

Article

Carbon Dioxide Enrichment Combined with Supplemental Light Improve Growth and Quality of Plug Seedlings of Astragalus membranaceus Bunge and Codonopsis lanceolata Benth. et Hook. f.

Ya Liu¹, Xiuxia Ren¹ and Byoung Ryong Jeong ^{1,2,3,*}

- ¹ Department of Horticulture, Division of Applied Life Science (BK21 Plus Program), Graduate School of Gyeongsang National University, Jinju 52828, Korea; liuya113@mails.ucas.ac.cn (Y.L.); renxiuxia@caas.cn (X.R.)
- ² Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea
- ³ Research Institute of Life Science, Gyeongsang National University, Jinju 52828, Korea
- * Correspondence: brjeong@gnu.ac.kr; Tel.: +82-0106-751-5489

Received: 29 September 2019; Accepted: 4 November 2019; Published: 5 November 2019



Abstract: Astragalus membranaceus Bunge and Codonopsis lanceolata Benth. et Hook. f. are two medicinal species used to remedy inflammation, tumor, and obesity in Eastern medicine. Carbon dioxide (CO_2) and supplemental lighting are two methods to enhance the growth, yield, and quality of crops. However, few studies have focused on the synergistic effects of CO_2 and the supplemental light source on plug seedlings of medicinal species. In this study, uniform seedlings were grown with no supplemental light (the control) or under one of three supplemental light sources [high pressure sodium (HPS), metal halide (MH), or mixed light-emitting diodes (LEDs)] combined with one of three levels of CO₂ (350, 700, or 1050 μ mol·mol⁻¹). The supplemental light (100 μ mol·m⁻²·s⁻¹ photosynthetic photon flux density) and CO₂ were provided simultaneously from 10:00 pm to 2:00 am every day. The results showed that the supplemental lighting (LEDs, MH, and HPS) greatly improved the seedling quality with greater dry weights (of the shoot, root, and leaf), stem diameter, leaf area, and Dickson's quality index (DQI) than those of the control in both species. An enriched CO_2 at 1050 µmol·mol⁻¹ accelerated the growth and development of plug seedlings, evidenced by the increased root and leaf dry weights, stem diameter, and DQI compared to the those from the other two CO₂ enrichment levels. Moreover, LEDs combined with 1050 μ mol·mol⁻¹ CO₂ not only increased the contents of soluble sugars but also the starch content. However, an enriched CO_2 at $700 \ \mu mol \cdot mol^{-1}$ was more suitable for the accumulation of total phenols and flavonoids. Furthermore, LEDs combined with 700 or 1050 μ mol·mol⁻¹ CO₂ increased the chlorophyll, quantum yield, and stomatal conductance at daytime and nighttime for A. membranaceus and C. lanceolata, respectively. In conclusion, the data suggest that LEDs combined with CO_2 at 1050 μ mol·mol⁻¹ is recommended for enhancing the growth and development of plug seedlings of A. membranaceus and C. lanceolata.

Keywords: carbon dioxide; growth; metabolite; quality; quantum yield; stomata; supplemental lighting

1. Introduction

Astragalus membranaceus Bunge and *Codonopsis lanceolata* Benth. et Hook. f. are two important medicinal herbs, mainly distributed in Asian countries, especially in Korea, China, and Japan [1,2]. In ancient China, those two species were used as the medicinal material for treating physiological disorders and illnesses, including inflammation, tumor, and obesity [3,4]. In addition to its medicinal



2 of 17

values, *C. lanceolata* has been a high-class vegetable consumed in Asian countries, especially in ancient Korea [5,6]. In recent years, more and more phytochemicals with positive effects on remedying diseases have been found in these two species [7,8]. However, most studies have focused on the separation, extraction, and characteristics of the phytochemicals in *A. membranaceus* and *C. lanceolata*. Not much research has focused on the cultivation of those two species to obtain high-quality plug seedlings in a glasshouse.

As a substrate of photosynthesis, carbon dioxide (CO_2) has a remarkable influence on the plant growth, yield, and quality [9–11]. Within a certain concentration, an enrichment in the CO_2 concentration leads to an increase in the intercellular CO_2 concentration, which leads to higher quality and yield in crops, as observed in rice [12], wheat [13], and maize [14]. However, excess CO_2 levels were also shown to have a negative effect on photosynthesis and yield [15,16], partly because of a lower activation of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) or a decreased enzyme content [17,18]. Therefore, providing a suitable concentration of CO_2 for specific species is the primary focus in the practice of CO_2 fertilization.

It is well known that photosynthesis is a chemical reaction driven by light. In artificial conditions, including those in a glasshouse, light insufficiency is common, resulting in a degradation of quality and a loss in yield [19,20]. Therefore, supplemental lighting has been employed in horticulture to improve crop growth, yield, and quality [21–23]. This is usually carried out at night, since the electricity price per unit kilowatt and energy cost can be lower with off-peak time-of-use (TOU). More importantly, a study reported that supplemental lighting during nighttime is more effective to promote plant growth and yield as compared with daytime both in summer and winter [23]. Furthermore, night-interruption not only increase leaf biomass and crop yield but also result in more compact and healthier plants [24–26]. In commercial production, supplemental light intensity is usually between 100 and 200 μ mol·m⁻²·s⁻¹ photosynthetic photon flux density (PPFD) [27,28]. Previous studies have reported the synergistic effects of CO₂ and supplemental light intensity [10,29,30]. Moreover, the effects of CO₂ and temperature on the plant growth and development were also demonstrated [12,29,31]. However, research on the synergistic effects of CO₂ and supplemental lighting source was insufficient, especially in medicinal plants.

As direct or indirect photosynthetic products, primary and secondary metabolites are strongly determined by photosynthesis [27]. Previous studies have proven that light intensity [32], photoperiod [33], temperature [34], and CO_2 [16,35] affect the biosynthesis and accumulation of primary and secondary metabolites such as sugar, starch, total phenols and flavonoids. However, more studies should be carried out to clarify how CO_2 and supplemental lighting source affect primary and secondary metabolites in medicinal plants. As valuable phytochemicals, total phenols and flavonoids have the potential to scavenge reactive oxygen species and protect human against diseases and physiological disorders [8,36]. Thus, contents of total phenols and flavonoids are necessary parameters to evaluate the medicinal quality. Therefore, more research is needed to provide the ideal supplemental CO_2 and lighting condition for medicinal plants.

High-quality plug seedlings are the primary objective of commercial production since a high quality means a high income and interest for growers. Previous study has reported a high quality of seedling defined by growth parameters, such as high dry matter, low shoot to root ratio, and high compactness [37,38]. However, no agreement has been achieved, since no single trait can evaluate plug-seedling quality well [39]. Recently, the Dickson's quality index (DQI) has been regarded as a promising indicator of plug-seedling quality [39,40]. As a parameter integrating the aspects of total biomass, the sturdiness quotient, and the ratio of stem diameter to shoot height, DQI explains overall plant potential for survival and growth in the field. Increasing DQI suggests the growing potential of plug seedlings for field establishment success after transplanting [39,40]. Therefore, in this study, the DQI was used as the most important parameter to evaluate the quality of plug seedlings, followed by biomass and stem diameter.

In this study, it was hypothesized that an enrichment of CO_2 and appropriate supplemental lighting could enhance the quality of plug seedlings of *A. membranaceus* and *C. lanceolata*. In order to test this hypothesis, uniform plug seedlings were cultured under no supplemental lighting (the control) or under one of three supplemental light sources [high pressure sodium (HPS), metal halide (MH), or mixed light-emitting diodes (LEDs)] combined with one of three levels of CO_2 enrichment (350, 700, or 1050 µmol·mol⁻¹). The growth, development and morphological characteristics of plug seedlings affected by the CO_2 enrichment and supplemental light source were investigated. Moreover, the contents of the primary and secondary metabolites such as soluble sugars, starch, total phenols, and flavonoids, were also determined. Furthermore, photosynthesis-related parameters were evaluated, such as the chlorophyll content, quantum yield, and stomatal conductance. These data could provide a theoretical and practical basis for improving the plug-seedling quality in *A. membranaceus* and *C. lanceolata*, and serve as useful information for growers to manage other medicinal plants.

2. Materials and Methods

2.1. Plant Materials and Treatments

Seeds of A. membranaceus and C. lanceolata were sown in 200-cell plug trays filled with the BioPlug Medium (FarmHannong Co. Ltd., Seoul, South Korea). After germination, uniform plug seedlings (720 seedlings) were selected as the plant material and then cultured under no supplemental light (the control) or under one of three supplemental light sources [high pressure sodium (HPS, BLV Licht- und Vakuumtechnik, Steinhöring, Germany), metal halide (MH, SunLumen Lighting Co. Ltd., Gyeongju, South Korea), or mixed light-emitting diodes (LEDs, red: blue 6:1, FL300, Senmatic A/S, Søndersø, Denmark) combined with one of three levels of CO₂ enrichment (350, 700, or 1050 μ mol·mol⁻¹). The supplemental light intensity used in this study was set at 100 µmol·m⁻²·s⁻¹ photosynthetic photon flux density (PPFD) (mean with five places) by using a portable photo/radiometer (HD-2102.2, Delta OHM, Padova, Italy), and the lighting duration (also the CO₂ enrichment time) was 4 h from 10:00 pm to 2:00 am every day. The CO₂ concentration during daytime is ambient level (350 μ mol mol⁻¹) among all treatments. The ambient level was chosen as control, and 2- and 3-fold increase were chosen as enriched concentration, according to previous study [41–43]. It was monitored and controlled by a crop-management system (SH-MV510, SOHA TECH Co, Ltd., Seoul, Korea). This experiment was carried out in Jinju, Gyeongsangnam-do, Korea, from 3 February to 2 March 2019. The mean day length is around 11 h, ranged from 10 h 32 min to 11 h 27 min during experiment. The photosynthetic light period is 15 h with approximate 4 h darkness before lighting treatments. The average daily maximum light intensity coming from the sunlight was about 568.9 μ mol·m⁻²·s⁻¹ PPFD. The culture environment had set points of 23/17 °C (day/night) temperatures and (70 ± 5)% relative humidity in a controlled environment inside a glasshouse. The plug seedlings were fertilized once two days with a multipurpose greenhouse nutrient solution [in mg·L⁻¹ Ca(NO₃)₂·4H₂O 737.0, KNO₃ 343.4, KH₂PO₄ 163.2, K₂SO₄ 43.5, MgSO₄·H₂O 246.0, NH₄NO₃ 80.0, Fe-EDTA 15.0, H₃BO₃ 1.40, NaMoO₄·2H₂O 0.12, MnSO₄·4H₂O 2.10, and ZnSO₄·7H₂O 0.44 (pH 6.5 and electrical conductivity 1.5 mS·cm⁻¹)] throughout the experiment [44]. The experimental design was of completely random design with three replications. Each replication included 20 plug seedlings (total 60 seedlings per treatment). After four weeks' cultivation, five plug seedlings were harvested at 10 am for measurements of the growth parameters and the others (55 plants per treatment) were frozen immediately in liquid nitrogen for further analysis. For measurements of the growth parameters, the whole plants were harvested and the roots were washed carefully with tap water. Growth parameters, such as fresh weight, length, and stem diameter, were measured directly whereas dry weight of shoot, root, and leaf were measure after 24 h drying at 60 °C in an oven. The Dickson's quality index was calculated according to a previous formula [45]. The formula is:

Dickson's quality index = Total DW/((shoot length/stem diameter) + (shoot DW/root DW)), (1)

where the DW means dry weight.

2.2. Contents of Soluble Sugar and Starch

The contents of soluble sugar and starch were measured according to the anthrone colorimetric method [46,47]. In brief, mixed samples (200 mg) of leaves were ground and homogenized in distilled water (14 mL), followed by extraction for 30 min at 100 °C. The solution was centrifuged at 3000 rpm for 15 min, and the supernatant was transferred into a new tube for the measurement of soluble sugars. The residue was collected and extracted in distilled water mixed with perchloric acid (2 mL, 52%) for the measurement of starch. The supernatant (0.5 mL) was mixed with distilled water (1.9 mL), anthrone (0.5 mL, 2%), and concentrated sulfuric acid (5 mL, 98%), and incubated for 15 min at 100 °C. The absorbance was recorded at 630 nm and 485 nm, respectively, by using a ultraviolet (UV)-spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK).

2.3. Contents of Total Phenols and Flavonoids

The contents of total phenols and flavonoids in leaves were measured according to the previous methods described by Manivannan et al. [36]. Briefly, total phenols and flavonoids were extracted with 80% methanol. For the assay of total phenols, the extract solution (100 μ L) was mixed with distilled water (900 μ L), phenol reagent (500 μ L, 1:1 water), and sodium carbonate (2.5%, 1 mL). The mixed solution was incubated for 40 min in dark, followed by recording the absorbance at 765 nm. For the assay of the total flavonoids, the extract solution (100 μ L) was mixed with methyl alcohol (80%, 900 μ L) and aluminum chloride (2%, 1 mL). After incubation for 30 min, the absorbance at 415 nm was recorded by using a UV-spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK). The total phenols and flavonoids were calculated from the standard gallic acid and quercetin calibration curve, respectively.

2.4. Assessment of the Chlorophyll Content, Quantum Yield, and Stomatal Conductance

The chlorophyll content was measured by using a Plus Chlorophyll Meter (SPAD 502, Konica Minolta Sensing Inc., Osaka, Japan). The quantum yield was determined with a FluorPen FP 100 (Photon Systems Instruments, PSI, Drásov, Czech Republic). The dark acclimatization (15 min) was conducted before taking measurements, according to the introduction for this instrument. The stomatal conductance was assessed at 10 am during daytime and at 12 am during nighttime, respectively, by using a Decagon Leaf Porometer SC-1 (Decagon Device Inc., Pullman, WA, USA).

2.5. Data Collection and Analysis

Data were collected and reported as the mean \pm standard error (n = 5 for growth parameters and n = 3 for other parametes). All data were processed and analyzed using the Statistical Package for the Social Sciences version 21 (SPSS Inc., Chicago, IL, USA). A two-way analysis of variance (two-way ANOVA) was performed to evaluate the significant differences among treatments, followed by a Duncan's multiple range test (p < 0.05). All figures were made with the OriginPro software version 9.0 (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Growth, Development, and Morphology

Plants grown with supplemental lighting had significantly higher shoot and root dry weights, stem diameter, leaf area, and Dickson's quality index (DQI), in comparison with plants grown without supplemental lighting (Figure 1, Tables 1 and 2). As an important parameter, DQI is a good indicator of seedling quality since changes in the strength and balance of the distribution of biomass are assessed, and several other important parameters are used to assess the quality [48]. Therefore, a significantly lower value of DQI under no supplemental lighting (Con) suggested any supplemental lighting greatly upgraded the quality of plus seedlings of *A. membranaceus* and *C. lanceolata*. Similarly, Lanoue et al. [49]

reported that the biomass production of tomato (Solanum lycopersicum L.) and lisianthus (Eustoma grandiflorum (Raf.) Shinners) were increased by all the supplemental light conditions than in the control. The enhancement of seedling quality is not only duo to supplemental lighting but also duo to night interruption. Similarly, Cao et al. [24] reported that night break not only increased stemdiameter, leaf number, and fruit fresh weight, but also resulted in more compact and healthier plant of tomato. Yoneda et al. [25] also found night interruption treatments (4 h) combined with 8 h photoperiod increased leaf biomass of tomato to amounts observed under LD conditions. Among the supplemental light sources, LEDs showed the greatest promotion of stem diameter and DQI in *C. lanceolata* plug seedlings, especially when combined with CO_2 at 1050 µmol·mol⁻¹, followed by MH and HPS, implying more compact and sturdy plug seedlings. Additionally, in A. membranaceus plug seedlings, the root dry weight is higher in LEDs combined with CO_2 at 1050 μ mol·mol⁻¹ than other treatments, while the stem diameter and DQI was increased by LEDs combined with CO₂ at 1050 µmol·mol⁻¹, only following the LEDs combined with 700 μ mol·mol⁻¹ CO₂. The results were in agreement with those of Park et al. [41] who reported that a mixture of red, blue and white (RBW, 8:1:1) LEDs promoted the vegetative growth of the shoot and root in lettuce (Lactuca sativa L.), compared to treatments with fluorescent lamps. Similar, Randall et al. [50] reported that DQI for Petunia, Salvia, and Violaw was significantly higher under LEDs than under HPS lamps. As a promising light source, LEDs can provide a narrow band of red and blue light, contributing to a high photosynthetic rate, photosynthetic products, yield, and quality [23,51]. Red and blue are two important spectral ranges, which can be absorbed efficiently by two photoreceptors, phytochromes and cryptochromes, respectively. Although plants can also absorb yellow and green light, the efficiency is pretty low, since there is no specific photoreceptor for those two kinds of light. Red and blue light greatly enhance photosynthesis as compared with others. Moreover, blue light can enhance the compactness by inhibiting the elongation of shoots. Compared with the LED used in this study, both MH and HPS possess a high ratio of yellow and green light which is not suited for improving photosynthesis and compactness.

	Light	Length (cm)		Dry Weight (mg)			Leaf Area	Stem	Dickson's
	(L)	Shoot	Root	Shoot	Root	Leaf	(cm ⁻²)	(mm)	Quality Index (×10 ⁻⁴)
	Con	8.4 ± 0.5 e z	5.0 ± 0.4 b	39.1 ± 2.5 e	6.4 ± 0.8 e	$8.2 \pm 0.6 \text{ g}$	$4.8\pm0.3~e^{~z}$	$0.99 \pm 0.02 \text{ d}$	$5.1 \pm 0.3 d$
250	LED	14.9 ± 0.6 a	$4.9 \pm 0.4 \text{ b}$	105.5 ± 9.6 bc	15.8 ± 1.6 ad	17.4 ± 1.6 be	7.9 ± 0.5 bd	1.37 ± 0.06 bc	$10.5 \pm 1.0 \text{ bc}$
350	MH	$14.7 \pm 0.5 \text{ ab}$	$4.7 \pm 0.3 \text{ b}$	103.8 ± 11.3 bc	15.8 ± 2.6 ad	$16.9 \pm 1.6 \text{bf}$	$8.0 \pm 0.5 \text{ bd}$	1.32 ± 0.09 bc	$10.4 \pm 1.8 \text{ bc}$
	HPS	$13.1\pm0.5~{\rm c}$	$4.7\pm0.3~b$	$84.6 \pm 8.0 \text{ cd}$	$11.4\pm1.4~\mathrm{ce}$	$14.1\pm1.8~df$	$7.6 \pm 0.6 \text{ bd}$	$1.25\pm0.07~{\rm c}$	$8.7 \pm 1.0 \text{ cd}$
-	Con	$10.7 \pm 0.3 d$	4.5 ± 0.3 b	$73.2 \pm 7.2 \text{ d}$	9.5 ± 1.8 de	11.8 ± 1.2 fg	6.7 ± 0.4 cd	1.24 ± 0.04 c	$8.8 \pm 1.1 \text{ cd}$
	LED	13.3 ± 0.3 bc	$4.1 \pm 0.2 \text{ b}$	134.8 ± 9.0 a	$17.0 \pm 3.7 \text{ ac}$	25.1 ± 2.0 a	$10.2 \pm 0.6 a$	1.62 ± 0.06 a	16.0 ± 1.7 a
700	MH	$13.3 \pm 0.5 \text{ bc}$	5.9 ± 0.4 a	$108.2 \pm 7.4 \text{ bc}$	16.4 ± 1.4 ac	19.3 ± 2.2 bd	9.3 ± 0.9 ab	$1.38 \pm 0.04 \text{ bc}$	12.1 ± 0.8 a-c
	HPS	13.4 ± 0.2 bc	$4.1\pm0.2~b$	$98.3\pm10.2~bd$	15.0 ± 2.2 ad	$16.9\pm2.5~bf$	$6.5 \pm 0.4 \text{ d}$	$1.28\pm0.06~c$	$10.4\pm1.4~bc$
	Con	12.2 ± 0.6 c	4.9 ± 0.2 b	76.8 ± 6.2 d	12.8 ± 1.2 be	13.5 ± 0.9 ef	7.3 ± 0.4 cd	1.23 ± 0.06 c	8.7 ± 1.2 cd
1050	LED	$12.7 \pm 0.7 c$	$4.5 \pm 0.2 \text{ b}$	111.2 ± 10.0 ac	20.4 ± 3.1 a	20.4 ± 1.9 ac	$8.2 \pm 0.5 \text{ b-d}$	$1.48 \pm 0.06 \text{ ab}$	15.5 ± 1.5 a
1050	MH	13.6 ± 0.3 ac	$4.0 \pm 0.2 \text{ b}$	121.4 ± 11.3 ab	$18.9 \pm 2.2 \text{ ab}$	21.4 ± 1.4 ab	$8.4 \pm 0.6 bc$	$1.48 \pm 0.10 \text{ ab}$	14.6 ± 2.3 ab
	HPS	$12.7\pm0.7~{\rm c}$	$4.1\pm0.4~b$	96.6 ± 5.6 bd	14.1 ± 1.1 ad	16.1 ± 0.9 cf	6.8 ± 0.3 cd	$1.30\pm0.03~bc$	$10.8\pm0.8~bc$
	С	NS	NS	**	**	**	*	**	***
F-test	L	***	NS	***	***	***	***	***	***
	C×L	***	***	NS	NS	NS	**	**	NS

Table 1. Effect of CO₂ and supplemental light source on the growth parameters of *A. membranaceus* plug seedlings.

^z Different letters indicate significant separation within columns by Duncan's multiple range test at a 0.05 level. NS, *, **, and ***, represent no significant or significant difference at p < 0.05, 0.01, or 0.001, respectively. Data were presented as the mean \pm standard error (n = 6). Con, control without supplemental lighting; LED, mixed light-emitting diodes; MH, metal halite; and HPS, high pressure sodium.

Moreover, a consistent trend between the CO_2 level and growth parameters was found under the same light source. An enriched CO_2 at 1050 µmol·mol⁻¹ accelerated the growth and development of plug seedlings, evidenced by the increased root and leaf dry weights, stem diameter, and DQI than those of plants grown with the other two levels of CO_2 (Tables 1 and 2). Wohlfahrt et al. [52] also found that the fresh biomass of grapevines were stimulated by elevated CO_2 . Therefore, among all treatments, LEDs combined with CO_2 at 1050 µmol·mol⁻¹ is recommended for the growth and development of *A. membranaceus* and *C. lanceolata* plug seedlings, when considering root biomass, stem diameter, and

DQI. This result is similar to that of a previous study that found that a combination of RBW LEDs and the highest concentration of CO_2 (1000 µmol·mol⁻¹) was the optimal condition for lettuce growth [41].

Table 2. Effect of CO_2 and supplemental light source on the growth parameters of *C. lanceolata* plug seedlings.

CO ₂ (C)	Light (L)	Length(cm)		Dry Weight (mg)			Leaf Area	Stem	Dickson's
		Shoot	Root	Shoot	Root	Leaf	(cm ⁻²)	(mm)	Quality Index $(\times 10^{-4})$
	Con	10.50.4 bd ^z	$4.4 \pm 0.3 a$	$30.6 \pm 4.7 \text{ f}$	$6.1 \pm 1.2 \text{ e}$	$5.6 \pm 0.4 d$	$4.6 \pm 0.2 \text{ f}$	$1.56 \pm 0.09 \; f$	$5.1 \pm 0.9 \text{ e}$
250	LED	11.3 ± 0.3 ac	$3.8 \pm 0.4 \text{ ab}$	52.9 ± 4.3 bd	$12.0 \pm 2.8 \text{ cd}$	8.9 ± 0.8 bc	7.2 ± 0.6 ac	$2.01 \pm 0.08 \text{ bc}$	10.8 ± 1.9 bc
350	MH	$10.0 \pm 0.6 d$	$3.0 \pm 0.3 \text{ b}$	49.1 ± 4.9 ce	12.5 ± 2.5 bd	8.2 ± 0.7 bc	$5.6 \pm 0.4 df$	1.69 ± 0.06 ef	9.7 ± 1.1 bd
	HPS	$10.5\pm0.3bd$	3.7 ± 0.2 ab	$46.5\pm2.2~de$	$12.8\pm1.4~bd$	$8.3\pm0.6bc$	7.0 ± 0.7 ad	$1.99\pm0.07~\rm{bc}$	$9.9 \pm 0.8 \text{ bd}$
	Con	10.2 ± 0.3 cd	4.4 ± 0.4 a	37.2 ± 2.2 ef	9.1 ± 0.7 de	$6.5 \pm 0.5 \text{ cd}$	$5.4 \pm 0.5 \text{ ef}$	1.73 ± 0.04 df	7.3 ± 0.5 ce
700	LED	$12.0 \pm 0.2 a$	$4.2 \pm 0.2 a$	61.9 ± 6.7 bc	16.2 ± 1.9 ac	9.7 ± 1.2 b	$7.3 \pm 0.5 \text{ ab}$	$2.04 \pm 0.04 \text{ b}$	$12.5 \pm 1.4 \text{ b}$
700	MH	11.0 ± 0.5 ad	$4.0 \pm 0.2 a$	$63.4 \pm 3.8 \text{ b}$	15.6 ± 1.8 ac	9.8 ± 0.4 b	7.1 ± 0.4 ad	1.81 ± 0.06 ce	$12.3 \pm 1.2 b$
	HPS	$11.6 \pm 0.5 \text{ ab}$	4.5 ± 0.4 a	$53.7 \pm 4.5 \text{ bd}$	$13.8 \pm 2.3 \text{ bd}$	7.5 ± 0.6 bd	5.7 ± 0.4 bf	$1.89\pm0.05~\mathrm{be}$	$10.2 \pm 0.7 \text{ bd}$
	Con	11.0 ± 0.5 ad	4.5 ± 0.3 a	36.5 ± 3.2 ef	10.4 ± 1.2 ce	$6.6 \pm 0.6 \text{ cd}$	5.7 ± 0.4 bf	$1.70 \pm 0.03 \text{ ef}$	6.8 ± 0.6 de
1050	LED	$12.2 \pm 0.2 a$	4.4 ± 0.3 a	80.3 ± 6.8 a	20.5 ± 1.4 a	12.1 ± 1.4 a	7.7 ± 0.9 a	2.30 ± 0.10 a	17.9 ± 2.1 a
1030	MH	$11.5 \pm 0.2 \text{ ab}$	4.1 ± 0.4 a	$61.7 \pm 4.6 \text{ bc}$	$18.3 \pm 2.5 \text{ ab}$	$9.5 \pm 0.7 \text{ b}$	6.7 ± 0.3 ae	$1.96 \pm 0.04 \text{ bc}$	$12.8 \pm 1.1 \text{ b}$
	HPS	$10.6 \pm 0.5 \text{ bd}$	4.5 ± 0.1 a	$45.1 \pm 2.8 \text{ de}$	15.0 ± 1.3 ad	6.7 ± 0.5 cd	5.6 ± 0.4 cf	1.90 ± 0.03 bd	$10.2 \pm 0.6 \text{ bd}$
	С	*	**	**	***	NS	NS	**	**
F-test	L	**	*	***	***	***	***	***	***
	C×L	NS	NS	*	NS	NS	NS	NS	NS

² Different letters indicate significant separation within columns by Duncan's multiple range test at a 0.05 level. NS, *, **, and ***, represent no significant or significant difference at p < 0.05, 0.01, or 0.001, respectively. Data were presented as the mean ± standard error (n = 5). Con, control without supplemental lighting; LED, mixed light-emitting diodes; MH, metal halite; and HPS, high pressure sodium.





Figure 1. Morphological characteristics of plug seedlings of *A. membranaceus* (**A**) and *C. lanceolata* (**B**) affected by the carbon dioxide (CO_2) and supplemental light source after four weeks of cultivation. Con, control without supplemental lighting; LED, mixed light-emitting diodes; MH, metal halite; and HPS, high-pressure sodium. Bar, 10 cm.

3.2. Contents of Soluble Sugar and Starch

Species

A. membranaceus

C. la

Factor

 $CO_2(C)$

Light(L)

Supplemental lighting including LEDs, MH, and HPS, increased the accumulation of soluble sugar in both species, compared to the control (Figure 2A,B, Table 3). Moreover, under the same lighting condition, the soluble sugar content presented an increasing trend with elevated CO_2 level in C. lanceolata plug seedlings, whereas it was observed to increase first and then decrease in A. membranaceus plug seedlings (except under LEDs). Analysis of variance showed significant interactions between supplemental lighting and CO₂ for soluble sugar in A. membranaceus (Table 3). This was in agreement with a report [10] that the interaction of supplementary light and CO_2 enrichment improves nutritional quality significantly in tomato. In details, under 350 or 1050 µmol·mol⁻¹ CO₂, LED largely increased the content of soluble sugar, followed by MH or HPS. However, HPS enhanced soluble sugar concentration more than two others did under 700 μ mol·mol⁻¹ CO₂ (Figure 2). The highest values of the soluble sugar content were $17.62 \pm 0.43 \text{ mg} \cdot \text{g}^{-1}$ fresh weight (FW) for A. membranaceus and $22.10 \pm 0.77 \text{ mg} \cdot \text{g}^{-1}$ FW for *C. lanceolata*, under LEDs combined with 1050 μ mol·mol⁻¹ CO₂. As an important signal, light, especially red and blue, can activate photosynthetic enzymes including Rubisco and improve stomata opening, which is the foundation of photosynthesis. Supplemental CO_2 increases CO_2 concentration in intercellular space, which lead to more CO_2 molecular is fixed by ribulose bisphosphate. This fixation step was catalyzed by Rubisco. Supplemental CO₂ and lighting promoted photosynthesis, increased carbohydrate content, and thus improved the quality of plug seedlings. Compared with MH and HPS, LEDs provided a high percentage of red and blue light, showing a greater promotion on soluble sugar content. Similarly, Terfa et al. [53] reported that plants grown under LEDs (red:blue 8:2) showed higher levels of soluble carbohydrates than those grown under HPS in Rosa \times hybrida. Dong et al. [54] found that elevated CO₂ increased the concentrations of carbohydrates, such as fructose, glucose, and total soluble sugars. During photosynthesis, sufficient CO_2 and light are needed as the substrates of photosynthesis, resulting in more photosynthetic products including soluble sugars. For plug seedlings of A. membranaceus and C. lanceolata, LEDs combined with 1050 μ mol·mol⁻¹ CO₂ were beneficial to the accumulation of soluble sugars.

	Č×L	***	***	***	***
	$CO_2(C)$	***	***	***	***
ıceolata	Light(L)	***	**	**	***
	$\breve{C} \times L$	NS	NS	***	***

Table 3. Analysis of variance for soluble sugar, starch, total phenols, and total flavonoids.

Starch

Total Phenols

NS

Total Flavonoids

Soluble Sugar

NS, **, and ***, represent no significant or significant difference at p < 0.01, or 0.001, respectively.

Regardless of CO₂ treatment with *A. membranaceus*, the supplemental lighting (LED, MH, and HPS) significantly increased starch content compared to that in the control (Figure 2C,D, Table 3). A significant interaction between supplemental lighting and CO₂ for starch was also shown in *A. membranaceus*, which was similar with soluble sugar. In *C. lanceolata*, the starch contents were increased with enriched CO₂ under the same light source (LED, MH, or HPS). The maximum starch contents were 72.57 \pm 0.31 mg·g⁻¹ FW in *A. membranaceus* and 37.40 \pm 1.41 mg·g⁻¹ FW in *C. lanceolata*, when grown under LEDs with 1050 µmol·mol⁻¹ CO₂. This is in agreement with the results of Li et al. [31], where elevated CO₂ also increased the concentration of carbohydrates including sucrose, sugar, starch and non-structural carbohydrates in dwarf bamboo. Importantly, contents of soluble sugar and starch are related to plug-seedling quality and survival of transplanting. Once transplanted, a plug seedling will suffer a hardest period to adapt its condition and recover growth. High consumption by respiration and low biosynthesis by photosynthesis will deplete the energy and health of plug seedlings. Thus, it is advantageous for plug seedlings with high contents of soluble sugar and starch to survive during a tough time.



Figure 2. Contents of soluble sugar and starch in plug seedlings of *A. membranaceus* (A,C) and *C. lanceolata* (B,D) as affected by the CO₂ level and supplemental light source. Data were presented as the mean \pm standard error (n = 3). Con, control without supplemental lighting; LED, mixed light-emitting diodes; MH, metal halite; and HPS, high pressure sodium. Different letters (a–g) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

3.3. Contents of Total Phenols and Flavonoids

Plug seedlings grown with elevated CO₂ and supplemental lighting (except LED combined $350 \ \mu mol \cdot mol^{-1} CO_2$, Figure 3C) had significantly higher contents of total phenols and flavonoids, in comparison with control (Figure 3, Table 3). Night interruption may contribute to the contents of secondary metabolites. A similar reported showed that night interruption (4 h) coupled with 8 h photoperiod resulted in higher steviol glycoside content than that under 12 h photoperiod [25]. In A. membranaceus, the highest total phenols level and the greatest flavonoids content were observed when grown under LEDs combined with 700 µmol·mol⁻¹ CO₂. Phenylalanine ammonia-lyase is the key enzyme controlling the translation from primary metabolites to secondary metabolites. This enzyme has been shown to be upregulated by blue light [55]. LEDs with a high percentage of blue light accounted for the higher contents of total phenols and flavonoids. Moreover, the higher contents of total phenols and flavonoids might be due to the stimulant effects of elevated CO_2 on the phenylpropanoid pathway, since more carbon molecules are available to synthesize phenols and flavonoids. [56]. As a non-enzyme antioxidant, total phenols and flavonoids can degrade the reactive oxygen species and reduce harm from abiotic stress, which is helpful for improving the quality of plug seedlings. Similarly, Park et al. [44] found that the total anthocyanin concentration in *Perilla frutescens* was greater under RBW LEDs than under fluorescent light. Ren et al. [35] reported that the production of anthocyanin, flavonoids and phenols increased with the CO_2 enrichment from 450 to 1200 μ mol·mol⁻¹, but decreased when the CO₂ level was raised from 1200 to 2000 µmol·mol⁻¹. Moreover, in C. lanceolata, elevated CO2 and/or supplemental lighting had improved the contents of total phenols and flavonoids

compared to those of the control combined with 350 μ mol·mol⁻¹ CO₂ (Figure 3B,D). However, no obvious trends were found in the contents of total phenol and flavonoids under different light sources (LEDs, MH, and HPS). Both the maximum total phenol and flavonoid levels were observed under the HPS combined with 700 μ mol·mol⁻¹ CO₂. Furthermore, analysis of variance for total phenols and flavonoids showed a significant synergy between CO₂ and supplemental lighting source in both *A. membranaceus* and *C. lanceolata* (Table 3). Therefore, the optimal CO₂ for total phenol and flavonoid varied with different light source and species, meanwhile the best light source changed with CO₂ concentration and species. More study with various combination in different species should be carried out to demonstrate this issue.



Figure 3. Contents of total phenols and flavonoids in plug seedlings of *A. membranaceus* (**A**,**C**) and *C. lanceolata* (**B**,**D**) as affected by the CO₂ level and supplemental light source. Data were presented as the mean \pm standard error (n = 3). Con, control without supplemental lighting; LED, mixed light-emitting diodes; MH, metal halite; and HPS, high pressure sodium. Different letters (a–g) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

3.4. Chlorophyll Content, Quantum Yield, and Stomatal Conductance

As shown in Figure 4A,B, all supplemental lighting increased the chlorophyll content in *A. membranaceus*, compared to that of seedlings grown without supplemental lighting, regardless of the CO₂ level (Table 4). This result did not agree with a study by Park et al. [26] who reported a decrease in chlorophyll under night interruption treatments (4 h) as compared with control. The discrepancy is from different photosynthetic light period. In their study, the photosynthetic light period of night interruption is 14 h (short day, 10 h), 2 h less than control (long day, 16 h). Analysis of variance showed a significant interactive effect of CO₂ and supplemental lighting source on chlorophyll. Under 350 or 700 µmol·mol⁻¹ CO₂, the LED increased chlorophyll, whereas MH did under 1,050 µmol·mol⁻¹ CO₂. The highest chlorophyll content observed in *A. membranaceus* was 37.4 ± 0.6 SPAD, grown under LEDs combined with 700 µmol·mol⁻¹ CO₂. In *C. lanceolata*, the chlorophyll content fluctuated with the supplemental CO₂ and lighting and was affected not only by supplemental lighting source but also the interaction (Table 4). The highest value was 27.0 ± 0.3 SPAD when grown under LEDs combined with 700 µmol·mol⁻¹ CO₂. These results agreed with those of a previous study. Mamatha et al. [9] found that the total chlorophyll content was higher when plants were grown with 380 and 550 ppm CO₂

than with 700 ppm CO₂. In photosynthesis, chlorophyll is a vital component in the light-harvesting complex (LHC), which absorbs and transports photons to the reaction center of photosystems [57]. Chlorophyll biosynthesis requires light and is affected by the light quality. Blue light has been shown to be favorable to the formation of chlorophyll [58,59]. Compared with MH and HPS, LEDs used in this study provided a narrow band of blue and red lights, resulting in an increase in the chlorophyll content. Elevated CO_2 has also increased the chlorophyll content [15]. The data in this study showed an increase in the chlorophyll content by supplemental lighting and/or elevated CO₂, implying an enhancement in the light capture capacity, which promotes the synthesis of photosynthetic products in plants [60]. This partly explains the higher contents of soluble sugar, starch, total phenols and flavonoids under LEDs and elevated CO₂ (Figures 2 and 3). However, some studies reported a reduction in chlorophyll content when plants are grown under elevated CO_2 levels [13,31]. The different results between ours and theirs are due to different species. Diversely, Ksiksi, et al. [61] reported there was no significant increase in total chlorophyll content in alfalfa under enriched CO₂ environments. A meta-analysis using 57 articles consisting of 1015 observations showed the concentrations of total chlorophyll, anthocyanins, and carotenoids were not influence by enriched CO₂ [54]. Therefore, more studies should be carried out to clarify how enriched CO₂ influences the biosynthesis, decomposition, and contents of chlorophyll at molecular level among species.



Figure 4. Effect of CO₂ level and supplemental light source on chlorophyll and quantum yield (*Fv/Fm*) of plug seedlings of *A. membranaceus* (**A** and **C**) and *C. lanceolata* (**B** and **D**). Data were presented as the mean \pm standard error (n = 3). Con, control without supplemental lighting; LED, mixed light-emitting diodes; MH, metal halite; and HPS, high pressure sodium. Different letters (a–e) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

Species	Factor	Chlorophyll	Quantum Yield	Stomatal Conductance	
-1	Tuctor		Quantum from	Day	Night
	CO ₂ (C)	***	**	NS	*
A. membranaceus	Light(L)	***	**	***	***
	C×L	**	NS	**	**
	$CO_2(C)$	NS	NS	**	**
C. lanceolata	Light(L)	***	**	NS	***
	Č×L	***	NS	NS	NS

Table 4. Analysis of variance for chlorophyll, quantum yield, and stomatal conductance.

NS, *, **, and ***, represent no significant or significant difference at p < 0.05, 0.01, or 0.001, respectively.

Plug seedlings of *A. membranaceus* grown with 1050 μ mol mol⁻¹ CO₂ showed higher quantum yield (QY) values in comparison with plants grown with 350 μ mol mol⁻¹ CO₂ in control and under the LEDs (Figure 4C). However, the CO₂ at 700 μ mol·mol⁻¹ improved the QY values of *A. membranaceus* grown under MH and HPS and of *C. lanceolata* grown under all the light sources (Figure 4C,D). Similarly, Moghaddam et al. [62] observed that a high CO₂ concentration (800 μ mol·mol⁻¹) improved the efficiency of photosystem II (PSII) in *Centella asiatica*. Ruhil et al. [63] also reported an increase in the quantum efficiency of Photosystem II with elevated CO₂ (585 μ mol·mol⁻¹) in mustard (*Brassica juncea* L. cv Pusa Bold). Therefore, supplemental CO₂ and lighting improved photosynthesis by increasing quantum yield, consequently contributing to higher contents of primary and secondary metabolites (Figures 2 and 3). However, Tisarum et al. [64] found that the quantum yield in *Hevea brasiliensis* cv. 'RRIT413' sharply declined by 39.0% under a CO₂-enriched condition (1500 μ mol⁻¹¹), compared with that in ambient CO₂ conditions. The probable reason for the decline in the quantum yield is the difference in the concentration of CO₂. In their study, 1500 μ mol·mol⁻¹ CO₂ was applied, which is much higher than the concentration used in this study. Another reason may be the species, since different species have different responses to the CO₂ concentration [42,65].

Importantly, LEDs had increased the QY in both species as compared to the control and MH under the same concentration of CO₂, even though the difference was not significant among all treatments. This agrees with a study where it was reported that the light quantum yield of PSII of pepper (*Capsicum frutescens* L., Sujiao No. 5) was higher (113.70%) under LED lighting than with no supplemental lighting [66]. Similarly, Bergstrand et al. [67] found that the specific photosynthetic capacity and maximum quantum yield of PSII (*Fv/Fm*) were higher in *Rosa* × *hybrida* leaves grown under LEDs than in the control. QY is the ratio of the number of photons emitted to the number of photons absorbed, and thus represents the efficiency of PS II. Therefore, these results imply that LEDs, as well as MH and HPS, have a positive effect on the photosynthetic efficiency as compared with control combined with 350 µmol·mol⁻¹ CO₂, resulting in high contents of primary and secondary metabolites. However, the QY presented in this study was lower as compared with a healthy range (0.70–0.85), which implied a stress. This can be explained by light intensity during daytime. In this study, the average daily maximum light intensity coming from the sunlight was about 568.9 µmol·m⁻²·s⁻¹ PPFD in winter, lower than that in growth season. Therefore, further study on the supplemental lighting intensity should be carried out in the near future.

Plug seedlings of A. membranaceus grown with control combined with 350 µmol·mol⁻¹ CO₂ showed a low value of stomatal conductance as compared with enriched CO_2 and supplemental lighting, although the difference was not significant among all treatments (Figure 5A,C). Among all treatments, the daytime stomatal conductance showed the maximum value under LEDs combined with 700 μ mol·mol⁻¹ CO₂ in *A. membranaceus*. However, the maximum value in *C. lanceolata* was under LEDs with 1050 μ mol·mol⁻¹ CO₂ (Figure 5), although it was not significantly different from LED or MH combined with 700 μ mol·mol⁻¹ CO₂ and MH or HPS combined with 1050 μ mol·mol⁻¹ CO₂. A similar feature was found in the nighttime stomatal conductance (Figure 5C,D). Importantly, in C. lanceolata, the stomatal conductance increased with enriched CO₂ under all light sources including LEDs, MH, and HPS (Figure 5B,D). This is in agreement with the results from other studies. For example, Wohlfahrt et al. [52] found that the stomatal conductance and transpiration rate were higher under elevated CO₂ for Vitis vinifera L. cvs. Riesling and Cabernet Sauvignon in three seasons. Prince et al. [68] reported that elevated CO_2 conditions increased the stomatal conductance and transpiration of *Phragmites australis*. However, in *A. membranaceus*, the stomatal conductance under LEDs and MH decreased when the CO₂ concentration was increased from 700 to 1050 μ mol·mol⁻¹. This is because of negative feedback regulation. Under high CO₂, a decrease in stomatal opening and reduction in stomatal conductance occurred in order to balance the rate of absorbing CO₂ and photosynthesis. Although the stomatal conductance decreased with elevated CO₂, the photosynthesis increased with CO_2 enrichment [9,13,69]. The different characteristics of stomatal conductance



between *A. membranaceus* and *C. lanceolata* may be because different species respond differently to the CO₂ concentration.

Figure 5. Effect of CO₂ level and supplemental light source on the stomatal conductance of plug seedlings of *A. membranaceus* (**A**,**C**) and *C. lanceolata* (**B**,**D**) at daytime (**A**,**B**) and nighttime (**C**,**D**). Data were presented as the mean \pm standard error (n = 3). Con, control without supplemental lighting; LED, mixed light-emitting diodes; MH, metal halite; and HPS, high pressure sodium. Different letters (a–f) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

Compared to MH and HPS under the same CO_2 concentration, LEDs combined 700 μ mol·mol⁻¹ in A. membranaceus or 1050 μ mol·mol⁻¹ in C. lanceolata led to larger improvements in the daytime and nighttime stomatal conductance, although difference was not significant among all treatments. The reason is higher percentage of blue light in LEDs than other light source. Blue light increases stomata opening with higher stomatal conductance, and consequently substrate of photosynthesis (water and (CO_2) exchanges more frequently, leading to more photosynthetic products and secondary metabolites (Figures 2 and 3). Similarly, Song et al. [70] reported that cucumber seedlings cultivated under white LEDs and RB LEDs had higher stomatal conductance values than those grown under conventional light sources (triphosphate fluorescent lamps and high-frequency fluorescent lamps). Borowski et al. [71] also found that leaves of lettuce grown under LEDs (R/B) had a higher stomatal conductance than those in other growth conditions. As conventional light sources, MH and HPS provided a broad-band spectrum for plant growth [72]. However, they have a lower efficiency for seedling growth compared to LEDs, since yellow and green light account for high proportions of the spectrum. LEDs provided a narrow band of red and blue lights, which can be absorbed by phytochromes and cytochromes, and used for photosynthesis efficiently. Moreover, previous studies have shown that blue light controls the opening and closing of stomata [73]. Therefore, a higher proportion of blue light in LEDs contributed to the stomata opening and greater stomatal conductance of seedlings [53].

Taking all treatments into consideration, LEDs combined with CO_2 at 1050 µmol·mol⁻¹ is recommended for growing the seedlings of *A. membranaceus* and *C. lanceolata*, since it enhanced the stomatal conductance at daytime and nighttime in those two species. Similarly, Park et al. [41] found that the stomatal conductance was the greatest in lettuce grown under RBW LEDs with 1000 µmol·mol⁻¹ CO_2 . As an estimation of the gas exchange rate (CO_2 uptake and water loss), the stomatal conductance indicates the rates of photosynthesis and transpiration. In this study, LEDs combined with CO_2 at 1050 µmol·mol⁻¹ increased the stomatal conductance, resulting in a higher photosynthetic rate and higher quality of plus seedlings.

4. Conclusions

In this study, the effects of the CO₂ and supplemental light source on the growth and development of plug seedlings of *A. membranaceus* and *C. lanceolata* were demonstrated. The data showed that LEDs combined with CO₂ at 1050 µmol·mol⁻¹ greatly enhanced the quality of plug seedlings, evident by the higher shoot, root, and leaf dry weights, stem diameter, leaf area, and Dickson' quality index compared to those in the control in both species. Moreover, a significant interaction of supplemental CO₂ and lighting source on metabolites was found in both *A. membranaceus* and *C. lanceolata*. The CO₂ and supplemental light source had a positive effect on primary metabolites, and the maximum contents of soluble sugar and starch were obtained when seedlings were grown under LEDs combined with 1050 µmol·mol⁻¹ CO₂. Furthermore, an enriched CO₂ at 700 µmol·mol⁻¹ is more optimal for accumulation of total phenols and flavonoids. In addition, LEDs combined with 700 or 1050 µmol·mol⁻¹ CO₂ enhanced the photosynthesis-related parameters such as the chlorophyll content, quantum yield, and stomatal conductance at daytime and nighttime. In conclusion, the data from this study suggest that LEDs combined with CO₂ at 1050 µmol·mol⁻¹ is the optimal condition for enhancing the quality of plug seedlings of *A. membranaceus* and *C. lanceolata*.

Author Contributions: Conceptualization, Y.L., X.R. and B.R.J.; Funding acquisition, B.R.J.; Investigation, Y.L. and X.R.; Project administration, X.R. and B.R.J.; Supervision, B.R.J.; Writing—original draft, Y.L.; Writing—review and editing, Y.L., X.R. and B.R.J.

Funding: This research was funded by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (Project No. 116057-03). Y.L. and X.R. were supported by a scholarship from the BK21 Plus Program, Ministry of Education, Korea.

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

References

- Tuan, P.A.; Chung, E.; Thwe, A.A.; Li, X.; Kim, Y.B.; Mariadhas, V.A.; Al-Dhabi, N.A.; Lee, J.H.; Park, S.U. Transcriptional profiling and molecular characterization of astragalosides, calycosin, and calycosin-7-o-beta-d-glucoside biosynthesis in the hairy roots of *Astragalus membranaceus* in response to methyl jasmonate. *J. Agric. Food Chem.* 2015, *63*, 6231–6240. [CrossRef] [PubMed]
- 2. Guo, W.L.; Gong, L.; Ding, Z.F.; Li, Y.D.; Li, F.X.; Zhao, S.P.; Liu, B. Genomic instability in phenotypically normal regenerants of medicinal plant *Codonopsis lanceolata* Benth. et Hook. F., as revealed by ISSR and rapd markers. *Plant Cell Rep.* **2006**, *25*, 896–906. [CrossRef] [PubMed]
- Yang, J.; Jia, M.M.; Zhang, X.J.; Wang, P.L. Calycosin attenuates MPTP-induced Parkinson's disease by suppressing the activation of TLR/NF-kappa B and MAPK pathways. *Phytother. Res.* 2019, *33*, 309–318. [CrossRef] [PubMed]
- 4. Han, A.Y.; Lee, Y.S.; Kwon, S.; Lee, H.S.; Lee, K.W.; Seol, G.H. *Codonopsis lanceolata* extract prevents hypertension in rats. *Phytomedicine* **2018**, *39*, 119–124. [CrossRef] [PubMed]
- Boo, H.O.; Kim, H.H.; Barasch, D.; Nemirovski, A.; Lee, M.S.; Gorinstein, S.; Ku, Y.G. *Codonopsis lanceolata* and *Nelumbo nucifera* Gaertn. Root extracts for functional food: Metabolic profiling by MS, FTIR and fluorescence and evaluation of cytotoxicity and anti-obesity properties on 3T3-L1 cell line. *Eur. Food Res. Technol.* 2017, 243, 689–700. [CrossRef]
- Moon, K.G.; Um, I.S.; Jeon, S.H.; Cho, Y.S.; Kim, Y.G.; Rho, I.R. Effect of organic fertilizer application on growth characteristics and saponin content in *Codonopsis lanceolata*. *Hortic. Environ. Biotechnol.* 2018, 59, 125–130. [CrossRef]
- Ghimire, B.K.; Seong, E.S.; Yu, C.Y.; Kim, S.H.; Chung, I.M. Evaluation of phenolic compounds and antimicrobial activities in transgenic *Codonopsis lanceolata* plants via overexpression of the gamma-tocopherol methyltransferase (gamma-tmt) gene. S. Afr. J. Bot. 2017, 109, 25–33. [CrossRef]

- Li, Y.; Guo, S.; Zhu, Y.; Yan, H.; Qian, D.W.; Wang, H.Q.; Yu, J.Q.; Duan, J.A. Flowers of *Astragalus membranaceus* var. *mongholicus* as a novel high potential by-product: Phytochemical characterization and antioxidant activity. *Molecules* 2019, 24, 434. [CrossRef]
- Mamatha, H.; Rao, N.K.S.; Laxman, R.H.; Shivashankara, K.S.; Bhatt, R.M.; Pavithra, K.C. Impact of elevated CO₂ on growth, physiology, yield, and quality of tomato (*Lycopersicon esculentum* Mill) cv. Arka Ashish. *Photosynthetica* 2014, 52, 519–528. [CrossRef]
- Pan, T.H.; Ding, J.J.; Qin, G.G.; Wang, Y.L.; Xi, L.J.; Yang, J.W.; Li, J.M.; Zhang, J.; Zou, Z.R. Interaction of supplementary light and CO₂ enrichment improves growth, photosynthesis, yield, and quality of tomato in autumn through spring greenhouse production. *HortScience* 2019, 54, 246–252. [CrossRef]
- Qiao, Y.; Zhang, H.; Dong, B.; Shi, C.; Li, Y.; Zhai, H.; Liu, M. Effects of elevated CO₂ concentration on growth and water use efficiency of winter wheat under two soil water regimes. *Agric. Water Manag.* 2010, 97, 1742–1748. [CrossRef]
- 12. Cheng, W.; Sakai, H.; Yagi, K.; Hasegawa, T. Interactions of elevated CO₂ and night temperature on rice growth and yield. *Agric. For. Meteorol.* **2009**, *149*, 51–58. [CrossRef]
- Wang, L.; Feng, Z.; Schjoerring, J.K. Effects of elevated atmospheric CO₂ on physiology and yield of wheat (*Triticum aestivum* L.): A meta-analytic test of current hypotheses. *Agric. Ecosyst. Environ.* 2013, 178, 57–63. [CrossRef]
- Meng, F.; Zhang, J.; Yao, F.; Hao, C. Interactive effects of elevated CO₂ concentration and irrigation on photosynthetic parameters and yield of maize in northeast China. *PLoS ONE* 2014, 9, e98318. [CrossRef] [PubMed]
- Li, X.; Brestic, M.; Tan, D.-X.; Zivcak, M.; Zhu, X.; Liu, S.; Song, F.; Reiter, R.J.; Liu, F. Melatonin alleviates low PSI-limited carbon assimilation under elevated CO₂ and enhances the cold tolerance of offspring in chlorophyll b-deficient mutant wheat. *J. Pineal Res.* 2018, *64*, e12453. [CrossRef]
- Aranjuelo, I.; Erice, G.; Sanz-Sáez, A.; Abadie, C.; Gilard, F.; Gil-Quintana, E.; Avice, J.C.; Staudinger, C.; Wienkoop, S.; Araus, J.L.; et al. Differential CO₂ effect on primary carbon metabolism of flag leaves in durum wheat (*Triticum durum* Desf.). *Plant Cell Environ.* 2015, *38*, 2780–2794. [CrossRef]
- 17. Bunce, J.A. Acclimation to temperature of the response of photosynthesis to increased carbon dioxide concentration in Taraxacum officinale. *Photosynth. Res.* **2000**, *64*, 89–94. [CrossRef]
- 18. Long, S.P.; Ainsworth, E.A.; Rogers, A.; Ort, D.R. Rising atmospheric carbon dioxide: Plants FACE the future. *Annu. Rev. Plant Biol.* **2004**, *55*, 591–628. [CrossRef]
- 19. Jiang, C.Y.; Johkan, M.; Hohjo, M.; Tsukagoshi, S.; Ebihara, M.; Nakaminami, A.; Maruo, T. Photosynthesis, plant growth, and fruit production of single-truss tomato improves with supplemental lighting provided from underneath or within the inner canopy. *Sci. Hortic.* **2017**, *222*, 221–229. [CrossRef]
- 20. Zhang, M.Z.; Whitman, C.M.; Runkle, E.S. Manipulating growth, color, and taste attributes of fresh cut lettuce by greenhouse supplemental lighting. *Sci. Hortic.* **2019**, *252*, 274–282. [CrossRef]
- 21. Bergstrand, K.J.; Schussler, H.K. Growth, development and photosynthesis of some horticultural plants as affected by different supplementary lighting technologies. *Eur. J. Hortic. Sci.* **2013**, *78*, 119–125.
- 22. Chen, X.L.; Wang, L.C.; Li, T.; Yang, Q.C.; Guo, W.Z. Sugar accumulation and growth of lettuce exposed to different lighting modes of red and blue led light. *Sci. Rep.* **2019**, *9*, 6926. [CrossRef] [PubMed]
- 23. Tewolde, F.T.; Lu, N.; Shiina, K.; Maruo, T.; Takagaki, M.; Kozai, T.; Yamori, W. Nighttime supplemental led inter-lighting improves growth and yield of single-truss tomatoes by enhancing photosynthesis in both winter and summer. *Front. Plant Sci.* **2016**, *7*, 448. [CrossRef] [PubMed]
- 24. Cao, K.; Cui, L.R.; Ye, L.; Zhou, X.T.; Bao, E.C.; Zhao, H.L.; Zou, Z.R. Effects of red light night break treatment on growth and flowering of tomato plants. *Front. Plant Sci.* **2016**, *7*, 527. [CrossRef] [PubMed]
- 25. Yoneda, Y.; Shimizu, H.; Nakashima, H.; Miyasaka, J.; Ohdoi, K. Effects of light intensity and photoperiod on improving steviol glycosides content in stevia rebaudiana (bertoni) bertoni while conserving light energy consumption. *J. Appl. Res. Med. Aroma.* **2017**, *7*, 64–73. [CrossRef]
- Park, Y.G.; Muneer, S.; Soundararajan, P.; Manivnnan, A.; Jeong, B.R. Light quality during night interruption affects morphogenesis and flowering in petunia hybrida, a qualitative long-day plant. *Hortic. Environ. Biotechnol.* 2016, 57, 371–377. [CrossRef]
- Ouzounis, T.; Frette, X.; Rosenqvist, E.; Ottosen, C.O. Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas. *J. Plant Physiol.* 2014, 171, 1491–1499. [CrossRef]

- Paradiso, R.; Meinen, E.; Snel, J.F.H.; De Visser, P.; Van Ieperen, W.; Hogewoning, S.W.; Marcelis, L.F.M. Spectral dependence of photosynthesis and light absorptance in single leaves and canopy in rose. *Sci. Hortic.* 2011, 127, 548–554. [CrossRef]
- 29. Shukla, M.; Tabassum, R.; Singh, R.; Dhar, D.W. Influence of light intensity, temperature and CO₂ concentration on growth and lipids in green algae and cyanobacteria. *Indian J. Exp. Biol.* **2016**, *54*, 482–487.
- 30. Correll, M.J.; Weathers, P.J. Effects of light, CO₂ and humidity on carnation growth, hyperhydration and cuticular wax development in a mist reactor. *In Vitr. Cell. Dev. Biol.-Plant* **2001**, *37*, 405–413. [CrossRef]
- Li, Y.; Zhang, Y.; Zhang, X.; Korpelainen, H.; Berninger, F.; Li, C. Effects of elevated CO₂ and temperature on photosynthesis and leaf traits of an understory dwarf bamboo in subalpine forest zone, China. *Physiol. Plant* 2013, 148, 261–272. [CrossRef]
- 32. Hwang, C.H.; Park, Y.G.; Jeong, B.R. Changes in content of total polyphenol and activities of antioxidizing enzymes in *Perilla frutescens* var. Acuta kudo and *Salvia plebeia* R. Br. as affected by light intensity. *Hortic. Environ. Biotechnol.* **2014**, *55*, 489–497. [CrossRef]
- Matsuda, R.; Ozawa, N.; Fujiwara, K. Leaf photosynthesis, plant growth, and carbohydrate accumulation of tomato under different photoperiods and diurnal temperature differences. *Sci. Hortic.* 2014, 170, 150–158. [CrossRef]
- 34. Liu, Y.; Ren, X.; Jeong, R.B. Night temperature affects the growth, metabolism, and photosynthetic gene expression in astragalus membranaceus and codonopsis lanceolata plug seedlings. *Plants* **2019**, *8*, 407. [CrossRef] [PubMed]
- 35. Ren, J.; Guo, S.S.; Xin, X.L.; Chen, L. Changes in volatile constituents and phenols from *Gynura bicolor* DC grown in elevated CO₂ and LED lighting. *Sci. Hortic.* **2014**, *175*, 243–250. [CrossRef]
- Manivannan, A.; Soundararajan, P.; Park, Y.G.; Jeong, B.R. In vitro propagation, phytochemical analysis, and evaluation of free radical scavenging property of *Scrophularia kakudensis* Franch tissue extracts. *Biomed Res. Int.* 2015, 2015, 480564. [CrossRef]
- 37. Kitaya, Y.; Niu, G.; Kozai, T.; Ohashi, M. Photosynthetic photon flux, photoperiod, and CO₂ concentration affect growth and morphology of lettuce plug transplants. *HortScience* **1998**, *33*, 58–62. [CrossRef]
- 38. Kim, H.M.; Hwang, S.J. The growth and development of 'mini chal' tomato plug seedlings grown under various wavelengths using light emitting diodes. *Agronomy* **2019**, *9*, 157. [CrossRef]
- 39. Yücedağ, C.; Bilir, N.; Özel, H.B. Phytohormone effect on seedling quality in Hungarian oak. *For. Syst.* **2019**, 28, 5. [CrossRef]
- 40. Elfadl, A.M. Growth performance and physiological characteristics of seedlings of six tropical dry land forest tree species in the sudan. *J. Nat. Resour. Environ. Stud.* **2013**, *1*, 25–33.
- Park, Y.G.; Park, J.E.; Hwang, S.J.; Jeong, B.R. Light source and CO₂ concentration affect growth and anthocyanin content of lettuce under controlled environment. *Hortic. Environ. Biotechnol.* 2012, 53, 460–466. [CrossRef]
- 42. Hoddinott, J.; Scott, R. The influence of light quality and carbon dioxide enrichment on the growth and physiology of seedlings of three conifer species. II. Physiological responses. *Can. J. Bot.* **1996**, *74*, 391–402. [CrossRef]
- 43. Yilmaz, O.; Kahraman, K.; Ozturk, L. Elevated carbon dioxide exacerbates adverse effects of mg deficiency in durum wheat. *Plant Soil* **2017**, *410*, 41–50. [CrossRef]
- Park, Y.G.; Oh, H.J.; Jeong, B.R. Growth and anthocyanin concentration of *Perilla frutescens* var. acuta Kudo as affected by light source and DIF under controlled environment. *Hortic. Environ. Biotechnol.* 2013, 54, 103–108. [CrossRef]
- 45. Dickson, A.; Leaf, A.L.; Hosner, J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries. *For. Chron.* **1960**, *36*, 10–13. [CrossRef]
- 46. Liu, Y.; Ren, X.; Jeong, H.K.; Wei, H.; Jeong, B.R. Growth and physiological responses of *Adenophora triphylla* (Thunb.) A.DC. plug seedlings to day and night temperature regimes. *Agronomy* **2018**, *8*, 173. [CrossRef]
- 47. Dreywood, R. Qualitative test for carbohydrate material. Ind. Eng. Chem. Anal. Ed. 1946, 18, 499. [CrossRef]
- 48. Santos-Moura, S.D.; Alves, E.U.; Ursulino, M.M.; Bruno, R.D.A.; dos Anjos Neto, A.P. Effect of shading on *Dimorphandra gardneriana* Tul. seedling production. *Biosci. J.* **2018**, *34*, 1147–1157. [CrossRef]
- 49. Lanoue, J.; Leonardos, E.D.; Ma, X.; Grodzinski, B. The effect of spectral quality on daily patterns of gas exchange, biomass gain, and water-use-efficiency in tomatoes and lisianthus: An assessment of whole plant measurements. *Front. Plant Sci.* **2017**, *8*, 1076. [CrossRef]

- 50. Randall, W.C.; Lopez, R.G. Comparison of supplemental lighting from high-pressure sodium lamps and light-emitting diodes during bedding plant seedling production. *HortScience* **2014**, *49*, 589–595. [CrossRef]
- 51. Zhang, T.; Shi, Y.; Piao, F.; Sun, Z. Effects of different led sources on the growth and nitrogen metabolism of lettuce. *Plant Cell Tissue Organ Cult.* **2018**, 134, 231–240. [CrossRef]
- 52. Wohlfahrt, Y.; Smith, J.P.; Tittmann, S.; Honermeier, B.; Stoll, M. Primary productivity and physiological responses of *Vitis vinifera* L. cvs. under free air carbon dioxide enrichment (FACE). *Eur. J. Agron.* **2018**, *101*, 149–162. [CrossRef]
- 53. Terfa, M.T.; Solhaug, K.A.; Gislerod, H.R.; Olsen, J.E.; Torre, S. A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of *Rosa* x *hybrida* but does not affect time to flower opening. *Physiol. Plant* 2013, 148, 146–159. [CrossRef] [PubMed]
- 54. Dong, J.L.; Gruda, N.; Lam, S.K.; Li, X.; Duan, Z.Q. Effects of elevated CO₂ on nutritional quality of vegetables: A review. *Front. Plant Sci.* **2018**, *9*, 924. [CrossRef] [PubMed]
- 55. Son, K.H.; Lee, J.H.; Oh, Y.; Kim, D.; Oh, M.M.; In, B.C. Growth and bioactive compound synthesis in cultivated lettuce subject to light-quality changes. *HortScience* **2017**, *52*, 584–591. [CrossRef]
- 56. Ebisawa, M.; Shoji, K.; Kato, M.; Shimomura, K.; Goto, F.; Yoshihara, T. Supplementary ultraviolet radiation b together with blue light at night increased quercetin content and flavonol synthase gene expression in leaf lettuce (*Lactuca sativa* L.). *Environ. Control Biol.* **2008**, *46*, 1–11. [CrossRef]
- 57. Melis, A. Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antennae to maximize efficiency. *Plant Sci.* **2009**, *177*, 272–280. [CrossRef]
- Fan, X.; Zang, J.; Xu, Z.; Guo, S.; Jiao, X.; Liu, X.; Gao, Y. Effects of different light quality on growth, chlorophyll concentration and chlorophyll biosynthesis precursors of non-heading chinese cabbage (*Brassica campestris* L.). *Acta Physiol. Plant.* 2013, *35*, 2721–2726. [CrossRef]
- 59. Wang, H.; Gu, M.; Cui, J.; Shi, K.; Zhou, Y.; Yu, J. Effects of light quality on CO₂ assimilation, chlorophyll-fluorescence quenching, expression of calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. *J. Photochem. Photobiol.* **2009**, *96*, 30–37. [CrossRef]
- Sezgin, A.; Altuntaş, C.; Demiralay, M.; Cinemre, S.; Terzi, R. Exogenous alpha lipoic acid can stimulate photosystem II activity and the gene expressions of carbon fixation and chlorophyll metabolism enzymes in maize seedlings under drought. *J. Plant Physiol.* 2019, 232, 65–73. [CrossRef]
- Ksiksi, T.S.; Ppoyil, S.B.T.; Palakkott, A.R. Co₂ enrichment affects eco-physiological growth of maize and alfalfa under different water stress regimes in the uae. *Physiol. Mol. Biol. Plants* 2018, 24, 251–259. [CrossRef]
 [PubMed]
- Moghaddam, S.S.; Ibrahim, R.; Damalas, C.A.; Noorhosseini, S.A. Effects of gamma stress and carbon dioxide on eight bioactive flavonoids and photosynthetic efficiency in *Centella asiatica*. J. Plant Growth Regul. 2017, 36, 957–969. [CrossRef]
- Ruhil, K.; Ahmad, A.; Iqbal, M.; Tripathy, B.C. Photosynthesis and growth responses of mustard (*Brassica juncea* L. cv *pusa* Bold) plants to free air carbon dioxide enrichment (FACE). *Protoplasma* 2015, 252, 935–946. [CrossRef] [PubMed]
- 64. Tisarum, R.; Samphumphung, T.; Theerawitaya, C.; Prommee, W.; Cha-um, S. In vitro photoautotrophic acclimatization, direct transplantation and ex vitro adaptation of rubber tree (*Hevea brasiliensis*). *Plant Cell Tissue Organ Cult.* **2018**, 133, 215–223. [CrossRef]
- 65. Bunce, J.A.; Ziska, L.H. Impact of measurement irradiance on acclimation of photosynthesis to elevated CO₂ concentration in several plant species. *Photosynthetica* **1999**, *37*, 509–517. [CrossRef]
- 66. Li, X.; Lu, W.; Hu, G.; Wang, X.C.; Zhang, Y.; Sun, G.X.; Fang, Z. Effects of light-emitting diode supplementary lighting on the winter growth of greenhouse plants in the Yangtze River Delta of China. *Bot. Stud.* 2016, 57, 2. [CrossRef]
- 67. Bergstrand, K.J.; Mortensen, L.M.; Suthaparan, A.; Gislerod, H.R. Acclimatisation of greenhouse crops to differing light quality. *Sci. Hortic.* **2016**, 204, 1–7. [CrossRef]
- Prince, C.M.; MacDonald, G.E.; Erickson, J.E. Effects of elevated temperature and carbon dioxide concentrations on the response of two common reed (*Phragmites australis*) haplotypes to glyphosate. *Invasive Plant Sci. Manag.* 2018, 11, 181–190. [CrossRef]
- Tom-Dery, D.; Eller, F.; Jensen, K.; Reisdorff, C. Effects of elevated carbon dioxide and climate change on biomass and nutritive value of kyasuwa (*Cenchrus pedicellatus* Trin.). J. Appl. Bot. Food Qual. 2018, 91, 88–95.

- 70. Song, J.X.; Meng, Q.W.; Du, W.F.; He, D.X. Effects of light quality on growth and development of cucumber seedlings in controlled environment. *Int. J. Agric. Biol. Eng.* **2017**, *10*, 312–318.
- 71. Borowski, E.; Michalek, S.; Rubinowska, K.; Hawrylak-Nowak, B.; Grudzinski, W. The effects of light quality on photosynthetic parameters and yield of lettuce plants. *Acta Sci. Pol.* **2015**, *14*, 177–188.
- 72. Liu, Y.; Ren, X.; Jeong, B.R. Supplementary light source affects growth, metabolism, and physiology of *Adenophora triphylla* (Thunb.) A.DC. seedlings. *Biomed. Res. Int.* **2019**, 2019, 16. [CrossRef] [PubMed]
- 73. Wang, J.; Lu, W.; Tong, Y.; Yang, Q. Leaf morphology, photosynthetic performance, chlorophyll fluorescence, stomata development of lettuce (*Lactuca sativa* L.) exposed to different ratios of red light to blue light. *Front. Plant Sci.* **2016**, *7*, 250. [PubMed]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).