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Blue light promotes germination and protocorm development in asymbiotic germination of *Phaius tankervilleae* (Banks) Blume 'Alba'

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ABSTRACT: *Phaius tankervilleae* 'alba' is a highly ornamental orchid species listed as a rare and endangered species in several countries. To develop an efficient in vitro propagation method, this study investigated the effects of light quality on seed germination and protocorm development. Seeds were cultured on VW medium under various light conditions, including darkness, cool white LED, cool white, warm white, blue, red, green and yellow fluorescent lamps, for three months. The highest germination rate (91.3%) was observed under blue fluorescent light, followed closely by red fluorescent light (86.0%), with no significant difference between the two. Notably, blue fluorescent light also promoted the highest percentage of advanced-stage protocorms (stage 3+4) at 28.3%. Although green fluorescent light resulted in a lower germination rate (61.0%), it induced a comparable rate of advanced-stage protocorms (25.0%), not significantly different from the blue light treatment. In contrast, seeds cultured in darkness exhibited the lowest germination rate (18.7%) and the highest proportion of dead protocorms (12.3%). Protocorms' mortality was also observed under cool white, green and yellow fluorescent lights. The results indicate that light quality significantly affects seed germination and protocorm development in *P. tankervilleae* 'alba', with blue fluorescent light being the most effective for promoting successful in vitro propagation.
Keywords: light quality; orchid; terrestrial orchid; light spectrum; VW medium.

A luz azul promove a germinação assimbiótica e o desenvolvimento do protocormo de *Phaius tankervilleae* (Banks) Blume 'Alba'

RESUMO: *Phaius tankervilleae* 'alba' é uma espécie de orquídea altamente ornamental, classificada como rara e ameaçada de extinção em diversos países. Para desenvolver um método eficiente de propagação in vitro, este estudo investigou os efeitos da qualidade da luz na germinação das sementes e no desenvolvimento dos protocormos. As sementes foram cultivadas em meio VW sob diferentes condições de luz, incluindo escuridão, LED branco frio, luz branca fria, luz branca quente, luz azul, luz vermelha, luz verde e luz amarela fluorescente, durante três meses. A maior taxa de germinação (91,3%) foi observada sob luz fluorescente azul, seguida de perto pela luz fluorescente vermelha (86,0%), sem diferença significativa entre as duas. Notavelmente, a luz fluorescente azul também promoveu a maior porcentagem de protocormos em estágios avançados (estágios 3 e 4), com 28,3%. Embora a luz fluorescente verde tenha resultado em menor taxa de germinação (61,0%), induziu uma taxa comparável de protocormos em estágios avançados (25,0%), sem diferença significativa em relação ao tratamento com luz azul. Em contraste, as sementes cultivadas no escuro apresentaram a menor taxa de germinação (18,7%) e a maior proporção de protocormos mortos (12,3%). A mortalidade dos protocormos também foi observada sob luz fluorescente branca fria, verde e amarela. Os resultados indicam que a qualidade da luz afeta significativamente a germinação das sementes e o desenvolvimento dos protocormos em *P. tankervilleae* 'alba', sendo a luz fluorescente azul a mais eficaz para promover a propagação in vitro.
Palavras-chave: qualidade da luz; orquídea; orquídea terrestre; espectro de luz; meio VW.

1. INTRODUCTION

Orchids (Orchidaceae) represent one of the largest and most diverse families of flowering plants, exhibiting remarkable variation in floral morphology that reflects adaptation to diverse habitats and environmental conditions (ZHANG et al., 2018). *Phaius tankervilleae* 'alba', a rare terrestrial orchid species, has garnered attention for its ornamental value due to its attractive appearance, ease of flowering, extended blooming period, and large inflorescences with showy flowers (CHENG et al., 2012; KURZWEIL, 2014). Orchid propagation relies heavily on seed germination (RASMUSSEN et al., 2015). Unlike most angiosperms, orchid seeds are exceptionally small, lack endosperm, and thus depend on symbiotic associations with mycorrhizal fungi during early germination and seedling development (ARDITTI; GHANI, 2000; AEWSAKUL et al., 2013; ZHAO et al., 2024a). Understanding the factors that influence seed germination is therefore critical for the conservation and propagation of these ecologically and horticulturally valuable plants.

In this context, asymbiotic seed germination has emerged as a powerful technique for orchid propagation and conservation. This in vitro method bypasses the need for fungal symbionts by providing a nutrient-rich, sterile environment that supports early orchid development (ZENG et al., 2016; KUNAKHONNURUK et al., 2018). Numerous studies have examined the factors affecting asymbiotic germination, including nutrient media composition (PAUL et al., 2012; AN et al., 2021), the role of plant growth regulators (VOGEL; MACEDO, 2011; CASTILLO-PÉREZ et al., 2021), and the influence of abiotic factors such as light and temperature (JOHNSON et al., 2011; KAUTH et al., 2011).

Among these factors, light is a critical environmental cue that influences seed germination and development in a wide range of plant species, including orchids (ZETTLER; MCINNIS,1994; NIKABADI et al., 2014; FLORES et al., 2016). Orchid species exhibited diverse, often species-specific, responses to light quality and quantity (GODO et al., 2011; TSUTSUMI et al., 2011; NIKABADI et al., 2014). For instance, *Cyrtopodium glutiniferum* showed the highest seed germination and protocorm development under green light (VOGEL; MACEDO, 2011). In *Bletilla ochracea*, green (525 nm) and orange (590 nm) light significantly enhanced germination rates, while orange and red (625 nm) light promoted rhizoid formation (GODO et al., 2011). In contrast, light spectra did not affect seed germination of *Brassavola perrinii*, but did influence protocorm development, with white fluorescent light being the most effective (PEREIRA et al., 2022).

Despite these findings, there remains a significant knowledge gap regarding the effects of light quality on the seed germination and protocorm development of *P. tankervilleae* ‘alba’, a horticulturally valuable color variant. To date, no study has explored its in vitro responses to light spectra, particularly under asymbiotic conditions.

Therefore, the objective of this study is to investigate the influence of different light spectra on the asymbiotic seed germination and protocorm development of *P. tankervilleae* ‘alba’. This work aims to contribute to the optimization of propagation protocols and to support conservation efforts for this underutilized orchid species.

2. MATERIAL AND METHODS

2.1. Capsule surface sterilization and seed culture

Flowers of *P. tankervilleae* ‘alba’ (Figure 1) from Romklao Botanical Garden under the Royal Initiative, Phitsanulok, Thailand, were hand-pollinated. Mature capsules (60 days after pollination) were surface-sterilized with 15% (v/v) Clorox® solution for 20 minutes, followed by five rinses with sterile distilled water. Seeds were then extracted and cultured on VW medium (VACIN; WENT, 1949) supplemented with 150 mL L^{-1} coconut water and 50 g L^{-1} potato extract, following the protocol of Kunakhonnuruk et al. (2018). The medium pH was adjusted to 5.2 and solidified with 8 g L^{-1} agar before autoclaving at 121 °C for 15 minutes.

Seed cultures were incubated at 25±2 °C under different lighting conditions: darkness, cool white LED lamp (Panasonic LDAHV7D67HAP), cool white fluorescent lamp (Philips TL-D Lifemax 18W/840), warm white fluorescent lamp (Philips Genie energy saver, 14W) and fluorescent lamps emitting blue, red, green and yellow (Lamptan Spiral Compact Colour, 13W each). Lighting was provided for 12 hours per day, with a photosynthetic photon flux density of approximately 18-22 µmol/m²/s for all light sources. The spectral profiles (Figure 2) and relative spectral distribution (Figure 3) were measured using a LI-180 spectrometer (LI-COR, Nebraska, USA).

Figure 1. Inflorescence (top) and flower (bottom) characteristics of *Phaius tankervilleae* ‘alba’ (left) compared to *P. tankervilleae* (right).
Figura 1. Características da inflorescência (acima) e da flor (abaixo) de *Phaius tankervilleae* ‘alba’ (à esquerda) em comparação com P. tankervilleae (à direita).

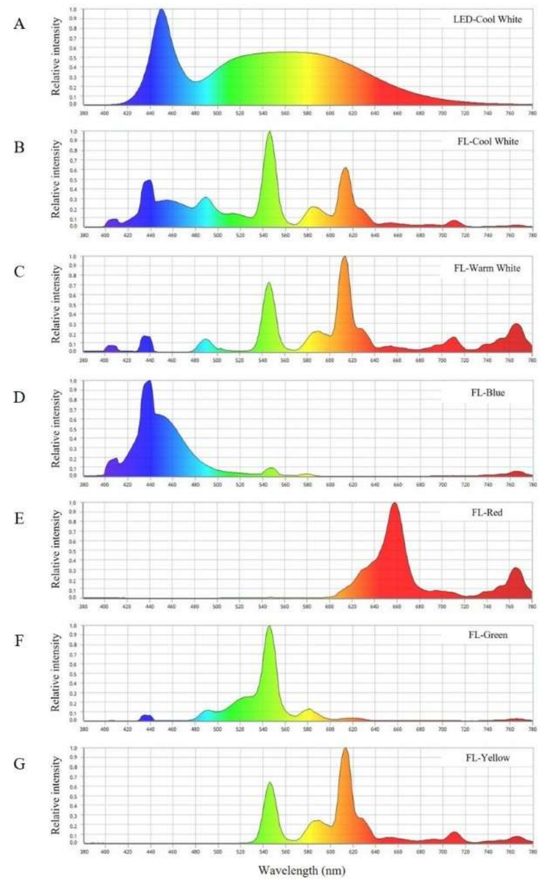

Figure 2. Spectral profiles of the light sources used in this study: (A) cool white LED lamp, (B) cool white fluorescent lamp, (C) warm white fluorescent lamp, (D) blue fluorescent lamp, (E) red fluorescent lamp, (F) green fluorescent lamp, and (G) yellow fluorescent lamp.

Figura 2. Perfis espectrais das fontes de luz utilizadas neste estudo: (A) lâmpada LED branca fria, (B) lâmpada fluorescente branca fria, (C) lâmpada fluorescente branca quente, (D) lâmpada fluorescente azul, (E) lâmpada fluorescente vermelha, (F) lâmpada fluorescente verde e (G) lâmpada fluorescente amarela.

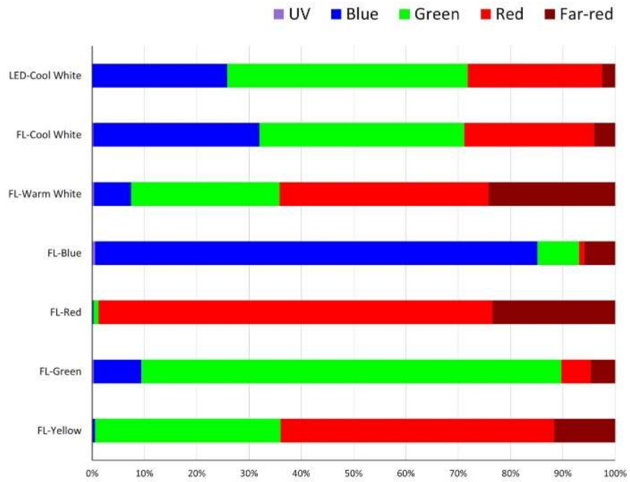

Figure 3. Proportion (%) of wavelength bands within the UV (380-400 nm), blue (400-500 nm), green (500-600 nm), red (600-700 nm), and far-red (700-780 nm) regions for each light source used in this study.
Figura 3. Proporção (%) das faixas de comprimento de onda nas regiões do ultravioleta (380-400 nm), azul (400-500 nm), verde (500-600 nm), vermelho (600-700 nm) e vermelho distante (700-780 nm) para cada fonte de luz utilizada neste estudo.

2.2. Examination of seed germination and protocorm development

After three months of culture, seed germination and protocorm development were observed under a stereomicroscope; each treatment included three replicates, with 100 seeds assessed per replicate. Germination percentage was calculated as the number of germinated seeds divided by the total number of seeds observed. Protocorm developmental stages were classified based on criteria adapted from Punjansing et al. (2021) with slight modifications. The percentage at each developmental stage was calculated as the number of protocorms at that stage divided by the total number of seeds examined.

2.3. Experimental design and data analysis

The experiment followed a completely randomized design. Data were analyzed using one-way analysis of variance (ANOVA), and significant differences among means were determined by Duncan's multiple range test (DMRT).

3. RESULTS

3.1. Protocorm development after asymbiotic germination of *P. tankervilleae* 'Alba'

The asymbiotic germination and subsequent development of *P. tankervilleae* 'alba' seeds were categorized into five distinct stages (Figure 4). Seeds with no visible development were classified as stage 0. In stage 1, embryos enlarge and form a cluster of cells, marking the initiation of germination. Stage 2 was characterized by the emergence of the apical meristem from the cluster of cells, resulting in protocorm formation. In stage 3, foliage development began with the appearance of leaf primordia - the transition to stage 4 involved root emergence, indicating that the seedling was fully developed.

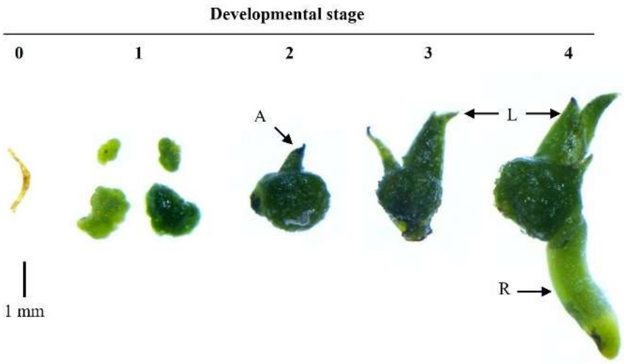

Figure 4. Protocorm developmental stages following asymbiotic germination of *Phaius tankervilleae* 'alba' seeds. Stage 0: no visible development. Stage 1: Embryo enlargement, forming a clump of cells (germination). Stage 2: Formation of the apical meristem (A). Stage 3: Emergence of the leaf (L). Stage 4: Emergence of the root (Ro), resulting in a mature seedling.
Figura 4. Estágios de desenvolvimento do protocormo após a germinação assimbiótica de sementes de *Phaius tankervilleae* 'alba'. Estágio 0: nenhum desenvolvimento visível. Estágio 1: Aumento do embrião, formando um aglomerado de células (germinação). Estágio 2: Formação do meristema apical (A). Estágio 3: Emergência da folha (L). Estágio 4: Emergência da raiz (Ro), resultando em uma plântula madura.

3.2. Protocorm development after asymbiotic germination of *P. tankervilleae* 'alba'

Seeds cultured on VW medium under eight different light conditions - cool white LED (LED-Cool White), cool white fluorescent (FL-Cool White), warm white fluorescent (FL-Warm White), blue fluorescent (FL-Blue), red fluorescent (FL-Red), green fluorescent (FL-Green), yellow fluorescent (FL-Yellow), and complete darkness—showed varied responses in germination and protocorm development after three months (Figure 5). FL-Blue supported the most advanced protocorm development, whereas FL-Cool White resulted in minimal progression. Dead protocorms, appearing dark brown, were most prominent under dark conditions.

The distribution of developmental stage under different light treatments is summarized in Table 1. Under FL-Cool White and dark conditions, most seeds (69.3% and 69.0%, respectively) remained at stage 0, indicating no visible development. In contrast, FL-Blue and FL-Red lights significantly reduced the proportion of undeveloped seeds, with only 8.7% and 14.0% remaining at stage 0, respectively. FL-Warm White light also enhanced early development, with only 26.0% at stage 0 - representing 2.0- and 2.7-fold lower values compared to LED-Cool White (52.3%) and FL-Cool White, respectively. Notably, FL-Cool White yielded a higher proportion of stage 0 protocorms than LED-Cool White, by approximately 1.3-fold. FL-Red light produced the highest proportion of protocorms at stage 1 (77.7%), followed by FL-Warm White (60.3%), FL-Blue (53.3%), and FL-Yellow (55.7%). Advanced development to stage 3 occurred most frequently under FL-Blue (24.0%) and FL-Green (20.3%), while progression beyond stage 2 was absent under FL-Cool White, FL-Yellow, and dark conditions. The highest mortality rate (12.3%) was observed under the dark treatment, whereas other light conditions resulted in minimal or no seed mortality.

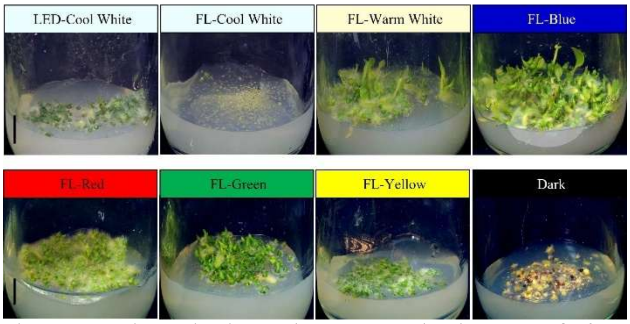

Figure 5. Seed germination and protocorm development of *Phaius tankervilleae* 'alba' after three months of asymbiotic culture on VW medium under various light treatments. Images show representative culture bottles illustrating the effects of different light conditions on developmental progression.

Figura 5. Germinação de sementes e desenvolvimento de protocormos de *Phaius tankervilleae* 'alba' após três meses de cultivo assimbiótico em meio VW sob diferentes condições de luminosidade. As imagens mostram frascos de cultura representativos, ilustrando os efeitos de diferentes condições de luz na progressão do desenvolvimento.

Light quality significantly influenced both seed germination and seedling development (stages 3 and 4) of *P. tankervilleae* 'alba' (Figure 6). FL-Blue and FL-Red yielded the highest germination percentage, reaching 91.3% and 86.0%, respectively, both significantly higher than those of other treatments ($p \leq 0.05$). These were followed by FL-Warm White (74.0%), FL-Green (61.0%), and FL-Yellow (55.7%). LED-Cool White (47.7%) and FL-Cool White (29.7%) were less effective, while germination was lowest in the dark (18.7%).

Protocorm progression to advanced stages (stages 3 and 4) was also influenced by light quality (Figure 6). FL-Blue and FL-Green supported the highest proportions of protocorms reaching these stages (28.3% and 25.0%, respectively), followed by FL-Warm White (10.7%). In contrast, FL-Red and LED-Cool White supported only 4.0% and 5.0%, respectively, while no seedlings reached these stages under FL-Cool White, FL-Yellow, or darkness. Interestingly, FL-Green light, despite inducing a moderate germination rate (61.0%), promoted a relatively high percentage of advanced-stage protocorms (25.0%). These findings demonstrate that light sources effective at stimulating germination may not necessarily support subsequent seedling development. For instance, although FL-Red was nearly as effective as FL-Blue in stimulating germination, its efficiency in inducing advanced-stage protocorm was approximately seven times lower. In contrast, FL-Green was less effective than FL-Blue in promoting germination but showed no statistically significant difference in advanced-stage development, suggesting comparable effectiveness in supporting later developmental stages.

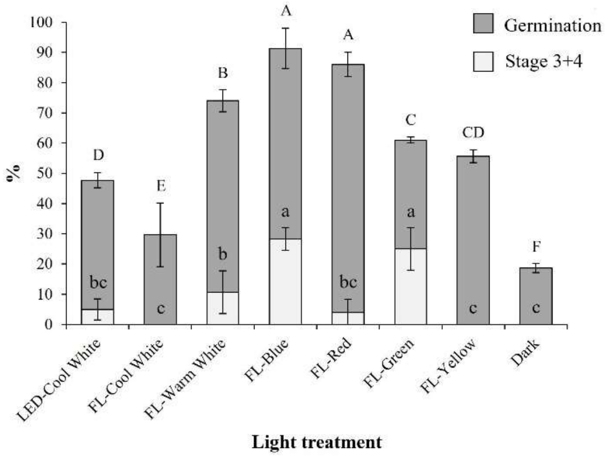

Figure 6. Germination percentage and proportions of protocorms at developmental stages 3 and 4 of *Phaius tankervilleae* 'alba' seeds cultured on VW medium under different light treatments for 3 months. Different uppercase letters above the bars indicate significant differences in germination percentage. In contrast, different lowercase letters within the bars indicate significant differences in the rate of protocorm at stages 3 and 4 ($p \leq 0.05$, DMRT). Bars represent means, and error bars represent SD from three replications (100 seeds per replication).

Figura 6. Percentagem de germinação e proporções de protocormos nos estágios de desenvolvimento 3 e 4 de sementes de *Phaius tankervilleae* 'alba' cultivadas em meio VW sob diferentes tratamentos de luz ao longo de 3 meses. Letras maiúsculas diferentes acima das barras indicam diferenças significativas na percentagem de germinação, enquanto letras minúsculas diferentes dentro das barras indicam diferenças significativas na percentagem de protocormos nos estágios 3 e 4 ($p \leq 0,05$, DMRT). As barras representam as médias e as barras de erro representam o desvio padrão de três repetições (100 sementes por repetição).

Table 1. Percentage distribution of protocorm developmental stages and dead protocorms of *Phaius tankervilleae* 'alba' after asymbiotic germination on VW medium under different light treatments for 3 months.

Tabela 1. Distribuição percentual dos estágios de desenvolvimento e dos protocormos mortos de Phaius tankervilleae 'alba' após germinação assimbiótica em meio VW, sob diferentes tratamentos de luz, durante 3 meses.

Light treatment	Percentage of developmental stage (%)					
	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Dead[1]
LED-Cool White	52.3 ± 2.5 b	42.7 ± 4.0 c	0.0 ± 0.0 b	4.3 ± 2.3 b	0.7 ± 1.2 ab	0.0 ± 0.0 b
FL-Cool White	69.3 ± 9.0 a	29.7 ± 10.5 d	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	1.0 ± 1.7 b
FL-Warm White	26.0 ± 3.6 d	60.3 ± 5.5 b	3.0 ± 1.0 b	7.0 ± 3.6 b	3.7 ± 4.7 ab	0.0 ± 0.0 b
FL-Blue	8.7 ± 6.7 e	53.3 ± 3.1 b	9.7 ± 6.7 a	24.0 ± 7.2 a	4.3 ± 3.5 a	0.0 ± 0.0 b
FL-Red	14.0 ± 4.0 e	77.7 ± 5.0 a	4.3 ± 3.5 b	3.3 ± 3.2 b	0.7 ± 1.2 ab	0.0 ± 0.0 b
FL-Green	38.3 ± 1.5 c	33.3 ± 3.8 d	2.7 ± 2.5 b	20.3 ± 7.8 a	4.7 ± 1.2 a	0.7 ± 1.2 b
FL-Yellow	44.0 ± 1.7 c	55.7 ± 2.1 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.3 ± 0.6 b
Dark	69.0 ± 1.0 a	18.7 ± 1.5 e	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	12.3 ± 2.5 a

Values are mean ± SD of three replicates (100 seeds per replicate). Values within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

[1] Dead protocorms refer to those that browned and ceased development after germination (see Figure 5).

4. DISCUSSION

Light quality significantly influenced both asymbiotic seed germination and subsequent protocorm development in *P. tankervilleae* 'alba'. Among the treatments tested, FL-blue light induced the highest germination percentage and the greatest progression to stage 3 and 4 protocorms. The enhanced performance under FL-Blue light could be attributed to its high proportion of blue wavelengths (84.64%, Figure 3), which are known to activate photoreceptors such as cryptochromes and phototropins (KANG et al., 2008).

These receptors regulate a suite of genes related to cell division, expansion, and differentiation (OKELLO et al., 2016), which are critical during protocorm development. Similar observations have been reported in other orchid species, such as *Dendrobium officinale*, where blue light promoted higher seedling growth and chlorophyll production (LIN et al., 2011; WANG et al., 2017). The study of *Epidendrum fulgens* also highlights the superior effect of the blue wavelength. Seeds cultured under a white LED lamp with a high blue-wavelength content produced more advanced-stage protocorms (FRITSCHE et al., 2022).

The FL-Green treatment also resulted in a relatively high percentage of protocorms at stages 3 and 4, despite its moderate overall germination. While green light has traditionally been considered less effective for plant development, recent findings suggest that it can penetrate deeper into plant tissues and influence cell division and elongation in the inner layers (FOLTA; MARUHNICH, 2007). This may explain the advanced protocorm progression observed in this treatment despite the absence of strong blue-light stimulation. Various studies have demonstrated the significance of green light in promoting plant growth and development (NAHAR et al., 2016; SCHENKELS et al., 2020; INTHIMA; SUPAIBULWATANA, 2025).

One possible reason why FL-Blue and FL-Green light promoted a high percentage of advanced-stage protocorm is the presence of a high fraction of UV-A wavelengths (0.55% and 0.30%, respectively. UV-A has been reported to promote seed germination and seedling growth in tomato (MARIZ-PONTE et al., 2018), and pretreatment of mung bean seeds with UV-A increased both seed germination and seedling growth (HAMID; JAWAID, 2011). Although FL-Warm White also had a relatively high fraction of UV-A (0.35%), progression to advanced-protocorm stages was significantly lower than under FL-Blue and FL-Green light. This may be due to the higher red and far-red wavelength fractions in this light source, which could have masked or limited the effect of UV-A.

Interestingly, FL-Red supported a high germination rate (86.0%) but resulted in limited progression beyond stage 2. This could be due to its dominant red and far-red spectral composition (75.31% red and 23.48% far-red; Figure 3), which may be effective for initiating germination via phytochrome-mediated pathways but insufficient to support morphogenesis and development in the absence of blue light. Similar effects have been observed in other orchid species, such as *Oreorchis patens*, where red light enhanced seed germination (BAE et al., 2014). Red light, primarily detected by phytochromes, plays a pivotal role in regulating germination. The conversion of phytochrome from the inactive (P_r) to the active (P_{fr}) form is a key step in signal transduction (LEGRIS et al., 2016). The active P_{fr} was then shown to activate gibberellin, auxin, and brassinosteroid synthesis, which promote seed germination (de LUCAS; PRAT, 2014; ZHAO et al., 2024b).

The poor development under FL-Yellow, FL-Cool White, and LED-Cool White treatments, despite varying degrees of germination, suggests that these spectra did not provide the optimal light signals necessary for full protocorm development. Notably, LED-Cool White light, which contained a higher fraction of green light (45.95%, Figure 3) compared to FL-Cool White, supported better development to stage 3 and 4 (5.0%, Figure 6), reinforcing the possibility of a positive role of green wavelengths when combined with minor blue and red components.

Meanwhile, FL-Warm White, with a balanced proportion of red, green, and far-red light, produced relatively high germination and moderate protocorm development (10.7% reaching stage 3+4). While its blue light component was low (7.29%), the spectral balance may have supported early morphogenesis to some extent, though not to the same degree as FL-Blue or FL-Green.

In contrast, seed germination was notably low in the dark treatment and did not proceed beyond stage 2. This finding aligns with previous studies in other orchid species, such as *Cattleya* (ISLAM et al., 1999) and *Dendrobium nobile* (SORGATO et al., 2020), in which light was essential not only for breaking seed dormancy but also for protocorm development.

Taken together, these results suggest that the quality of light, particularly the enrichment of blue and green wavelengths, plays a crucial role in stimulating both seed germination and morphogenesis in *P. tankervilleae* 'alba'. The mechanism underlying this effect warrants further study. Future studies could examine gene expression profiles or hormonal dynamics under different light treatments to elucidate the molecular mechanisms governing protocorm differentiation in response to specific light cues.

Moreover, understanding light requirements during early orchid development also provides insight into the ecological preferences of *P. tankervilleae* 'alba' in its natural habitat, which could inform reintroduction strategies in conservation areas. The findings offer valuable insights for developing efficient, species-specific in vitro protocols for the conservation and mass production of this orchid, which is especially important for sustainable utilization and ex situ conservation of rare or endangered varieties.

Based on the observed spectral responses, custom-designed LED systems enriched in blue and green light could be developed to enhance protocorm development efficiency in commercial micropropagation settings. Such spectral optimization could improve both productivity and energy efficiency in large-scale orchid tissue cultures.

5. CONCLUSIONS

This study demonstrates the critical role of light in the asymbiotic germination and protocorm development of *P. tankervilleae* 'alba' on VW medium. Exposure to blue and red fluorescent light significantly enhanced germination rates, while blue and green fluorescent light promoted advanced protocorm development. These findings underscore the importance of specific light spectra not only in triggering germination but also in supporting early seedling growth.

The optimized light conditions identified here offer practical applications for improving in vitro propagation efficiency, with potential benefits for both conservation and commercial cultivation of *P. tankervilleae* 'alba'. Further studies should explore the influence of light spectra on subsequent development and ex vitro acclimatization to optimize large-scale propagation strategies.

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