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RESEARCH ARTICLE

Optimization of growth regulators for efficient callus-mediated organogenesis in ornamental *Cordyline*

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Abstract

An efficient *in vitro* regeneration protocol was developed for *Cordyline terminalis* via indirect organogenesis using different explants and plant growth regulator (PGR) combinations. Nodal segments, shoot tips and leaf bases were cultured on Murashige and Skoog (MS) medium supplemented with varying 6-Benzylaminopurine (BAP), α-Naphthaleneacetic acid (NAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D) concentrations. Among the explants, nodal segments showed the highest morphogenic response with early shoot initiation and maximum shoot production. The treatment with BAP 1.0 mg L^{-1} + NAA 0.5 mg L^{-1} (H_3) recorded the highest callus induction (83.33 %), highest callus intensity (4.40) and callus index (367.00). Media supplemented with 2,4-D promoted early and dense callus formation but resulted in limited shoot regeneration. Callus tissues were subcultured at 3-week intervals onto fresh regeneration medium containing BAP (1.0 mg L^{-1}) + NAA (0.5 mg L^{-1}) to promote shoot differentiation. It resulted in the regeneration of multiple shoot primordia (up to 4-5 shoots per culture). Correlation analysis indicated a strong positive relationship between callus intensity and shoot proliferation efficiency. These results highlight the critical role of explant selection and hormonal balance in optimizing callus-mediated regeneration. The standardized protocol developed in this study can be effectively utilized for the large-scale propagation and conservation of *Cordyline terminalis*.

Keywords: 2,4-D; BAP; callus; *Cordyline terminalis*; indirect organogenesis; *in vitro* regeneration; micropropagation; NAA

Introduction

Cordyline originated in the tropical and subtropical regions of the world, including the eastern Himalayas, southern China, Malaysia and northern Australia. *Cordyline*, often known as cabbage palm, derives its generic name from the Greek word 'Kordyle', meaning club or cudgel, referring to the shape of its thickened roots (1). The genus *Cordyline* belongs to the family Asparagaceae (formerly Agavaceae) (2).

Cordyline is remarkably similar to *Dracaena*, differing mainly in the shape of the ovary. The plant is widely grown as an ornamental foliage species in homes, offices, shopping complexes, banks, hotels, restaurants, clubs, hospitals and schools. It can also be planted in terrariums, glass containers and other decorative arrangements for aesthetic indoor landscaping (3).

Owing to its colorful foliage, ease of maintenance and market demand, *Cordyline* has become one of the most popular ornamental plants traded in the global floriculture market. Tissue culture has emerged as a reliable tool for the mass multiplication of ornamental species, including *Cordyline*, with a growing demand for uniform, disease-free and elite cultivars.

These foliage plants are commercially propagated by vegetative methods such as sucker division, air layering and stem cuttings. However, conventional propagation methods are slow, seasonal and produce limited plantlets from a single mother plant. Micropropagation overcomes these limitations by enabling large-scale production of true-to-type plants in a relatively short time. It is also a cost-effective method for rapid clonal multiplication where space and labor are limited. Moreover, *in vitro* culture allows year-round production of disease-free planting material in controlled quantities, independent of seasonal constraints.

Plantlets produced through tissue culture can be directly transferred to pots, ensuring rapid establishment and higher survival. In contrast, conventional propagation yields only a few daughter plants from each mother plant (4). Micropropagation through *in vitro* techniques is gaining importance in enhancing multiplication rates and reducing dependence on field-grown stock

plants (5).

Among *in vitro* techniques, indirect organogenesis via callus culture provides greater flexibility for manipulating morphogenesis, improving regeneration efficiency and serving as a foundation for genetic improvement and soma clonal variation studies.

The present study aimed to develop and standardize an efficient callus-mediated regeneration protocol for *Cordyline terminalis* by assessing the influence of explant type and different combinations of BAP, NAA and 2,4-D on callus induction and shoot regeneration under *in vitro* conditions.

Materials and Methods

Plant material

The explant was collected from the plants maintained at the Biotechnology-cum-Tissue Culture Centre in Baramunda under the Floriculture and Landscaping Department, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar and the experiments were conducted during the years 2022 and 2023.

Explants selection and surface sterilization

Young shoots were excised from greenhouse-grown *Cordyline* plants and transported in sterile polythene bags to the laboratory. The shoots were washed with running water. All leaves were removed using a sterile scalpel (Fig. 1). After surface sterilization, shoot tips, nodal segments and leaf bases were removed and inoculated onto the medium with the cut surface in contact with the medium. Surface sterilization was performed using 70 % ethanol (30 sec) followed by 0.1 % mercuric chloride ($HgCl_2$) for 3-5 min (or 0.5 % sodium hypochlorite for 10 min), then rinsed three times with sterile distilled water. The Murashige and Skoog (MS) basal medium was used for all culture experiments.

Preparation of stock solution

Stock solutions for macronutrients, micronutrients, iron and vitamins (hereafter referred to as MS-A, MS-B, MS-C and MS-D, respectively) were prepared separately following the composition of Murashige and Skoog (1962). MS-A (macronutrients) was prepared at 10 × strength, whereas MS-B (micronutrients) and MS-D (vitamins) were prepared at 10 × and 50 × concentrations, respectively. Chemicals were weighed using an electronic balance and dissolved in small amounts of double-distilled water. Each stock's components were blended and double-distilled water was added to make the final volume. Prepared stocks were stored in labelled glass bottles at 4 °C, while the iron-EDTA stock (MS-C) was stored separately in an amber bottle to prevent photodegradation.

Growth regulator preparation

Stock solutions of all growth regulators were prepared at 100 mg L^{-1}. To prepare a BAP stock solution, 10 mg of BAP was dissolved in a few drops of 1 N sodium hydroxide (NaOH) and the volume was made up to 100 mL with double-distilled water. For NAA and 2,4-D, 10 mg of each was dissolved in a few drops of absolute ethanol before making up the volume to 100 mL with double-distilled water. All growth regulator stocks were stored at 4 °C until use.

Culture media preparation

The required quantities of stock solutions, growth regulators and 3 % (w/v) sucrose were mixed and the volume was adjusted to 1 L with double-distilled water. The pH of the medium was adjusted to 5.6-5.8 using a digital pH meter with continuous stirring. To attain the desired pH, 0.1 N NaOH was added dropwise if the medium was too acidic, or 0.1 N hydrochloric acid (HCl) if it was too alkaline. After pH adjustment, 0.7 % (w/v) agar was added, the medium was gently heated to dissolve the agar completely and approximately 30 mL of the medium was dispensed into 300 mL culture vessels. The media were autoclaved at 121 °C and 15 psi for 20 min.

Sterilization methods

To ensure aseptic conditions, all instruments and culture vessels were autoclaved at 121 °C and 15 psi for 20 min before use. Before inoculation, the working area inside the laminar airflow cabinet was sterilized by exposure to ultraviolet (UV) light for 20 min and the airflow was then switched on to maintain aseptic conditions during culture handling. All inoculations and subculturing were carried out under laminar airflow using sterile scalpels, forceps and needles.

Establishment of culture

Explant inoculation was carried out under aseptic conditions inside the laminar airflow cabinet. Culture tubes or jars were incubated at 25 ± 2 °C under a 16 hr photoperiod with a light intensity of 2000-2500 lux, followed by 8 hr of darkness. Callus tissues were subcultured every 21 days onto fresh regeneration medium (MS + 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA) to promote shoot differentiation. Emerging shoots

Fig. 1. Three different explants of *Cordyline terminalis* used for indirect organogenesis (A - shoot tip, B - nodal segment, C - leaf base).

were later transferred to elongation or rooting medium for further growth.

Experimental details

The experiment was conducted following a completely randomized design (CRD) with ten culture tubes per treatment, each replicated three times. The effects of different explants (shoot tip, nodal segment and leaf base) and combinations of growth regulators were studied for their influence on callus induction and shoot regeneration. MS medium supplemented with cytokinins (BAP) and auxins (NAA and 2,4-D) was used to evaluate morphogenic response, while different concentrations of GA_3 were tested to enhance shoot elongation. 2,4-D was included as an auxin source specifically for callus induction.

Observation recorded

- Percentage of cultures exhibiting callus formation (%) :(Number of explants producing callus / Total number of explants cultured) × 100.
- Days taken for callusing: The time elapsed between explant inoculation and callus formation.
- Callus intensity: Length of callus (E-W) cm × breadth of callus (N-S) cm, i.e. volume of callus (Callus spread).
- Callus index (CI): CI = P × I, where P is the percentage of cultures and I is the Callus intensity, i.e. Mean ± SE.
- Callus texture: Texture of callus in the entire growth phase.
- Callus color: Color of callus in the entire growth phase.
- Days to callus differentiation: The duration from callus mass initiation to callus differentiation through subculture for shoot proliferation.
- Number of days for shoot initiation.
- Number of shoot primordia: It is the time between the inoculation of the callus mass and the start of the shoot.

Statistical analysis

The experiment was laid out in a CRD with three replications per treatment. Percentage data were subjected to arcsine transformation before analysis to stabilize variance. Analysis of variance (ANOVA) was performed following a standardized procedure to assess the significance of treatment effects (6). When the F-value was significant at the 5 % probability level ($p \leq 0.05$), mean separation was carried out using the critical difference (CD) test. The coefficient of variation (CV) and standard error of the mean [SE(m)] were also computed to determine experimental precision. All results are presented as mean ± standard error (SE).

Results and Discussion

Standardization of different explants for culture establishment

Table 1 summarizes the response of different explants in terms of shoot initiation days, shoot production and percentage response. Among the three explant types evaluated, nodal segments (E_1) exhibited the best establishment response, recording the highest culture establishment rate (78.63 ± 0.09 %), maximum shoot production (1.8 ± 0.06 shoots per explant) and the earliest shoot initiation (14.2 ± 0.12 days).

Shoot tips (E_2) responded moderately, whereas leaf bases (E_3) failed to initiate culture growth. The superior performance of nodal segments may be attributed to the presence of pre-formed axillary meristems and reduced phenolic exudation, which minimize tissue necrosis and provide a more stable morphogenic environment.

In *Cordyline terminalis*, earlier studies reported regeneration up to 95 % from shoot-apex explants cultured with adenine sulfate and BAP (7), supporting the high regenerative potential of nodal or meristematic tissues. Similarly, *Dracaena sanderiana* nodal explants exhibited significantly higher shoot multiplication and survival than leaf explants on MS medium supplemented with BAP and NAA (8). Comparable trends have been observed in other monocots where nodal segments provide a reliable source of explants due to their active meristematic zones.

Effect of BAP and NAA combinations on callus induction in *Cordyline terminalis*

Significant variation was observed among treatments for callus induction parameters (Table 2). The maximum callus induction (83.33 ± 1.67 %), highest callus intensity (4.40 ± 0.31) and callus index (367.00 ± 3.04) were obtained in treatment H_3 (MS + BAP 1.0 mg L^{-1} + NAA 0.5 mg L^{-1}). Days to callusing were minimum (11.33 ± 0.88 days) in the same treatment, indicating faster initiation. Treatments with higher cytokinin levels (H_6 and H_7) resulted in poor or no callus induction, possibly due to hormonal imbalance.

The induced callus was predominantly soft-friable with a cream to light-green coloration (Fig. 2), characteristic of actively growing, morphogenically competent tissue. Statistical analysis confirmed significant differences ($p \leq 0.05$) among treatments using the CD (5 %) test.

Correlation analysis (Fig. 3) revealed strong positive associations between percentage callusing and callus intensity (r = 0.95) as well as callus index (r = 0.91). Callus intensity and callus index were almost perfectly correlated (r = 0.99), indicating their reliability in assessing callogenic efficiency. In contrast, days to callusing showed weak correlation with other traits, suggesting that faster initiation does not necessarily result in more vigorous callus development.

Table 1. Effect of different explants on *in vitro* culture establishment of *Cordyline terminalis*

Treatment (Explant type)	Days to shoot initiation (days)	Mean no. of shoots per explant (± SE)	Culture establishment (%)
E_1- Nodal segment	14.2 ± 0.11[a]	1.8 ± 0.06[a]	95.6 (78.64) ± 0.09[a]
E_2- Shoot tip	7.8 ± 0.11[b]	1.4 ± 0.06[b]	75.2 (60.23) ± 0.15[b]
E_3- Leaf base	-	-	-
SE (m)	**0.11**	**0.06**	**0.09**
CD (5 %)	**0.32**	**0.16**	**0.24**

Note: Values in parenthesis are arc sine converted values (°). Leaf base explants failed to show a response.

Table 2. Effect of BAP and NAA Combinations on callus induction in *Cordyline terminalis*

Treatment No.	Treatment details (mg L^{-1})	Callus induction (%)	Days to callusing (days)	Callus intensity (± SE)	Callus index (± SE)	Callus texture	Callus colour
H_1	Control (MS media)	NC	NC	NC	NC	NC	NC
H_2	MS + BAP 0.5 mg L^{-1} + NAA mg L^{-1}	63.33 ± 0.88^{b}	14.33 ± 0.34^{ab}	3.50 ± 0.12^{b}	221.67 ± 5.64^{b}	Soft-friable	Cream
H_3	MS + BAP 1.0 mg L^{-1} + NAA mg L^{-1}	83.33 ± 1.67^{a}	11.33 ± 0.88^{d}	4.40 ± 0.31^{a}	367.00 ± 3.04^{a}	Soft-friable	Cream
H_4	MS + BAP 1.5 mg L^{-1} + NAA 0.5 mg L^{-1}	60.00 ± 1.00^{c}	15.00 ± 0.32^{a}	2.00 ± 0.31^{d}	120.00 ± 7.01^{d}	Soft-friable	Cream
H_5	MS + BAP 2.0 mg L^{-1}+ NAA 0.5 mg L^{-1}	53.33 ± 0.88^{d}	14.00 ± 0.35^{ab}	1.50 ± 0.17^{e}	78.33 ± 3.10^{de}	Soft-friable	Light green
H_6	MS + BAP 2.5 mg L^{-1} + NAA 0.5 mg L^{-1}	33.33 ± 1.20^{f}	13.00 ± 0.16^{bc}	2.0 ± 0.36^{de}	66.67 ± 2.19^{e}	Loose-friable	Whitish-green
H_7	MS + BAP 3.0 mg L^{-1} + NAA 0.5 mg L^{-1}	50.00 ± 1.53^{e}	12.00 ± 0.43^{cd}	3.00 ± 0.20^{c}	150.00 ± 5.29^{c}	Soft-friable	Cream
SE(m) ±		1.15	0.27	0.15	2.71		
CD (5 %)		2.51	0.59	0.32	5.91		
CV (%)		2.15	3.45	9.5	5.62		

Note: Each value represents the mean of three replications, with 10 test tubes per replication under CRD ± SE indicates standard error of replication. Means followed by different letters differ significantly at $p \leq 0.05$ as determined by the critical difference (CD) test. NC- No change in the test tube.

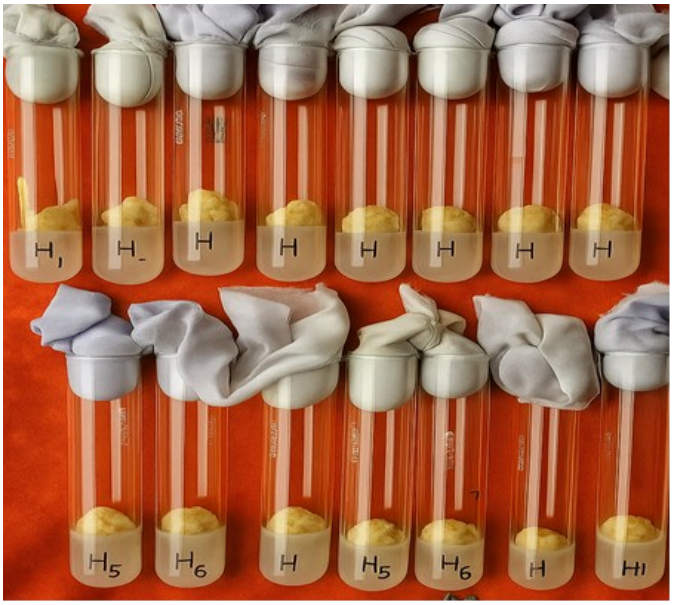

Fig. 2. Variation in callus morphology in *Cordyline terminalis* under different BAP, NAA and 2,4-D treatments.

Note: H_1–H_7 correspond to treatment combinations listed in Table 2.

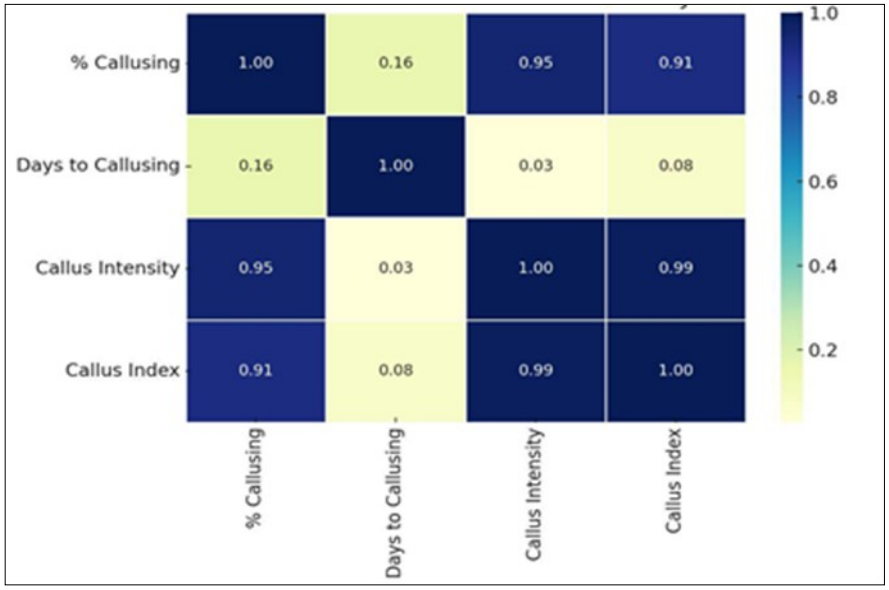

Fig. 3. Pearson's correlation matrix among callus-treated traits in *Cordyline terminalis* as influenced by different concentrations of BAP in combination with NAA. A strong positive correlation was observed between % culture callusing, callus intensity and callus index, while days to callusing showed negligible correlation with other traits. Values range from -1 to +1.

MS medium containing 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA produced the highest mean number of shoots (2.33 ± 0.17 per explant), significantly higher than other treatments. This finding agrees with reports that axillary bud development was greatest in media containing 0.5-1.0 mg L^{-1} BAP (9).

Effect of different concentration of 2,4-D on callus parameters in *Cordyline terminalis*

Significant variation was observed among treatments with different concentrations of 2,4-D (Table 3, Fig. 4). The percentage of cultures showing callusing ranged from 46.67 ± 0.73 % (D_1) to 66.67 ± 0.87 % (D_4). Treatment D_4 (MS + 2.0 mg L^{-1} 2,4-D) produced the highest callus index (141.67 ± 1.45) and callus intensity (2.50 ± 0.06), indicating an optimum auxin concentration for callogenesis. Days to callusing were shortest (12.0 ± 0.23 days) in D_4, whereas D_2 required the longest period (16.0 ± 0.17 days).

Although 2,4-D effectively promoted callus proliferation, calli derived from 2,4-D alone failed to regenerate shoots, confirming the necessity of cytokinin supplementation for organogenic differentiation. These results align with earlier findings that callus initiation from stem explants was effective at 1–2 mg L^{-1} 2,4-D (10, 11) and shoot differentiation occurred only when the callus was transferred to MS medium containing both auxins (NAA or 2,4-D) and cytokinins (BAP) (12-14). Hence, MS + 2.0 mg L^{-1} 2,4-D (D_4) may be optimal for vigorous and early callus induction in *Cordyline terminalis*, but not for subsequent shoot regeneration.

Influence of plant bioregulators on callus differentiation and shoot proliferation in *Cordyline terminalis* via sub-culturing

Based on the callus induction results, H_3 (MS + BAP 1.0 mg L^{-1} + NAA 0.5 mg L^{-1}) was selected as the regeneration medium for sub-culturing callus tissues to induce shoot formation (Table 4, Fig. 5 & 6). Data were recorded at 21 day intervals. The earliest visible shoot primordia appeared after 19 ± 0.33 days in T_3, followed by T_7 (BAP 3.0 mg L^{-1} + NAA 0.5 mg L^{-1}). The control (MS only) showed no regeneration response, reaffirming the requirement of exogenous growth regulators for morphogenesis. Longer induction periods (27 days) were noted in treatments with supra-optimal BAP concentrations (e.g. T_6, BAP 2.5 mg L^{-1} + NAA 0.5 mg L^{-1}), possibly due to cytokinin-induced inhibition of cell differentiation.

The number of shoot primordia varied significantly among the treatments (Table 4). The highest number of shoot primordia (4.00 ± 0.23 per explant) was recorded in T_3 (BAP 1.0 mg L^{-1} + NAA 0.5 mg L^{-1}), followed by T_8 (1.67 ± 0.06), whereas control and higher BAP levels produced few or no shoots. Treatments with 2,4-D alone (T_8-T_{11}) induced callus proliferation but limited shoot initiation, indicating that auxin dominance favors callogenesis but suppresses organogenesis.

Table 3. Effect of different concentrations of 2,4-D on callus induction in *Cordyline terminalis*

Treatment No.	Treatment details	Callus induction (%)	Days to callusing (days)	Callus intensity (± SE)	Callus index (± SE)	Callus texture	Callus colour
D_1	MS + 2,4-D 0.5 mg L^{-1}	46.67 ± 0.73^{c}	13.00 ± 0.10^{b}	2.00 ± 0.06^{a}	93.33 ± 1.2^{c}	Dense-glossy	Cream
D_2	MS + 2,4-D 1.0 mg L^{-1}	50.00 ± 0.43^{bc}	16.00 ± 0.17^{c}	2.00 ± 0.16^{a}	100.00 ± 1.1^{bc}	Soft-friable	Cream
D_3	MS + 2,4-D 1.5 mg L^{-1}	56.67 ± 0.37^{ab}	13.00 ± 0.06^{b}	2.00 ± 0.00^{a}	113.33 ± 1.7^{ab}	Soft-friable	Cream
D_4	MS + 2,4-D 2.0 mg L^{-1}	66.67 ± 0.86^{a}	12.00 ± 0.23^{a}	2.50 ± 0.06^{a}	141.67 ± 1.4^{a}	Dense-glossy	Light green
SE(m) ±		0.63	0.15	0.07	1.00		
CD (5 %)		1.46	0.36	0.16	2.31		
CV (%)		2.00	1.99	5.51	1.54		

Note: Values represent the mean of 3 replicates. Mean values followed by the same letter in a column are not significantly different at 5 % level using the critical difference (CD) test.

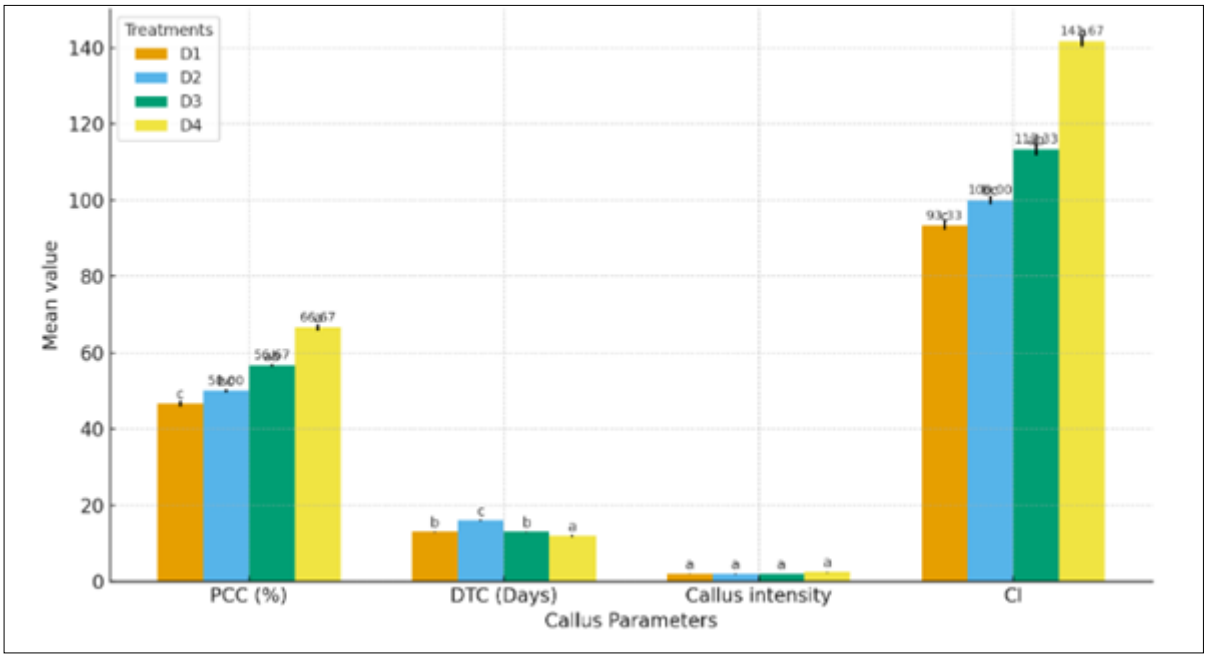

Fig. 4. Effect of 2,4-D on callus parameters in *Cordyline terminalis*, bars represent mean values of three replicates (n = 3) ± SE. Means followed by different letters are significantly different at $p \leq 0.05$ according to DMRT.

Table 4. Influence of plant bioregulators on callus differentiation and shoot proliferation in *Cordyline terminalis* via sub-culturing

Treatment No.	Sub-culture treatment details	Number of days taken for callus differentiation	No of shoots primordia (± SE)	Nature of response
T_1	Control (Full MS media)	NR	6.00 ± 0.23^{a}	Direct organogenesis and shoot regeneration
T_2	MS + BAP 0.5 mg L^{-1}+ NAA 0.5 mg L^{-1}	21.00 ± 0.29^{abc}	1.00 ± 0.06^{cd}	Callus-mediated and shoot proliferation
T_3	MS + BAP 1.0 mg L^{-1}+ NAA 0.5 mg L^{-1}	19.00 ± 0.29^{a}	2.33 ± 0.17^{b}	Callus proliferation and multiple shoot growth
T_4	MS + BAP 1.5 mg L^{-1} + NAA 0.5 mg L^{-1}	22.33 ± 0.27^{bc}	0.33 ± 0.06^{d}	Callus-mediated and shoot proliferation
T_5	MS + BAP 2.0 mg L^{-1} + NAA 0.5 mg L^{-1}	22.33 ± 0.17^{bc}	1.33 ± 0.15^{c}	Callus-mediated and shoot proliferation
T_6	MS + BAP 2.5 mg L^{-1} + NAA 0.5 mg L^{-1}	27.00 ± 0.29^{d}	0.00 ± 0.00^{e}	Callus turned green, no shoot growth
T_7	MS + BAP 3.0 mg L^{-1} + NAA 0.5 mg L^{-1}	19.00 ± 0.29^{a}	0.67 ± 0.058^{d}	Callus-mediated and shoot proliferation
T_8	MS + 2, 4-D 0.5 mg L^{-1}	20.00 ± 0.29^{ab}	1.67 ± 0.21^{bc}	Callus-mediated and shoot proliferation
T_9	MS + 2, 4-D 1.0 mg L^{-1}	22.67 ± 0.44^{bc}	1.33 ± 0.12^{c}	Callus-mediated and shoot proliferation
T_{10}	MS + 2, 4-D 1.5 mg L^{-1}	19.33 ± 0.17^{a}	1.33 ± 0.21^{c}	Callus-mediated and shoot proliferation
T_{11}	MS + 2, 4-D 2.0 mg L^{-1}	19.67 ± 0.17^{ab}	1.33 ± 0.15^{c}	Callus-mediated and shoot proliferation
SE(m) ±		1.15	0.15	
CD (5 %)		0.34	0.43	
CV (%)		2.17	14.14	

Note: All treatments were sub-cultured on MS medium supplemented with BAP (1 mg L^{-1}) and NAA (0.5 mg L^{-1}); values represent the mean of three replications ± SE. Means followed by the same letter do not differ significantly at $p \leq 0.05$ (DMRT). T_1- NR (No response; excluded from analysis).

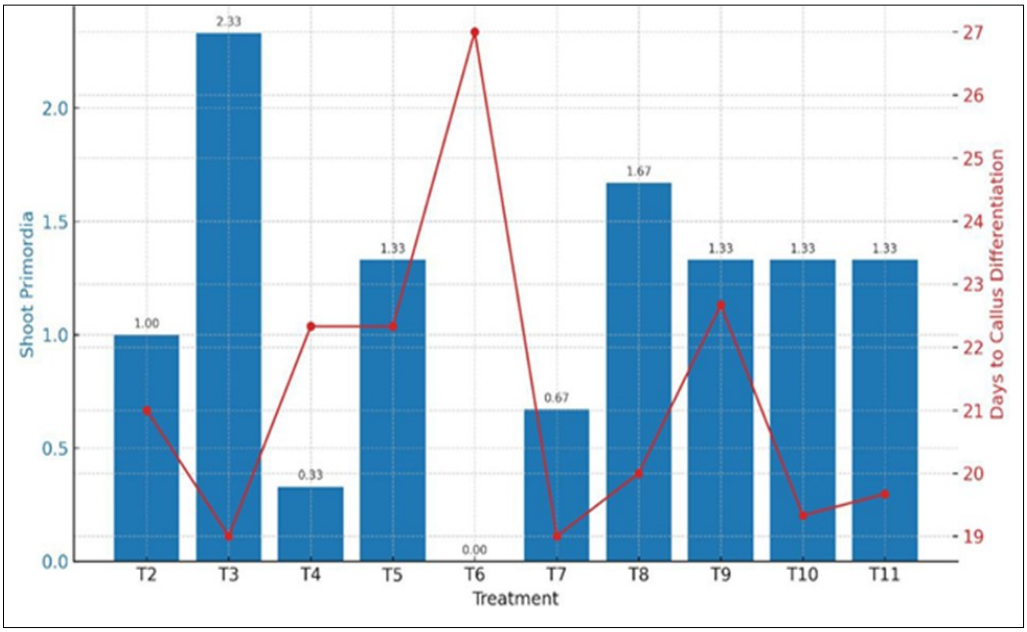

Fig. 5. Effect of plant growth regulators on shoot primordia and callus differentiation in *Cordyline terminalis*.

Note: Treatments correspond to details in Table 4; bars = shoot primordia, line = days to callus differentiation.

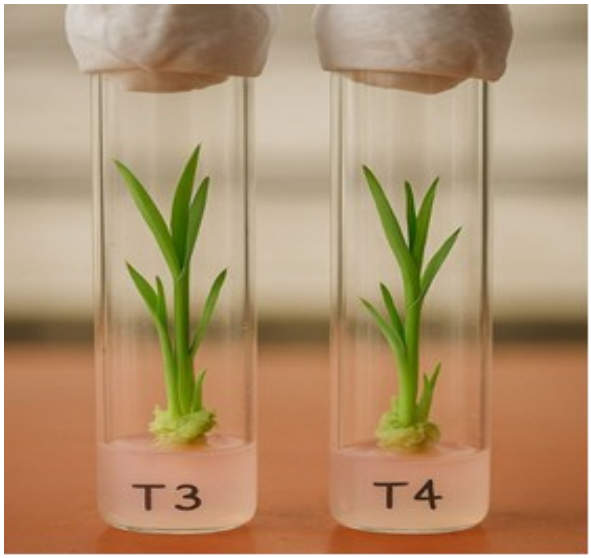

Fig. 6. Sub-culturing of callus for shoot regeneration in *Cordyline terminalis* under plant growth regulators treatment.

Statistical analysis showed significant ($p \leq 0.05$) differences among treatments (CD = 0.33, SE(m) = 0.11, CV = 14.14 %), confirming acceptable experimental precision. Mean grouping revealed that T_3 was significantly superior, forming group 'a', followed by T_8 ('b').

Multiple shoot formation (4-5 shoots per callus) was observed in calli sub-cultured on MS + BAP 1.0 mg L^{-1} + NAA 0.5 mg L^{-1} (Fig. 7, 8), demonstrating the efficacy of cytokinin-auxin interaction in promoting indirect organogenesis. The synergistic effect of BAP and NAA likely breaks apical dominance and stimulates meristematic activity within the callus tissue. These results concur with previous findings where BAP-enriched media enhanced shoot bud differentiation in *Cordyline* and other monocots (4, 15-16). Therefore, BAP (1.0 mg L^{-1}) + NAA (0.5 mg L^{-1}) can be regarded as the most effective hormonal combination for indirect shoot regeneration in *Cordyline terminalis*.

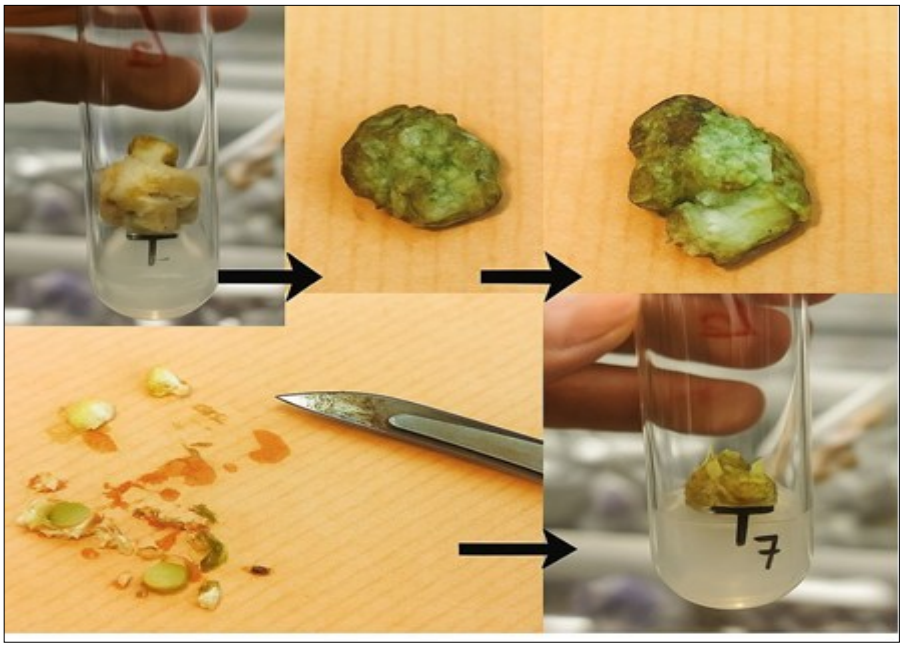

Fig. 7. Shoot proliferation from callus mass under regeneration media T_3 (MS + 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA) and T_4 (MS + 1.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA).

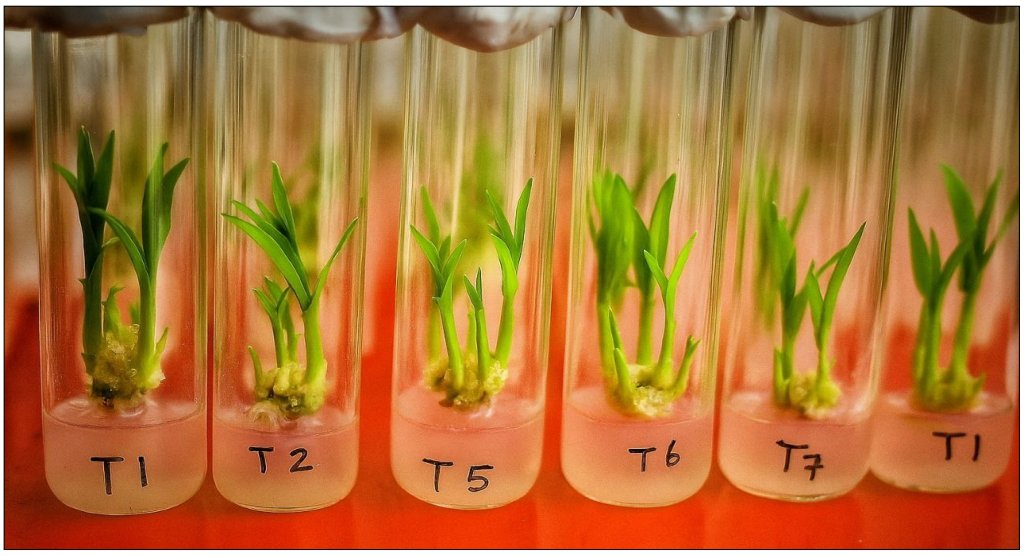

Fig. 8. Callus-mediated multiple shoot formation in *Cordyline terminalis* under optimized regeneration medium (H_3 = MS + 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA). Multiple shoot buds (4-5 per callus) are visible arising from friable callus after 21 days of sub-culture.

Conclusion

The present study standardized an efficient and reproducible protocol for callus-mediated organogenesis in *Cordyline terminalis*. Nodal segments exhibited the highest morphogenic competence among the explants tested, with the highest callus induction and shoot regeneration responses. The combination of BAP (1.0 mg L^{-1}) and NAA (0.5 mg L^{-1}) proved to be the most effective hormonal balance, yielding compact, friable callus and multiple shoots (4-5 per explant) upon subculture. Treatments containing 2,4-D induced callus formation but did not support subsequent shoot differentiation, reaffirming the essential role of cytokinin-auxin interaction in organogenic regeneration.

The optimized protocol provides a reliable method for large -scale clonal propagation, germplasm conservation and future genetic transformation studies in *Cordyline terminalis*. This system can serve as a foundation for rapid commercial multiplication of elite ornamental varieties and for the development of improved cultivars through *in vitro* mutagenesis or biotechnological interventions.

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Authors' contributions

SSJ carried out the entire tissue culture protocol work, drafted the manuscript and performed statistical analysis. SSJ, LT, KM, SB and PJ conceived the study, participated in its design, coordination and

carried out the revision of the manuscript. LT, SB, SKP, PJ, WM and SBN 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 conflicts of interest to declare.

Ethical issues: None

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