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Research Article

Effect of Media Composition and Light Emitting Diodes (LEDs) on the Induction of Protocorm-like Bodies (PLBs) from Thin Cell Layers (TCLs) of *Ludisia discolor*

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ABSTRACT

Ludisia discolor is a terrestrial, shade-loving orchid species highly valued for its striking foliage and medicinal properties. Conventional propagation of the orchid is limited due to slow growth and low germination rates. This study aimed to assess the induction of protocorm-like bodies (PLBs) using transverse thin cell layers (tTCLs) derived from *in vitro* nodal segments as an alternative method of propagating the orchid. Parameters assessed included the influence of light-emitting diode (LED) wavelengths and culture medium strength on PLB formation and overall plant development. Transverse TCL explants were cultured on full- and half-strength Mitra media with plant growth regulators (PGRs) and then exposed to five light treatments: complete darkness, blue (460–470 nm), red (610–625 nm), green (515–525 nm), and cold white (6000–6500 K) LEDs. Data on PLB induction, root and callus formation, and the duration required for PLB and root development were collected over a span of six weeks. The highest PLB induction (22.22 ± 4.65%) was observed in explants cultured on full-strength Mitra medium under darkness. The greatest callus formation occurred under blue LEDs (8.89 ± 3.09%), while green LEDs combined with half-strength Mitra medium led to the highest root production (13.33 ± 2.36%). Stem tTCLs cultured on half-strength Mitra medium supplemented with 1.0 mg L^{-1} 6-benzylaminopurine (BAP) resulted in (20.00 ± 6.70%) PLB induction. Histological analysis confirmed direct PLB development from the cut surface of tTCLs within two to four weeks without intermediate callus formation. These findings demonstrate that medium composition and light quality significantly influence morphogenic responses in *L. discolor*. The optimised tTCL protocol supports future applications in conservation and commercial mass production of this valuable orchid species.

Key words: Light-emitting diodes, *Ludisia discolor*, orchid, protocorm-like bodies, thin cell layers

INTRODUCTION

The monocotyledonous Orchidaceae, a family with over 30,000 species in approximately 750 genera (Kong *et al*., 2003), are known for their aesthetics, medicinal values, and their role as ecological indicators (Joshi *et al*., 2009). Orchids are economically cultivated for ornamental and commercial uses (Billore *et al.,* 2017). Among these, the Jewel orchids of tribe *Cranichideae* and subtribe *Goodyerinae* are especially prized for their leaves rather than flowers (Hu *et al.,* 2016). *Ludisia discolor* is notably sought-after for its leaves (Chen & Shiau, 2015), but faces challenges like slow seed growth, low germination rates and environmental sensitivity (Kauth *et al*., 2008).

Traditionally, orchids play a vital role in medicine; species like *Anoectochilus koshunensis, A. roxburghii,* and *A. formosanus* are used for various ailments including heart disease, diabetes, and hypertension (Pant, 2013; Bhattacharyya & Kumaria, 2015; Poobathy *et al*., 2018). *Ludisia discolor* can soothe nerves, strengthen the spleen, and moisturise the lungs (Liu *et al*., 2021). Micropropagation supports the pharmaceutical industry and conservation efforts by increasing orchid numbers (da Silva, 2013; Bhattacharyya & Kumaria, 2015).

Various factors influence successful *in vitro* propagation, including media and plant growth regulators (da Silva, 2013). *In vitro* cultures were successful in producing PLBs from various vegetative organs of orchids, including leaves, stems, and existing protocorms (Kiaheirati *et al*., 2024). The PLB is favoured in orchid propagation due to its rapid regeneration capability and organised structure. Studies on various orchid species, particularly *Cymbidium*, *Phalaenopsis*, *Dendrobium*, and *Paphiopedilum* indicate that effective plant growth regulators (PGRs) for callus and PLB induction include N-phenyl-N'-1,2,3-thidiazol-5-yl-urea (TDZ) and 2,4-dichlorophenoxyacetic acid (2,4-D; Cardoso *et al*., 2020; Guo *et al*., 2024; Kiaheirati *et al*., 2024).

Established protocols for *in vitro* plant regeneration have shown that light significantly influences orchid growth (da Silva & Dobránszki, 2014), with advancements in LED technology providing alternative light sources (Gupta & Jatothu, 2013). Despite the economic value of *L. discolor*, limited research exists on PLB induction within this species. This study explores the induction of PLBs from *in vitro* leaves and nodal segments of *L. discolor* by the thin cell layer (TCL) method (da Silva & Dobránszki,

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2015), evaluating various PGR combinations and analysing PLB developmental stages through histological observations using toluidine blue O staining.

MATERIALS AND METHODS

Plant material

Ludisia discolor plantlets were propagated and maintained *in vitro* at Quest International University, Perak, Malaysia in half-strength semi-solid MS medium, supplemented with 3% (w/v) sucrose, 0.2% (w/v) activated charcoal, 8% (w/v) Mas banana cultivar homogenate, 1.0 mg L^{-1} 1-NAA, 0.1 mg L^{-1} TDZ and 3.5 g L^{-1} Gelrite (Poobathy *et al*., 2019). The plants were incubated for six weeks at 24 ± 2 °C under cool white LED tubes (6,500 K) at 16 hours photoperiod.

Media preparation and tissue culture experiment design

This study employed semi-solid media consisting of half-strength Murashige & Skoog (MS, 1962) and half-strength or full-strength Mitra (Mitra *et al*., 1976) components supplemented with different PGR combinations listed in Tables 1 and 2. All media were supplemented with 3% sucrose. The pH of the media was adjusted to 5.75 ± 0.05, followed by the addition of a solidifying agent (Gelrite). The media were autoclaved at 121 °C and 15 psi for 20 minutes. Pour plates were prepared by aliquoting 15 mL of the autoclaved media into 9 cm Petri plates, while culture tubes were prepared by aliquoting 15 mL of the autoclaved media into boiling tubes.

The stem and leaf tTCLs were subjected to seven (Table 1) and five (Table 2) different PGR treatment combinations, respectively. In this study, each PGR was tested individually at different concentrations (0 to 2.0 mg L^{-1}). This approach was used as an initial screening strategy to identify suitable PGR types and concentrations and to evaluate their independent effect on PLB induction from stem and leaf tTCLs in *L. discolor*.

Table 1. Combinations of PGRs used in the induction of PLBs from stem tTCLs of *L. discolor*

Treatment	PGR concentration (mg L^{-1})			
	1-naphthaleneacetic acid (NAA)	6- benzylaminopurine (BAP)	2,4-Dichlorophenoxyacetic acid (2,4-D)	Thidiazuron (TDZ)
1	0	0	0	0
2	0.25	0.25	0.25	0.25
3	0.50	0.50	0.50	0.50
4	0.75	0.75	0.75	0.75
5	1.00	1.00	1.00	1.00
6	1.50	1.50	1.50	1.50
7	2.00	2.00	2.00	2.00

Table 2. Combinations of PGRs used in induction of PLBs from leaf tTCLs of *L. discolor*

Treatment	PGR concentration (mg L^{-1})			
	1-naphthaleneacetic acid (NAA)	6- benzylaminopurine (BAP)	2,4-Dichlorophenoxyacetic acid (2,4-D)	Thidiazuron (TDZ)
1	0	0	0	0
2	0.25	0.25	0.25	0.25
3	0.50	0.50	0.50	0.50
4	0.75	0.75	0.75	0.75
5	1.00	1.00	1.00	1.00

Thin cell layers (TCL) of *L. discolor*

Stem segments were sliced into internodal segments with a scalpel, and then into 0.5 mm to 1.0 mm transverse sections using thin razor blades guided by graph paper (da Silva & Dobránszki, 2015). Leaf segments were sliced from the youngest leaf of each plantlet, and then further sliced into transverse sections. All tTCLs were then placed onto their designated treatment media, followed by incubation under 16 hours photoperiod at 24 ± 2°C in an LED chamber designed by Cheong (2019; Table 3).

Table 3. Characteristics and wavelengths of the 5050 SMD LED (Cheong, 2019)

Code	Colour	Wavelength (nm)	LM (M) (lm/m)
5100	Red	610-625	180
5102	Blue	460-470	170
5103	Green	515-525	550
5104	Cold White	6000-6500K	1080

Freehand sectioning and toluidine blue O staining

Protocorm-like bodies produced from tTCLs of *L. discolor* were selected for observations (Gould *et al*., 2002; Shanmugam, 2019). Freehand sections of the PLBs were prepared using double-edged razor blades, stained, and observed under a compound microscope (O'Brien *et al*., 1964; Yeung, 1998; Poobathy, 2017; Shanmugam, 2019). Images of the sections were captured using a light microscope affixed to a colour video camera and analysed using the cellSens imaging software (Poobathy, 2017; Shanmugam, 2019).

Statistical analyses

Results were compiled after six weeks of culture, with each treatment, consisting of three replicates, repeated twice. Data obtained from this study, compiled as mean ± standard error of mean (SE), were analysed with the SPSS software (version 20). Means were analysed using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test in the first part of the study to allow conservative pairwise comparisons among treatments, and Duncan's Multiple Range Test (DMRT) in the second part to provide greater sensitivity for distinguishing and ranking treatment means during the optimisation phase, with the probability value set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of media and PGR composition on PLB, plantlet, and root induction from stem tTCLs of *L. discolor*

Significant differences were detected in the PLB induction percentages (Table 4). The highest PLB induction was observed in stem tTCLs cultured on Mitra basal medium supplemented with 1.0 mg L^{-1} BAP (20.00 ± 6.70%), followed by media added with 0.5 mg L^{-1} BAP (13.30 ± 5.80%) and 0.75 mg L^{-1} NAA (11.10 ± 4.80%). No PLBs were generated from stem tTCLs cultured on MS medium within the six-week observation period.

Table 4. Percentage (%) of PLB induction from tTCLs of *L. discolor* as a function of medium type, PGR type and PGR concentration

Medium	PGR type	PLB induction rate (%)						
		PGR concentration (mg L^{-1})						
		0	0.25	0.50	0.75	1.00	1.50	2.00
MS	NAA	0c	0c	0c	0c	0c	0c	0c
	BAP		0c	0c	0c	0c	0c	0c
	2,4-D		0c	0c	0c	0c	0c	0c
	TDZ		0c	0c	2.22 ± 2.22bc	0c	0c	0c
Mitra	NAA	0c	2.22 ± 2.22 bc	6.70 ± 4.70 bc	11.10 ± 4.80 abc	6.70 ± 3.30 bc	2.22 ± 2.22 bc	0c
	BAP		6.60 ± 4.70 bc	13.30 ± 5.80 ab	2.22 ± 2.22 bc	20.00 ± 6.70 a	0c	4.40 ± 3.00 bc
	2,4-D		0c	0c	0c	2.22 ± 2.22 bc	0c	0c
	TDZ		0c	2.22 ± 2.22 bc	4.44 ± 3.00 bc	2.22 ± 2.22 bc	2.22 ± 2.22 bc	2.22 ± 2.22 bc

Note: Values represent M ± SE. Means with same alphabets are not significantly different from one another.

A study conducted by Gomes *et al.* (2015) emphasised the significant role of cytokinins in improving the induction of protocorm-like bodies (PLBs) from stem tip tissue culture lines (tTCLs). Sheelavanthmath *et al*. (2005) demonstrated that 1.0 µM (0.23 mg L^{-1}) BAP effectively stimulated PLB formation, yielding an average of 49.1 PLBs per explant. Similarly, Gomes *et al*. (2015) found that 1.0 µM (0.23 mg L^{-1}) BAP resulted in the highest average PLB production (9.3 PLBs per tTCL) when cultured on Woody Plant Medium (WPM). The use of TDZ was also observed to induce PLBs from stem tTCLs, although the outcomes did not significantly differ from those obtained with BAP. Malabadi *et al*. (2009) reported that the most PLBs were generated in shoot tTCLs of *Aerides maculosum* cultured on Mitra basal medium supplemented with 13.62 µM (3.0 mg L^{-1}) TDZ. Similarly, Mulgund *et al*. (2011) found that 11.35 µM (2.5 mg L^{-1}) TDZ resulted in the highest PLB production in *Xenikophyton smeeanum*. These concentrations are comparable to those tested in this study, indicating that TDZ commonly promotes PLB induction in orchids. However, TDZ was less effective than BAP in inducing PLBs in *L. discolor*. This difference may reflect species-specific hormonal sensitivity, explant type, or culture conditions, suggesting that cytokinin responsiveness varies among orchid species and tissues.

A study conducted by Guo *et al*. (2024) reported that half-strength MS and 0.025 mg L^{-1} 2,4-D was suitable for the proliferation of PLBs, while half-strength MS added with 10% coconut water (cw, v/v) and 0.5 g L^{-1} activated carbon (AC) was suitable for PLB differentiation of *Paphiopedilum* SCBG Huihuang 90. According to a study by Kiaheirati *et al*. (2024), the highest number of PLBs of *Phalaenopsis circus* was achieved using 1.0 mg L^{-1} 2,4-D. The highest number of plantlets were obtained using two to three cell-layered tTCLs of *Phalaenopsis circus* cultured on medium supplemented with 0.5 mg L^{-1} IBA and 1.0 mg L^{-1} TDZ. In contrast, although TDZ has been reported as effective in other orchids, it showed limited effectiveness in this study, indicating species-specific responses. Moderate induction observed with NAA suggests that auxin may support early dedifferentiation, but it was less effective than BAP. The lack of response on MS medium may be related to differences in nutrient composition, further highlighting the importance of basal medium selection (Tikendra *et al*., 2025).

In this study, the PLBs were mostly induced from the stem tTCLs without any intermediate callus stage (Figure 1). A slight expansion of stem tTCLs was observed after two weeks when the colour of tTCLs changed from green to brown (Figure 1). The PLB formation was first detected as the formation of a small detachable globular white protuberance on the epidermal surface of the stem tTCL after four weeks of culture. These globular structures further differentiated into mature PLBs between the four to six-week mark and further differentiated into plantlets (Figure 1).

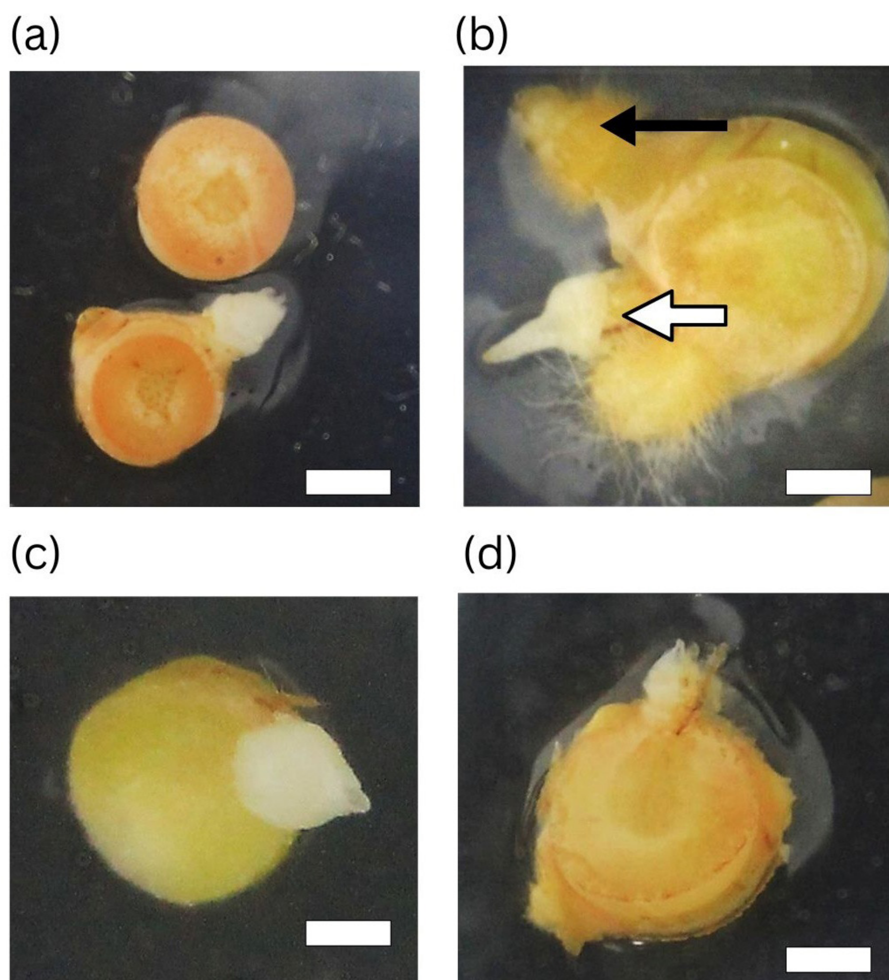

Fig 1. (a) Protocorm-like body as well as (b) shoot (white arrow) and root (black arrow) development from stem tTCLs of *in vitro L. discolor* cultured on full-strength Mitra medium supplemented with 1.0 mg L^{-1} BAP. (c and d) protocorm-like body development from stem tTCLs of *in vitro L. discolor* cultured on half-strength Mitra medium supplemented with 1.0 mg L^{-1} BAP. Bar = 1 cm.

In this study, plantlet and root induction from stem tTCLs of *L. discolor* was generally low and inconsistent across treatments (Tables 5 & 6). Several treatments showed 0% induction, indicating limited morphogenic competence for complete plantlet regeneration within the six-week culture period. Although some explants produced shoots and roots, the responses were infrequent and not consistent across PGR types or concentrations. Root hairs appeared as fine white structures emerging from nodal regions and later turned light brown or yellow, while shoots showed a bleached appearance, likely due to reduced chlorophyll accumulation under certain culture conditions (Figure 1[b]). Multiple shoot clusters were not observed. These low regeneration frequencies suggest that stem tTCLs of *L. discolor* may possess limited endogenous hormonal balance for complete organogenesis, similar to recalcitrant responses reported in other orchid species. For example, *Paphiopedilum* species exhibit poor in vitro regeneration and slow growth, while PLB induction in *Phalaenopsis* and *Dendrobium* varies depending on explant type and hormonal conditions. Thus, these observations indicate that morphogenic responses in orchids are highly species-specific, highlighting the importance of optimising culture conditions for individual species (da Silva, 2013; Cardoso *et al*., 2020).

Table 5. Percentage (%) of plantlet induction from tTCLs of *L. discolor* as a function of medium type, PGR type and PGR concentration

Medium	PGR type	Plantlet induction rate (%)						
		PGR concentration (mg L^{-1})						
		0	0.25	0.50	0.75	1.00	1.50	2.00
MS	NAA	8.90 ± 4.80	4.44 ± 2.90	0	2.22 ± 2.22	2.22 ± 2.22	4.44 ± 3.00	8.90 ± 3.50
	BAP		8.90 ± 3.50	2.22 ± 2.22	2.22 ± 2.22	8.90 ± 3.50	8.90 ± 3.50	4.44 ± 2.90
	2,4-D		2.22 ± 2.22	13.30 ± 6.70	4.44 ± 2.90	8.90 ± 3.50	4.44 ± 2.90	6.70 ± 3.30
	TDZ		2.22 ± 2.22	6.70 ± 6.70	6.70 ± 6.70	4.44 ± 2.90	4.44 ± 2.90	6.70 ± 3.30
Mitra	NAA	6.70 ± 3.30	4.44 ± 2.90	2.22 ± 2.22	0	2.22 ± 2.22	2.22 ± 2.22	4.44 ± 2.90
	BAP		13.30 ± 4.70	4.40 ± 2.90	4.44 ± 2.90	0	4.44 ± 2.90	6.70 ± 6.70
	2,4-D		8.90 ± 3.50	6.70 ± 3.30	15.60 ± 5.60	0	8.90 ± 3.50	6.70 ± 3.30
	TDZ		0	4.40 ± 2.90	4.40 ± 2.90	8.90 ± 3.50	0	0

Note: Values represent M ± SE. Means are not significantly different from one another.

Table 6. Percentage (%) of root induction from tTCLs of *L. discolor* as a function of medium type, PGR type and PGR concentration

Medium	PGR type	Root induction rate (%)						
		PGR concentration (mg L^{-1})						
		0	0.25	0.50	0.75	1.00	1.50	2.00
MS	NAA	0	0	0	0	0	0	0
	BAP		2.22 ± 2.22	0	0	2.22 ± 2.22	0	0
	2,4-D		0	0	0	0	0	0
	TDZ		0	0	0	0	4.44 ± 2.90	0
Mitra	NAA	6.70 ± 4.70	0	0	0	0	11.10 ± 4.80	6.70 ± 4.70
	BAP		4.44 ± 4.44	2.22 ± 2.22	2.22 ± 2.22	0	2.22 ± 2.22	8.90 ± 6.80
	2,4-D		6.70 ± 4.70	0	6.70 ± 4.70	4.44 ± 2.90	0	2.22 ± 2.22
	TDZ		0	0	0	0	0	2.22 ± 2.22

Note: Values represent M ± SE. Means are not significantly different from one another.

A three-way analysis of variance (ANOVA) showed that media type significantly influenced the outcomes of PLB, plantlet, and root induction compared to PGR type and concentration. Additionally, interactions were noted between media type, PGR type, and concentration in the induction process of PLBs, plantlets, and roots from stem tTCLs of *L. discolor* (Table 7).

Table 7. Analysis of variance for PLB, plantlet and root production from stem tTCLs of *L. discolor* as a function of medium type, PGR type and PGR concentration

Variable and source	*df*	*MS*	*F*	*p*
Media				
TCL producing PLBs (%)	1	1267.013	35.997	0.000
TCL producing plantlets (%)	1	85.239	0.783	0.377
TCL producing roots (%)	1	963.140	20.186	0.000
PGR				
TCL producing PLBs (%)	3	230.711	6.555	0.000
TCL producing plantlets (%)	3	296.571	2.724	0.044
TCL producing roots (%)	3	37.540	0.787	0.502
PGR concentration				
TCL producing PLBs (%)	6	142.535	4.050	0.001
TCL producing plantlets (%)	6	88.438	0.812	0.561
TCL producing roots (%)	6	80.403	1.685	0.123
Media * PGR				
TCL producing PLBs (%)	3	248.600	7.063	0.000
TCL producing plantlets (%)	3	46.526	0.427	0.733
TCL producing roots (%)	3	66.071	1.385	0.247
Media * PGR concentration				
TCL producing PLBs (%)	6	134.614	3.825	0.001
TCL producing plantlets (%)	6	94.850	0.871	0.516
TCL producing roots (%)	6	92.819	1.945	0.072
PGR * PGR concentration				
TCL producing PLBs (%)	18	70.616	2.006	0.009
TCL producing plantlets (%)	18	113.272	1.041	0.412
TCL producing roots (%)	18	42.568	0.892	0.589
Media * PGR * PGR concentration				
TCL producing PLBs (%)	18	67.623	1.921	0.013
TCL producing plantlets (%)	18	108.530	0.997	0.462
TCL producing roots (%)	18	51.033	1.070	0.381

Note: Values are statistically significant when $p \leq 0.05$.

Significant differences were detected in the mean percentage of PLB induction from stem tTCLs of *L. discolor* (Table 8), with an interaction observed between medium and lighting types (Table 9). Protocorm-like bodies were induced within the first week in tTCLs placed in full-strength Mitra medium and complete darkness when compared to the other treatments (11.11 ± 5.39%), followed by half-strength Mitra medium with white LEDs (4.44 ± 2.42%). By Week 6, the highest PLBs induction was observed in tTCLs placed in full-strength Mitra medium and complete darkness (22.22 ± 4.56%, Table 8) while the lowest was observed in full-strength Mitra medium and white LEDs (8.89 ± 3.89%, Table 8). By Week 6, the highest PLB induction was observed in tTCLs placed in full-strength Mitra medium under complete darkness, possibly due to the adaptation of the tTCLs influenced by both the medium and lighting types. Enhanced PLB induction under complete darkness suggests that reduced light favours dedifferentiation and early morphogenic events by suppressing photomorphogenic pathways. Consistent with this, Hussien *et al*. (2025) reported that dark conditions significantly promoted protocorm development and seedling formation in *Dactylorhiza fuchsii*. In addition, full-strength Mitra medium may provide a more suitable mineral balance than MS medium, supporting the metabolic activity required for PLB induction.

Table 8. Percentage (%) of PLB induction from tTCLs of *L. discolor* as a function of medium and lighting type at Weeks 1, 2 and 6 of treatment.

Medium	Lighting Type	% of PLB Induction		
		Week 1	Week 2	Week 6
Full-strength Mitra	PTC room control	0.00 ± 0.00 b	4.44 ± 2.42 cd	10.00 ± 2.89 bc
	Darkness control	11.11 ± 5.39 a	12.22 ± 5.47 abc	22.22 ± 4.56 a
	Blue LED	0.00 ± 0.00 b	14.44 ± 1.76 ab	21.11 ± 2.00 a
	Red LED	0.00 ± 0.00 b	17.78 ± 3.64 a	20.00 ± 3.33 ab
	Green LED	2.22 ± 1.47 b	6.67 ± 2.36 bcd	16.67 ± 4.08 abc
	White LED	0.00 ± 0.00 b	3.33 ± 2.36 cd	8.89 ± 3.89 c
Half-strength Mitra	PTC room control	0.00 ± 0.00 b	2.22 ± 1.47 d	14.44 ± 1.75 abc
	Darkness control	1.11 ± 1.11 b	1.11 ± 1.11 d	10.00 ± 2.88 abc
	Blue LED	0.00 ± 0.00 b	8.89 ± 3.51abcd	16.67 ± 3.73 abc
	Red LED	0.00 ± 0.00 b	5.56 ± 2.42 bcd	12.22 ± 2.22 bc
	Green LED	0.00 ± 0.00 b	6.67 ± 2.36 bcd	12.22 ± 2.22 abc
	White LED	4.44 ± 2.42 b	10.00 ± 4.41 abcd	20.00 ± 4.08 ab

Note: Values represent M ± SE. Means with the same alphabet within the same column are not significantly different from one another.

Table 9. Analysis of variance for PLB production from stem tTCLs of *L. discolor* as a function of medium and lighting type at Weeks 1, 2 and 6 of treatment

Variable and source	*df*	*MS*	*F*	*p*
Medium				
Week 1 (%)	1	45.37	1.58	0.21
Week 2 (%)	1	448.15	5.44	0.02
Week 6 (%)	1	133.33	1.38	0.24
Light type and LED wavelength				
Week 1 (%)	5	103.15	3.59	0.00
Week 2 (%)	5	193.33	2.35	0.05
Week 6 (%)	5	90.37	0.94	0.46
Medium * Light type and LED wavelength				
Week 1 (%)	5	103.15	3.59	0.00
Week 2 (%)	5	228.15	2.77	0.02
Week 6 (%)	5	326.67	3.38	0.00

Note: Values are statistically significant when p ≤ 0.05.

Significant differences were detected in the mean percentages of callus induction from stem tTCLs of *L. discolor* on Week 6 of treatment (Table 10), with an interaction observed between both the medium and lighting type (Table 11). Three treatments induced PLBs via a callus-intermediate stage. The highest callus induction was detected in tTCLs treated with full-strength Mitra medium and blue LEDs (8.89 ± 3.09%, Table 10), followed by full-strength Mitra medium and green LEDs (2.22 ± 1.47%), and half-strength Mitra medium with white LED (2.22 ± 2.22%). Differences among LED treatments likely reflect wavelength-specific activation of plant photoreceptors. Blue light, perceived by cryptochromes and phototropins, may enhance cell division and callus formation, while red light, mediated by phytochromes, could influence early root initiation (Wongsa *et al*., 2025). However, the modest effects suggest that light quality plays a secondary role to basal medium composition in regulating PLB induction in *L. discolor*.

Table 10. Percentage of callus induction from tTCLs of *L. discolor* as a function of medium and lighting type at Week 6 of treatment

Light type	% of callus induction	
	Full-strength Mitra medium	Half-strength Mitra medium
PTC room control	0.00 ± 0.00b	0.00 ± 0.00 b
Darkness control	0.00 ± 0.00 b	0.00 ± 0.00 b
Blue LED	8.89 ± 3.09 a	0.00 ± 0.00 b
Red LED	0.00 ± 0.00 b	0.00 ± 0.00 b
Green LED	2.22 ± 1.47 b	0.00 ± 0.00 b
White LED	0.00 ± 0.00 b	2.22 ± 2.22 b

Note: Values represent M ± SE. Means with the same alphabets are not significantly different from one another.

Table 11. Analysis of variance for callus induction from stem tTCLs of *L. discolor* as a function of medium and lighting type at Week 6 of treatment

Variable and source	*df*	*MS*	*F*	*p*
Medium	1	59.26	4.74	0.03
Light type	5	53.33	4.27	0.00
Medium * Light type	5	68.15	5.45	0.00

Note: Values are statistically significant when p ≤ 0.05.

It was observed that other treatments induced PLBs via direct embryogenesis from the stem. The callus formation was first detected as the formation of an amorphous mass of loosely arranged thin-walled parenchymatous cells arising from the epidermal surface of the stem tTCL after three weeks of culture (Figure 2).

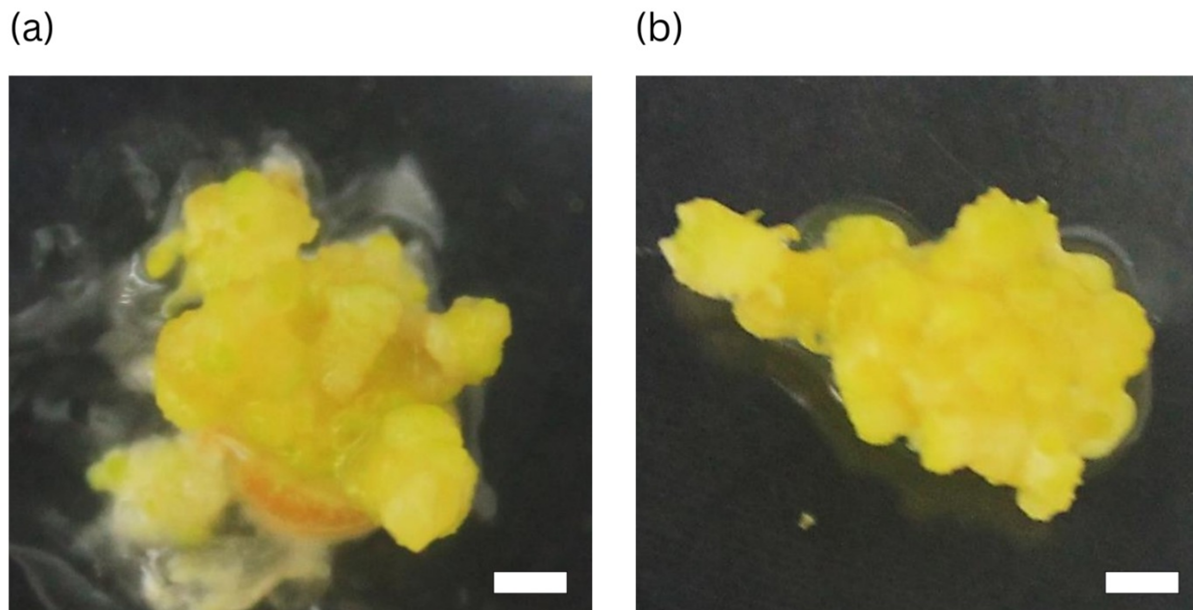

Fig. 2. (A) Callus induction from stem tTCLs of *in vitro L. discolor* cultured on Mitra medium supplemented with 1.0 mg L^{-1} BAP (a) full-strength Mitra. (b) half-strength Mitra medium. Bar = 1 cm.

da Silva (2008) reported PLB formation was predominantly influenced by the type of medium, with minimal callus induction. Almeida *et al*. (2019) reported that the use of LED lamps with white and medium-blue wavelengths led to significant increase in somatic cotyledonary embryos in *Carica papaya* compared to fluorescent lamps, highlighting the light source's impact on callus production. Medium-blue LEDs resulted in highest number of matured somatic embryos in sugarcane (Heringer *et al*., 2017). Blue LEDs light achieved the greatest conversion rate of somatic embryos to plantlets in *V. vinifera* (Tittmann *et al*., 2013).

For root production, tTCLs treated with full-strength Mitra medium and red LEDs showed the highest initial root formation (6.67 ± 2.89%, Table 12). Significant differences in root production were observed in Week 1, with interactions between medium and lighting types (Table 13). In Week 2, the highest root production was observed in tTCLs of *L. discolor* treated with half-strength Mitra medium and white LEDs (8.89 ± 2.61%, Table 12). From Weeks 3 to 6, no significant differences in root production were observed (Table 12). These early responses likely reflect the influence of light-specific photoreceptors and nutrient availability on initial root initiation. From Weeks 3 to 6, root development showed no significant differences, suggesting later growth was less dependent on light or medium composition.

Table 12. Percentage (%) of root production from tTCLs of *L. discolor* as a function of medium and lighting type at Weeks 1, 2 and 6 of treatment

Medium	Light type	Weeks		
		1	2	6
Full-strength Mitra	PTC room control	0.00 ± 0.00 b	0.00 ± 0.00 b	5.56 ± 2.42
	Darkness control	2.22 ± 1.47 ab	2.22 ± 1.47 ab	11.11 ± 3.51
	Blue LED	4.44 ± 2.94 ab	4.44 ± 2.94 ab	11.11 ± 2.00
	Red LED	6.67 ± 2.89 a	6.67 ± 2.89 ab	12.22 ± 2.78
	Green LED	0.00 ± 0.00 b	0.00 ± 0.00 b	10.00 ± 1.67
	White LED	0.00 ± 0.00 b	0.00 ± 0.00 b	10.00 ± 3.33
Half-strength Mitra	PTC room control	1.11 ± 1.11 b	0.00 ± 0.00 b	12.22 ± 2.78
	Darkness control	4.44 ± 2.42 ab	6.67 ± 2.35 ab	7.78 ± 2.78
	Blue LED	2.22 ± 1.47 ab	4.44 ± 2.93 ab	12.22 ± 2.78
	Red LED	0.00 ± 0.00 b	6.67 ± 2.89 ab	11.11 ± 2.00
	Green LED	1.11 ± 1.11 b	4.44 ± 1.76 ab	13.33 ± 2.36
	White LED	4.44 ± 1.76 ab	8.89 ± 2.61 a	11.11 ± 3.09

Note: Values represent M ± SE. Means with the same alphabets or without alphabets within the same column are not significantly different from one another.

Table 13. Analysis of variance for root production from stem tTCLs of *L. discolor* as a function of medium and lighting type at Weeks 1, 2 and 6 of treatment

Variable and source	*df*	*MS*	*F*	*p*
Medium				
Week 1 (%)	1	0.00	0.00	1.00
Week 2 (%)	1	237.04	6.13	0.02
Week 6 (%)	1	45.37	0.70	0.40
Light type and LED wavelength				
Week 1 (%)	5	33.33	1.36	0.25
Week 2 (%)	5	94.82	2.45	0.04
Week 6 (%)	5	27.59	0.43	0.83
Medium * light type and LED wavelength				
Week 1 (%)	5	68.89	2.81	0.02
Week 2 (%)	5	59.26	1.53	0.19
Week 6 (%)	5	54.26	0.84	0.53

Note: Values are statistically significant when $p \leq 0.05$.

Histological observations of PLB formation from stem tTCLs of *L. discolor*

Longitudinal sections of stem tTCLs of *L. discolor* revealed direct induction of PLBs without an intermediate callus stage. It was noted that the callus induction led to PLB generation. Root hair formation was also observed on the tTCLs (Figure 3[a]).

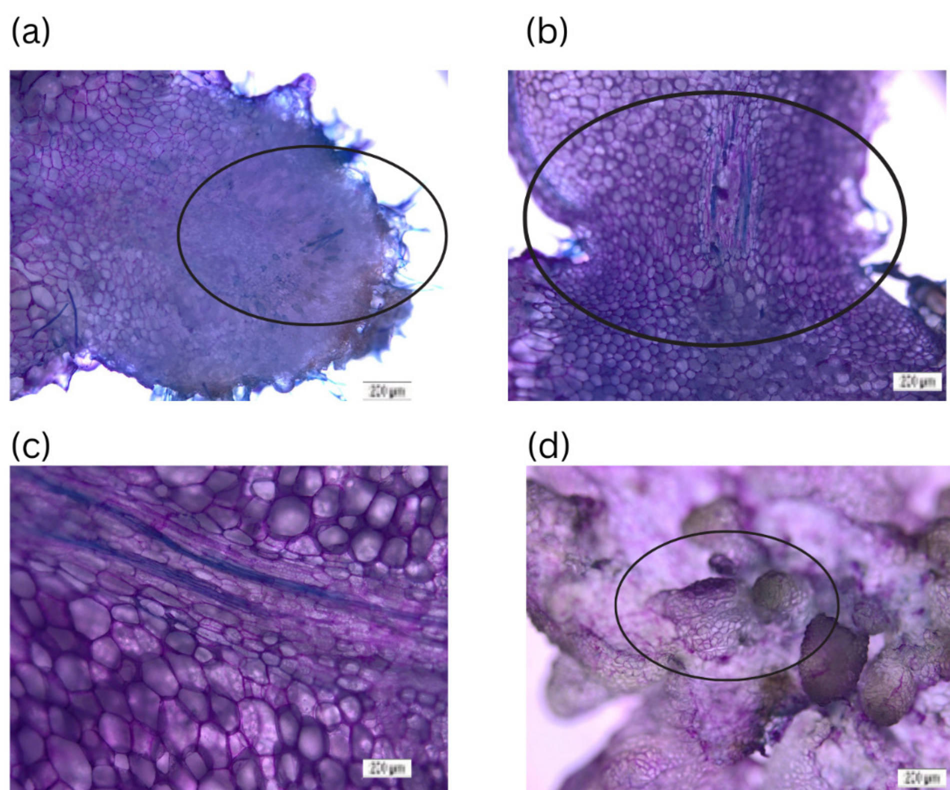

Fig. 3. Observations of tissues formed from stem tTCLs of *L. discolor*, circled in black. (a) Root formation from tTCL. (b) PLB formation with separate vasculature system from the original explant (c) The vascular system of the induced shoots was directly connected to the stem tTCLs. (d) Callus induction with potential PLB formation. Bar = 200 μm

The PLBs were observed to mostly have separate vascular systems which were not connected to the explant (Figure 3[b]), while some had vascular systems directly connected to that of the TCL (Figure 3[b]). The shoot apical meristem (SAM) can be observed after three weeks of culture. The SAM contains closely-packed actively-dividing small-sized cells. The early proliferation of SAM can be recognised by the existence of dense cytoplasm (Gantait & Sinniah, 2012). The protrusions that formed on PLBs are the rapidly dividing meristematic cells that later differentiated into the shoot and finally leaf.

Similar observations were reported by Bustam *et al*. (2013) for PLBs of *Dendrobium* Shavin White that were five to six weeks old. According to Antony *et al.* (2013), the meristematic cells in PLBs originate from wounded surfaces and eventually develop into the shoot primordia and plantlets. In contrast to PLBs regenerated from callus, Sheelavanthmath *et al*. (2005) reported that PLBs regenerated directly from orchid explants enhance the genetic stability of the orchid clones.

CONCLUSION

The best results in the induction of PLBs from stem tTCLs of *L. discolor* were obtained using Mitra basal medium supplemented with 1.0 mg L^{-1} BAP, followed by 0.5 mg L^{-1} BAP and 0.75 mg L^{-1} NAA. When LEDs were included in this study, the best results in the induction of PLBs from stem tTCLs of *L. discolor* were observed in samples cultured in full-strength Mitra medium under complete darkness, followed by full-strength Mitra medium and blue LEDs. Globular-shaped PLBs were induced within two weeks of the treatment, indicating that the TCL technique can be used in the propagation of *L. discolor*. Histological observations confirmed that the PLBs were induced directly from the stem tTCLs without intermediate callus formation. Although plantlet and root induction frequencies were generally low, the results indicate that the TCL technique represents a promising alternative approach for the propagation of this economically valuable jewel orchid. Further optimisation of hormonal balance and culture conditions may improve regeneration limitations.

ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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