitro rooting was observed in hormone free MS medium in *Agave tequilana* (21). *In vitro* rooting stage in *C. terminalis* cv. Red top was seen by culturing newly formed shoots from multiplication stage on MS PGRs-free medium gelled with 2 % (w/v) gelrite (19). Shorter durations (T_2 and T_4) induced moderate rooting responses, while longer exposures (T_5-T_7), resulted in browning and necrosis, indicating toxicity of prolonged $HgCl_2$ exposure. Overexposure to sterilants has been reported to cause oxidative stress and cell death in explants, which could explain the observed decline in root formation in those treatments (22).

Thus, the combination of optimized PGR concentrations, proper nutrient balance and effective surface sterilization significantly improved the micropropagation efficiency of *C. terminalis*. The results establish a reproducible and scalable *in vitro* regeneration and rooting protocol that can contribute to the sustainable commercial propagation of this ornamental foliage plant.

Planting out of tissue culture plants and acclimatization

The rooted plants were carefully removed from the test tubes and morphological observations were done. The height of plantlets at the time of planting out ranged from 3.0 cm to 5.5 cm with a mean height of 4.25 cm. The number of leaves per shoots ranged from 7.00 to 9.00 with a mean value of 8.00. The number of roots ranged from 3.00 to 5.00 with a mean value of 4.00. The length of the roots ranged from 5.00 to 8.00 cm with a mean value of 6.5 cm. The plantlets were treated with 0.2 % bavistin solution for 10 min. These plantlets were then subjected to acclimatization treatments and the results were observed. When the plants were kept in portrays with coco peat as

Table 2. Effect of $HgCl_2$ sterilization duration on *in vitro* rooting of nodal segments of *Cordyline terminalis* on full-strength MS medium without PGRs

Duration: 9 weeks		Basal medium: MS				
Treatment No.	Treatments	Number of shoots/ explants	Time taken for root initiation (days)	Root length (cm)	Number of roots/ explants	Nature of roots	Color of leaves
T_1	Control (tap water)	5.00±0.18^{bc}	NC	NC	NC	NC	Light green
T_2	0.1 % $HgCl_2$ for 4 min	5.67±0.22^{ab}	54.67±0.32	5.07±0.07	2.67±0.08	Long, creamy white root with root hairs	Light green
T_3	0.1 % $HgCl_2$ for 5 min	4.00±0.15^{d}	NC	NC	NC	NC	Whitish green
T_4	0.1 % $HgCl_2$ for 6 min	5.33±0.19^{ab}	50.00±0.17	5.53±0.17	3.33±0.12	Long, creamy white root with root hairs	Light green
T_5	0.1 % $HgCl_2$ for 7 min	3.67±0.14^{d}	NC	NC	NC	NC	Whitish green
T_6	0.1 % $HgCl_2$ for 8 min	4.33±0.17^{cd}	NC	NC	NC	NC	Light green
T_7	0.1 % $HgCl_2$ for 9 min	4.67±0.18^{bc}	NC	NC	NC	NC	Light green
T_8	0.1 % $HgCl_2$ for 10 min	6.00±0.23^{a}	47.33±0.14	7.67±0.18	5.33±0.06	Long, creamy white root with root hairs	Light green
SE(m)±		0.14	0.225	0.23	0.09		
CD (5 %)		0.42	0.78	0.54	0.33		

Note: NC- no change, CD = critical difference at 5 %; SE(m) = standard error. Data are means of three replicates.

Fig. 2. *In vitro* rooting in *Cordyline terminalis.*

the media under polytunnels covered greenhouse maintaining optimum environmental conditions, 100 % survival rates were observed for 2 weeks. It was watered at 2 days interval. After 2 weeks, it was planted in fine sand treated with 0.2 % carbendazim (50 % WP) and 50 % survival was recorded. When plantlets were kept in shade net house, survival percentage of 50.00 %, 30.00 % and 10.00 % were recorded up to 8 weeks in the potting media (sand + peat + vermiculite). This is in accordance with the study where hardening techniques were modified in micropropagated banana plants with better results, when the roots of the plantlets were dipped in 0.2 % bavistin for 5 min, the plantlets covered with microscope cover after planting (23).

Ex vitro rooting and acclimatization

The *ex vitro* rooting response of *C. terminalis* plantlets was significantly influenced by both the concentration of IBA and the type of potting substrate (Table 3; Fig. 3). Among the tested treatments, the combination of IBA at 25 ppm with potting mixture (likely composed of cocopeat and vermicompost) showed the highest rooting percentage (96.6 %), the maximum number of roots per plantlet (5.10) and the greatest root length (5.77 cm). In contrast, the lowest rooting efficiency (70.3 %) and shorter roots (2.00 cm) were observed in peat medium with 10 ppm IBA.

The peat alone consistently outperformed potting mixture, suggesting that improved aeration, moisture retention and microbial interaction may have contributed to enhanced rooting and early acclimatization. The findings are in line with earlier reports where exogenous auxin treatments during the transfer phase improved root formation and survival in ornamental foliage plants (12). The superiority of higher IBA concentrations (20–25 ppm) is likely due to their stronger role in inducing adventitious roots by promoting cell differentiation at the basal cut ends (5). This phase also contributed to successful hardening, as roots formed directly in acclimatization substrates are known to adapt better to *ex vitro* conditions. Therefore, the current protocol provides a cost-effective, hormone-efficient method for mass-scale propagation and acclimatization of *C. terminalis*.

Table 3. Effect of IBA Concentration and Substrate type on *ex vitro* rooting and root traits of *Cordyline terminalis* plantlets

Treatment No.	Treatment details	% Rooting		Mean	Number of Roots		Mean	Root length (cm)		Mean
	IBA Concentration (C)	Peat	Potting media (M)		Peat	Potting media (M)		Peat	Potting media (M)	
T_1	IBA 10 ppm	86.6 (72.3)	70.3	80.0	2.67	2.00	2.37	4.03	3.83	3.93
T_2	IBA 15 ppm	80.0 (63.4)	80.0	80.0	2.67	2.07	2.33	4.23	4.23	4.23
T_3	IBA 20 ppm	93.3 (81.1)	86.7	90.0	3.47	2.50	2.98	5.10	4.90	5.00
T_4	IBA 25 ppm	96.6 (79.01)	86.7	90.0	5.10	4.07	3.55	5.77	5.33	5.55
Mean		88.3 (74.5)	81.7		3.21	2.41	2.81	4.78	4.57	4.66
Factors		SE(m)±	CD at 1 %		SE(m)±	CD at 1 %		SE(m)±	CD at 1 %	
Media (M)		3.57	ns		0.11	0.46		0.09	ns	
Concentration (C)		5.06	ns		0.16	0.65		0.13	0.52	
C × M		7.15	ns		0.22	ns		0.18	ns	

Note: value in Parathesis is Arc-sine transformed values, ns ⊠ non-significant at $p > 0.05$.

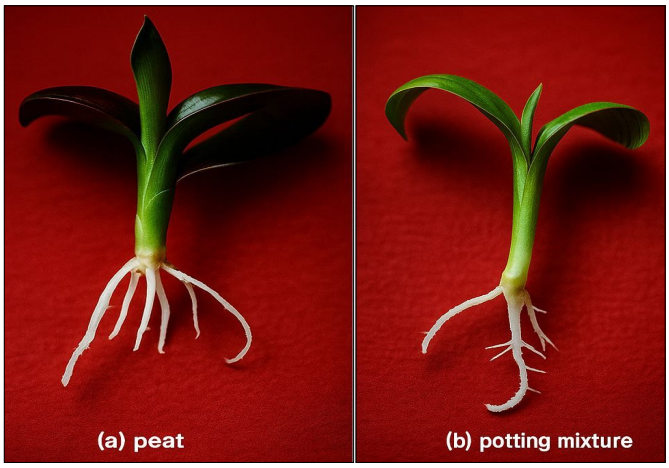

Fig. 3. *Ex vitro* rooting response of *Cordyline terminalis* in different media.

Fig. 4. Acclimatization and hardening of *Cordyline terminalis* plantlets in polybags under green house.

Effect of hardening techniques on post transplanting survival of tissue cultured plantlets of cordyline

The success of tissue-cultured plantlets depends significantly on post-transplanting acclimatization. In the present study, plantlets were subjected to various hardening environments (open, plastic cover and net house) with different substrates and antifungal treatments. The percentage of surviving plantlets was recorded up to 8 weeks (Fig. 4). After 8 weeks, the highest survival percentage (40 %) was observed under net house conditions with vermiculite + sand, where no chemical treatments were applied. This suggests that substrate porosity and water-holding capacity played a critical role in the survival of delicate plantlets. Other effective treatments included peat + sand under net house and vermiculite + sand under plastic cover, each achieving 30 % survival.

In contrast, all plantlets maintained under open conditions across all substrates showed 0 % survival, indicating that uncontrolled environmental exposure leads to transplant shock and rapid desiccation (3). This study agrees with the optimal media for cordyline was pine bark + garden soil + sand (1:1:1 w/w) (24). Treatments with triadimefon (20 mg L^{-1}) as a foliar spray or soil drench, intended to reduce microbial stress, resulted in moderate survival percentages of 10 %-20 %, but were inferior to the physical protection offered by net houses. These results are in agreement with, who reported that microclimatic control was more effective than chemical interventions during early acclimatization (25). The use of polythene tents, misting and fogging are ways for regulating relative humidity for micropropagated plants (26).

The radar graph pattern, clearly shows the relative superiority of net house conditions over other environments, highlighting the combined importance of substrate quality, humidity regulation and partial shade in ensuring transplant success in *C. terminalis* (Fig. 5). The significance of gradual acclimatization and the use of semi-controlled structures such as net houses in ornamental plant propagation (27).

Standardization of nutritional requirements of micropropagated plantlets

The present study evaluated the response of *C. terminalis* plantlets to different concentrations and modes of NPK (19:19:19) application through soil drenching and foliar spray, assessed at 30 and 60 days post-treatment (Table 4).

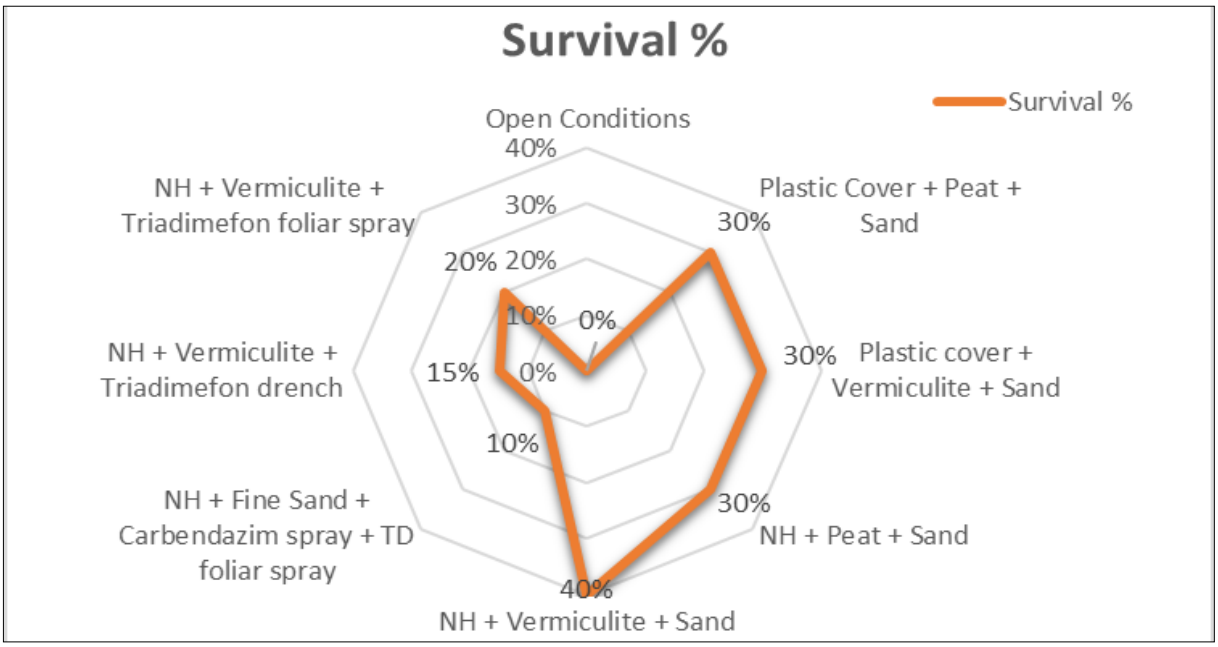

Fig. 5. Radar chart showing the survival percentage of *Cordyline terminalis* plantlets 8 weeks after transplanting under different hardening treatments and environmental conditions.

Table 4. Standardization of nutritional requirements of micropropagated plants of *Cordyline terminalis* Medium: Cocopeat + Vermicompost

Treatments 19:19:19 NPK (g/plant)		Increment after 30 days				Increment after 60 days			
		Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf breadth (cm)	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf breadth (cm)
Control	0.00	1.15	2.0	0.34	0.18	2.90	5.1	0.42	0.23
	0.50	1.20	2.2	0.38	0.18	2.96	5.5	0.64	0.30
Soil drenching (X)	0.75	2.20	3.1	0.38	0.22	4.80	6.0	0.66	0.35
	1.00	1.88	3.0	0.36	0.20	4.12	5.6	0.65	0.33
	0.25	1.18	2.2	0.36	0.18	2.91	6.2	0.65	0.31
Foliar Spray (Y)	0.50	1.18	2.6	0.34	0.20	2.88	6.3	0.62	0.39
	0.75	1.82	3.0	0.38	0.22	3.90	6.3	0.72	0.35
CD (0.05)		0.405	0.40	0.014	0.016	0.717	0.429	0.087	0.046
SE(m)±		1.66	0.162	0.006	0.0068	0.293	0.175	0.035	0.19
CV (%)		28.91	16.6	4.13	9.08	22.21	7.94	15.19	15.61
Mean		1.52	2.59	0.365	0.197	3.49	5.855	0.622	0.322
Interaction Effect (X-Y)		+0.37	+0.17	+0.006	0.00	+0.73	-0.57	-0.013	-0.023

Note: CD = critical difference at 5 %; SE(m) = standard error; CV = coefficient of variation. Interaction effect = soil drenching (X) – foliar Spray (Y).

Plant height after 30 days

Plant height at 30 days varied significantly across treatments. The highest increase (3.88 cm) was recorded with 0.75 g per plant soil drenching, which was significantly superior to all other treatments ($CD_{0\text{-}05}$ = 0.462 cm). This was followed by 0.75 g per plant foliar spray (3.18 cm) and 0.5 g per plant soil drenching (3.12 cm), while the lowest plant height (1.16 cm) was observed in the untreated control. Treatments like 0.5 g foliar spray and 0.25 g foliar spray were statistically at par with the control. These findings highlight the superior efficiency of soil-applied macronutrients in early vegetative development, which aligns with previous studies emphasizing root-mediated nutrient uptake during the initial growth phases (28).

A positive interaction effect (+ 0.17) between the nutrient dose and method of application further supports the synergistic role of optimized drenching at higher NPK levels. Similar enhancement in plant vigor with soil-based nutrient delivery systems has been reported in ornamental crops such as *Dieffenbachia* and *Chlorophytum* (29).

Leaf number, length and breadth after 30 days

The number of leaves after 30 days increased significantly from 2.91 (control) to a maximum of 4.80 leaves in 0.75 g/plant soil drenching, which was statistically comparable with 0.5 g/plant soil drenching (4.12) and 0.75 g foliar spray (4.10). Lower values were recorded in 0.25 g foliar spray and control. The leaf length and breadth ranged from 2.906 cm–3.98 cm and 0.30 cm–0.39 cm, respectively, but did not show statistically significant differences ($CD_{0\text{-}05}$ = NS). These moderate variations indicate that leaf morphometry is less sensitive than height and leaf number to NPK dosage, especially at early growth stages (5).

The coefficient of variation for leaf number and length ranged from 6.16 % to 10.31 %, indicating moderate variability among treatments. A positive interaction effect (+ 0.13) was noted for leaf number, suggesting a beneficial cumulative influence of higher soil-applied nutrients, while leaf breadth showed a slight negative effect (-0.04) between foliar and soil treatments, similar to trends noted in *C. fruticosa* (30).

Plant height and leaf traits after 60 days

After 60 days, the highest plant height (6.16 cm) was again noted in 0.75 g per plant soil drenching, followed by 0.75 g per plant foliar spray (5.85 cm). The control showed the lowest growth (2.90 cm). The number of leaves followed a similar trend, with the maximum (5.6 leaves) under 0.75 g per plant drenching. These results underscore the long-term benefit of direct root-zone NPK availability, likely due to sustained release and uptake (31).

Interestingly, leaf length and breadth were higher in foliar applications (up to 6.3 cm length and 0.72 cm breadth in 0.75 g foliar spray), suggesting that foliar sprays promote laminar expansion, possibly due to more direct and rapid nutrient absorption by foliage (32). However, a negative interaction effect (-0.57) for plant height and minor negative values (-0.01 to -0.02) for leaf traits indicate that while foliar sprays favour leaf quality, soil drenching better supports structural growth.

From the overall response, 0.75 g per plant NPK through soil drenching emerged as the most effective treatment for increasing plant height and number of leaves in *C. terminalis*. Foliar spray at the same dose performed better for leaf length and breadth, especially during the later stages. These findings suggest that dual strategies combining drenching for structural growth and foliar feeding for foliage quality may be optimal for ornamental plant production.

Conclusion

The present study successfully established an efficient *in vitro* rooting and *ex vitro* acclimatization protocol for *C. terminalis*, a commercially valuable ornamental foliage plant. The optimized micropropagation system demonstrated that MS medium supplemented with low concentrations of BAP and NAA induced multiple shoot formation and early rhizogenesis, while effective rooting was achieved both *in vitro* (with optimized sterilization and PGR-free media) and *ex vitro* (IBA quick dips and substrate selection). *Ex vitro* rooting using 25 ppm IBA and potting mixture significantly enhanced rooting percentage (96.6 %), root number and root length, facilitating better acclimatization. Hardening under net house conditions using vermiculite + sand substrate resulted in the highest post-transplant survival (40 %), outperforming chemical treatments and open-air environments. Moreover, nutritional standardization revealed that 0.75 g per plant NPK through soil drenching was most effective for enhancing plant height and leaf number, while foliar application improved leaf dimensions. Together, these findings provide a reproducible, cost-effective protocol for large-scale propagation of *C. terminalis*, which can benefit commercial floriculture and landscape horticulture industries. Further research may explore genotype-specific responses, secondary metabolite profiles and field performance post-transfer.

Acknowledgements

The Authors appreciate Biotechnology-cum-Tissue Culture Centre in Baramunda, Floriculture and Landscaping Department, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar for providing planting materials, equipment and chemical resources to carry out the research work.

Authors' contributions

SSJ carried out the entire protocol generation of standardization and micropropagation work for rooting in Cordyline successfully, designed the treatment details, performed the statistical analysis and drafted the manuscript. LT assisted in the successful completion of entire Protocol as a Supervisor, verified the design of study and helped in overall coordination of the research work. PJ helped in carrying out the experiment, assisted in drafting manuscript, technical help and helped in statistical analysis. HRKT, VP, JP, MSR, LU, YS and KM provided support and guidance in this research work. All authors read, revised and approved the final version of manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Das A, Sahu SC, Beuria R, Sahu DK, Mishra A, Panigrahi A, Pati A, Priyadarshini P. Investigating air temperature changes in Bhubaneswar city over 123 years from 1901 to 2023. Sci Rep. 2025;15 (1):27389. https://doi.org/10.1038/s41598-025-12746-5
2. Huxley A, Griffiths M, Levy M, editors. New Royal Hortic Soc dict gardening. London: Macmillan; 1992.
3. Rout GR, Samantaray S, Das P. In vitro manipulation and propagation of medicinal plants. Biotechnol Adv. 2000;18(2):91–120. https://doi.org/10.1016/S0734-9750(99)00026-9
4. Martin K. Rapid propagation of Holostemma ada-kodien Schult. through axillary bud multiplication and indirect organogenesis. Plant Cell Rep. 2002;21(2):112–7. https://doi.org/10.1007/s00299-002-0483-7
5. George EF, Hall MA, De Klerk GJ. Plant propagation by tissue culture. 3rd ed. Dordrecht: Springer; 2008. p. 65–175.
6. Holttum RE, Enoch IC. Gardening in the tropics. Singapore: Malayan Nature Soc; 1991.
7. George EF, Sherrington PD. Plant propagation by tissue culture. Basingstoke: Exegetics Ltd; 1993. p. 709.
8. Bhojwani SS, Dantu PK. Plant tissue culture: an introductory text. New Delhi: Springer; 2013. https://doi.org/10.1007/978-81-322-1026-9
9. Hazarika BN. Morpho-physiological disorders in in vitro culture of plants. Sci Hortic. 2006;108(2):105–20. https://doi.org/10.1016/j.scienta.2006.01.038
10. Kadleček P, Tichá I, Haisel D, Čapková V, Schäfer C. Importance of in vitro pretreatment for *ex vitro* acclimatization and growth. Plant Sci. 2001;161 (4):695–701. https://doi.org/10.1016/S0168-9452(01)00456-3
11. Jena SS, Tripathy L, Beura S, Dash SK, Maharana K, Jena P. In vitro optimization of protocol for micropropagation in Cordyline terminalis. J Adv Biol Biotechnol. 2025;28(6):880–912. https://doi.org/10.9734/jabb/2025/v28i62449
12. Ray T, Saha P, Roy SC. Micropropagation of *Cordyline terminalis*. In: Lambardi M, Ozudogru EA, Jain SM, editors. Protocols micropropag selected econ important hortic plants. Totowa: Humana Press; 2012. p. 269–77. https://doi.org/10.1007/978-1-62703-074-8_21
13. Fisher JB, Tomlinson PB. Vegetative morphology of *Cordyline terminalis*. J Arnold Arbor. 1972;53(1):113–27. https://doi.org/10.5962/p.185779
14. Chisti Y. Biotechnology of pollution control. In: Doble M, Kumar A, editors. Biotreatment industrial effluents. Oxford: Elsevier; 2005.
15. Pospíšilová J, Tichá I, Kadleček P, Haisel D, Plzáková Š. Acclimatization of micropropagated plants to ex vitro conditions. Biol Plant. 1999;42 (4):481–97. https://doi.org/10.1023/A:1002688208758
16. Noor El-Deen TM, Abdul-Moneem NE. Evaluating plant growth regulators, medium strength and supporting method for optimum micropropagation of Cordyline 'Atom'. Hortic Res J. 2025;3(2):15–30. https://doi.org/10.21608/hrj.2025.424687
17. Sanjeewa TA, Peiris SE. In vitro propagation of *Cordyline terminalis* cvs. Gold-Compacta and Red-Compacta. 2nd Annual Research Symposium; 2011. p. 28-31.
18. Mei-Lan L, Murthy HN, Park KY. Photoautotrophic culture conditions and photon flux influence growth of *Lilium* bulblets *in vitro*. *In Vitro* Cell Dev Biol Plant. 2003;39(5):532–535. https://doi.org/10.1079/IVP2003440
19. Eltorky MG, Elmahrouk ME, Elmokadem H, El-Sammak H. Micropropagation and genetic variations of *Cordyline terminalis* cv. Red Top. J Adv Agric Res. 2018;23(2):328–49.
20. Khan SA, Naz SH, Saeed BU. *In vitro* production of *Cordyline terminalis* for commercialization. Pak J Bot. 2004;36(4):757–61.
21. Valenzuela-Sánchez KK, Juárez-Hernández RE, Cruz-Hernández A, Olalde-Portugal V, Valverde ME, Paredes-López O. Plant regeneration of Agave tequilana by indirect organogenesis. In Vitro Cell Dev Biol Plant. 2006;42(4):336–40. https://doi.org/10.1079/IVP2006788
22. Tematio Fouedjou R, Tsakem B, Siwe-Noundou X, Dongmo Fogang HP, Tiombou Donkia A, Kemvoufo Ponou B, et al. Ethnobotany, phytochemistry and biological activities of the genus *Cordyline*. Biomolecules. 2023;13(12):1783. https://doi.org/10.3390/biom13121783
23. Mini B. Induction of genetic variability in Musa sp. var. Nendran by in vitro methods. PhD thesis. Thrissur: Kerala Agric Univ.
24. Lavanya R. Blast. BSc Hortic thesis.
25. Krishna H, Hebbar S, Kumar P, Sharma S, Kumar R, Tiwari SK, et al. Navigating challenges and prospects in off-season vegetable production. Veg Sci. 2024;51:97-105. https://doi.org/10.61180/vegsci.2024.v51.spl.09
26. Hu CY, Wang PJ. Meristem, shoot tip and bud culture. In: Evans DA, Sharp WR, Ammirato PV, Yamada Y, editors. Handb plant cell cult. Vol 1. New York: Macmillan; 1983. p. 177–227.
27. Samal M, Abass S, Parveen R, Ahmad S, Iqbal M. Effect of abiotic stress on production of secondary metabolites in plants. In: Plants as medicine and aromatics. Boca Raton: CRC Press; 2023. p. 145–72. https://doi.org/10.1201/9781003226925-11
28. Abdullaha M, Waraich E, Ahmad M, Hussain S, Haider A, Ahmad Z, et al. Enhancing drought stress tolerance in soybean through silicon-induced modulations.
29. Ghosh SN, Tarai RK, Ahlawat TR. Plant growth regulators in tropical and subtropical fruit crops. Boca Raton: CRC Press; 2022. https://doi.org/10.1201/9781003300342
30. Basvantrao MC. Effect of foliar application of nutrients on growth, yield and quality of wheat (*Triticum aestivum* L.). PhD thesis. Rahuri: MPKV.
31. Deshmukh SK, Kochar M, Kaur P, Singh PP, editors. Nanotechnology in agriculture and environmental science. Boca Raton: CRC Press; 2023. https://doi.org/10.1201/9781003323945
32. Ding Y, Zhao W, Zhu G, Wang Q, Zhang P, Rui Y. Recent trends in foliar nanofertilizers: A review. Nanomaterials. 2023;13(21):2906. https://doi.org/10.3390/nano13212906

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc

See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.