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Research Article
Influence of LED Light, Salicylic Acid and Yeast Extract on Growth and Phenolic Content of *Dendrobium officinale* Plantlets

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Abstract

Background and Objective: *Dendrobium officinale* Kimura et Migo was a precious plant used as a folk food and traditional medicine. It has proven effective in the treatment of diabetes, anticancer and enhancement of the immune system. To enhance the growth and accumulation of bioactive compound content, the effect of Light-Emitting Diode (LED), salicylic acid (SA) and yeast extract (YE), individual or in combination on the growth and total phenolic content (TPC) of *in vitro D. officinale* plantlets was studied. **Materials and Methods:** *D. officinale* shoots (0.5 cm) were incubated in various ratios of red LED and blue LED, the fluorescent was considered as the control treatment. Continuously, the effect of LED in combination with elicitors like SA or YE on TPC accumulation and the growth of *D. officinale* shoots cultured *in vitro* was studied. **Results:** The results showed that the growth and the TPC were changed with incubation time in various light conditions. After 45 days of culture, the plantlet height of *D. officinale* was the highest in 90% red LED and 10% blue LED (9R1B) conditions, meanwhile, the TPC was the most biosynthesised after 35 days of culture. The addition of elicitors to the culture medium and placed in the 9R1B condition increased the TPC, which was higher than the elicitors-free medium after 30 days of culture. **Conclusion:** LED light, SA and YE caused significant changes in TPC accumulation of *in vitro D. officinale* plantlets. The highest TPC were from plantlets grown in MS medium supplemented with 1.5 mg L^{-1} SA and incubated under 9R1B condition. Regarding YE, the TPC was highest when plantlets were cultured on MS medium supplemented with 1.0 g L^{-1} YE and incubated under the 9R1B condition.

Key words: LED, salicylic acid, yeast extract, *Dendrobium officinale*, total phenolic content, Murashige and Skoog medium, flavonoids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dendrobium officinale Kimura et Migo (*D. officinale*) belongs to the *Dendrobium* genus and is the most important species of this genus. *D. officinale*, well-known as "Thạch hộc tía" in Vietnam or "Tiepishihu" in China, is distributed in several countries in the world such as Japan, Australia, the United States and more widely in China[1]. In addition to being an ornamental plant, *D. officinale* is used as a folk food and traditional medicine. Many reports have shown that *D. officinale* contains at least 190 biologically active compounds such as the flavonoids, alkaloids and poly saccharides[2]. *Dendrobium officinale* has major (significant) pharmacological activity such as hepatoprotective, anticancer, hypoglycemic, gastric ulcer protective, anti-inflammatory, anti-ageing (anti-ageing) skin, strengthening the immune system and protecting the eyesight of children[3]. The medicinal value of *D. officinale* has been demonstrated in many clinical experiments on animals. Recent studies have confirmed that polysaccharide is a major compound present in *D. officinale*, however, there are still some other critical secondary compounds such as alkaloids, flavonoids, especially phenolics[4], which as well as related pharmacological activities have been studied in the *D. officinale*[5,6].

In recent years, *D. officinale* has been over-exploited, leading to a decrease in the number of plants in nature. Similar to other orchids, *D. officinale* can be propagated through seeds, but they are difficult to develop into seedlings in nature because seeds lack endosperm and nutrients[7]. Cell tissue culture methods have been studied and applied to propagate and preserve this precious orchid to overcome these difficulties. Research in recent years has demonstrated success in applying this technique to the production of seedlings as well as biomass of *D. officinale*[8].

Interestingly, changes in medium elements and culture conditions can the increase growth, multiplication rate, biomass, as well as metabolite compounds accumulation in *D. officinale*[9,10]. The content of phenolics, flavonoids, or other compounds can be related to the response of the plant to particular environmental conditions, especially light[11]. Light is one of the most important factors of the culture condition for the growth and metabolism process in plants, especially under *in vitro* culture conditions. Nowadays, the use of Light-Emitting Diodes (LEDs) as a source of radiation for plants has gained a lot of attention for plant cell tissue culture. The wavelength of LED can be easily controlled and changed to be suitable for plants, as a result, the growth and biosynthesis way will be stimulated[12]. Several studies indicated that red LED and blue LED light have affected metabolism and morphology in *D. officinale*[9], *Rehmannia glutinosa*[13], herb microgreens[14] and *Myrtus communis*[15].

Along with changing the light conditions, the addition of elicitors (including biotic and abiotic elicitors) to the culture medium also enhanced the accumulation of bioactive compounds in herbal plants cultured *in vitro*[16]. Elicitors, such as SA or YE, act as signalling molecules, which play an important role in the signal transduction system and induce the activation of genes involved in the process of secondary metabolites biosynthesis[17,18]. Besides, elicitors are non-toxic and often used at low concentrations to promote the accumulation of secondary compounds[16]. With these advantages, the use of elicitors in the production of bioactive compounds in biomass culture is more popular. A few studies have been conducted on *D. officinale* to investigate the effect of elicitors on the accumulation of some of the bioactive compounds such as alkaloids, polysaccharides and flavonoids[19,20]. However, to investigate some other factors to promote growth as well as to accumulate compounds, studies on this species still need to be continued.

In this study, the effect of LEDs with different ratios of blue and red light on the growth and content of phenols of *in vitro D. officinale* plantlets were evaluated. Simultaneously, the combined effects of LED lights and 2 types of elicitors, abiotic (SA) or biotic (YE) elicitors on the accumulation of total phenolic content of this precious orchid were also studied.

MATERIALS AND METHODS

Study area: The study was carried out from June, 2020 to March, 2022 at the Plant Cell Technology Department of the Institute of Tropical Biology (VAST, Ho Chi Minh City, Vietnam).

Light conditions: The explants were incubated under different LED light conditions with the ratio of red to blue LED light, respectively as follows: 100% red LED (R), 100% blue LED (B), 90% red LED and 10% blue LED (9R1B), 80% red LED and 20% blue LED (8R2B), 70% red LED and 30% blue LED (7R3B), 60% red LED and 40% blue LED (6R4B), 50% red LED and 50% blue LED (5R5B). Using fluorescent lamps (FL) (Philips, Vietnam) for the control treatment. All the explants were maintained at 24±2°C under a 12 hrs photoperiod and 55-60% relative humidity. The detailed wavelengths of the different light conditions were expressed in Fig. 1a-h.

Plant material and establishment of plant culture: *Dendrobium officinale* protocorm like-bodies (PLBs) were cultured on the Murashige and Skoog[21] (MS) medium

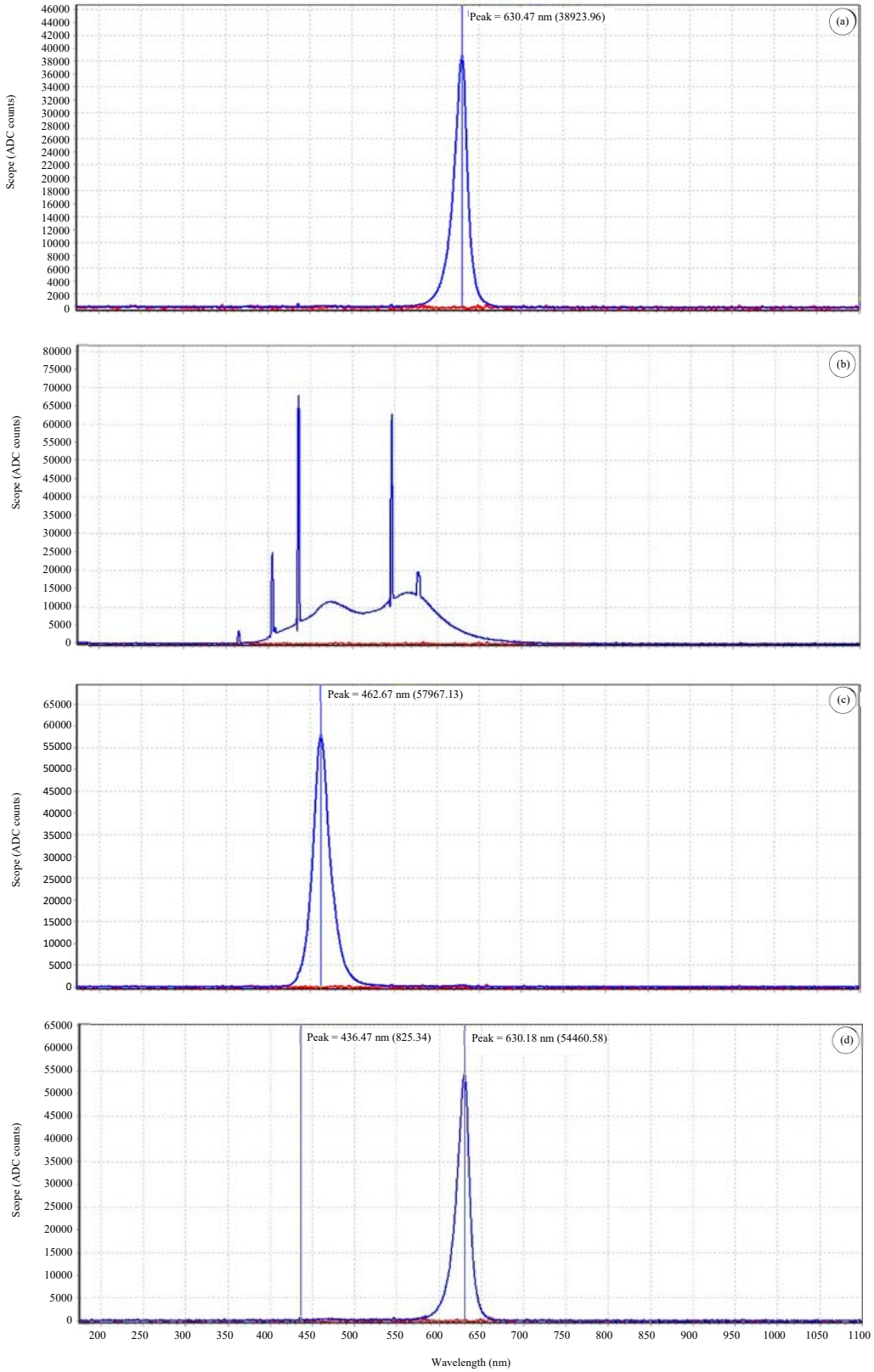

Fig. 1(a-h): Continuous

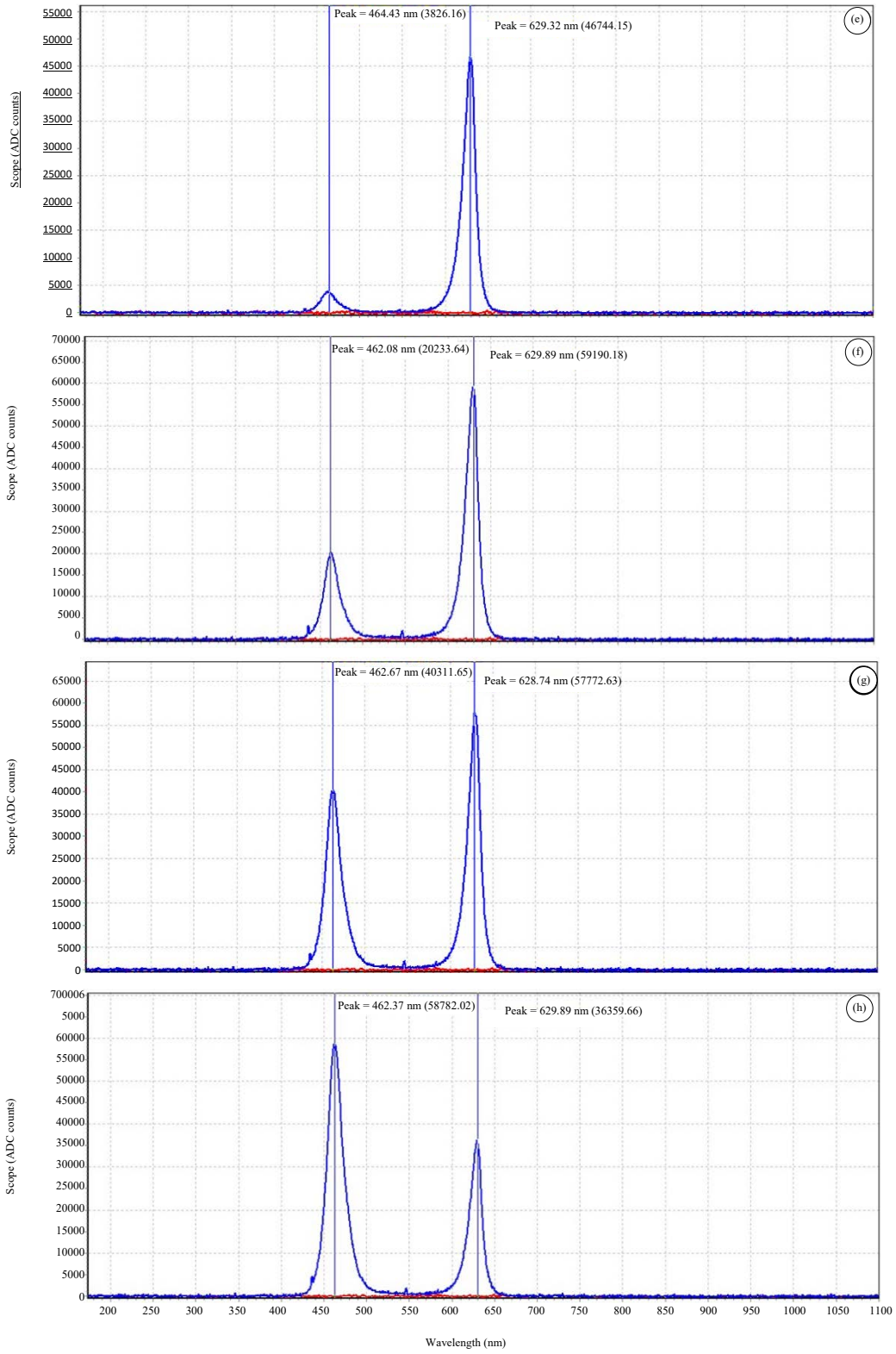

Fig. 1a-h: The wavelength of the different light conditions tested in this study, (a) 100% red LED (R), (b) Fluorescent light, (c) 100% blue LED (B), (d) 90% red LED and 10% blue LED (9R1B), (e) 80% red LED and 20% blue LED (8R2B), (f) 70% red LED and 30% blue LED (7R3B), (g) 60% red LED and 40% blue LED (6R4B) and (h) 50% red LED and 50% blue LED (5R5B)
Wavelength of light was measured by using spectrometers (Avantes, Netherland)

supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar, 30 mL L^{-1} coconut water[10] (MS1 medium) for shoot regeneration. For maintenance, PLBs were subcultured in the same medium every month with a 12 hrs photoperiod by using fluorescent lamps at a light intensity of 40±2 µmol/m^2/s. All the media was autoclaved at 121°C, 1 atm in 20 min. The shoots derived from PLBs were used as explants for experiments in this study.

In vitro D. officinale shoots (0.5 cm of height) derived from PLB were cultured in the PGR-free MS medium supplemented with 8 g L^{-1} agar, 30 g L^{-1} sucrose and 80 g L^{-1} banana crude extract (MS2 medium). Fresh weight (g), dry weight (g), plantlet height (cm), the average numbers of the shoot and TPC (mg GAE/g DW) were determined after every 5 day culture and the growth curve was established to determine the appropriate lighting time for the growth and TPC accumulation of *in vitro D. officinale* plantlets.

Salicylic acid and yeast extract experiments design: SA (Duchefa, Netherland) or YE (Himedia, India) was added to the MS2 medium at different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1}) to evaluate the effect of these elicitors on the growth and TPC of *D. officinale* plantlets. The MS2 medium without SA and YE was used as a control treatment. Plantlets were placed under the optimal lighting conditions determined in the previous experiment. Fresh weight (g), dry weight (g), the average numbers of the shoot, the plantlet height (cm) and TPC (mg GAE/g DW) of plantlets were collected after 30 days of culture.

Determination of total phenolic content method: *Dendrobium officinale* plantlets were dried in shade at 40°C, then chopped and ground to a fine powder. Dried plantlets powder (0.3 g) was extracted with 10 ml methanol at room temperature for 8 hrs. The extract was filtered through Whatman filter paper No. 1, this extraction method was repeated 3 times. The filtrates were collected and the concentrated under reduced pressure at 40°C. The extract was dried and stored at 4°C in storage vials for experimental use.

The TPC of the extract was determined by the Folin-Ciocalteu method[22]. The crude extract was serially diluted with distilled water to final concentrations of 1.0, 0.5 and 0.25 mg mL^{-1}. About 100 µL of the water diluted extract was mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent for 5 min, followed by the addition of 400 µL of 7.5% sodium carbonate solution. The mixture was allowed to stand for a further 60 min in the dark at 25°C and then was centrifuged and aliquoted to a 96-well plate. The absorbance was measured at 760 nm by a microplate reader. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per gram dry weight (mg GAE/g DW).

Statistical analysis: The experiments were arranged in a completely randomized design (RCD) with three replications. All data were analyzed statistically using the Statgraphics software (version 18.0) and the graph was drawn by Microsoft Office Excel 2010 Software. Significant differences among the treatments were determined using Tukey's test[23] at $p<0.05$. The results were expressed as the Mean±SE of the repeated experiments.

RESULTS

Effect of LED light on the growth of *D. officinale* plantlets: Considering the fresh and the dry weight of *D. officinale* plantlets after 5 day intervals in various light conditions, the fresh and dry weight of *D. officinale* incubated in the red LED, blue LED or red LED in combination with blue LED was higher than the control ($p<0.05$, Fig. 2a-b). In all tested light conditions, fresh and dry weights were significantly increased with incubation time ($p<0.05$). During the stage from 5-30 days after culture, the fresh and dry weight explants in the treatments were not statistically significantly different. The fresh weight was only different on the 30th day of culture, while the dried weight was on the 35th day of culture. After 30 days of culture, the explants were placed under B condition and had the highest fresh weight (0.302 g), 1.14 times higher than FL (0.265 g). After 35 days of culture, the highest dry weight was 0.025 g per explant under the 5R5B condition, 1.25 times higher than the control (0.02 g). After 30 days of culture, the explants grew well under the 5R5B condition, higher than the other treatments at corresponding periods. In this experiment, the fresh weight increased significantly on the day 35th, 40th and 45th with the fresh weight achieving 0.306, 0.330 and 0.346 g, respectively, higher than the rest of the treatments at the respective stages. The dry weight of plantlets incubated in 5R5B condition on the days of 35th, 40th and 45th reached higher values, respectively 0.025, 0.026 and 0.028 g compared to other treatments at the same time point. The results showed that the combination of a 50% red LED and a 50% blue LED positively affected the increase in biomass of *D. officinale* after 45 days of culture.

The plantlet height of *D. officinale* significantly increased according to the incubation time under various light conditions was observed ($p<0.05$, Fig 2c). At the first stage (from 0-20 days after culture) the height of plantlets incubated

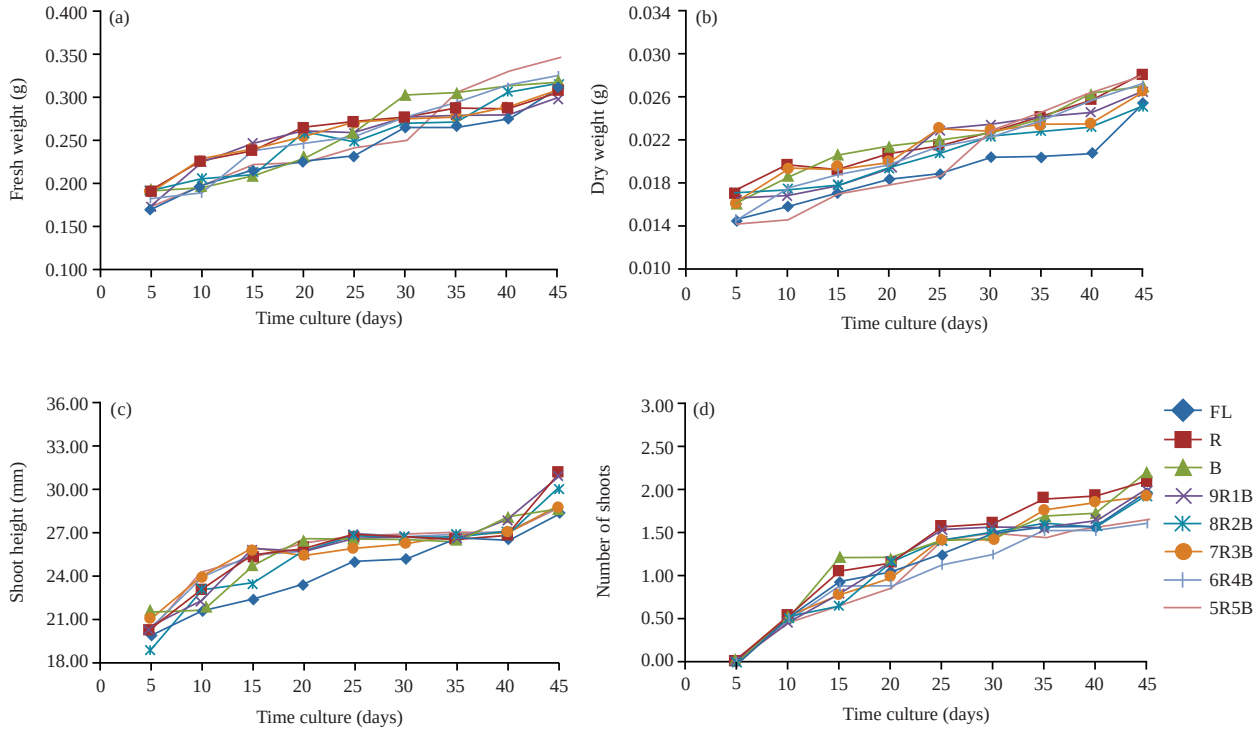

Fig. 2(a-d): Effect of the various light condition on the (a) Fresh weight, (b) Dry weight, (c) Shoot height and (d) Number of shoots of *D. officinale* plantlets from 5-45 days

in the LED conditions was higher than they were incubated in fluorescent. The second stage was from the 20-40 days of culture, at this stage, the plantlet height increased more slowly than in the previous stage. And the last stage after 40 days of culture, the plantlet height increased rapidly. In particular, the plantlet height was the highest when explants were incubated in 9R1B on the 45th day of culture. These results showed that the red LED significantly affected the height of the *D. officinale* plantlets increased with the increase of the red-light ratio in this experiment.

The change in the number of shoots per explant after 45 days of culture was recorded and shown in Fig. 2d. From the 5th to the 35th day, the number of shoots per explant was not statistically significantly different in all treatments. It was only different from the 40th day. On the 45th day, the explants were placed under 100% blue LED and had the highest number of shoots (2.2 shoots per explant). This result showed that the blue LED effectively affected the number of shoots.

Effect of light condition on the total phenolic content of *D. officinale* plantlets: After 45 days of culture, we observed that the TPC of *D. officinale* was different according to incubation time under various light conditions ($p<0.05$, Table 1). The results showed that the accumulation of total phenolic content in *D. officinale* plantlets could be divided into four phases. The first phase was 15 days after culture and TPC increased significantly on the 15th day. The highest TPC was recorded on the 15th day at 4.751 mg GAE/g DW in 8R2B, 1.24 times higher than the control treatment (FL condition). The second stage started from the 15th-30th days after culture, the TPC increased with the period time of culture. The TPC of the explants did not change significantly from the 20th to the 30th day. In the third stage, the accumulation of phenolic compound (phenolic compound accumulation) had maximum increases in all the lighting conditions tested (except the control) on the 35th day of culture. The TPC reached the highest value of 6.240 mg GAE/g DW in 9R1B, 1.57 times higher than the control. However, the TPC decreased after 35 days of culture. This can be considered the decline stage in the TPC biosynthesis of *in vitro D. officinale* shoots.

Effects of salicylic acid on the growth and the total phenolic content of *D. officinale* plantlets under 9R1B light conditions: In our study, results showed that the impact of various concentrations of SA made a difference among treatments after 30 days of culture ($p<0.05$). This proved that SA has a positive effect on the growth of *D. officinale*

Table 1: Effect of different light conditions on total phenolics content (mg GAE/g DW) of *in vitro D. officinale* plantlets after every 5 days of culture

	Time of culture (day) (B)								
Light condition (A)	5	10	15	20	25	30	35	40	45
FL	2.411^{d}	2.895^{d}	3.318^{b}	3.789^{abc}	3.778^{ab}	3.686^{c}	3.928^{d}	4.892^{a}	3.980^{a}
R	3.725^{bc}	3.415^{b}	3.556^{ab}	4.064^{a}	4.195^{a}	4.788^{a}	5.138^{b}	3.842^{c}	3.272^{c}
B	4.215^{a}	3.890^{a}	3.530^{ab}	3.580^{bcd}	3.930^{ab}	4.352^{b}	4.152^{cd}	4.448^{b}	3.680^{ab}
9R1B	3.261^{c}	3.404^{b}	3.81^{a}	3.990^{ab}	4.202^{a}	4.388^{b}	6.190^{a}	4.508^{b}	3.530^{bc}
8R2B	3.384^{bc}	3.298^{bc}	3.562^{ab}	3.632^{abc}	4.008^{a}	4.702^{ab}	5.264^{b}	2.838^{d}	3.316^{c}
7R3B	3.302^{c}	2.842^{d}	3.660^{a}	3.980^{ab}	4.064^{a}	4.508^{ab}	5.128^{b}	3.084^{d}	3.508^{bc}
6R4B	3.909^{ab}	2.944^{cd}	3.490^{ab}	3.330^{cd}	3.774^{ab}	4.360^{b}	5.056^{b}	3.718^{c}	3.556^{bc}
5R5B	3.546^{bcd}	2.805^{d}	3.327^{b}	3.154^{d}	3.574^{b}	3.704^{c}	4.436^{c}	3.050^{d}	3.404^{bc}

Different letters (a-d) in each column differ significantly according to Tukey's multiple range tests at $p<0.05$

Table 2: Effect of salicylic acid on the growth and total phenolics content of *in vitro D. officinale* plantlets after 30 days of culture under 9R1B condition

SA (mg L^{-1})	Fresh weight (g)	Dry weight (g)	Height of the plantlet (mm)	No. of shoots	TPC (mg GAE/g DW)
Control	0.277±0.015^{b}	0.023±0.002^{bc}	26.76±1.62^{ab}	1.56±0.22^{ab}	4.388±0.326^{cd}
0.5	0.280±0.029^{b}	0.023±0.002^{bc}	26.08±2.04^{abc}	1.80±0.35^{ab}	5.410±0.680^{abc}
1.0	0.271±0.234^{b}	0.020±0.002^{c}	22.88±2.24^{c}	1.32±0.23^{b}	5.244±0.453^{bc}
1.5	0.330±0.018^{a}	0.030±0.002^{a}	29.36±0.86^{a}	2.00±0.20^{a}	5.438±0.690^{abc}
2.0	0.285±0.021^{ab}	0.024±0.003^{b}	25.56±2.09^{bc}	1.76±0.26^{ab}	5.788±0.805^{ab}
2.5	0.256±0.028^{b}	0.021±0.001^{bc}	25.92±0.68^{bc}	1.68±0.23^{ab}	6.680±0.723^{a}
3.0	0.255±0.033^{b}	0.022±0.001^{bc}	25.32±1.40^{bc}	1.60±0.20^{ab}	3.670±0.649^{d}

Values represent Mean±SE, different letters (a-d) in each column differ significantly according to Tukey's multiple range tests at $p<0.05$ and Control: SA-free medium

Table 3: Effect of yeast extract on the growth and total phenolics content of *in vitro D. officinale* plantlets after 30 days of culture under 9R1B light condition

YE (g L^{-1})	Fresh weight (g)	Dry weight (g)	Height of the plantlet (mm)	No. of shoots	TPC (mg GAE/g DW)
Control	0.277±0.015^{bc}	0.023±0.002^{bc}	26.76±1.62^{bc}	1.56±0.22^{bc}	4.388±0.326^{e}
0.5	0.207±0.024^{d}	0.019±0.002^{d}	25.32±1.23^{bc}	1.44±0.17^{bc}	6.451±0.507^{abc}
1.0	0.230±0.021^{cd}	0.023±0.001^{bc}	21.72±1.06^{d}	1.24±0.22^{c}	6.918±0.567^{a}
1.5	0.248±0.03^{bcd}	0.020±0.001^{cd}	27.74±0.95^{ab}	2.12±0.23^{a}	6.692±0.395^{ab}
2.0	0.260±0.023^{bc}	0.021±0.001^{bcd}	24.30±0.95^{c}	1.80±0.14^{ab}	5.538±0.588^{cd}
2.5	0.362±0.036^{a}	0.027±0.002^{a}	29.36±1.28^{a}	1.68±0.18^{b}	5.860±0.243^{bcd}
3.0	0.284±0.029^{b}	0.024±0.002^{ab}	25.28±1.40^{bc}	1.60±0.20^{bc}	5.134±0.716^{de}

Values represent Mean±SE, different letters (a-e) in each column differ significantly according to Tukey's multiple range tests at $p<0.05$ and Control: YE-free medium

plantlets. The fresh weight, as well as the dry weight of the explants gradually increased when the SA concentration increased from 0.5-1.5 mg L^{-1} and reached a maximum at the concentration of 1.5 mg L^{-1} SA, with the fresh weight was 0.33 g, dry weight was 0.03 g, the shoot height was 29.36 mm and the numbers of the shoot was 2.0 per explant. After that, these observation targets tend to decrease gradually when the concentration of SA continuously increases (from 2.0-3.0 mg L^{-1}).

The TPC of *D. officinale* plantlets after 30 days of culture was recorded (Table 2). The TPC increased as the SA concentration increased from 0.5-2.5 mg L^{-1} and reached the highest value (6.680 mg GAE/g DW) at 2.5 mg L^{-1} SA (1.52-fold as compared with control). However, the TPC was significantly reduced and lower than the control when SA concentration increased to 3.0 mg L^{-1}.

Effect of yeast extract on the growth and the total phenolic content of *D. officinale* plantlets under 9R1B light conditions: In this experiment, the MS medium supplemented with YE at a concentration of 2.5 g L^{-1} was optimal for the growth of *D. officinale* plantlets, with the fresh weight (0.362 g), dry weight (0.027 g) and shoot height (29.36 mm). The growth of the explants tended to decrease on the MS medium supplemented with YE at a high concentration (3.0 g L^{-1} YE).

It can be seen that the addition of YE to the medium enhanced the phenolic accumulation of all treatments (Table 3). The TPC was the highest at YE concentration of 1.0 g L^{-1} (TPC of 6.918 mg GAE/g DW), 1.58-fold as compared with control (4.388 mg GAE/g DW). However, TPC decreased when YE concentration increased from 2.0-3.0 g L^{-1}.

DISCUSSION

Light plays a major role in plant growth and the accumulation of secondary metabolites[11]. Nowadays, there have been many studies on LED as a new artificial light source for the growth and development of different plant species such as *D. officinale*[9], *Panax vietnamensis*[24] and *Camelia japonica*[25]. However, to the best of our knowledge, there are few investigations on *D. officinale* using LED, as there have

not been studies on the effects of LED combined with SA or YE on the growth and TPC in *D. officinale* plantlets.

In this study, experimental results also showed that the impact of various lights made a difference among treatments after each different incubation period ($p<0.05$). Results showed that after 45 days of culture, growth parameters such as the average plantlet height and the average number of shoots in the treatment using a high red wavelength ratio (from 80-100%) was higher than the average number of shoots in the treatments using FL, B and LED with lower red LED percentage (from 50-70%). The plantlet height of *D. officinale* was the highest in 9R1B and R conditions. The results of this study were like those of Naznin *et al.*[26]. The average height of kale and lettuce increased with the increasing red LED ratio and the highest plantlet height was observed in the treatment using 100% red LEDs. Similarly, Frąszczak *et al.*[27] reported that the average height of *Anethum graveolens* was proportional to the amount of red light after 28 days of culture, that value was highest in the treatment using a combination of 70% red LED and 10% blue LED. Several other studies have also shown that red LED increased the height of various plantlet species such as *Rehmannia glutinosa*[13], grape[28] and *Brosimum gaudichaudii*[29]. The differences between treatments could be explained by the interactions between the red and blue light receptors. Phytochromes can regulate stem and leaf growth, while cryptochromes have an inhibitory effect on stem elongation. Interactions among receptors can either stimulate or inhibit stem elongation depending on the specific species[30].

Regarding the weight of *D. officinale* plantlet, the increase in fresh weight was proportional to the blue light ratio used in each treatment. Fresh weight was the highest in treatment 5R5B. Similar results have previously been observed by Li *et al.*[31] and Chen *et al.*[32], the authors reported that the fresh weight of *Gossypium hirsutum* and lettuce was the highest under the 5R5B condition. However, in other species such as petunia, tomato, or salvia, the highest biomass was obtained in the treatment using 100% red LED light[33] and Li *et al.*[28] also showed that the plantlet biomass obtained from cultures placed under white fluorescent was higher than that of the other LEDs. Besides, depending on different growth stages, the biomass of *D. officinale* was affected by different types of light. For example, at the PLBs stage, 50% red LED combined with 50% blue LED was suitable for the increase of PLBs biomass as well as the regeneration shoots rate from PLBs of *D. officinale*[9]. The above results have shown that the influence of light wavelength on plant growth is complex and different, depending on plant species or cultivar and the stage of plant growth[34]. The increase in sample biomass can be explained based on the mechanism of action of cryptochrome and phototropin[35]. Cryptochromes interact with signalling proteins to alter gene expression at the transcriptional or post-transcriptional level, stimulating cell division and leading to growth and metabolism in plants. Along with that, phototropin receptors regulate the opening and closing of stomata, leading to increased efficiency of photosynthesis and the accumulation of essential compounds in plants[35].

LED not only has a positive effect on growth but also affects the accumulation of secondary compounds in plants[36]. Many studies showed that the use of red LED or blue LED or in combination with different ratios affects the increase of phenolic content in *Lactuca sativa*[37], *Aronia melanocarpa*, *Aronia arbutifolia*, *Aronia*× *prunifolia*[38] and *Nasturtium officinale*[39]. In current results, the red LED had a greater impact than the blue LED on the TPC change in *in vitro D. officinale* shoots, especially, the combination of red and blue LED has the most effect on the TPC accumulation. The TPC content of *D. officinale* shoots reached the highest value under the 9R1B condition after 35 days of culture. This result is similar to the studies of Samuolienė *et al.*[14] and Cioć *et al.*[15], the authors reported red LED light increased phenols content. The results also showed that the addition of blue wavelength in the spectrum reduced TPC content and the use of 100% blue LED light caused the lowest TPC contents in the extracts. Zheng and van Labeke[40] reported that TPC in the leaves of *Dendranthema morifolium* was suppressed under blue LED. However, some other studies have shown that blue LED is more effective than red LED in the process of phenol accumulation for sprouts[41] and lettuce[42]. This is also evidence showing this effect depends on plant species. According to the results of research by Lobiuc *et al.*[43] for acyanic và cyanic *Ocimum basilicum*, the green cultivar was most stimulated by a higher proportion of red light, while the red cultivar, by higher ratios of blue light. Different wavelengths, such as B (450-495 nm) and R (620-750 nm), regulated the synthesis of phenols either directly or indirectly through signalling, which led to the expression of key enzymes or regulated the synthesis of shikimic acid, a precursor to phenolic acid[44]. Most plant phenolics are derived from phenylalanine via eliminating an ammonia molecule to form cinnamic acid, this reaction is catalyzed by the phenylalanine ammonia-lyase (PAL) enzyme[45]. The presence of these enzymes can help cells enhance the ability to metabolize precursors such as phenylalanine into necessary intermediates for the synthesis of phenolic compounds[46].

These results showed that the treatment using 90% red and 10% blue LED was suitable for the growth and TPC accumulation in *D. officinale* plantlets, therefore this light

condition was used for the subsequent experiments. Based on the change of TPC over time of culture, the 35th day of culture was a suitable time for collecting phenolic compounds.

Continuously, the combination of LED and SA or YE on TPC accumulation as well as the growth of *D. officinale* plantlets was also evaluated. The results showed that the addition of elicitors to the culture medium and placed under the 9R1B condition increased the TPC of the explants, which was higher than that of the control after 30 days of culture. Specifically, in the treatments using SA at concentrations from 0.5-2.5 mg L^{-1}, the TPC was 1.2-1.5 times as high as the treatment without SA. The highest phenolic content was obtained in the treatment using 2.5 mg L^{-1} SA, with a TPC of 6.680 mg GAE/g DW. Salicylic acid (SA) is an abiotic elicitor commonly used in plant cell culture and plays an important role in the plant response to abiotic stresses such as drought, heat, chilling, heavy metal, salinity, UV radiation and ozone stress[47]. According to the study by Dong *et al.*[48], SA activates the antioxidant mechanism in plants by enhancing the activity of antioxidant enzymes, thereby stimulating the synthesis of secondary compounds and preserving protecting plant cells against oxidative stress. In addition, SA also regulates the activity of the PAL enzyme, a key enzyme in the process of phenolic biosynthesis, which promoted the accumulation of this compound. In a study on *Lavandula angustifolia* Mill by Miclea *et al.*[49], most explants grew on the medium culture supplemented with SA had TPC higher than explants were culture on an SA-free medium. Tajik *et al.*[50] also reported that SA pretreatment increased the activity and expression of the PAL enzyme, which LED to an increase in the phenolic and flavonoid content of *Crocus sativus*.

TPC of explants cultured on a medium supplemented with YE was 1.2-1.6 times as high as the control. The highest TPC was obtained in the treatment using YE 1.0 g L^{-1}, with a TPC of 6.918 mg GAE/g DW. Sahu *et al.*[51] reported that the use of some elicitors such as methyl jasmonate (MJ), SA and YE increased rosmarinic acid (RA) content, in which SA and YE were used at a concentration of 50 mM, 100 mg mL^{-1} increased RA content by 1.4, 1.5 times after 24 hrs of culture, respectively. This increase in RA was due to the effect of these elicitors on the main enzymes in the synthesis of RA such as phenylalanine ammonia-lyase, tyrosine aminotransferase and hydroxyl-phenylpyruvate reductase. Veerashree *et al.*[52] studied the effect of elicitor on gymnemic acid synthesis by using the method of cell suspension culture of *Gymnema sylvestre*. Gymnemic acid content reached the highest in treatment with 0.5 g L^{-1} YE after 20 days of culture, it was 5.25 times as high as the control. In the cell culture of *Psoralea corylifolia* for psoralen production, treatment with 1.5% YE increased the psoralen content by 4 times compared with the control without YE[53]. YE, an abiotic elicitor, was added to the culture medium to enhance the biosynthesis of natural compounds in plants. Fungal elicitors have been widely used to stimulate the production of many classes of secondary metabolites, such as terpenoids, alkaloids and flavonoids. Besides, YE has also been employed to analyze the interactions between plants and microorganisms as well as plant defense responses[54].

In this study, the addition of SA or YE to the culture medium not only increased the TPC content but also promoted the growth of *D. officinale* plantlets. In the treatment using 1.5 mg L^{-1} SA, growth parameters such as fresh weight, dry weight and shoot height were obtained higher than those of the control. Similarly, regarding YE, fresh weight, dry weight and shoot height obtained in the medium supplemented with 2.5 g L^{-1} YE were also higher than the control. Apart from amino acids, vitamins and minerals, YE contains a range of chemicals and elicitation effects could be owing to the presence of cations such as Zn, Ca and Co in the YE[55], which had a positive effect on the growth, development and physiological responses in plants. SA acts as a plant growth regulator, enhances photosynthetic activity, stimulates leaf area expansion and cell biomass accumulation and increases the height. According to Vicente and Plasencia[56], the role of SA in plant growth and development is different, which depends on concentration and developmental phase. However, SA used at high concentrations will inhibit plant growth[47]. In general, this high concentration depends on the plant species[57]. In this study, 1.5 g L^{-1} SA is the suitable concentration for the *in vitro* growth of *D. officinale* shoots, when the SA concentration increased from 2.0-3.0 g L^{-1}, the growth of *D. officinale* plantlets was decreased. Similar to current results, some previous studies also showed that SA and YE affected the growth of some plant species such as *Stevia rebaudiana*[58,49] and *Lavandula angustifolia* Mill[59]. The result of this study also showed that the combination of LED (9R1B condition) and elicitors (SA, yeast extract) promoted the growth and the accumulation of total phenolic content of *D. officinale in vitro*. This is also one of the first studies using a combination of LED light and elicitors on the *D. officinale*. The results in this study can serve as a premise for further studies on *D. officinale* and especially other medicinal plants.

CONCLUSION

The red and blue LED lights had a positive effect on the growth and the accumulation of phenolic compounds in *Dendrobium officinale* Kimura et Migo plantlets. The growth

and the TPC were the highest after 45 days of culture under the 9R1B condition. Moreover, the combination of LED (9R1B) and elicitors promoted growth, as well as increased the TPC content of *D. officinale* plantlets. The culture medium supplemented with SA at the concentration of 1.5 mg L^{-1} was suitable for the growth and the accumulation of phenolic compounds in the *D. officinale* plantlets. The growth of *D. officinale* plantlets on medium supplemented with YE was better than that cultured on medium without YE. The growth of *D. officinale* was suitable at the concentration of 2.5 g L^{-1} YE and the TPC was the highest at the concentration of 1.0 g L^{-1} YE.

SIGNIFICANCE STATEMENT

This study evaluated the effect of LEDs (with different ratios of blue and red light), salicylic acid and yeast extract on the growth and phenolics content of *in vitro D. officinale* plantlets. The results indicated the phenolics content of *in vitro D. officinale* plantlets increased when *in vitro* plantlets were cultured in the MS medium supplemented elicitors and incubated under the 9R1B condition.

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