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RESEARCH ARTICLE

Standardization of sterilization protocol and culture medium for growth regulator-free direct organogenesis in *Cordyline terminalis* (L.) Kunth

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Abstract

The study was conducted at the Biotechnology-cum-Tissue Culture Centre, Baramunda, AICRP of Floriculture and Landscaping, Odisha University of Agriculture and Technology, Bhubaneswar, India to standardize surface sterilization protocols and assess direct organogenesis in 3 explants-nodal segments, shoot tips and leaf bits under free plant growth regulators (PGR) conditions in *Cordyline terminalis* (L.) Kunth. Six sterilization treatments involving ethanol, sodium hypochlorite and mercuric chloride ($HgCl_2$) at varying concentrations and durations were evaluated. Among all, 0.1 % $HgCl_2$ for 10 min (T_8) was most effective, resulting in the highest survival (75.03 ± 0.17 %), minimal fungal (10.52 ± 0.16 %) and bacterial (0 %) contamination and maximum aseptic cultures (79.48 ± 0.14 %). Nodal segments consistently outperformed other explants in survival and regeneration. Subsequent culturing of nodal segments on MS basal medium devoid of PGRs resulted in direct shoot and root formation in all treatments (T_1–T_8), with T_8 showing maximum cultures with direct organogenesis (6.67), shoots (6.00/explant), leaves (9.00/shoot), root length (7.67 cm) and number of roots (5.33). Additionally, seasonal influence was significant for explants collected during the winter season (November-December) with higher survival and lower contamination compared to those from other months. The findings establish an efficient, hormone-free regeneration protocol for *C. terminalis*, emphasizing the importance of optimized sterilization and seasonal timing. This approach offers a simplified and cost-effective alternative for commercial micropropagation of *C. terminalis*.

Keywords: *Cordyline terminalis*; direct organogenesis; explants; micropropagation; MS medium; sterilants

Introduction

Cordyline terminalis (L.) Kunth, commonly known as Ti plant, is a widely cultivated ornamental foliage plant belonging to the family Asparagaceae. Native to Southeast Asia and the Pacific Islands, it is valued for its vividly coloured leaves and is commonly used in landscaping, indoor decoration and traditional rituals (1). Despite its popularity and commercial potential, conventional propagation methods such as sucker division or stem cuttings are slow, season-dependent and limited in multiplication rate (2). Micropropagation offers a powerful alternative for the rapid, year-round production of true-to-type and disease-free planting material in *Cordyline* and other ornamentals (3, 4). However, 2 critical factors influencing the success of *in vitro* propagation are the establishment of aseptic cultures through effective surface sterilization and the optimization of culture media for shoot regeneration and multiplication. Surface sterilization is the first and most vital step in establishing contamination-free cultures. Explants collected

from field-grown plants often harbour high levels of microbial contaminants, especially under humid or rainy seasonal conditions. The choice of sterilant, its concentration and exposure duration significantly impact the explant's survival and aseptic establishment (5). Commonly used sterilants such as ethanol, sodium hypochlorite and mercuric chloride vary in their effectiveness and phytotoxicity depending on the tissue type and species (6). Furthermore, seasonal variation affects microbial load and the physiological condition of explants, which in turn influences *in vitro* response (7). In addition to surface disinfection, the choice and combination of plant growth regulators (PGRs) in the culture medium are crucial for successful shoot induction and direct organogenesis. Cytokinins such as benzyl aminopurine (BAP) and kinetin have been shown to promote multiple shoot formation in ornamental species, while auxins like NAA and IAA play a role in root initiation and morphogenesis(4, 8). However, exogenous application of PGRs increases production cost and may introduce variability, especially during extended culture periods. In contrast, PGR-free media offer a simpler and potentially more stable system, provided that the endogenous hormone levels and culture conditions are favourable. Hence, species-specific media optimization is essential for reproducible and efficient regeneration. A critical prerequisite for successful tissue culture is the establishment of aseptic cultures. Surface sterilization is especially challenging in ornamental species like *C. terminalis*, which have waxy or fibrous tissue surfaces prone to contamination. Optimization of sterilization protocols is therefore essential to ensure high survival rates and morphogenic response without tissue damage. Although previous studies on *C. terminalis* have explored aspects of callus induction and shoot regeneration, comprehensive protocols integrating sterilization, seasonal influence and PGR-free regeneration remain limited (9). Hence, the present study aimed to evaluate the effect of different sterilization treatments, durations, seasonal explant-collection periods and explant types on *in vitro* survival and contamination and to assess direct organogenic response from nodal explants of *C. terminalis* on Murashige and Skoog (MS) medium devoid of growth regulators.

Materials and Methods

Material and explant preparation

Healthy, vigorously growing plants of *Cordyline terminalis* L. (Ti plant) were selected from field-grown mother stock of a local ornamental type (Fig. 1) maintained at the Biotechnology-cum-Tissue Culture Centre in Baramunda, Floriculture and Landscaping Department, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar in the year 2022 and 2023. Nodal segments, shoot tips and leaf bits were excised and washed thoroughly under running tap water for 15-20 min to remove surface dust and debris. Explants were then pre-soaked in a liquid detergent solution (Teepol 5 %) for 10 min and rinsed with double distilled water multiple times (Fig. 2).

Surface sterilization treatments

Surface sterilization was conducted under aseptic conditions inside a laminar airflow cabinet. Explants were subjected to combinations of sterilants including 70 % ethyl alcohol (for 2 min), sodium hypochlorite (0.5 %, 1.0 %, 2.0 %) for 5-30 min and mercuric chloride

Fig. 1. *Cordyline terminalis* mother plant.

Fig. 2. Explants preparation (Nodal segments, Shoot tips and Leaf bits).

Fig. 3. Surface sterilization of explants (nodal segments, shoot tips and leaf bits) under different sterilant treatments.

($HgCl_2$ at 0.05 % and 0.1 %) for 4-15 min (Fig. 3). In some treatments, explants were pre-wiped with ethanol before immersion in chemical disinfectants. Treated explants were rinsed thrice with sterile distilled water to remove residual chemicals.

Seasonal collection study

To evaluate the effect of seasonal variation, explants were collected monthly over a one-year period (January to December). The same sterilization protocol and culture conditions were applied to each batch. Contamination and survival percentages were recorded after 3 weeks of culture.

MS media preparation and culture conditions

Murashige and Skoog (MS, 1962) basal medium was used for all experiments. To standardize direct organogenesis, explants were cultured on MS medium. The research study used Excel R grade analytical reagents from Titan Biotech Ltd., Ranbaxy Laboratory Ltd., Merck (India), Qualigen Fine Chemicals and Himedia Laboratories Ltd. (India). Sigma (USA) supplied auxins, cytokinins, myo-inositol and Fe-EDTA, while Ranbaxy Laboratory Limited provided agar. MS Medium was used for the investigation. Macronutrients, micronutrients, Fe-EDTA, vitamins and plant bioregulators were taken from the stock solution and sucrose dissolved in distilled water was added to the medium. The solution's pH was adjusted to 5.7 ± 0.1 by adding 0.1 N NaOH or 0.1 N HCL. The capacity was then increased to 1 L by adding distilled water. Sucrose (3 %) and Agar (0.8 % w/v) were added to the prepared medium before pouring it into culture flasks and capping them. Culture bottles with culture medium were autoclaved for 20 min at 121 °C and 15 Psi pressure. The autoclaved medium was cooled using a laminar air flow bench. Glassware was soaked in a detergent solution overnight and then washed with running water. They were rinsed with distilled water before being dried in an oven set to 150 °C for 2 hr. Forceps, Petri dishes and scalpels were washed with isopropanol or wrapped in paper before being sterilized in an autoclave at 15 pressure and 121 °C for 20 min.

The operating chamber of the laminar air flow cabinet was cleaned with isopropanol. To prevent particulates from settling in the working area, filtered air (80-100 cft/min) was blown for 5 min. The sterilized materials (except living tissue) were placed in the chamber and exposed to UV radiation for 30 min. The laminar air flow cabinet provided constant filtered air while working. All cultures were incubated in a controlled growth chamber maintained at 25 ± 2 °C under a 16 hr photoperiod with a light intensity of 2000-2500 lux provided by cool white fluorescent lamps (3000-3200 Lux) at 80 % relative humidity. Cultures were monitored regularly and observations were recorded at appropriate intervals (Fig. 4).

Observations recording

The following parameters were recorded:

Percentage survival of explants

Percentage necrosis or death (%): The explant turns brown or dies inside media.

Percentage contamination (fungal and bacterial): Fungal growth appears as a fuzzy growth (either white or black) whereas bacterial growth appears as slimy brownish layer at bottom of media. It is calculated as

$$\frac{\text{Total infection observed in each test tube/Treatment}}{\text{Total number of cultured test tube/Treatment}} \times 100$$

Aseptic culture percentage: Test tube free from any microbial contamination.

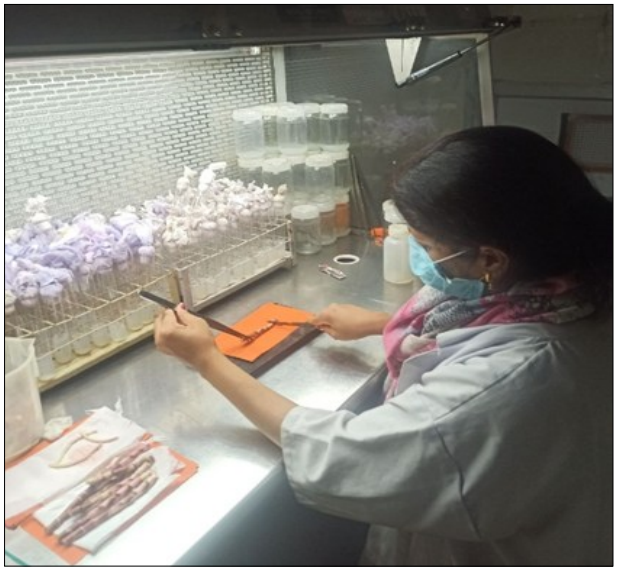

Fig. 4. Conduct of sterilization and direct organogenesis experimental procedure under laminar air flow cabinet.

Number of cultures showing direct organogenesis: This involves direct shoot growth from explant without callus formation phase in media.

Time taken for organogenesis (days): It is the time interval between explant inoculation and direct shoot regeneration.

Time taken for bud emergence (days): It is the time that elapses between explant inoculation and bud initiation.

Time taken for shoot elongation (days): It is the time between inoculation of mass to complete shoot elongation.

Shoot length (cm): It is the length of shoots after development.

Number of leaves/shoots: It is the total number of leaves formed after entire proliferation.

Number of shoots/explants: It is the total number of multiple shoots developed from the date of inoculation.

Time taken for root initiation (days): It is the time taken for initiation of root.

Root length (cm): Length of the roots developed in each cultured media.

Number of roots/explants: Total number of roots primordia formed in each test tube during growth period.

Nature of roots: It is nature of roots during entire growth phase.

Colour of the leaves: It is the colour of leaves during entire growth phase.

Statistical analysis

The experiment was conducted using a Completely Randomized Design (CRD) with 3 replications per treatment, each consisting of 10 cultures. The treatment effects on direct organogenesis, shoot and root traits were analysed using one-way ANOVA at a 5 % level of significance. The standard error of mean (SEm) and critical difference (CD) values were calculated to assess treatment-wise comparisons. Polynomial regression was performed using Microsoft Excel to evaluate the relationship between sterilant exposure duration and culture response variables such as survival, contamination and regeneration efficiency. Regression equations and corresponding R^2 values were recorded.

Results and Discussion

Effect of sterilant type and exposure duration

The response of *Cordyline terminalis* explants to different sterilants and their exposure durations is summarized in Table 1.

Survival percentage (%) of shoot tip

The survival of shoot tip explants was significantly influenced by the type of sterilant, its concentration and exposure duration (Table 1). The highest survival (100 %) was recorded with 70 % ethanol + 0.1 % $HgCl_2$ for 18 min, which was significantly superior to all other treatments. This was followed by ethanol + NaOCl (1.0 % and 1.5 %) at longer durations, which also showed high survival (70 %-80 %) and were statistically distinct. Moderate survival (20 %-60 %) was observed in several treatments using $HgCl_2$ (0.1 %) or ethanol + NaOCl. Treatments involving only ethanol, NaOCl, or short-duration $HgCl_2$ showed poor or no survival. These differences were statistically significant (CD = 1.15, SE(m) = ± 0.58).

Survival percentage (%) of nodal segment

Nodal segment survival in *Cordyline terminalis* varied significantly with sterilant type, concentration and exposure duration (Table 1). The highest survival (100 %) was achieved with 0.1 % $HgCl_2$ for 18 min + 70 % ethanol (T_{16} : 70 % ethanol (30 sec) + 0.1 % $HgCl_2$ (18 min), significantly outperforming all treatments. High survival (90 %-93.33 %) was also observed in T_5 (0.1 % $HgCl_2$, 4 min), T_7 (0.1 % $HgCl_2$, 6 min), T_{11} (0.1 % $HgCl_2$, 10 min) and T_{13}-T_{15} (70 % ethanol + 0.1 % $HgCl_2$, 10-15 min), highlighting the effectiveness of $HgCl_2$ with ethanol at longer durations. Moderate survival (66.67 %-70 %) occurred with 0.1 % $HgCl_2$ (7-9 min), while ethanol + NaOCl combinations (0.5 %–2.0 %) at 15-20 min gave 60 % survival and 13 treatments recorded 0 %. Overall, $HgCl_2$ (0.1 %) combined with ethanol and increased exposure duration significantly improved nodal viability (SE(m): ± 0.54; CD = 1.08).

Survival percentage (%) of leaf bits

Leaf bit survival in *C. terminalis* varied widely across treatments (Table 1). The highest survival (100 %) was observed with 0.1 % $HgCl_2$ for 10 min, which was significantly superior to all others. Ethanol + $HgCl_2$ for 15 min resulted in 50 % survival, followed by 30 % and 20 % with 0.05 % $HgCl_2$ at 15 and 12 min respectively. No survival was recorded with 0.05 % $HgCl_2$ for 10 min and 2.0 % NaOCl. These differences were statistically significant (SE(m): ± 2.91; CD = 6.35).

Comparative analysis (Fig. 5) shows, among the three explants, nodal segments performed best with 93.33 % survival rate while shoot tips recorded 66.67 % survival percentage, both having maximum survival in 70 % ethanol wiping + 0.1 % $HgCl_2$ for 18 min. Leaf bit explants showed 50 % survival among the three explants in 0.1 % $HgCl_2$ for 10 min.

Mercuric chloride is known for its broad-spectrum antimicrobial activity and ability to eliminate both surface and sub-surface contaminants. However, its phytotoxicity at prolonged exposures necessitates precise standardization. The current findings are in agreement with earlier reports that 0.1 % $HgCl_2$ effective in *C. terminalis* and similar trends in *Dracaena* (10, 11). The efficacy of $HgCl_2$ (0.1 % for 10 min) in T_8 aligns with the previous findings which observed improved explant survival and rapid bud release in *C. terminalis* when nodal segments were sterilized using 0.1 % $HgCl_2$ for 10-18 min (12). This treatment likely preserved endogenous

Table 1. Effect of different surface sterilants on survival of *Cordyline* explants after 30 days of culture

Treatment No.	Treatments detail	Concentration(%)	Duration (Minutes)	Survival (%)		
				Shoot tips	Nodal segments	Leaf bits
T_1	Ethyl Alcohol	70	2	0.00	0.00	-
			10	-	-	0.00^{e}
		0.05	12	-	-	20.00^{d}
			15	-	-	30.00^{c}
			4	-	90.00^{b}	-
T_2	Mercuric Chloride		5	-	60.00^{e}	-
			6	-	90.00^{b}	-
		0.10	7	-	70.00^{d}	-
			8	-	70.00^{d}	-
			9	0.00	66.67^{de}	-
			10	0.00	93.33^{ab}	100.00^{a}
T_4	Sodium Hypochlorite	2.0	10	0.00	0.00	0.00^{e}
			10	0.00	93.33^{ab}	-
		70.00 + 0.1	12	10.00^{f}	90.00^{b}	-
T_5	Ethyl Alcohol wiping + Mercuric Chloride		15	20.00^{ef}	90.00^{b}	50.00^{b}
			18	100.00^{a}	100.00^{a}	-
			20	60.00^{c}	80.00^{c}	-
		70.00 + 0.50	5	10.00^{f}	0.00	-
			10	10.00^{f}	0.00	-
			15	60.00^{c}	60.00^{e}	-
			20	10.00^{f}	20.00^{f}	-
		70.00 + 1.00	5	0.00	0.00	-
			10	20.00^{ef}	0.00	-
			15	60.00^{c}	60.00^{e}	-
T_6	Ethyl Alcohol wiping + Sodium Hypochlorite		20	80.00^{b}	60.00^{e}	-
		70.00 + 1.50	5	0.00	0.00	-
			10	30.00^{e}	0.00	-
			15	70.00^{bc}	60.00^{e}	-
			20	60.00^{c}	60.00^{e}	-
		70.00 + 2.00	5	50.00^{d}	60.00^{e}	-
			10	20.00^{ef}	60.00^{e}	-
			15	20.00^{ef}	60.00^{e}	-
			20	20.00^{ef}	60.00^{e}	-
		SE(m) ±		**0.58**	**0.54**	**2.91**
		CD (5 %)		**1.15**	**1.08**	**6.35**

Notes: Data represents average of 10 cultures, duration-30 days and basal MS medium.

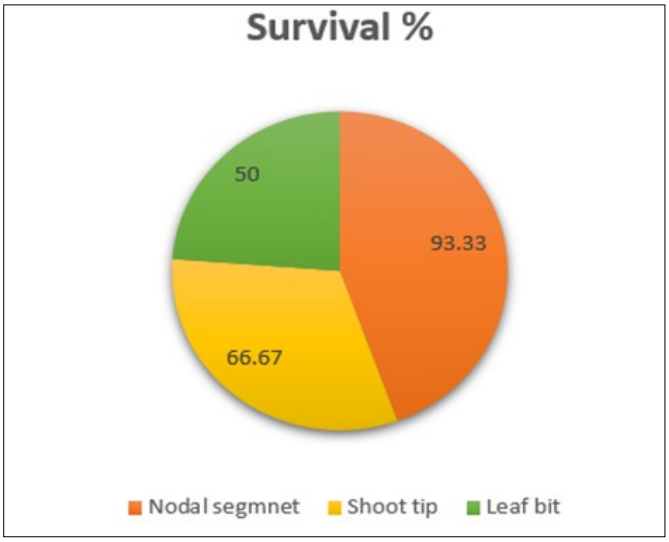

Fig. 5. Explant-wise survival percentage under optimized sterilization.

meristems while ensuring asepsis, facilitating direct shoot initiation without the need for exogenous PGRs. Among the explants tested (shoot tips, nodal segments and leaf bits), nodal segments performed best, which is in accordance with the results of the studies in *Cordyline* (10, 13) and in *D. fragrans* (14). The results are in accordance with the use of Sodium hypochlorite (10) in *Cordyline* and the sterilization of explants in *Agave angustifolia* with 0.1 % $HgCl_2$ (15).

Microbial contamination control with $HgCl_2$ exposure

Table 2 presents the effect of varying durations of 0.1 % $HgCl_2$ on microbial contamination. A 10 min exposure resulted in the lowest fungal contamination (3.33 %) and highest aseptic culture percentage (96.67 %). Shorter exposures (4-6 min) were insufficient in some replicates, while longer durations (8-9 min) showed slightly reduced survival, likely due to chemical stress.

Interestingly, bacterial contamination remained relatively low across all $HgCl_2$ treatments, but fungal contamination increased when exposure dropped below 6 min or exceeded 9 min (Fig. 6). Thus, a 10 min exposure was statistically optimal (CD at 5 % = 13.8 for survival). The result was in accordance with the findings where surface sterilization of the explants of *C. terminalis* with 0.1 % $HgCl_2$ for 10 min was accomplished (16).

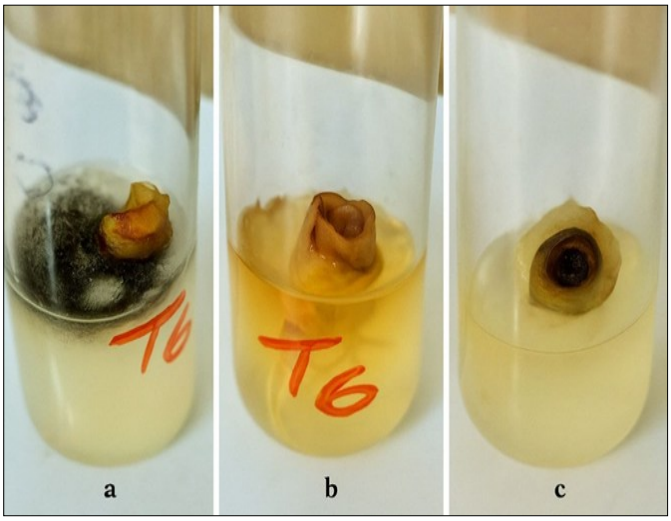

Fig. 6. Contamination of explants in MS media (a-fungal infection, b-bacterial infection, c-death/necrosis of explant).

Table 2. Effect of Mercuric chloride sterilant timings on *in vitro* microbial contamination and survival of nodal segments explant of *Cordyline terminalis*

Treatment No.	Treatments concentration	Time	Fungal (%)	Bacterial (%)	Death (%)	Aseptic (%)	Survival (%)
T_1	Control (tap water)	-	23.33 (28.88 ± 0.44) ^{a}	6.67 (14.96 ± 0.14) ^{a}	6.67 (14.96 ± 0.23) ^{c}	63.33 (52.73 ± 0.12) ^{c}	63.33 (52.73 ± 0.17) ^{c}
T_2	0.1 % $HgCl_2$	4 min	6.67 (14.96 ± 0.44) ^{b}	0.00 (0.00 ± 0.00) ^{c}	3.33 (10.52 ± 0.12) ^{d}	93.33 (75.03 ± 0.27) ^{a}	90.00 (71.56 ± 0.23) ^{a}
T_3	0.1 % $HgCl_2$	5 min	20.00 (26.56 ± 0.35) ^{a}	6.67 (14.96 ± 0.09) ^{a}	10.00 (18.43 ± 0.32) ^{b}	60.00 (50.77 ± 0.20) ^{c}	60.00 (50.76 ± 0.20) ^{c}
T_4	0.1 % $HgCl_2$	6 min	6.67 (14.96 ± 0.26) ^{b}	0.00 (0.00 ± 0.00) ^{c}	3.33 (10.52 ± 0.07) ^{d}	90.00 (68.58 ± 0.14) ^{ab}	90.00 (68.58 ± 0.12) ^{ab}
T_5	0.1 % $HgCl_2$	7 min	16.67 (24.09 ± 0.56) ^{a}	6.67 (14.96 ± 0.20) ^{a}	6.67 (14.96 ± 0.14) ^{c}	70.00 (56.79 ± 0.17) ^{bc}	70.00 (56.79 ± 0.14) ^{bc}
T_6	0.1 % $HgCl_2$	8 min	10.00 (18.43 ± 0.55) ^{a}	6.67 (14.96 ± 0.23) ^{a}	13.33 (21.42 ± 0.27) ^{a}	70.00 (56.79 ± 0.23) ^{bc}	70.00 (56.79 ± 0.06) ^{bc}
T_7	0.1 % $HgCl_2$	9 min	20.00 (26.56 ± 0.14) ^{a}	3.3 (10.52 ± 0.14) ^{b}	10.00 (18.43 ± 0.29) ^{b}	66.67 (54.73 ± 0.20) ^{bc}	66.67 (54.73 ± 0.12) ^{bc}
T_8	0.1 % $HgCl_2$	10 min	3.33 (10.52 ± 0.16) ^{b}	0.00 (0.00 ± 0.00) ^{c}	3.33 (10.52 ± 0.17) ^{d}	96.67 (79.48 ± 0.14) ^{a}	93.33(75.03 ± 0.17) ^{a}
SE(m) ±			**4.55**	**4.29**	**2.44**	**6.31**	**5.72**
CD (5 %)			**10.76**	**10.15**	**5.77**	**14.92**	**13.80**

Note: Values in parenthesis are arc sine transformed values (^{0}), Data represents average of 10 cultures, duration-30 days and basal medium-Murashige and Skoog (MS) medium.

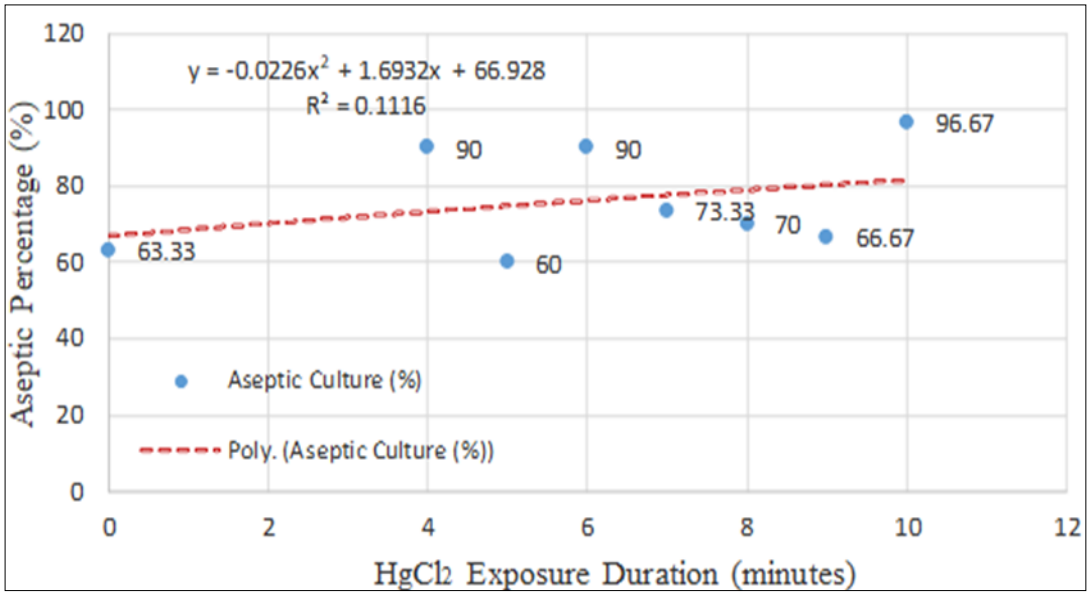

Fig. 7. Polynomial regression between $HgCl_2$ exposure time and survival percentage in *Cordyline* explants.

Regression analysis between $HgCl_2$ exposure duration and explant response

To determine the effect of sterilant exposure time on explant viability and contamination, polynomial regression analysis was performed using the mean values of survival, aseptic culture and microbial contamination (fungal and bacterial) across different $HgCl_2$ durations. A second-order polynomial equation was fitted for survival percentage against $HgCl_2$ exposure time. A polynomial regression curve further illustrates the quadratic relationship between $HgCl_2$ exposure time and explant survival (Fig. 7). The analysis showed a strong quadratic relationship (R^2 = 0.97), with survival increasing up to 10 min (T_8) and decreasing beyond that duration. The regression equation was: > $Y = -0.0577 x^2 + 1.8245 x + 66.919$ ($R^2 = 0.0883$) (where Y = Survival %, x = exposure time in min).

Similarly, aseptic culture percentage showed a positive correlation up to 10 min (T_8) with a peak at that point (Fig. 8), following the equation: > $Y = -0.0226 x^2 + 1.6932 x + 66.928$ (R^2 = 0.1116).

Fungal and bacterial contamination along with mortality (%) showed the opposite trend (Fig. 9), decreasing with increasing exposure time up to 10 min, then plateauing. This confirms 10 min as the statistically optimal exposure duration for disinfection with 0.1 % $HgCl_2$ in *C. terminalis* explants.

Seasonal influence on explant viability

Seasonal variation significantly influenced the *in vitro* survival of *C. terminalis* explants (Table 3; Fig. 10). The highest survival (up to 95.00 ± 0.29 %) was observed during November-December in nodal segments, followed by shoot tips (65.00 ± 0.29 to 0.58 %) and leaf bits (35.00 ± 0.29 %). In contrast, the lowest survival was recorded during June-July, with leaf bits showing only 10.00 ± 0.58 % and shoot tips around 30.00 ± 0.00 to 0.58 %. Nodal segments maintained relatively higher survival throughout all seasons. These findings confirm that explant viability is strongly seasonal, with winter months favoring better survival, possibly due to reduced microbial load and better physiological condition of explants during the dry period. Similar seasonal effects have been documented in other ornamental species (17), reinforcing the importance of explant collection timing in successful *in vitro* establishment. The optimal environmental conditions in the tissue culture lab, including a temperature of 25 °C-30 °C, relative humidity of 60 %-70 % and low light intensity, resulted in green and tall shoots in test tubes. This confirms earlier findings that the culture environment should be maintained under white, fluorescent light for 16 hr photoperiod at 25 ± 2 °C and 55 %-60 % relative humidity (16).

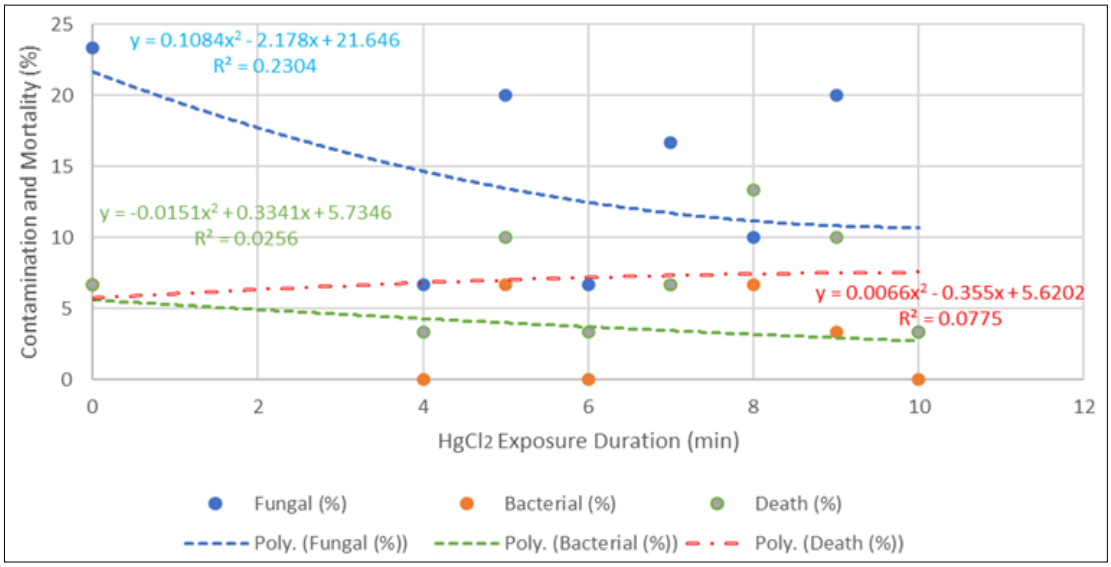

Fig. 8. Polynomial regression between $HgCl_2$ exposure time and aseptic percentage in *Cordyline* explants.

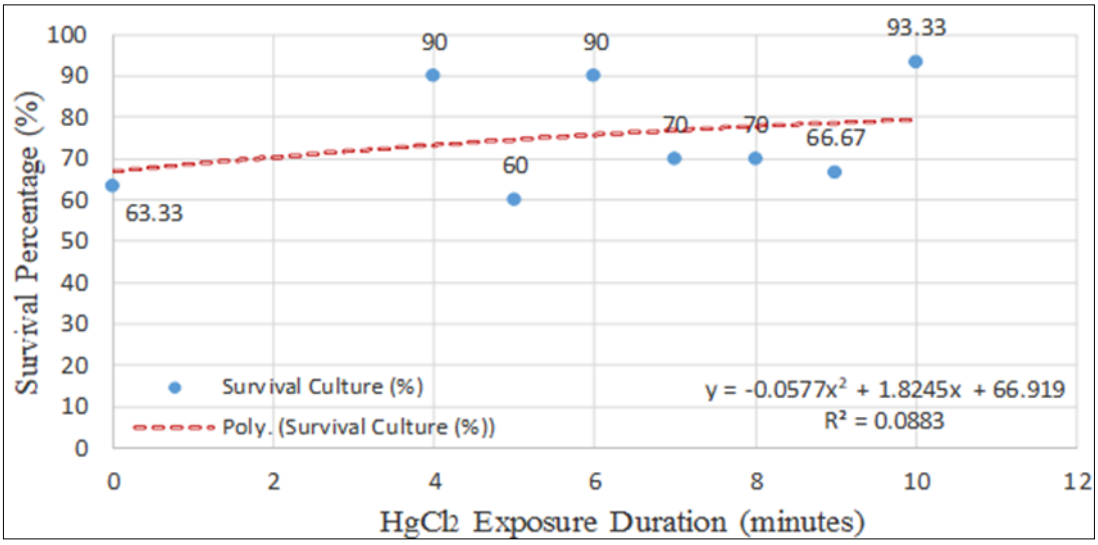

Fig. 9. Polynomial regression between $HgCl_2$ exposure time and contamination percentage in *Cordyline* explants.

Table 3. Seasonal influence on contamination and survival of different explants of *Cordyline terminalis* cultured on MS medium (3-week period)

Months	Nodal segments		Leaf bits		Shoot tips	
	Contamination (%)	Survival (%)	Contamination (%)	Survival (%)	Contamination (%)	Survival (%)
January	10.00 ± 0.29^{e}	85.00 ± 0.29^{c}	65.00 ± 0.29^{d}	30.00* ± 0.29^{b}	20.00 ± 0.29^{g}	60.00* ± 0.29^{b}
February	10.00 ± 0.29^{e}	90.00 ± 0.29^{b}	60.00 ± 0.29^{e}	30.00* ± 0.29^{b}	20.00 ± 0.58^{g}	60.00* ± 0.58^{b}
March	10.00 ± 0.29^{e}	90.00 ± 0.69b	65.00 ± 0.29^{d}	25.00* ± 0.29^{c}	25.00 ± 0.58^{f}	55.00* ± 0.58^{c}
April	30.00 ± 0.41^{d}	70.00 ± 0.41^{d}	70.00 ± 0.29^{c}	20.00* ± 0.29^{d}	30.00 ± 0.58^{e}	45.00* ± 0.29^{d}
May	35.00 ± 0.29^{c}	60.00* ± 0.29^{f}	75.00 ± 0.29^{b}	15.00* ± 0.29^{d}	35.00 ± 0.58^{d}	40.00* ± 0.29^{e}
June	55.00 ± 0.58^{a}	45.00 ± 0.29^{h}	80.00 ± 0.58^{a}	10.00* ± 0.29^{f}	55.00 ± 0.58^{a}	30.00* ± 0.58^{f}
July	55.00 ± 0.58^{a}	45.00 ± 0.58 ^{h}	80.00 ± 0.58^{a}	10.00* ± 0.58^{f}	55.00 ± 0.00^{a}	30.00* ± 0.00^{f}
August	50.00 ± 0.41^{b}	50.00 ± 0.41^{g}	75.00 ± 0.29^{b}	10.00* ± 0.29^{f}	50.00 ± 0.58^{b}	30.00* ± 0.29^{f}
September	35.00 ± 0.29^{c}	65.00 ± 0.29^{e}	70.00 ± 0.29^{c}	15.00* ± 0.29^{e}	40.00 ± 0.58^{c}	40.00* ± 0.58^{e}
October	35.00 ± 0.29^{c}	60.00* ± 0.29^{f}	65.00 ± 0.29^{d}	20.00* ± 0.29^{d}	40.00 ± 0.00^{g}	40.00* ± 0.00^{e}
November	5.00 ± 0.58^{f}	95.00 ± 0.58^{a}	55.00 ± 0.58^{f}	35.00* ± 0.58^{a}	15.00 ± 0.58^{h}	65.00* ± 0.58^{a}
December	5.00 ± 0.29^{f}	95.00 ± 0.29^{a}	55.00 ± 0.29^{f}	35.00* ± 0.29^{a}	15.00 ± 0.29^{h}	65.00* ± 0.29^{a}
SE(m) ±	**1.54**	**0.71**	**0.50**	**0.68**	**0.50**	**0.44**
CD (5 %)	**3.18**	**1.47**	**1.03**	**1.40**	**1.03**	**1.91**

Note: *The remaining explants dried inside the test tube.

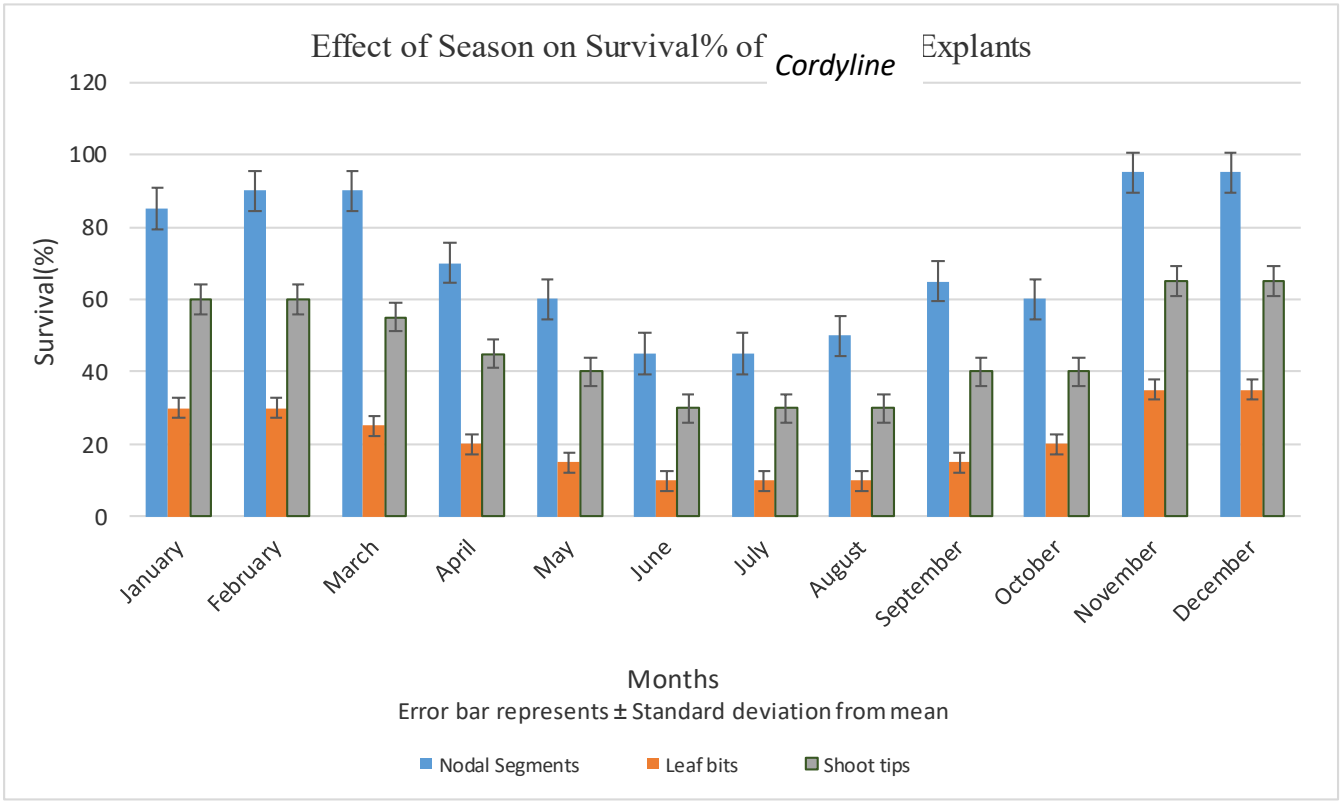

Fig. 10. Seasonal variation in survival percentage of *Cordyline* explants cultured on MS medium.

Table 4. Effect of MS media on the culture establishment of nodal segments for direct organogenesis

Treatment No.	Treatments	Number of cultures showing direct organogenesis	Time taken for organogenesis (days)	Time taken for bud emergence (days)	Time taken for shoot elongation (days)	Shoot length (cm)	Number of leaves/ shoots	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)	5.33 ± 0.17^{a}	51.67 ± 0.33^{f}	20.00 ± 0.06^{cd}	30.00 ± 0.49^{bc}	2.73 ± 0.09^{c}	8.67 ± 0.17^{ab}	5.00 ± 0.18^{bc}	NC	NC	NC	NC	Light green
T_2	0.1% $HgCl_2$ for 4 min	5.67 ± 0.17^{a}	53.33 ± 0.17^{d}	20.33 ± 0.19^{d}	31.67 ± 0.33^{a}	3.03 ± 0.11^{ab}	8.00 ± 0.21^{bc}	5.67 ± 0.22^{ab}	54.67 ± 0.38	5.07 ± 0.15	2.67 ± 0.19	Long, creamy white root with root hairs	Light green
T_3	0.1% $HgCl_2$ for 5 min	5.33 ± 0.17^{a}	53.67 ± 0.17^{cd}	20.33 ± 0.14^{d}	30.67 ± 0.44^{ab}	2.70 ± 0.08^{c}	8.33 ± 0.15^{ab}	4.00 ± 0.15^{d}	NC	NC	NC	NC	Whitish green
T_4	0.1% $HgCl_2$ for 6 min	6.00 ± 0.12^{a}	53.00 ± 0.29^{e}	20.00 ± 0.19^{abc}	29.00 ± 0.29^{cd}	3.00 ± 0.07^{ab}	7.67 ± 0.12^{cd}	5.33 ± 0.19^{ab}	50.00 ± 0.26	5.53 ± 0.21	3.33 ± 0.21	Long, creamy white root with root hairs	Light green
T_5	0.1% $HgCl_2$ for 7 min	6.33 ± 0.09^{a}	54.33 ± 0.17^{c}	19.33 ± 0.09^{bcd}	29.33 ± 0.24^{cd}	3.10 ± 0.10^{a}	7.33 ± 0.11^{d}	3.67 ± 0.14^{d}	NC	NC	NC	NC	Whitish green
T_6	0.1% $HgCl_2$ for 8 min	6.00 ± 0.06^{a}	57.33 ± 0.17^{b}	19.67 ± 0.19^{a}	28.67 ± 0.18^{d}	2.97 ± 0.06^{ab}	8.33 ± 0.18^{ab}	4.33 ± 0.17^{cd}	NC	NC	NC	NC	Light green
T_7	0.1% $HgCl_2$ for 9 min	6.33 ± 0.17^{a}	57.67 ± 0.17^{ab}	18.67 ± 0.12^{ab}	28.33 ± 0.33^{d}	2.80 ± 0.09^{bc}	8.67 ± 0.19^{ab}	4.67 ± 0.18^{bc}	NC	NC	NC	NC	Light green
T_8	0.1% $HgCl_2$ for 10 min	6.67 ± 0.09^{a}	58.00 ± 0.12^{a}	19.00 ± 0.03^{ab}	29.33 ± 0.21^{cd}	3.07 ± 0.08^{a}	9.00 ± 0.16^{a}	6.00 ± 0.23^{a}	47.33 ± 0.31	7.67 ± 0.28	5.33 ± 0.26	Long, creamy white root with root hairs	Light green
	SE(m) ± CD (5 %)	**0.56 1.41**	**0.17 0.51**	**0.18 0.53**	**0.38 1.07**	**0.16 0.22**	**0.12 0.35**	**0.14 0.42**	**0.22 1.14**	**0.18 0.66**	**0.23 0.54**		

Notes: Culture period: 9 weeks, Average of 10 cultures and Basal MS medium. NC: No Change.

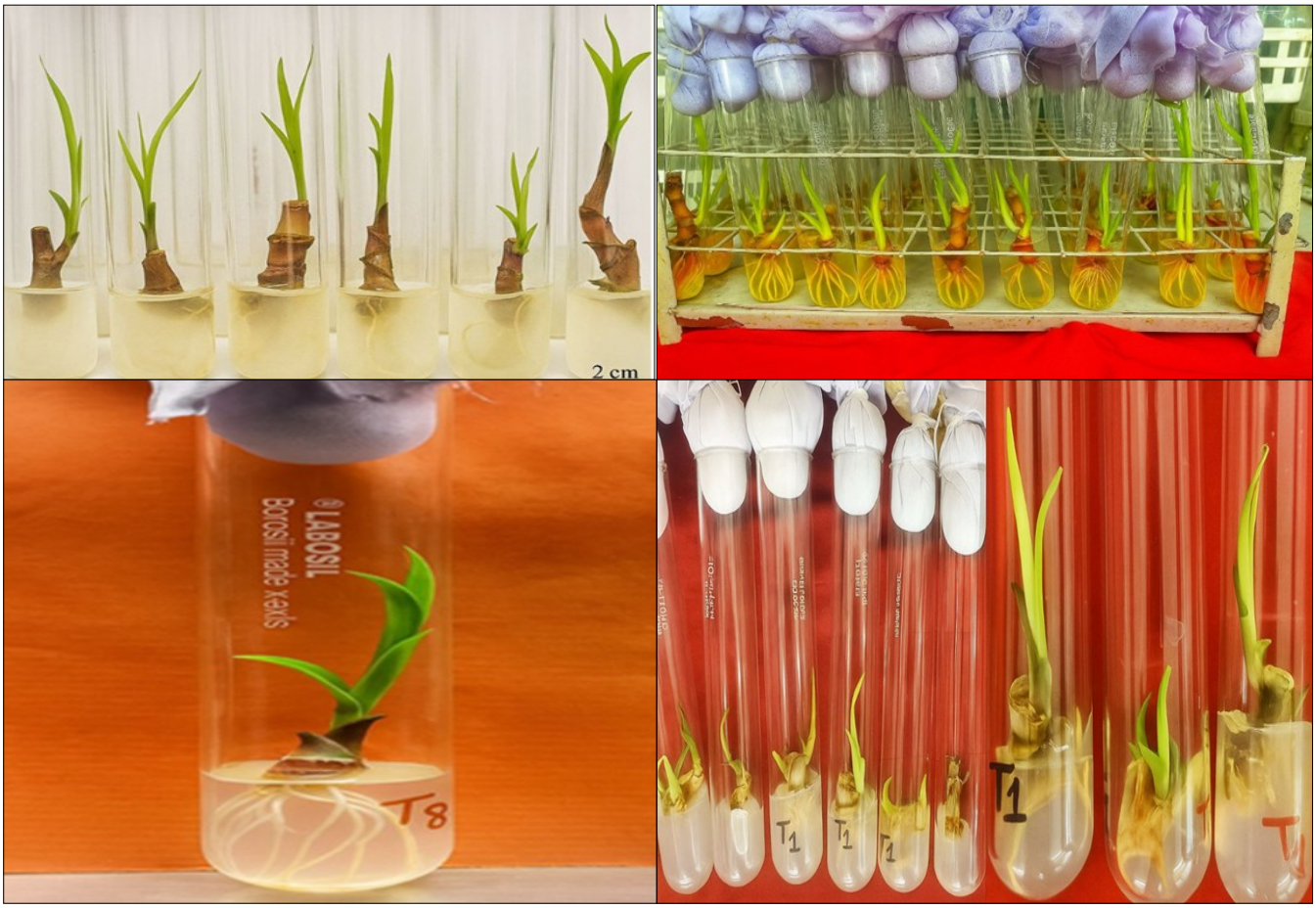

Fig. 11. Direct organogenesis of *Cordyline* explants cultured in growth regulator free MS medium with shoot and root growth having > 90 % survival percentage (A-T_8 with $HgCl_2$ sterilant for 10 min).

Explant response for direct organogenesis under growth regulator free conditions

Under growth regulator-free MS medium, T_8 (0.1 % $HgCl_2$ for 10 min) delivered consistently superior outcomes (Table 4; Fig. 11).

Direct organogenesis frequency

The highest number of cultures exhibiting direct organogenesis (6.67) was recorded in T_8 (0.1 % $HgCl_2$ for 10 min), which was statistically at par with T_5 (0.1 % $HgCl_2$ for 7 min) and T_7 (0.1 % $HgCl_2$

for 9 min) (means 6.33), but significantly higher than T_1 (control - unsterilized explants) ($CD_{0.05}$ = 1.41).

Shoot elongation time

With the earliest elongation (~28.33 days in T_7 [0.1 % $HgCl_2$ for 9 min]) and shortest duration in T_6–T_8 [T_6: 0.1 % $HgCl_2$ for 8 min; T_7: 0.1 % $HgCl_2$ for 9 min; T_8: 0.1 % $HgCl_2$ for 10 min] (based on $CD_{0.05}$ = 1.07, SE_m = ± 0.38).

Shoot length and leaf count

T_5 (0.1 % $HgCl_2$ for 7 min) (3.10 cm) and T_8 (0.1 % $HgCl_2$ for 10 min) (3.07 cm) had highest shoot lengths; T_8 showed maximum leaves/shoot (9.00), significantly superior to lower performers ($CD_{0.05}$ = 0.35).

Rooting traits

Among only three treatments showing roots, T_8 (0.1 % $HgCl_2$ for 10 min) had earliest root initiation (47.33 days, group "c"), longest root length (7.67 cm, group "a") and maximum roots per explant (5.33, group "a") - all significantly higher than others ($CD_{0.05}$ ranged 0.62-1.14).

These results underscore that direct organogenesis can occur effectively in PGR-free MS medium when explants are sterilized optimally (T_8: 0.1 % $HgCl_2$ for 10 min), which minimized tissue damage yet prevented contamination (Fig. 9). This mirrors the previous findings having advocated the same $HgCl_2$ protocol for *C. terminalis* nodal segments, achieving high survival and rapid budding when transferred to MS medium-with or without cytokinins (12). Protocols reported typically include cytokinins such as BAP (2 mg L^{-1}), adenine sulphate (80 mg L^{-1}) and IAA (0.1 mg L^{-1}), yielding ~ 95 % regeneration and ~ 60 shoots per explant (16). These findings demonstrate that under optimal sterilization and culture conditions, PGR-free regeneration is viable highlighting the potential for endogenous hormone activity to drive organogenesis in *C. terminalis*. This PGR-free approach thus highlights a cost-effective, simpler path to organogenesis, potentially driven by endogenous phytohormones and contingent on explant health and aseptic conditions rather than exogenous regulators.

Growth and rooting traits

The high shoot length (3.07-3.10 cm) and leaf number (up to 9 leaves/shoot) achieved under regulator-free regeneration is noteworthy, particularly given that other studies used multiple PGRs to optimize these traits. Moreover, T_8 (0.1 % $HgCl_2$ for 10 min) produced significantly longer roots (7.67 cm) and greater root numbers per explant (5.33), surpassing T_2 (0.1 % $HgCl_2$ for 4 min) and T_4 (0.1 % $HgCl_2$ for 6 min) which contrast with most *in vitro* rooting protocols that supplement IBA/NAA.

Biological implications

Direct regeneration via axillary bud meristem activation (A×M) observed here and in *Cordyline* studies is preferred to callus-based organogenesis to reduce soma clonal variation (18). Histological analyses have documented A×M activation in *Cordyline* under low PGR conditions, reinforcing that PGR-free culture can support direct organogenesis.

Practical applications

This fast protocol supports a simpler, cost-effective propagation strategy suitable for mass production of *Cordyline* cultivars. By eliminating exogenous cytokinins and auxins, the method reduces complexities related to regulatory compliance, hormonal residues and production cost.

Future work is recommended to analyse endogenous phytohormone levels, perform molecular fidelity assessments (e.g., APD, isozyme markers) and evaluate seasonal or explant-source effects on regeneration efficiency (16).

Conclusion

The present study established that *C. terminalis* nodal explants can respond efficiently to growth regulator-free MS medium when sterilized optimally. Among all the explants used, nodal segments performed better than shoot tips and leaf bits. Among the sterilant, $HgCl_2$ (0.1 % for 10 min) for leaf bits and 70 % ethyl alcohol wiping followed by 0.1 % $HgCl_2$ gave 100 % survival in nodal segments and shoot tips explants. For direct organogenesis without callus, T_8 (0.1 % $HgCl_2$ for 10 min) showed the best response in terms of surface sterilization, direct organogenesis, shoot and root traits and early regeneration. This highlights the importance of sterilization conditions in influencing morphogenic responses without exogenous hormonal input. The protocol offers a simple, cost-effective method for *in vitro* propagation of *C. terminalis*.

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Authors' contributions

SSJ carried out the entire Micropropagation protocol work, drafted the manuscript and performed statistical analysis. SSJ, LT and PJ conceived the study, participated in its design, coordination and carried out the revision of manuscript. SSJ, MS, AS, BB, SS, RN, SKS, PJ and AKS provided support, guidance and overall supervision in this research work. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

References

1. Beura S, Samal P, Jagadev PN. Preliminary studies of *in vitro* cloning of Dracaena (*Dracaena sanderiana*). Acta Hortic. 2007;760:241-6. https://doi.org/10.17660/ActaHortic.2007.760.31
2. Evaldsson IE, Welander NT. The effects of medium composition on *in vitro* propagation and *in vivo* growth of *Cordyline terminalis* cv. Atoom. J Hortic Sci. 1985;60(4):525-30. https://doi.org/10.1080/14620316.1985.11515660

3. Pierik RLM. *In vitro* culture of higher plants. Dordrecht: Springer; 1997. https://doi.org/10.1007/978-94-017-1854-7

4. 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

5. Dhyani SK, Kumar RV, Ahlawat SP. *Jatropha curcas*: a potential biodiesel crop and its current R & D status. Indian J Agric Sci. 2011;81(4):295-308.

6. Bharathi JK, Prakash MA. Micropropagation of vetiver grass-A review. In: Micropropagation of medicinal plants. 2024. p.283-96. https://doi.org/10.2174/9789815196146124010017

7. Rani A, Kumar M, Kumar S. *In vitro* propagation of *Withania somnifera* (L.) Dunal from shoot apex explants. J Appl Nat Sci. 2014;6(1):159. https://doi.org/10.31018/jans.v6i1.393

8. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15(3). https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

9. Jena SS, Tripathy L, Beura S, Dash SK, Maharana K, Jena P. *In vitro* optimization of protocol for micropropagation in *Cordyline terminalis* (L.) Kunth. J Adv Biol Biotechnol. 2025;28(6):880-912. https://doi.org/10.9734/jabb/2025/v28i62449

10. Khan SA, Naz SH, Saeed BU. *In vitro* production of *Cordyline terminalis* for commercialization. Pak J Bot. 2004;36(4):757-61.

11. Dewir YH, El-Mahrouk ME, El-Banna AN. *In vitro* propagation and preliminary results of *Agrobacterium*-mediated genetic transformation of *Cordyline fruticosa*. S Afr J Bot. 2015;98:45-51. https://doi.org/10.1016/j.sajb.2015.01.017

12. 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

13. Ye Y, Du C, Xie H, Yang D, Li G, Jiang S, et al. First report of *Colletotrichum cordylinicola* causing anthracnose on *Cordyline fruticosa* in China. Plant Dis. 2024;108(6):1887. https://doi.org/10.1094/PDIS-09-23-1915-PDN

14. Vinterhalter DV. *In vitro* propagation of green-foliaged *Dracaena fragrans* Ker. Plant Cell Tissue Organ Cult. 1989;17(1):13-19. https://doi.org/10.1007/BF00042277

15. Abdallah S. *In vitro* micro-propagation of cordyline and dieffenbachia plants. Hortscience J Suez Canal Univ. 2015;4(1):17-24.

16. Ray T, Saha P, Roy SC. Micropropagation of *Cordyline terminalis*. In: Protocols for micropropagation of selected economically important horticultural plants. Totowa: Humana Press; 2012. p. 269-77. https://doi.org/10.1007/978-1-62703-074-8_21

17. Noor El-Deen TM, Abdul-Moneem NE. Evaluating some 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

18. Scowcroft WR, Larkin PJ. Somaclonal variation. Ciba Found Symp. 2007;137:21-35. https://doi.org/10.1002/9780470513651.ch3

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