Research Article

Evaluating the Impact of Various LED Light Spectrums on *Dendrobium officinale* Tissue Culture Seedlings

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Abstract | Light plays a crucial role in plant growth and development and extends mere illumination it's a pivotal environmental factor shaping plant physiology and morphology. To gain a comprehensive understanding, an experiment was designed to investigate the impact of various light-emitting diode (LED) spectrums including red, blue, yellow, green, and their composite lights on the plant growth parameters, physiological characteristics, and antioxidant activity of *Dendrobium officinale* tissue culture seedlings. The fluorescent white light served as control. The tissue culture seedlings were grown in simple light and transplanted about 0.1 cm long into a solid culture medium. The plants were subjected to various LED spectra for a duration of 14 hours each day and continuous subsequent 100 days. The experiment was completely randomized design with nine treatments and each treatment have ten replications. The SPSS statistics software was used to analyses experimental data. In addition, the result of the current study revealed that the plant height, leaf length, leaf width and root number were significantly increased in the red, blue and white (RBW) composite treatment, while composite red, blue and yellow (RBY) subjected seedlings exhibited maximum leaf number, root length, and proliferation rate as compared to the others. Therefore, seedlings exposed to the light combination of red and blue (RB) exhibited the highest chlorophyll a and b, total chlorophyll content, catalase and root activity. The soluble sugar, soluble protein, superoxide dismutase and peroxidase levels increased significantly under compound red, blue and green (RBG) light conditions, while malondialdehyde content was highly decreased in the same treatment (RBG) as compared to control. The study indicated that the various light qualities could enhance the growth parameters of *D. officinale* seedlings by improving biochemical features and their enzymatic activities.

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Keywords | *Dendrobium officinale,* Tissue culture seedlings, Light quality, Growth parameters, Antioxidant enzyme, Chlorophyll content

Introduction

Dendrobium officinale belong to the family Orchidaceae, is an important medicinal plant, thriving primarily in the moist and cool climates of southeastern and southwestern of China (Mudoi *et al.*, 2023). It plays an important role to boost immune system, control blood pressure, enhance life span

and relive from stomach diseases (Chen *et al*., 2014). The stems of this plant wield a powerful influence, promoting Yin nourishment, lung moisturization for blood glucose reduction, immune system enhancement, relief from throat inflammation, fever reduction, facilitated expectoration, and potent antioxidant effects (Zhang *et al*., 2006). The convention of international trade in Endangered Species of Wild Fauna and Flora (CITES) included this plant into endanger plant species (Hinsley *et al*., 2018). The slow growth, low natural reproduction rate by seed, and high market demand, further increase the demand due to uncontrolled collection for medical uses (Jin *et al*., 2016; Iqbal *et al*., 2023a). *In vitro* propagation of *D. officinale* is pressing need to content the demand of medicinal market and to decrease the pressures on natural populations from wild harvesting (Hou *et al*., 2012). Previous study demonstrate that *in vitro* plant propagation technique is most important to promote the rapid growth and reproduction rate of plants (Jin *et al*., 2009). Tissue culture techniques must be used to cultivate this plant, ensuring its sustainable availability and economic significance. Previous research has predominantly explored artificial cultivation and tissue culture methods for *D. officinale*. While studies on *D. officinale* tissue culture have predominantly centered on aspects such as the selection of culture medium, hormone type, explant type, and culture formula (Deng *et al*., 2013; Khan *et al*., 2019; Gao *et al*., 2020; Iqbal *et al*., 2023b), still now limited attention has been given to quality-related considerations. Consequently, there is an urgent imperative for continued research to promote the quality growth of *D. officinale* using tissue culture technique. Environmental factors, including light quality, temperature, and humidity, play pivotal roles as key factors in tissue culture which significantly influencing the growth and development of plant tissue culture seedlings (Batista *et al*., 2018; Yaseen and Hájos, 2021). Light plays an important role on the plant growth and development, as well as effect of plant photosynthesis, propagation, chlorophyll synthesis, photomorphogenesis, metabolism, gene expression, and modulation of antioxidant enzyme activity (Yang *et al*., 2018). Light quality, intensity, and duration regulate plant physiology, steering adaptive responses to environmental dynamics and influencing biochemical and morphological changes (Wei *et al*., 2023). In the realm of *in vitro* culture, the conventional use of fluorescent white light is giving way to the ascendance of light-emitting diodes (LEDs) as a superior energy source. LEDs boast numerous advantages, encompassing a long lifespan, wavelength specificity, small size, precise spectra, durability, and cool emitting surfaces, thereby underlining the increasing preference for LED lighting systems in optimizing plant tissue culture environments (Nardelli *et al*., 2017).

In the previous study, monochromatic and composite light enhance plant growth and physiological metabolism, and these effects differ based on the plant species and their various growth stages (Wang *et al*., 2017; Zhao *et al*., 2020). In addition, previously research reported that due to the red-light effect tissue culture seedling showed maximum growth parameters such as; height of plant, leaf and root length, soluble sugar content, root activity, and decreased MDA content (Kurilčik *et al*., 2008; Shang *et al*., 2013; Li *et al*., 2010; Mengxi *et al*., 2011; Li *et al*., 2019). While blue light can enhance the number of leaves (Ramírez-Mosqueda *et al*., 2017a), synthesis of chlorophyll content (Lim *et al*., 2023), soluble protein (Liu *et al*., 2020), and CAT activity (Ye and Shao, 2017). The aim of the present study was to check the effect of different light quality on tissue culture seedling of *D. officinale,* seeking an optimal light spectrum for enhancing their *in vitro* growth and development. Utilizing energy-efficient LED lamps, the investigation assessed the impact of diverse light qualities on seedling growth, chlorophyll levels, soluble proteins, soluble sugar, root activity, and antioxidant enzyme activity, aiming to identify the most conducive light conditions for optimal growth and development in tissue-cultured *D. officinale* seedlings.

Materials and Methods

Plant materials, culture methods and lighting condition

The *Dendrobium officinale* tissue culture seedlings were grown in the tissue culture Lab of the Institute of Flower, Yunnan Agricultural University, China. Murashige and Skoog medium (1/2 MS) supplemented with indole acetic acid (IAA 0.3 milligram per liter), Thidiazuron TDZ (1.25 mg/l), Potato (50 g/l), Banana (50 g/l), Sucrose (30 g/l), Agar (7 g/l), Huabao (No.1) (1 g/l) and activated charcoal (1 g/l). The pH was modified to 5.8 using NaOH solution and pour into sterilized bottles and autoclaving at 121˚C for 20 min. A tissue culture seedling (approximately 0.1 cm high) was placed on the MS medium with three seedlings per bottle. The experiment was completely

randomized design with nine treatment and each treatment have ten replications. The materials were pre-cultured under fluorescent lamps for 7 days. Next, bottles were transferred to culture rack with a consistent light source, maintaining a 14 h/d. The culture room's relative humidity was adjusted to (75±5%), with temperature (25±2). The seedlings were grown under nine different light sources (Table 1) for 100 days and the growth parameters, physiological characteristics and antioxidant enzymes activity were recorded.

Growth parameters

Ten seedlings were randomly collected from each treatment and the growth parameters including, seedling height, leaf number, leaf length, leaf width, root number and root length were measured using scale method given by (Huang *et al.*, 2023). The proliferation rate was calculated by counting the number of newly developed buds.

Determination of soluble sugar and protein content

One gram of the samples originating from different light treatments was dipped sterilized water and boil 30 minutes. The extracts were boiled, filtered and adjusted to 100 ml by diluting with sterilized distilled water. 0.3 ml of the extract was transferred to 10 ml test tube containing 0.5 ml anthrone ethyl acetate, and 5 ml H_2SO_4. The soluble sugar content was measured through anthrone-sulfuric acid method (Hernandez and Hernandez, 1994). For protein content, two-gram of the sample was finely homogenized and centrifuged for 20 minutes at 4 °C and 12,000 rpm. The supernatant was mixed with 5 ml Coomassie Brilliant blue G-250 solution and incubated for 2 minutes. The protein content was calculated with formula given by Ku *et al.* (2013).

$$Soluble\ sugar\ content\ (\%) = \frac{m^1 * V * N}{Vs * m * 10\text{^}6)}\ X\ 100$$

M^1= represent of sugar mass, V= represent volume in milliliter, N= represent dilution factor, Vs= represent volume of sample, M= represent tissue mass sample.

$$Soluble\ protein\ content\ \left(\frac{mg}{g}\right) = \frac{m^1 * V}{Vs * m * 1000}$$

M^1= represent of protein mass, V= represent volume in milliliter, Vs= represent sample volume, M= represent tissue mass sample.

Chlorophyll contents and root activity

A 0.1g fresh leaves were chopped and ground with 80% acetone and placed in the experiment room overnight at room temperature. Spectrophotometer was used for measuring the absorbance wavelength. The chlorophyll content was recorded with the following method described by Cao *et al.* (2007). The roots of the seedlings were cut into small pieces and dipped into 20 ml TTC solution for 24 hours followed by rising in sterilized distilled water and soaked in 95% ethanol solution and then placed in the water bath at 85 °C for 10 minutes to extract the triphenyl methyl hydrazone (TTF). Root activity was recorded using the triphenyl tetrazolium chloride (TTC) staining method (Wang and Huang, 2006). The absorbance was measured at 485 nm, and root activity was quantified as OD g^{-1} FW.

Table 1: *Detail of optical combination system of the light spectrum.*

Spectrum processing	**PPFD (umol.m^{-2}s^{-1})**						**Total PPFD (umol.m^{-2}s^{-1})**	**(W)**
Treatment	**CK**	**R660**	**Y590**	**G520**	**B440**	**W**	**50**	**60**
CK	50	--	--	--	--	--	50	60
R660	--	50	--	--	--	--	50	60
Y590	--	--	50	--	--	--	50	60
G520	--	--	--	50	--	--	50	60
B440	--	--	--	--	50	--	50	60
R660B	--	36.49	--	--	13.51	--	50	60
R660BG	--	33.75	--	3.75	12.5	--	50	60
R660BY	--	33.75	3.75	--	12.5	--	50	60
R660BW	--	33.75	--	--	12.5	3.75	50	60

CK is the control group, R660 red light, Y590 yellow light, G520 green light, B440 blue light, R660B red and blue combination, R660BG

red, blue and green combination, R660BY red, blue and yellow combination, R660BW red, blue and white combination and represent empty.

$$Chl\ A = 12.21\ A_{663} - 2.81\ A_{646}$$
$$Chl\ B = 20.13\ A_{646} - 5.03\ A_{663}$$

Chl A and Chl B is the concentration of chlorophyll A and B, respectively, mg/l.

Superoxide dismutase, peroxidase, catalase enzyme activity and malondialdehyde content

A 0.2 g leaf sample was ground with a mortar and pestle and homogenized with 2ml phosphate buffer (pH= 7.8). The homogenized sample was centrifuged at 10,000 rpm for 20 min and 20 microliter of the supernatant was mixed with 2.5 ml reaction solution in 5 ml test tube. The mixture was placed in 4,000 Lux illumination for 30 min and the absorbance was measured at 560 nm for Superoxide dismutase (SOD). For Peroxidase (POD), the different reaction solution was used and the absorbance was recorded at 470 nm. A 0.1 ml supernatant was mixed in 5 ml test tube with 2.5 ml of the reaction solution and the CAT absorbance was measured with 240 nm. A malondialdehyde (MDA) content was measured using thiobarbituric acid (TBA). 1 ml supernatant was mixed with 2 ml of TBA 0.6% solution and heat in water bath for 15 min at 80 °C followed by centrifugation at 8,000 rpm for 15 min. The color was measured at 600 nm, 532 nm, and 450 nm wavelengths. The calculation methods of (SOD, POD, CAT and MDA) was described by Wang (2018).

$$\text{SOD activity } (\text{U} \cdot g^{-1}) = \frac{(A_{CK} - A_{560}) * V_T}{0.5 * A_{CK} * \text{W} * V_S}$$

ACK= represents control absorbance; A560= represents sample tube absorbance, VT= represents total volume of extracted enzyme solution, W= sample fresh weight, Vs= volume of enzyme solution.

$$POD\ activity\ [U.(min.g)^{-1}] = \frac{\Delta A_{470} * V_T}{W * V_S * 0.01 * t}$$

ΔA470= represents change value of absorbance of sample, VT= total volume, W= fresh weight, VS= volume of enzyme solution, t= represent the reaction time.

$$CAT\ activity\ [U.(min.g)^{-1}] = \frac{\Delta A_{240} * V_T}{W * V_S * 0.1 * t}$$

ΔA240= represents change value of absorbance of sample, VT= total volume, W= fresh weight, VS= volume of enzyme solution, t= represent the reaction time.

$$MDA\ content\ \left(\frac{\mu mol}{g} FW\right) = \frac{(6.45 \times (D_{532} - D_{600}) - 0.56\ D_{450}) \times 0.015}{W}$$

W= represents sample fresh weight, D532= absorbance value of the sample tube at 532 nm; D600= refers to the absorbance value of the sample tube at 600 nm, D450= absorbance value of the sample tube at 450 nm.

Data analysis

Statistical analysis of the data was carried out using the one-way ANOVA and Duncan multiple comparisons. IBM SPSS software version 26.0 (IBM Inc., Chicago, IL, USA), and GraphPad Prism version 8.0.1 was used for graph plotting.

Results and Discussion

Morphological parameters

In the present study, the effects of various light sources, including monochromatic (red, blue, yellow, green), composite lights (RB, RBY, RBG, RBW), and fluorescent white light as a control (CK), were investigated on the growth and chlorophyll content of *Dendrobium officinale* tissue culture seedlings (Figure 1A-I). The results showed that, maximum plant height was observed in RBW treatment followed by RBY and RB compared treatments and control (Figure 2 A-I). The highest leaf length was recorded in RBW followed by blue and RBY while RBY, yellow and RBW exhibited the higher leaf width. The largest root length was recorded in the RBY treatment followed by RBW and RBG treatment compared with other treatments. The root number significantly increased under RBW, RBG and blue treatments. The proliferation rate was significantly reduced under RBY, blue and yellow treatments (Table 2). Previous study has demostreated that RBW increase the plant height, leaf length and leaf width of lettuce plants (Park *et al.*, 2012). Furthermore, studies have shown that red and combined RB light increase the growth of *Chrysanthemum*, grapes, *Gerbera jamesonii* and *Anthurium andraeanum* plantlets under laboratory condition (Poudel *et al.*, 2008; Chen *et al.*, 2013; Kurilčik *et al.*, 2008; Lim *et al.*, 2023). Previous study demonstrates that the RBY light increase plant weight, root number and root length under laboratory

conditions (Li *et al.*, 2018). Additionally, maximum proliferation rate was observed under RBY light and minimum under red light as compared to CK which is in accordance with the previous finding (Cavallaro *et al.*, 2023). Another study reported a higher proliferation rate under combined red and blue (RB) light (Naznin *et al.*, 2019).

Table 2: *Morphological indexes, including seedling height, leaf length, leaf width, leaf no., root length, root no., and proliferation rate of D. officinale tissue culture seedlings under different light qualities.*

Light treatment	Seedling height	Leaf length	Leaf width	Leaf number	Root length	Root number	Proliferation rate
Red	2.95 ± 0.25 de	0.90 ± 0.27 d	0.24 ± 0.05 b	18.6 ± 3.23 bc	1.11 ± 0.22 d	9.50 ± 2.17 cd	12.0 ± 3.52 f
Blue	3.33 ± 0.39 bc	1.65 ± 0.34 ab	0.32 ± 0.08 a	21.4 ± 3.40 ab	0.79 ± 0.16 e	12.3 ± 3.05 bc	34.0 ± 4.73 b
Yellow	3.23 ± 0.33 cd	1.27 ± 0.29 c	0.33 ± 0.06 a	19.1 ± 3.57 abc	0.97 ± 0.18 de	8.90 ± 2.07 d	28.0 ± 6.41 cd
Green	3.52 ± 0.37 bc	1.32 ± 0.26 c	0.33 ± 0.06 a	16.7 ± 2.40 cd	0.85 ± 0.20 e	10.5 ± 2.22 cd	24.0 ± 3.74 de
RB	3.47 ± 0.28 bc	1.42 ± 0.19 bc	0.28 ± 0.08 ab	17.4 ± 2.54 cd	1.42 ± 0.26 c	11.1 ± 2.68 cd	20.0 ± 3.36 e
RBY	3.64 ± 0.35 b	1.62 ± 0.22 ab	0.29 ± 0.08 ab	21.7 ± 2.26 a	2.45 ± 0.25 a	12.1 ± 3.60 bc	39.0 ± 3.82 a
RBG	2.82 ± 0.27 e	1.45 ± 0.21 bc	0.30 ± 0.08 ab	14.8 ± 2.48 d	1.71 ± 0.30 b	14.5 ± 3.17 b	15.0 ± 5.20 f
RBW	4.21 ± 0.39 a	1.75 ± 0.19 a	0.34 ± 0.06 a	20.8 ± 2.20 ab	2.35 ± 0.31 a	17.9 ± 2.64 a	27.0 ± 4.16 cd
CK	3.23 ± 0.42 cd	0.99 ± 0.31 d	0.29 ± 0.03 ab	18.4 ± 4.45 bc	1.37 ± 0.18 c	11.8 ± 3.15 bc	29.0 ± 6.89 c

Different letters (a, b, c, d, e, f) in the same column indicate significant differences between treatments at P≤0.05 by using Duncan's test. Data are the means of ten replicates ± standard deviation.

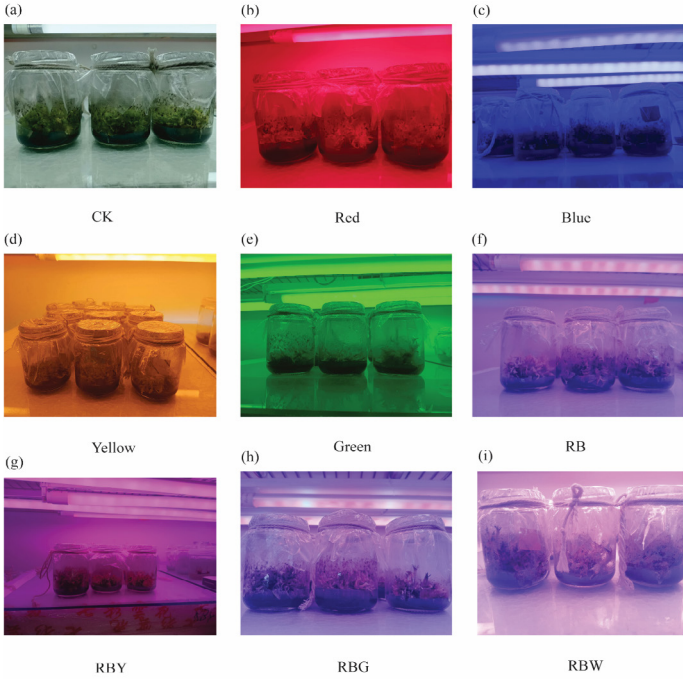

Figure 1: *Dendrobium officinale tissue culture seedlings were illuminated under different LED light qualities condition. Different letters indicate various color of light spectrum.*

Chlorophyll contents

The effect of different light quality significantly increased chlorophyll (Chl) contents of *Dendrobium officinale* seedlings. The maximum chlorophyll a, chlorophyll b and total chlorophyll content were recorded in RB treatment, while RBW showed moderate impact followed by RBW and blue light treatments (Figure 3A-C). However, the chlorophyll A/B ratio was higher under RBW treatment followed by red and RBY, respectively (Figure 3D). blue light enhances chlorophyll A/B and total chlorophyll contents, while the combination of RB significantly increases chlorophyll A/B ratio Wang *et al.* (2017). Similarly, the RB combination has been reported to increased chlorophyll A/B and total chlorophyll content *Doritaenopsis, Peony plantlets*, *G. jamesonii* and *Stevia rebaudiana* tissue culture seedlings (Shin *et al.*, 2008; Pawłowska *et al.*, 2018; Ramírez-Mosqueda *et al.*, 2017). In another study, blue light showed highly chlorophyll A/B and total chlorophyll content of *D. officinale* tissue culture seedlings (Lin *et al.*, 2011).

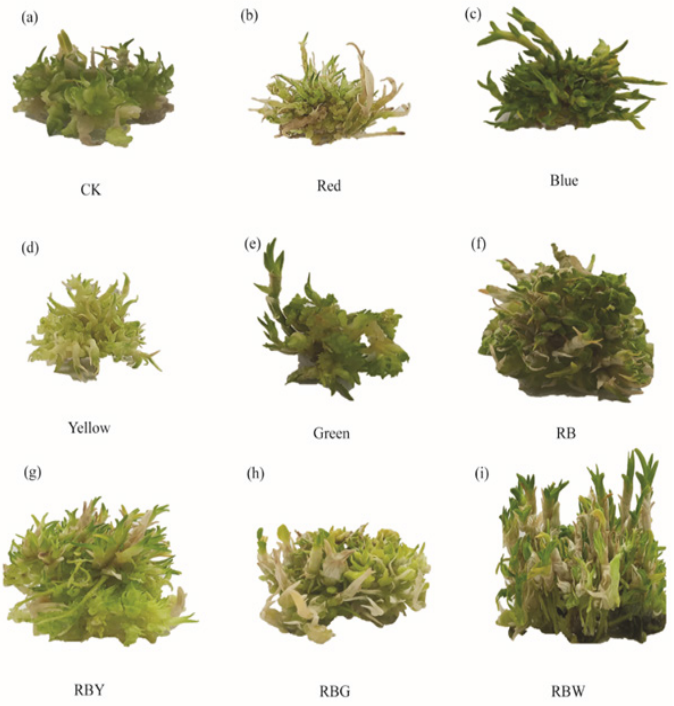

Figure 2: *Dendrobium officinale tissue culture seedlings were irradiated under different light sources, including red, blue, yellow, green, combined red and blue (RB), mixture of red, blue, and yellow*

(RBY), a combination of red, blue, and green (RBG), a blend of red, blue, and white (RBW) compared with fluorescent white light (CK).

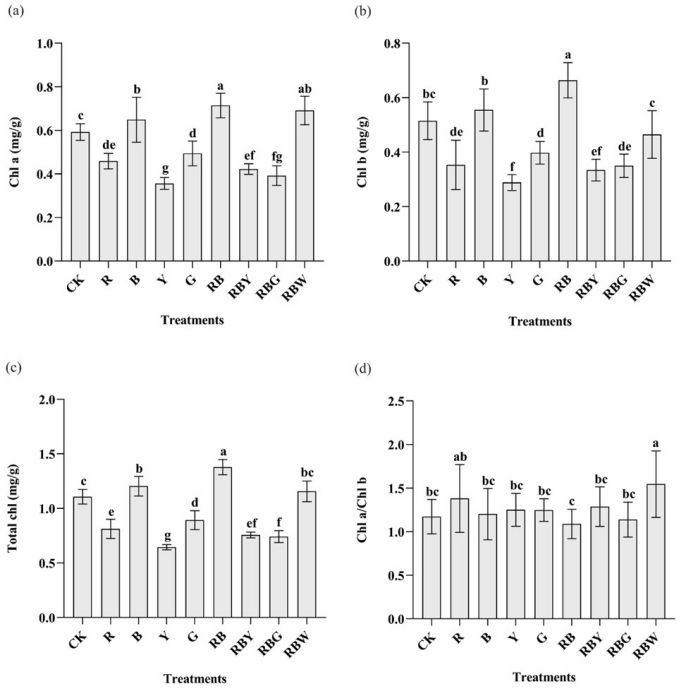

Figure 3: *Soluble sugar content, soluble protein content, and root activity in tissue culture seedlings of D. officinale under different light qualities. Different letters (a, b, c, d, e, f) indicate significant differences between treatments at P≤0.05 by using Duncan's test. Vertical bars represent the standard deviation of the means (n = 10) in soluble sugar and protein, whereas the standard deviation of the means (n = 6) in root activity.*

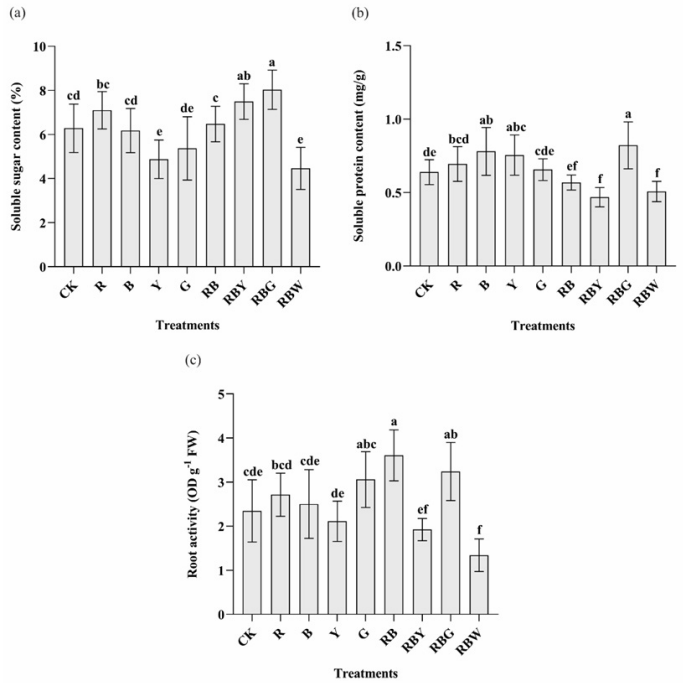

Figure 4: *Chlorophyll a, chlorophyll b, total chlorophyll, and Chl a/Chl b ratio in tissue culture seedlings of D. officinale under different light qualities. Different letters (a, b, c, d, e, f, g) indicate significant differences between treatments at P≤0.05 by using Duncan's test. Vertical bars represent the standard deviation of the means (n = 10).*

Soluble sugar content, soluble protein content, and root activity

The soluble sugar content (SSC), soluble protein content (SPC), and root activity of the *D. officinale* tissue culture seedlings were significantly affected by light treatments. As shown in Figure 4, the maximum SSC was highly observed in RBG treatment seedlings followed by RBY and red light treated plants (Figure 4A). In addition, the RBY was showed minimum SPC followed RBW compared with other treatments. In comparison, RBG showed highly SPC in seedlings of *D. officinale* (Figure 4B). These suggest that variations of light could increase the root activity, soluble sugar content and soluble protein contain and the maximum root activity was recorded with RB followed by RBG, treated seedlings (Figure 4C). Previous study in *Solanum tuberousm* L. reported the highest SSC and SPC under RBG treatment (Ma *et al*., 2015). According to Shang *et al*. (2013), the combination of RB light plays a crucial role to enhance the root activity of *D. officinale* seedlings. According to Zhou *et al*. (2020), the combined RB light positively impacts and causes an increase in root activity, favorably contributing to the improvement of the root system.

Superoxide dismutase, peroxidase, catalase enzyme activity and malondialdehyde content

Antioxidant enzymes play a crucial role in eliminating reactive oxygen species in plants, and their activity serves as an indicator of both physiological activity and senescence in plants (Molassiotis *et al*., 2004). Under different light treatments, Superoxide dismutase (SOD), Peroxidase (POD) and Catalase (CAT) significantly increased, while Malondialdehyde (MDA) content decreased. The highest SOD was recorded with RBG treatment followed by RBW and RBY light treated seedlings. In comparison, the yellow light showed least effect on SOD (Figure 5A). Similarly, the RBG light showed maximum effect on tissue culture seedlings and increase the POD activity followed by RB treatment (Figure 5B). Furthermore, CAT was significantly increase in RB light treatment followed by RBG and red treated seedlings (Figure 5C). Previous study showed the maximum SOD and POD activity in RBG light treatment (Mengxi *et al*., 2011). RB light treatment enhances the CAT activity of *D. officinale* seedlings (Wang *et al*., 2017). The RB also enhance tomato plantlets under in vitro condition (Khattak *et al*., 2007; Naznin *et al*., 2019). These studies results suggest that different light qualities have significant effect on *D. officinale* tissue

culture seedlings. Moreover, the MDA content was highly decreased in RBG treated seedlings followed by RBW as compared to others. In comparison, the MDA content was significantly increase in red treated seedlings (Figure 5D). Similarly, the red treated seedlings showed minimum MDA content in *G. jamesonii* under laboratory condition (Meng *et al*., 2019). Feng *et al*. (2021) reported that the treated seedling of *D. officinale* showed highly decreased MDA content as compared to control.

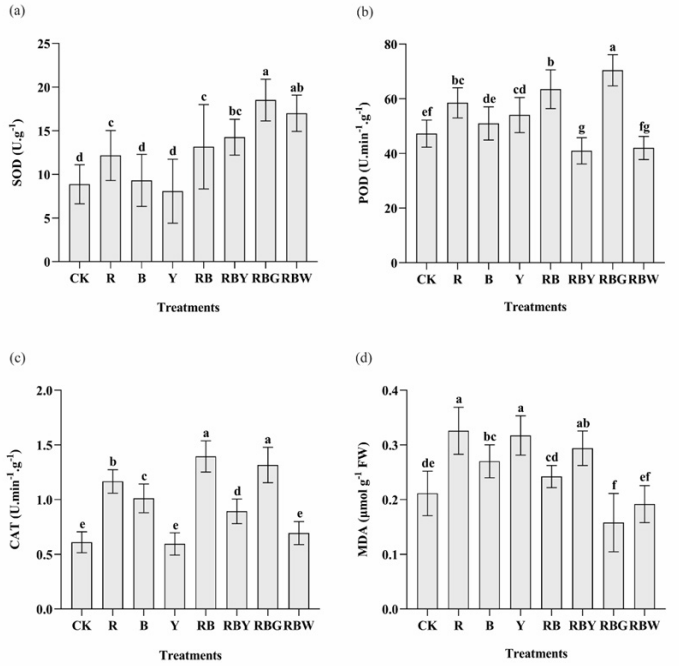

Figure 5: *Superoxide dismutase, peroxidase, catalase, and malondialdehyde enzyme activity in tissue culture seedlings of D. officinale under different light qualities. Different letters (a, b, c, d, e, f, g) indicate significant differences between treatments at P≤0.05 by using Duncan's test. Vertical bars represent the standard deviation of the means (n = 10) in superoxide dismutase, peroxidase, and catalase whereas the standard deviation of the means (n = 6) in malondialdehyde.*

Conclusions and Recommendations

The role of lights on plant growth is vital. It's a critical environmental factor shaping plant physiology and morphology. Many researchers have made much contribution to achieve adequate plant growth through different lights to control environmental factors. On contrary our research has been much focused on the different light qualities aspects. To gain a comprehensive understanding, an experiment was designed to investigate the impact of various light-emitting diode (LED) spectrums including red, blue, yellow, green, and their composite lights with fluorescent white light as a control on the plant growth parameters, physiological characteristics, and antioxidant activity of *Dendrobium officinale* tissue culture seedlings. According to our results, we conclude that the combination of Red, Blue and White (RBW) light enhanced the growth parameters of tissue culture seedlings. Also, the combination of Red and Blue (RB) and Red, Blue and Green (RBG) increased SSC, SPC, chlorophyll contents, SOD, POD, CAT, and root activity. It should be possible; therefore, evaluating these different lights in combination could enhance the growth and quality of *D. officinale* seedlings.

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Novelty Statement

This study checked the effect of different light qualities on *Dendrobium officinale* seedlings under in vitro condition. As compared to other studies, the current study clearly highlights the effect of different light qualities on *D. officinale* seedlings under in vitro conditions. The evidence from this study will assist in considering the best light quality for optimum growth and physiological characteristics that will improve the quality and stimulate the growth of *D. officinale* seedlings.

Author's Contribution

Hafiz Ishtiaq Ahmad: Conducted research and write original draft of manuscript.
Jinlong Zhang: Re- analyzed data.
Fuxun Luo: Revise and editing part of the manuscript.
Owais Iqbal: Evaluate and revise the final version of manuscript.
Yuying Wang: Supervised, editing and approved manuscript.

Conflicts of interest
The authors have declared no conflict of interest.

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