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Blue light dosage affects photosynthesis, chlorophyll, and antioxidant properties of *Mesembryanthemum crystallinum*

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Abstract

Mesembryanthemum crystallinum is an annual succulent plant that is being used as an emerging healthy leafy vegetable. To investigate the growth and physiological response of *M. crystallinum* to artificial lighting, five different light treatments were applied at 150 μmol(photon) m^{-2} s^{-1}, which were white (W), different rations of red/blue (B) (15, 40, and 70%B), and blue (100%B), respectively. Our results showed that plants could gain as much as edible leaf area and dry mass with a certain ratio of blue (40%) in comparison with W. Plants grown under 100%B resulted in reduced photosynthetic rate, leaf area, and fresh mass compared with W. Adding blue fraction in the light regime enhanced the photosynthetic performance by influencing the amount of chlorophyll (Chl), Chl *a*/*b*, and specific leaf area. Under red/blue treatments, the electron transport rate and effective quantum yield of both PSII and PSI increased, while the nitrate content was reduced and flavonoids and total antioxidant capacity were unaffected.

Keywords: common ice plant; controlled environment; gas exchange; light quality.

Introduction

Light is one of the most important environmental factors for plants; it is not only the source of energy for photosynthetic activity but also acts as a key environmental signal that regulates morphological and physiological responses (Kami *et al.* 2010). Light quality, light quantity, light duration, and even direction are the key components of light conditions that affect plant growth, photomorphogenesis, and phytochemical metabolism (Fankhauser and Chory 1997, Zoratti *et al.* 2014, Bantis *et al.* 2018).

Nowadays, artificial lighting is widely accepted for horticultural crops produced under controlled environments such as plant factories and space agriculture (Graamans

Highlights

- Monochromatic blue reduced photosynthetic rate, leaf area, and fresh mass of ice plants
- Φ_{PSII}, Φ_{PSI}, and P_N increased with the proportion of blue in the dichromatic treatments
- Narrow-band treatments reduced the nitrate content accumulation in the leaves of ice plants

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Abbreviations: AQE – apparent quantum efficiency; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DM – dry mass; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry in the dark-adapted state; g_s – stomatal conductance; LA – leaf area; LCP – light-compensation point; MDA – malondialdehyde content; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; R_D – dark respiration rate; Φ_{NA} – quantum yield of nonphotochemical energy dissipation in PSI in the light-adapted state due to the acceptor-side limitation; Φ_{ND} – quantum yield of nonphotochemical energy dissipation in PSI in the light-adapted state due to the donor-side limitation; Φ_{NO} – quantum yield of nonregulated fluorescence quenching; Φ_{NPQ} – quantum yield of nonphotochemical quenching; Φ_{PSI} – effective quantum yield of PSI photochemistry in the light-adapted state; Φ_{PSII} – effective quantum yield of PSII photochemistry in the light-adapted state.
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et al. 2018). The recent development of LEDs (light-emitting diodes), which allows targeted manipulation of the spectrum composition and intensity for artificial lighting, has provided an opportunity to maximize crop productivity and accumulation of health beneficial compounds for both commercial and research perspectives in greenhouse crop cultivation (Kozai 2016, Pattison *et al*. 2018, Kusuma *et al*. 2020, Paradiso and Proietti 2021). Red light photons generally provide the highest spectrum-dependent quantum yield (McCree 1971, Zheng *et al*. 2019), whereas additional blue light is necessary because of its positive influences on chlorophyll (Chl) biosynthesis and stomatal movement (Im *et al*. 2006, Ilić and Fallik 2017). The previous investigation reported that adding blue light to the light spectrum increased the photosynthetic rate of rice leaves, which promoted the accumulation of nitrogen content in the leaves and dry matter production (Matsuda *et al*. 2004); this was also confirmed in other plant species, such as cucumber (Hogewoning *et al*. 2010), lettuce (Wang *et al*. 2016), and chrysanthemum (Jeong *et al*. 2014). Although the necessity of blue light is commonly accepted, the optimal red/blue ratio could be species-dependent (Piovene *et al*. 2015).

Mesembryanthemum crystallinum (the common ice plant) is an annual succulent plant native to the southwest African desert; it employs C_3 photosynthesis under well-watered conditions but optionally shifts to CAM (crassulacean acid metabolism) pathway when exposed to stress conditions such as drought, salinity, and high temperature (Lee *et al*. 2019). *M. crystallinum* is rich in minerals, antioxidants, and polyols, therefore it has important functions in disease prevention and health promotion (Loconsole *et al*. 2019, Sánchez-Faure *et al*. 2020). Nowadays, *M. crystallinum* has been entered into cosmetics because of its good moisturizing effect and medicinal value, whereas it is also being used as an emerging healthy fresh leafy vegetable in many countries (Rahman *et al*. 2011, Kim *et al*. 2021).

Light quality manipulation might be a safe and more reliable way to improve the yield formation and nutritional composition for commercial ice plant cultivation. Aimed to provide a theoretical basis on light environment control for the production of ice plants under artificial lighting conditions, this study was performed to investigate the growth, photomorphogenesis, photosynthetic efficiency, and antioxidant capacity of *M. crystallinum* grown under different combinations of blue and red light, as well as white light.

Materials and methods

Plant material and treatments: The experiments were conducted in a climate-controlled growth chamber at China Agriculture University (Haidian Beijing, China). Seeds of *Mesembryanthemum crystallinum* were pre-germinated and sown in plug trays, and then allowed to grow under white light for four weeks to obtain the young seedlings. Uniform seedlings were selected and transplanted into individual plastic pots (300 mL) with a peat-based substrate, and then subjected to different light treatments. The light intensity at canopy level was set at 150 µmol(photon) m^{-2} s^{-1} by adjusting the forward current of LED lamps and a photoperiod of 14 h per day was given. The day and night temperatures in the growth chamber were maintained at 25 and 18°C, relative humidity was set at 70–75%. Plants received irrigation with 1/2 dose Hoagland's nutrient solution every 2 d for the first 10 d, once a day for the later days.

Five light quality groups were set for the experiment as follows: white light control (W); a combination of red (R) and blue (B) light (15, 40, and 70%B, respectively); monochromatic blue light (100%B). The light was supplied with LEDs (R: peak at 660 nm; B: peak at 460 nm), the light spectra (Fig. 1) were recorded with a spectrometer (*AvaSpec-ULS2048*, *Avantes Inc.*, Apeldoorn, NL). The seedlings were cultivated under different light qualities until the adult stage for 30 d, then the gas exchange and Chl fluorescence measurement were conducted on the second fully expanded leaf. After that, the same leaf was immediately frozen with liquid nitrogen and stored at –80°C until further biochemical assay.

Total leaf area, dry mass, and fresh mass: The fully expanded leaves of each plant were excised and scanned with a flatbed image scanner (*Epson Perfection V19*, *Epson*, Japan), the total leaf area was determined with *Image J* (*NIH*, Bethesda, MD, USA), specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry mass. Plants were harvested and weighted with an analytical balance to determine their fresh mass (FM), then sequentially oven-dried at 105°C for 3 h and 85°C for 3 d until the constant mass was reached to obtain the dry mass (DM).

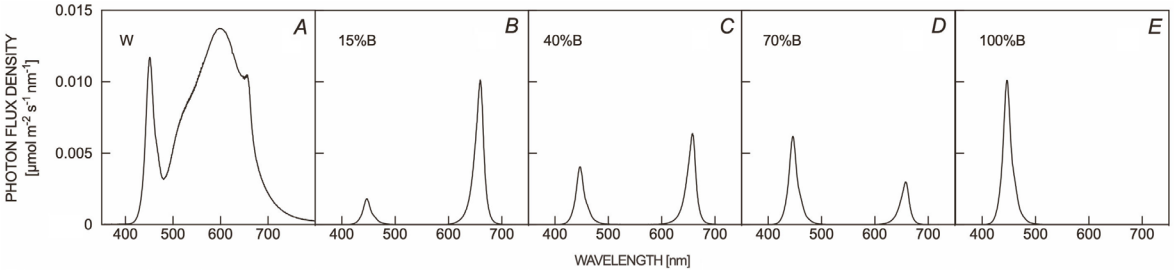

Fig. 1. Light spectrum of the different light quality treatments (W, 15%B, 40%B, 70%B, 100%B). Spectral scan was performed each 0.6 nm with an optical fiber spectrometer (*AvaSpec-ULS2048*, *Avantes Inc.*, Apeldoorn, NL).

Leaf pigment contents: The homogenized leaf sample was extracted with 10 mL of 80% aqueous acetone at 4°C for 24 h under dark conditions. The absorbance at 470, 645, 663 nm of the supernatant was measured using a UV-VIS spectrophotometer (*UV-2802*, *Unico Inc.*, Shanghai, China). The Chl *a*, Chl *b*, and carotenoid (Car) contents were calculated using the following formulas referring to Wellburn and Lichtenthaler (1984): Chl *a* = 12.25 A_{663} – 2.79 A_{645}, Chl *b* = 21.5 A_{645} – 5.1 A_{663}, Car = (1,000 A_{470} –1.82 Chl *a* – 85.02 Chl *b*)/198.

Phytochemicals content: The malondialdehyde (MDA) content was determined following the method described by Hodges *et al.* (1999). The homogenized leaf sample was extracted with 10 mL of 80% ethanol and centrifuged at 10,000 rpm. The supernatant (0.5 mL) was added to either solution without TBA (20% trichloroacetic acid, 0.01% butylated hydroxytoluene) or solution with TBA (20% trichloroacetic acid, 0.01% butylated hydroxytoluene, 0.65% TBA), respectively. The mixture was vigorously shaken and incubated at 95°C for 25 min and immediately cooled down on an ice bath. The absorbance of both extracts was measured at 440, 532, and 600 nm. The MDA content was calculated as: A = ($A_{532+TBA}$ – $A_{600+TBA}$) – ($A_{532-TBA}$ – $A_{600-TBA}$), B = ($A_{440+TBA}$ – $A_{600+TBA}$) × 0.0571, MDA equivalents [nmol ml^{-1}] = (A – B/157,000) × 10^6.

The nitrate content was determined following Li *et al.* (2016). The mixture composed of 0.4 mL of 5% salicylic acid–sulfuric acid solution, 9.5 mL of 8% NaOH, and 0.1 mL of leaf extract was boiled in a water bath for 30 min. The liquid supernatant was measured spectrophotometrically (*UV-2802*, *Unico Inc*., Shanghai, China) at 410 nm to determine the nitrate content. The nitrate content was calculated as (C × V)/W, where C is the nitrate concentration calculated using a standard curve (C = 0.0074 A + 0.0049) [μg mL^{-1}], A is the absorbance at 410 nm, V is the volume for sample extraction [mL], W is the sample mass [g].

The total protein content was determined with the *Coomassie Brilliant Blue* dye method (Bradford 1976). Homogenized leaves were extracted with 3 mL of distilled water and centrifuged at 10,000 rpm for 10 min at 4°C, then 1 mL of the supernatant was mixed well with 5 mL of *Coomassie Brilliant Blue G-250* (0.1 g L^{-1}). The absorbance at 595 nm was measured using a spectrophotometer (*UV-2802*, *Unico Inc*., Shanghai, China); the total soluble protein was calculated as mg g^{-1}(FM) using the calibration curve (C = 0.0041 A + 0.095).

Total phenolic content was determined with the Folin–Ciocalteu phenol reagent (Kaulmann *et al*. 2014). Briefly, leaf material (250 mg) was extracted with 10 mL of 80% methanol. The extract (200 μL) was added to 1.5 mL of Folin–Ciocalteu (1:10) reagent. After 4 min, 800 μL of 7.5% Na_2CO_3 was added and mixed well, the mixture was incubated at room temperature in the dark for 2 h. The absorbance of the mixture at 765 nm was recorded. Total phenolic content calculated with an external calibration curve of gallic acid and expressed as mg(gallic acid equivalent) g^{-1}(FM).

Total flavonoid content was evaluated using the aluminum chloride method (Kaulmann *et al*. 2014) with minor modifications. The extract (400 μL) was mixed well with 600 μL of distilled water and 60 μL of 5% $NaNO_2$ in a 15-mL tube. After 5 min, 60 μL of 10% $AlCl_3$ was added. After 6 min, 0.4 mL of 1.0 M NaOH and 0.4 mL of distilled water were added and mixed thoroughly. The absorbance at 510 nm was read after 15 min. The total flavonoid content was calculated with a rutin external calibration curve and expressed as mg(rutin equivalent) g^{-1}(FM).

Total antioxidant activity was determined with the same extract for total flavonoids assay following the method described by Re *et al.* (1999). The reaction mixture was composed with 0.1 mL of the extract and 0.9 mL of ABTS-working solution included 75 mM KH_2PO_4, 20 mM 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horseradish peroxidase (HRP) stock (aqueous solution, which contains HRP and ammonium sulphate with concentration at 0.08 and 13 mg ml^{-1}, respectively), and H_2O_2 stock (35% aqueous solution). The absorbance at 734 nm was measured using a UV-VIS spectrophotometer (*UV-2802*, *Unico Inc*., Shanghai, China) after incubating for 6 min, and the total antioxidant capacity was expressed as μmol(L-AA equivalent) g^{-1}(FM). The total antioxidant activity was calculated as (C × V)/W, where C is the concentration of L-AA calculated using a standard curve (C = –20.69A + 0.4649) [μmol L^{-1}], A is the absorbance at 734 nm, V is the volume of the supernatant used for absorbance determination [μL], and W is the mass of sample [g].

Gas-exchange parameters: Net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were determined using an *LI-6400XT* gas-exchange analyzer (*Li-Cor Inc*., Lincoln, NE, USA). Apparent mesophyll conductance (the conductance of CO_2 from the stomatal cavity to the chloroplast) was calculated as the ratio of P_N to C_i (Fischer *et al*. 1998). The measuring area of the standard leaf chamber was 4 cm^2, the CO_2 concentration was adjusted to 400 μmol mol^{-1} supplied by a CO_2 container, and the flow rate of 500 μmol s^{-1}. Leaf temperature and PPFD were maintained under the same conditions as that of the plant growth conditions at 25°C and 150 μmol m^{-1} s^{-1}, respectively. The gas-exchange parameters were obtained by averaging the records of a 30-s period upon a stable photosynthetic rate was reached (approximately 5 min). P_N–PPFD curve was fitted using a modified rectangular hyperbola model with the P_N data and light intensity to calculate the light-saturated maximum photosynthetic rate (P_{Nmax}), apparent quantum efficiency (AQE), light-compensation point (LCP), and dark respiration rate (R_D) according to Ye *et al.* (2013). The interval of light intensities of the P_N–PPFD curve was ranging from 2,000 to 0 μmol m^{-2} s^{-1}, the minimum waiting time was set to 120 s, and the maximum waiting time was set to 200 s.

Chl fluorescence and P_{700} measurement of the fully developed leaf were determined simultaneously using a

PAM fluorometer (*Dual-PAM-100*, *Walz GmbH*, Effeltrich, Germany). Plants were pre-darkened for 30 min before determination. F_m and P_m were obtained by applying two saturation pulses. The leaf was then subjected to a light adaption phase with actinic light intensity at 150 μmol(photon) m^{-2} s^{-1}, saturating light pulses were given every 60 s, and a far-red pulse after the actinic light was turned off. F_s represents steady-state chlorophyll fluorescence for the light-adapted state, while F_m' is the maximum fluorescence in the light-adapted state. The PSII parameters were determined as described by Lazár (2015). The maximum photochemical efficiency of PSII for the dark-adapted state was calculated as $F_v/F_m = (F_m - F_0)/F_m$, the effective quantum yield of PSII photochemistry for the light-adapted state as $\Phi_{PSII} = (F_m' - F_s)/F_m'$, the quantum yield of nonphotochemical fluorescence quenching was calculated as $\Phi_{NPQ} = F_s/F_m' - F_s/F_m$, and the quantum yield of nonregulated fluorescence quenching in PSII was calculated as $\Phi_{NO} = F_s/F_m$, where $\Phi_{PSII} + \Phi_{NPQ} + \Phi_{NO} = 1$.

The PSI parameters were measured according to Klughammer and Schreiber (1994) as follows: $\Phi_{PSI} = (P_m' - P)/P_m$, $\Phi_{ND} = P/P_m$, and $\Phi_{NA} = (P_m - P_m')/P_m$. P is the relative electron transfer efficiency, while P_m' is the maximum relative electron transfer efficiency. Φ_{PSI}, Φ_{ND}, and Φ_{NA} represent the effective quantum yield of PSI photochemistry in the light-adapted state, quantum yield of nonphotochemical energy dissipation in PSI in the light-adapted state due to the donor-side limitation, and quantum yield of nonphotochemical energy dissipation in PSI in the light-adapted state due to the acceptor-side limitation, respectively.

Statistical analysis: Five random individual plants were considered as five biological replicates, except quadruplicates for biomass and Chl fluorescence measurement were conducted. Statistical analysis was performed with *SPSS 26.0* (*SPSS Inc.*, Chicago, IL, USA). After verifying homoscedasticity by *Levene*'s test, a one-way analysis of variance (*ANOVA*) was conducted to test for significance between different treatments and *Turkey*'s multiple range test at $p<0.05$ for *ad hoc* analysis. Correlations between traits were tested using *Pearson*'s correlation coefficient.

Results

Plant growth and morphology: The greatest DM was observed under 40%B, although no significant difference was detected compared to other treatments (Table 1). The greatest FM was recorded for 40%B and W, while it was reduced under 15%B and 70%B, and significantly decreased under 70%B.

The total leaf area was the greatest under W; compared with W, no statistical difference was found for 15%B and 40%B treatments but it was reduced under 70%B and significantly decreased under 100%B. The plants grown under white light resulted in the greatest SLA, which was not statistically different from that under 15%B. SLA under 40%B and 15%B was lower than that of W though there was no significant difference between them, whereas the plants under 100%B resulted in the lowest specific leaf area.

Pigment content: Leaf Chl and carotenoids (Car) contents were influenced by light quality (Table 2). Overall, the

Table 1. Plant growth and leaf morphology of *Mesembryanthemum crystallinum* grown under different combinations of red and blue LED light. Values are means ± SE. *Different letters* indicate significant difference, as determined by *Tukey*'s multiple comparison tests ($P<0.05$, $n = 4$).

	Fresh mass [g per plant]	Dry mass [g per plant]	Leaf area [cm² per plant]	Specific leaf area [cm^2 g^{-1}]
W	43.38 ± 5.81^{a}	0.44 ± 0.07^{a}	349.44 ± 34.74^{a}	790.0 ± 99.0^{a}
15%B	32.15 ± 3.02^{ab}	0.39 ± 0.03^{a}	288.01 ± 26.90^{a}	736.6 ± 47.9^{a}
40%B	41.97 ± 3.40^{a}	0.54 ± 0.03^{a}	336.25 ± 19.00^{a}	633.2 ± 53.3^{ab}
70%B	28.70 ± 2.48^{ab}	0.45 ± 0.03^{a}	253.85 ± 18.59^{ab}	568.6 ± 6.7^{ab}
100%B	20.47 ± 0.48^{b}	0.39 ± 0.02^{a}	174.45 ± 5.53^{b}	449.9 ± 32.9^{b}

Table 2. Chlorophyll and carotenoid contents of *Mesembryanthemum crystallinum* grown under different combinations of red and blue LED light. Values are means ± SE. *Different letters* indicate significant difference, as determined by *Tukey*'s multiple comparison tests ($P<0.05$, $n = 4$).

	Chl content [mg g^{-1}(FM)]	Car content [mg g^{-1}(FM)]	Chl *a*/*b*	Chl/Car
W	0.219 ± 0.009^{bc}	0.047 ± 0.002^{bc}	3.41 ± 0.05^{b}	4.65 ± 0.06^{a}
15%B	0.228 ± 0.015^{bc}	0.046 ± 0.002^{bc}	3.58 ± 0.04^{ab}	4.99 ± 0.13^{a}
40%B	0.246 ± 0.013^{b}	0.050 ± 0.002^{ab}	3.69 ± 0.06^{a}	4.94 ± 0.22^{a}
70%B	0.284 ± 0.011^{a}	0.057 ± 0.003^{a}	3.70 ± 0.05^{a}	4.98 ± 0.07^{a}
100%B	0.191 ± 0.006^{c}	0.040 ± 0.002^{c}	3.79 ± 0.08^{a}	4.75 ± 0.08^{a}

total Chl and Car content increased with increasing blue light proportion for the dichromatic light treatments, and plants under 70%B resulted in the greatest total Chl and Car content. Monochromatic B led to the lowest Chl and Car content, which was significantly reduced compared with the W control. For the Chl *a*/*b* ratio, narrow-band light treatments induced a greater ratio of Chl *a*/*b* in comparison with W. There were no significant differences in the Chl/Car ratio between all the treatments.

Gas-exchange parameters: Evaluation of leaf gas exchange showed that red/blue light influenced the photosynthesis-related parameters (Fig. 2). P_N was the lowest under W and increased after the treatment of 40%B, while it was significantly greater under 70%B. C_i was higher for the dichromatic light treatments than that of control, 100%B showed no difference from W. The greatest value of stomatal conductance (g_s) was recorded under 40%B treatment, while it was not different from the W, 15%B, and 100%B, and was significantly lower for 70%B treatment.

Compared with W, the dark respiration rate (R_D) and LCP increased for all the dichromatic and monochromatic groups (Fig. 3). There was no significant difference found for P_{Nmax} for all the treatments (Fig. 3), AQE was the highest under 40%B and lower under W, 15%B, and 70%B without a statistical difference, and significantly lower under 100%B.

Chl fluorescence and P_{700}: Generally, in comparison with W-grown plants, both Φ_{PSII} and Φ_{PSI} were higher under the mixture of narrow-band R and B treatments (Fig. 4), and no statistical difference was observed between these treatments. There was no significant difference for Φ_{NPQ} and Φ_{NA} under different light qualities. Φ_{NO} and Φ_{ND} were the highest under W, Φ_{NO} decreased with the increase of

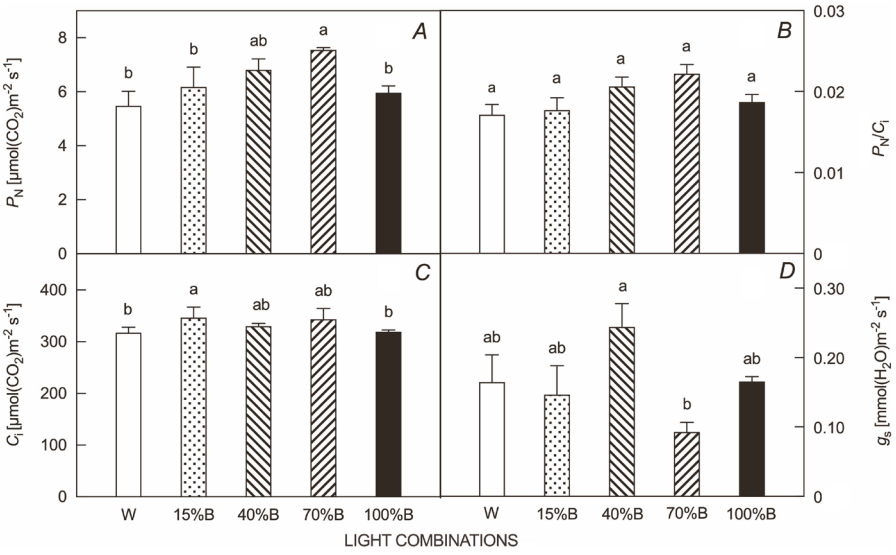

Fig. 2. Net photosynthetic rate (P_N) (*A*), P_N/C_i ratio (*B*), intercellular CO_2 concentration (C_i) (*C*), and stomatal conductance (g_s) (*D*) of *Mesembryanthemum crystallinum* grown under different combinations of red and blue light. Values are means ± SE. *Different letters* indicate statistical differences as determined by *Tukey*'s multiple comparison tests (P<0.05, n = 5).

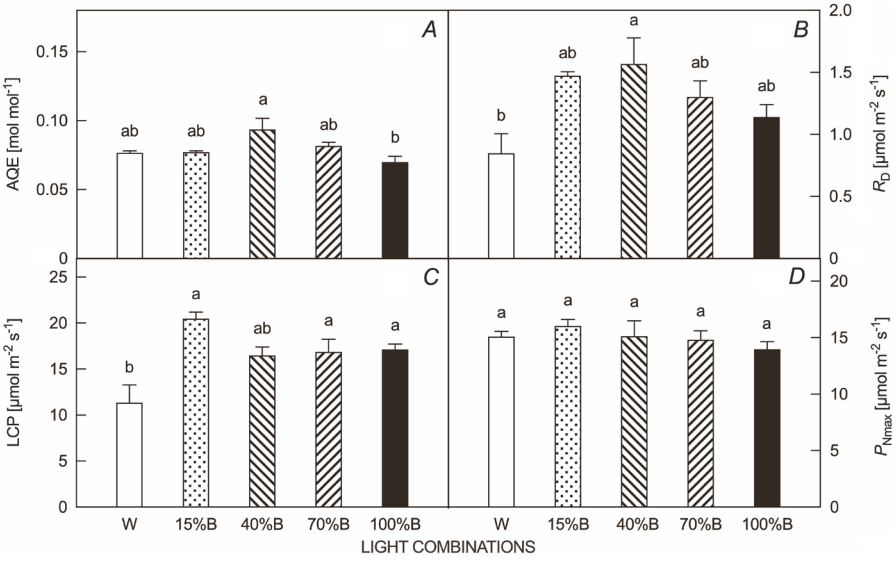

Fig. 3. Apparent quantum efficiency (AQE) (*A*), dark respiration rate (R_D) (*B*), light-compensation point (LCP) (*C*), maximum net photosynthetic rate (P_{Nmax}) (*D*) of *Mesembryanthemum crystallinum* grown under different combinations of red and blue light. Values are means ± SE. *Different letters* indicate statistical differences as determined by *Tukey*'s multiple comparison tests (P<0.05, n = 5).

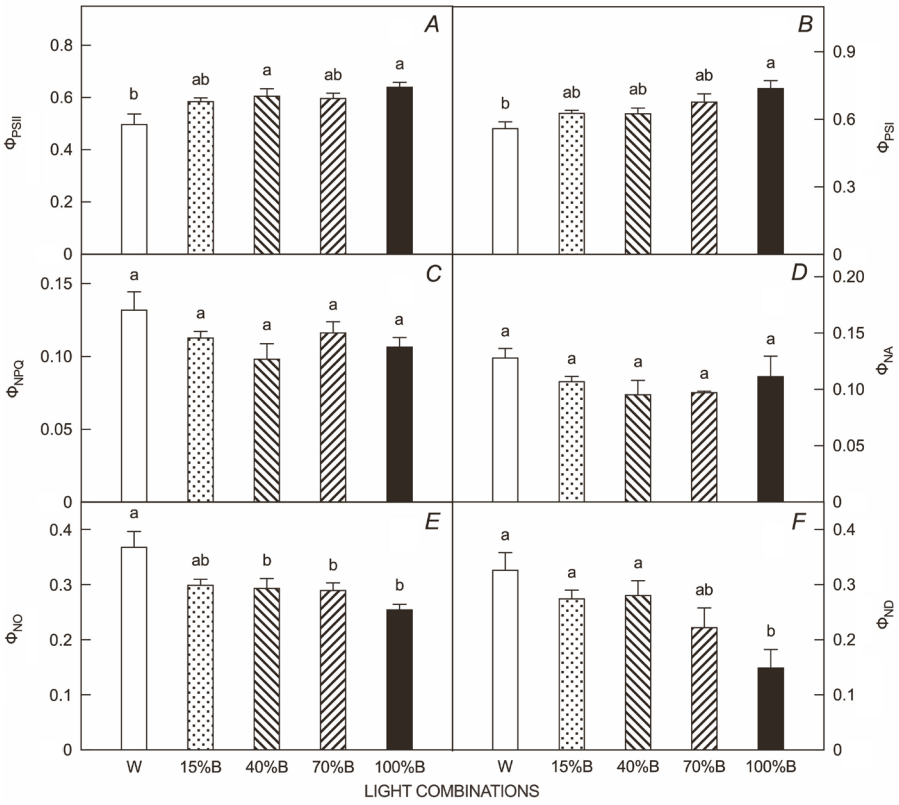

Fig. 4. The quantum yield fractions of three processes: effective quantum yield of PSII photochemistry in light-adapted state (Φ_{PSII}) (*A*), regulated nonphotochemical heat dissipation (Φ_{NPQ}) (*C*), and other nonphotochemical losses (Φ_{NO}) (*E*); effective quantum yield of PSI photochemistry in light-adapted state (Φ_{PSI}) (*B*), quantum yield of nonphotochemical energy dissipation in PSI in light-adapted state due to the acceptor-side limitation (Φ_{NA}) (*D*), and quantum yield of non-photochemical energy dissipation in PSI in light-adapted state due to the donor-side limitation (Φ_{ND}) (*F*) of *Mesembryanthemum crystallinum* grown under different combinations of red and blue light. Values are means ± SE. *Different letters* indicate statistical differences as determined by *Tukey*'s multiple comparison tests (P<0.05, n = 5).

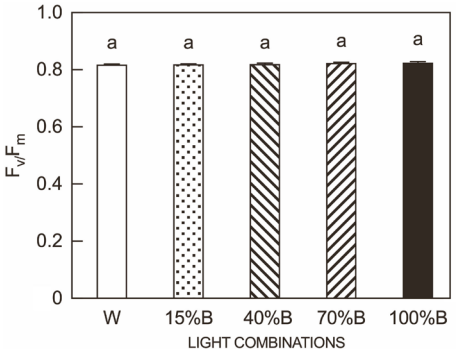

Fig. 5. Maximal quantum yield of PSII photochemistry in dark-adapted state (F_v/F_m) of *Mesembryanthemum crystallinum* grown under different combinations of red and blue light. Values are means ± SE. *Different letters* indicate statistical differences as determined by *Tukey*'s multiple comparison tests (P<0.05, n = 5).

blue light ratio, and was significantly lower under 40%B, 70%B, and 100%B while Φ_{ND} decreased for the plants under 70%B and 100%B.

The ice plants grown under 100%B showed the highest F_v/F_m value (Fig. 5), with no statistical difference in F_v/F_m values between the plants grown under white light and red/blue-combined light treatments.

MDA, soluble protein, and nitrate content: The plants grown under W resulted in the lowest MDA content and soluble protein content, while MDA content increased for all the plants grown under combined red and blue light (Fig. 6). *M. crystallinum* grown under 70%B had the greatest protein content. The W control resulted in the greatest nitrate content, which was approximately 1.80 mg g^{-1} and significantly greater than the other red/blue light treatments.

Total antioxidant capacity, total phenol and flavonoid content: Total antioxidant activity of the leaves under W and R/B combined treatments showed no statistical difference (Fig. 6); the greatest value was recorded in the 40%B treatment [24.20 μmol(L-AA) g^{-1}(FM)], while the lowest value was in 70%B treatment [20.86 μmol(L-AA) g^{-1}(FM)]. There was no significant difference between the different light treatments for the total flavonoid contents. The total phenolics content was the lowest under monochromatic blue light, while it was greater under 40%B, 70%B, and W, and significantly greater for the 15%B treatment.

Discussion

Plant biomass accumulation under a controlled environment is largely dictated by the light energy interception during growth. In this study, the growth and morphogenesis of the ice plants were strongly influenced by the different combinations of red and blue light. Under monochromatic blue light, plant DM, FM, and total LA were the lowest in comparison with red/blue treatments and W. This result is not surprising as amount of previous studies have proved that monochromatic blue or red light is not conducive to

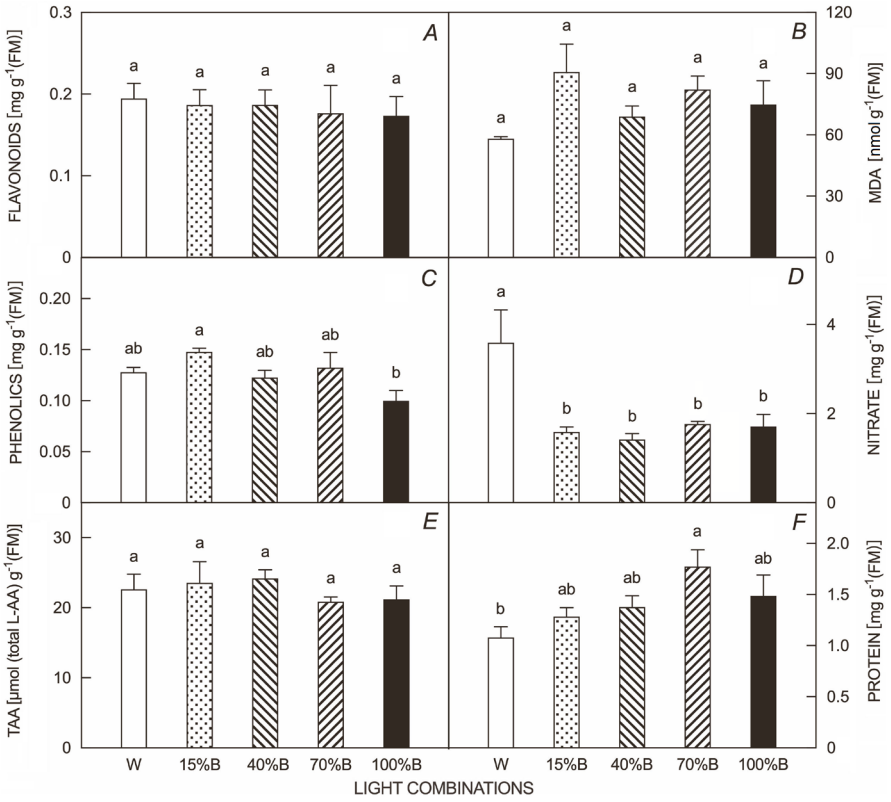

Fig. 6. Flavonoids content (*A*), malondialdehyde (MDA) content (*B*), phenolics content (*C*), nitrate content (*D*), total antioxidant activity (*E*), and soluble protein content (*F*) of *Mesembryanthemum crystallinum* grown under different combinations of red and blue LED light. Values are means ± SE. *Different letters* indicate statistical differences as determined by *Tukey*'s multiple comparison tests ($P<0.05$, $n = 5$).

plant growth and physiological performance (Wang *et al.* 2009, Hogewoning *et al.* 2010, Chang *et al.* 2016, Liu *et al.* 2020). B (blue light) is normally associated with inhibition of cell division or expansion (Dougher and Bugbee 2004, Okello *et al.* 2016, Izzo *et al.* 2020), which in consequence lead to the reduced LA under B alone in this study (Table 1). Consistent with our findings, combing red and blue lights could promote leaf expansion and biomass accumulation in comparison with monochromatic R or B plants of leafy lettuce and rapeseed (Chang *et al.* 2016, Wang *et al.* 2016).

SLA showed a strong negative correlation with the blue light proportion for all the groups ($r = –0.926$), which is in line with previous reports on other species including cucumber (Hogewoning *et al.* 2010), tomato (Zhang *et al.* 2019), and lettuce (Lin *et al.* 2013). In our study, the P_N of leaves increased in response to the increasing proportion of blue light ($r = 0.913$; Fig. 4), and this effect was not seen in monochromatic blue light. Lower SLA (*i.e.*, thicker leaf) generally indicates for 'sun-type' leaves with an increased amount of photosynthesis-related pigments and enzymes, which could enhance the photosynthetic capacity per area (Evans and Poorter 2001, Vile *et al.* 2005), P_{Nmax} showed a positive relation with SLA in our observation though it was not statistically significant ($r = 0.805$, $P=0.100$).

Light quality plays an important role in regulating the biosynthesis of photosynthesis-related pigments. Chl biosynthesis can be stimulated by the existence of blue photons (Sood *et al.* 2005), though this was also suggested to be a coaction of cryptochrome and active phytochrome (Cope *et al.* 2014, Hernández and Kubota 2016), absence of phytochrome in its active form might cause in case of the 100%B the decrease in Chl content in our study (Table 2). The high proportion of blue light (59%B, 47%B) can lead to a higher SPAD value of leaf lettuce (Son and Oh 2013), which is consistent with our results that the Chl and Car contents were greater for 40%B and 70%B treatments. The higher Chl *a*/*b* ratio normally indicates a high light-adapted photosynthetic apparatus with higher capacity for electron transport (Evans 1988); we also found an upward trend of the ratio of Chl *a*/*b* with the increasing percentage of blue light ($r = 0.752$). With a lower SLA and higher Chl *a*/*b* ratio, the ice plant leaves developed under a higher proportion of B could absorb light energy within the photosynthetic active range more efficiently (Zhen *et al.* 2019).

Blue light has been characterized as an environmental signal inducing stomatal and chloroplast movement mediated by its specific photoreceptors (Ichiro *et al.* 2016, Inoue and Kinoshita 2017). Correlation analysis between P_N, g_s, and P_N/C_i revealed that P_N was unrelated with g_s ($P=0.666$) but significantly correlated with P_N/C_i ($r = 0.959$, $P=0.010$), indicating that the increase of net photosynthetic rate with the blue light fraction was due to nonstomatal limitations and mainly resulted from the changed efficiency of available CO_2 to the chloroplasts in mesophyll cells (Dias and Brüggemann 2010, Arena *et al.* 2016). The present study shows that the net photosynthetic rate was also positively correlated with total soluble protein content ($r = 0.853$, $P=0.066$). Since Rubisco is the main protein component in plant leaves, this result suggests that the increase of total soluble protein may contribute to

the enhancement of photosynthetic ability under a certain portion of blue light. This result is in agreement with the previous investigation that the enhanced photosynthetic rates induced by the blue light supplement to red light control were associated with higher total N content of leaves including Rubisco and LHCII (Matsuda *et al*. 2004).

F_v/F_m of most unstressed plant species usually fluctuates between 0.75 and 0.85 (Quiles 2005). Our results indicated that the light treatments did not cause different degrees of stress on the ice plants. Φ_{PSII} and Φ_{PSI} reflect the fraction of absorbed light energy used for photosynthetic electron transfer. In agreement with previous reports (Hogewoning *et al*. 2010, Kaiser *et al*. 2019), both Φ_{PSII} and Φ_{PSI} were the highest under blue light and the lowest under white light, indicating the greatest percentage of functional PSII centers and electron transport capacity for the plants under blue light in the present study. Φ_{NPQ} and Φ_{NO} reflect the quantum yield for dissipation by down-regulation and other nonphotochemical losses, respectively (Dreuw *et al*. 2005). Plants employ NPQ mechanisms to protect themselves from excessive excitation energy by harmless heat dissipation. In the present study, there was no significant difference in Φ_{NPQ} between all treatments, indicating that the ability of regulated dissipation in the form of heat was unaffected by light quality. Decreased Φ_{NO} for the narrow-band R/B or B treatments indicates the increase of PSII photochemical energy conversion by reducing the fraction of nonprotective light dissipation (Shibaeva and Markovskaya 2013).

Blue light radiation, due to its relatively high photon energy, could stimulate the accumulation of reactive oxygen species, thereby causing the synthesis of protective secondary metabolites (Ort 2001, Wu *et al*. 2007, Jaleel *et al*. 2009). MDA content represents the degree of membrane lipid peroxidation and it provides an estimation of the oxidative stress level of the plants. In this study, MDA content in the red/blue light treatments was numerically greater than that in the control group, which may be attributed to the free radicals accumulation induced by these light treatments. Jing *et al*. (2018) have demonstrated that blue light induces the accumulation of hydrogen peroxide, which induced phenylalanine metabolism and resulted in the synthesis of phenolics and flavonoids.

Previous observation showed that compared with white light, the total phenol content of Chinese kale under red light decreased significantly, while the total phenol content under LED blue light increased (Qian *et al*. 2016). In this study, there was no significant difference in the total flavonoids under different blue light ratios. Adding blue light generally enhances the phenolics and flavonoids content in plants as reported in many previous reports (Ouzounis *et al*. 2014, Fazal *et al*. 2016, Zheng and Van Labeke 2017), which is mediated by regulation of the expression of key genes in their metabolic pathway (Thwe *et al*. 2014). This effect could be species- or genotype-dependent as confirmed in our previous investigation on chrysanthemum, in which three of eight cultivars studied resulted in unaffected phenolics content in response to different light quality (Zheng and Van Labeke 2017).

Phenolic compounds and flavonoids as their subgroup in the plants are known as antioxidants with the ability in scavenging free radicals (Chen and Chen 2013). There was a positive correlation between flavonoids content and total antioxidant activity (r = 0.886, P=0.057) in the present study. Nitrate is necessary for plant development, though it can be reduced into nitrite-nitrogen, especially in leafy vegetables, which is a potential health hazard for humans (Bian *et al*. 2018). In comparison with W, the red/blue light treatment reduced the lowest nitrate-nitrogen content in the leaves (Fig. 6), indicating the combination of the red and blue spectrum as a promising method in the production of the ice plants for preventing nitrate accumulation. This is in agreement with the conclusion of Zhou *et al*. (2013), who reported that pre-harvest exposure to 48 h continuous LED light with 20%B could effectively reduce nitrate accumulation in lettuce.

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