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LEDs Combined with CHO Sources and CCC Priming PLB Regeneration of *Phalaenopsis*

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Abstract: Throughout this study, the objective was to determine the most effective carbohydrate (CHO) sources under different light-emitting diodes (LEDs), and the impact of chlorocholine chloride (CCC), for the in vitro regeneration of the protocom-like bodies (PLBs) in *Phalaenopsis* 'Fmk02010'. We applied 15 LEDs combined with three CHO sources and five CCC concentrations in the study. Organogenesis of PLBs was very poor in maltose both for the number of PLBs and their fresh weight (FW) compared to media containing sucrose and trehalose. Sucrose was the best CHO source under the red-white (RW) LED for the in vitro organogenesis of PLBs (PLBs: 54.13; FW: 0.109 g), while trehalose was best under the blue-white (BW) LED (PLBs: 36.33, FW: 0.129 g). The red-blue-white (RBW)-trehalose combination generated a suitable number of PLBs (35.13) with the highest FW (0.167 g). CCC at 0.01, 0.1, and 1 mgL^{-1}CCC had no effect on PLB formation or FW, but 10 mg L^{-1} reduced both. RW-sucrose, BW-trehalose, and RBW-trehalose were the best combinations for PLB organogenesis. The addition of low concentrations of CCC in the plant culture medium are unnecessary.

Keywords: protocorm-like bodies; light-emitting diode; trehalose; maltose; CCC; correlation; growth retardants

1. Introduction

Phalaenopsis is the most important and valuable commercial orchid in the Orchidaceae family. It is widely accepted both as cut and pot flowers. Unlike most flowering plants, orchids have a very unique reproductive system. Propagation of *Phalaenopsis,* either vegetatively or by seed, is quite difficult. Tissue culture is the common method due to its successful and rapid propagation. PLB regeneration is the best and most efficient technique for orchid micropropagation [1], because it has a rapid proliferation capacity for producing a large number of protocorm-like bodies PLBs within a short period [2]. They can be induced directly from explants, such as shoot tips [3], flower stalk buds [4], root tips [5], and leaf segments [6]. The indirect regeneration of PLBs can be done by embryogenic callus culture using solid [7] or liquid [8] suspension cultures. Proper media compositions with optimum culture conditions are among the significant factors for fast and high quality plantlet regeneration through PLBs [9,10]. PLBs are the sole form of somatic embryo that imitates the zygotic embryo in natural seed, but unlike the zygotic embryo, it can grow continuously without any dormancy [11].

Media ingredients are the key factor for successful PLB regeneration in vitro. Plant tissue culture media generally have mineral salts, vitamins, growth regulators, and water [12]; another important component is the carbon source to supply energy [13]. There are many carbon sources like sucrose, fructose, glucose, trehalose, maltose, and sorbitol [8,14] used for plant tissue culture that might be in simple or complex forms [15]. It is well known that plants are sensitive to light. Light also affects PLB regeneration through photosynthetic and phototropic responses and may depend on light quality

and photoperiod [16]. Fluorescent lights are commonly used. LED lights are currently used for in vitro cultivation. Power consumption of fluorescent lights is greater, and they produce a wide range of wavelengths (350–750 nm) that are unnecessary for plant development. Monochromatic LEDs emit light at specific wavelengths. LEDs are used commercially in plant tissue culture due to their numerous advantages compared to conventional light systems; such as wavelength specificity, durability, small size, long operating time, relatively cool emitting surface, and the ability to control spectral composition [17–19]. Concerning economic viability, the use of LEDs is increasing rapidly in agriculture due to their huge capacity to save electrical energy. LEDs are more efficient in in vitro culture than white fluorescent light. It is stipulated that they have the specific wavelengths that fits plants exact need for morphogenic responses [20]. The wavelength a plant needs varies according to the species.

LEDs are a unique type of semiconductor diode that have several technical benefits over usual light sources for photosynthesis [21]. LEDs allow wavelengths to be matched to the plant photoreceptors to influence plant morphology and metabolic composition [22–24]. Plant pigments absorb red wavelengths (600–700 nm) efficiently, with the most efficient being660 nm, which is close to the chlorophyll absorption peak, whereas the blue region includes the visible spectrum (400–500 nm) [25]. It has also been reported that red light has a significant role in starch accumulation through photosynthesis [26] and blue light in chloroplast development, chlorophyll formation, and stomatal opening [27]. Red and blue light are the best to drive photosynthetic metabolism. Green wavelength effects are opposite those of red and blue wavelengths [28].

A number of in vitro studies have reported vigorous plant growth under LEDs. LED lights have been previously used for PLB organogenesis in *Cymbidium finlaysonianum* [29], *Dendrobiumkingianum* [30,31], hybrid *Cymbidium* [32,33], and plantlet regeneration in gerbera [34]. A series of studies have already been conducted to improve the tissue culture of *Phalaenopsis* using a number of factors. We previously used growth regulators and elicitors for the PLB regeneration of *Phalaenopsis* [35,36]. The effects of light spectral quality on the photosynthetic ability varied by plant species [37]. Many plant species do not respond well under a sole LED color, and this limitation can be overcome by combining different colors. On the other hand, many researchers have reported the long-term effect of growth retardants on in vitro growth and development [38,39]. Chlorocholine chloride (CCC: (2-chloroethyl) trimethyl-ammonium chloride) can be used to manipulate plant growth [40–42]; it inhibits gibberellic acid biosynthesis [43,44]. Gibberellic acid reduces adenosine diphosphate-glucose pyrophosphorylase (AGPase) activity, which is responsible for the reduction of starch synthesis [45]. Application of CCC can counteract this starch synthesis reduction by blocking gibberellic acid synthesis. CCC may have an impact on plant growth by altering the hormone content.

The purpose of this study was to determine the best CHO source and LED light combination for successful PLB regeneration of *Phalaenopsis* 'Fmk02010'. In addition, our goals was also to assess the impact of CCC priming in in vitro PLB propagation of *Phalaenopsis*.

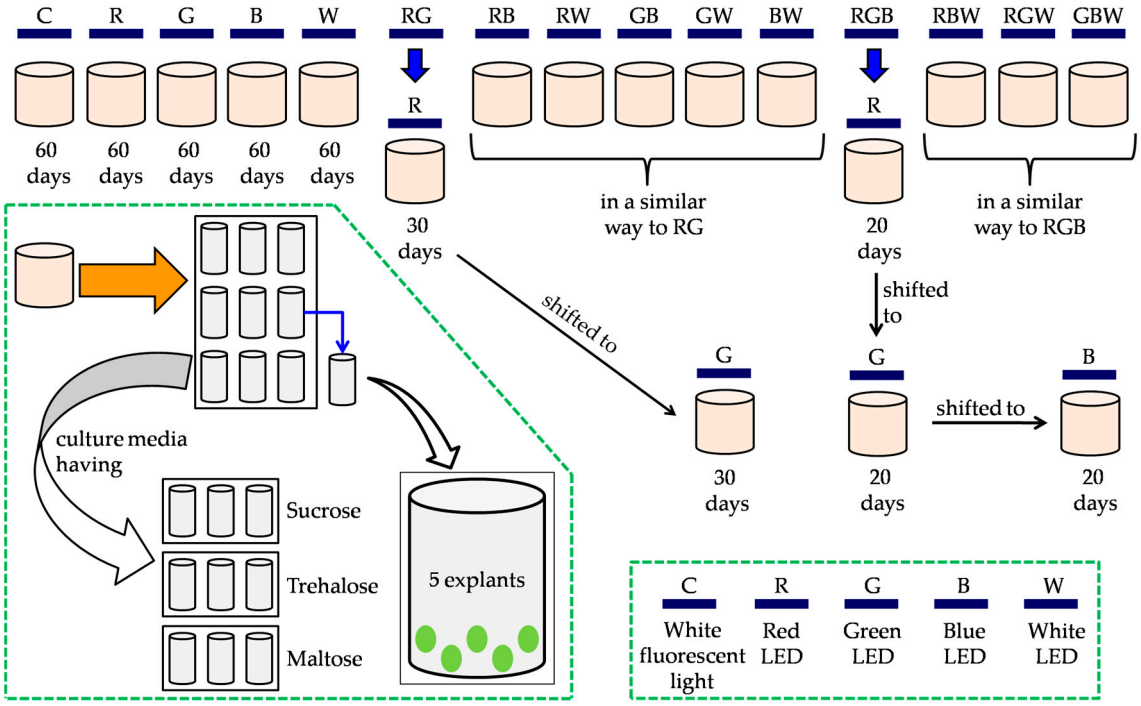
2. Materials and Methods

2.1. Plant Materials and Culture Conditions

PLBs of *Phalaenopsis* 'Fmk02010' were multiplied in 2.2 gL^{-1} of Phytagel™ (Sigma-Aldrich®, Tokyo, Japan) solidified MS medium (modified) [46] at the Lab of Vegetable and Floricultural Science, Faculty of Agriculture and Marine Science, Kochi University, Japan. We added two major salts, ammonium nitrate (412.5 mgL^{-1}) and potassium nitrate (950.0 mgL^{-1}), to the MS medium for the modification. We excised single PLBs to use as explants. The pH was adjusted to 5.5–5.8 using 1 mM 2-(N-morpholino) ethanesulfonic acid sodium salt (MES-Na) prior to autoclaving. We used 30 mL of culture media in each 250-mL culture bottle (UM culture bottle: AsOne, Japan) and autoclaved at 121 °C for 15 min at 117.1 KPa.

2.2. CHO Sources and LED Lights

Sucrose, trehalose, and maltose (20 g/L) (Sigma-Aldrich®, Tokyo, Japan) were used as CHO sources before autoclaving. The PLBs for organogenesis were placed under fourteen different LED light sources with a control. These were: (1) control (C: white fluorescent light); (2) R (red LED); (3) G (green LED); (4) B (blue LED); (5) W (white LED); (6) RG (red → green LED); (7) RB (red → blue LED); (8) RW (red → white LED); (9) GB (green → blue LED); (10) GW (green → white LED); (11) BW (blue → white LED); (12) RGB (red → green → blue LED); (13) RBW (red → blue → white LED); (14) RGW (red → green → white LED); and (15) GBW (green → blue → white LED). All LED lamps were monochromic. We did not use two different monochromic LEDs together. A monochromic light supplemented with ≥1 monochromic light had a dissimilar light effect. For example, red LEDs supplemented with blue fluorescent were equivalent to cool-white fluorescent plus incandescent lamps [47]. The technique for the sole, double, and triple LED light combinations used in this study is shown in Scheme 1.



Scheme 1. Visualization of the experimental layout. C, control.

2.3. CCC Concentrations

Four different CCC concentrations with the control (BioReagent, Sigma-Aldrich®, Tokyo, Japan) were used. They were 0 (control), 0.01, 0.1, 1, and 10 mgL^{-1}. Sucrose was used in the culture media, while other culture conditions were similar as described in Section 2.1.

2.4. PLB Culture, Data Collection, and Data Analysis

Experiments were organized in a randomized complete block design. Each bottle contained five PLBs (with three replications). PLBs were cultured 60 days for the LED-carbon source experiments and 42 days for the CCC-treated PLBs. The explants were cultured at 25 ± 2 °C with a 16-h photoperiod with 54 $\mu mol/m^{-2}$ s^{-1} of irradiance. The number of PLBs (including budding PLBs), shoots, and roots were counted (Figure 1a). The length of shoots and the fresh weight (FW) of PLBs were measured. The average numbers and percentages were calculated as follows.

- Average number = Number of cultured explants with new PLBs/Total number of cultured explants

- Percentage of PLB (%) = (Number of cultured explants with new PLBs/Total number of cultured explants) × 100

Data are presented as the mean ± the standard error (SE). One-way ANOVA was analyzed by Minitab®17 (Minitab Inc., Pennsylvania 16801-3008, USA, 2017) using Tukey's multiple comparisons test method with the 95% confidence interval.

3. Results

3.1. CHO Sources and LED Lights

RW-sucrose and the control did not significantly differ (Table 1). However, all other LED-sucrose combinations produced a significantly lower number of PLBs than the RW-sucrose, some significantly lower than the control as well. Within trehalose treatments, BW-trehalose performed well for the mean number of PLBs (36.33), which was closely followed by RBW-trehalose (35.13) (Table 1). Maltose showed the worst overall performance for PLB regeneration with all LED combinations (Table 1). PLBs under different LEDs showed statistically identical fresh weights for mediums with sucrose and most with trehalose. However, the medium with maltose showed significant differences among the different LEDs. Maximum mean fresh weight was found for RBW-trehalose (0.167 g), RBW-maltose (0.112 g), and RW-sucrose (0.109 g) (Table 1). The RBW-trehalose combination also produced a satisfactory number of PLBs (35.13) (Table 1). The CHO source-LED combinations with the first, second, and third highest values for the number of PLBs within each CHO source group (Figure 2) and fresh weight (Figure 3) are shown. Sucrose produced the highest and second highest numbers of PLBs, and trehalose was best for the fresh weight as the CHO source in the culture medium (Figures 2 and 3).

After 60 days of culture, some treatments tended to produce shoots. Trehalose had a greater tendency for shoot growth under LED lights except GW, RGB, RBW, RGW, and GBW (Table 2). There were no shoots for trehalose under white fluorescent light (control). Shoots were produced with W-sucrose and RGW-sucrose, as well as with RG-maltose. Root formation was not observed in any of the treatment combinations except trehalose-RG (number: 0.03; length: 0.03 cm; data are not shown).

3.2. CCC Concentrations

The number of PLBs, PLB formation rate, and fresh weight of *Phalaenopsis* 'Fmk02010' with different concentrations of CCC in the culture medium are shown in Table 3. The number of PLBs and fresh weight were significantly lower at 10 mgL^{-1}CCC. The maximum number of PLBs were produced in the culture medium treated with 0.01 mgL^{-1} of CCC. In this treatment, there was a 100% PLB formation rate. The PLB formation rates were 93.33%, 93.33%, 80.00%, and 33.33% at 0, 0.01, 1, and 10 mgL^{-1} of CCC, respectively. The maximum fresh weight was from the culture media having 0.01 mgL^{-1} of CCC, whereas the minimum fresh weight was found at 10 mgL^{-1} of CCC (Table 3). CCC at 0.01, 0.1, and 1 mg L^{-1} did not differ from the control values for number of PLBs or fresh weight. In the scatter plot (Figure 4), the relationship between PLB organogenesis and CCC concentration are shown. The R^2 of the correlation was high for both number of PLBs (R^2 = 0.915) and fresh weight (R^2 = 0.747) to the different CCC concentrations. There was a negative relationship in both cases, suggesting that the number of PLBs and fresh weight would decrease with increasing CCC concentration in the culture medium.

Table 1. Mean number of PLBs and fresh weight of *Phalaenopsis* 'Fmk02010'withdifferent CHO sources and LED lights.

Light [z]	Mean Number of PLBs			Fresh Weight (g)		
	Sucrose	Trehalose	Maltose	Sucrose	Trehalose	Maltose
Control	37.73 ± 4.40 [y] ab	28.13 ± 4.87 abcd	1.60 ± 0.53 b	0.098 ± 0.057 a	0.059 ± 0.033 ab	0.043 ± 0.023 ab
R	26.20 ± 3.38 bc	15.87 ± 2.89 bcde	2.73 ± 0.97 b	0.062 ± 0.042 a	0.059 ± 0.032 ab	0.033 ± 0.019 b
G	21.93 ± 3.76 bcd	8.87 ± 1.55 de	6.00 ± 1.35 b	0.066 ± 0.045 a	0.041 ± 0.022 ab	0.033 ± 0.018 b
B	20.47 ± 3.92 bcd	27.67 ± 3.21 abcd	2.27 ± 0.76 b	0.043 ± 0.024 a	0.137 ± 0.074 ab	0.017 ± 0.009 b
W	24.80 ± 4.49 bcd	22.80 ± 2.92 abcde	3.60 ± 0.67 b	0.071 ± 0.039 a	0.079 ± 0.042 ab	0.028 ± 0.017 b
RG	11.07 ± 3.97 cd	19.33 ± 3.44 abcde	4.47 ± 1.04 b	0.029 ± 0.022 a	0.063 ± 0.034 ab	0.043 ± 0.023 ab
RB	11.60 ± 3.11 cd	15.33 ± 5.55 bcde	1.47 ± 0.45 b	0.026 ± 0.020 a	0.091 ± 0.065 ab	0.024 ± 0.013 b
RW	54.13 ± 8.85 a	12.13 ± 2.82 cde	14.47 ± 3.39 a	0.109 ± 0.063 a	0.027 ± 0.020 b	0.066 ± 0.041 ab
GB	19.93 ± 4.83 bcd	22.73 ± 3.67 abcde	2.27 ± 1.03 b	0.061 ± 0.035 a	0.117 ± 0.062 ab	0.020 ± 0.011 b
GW	26.40 ± 3.60 bc	8.73 ± 2.19 de	4.80 ± 1.11 b	0.080 ± 00.047 a	0.047 ± 0.026 ab	0.051 ± 0.027 ab
BW	10.67 ± 4.11 cd	36.33 ± 5.08 a	3.80 ± 1.76 b	0.020 ± 0.020 a	0.129 ± 0.071 ab	0.045 ± 0.024 ab
RGB	5.47 ± 1.98 d	5.00 ± 1.70 e	8.40 ± 1.85 a	0.015 ± 0.015 a	0.066 ± 0.048 ab	0.079 ± 0.047 ab
RBW	13.53 ± 2.88 cd	35.13 ± 4.36 ab	13.73 ± 2.09 a	0.034 ± 0.024 a	0.167 ± 0.098 a	0.112 ± 0.068 a
RGW	19.07 ± 2.60 bcd	29.40 ± 4.45 abc	1.47 ± 0.35 b	0.030 ± 0.021 a	0.090 ± 0.048 ab	0.022 ± 0.013 b
GBW	12.20 ± 2.79 cd	32.00 ± 7.77 ab	4.73 ± 1.27 b	0.056 ± 0.038 a	0.088 ± 0.063 ab	0.030 ± 0.017 b

[z] Control (C: white fluorescent light); R (red LED); G (green LED); B (blue LED); W (white LED); RG (red → green LED); RB (red → blue LED); RW (red → white LED); GB (green → blue LED); GW (green → white LED); BW (blue → white LED); RGB (red → green → blue LED); RBW (red → blue → white LED); RGW (red → green → white LED); and GBW (green → blue → white LED). [y] Mean± SE values that do not share a letter are significantly different within each column, and those sharing a letter are statistically similar at $P \leq 0.05$.

Table 2. Shoot growth withdifferent CHO sources and LED lights during PLB organogenesis of *Phalaenopsis* 'Fmk02010'.

Light [z]	Number of Shoots			Mean Shoot Length		
	Sucrose	Trehalose	Maltose	Sucrose	Trehalose	Maltose
Control	0	0	0	0	0	0
R	0	0.38 ± 0.14	0	0	0.26 ± 0.10	0
G	0	0.38 ± 0.14	0	0	0.04 ± 0.02	0
B	0	0.75 ± 0.29	0	0	0.11 ± 0.05	0
W	0.13 ± 0.07	0.25 ± 0.09	0	0.05 ± 0.05	0.08 ± 0.03	0
RG	0	0.13 ± 0.07	0.13 ± 0.13	0	0.08 ± 0.04	0.03 ± 0.03
RB	0	0.25 ± 0.13	0	0	0.05 ± 0.03	0
RW	0	0.13 ± 0.07	0	0	0.03 ± 0.01	0
GB	0	0.13 ± 0.07	0	0	0.05 ± 0.03	0
GW	0	0	0	0	0	0
BW	0	0.38 ± 0.14	0	0	0.12 ± 0.04	0
RGB	0	0	0	0	0	0
RBW	0	0	0	0	0	0
RGW	0.25 ± 0.13	0	0	0.03 ± 0.03	0	0
GBW	0	0	0	0	0	0

[z] Control (C: white fluorescent light); R (red LED); G (green LED); B (blue LED); W (white LED); RG (red → green LED); RB (red → blue LED); RW (red → white LED); GB (green → blue LED); GW (green → white LED); BW (blue → white LED); RGB (red → green → blue LED); RBW (red → blue → white LED); RGW (red → green → white LED); and GBW (green → blue → white LED).

Table 3. Role of CCC concentrations forthe in vitro PLB production of *Phalaenopsis* 'Fmk02010'.

CCC (mgL^{-1})	Number of PLBs	PLB Formation (%)	Fresh Weight (g)
0	12.53 ± 1.71 [z] a	93.33	0.175 ± 0.028 a
0.01	15.67 ± 1.01 a	100.00	0.211 ± 0.018 a
0.1	13.73 ± 1.62 a	93.33	0.191 ± 0.022 a
1	11.07 ± 2.08 ab	80.00	0.182 ± 0.027 a
10	4.40 ± 1.74 b	33.33	0.049 ± 0.019 b

[z] Mean ± SE values that do not share a letter within each column are significantly different at $P \leq 0.05$.

(**a**) (**b**) (**c**)

Figure 1. PLB organogenesis of *Phalaenopsis*: (**a**) PLBs, shoots, and roots, (**b**) RW-sucrose; and (**c**) BW-trehalose.

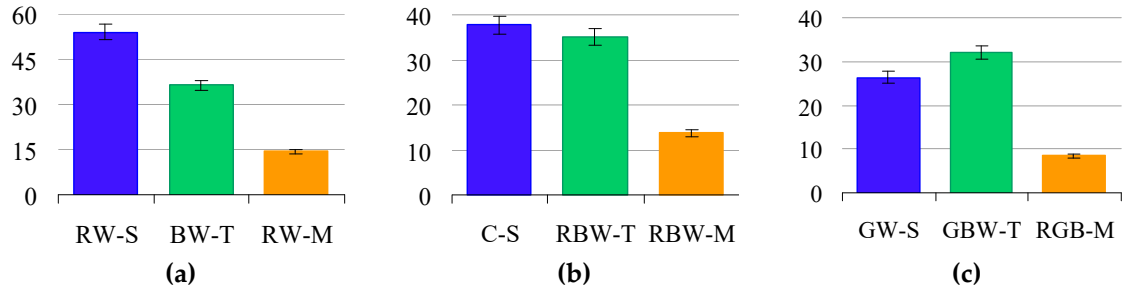


Figure 2. Comparison of PLB production for the (**a**) highest (**b**) second highest and (**c**) third highest CHO source –LED combination within each CHO source. The X-axis the treatment combination, the Y-axis represents the number of PLBs, and the error bar represents the 95% confidence intervals. See text for meanings of abbreviations.

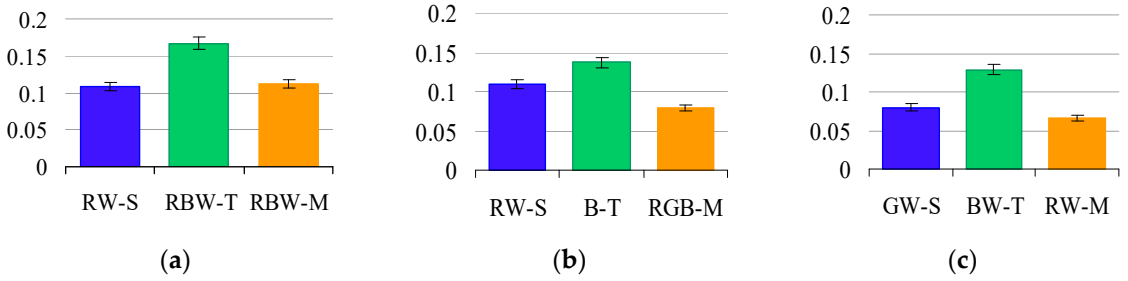


Figure 3. Comparison of fresh weight for the (**a**) highest (**b**) second highest and (**c**) third highest within CHO source –LED combination each CHO source. The X-axis the treatment combination, the Y-axis represents the number of PLBs, and the error bar represents the 95% confidence intervals. See text for meanings of abbreviations.

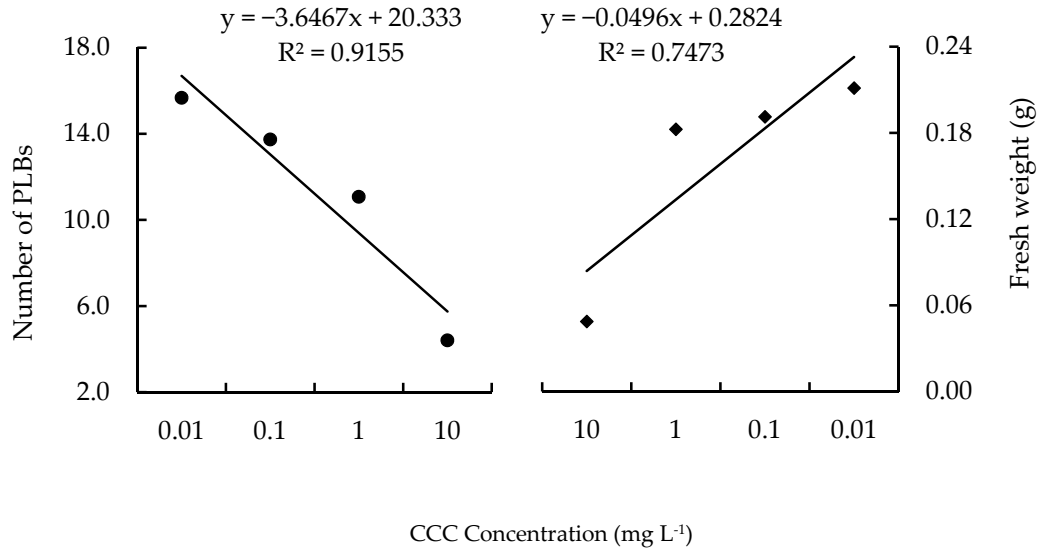


Figure 4. Correlation between the number of PLBs (left) and fresh weight (right) with CCC concentrations of the culture medium. Mean data were used for these analyses.

4. Discussion

Among the combinations, RW-sucrose produced the maximum number of PLBs, but the fresh weight not the highest (Table 1). On the other hand, the BW-trehalose combination produced comparatively fewer PLBs than that of RW-sucrose, but the fresh weight was higher. Sucrose was the best CHO source for number of PLBs (Figure 2), whereas trehalose was the best regarding the

fresh weight (Figure 3). Both number of PLBs and fresh weight are very important for successful and healthy PLB regeneration. Using trehalose in culture media was more effective than sucrose for friable callus formation in *Phalaenopsis* [48]. RW-sucrose, BW-trehalose, and RBW-trehalose combinations may be better for PLB organogenesis of *Phalaenopsis* considering both the number of PLBs and fresh weight. These three combinations were cultured in the white LED on the last 20 or 30 days; the first 20 or 30 days they were cultured under red, or blue, or red and blue LEDs. Results suggested that a white LED was important for rapid and healthy PLB growth, because the plant may have the ability to produce more chlorophyll under white light [49]. PLBs cultured under red LEDs for the early period showed a tendency to generate more PLBs. Plant growth was fragile under red light [50,51], and it stimulated endogenous gibberellins that cause cell proliferation and mitosis [52]. Red light increased multiplication rate [53], and our study also confirmed the increased multiplication of PLBs under red light. The red wavelengths (between 600 and 700 nm) were absorbed by plant pigments [23]. Hormones responsible for inflorescence formation, inflorescence elongation, and bud breakage were stimulated by red light [54]. PLBs cultured under blue LEDs in the early period produced a higher fresh weight. Trehalose-BW produced the maximum fresh weight of PLBs, and blue LEDs robustly encouraged PLB growth. Tanaka et al. [55] found blue LEDs to be effective for PLB formation in *Phalaenopsis*.

LEDs are an effective light source for plants [52,56], and light quality plays apart in the vital function of photosynthesis. The mechanism is that in which light is absorbed by chlorophyll [57]. Blue light plays an important role in chlorophyll biosynthesis [58–60] that may affect both the number of PLBs and fresh weight with a white LED. Chlorophyll contents are correlated with plant species or cultivar when grown under different light qualities [61]. Anuchai and Hsieh [62] found significantly higher chlorophyll (both a and b) and carotenoid content under blue light in *Phalaenopsis*. They also found a higher number of stems, fresh weight, and leaf length under red LEDs and higher RuBisCO enzyme activity. PLBs cultured under red LEDs in the early period, and then shifted to blue LEDs and white LEDs, showed significantly better results both for number of PLBs and fresh weight. The results suggested that red, or blue, or red and blue LEDs should be used initially, and then shifted to white LEDs for successful and healthier PLB regeneration of *Phalaenopsis* 'Fmk02010', but their effects also depend on the CHO sources in the culture medium. In our study, trehalose was better for available CHO for PLB organogenesis with BW LEDs (i.e., 30 days under a blue LED → 30 days under a white LED). Similarly, sucrose was better for available CHO under RW LEDs (i.e., 30 days under a red LED → 30 days under a white LED). Conversely, BW-trehalose produced the second largest number of shoots (Table 2). LEDs have been successfully applied in vitro in various plant species [20,29–33]. The ideal light stipulation for each plant species is unique. The response to the spectral composition of one plant species in vitro may not be similar for another plant species [63].

In our previous study, we used a number of growth regulators for PLB regeneration [35,36], so included the growth retardant CCC in the current study. The concentration of CCC played an important role in PLB organogenesis of *Phalaenopsis* "Fmk02010" 'Fmk02010'. Growth retardants were extensively used in vivo to improve floricultural characteristics, especially to control plant height. Application of CCC seemed to be effective with a very low concentration. PLB formation was very sensitive to a high concentration (>0.01 mgL^{-1}). Plant growth retardants like CCC could improve carbohydrate accumulation by increasing photosynthetic capacity and altering endogenous hormones [64,65]. CCC treatment can promote nutrient uptake, water balance, and protein synthesis in growing organs [66]. An increasing concentration of CCC resulted in a decreased number and percentage of PLB formation. The addition of CCC to the in vitro medium enhanced tuberization [67–69]. We found an effect of a higher concentration CCC on PLB organogenesis in culture media through the investigation of the relationship between CCC with PLB organogenesis. Similar relationships have also been studied previously [70–72]. CCC is an anti-gibberellin growth regulator that inhibits an early step in gibberellic acid (GA) biosynthesis [43]. Treatments with CCC counteract the reduction in starch synthesis by blocking GA synthesis. Plant growth retardants like CCC and paclobutrazol are able to inhibit gibberellin biosynthesis or action [73,74], and can control excessive vegetative growth [75,76]

which ultimately increases quality attributes such as dry matter content [77]. CCC treatment is mostly effective for tuberous and bulbous pants. PLBs are tuber-like bodies. We observed that the addition of the growth retardant CCC had no effect on PLB formation or fresh weight except for a reduction at the highest concentration (Table 3).

5. Conclusions

Sucrose and trehalose can be used as excellent CHO sources in the culture media for PLB regeneration of *Phalaenopsis*. RW-sucrose was the best combination to produce the maximum number of PLBs. However, the combination of BW-trehalose also produced a large number with healthier PLBs; it also had a tendency to produce a greater number of shoots that would need immediate subculture for future preservation. RBW-trehalose generated a satisfactory number of PLBs with a higher fresh weight and did not generate any shoots. An excessive concentration of CCC (10 mgL^{-1}) caused enormous reduction in the number of PLBs, the percentage of PLB formation, as well as fresh weight.

Author Contributions: H.M. (Hasan Mehraj) conceptualized and executed the study, as well as prepared the original draft of the manuscript; M.M.A. and H.M. (Hasan Mehbub) were responsible for data collection, compilation, and formal analysis; the manuscript was edited by S.U.H.

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Conflicts of Interest: The authors declare that there is no conflict of interest.

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