Article

Shortening the Vegetative Growth Stage of *Phalaenopsis* Queen Beer ‘Mantefon’ by Controlling Light with Calcium Ammonium Nitrate Levels under Enriched CO₂

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Abstract: The vegetative growth, photosynthetic, and stomatal characteristics were investigated in *Phalaenopsis* Queen Beer ‘Mantefon’ to determine light’s influence with calcium ammonium nitrate (CAN) levels under 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂. Two lights (150 ± 20 and $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and CAN levels were employed for 40 weeks: calcium, ammonium, and nitrate levels by 0.90, 0.55, and 2.97 mmol·L⁻¹ (CAN1), 8.63, 1.11, and 6.05 mmol·L⁻¹ (CAN2), 12.80, 1.72, and 9.13 mmol·L⁻¹ (CAN3), and 18.80, 2.27, and 12.20 mmol·L⁻¹ (CAN4), respectively. The number of leaves increased in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 compared to control. Plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN4 had the lowest number of leaves among all plants. The time to the mature leaf span decreased in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1. The net CO₂ uptake was higher in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ than those grown at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–3 conditions. The water-use efficiency is higher in the plants grown with CAN1 than those with CAN2–4 at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The maximum stomatal aperture was the largest in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–2 among all plants. Consequently, light levels of $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in *Phalaenopsis* Queen Beer ‘Mantefon’ must be accompanied by nutrient CAN1 to improve photosynthesis and stomatal activity and promote leaf growth under 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ conditions.

Keywords: CO₂ enrichment; cultivation duration; light intensity; nutrient level; orchid

Citation: Cho, A.R.; Chung, S.W.; Kim, Y.J. Shortening the Vegetative Growth Stage of *Phalaenopsis* Queen Beer ‘Mantefon’ by Controlling Light with Calcium Ammonium Nitrate Levels under Enriched CO₂.

Horticulturae **2022**, *8*, 157.
<https://doi.org/10.3390/horticulturae8020157>

Academic Editor: Jiafu Jiang

Received: 10 December 2021

Accepted: 9 February 2022

Published: 11 February 2022

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1. Introduction

Phalaenopsis is a genus of epiphytic orchids found mainly in tropical Asia [1,2]. *Phalaenopsis* Queen Beer ‘Mantefon’ is an essential plant in Asia, especially China, Japan, and Korea, due to its intense red color flower petals and many small flowers in one flower spike. Many *Phalaenopsis* species exhibit an obligate CAM pattern characterized by day/night fluctuations of photosynthetic characteristics [3] and nocturnal CO₂ absorption [2].

The vegetative growth stage, during which a plant is insensitive to conditions that promote floral initiation [4], varies among different plant species. For *Kalanchoe* spp., the period of the vegetative growth stage is 44–55 days [5], whereas the average length of the vegetative growth stage in *Cymbidium* orchid is between 2 and 3 years [6]. Suppose a plant is prematurely exposed to reproductive growth conditions before the end of the vegetative growth stage. In that case, the plant may not support quality flowers and thereby decrease the uniformity of flowering [7]. Vigorous vegetative growth of five leaves and 25 cm leaf span is desirable to induce flowering in *Phalaenopsis* [8]. Meeting the minimum leaf formation is important before floral initiation for plant quality [9].

Elevated heating and cooling often generate a large amount of surplus CO₂. The surplus CO₂ can be used in ornamental greenhouse production by high efficient distributed power generation system [10]. Surplus CO₂ enhanced photosynthetic carbon

assimilation, growth, and flowering of *Phalaenopsis* Queen Beer ‘Mantefon’ in a commercial greenhouse [11]. The number of leaves, leaf length, and leaf width displayed more significant increases when *Phalaenopsis* ‘Fuller’s Pink Swallow’ plants were grown under 1600 and 2400 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 than under 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 [12]. Net CO_2 assimilation rate increased in *Phalaenopsis* Queen Beer ‘Mantefon’ grown under 800 and 1600 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 compared to those grown under 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 [13]. Since *Phalaenopsis* is an ornamental CAM plant, photosynthesis requires 800–1000 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 to reach normal saturation [13,14]. Commercial production of *Phalaenopsis* cultivated at 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 in a greenhouse, resulting in a significant increase in plant growth and yield of good quality flowers.

The photosynthesis of *Phalaenopsis* saturates at a light level of 130–180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [15,16]. Photoinhibition occurs when plants are exposed to a light level higher than 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ than those to a light level below 125 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in *Phalaenopsis amabilis* (L.) Blume [17] at an ambient CO_2 concentration. Naing et al. (2016) [18] suggested the leaf growth rate increased in the plants grown with combined CO_2 enrichment, 1000 $\mu\text{mol}\cdot\text{mol}^{-1}$ and light level, 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Similarly, the combined effect improved seedling growth and yield of chrysanthemum [19]. Cho et al. (2019) [20] observed more floral buds and flowers in *Phalaenopsis* Queen Beer ‘Mantefon’ when plants were grown under a light level of 260 ± 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 than when they were grown under 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 .

Phalaenopsis Tam Butterfly plants fertigated with nitrogen (N) concentration of 200 $\text{mg}\cdot\text{L}^{-1}$ had a wider leaf spread, produced more and larger leaves, and had greater total leaf areas than those with N of 100 $\text{mg}\cdot\text{L}^{-1}$ [21]. Increasing the N concentration from 50 to 200 $\text{mg}\cdot\text{L}^{-1}$ promoted leaf production in white-flowered *Phalaenopsis* hybrid [*P. amabilis* (L.) Blume $\times$ *P. Mount Kaala* ‘Elegance’] [22]. Plant photosynthetic responses under enriched CO_2 conditions require additional nutrients, including N, phosphorus, and potassium [23–25]. Higher production due to CO_2 enrichment increases the nutrient consumption of plants and therefore lowers the salinity level in nutrient solution [26]. Calcium ammonium nitrate (CAN) is a granulated nitrogenous inorganic fertilizer that supplies 25–28% with the lowest carbon footprint of any fertilizer product [27]. Exogenous calcium (Ca) treatment can increase a plant’s tolerance to adverse environments by regulating N metabolism [28]. Limiting nutrients such as Ca deficiency could reduce shoot dry mass and cause abortion of the apical meristem in *Eustoma* cultivars [29]. However, the loss of photosynthetic capacity in *Phalaenopsis* ‘Ney Shan Gu Niang’ grown at enriched CO_2 could be improved by adding N to the nutrient supply in proportion to the relative growth rate of the plant [30,31].

We examined the effects of the combination of light and CAN levels on the vegetative growth under 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 on the leaf growth, photosynthesis, and chlorophyll a fluorescence and stomatal characteristics of *Phalaenopsis* Queen Beer ‘Mantefon’. This study may provide information for using surplus CO_2 in floriculture crop production.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Phalaenopsis Queen Beer ‘Mantefon’ plants were purchased from Orchid Nursery in Paju, Korea (latitude 37° N, longitude 126° E) and transplanted into 10 cm pots (0.5 L container) after 36 weeks of growth. The pots contained 100% Chilean dried *Sphagnum moss* (Lonquen, Ltd., Puerto Montt, Chile). Mean temperature, relative humidity, and solar light levels were 29 ± 2 °C, $70 \pm 10\%$, and 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. The plants had a mean of 3.6 leaves and a leaf span of 18.3 cm (longest opposing leaf to a horizontal position).

Purchased plants were moved to the growth chambers (HB-303DHC, Hanbaek Scientific Co., Ltd., Suwon, Korea) of Seoul Women’s University (Seoul, Korea) on 18 March 2017, acclimated for two weeks before the treatments. Mean day/night temperatures,

relative humidity, and daylength were 29/25 °C, 70% at constant, and 12 h (06:00–18:00 HR), respectively.

Atmospheric CO₂ concentrations of the growth chambers were maintained at an ambient concentration (approximately 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂) from 06:00 to 00:00 HR. The CO₂ concentration was enriched during 00:00–06:00 HR, the last 6 h of the dark period. The 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ was supplied with pure CO₂ from compressed gas cylinders (Bottle CO₂ $\geq$ 99.9%) (Seoul Specialty Gases Co., Ltd., Seoul, Korea) with 50 mL·min^{−1} flow, controlled with an infrared gas analyzer (KCD-HP, Korea Digital, Seoul, Korea) and monitored with a CO₂ analyzer (AM-21A, WISE Sensing Inc., Yongin, Korea). The experiment was conducted from 1 April 2017 to 17 January 2018 (40 weeks).

2.2. The Combination of Light and Calcium Ammonium Nitrogen Levels

The two-factor design was conducted to determine the effects of the pairwise combinations between light and calcium ammonium nitrogen (CAN) with 22 replicates at each treatment. Eight treatments were configured with the two levels of lights, 150 ± 20 and 300 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the four levels of CAN (Table 1) as follows: 150 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 (Control), 150 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN2, 150 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN3, 150 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN4, 300 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1, 300 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN2, 300 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN3, and 300 ± 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN4.

Table 1. The total nitrogen (N) concentration, the elements concentrations of calcium (Ca), ammonium (NH₄), nitrate (NO₃), phosphate (P), potassium (K), and sulfur (S), electrical conductivity (EC), and pH as CAN levels.

Level	Total N (mg·L ^{−1})	Elements (mmol·L ^{−1})						EC (dS·m ^{−1})	pH
		Ca	NH ₄	NO ₃	P	K	S		
CAN1	200	0.90	0.55	2.97	2.74	4.37	0.81	1.6 $\pm$ 0.1	5.9
CAN2	400	8.63	1.11	6.05	2.74	4.37	0.81	2.1 $\pm$ 0.1	6.0
CAN3	600	12.80	1.72	9.13	2.74	4.37	0.81	3.0 $\pm$ 0.1	6.1
CAN4	800	18.80	2.27	12.20	2.74	4.37	0.81	4.9 $\pm$ 0.1	6.1

The light source was three-wave cool white fluorescent lamps (Triband phosphor fluorescent lamps, Dulux L 36 W, Osram Korea Co., Ansan, Korea) and metal halide lamp (MH; GEO–MH 100W–L/P, Geo Lighting, Anseong, Korea), and the photosynthetic photon flux (PPF) was measured using a quantum light meter (3415FSE, Spectrum Technologies Inc., Aurora, IL, USA).

The four CAN levels were adjusted using 0N–52P–35K (Krista MKP, Yara, Oslo, Norway), 12N–61P–0K (Krista MAP, Yara), 11N–0P–0K (Krista MAG, Yara), and 14N–0P–46K (Krista K, Yara). Calcium (Ca), ammonium (NH₄), and nitrate (NO₃) were 0.90, 0.55, and 2.97 mmol·L^{−1} (CAN1); 8.63, 1.11, and 6.05 mmol·L^{−1} (CAN2); 12.80, 1.72, and 9.13 mmol·L^{−1} (CAN3); and 18.80, 2.27, and 12.20 mmol·L^{−1} (CAN4), respectively (Table 1). The levels of total N, electrical conductivity (EC), and pH of each CAN level were also described in Table 1. The EC and pH were measured with a portable EC/pH/TDS meter (HI9811-5, Hanna Instruments, Woonsocket, RI, USA). Each plant was fertigated with 150 mL of nutrient solution weekly during the experimental period.

2.3. Measurements of Leaf Growth

The number of leaves, time to the fifth leaf emergence, and time to the mature leaf span were measured weekly for each plant. The number of leaves with lengths of ≥ 0.5 cm was measured for each plant every four weeks for 40 weeks. The time to the fifth leaf emergence from the start of the treatments, along with the leaf number above 5, was measured. The time to the mature leaf span was when the leaf span reached 25 cm. The leaf growth was measured with 13 replicated plants per treatment.

2.4. Measurements of Gas Exchange and Chlorophyll a Fluorescence

Gas exchanges were measured on the uppermost fully opened mature leaf at 20 weeks after treatments (WAT) using a portable photosynthesis system (Li-6400XT, Li-Cor Inc., Lincoln, NE, USA) equipped with an infrared gas analyzer. The leaf temperature was kept at 29 and 25 °C during the day and night, respectively. Relative humidity in the leaf chamber ranged from 55% to 70%. The CO₂ concentration inside the leaf chamber was maintained at 800 µmol·mol⁻¹ CO₂, which equals the level in the growth chamber. The net CO₂ uptake, water-use efficiency (WUE), stomatal conductance (g_s), and transpiration rate (t_r) were measured during 00:00–03:00 HR. The instantaneous WUE was calculated as $WUE = \text{net CO}_2 \text{ uptake} / t_r$ [32]. Gas exchanges were measured with three replicated plants per treatment.

Pulse-amplitude modulated (PAM) fluorometry has become a powerful tool in the study of plant photosynthesis and is increasingly being used for ecological monitoring in situ [22,33,34]. Chlorophyll a fluorescence was simultaneously measured on the same leaves used to measure gas exchanges using chlorophyll a fluorometer (JUNIOR PAM, Walz, Effeltrich, Germany) at 20 WAT. The maximum photochemical efficiency (F_v/F_m), representing the energy capture efficiency by open photosystem II (PSII), was estimated after 15 min dark adaptation. The actual quantum yield of PSII (Φ_{PSII}), described as the fraction of absorbed light utilized through photochemistry, and non-photochemical quenching (NPQ) were measured after 15 min light adaptation at an actinic PPF of 275 µmol·m⁻²·s⁻¹. Chlorophyll a fluorescence was measured at the middle of the day during 11:00–13:00 HR, considering that Φ_{PSII} of *Phalaenopsis* increased from the sunrise and declined at the end of a day at 12 h photoperiod in a greenhouse [35]. Chlorophyll a fluorescence was measured with three replicated plants per treatment.

2.5. Stomatal Aperture Width, Length, Maximum Stomatal Aperture, and Stomatal Index Measurements

Nail varnish (Shine topcoat, Missha, Korea) was applied to abaxial surfaces of fully expanded leaves, and nail varnish peels were taken from leaves. Cell counts were taken from the broadest area of one leaf, each from at least three replicated plants per treatment. The stomatal aperture width, length, and maximum stomatal apertures were determined by light microscopy (Nikon-YS100, Nikon Co., Tokyo, Japan), using a fitted camera (Smart-adaptor, AT microscope, Gyeonggi-do, Korea), and measured using ImageJ software v. 1.8.0.

The maximum stomatal aperture (a_{max}) was estimated from

$$a_{max} = \frac{\pi \times W_a \times L_a}{4} \quad (1)$$

where W_a is the stomatal aperture width and L_a is the stomatal aperture length.

The stomatal index (SI) was estimated from

$$SI(\%) = \frac{S}{S + E} \times 100 \quad (2)$$

where S is the number of stomata and E is the number of epidermal cells.

2.6. Statistical Analysis

Statistical analyses were performed using the SAS system for version 9.4 (SAS Inst., Inc., Cary, NC, USA). Duncan's multiple range test assessed differences among the treatments at $p \leq 0.05$. The graph module analysis was made by SigmaPlot program version 10.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Leaf Growth Characteristics

The number of leaves on the plants grown at 300 ± 20 µmol·m⁻²·s⁻¹ conditions was higher than that of 150 ± 20 µmol·m⁻²·s⁻¹ at 12–20 WAT, regardless of CAN lev-

els (Figure 1a). The number of leaves was significantly greater in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 than those grown under control conditions at 24–32 WAT. At 36–40 WAT, the number of leaves was the highest on the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ condition with CAN1 than those grown with CAN2–4 conditions. However, the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN4 exhibited the lowest leaves appearing at 36–40 WAT.

Figure 1. The number (No.) of leaves (a), time to the fifth leaf emergence (b), and time to the mature leaf span (c) in *Phalaenopsis* Queen Beer ‘Mantefon’ grown under two light levels of 150 ± 20 and $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with four calcium ammonium nitrate (CAN) level under $800 \mu\text{mol}\cdot\text{mol}^{-1} \text{CO}_2$. The elements concentrations of calcium, ammonium, and nitrate were 0.90 , 0.55 , and $2.97 \text{ mmol}\cdot\text{L}^{-1}$ (CAN1); 8.63 , 1.11 , and $6.05 \text{ mmol}\cdot\text{L}^{-1}$ (CAN2); 12.80 , 1.72 , and $9.13 \text{ mmol}\cdot\text{L}^{-1}$ (CAN3); and 18.80 , 2.27 , and $12.20 \text{ mmol}\cdot\text{L}^{-1}$ (CAN4), respectively. The time to the mature leaf span is based on a 25 cm leaf span emergence time from any leaf initiation. Vertical bars are standard errors of the means ($n = 13$), and values followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

The plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ emerged the fifth leaf within 12 WAT regardless of CAN levels, whereas none of the plants appeared the fifth leaf at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ conditions within 12 WAT (Figure 1b). The time to the mature leaf span was 20.0 , 21.2 , 16.0 , 20.3 , and 21.3 WAT, respectively, in the plants grown under control, at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN2, and at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–3 conditions (Figure 1c). None of the plants had a mature leaf span when they grew at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN3–4 and $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN4 during the experiment (40 weeks) (Figure 1c).

3.2. Photosynthetic Characteristics

The net CO_2 uptake was significantly greater in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–3 conditions than those grown at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–3 (Figure 2a). Maximum net CO_2 uptake, $4.3 \mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, showed in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 condition. The net CO_2 uptake was higher in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–2 than those grown under control conditions. The decrease of WUE at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–3 conditions was more apparent in the plants than in the plants grown at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–3 conditions (Figure 2b). The changes of g_s and t_r did not completely conform to the net CO_2 uptake (Figure 2a,c,d).

Figure 2. The net CO_2 uptake (a), water-use efficiency (WUE) (b), stomatal conductance (g_s) (c), and transpiration rate (t_r) (d) in *Phalaenopsis* Queen Beer 'Mantefon' grown under two light levels of 150 ± 20 and $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with four calcium ammonium nitrate (CAN) levels under $800 \mu\text{mol}\cdot\text{mol}^{-1} \text{CO}_2$. The elements concentrations of calcium, ammonium, and nitrate were $0.90, 0.55$, and $2.97 \text{ mmol}\cdot\text{L}^{-1}$ (CAN1); $8.63, 1.11$, and $6.05 \text{ mmol}\cdot\text{L}^{-1}$ (CAN2); $12.80, 1.72$, and $9.13 \text{ mmol}\cdot\text{L}^{-1}$ (CAN3); and $18.80, 2.27$, and $12.20 \text{ mmol}\cdot\text{L}^{-1}$ (CAN4), respectively. Measurements were taken at 20 weeks after treatment. Vertical bars are standard errors of the means ($n = 3$), and values followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

3.3. Chlorophyll a Fluorescence

The ratios F_v/F_m of dark-adapted samples determine the maximal quantum efficiency of PSII. The values detected on the adaxial surface all appeared to be ranged from 0.617 – 0.715 (Table 2), suggesting that all PSII functioned not normally, respective of the light condition of growth. The plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN4 condition had the lowest F_v/F_m . The Φ_{PSII} represents a measure of the fraction of absorbed light utilized through photochemistry. The Φ_{PSII} was greater in the plants grown at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ than those with $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ treatments, regardless of CAN levels (Table 2). The NPQ is related to the energy dissipation of the excess radiation to heat in the antenna of PSII in the light-adapted state. There were no significant differences in the NPQ among the treatments (Table 2).

Table 2. Chlorophyll a fluorescence parameters of the maximal quantum efficiency (Fv/Fm), actual quantum yield of photosystem II (Φ_{PSII}), and non-photochemical quenching (NPQ) of chlorophyll a fluorescence in *Phalaenopsis* Queen Beer ‘Mantefon’ grown under two light levels of 150 ± 20 and $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with four calcium ammonium nitrate (CAN) levels under $800 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 . The elements concentrations of calcium, ammonium, and nitrate were 0.90, 0.55, and 2.97 $\text{mmol}\cdot\text{L}^{-1}$ (CAN1); 8.63, 1.11, and 6.05 $\text{mmol}\cdot\text{L}^{-1}$ (CAN2); 12.80, 1.72, and 9.13 $\text{mmol}\cdot\text{L}^{-1}$ (CAN3); and 18.80, 2.27, and 12.20 $\text{mmol}\cdot\text{L}^{-1}$ (CAN4), respectively.

Light Levels ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	CAN Levels	Fv/Fm	Φ_{PSII}	NPQ
150 ± 20	CAN1	$0.709 \pm 0.011 \text{ ab}^z$	$0.299 \pm 0.009 \text{ ab}$	$1.101 \pm 0.084 \text{ a}$
	CAN2	$0.715 \pm 0.001 \text{ a}$	$0.330 \pm 0.025 \text{ a}$	$0.896 \pm 0.189 \text{ a}$
	CAN3	$0.697 \pm 0.019 \text{ ab}$	$0.256 \pm 0.016 \text{ bc}$	$1.010 \pm 0.051 \text{ a}$
	CAN4	$0.688 \pm 0.053 \text{ ab}$	$0.251 \pm 0.026 \text{ bc}$	$0.862 \pm 0.089 \text{ a}$
300 ± 20	CAN1	$0.647 \pm 0.015 \text{ ab}$	$0.211 \pm 0.018 \text{ cd}$	$0.835 \pm 0.139 \text{ a}$
	CAN2	$0.673 \pm 0.034 \text{ ab}$	$0.230 \pm 0.017 \text{ cd}$	$0.890 \pm 0.147 \text{ a}$
	CAN3	$0.671 \pm 0.021 \text{ ab}$	$0.233 \pm 0.022 \text{ cd}$	$1.081 \pm 0.032 \text{ a}$
	CAN4	$0.617 \pm 0.030 \text{ b}$	$0.183 \pm 0.024 \text{ d}$	$0.839 \pm 0.274 \text{ a}$
Significance ^y				
Light		*	*	ns
CAN		ns	ns	ns
Light $\times$ CAN		ns	ns	ns

^z Average values in a column with a different letter are significantly different according to Duncan’s multiple range test at $p \leq 0.05$ ($n = 3$). ^y, * denotes significance at $p \leq 0.05$; ns denotes not significant (Duncan’s multiple range test).

3.4. Stomatal Aperture Width, Length, Maximum Stomatal Aperture, and Stomatal Index

The stomatal aperture width and length were promoted in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–2 conditions (Table 3). This significant promotion of average stomatal aperture width and length might maximize the stomatal aperture (Table 3). There were no significant differences in the stomatal index depending upon light levels. The stomatal index decreased in the plants grown at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN2 and those at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN3–4 compared to those under control (Table 3).

Table 3. The stomatal aperture width, length, maximum stomatal aperture, and stomatal index in *Phalaenopsis* Queen Beer ‘Mantefon’ grown under two light levels of 150 ± 20 and $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with four calcium ammonium nitrate (CAN) levels under $800 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 . The elements concentrations of calcium, ammonium, and nitrate were 0.90, 0.55, and 2.97 $\text{mmol}\cdot\text{L}^{-1}$ (CAN1); 8.63, 1.11, and 6.05 $\text{mmol}\cdot\text{L}^{-1}$ (CAN2); 12.80, 1.72, and 9.13 $\text{mmol}\cdot\text{L}^{-1}$ (CAN3); and 18.80, 2.27, and 12.20 $\text{mmol}\cdot\text{L}^{-1}$ (CAN4), respectively.

Light Levels ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	CAN Levels	Stomatal Aperture Width (μm)	Stomatal Aperture Length (μm)	Maximum Stomatal Aperture (μm^2)	Stomatal Index (%)
150 ± 20	CAN1	$3.0 \pm 0.4 \text{ b}^z$	$12.6 \pm 0.6 \text{ bc}$	$27 \pm 5 \text{ b}$	$6.23 \pm 0.15 \text{ a}$
	CAN2	$3.3 \pm 0.3 \text{ b}$	$12.9 \pm 0.4 \text{ bc}$	$29 \pm 3 \text{ b}$	$5.07 \pm 0.22 \text{ c}$
	CAN3	$2.7 \pm 0.4 \text{ bc}$	$14.1 \pm 0.3 \text{ ab}$	$28 \pm 3 \text{ b}$	$5.93 \pm 0.28 \text{ ab}$
	CAN4	$1.9 \pm 0.1 \text{ c}$	$13.6 \pm 0.2 \text{ bc}$	$20 \pm 1 \text{ b}$	$5.91 \pm 0.10 \text{ ab}$
300 ± 20	CAN1	$5.7 \pm 0.3 \text{ a}$	$13.8 \pm 0.5 \text{ ab}$	$58 \pm 5 \text{ a}$	$6.18 \pm 0.26 \text{ a}$
	CAN2	$6.1 \pm 0.5 \text{ a}$	$15.1 \pm 0.5 \text{ a}$	$70 \pm 10 \text{ a}$	$5.25 \pm 0.09 \text{ bc}$
	CAN3	$3.0 \pm 0.1 \text{ b}$	$11.0 \pm 0.8 \text{ d}$	$23 \pm 3 \text{ b}$	$4.03 \pm 0.55 \text{ d}$
	CAN4	$3.1 \pm 0.5 \text{ b}$	$12.1 \pm 0.5 \text{ cd}$	$26 \pm 5 \text{ b}$	$6.08 \pm 0.16 \text{ ab}$
Significance ^y					
Light		***	ns	***	*
CAN		***	*	***	***
Light $\times$ CAN		**	***	***	**

^z Average values in a column with a different letter are significantly different according to Duncan’s multiple range test at $p \leq 0.05$ ($n = 3$). ^y, *, **, and *** denote significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$; ns denotes not significant (Duncan’s multiple range test).

4. Discussion

Sexual reproduction is delayed until plants reach leaf numbers and sizes (leaf span) sufficient to maintain the energetic demands of flowering. Increasing light level with night interruption increased the number of leaves, leaf length, decreased time to flowering, and improved final plant quality in *Cymbidium* ‘Red Fire’ and ‘Yokihi’ [6]. Compared to plants in the control group, those grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ conditions had more significant numbers of leaves at 24–32 WAT, along with decreased time to the fifth leaf emergence and mature leaf span during the vegetative growth stage (Figure 1). Oh et al. (2008) [36] reported that the sufficient plant growth of 8 leaves and 28 flower numbers in *Cyclamen* could be achieved by increasing the net CO_2 uptake with a light level of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Leaf span is increased when either cell expansion or cell division is enhanced [37]. Increased cell division appears to be supported by increased translocate of assimilates and carbohydrate availability in shoot apical meristems [38]. This experiment shows that for *Phalaenopsis*, increasing light level with CAN1 can be considered a method to promote growth rate than the control plants. This may be because *Phalaenopsis* Queen Beer ‘Mantefon’ grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 fixes more large amounts of carbon in photosynthesis than other levels. The net CO_2 uptake in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–3 was 2.5 to 5.5 times greater than those grown at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under enriched CO_2 conditions (Figure 2a).

Monitoring photochemical efficiency of PSII is considered the most sensitive part of the photosynthetic pathway to plant stress [39]. Pulse-amplitude modulated fluorescence measurements have been used to identify changes in the physiological status of plants in response to changing environments before morphological changes are evident [33]. In non-stressed plants, the F_v/F_m is about 0.77–0.81 for most plant species [40]. When plants are exposed to stressful conditions, the values of the F_v/F_m drop significantly [41]. Decrease of the F_v/F_m and Φ_{PSII} has indicated either down-regulation or recovery of PSII and photoinhibition [32], which adjustments in photochemical capacity can cause the thermal dissipation capacity or both. Decrease in the F_v/F_m due to diurnal photo-inhibition may improve more rapidly in many environments as light levels and CO_2 concentration, and nitrogen [42]. Plants are adapted to environments by allocating excessive energy and increasing energy dissipation through the NPQ, which may serve as a photo-protection response [43]. This might be related to the fact that our study site $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN4 level exceeded the tolerance threshold levels (Table 2), resulting in a decrease in the photochemical efficiency of *Phalaenopsis* Queen Beer ‘Mantefon’ under $800 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 .

In commercial production, the ability to schedule potted *Phalaenopsis* to flower during periods of high energy cost is desirable because it allows precise ornamental plants’ scheduling and improves production efficiency. CO_2 enrichment allows *Phalaenopsis* to continue to initiate leaf. Enriched CO_2 of $800\text{--}1600 \mu\text{mol}\cdot\text{mol}^{-1}$ promoted the leaf growth and net CO_2 uptake in many *Phalaenopsis*, including ‘Fuller’s Pink Swallow’ [12], ‘Ney Shan Gu Niang’ [30], and Queen Beer ‘Mantefon’ [13,14]. According to Lin and Tsu (2004) [17], the threshold light level for photosynthesis and starch accumulation in *Phalaenopsis amabilis* (L.) Blume was $< 200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Only the plants were flowered when they were grown at $260 \pm 40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ compared to that grown at $90 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under CO_2 of $800 \mu\text{mol}\cdot\text{mol}^{-1}$ conditions in *Phalaenopsis* Queen Beer ‘Mantefon’ [20]. In the present study, leaf emergence is fastened in the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ along with $800 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 compared to that grown at $150 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, regardless of CAN level (Figure 1b). Shortening the fifth leaf emergence can be promoted flowering [8].

Stomatal morphogenesis and behavior are controlled by genetic and environmental factors such as light [44,45] and CO_2 enrichment [46]. The status of stomata governs the overall net CO_2 uptake and WUE from plants [45]. The fine regulation of opening and closure of stomata in response to light and nutrients is crucial to ornamental crop production. The increase of stomatal aperture length and width and maximum stomatal aperture indicated a rise in overall stomatal pore area through the regulations of membrane

transport of guard cells [45]. The stomatal index represents the ratio of the number of stomata to the number of stomata and epidermal cells per unit leaf area, serving as a sensitive parameter detecting stomatal frequency changes [47]. Increasing light levels from 90 to 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased tobacco leaf stomatal aperture length and stomatal index. An increase in the stomatal aperture with increasing light level from 10% sunlight to full sunlight has also been previously noted in two *Eucalyptus globulus* ssp. [48]. The larger stomata in 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provide higher CO_2 intake and enhanced leaf cooling through transpiration water loss [49]. The plants have adapted to certain light levels by maintaining low or high stomatal index [45]. Here, *Phalaenopsis* Queen Beer ‘Mantefon’ stomata showed an increased response to the light level of $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1–2 by significantly increasing the stomatal aperture width and length (Table 3). Those findings indicated that the stomatal aperture might be a critical determinant for photosynthesis and *Phalaenopsis* Queen Beer ‘Mantefon’ leaf growth.

The enriched CO_2 increased plant growth only when there was high nutrient availability [23,24]. Adding Ca fertilizer can promote plant growth and significantly increase plant yield in tomatoes [50] and cucumber and melon [51]. This may be connected with the findings that Ca promotes N’s absorption by plants, increases NO_3 enzyme activity in the leaf, and enhances plant photosynthetic capacity [50,52]. But ^{15}N tracer of N results confirmed that rising of Ca levels forms antagonistic effects under high levels, affecting N absorption, distribution, or utilization in plants, and finally influencing the plant growth of apples [52]. Ca and N added into irrigation water can significantly improve the plant growth, fruit yield, and membrane permeability affected by high salinity and can also correct both Ca and N deficiencies in tomato and pepper fruit [53]. However, the lowest ^{15}N absorption was observed in Ca-free treatment, and the highest uptake was under 6 $\text{mmol}\cdot\text{L}^{-1}$ Ca supply [52]. In the present study, the WUE of the plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 condition was approximately 41–73% greater than those grown with CAN2–4 conditions at the same light level (Figure 2b). Water use decreased when saline water was used [53]. Sonneveld and Voogt (1999) [51] have reported that the WUE in tomatoes was reduced with high NaCl. A moderate Ca fertilizer supply of CAN1 consisting of 0.90 $\text{mmol}\cdot\text{L}^{-1}$ is crucial to ensure better growth and improve N absorption in the plants.

5. Conclusions

Plants grown at $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 should have vigorous vegetative growth that will rapidly attain the minimum leaf size required for flowering and have leaves greater in number than those grown under control conditions and all the plants grown with CAN2–4. In this study, $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 condition increased *Phalaenopsis* net CO_2 uptake and maximum stomatal aperture. This data suggest that crop time can be decreased when the light level of $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with CAN1 consisting of 0.90 $\text{mmol}\cdot\text{L}^{-1}$ Ca, 0.55 $\text{mmol}\cdot\text{L}^{-1}$ NH_4 , and 2.97 $\text{mmol}\cdot\text{L}^{-1}$ NO_3 for *Phalaenopsis* cultivation than the control conditions. Consequently, light levels of $300 \pm 20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in *Phalaenopsis* Queen Beer ‘Mantefon’ must be accompanied by nutrient CAN1 to improve the photosynthesis and stomatal activity and promote the leaf growth under 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 condition.

Author Contributions: Conceptualization, A.R.C. and Y.J.K.; methodology, A.R.C., S.W.C. and Y.J.K.; validation, A.R.C., S.W.C. and Y.J.K.; formal analysis, A.R.C. and S.W.C.; investigation, A.R.C.; writing—original draft preparation, A.R.C., S.W.C. and Y.J.K.; writing—review and editing, S.W.C. and Y.J.K.; visualization, A.R.C. and Y.J.K.; supervision, Y.J.K.; project administration, Y.J.K.; funding acquisition, Y.J.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research Foundation of Korea, grant number “NRF-2018R1A2B6007834” and Seoul Women’s University “2021–0104”.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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