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Growth, Development, and Chemical Constituents of Edible Ice Plant (*Mesembryanthemum crystallinum* L.) Produced under Combinations of Light-emitting Diode Lights

Thitipat Weeplian and Tsair-Bor Yen
Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan

Yunn-Shy Ho[1]
Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan

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Abstract. **To investigate the effects of light treatments on the growth morphology and chemical constituents of *Mesembryanthemum crystallinum* L. plants, red (R), blue (B), far red (Fr), and white (W) light-emitting diodes (LEDs) were configured to provide different combinations of light spectra and photosynthetic photon flux densities (*PPFDs*). In Expt. 1, five light spectra of red/white (RW), red/white/far red (RWFr), red/white/high-intensity far red (RWFrD), red/blue (RB), and red/blue/far red (RBFr) were set up in two 3-layered racks with circulating hydroponic systems. In each light spectrum treatment, the distance between the LED lamps and the transplanting board was regulated to provide low *PPFD* and high *PPFD* treatments. In Expt. 2, the effect of Fr was further investigated in plants in the early and late growth stages. RWFr light was modified by covering the Fr lamps to become red/white without far red (RW − Fr) light during the early growth stage, and then removing the covers to provide the Fr spectrum red/white with far red (RW+Fr) during the later growth stage. This study suggested that high *PPFD* was not beneficial for promoting plant growth in any light spectrum treatment. Among light spectrum treatments at a *PPFD* of 215 ± 15 μmol·m^{-2}·s^{-1}, RW light produced higher vegetative growth. In the late growth stage, RW and RB combined with Fr light promoted reproductive growth, antioxidant activities, and secondary compounds, such as phenolic compounds, pinitol accumulation, and betacyanins. Therefore, RW (227 μmol·m^{-2}·s^{-1}), RW − Fr (162 μmol·m^{-2}·s^{-1}), and RB (162 μmol·m^{-2}·s^{-1}) are suggested for the early growth stage to promote vegetative growth. Then additional Fr light can be applied in addition to RW for secondary metabolite induction in the late growth stage.**

These days, consumers increasingly demand a diet for human health and well-being that includes high-quality vegetables that are free of pesticides and other harmful residues. Controlled environmental agriculture, such as plant factories and application of advanced electronic facilities, has become the new production system to meet those requirements (Albright and Langhans, 1996). In recent years, food and pharmaceutical companies have spent great efforts in developing natural products extracted from plants to produce high-quality food and remedies that are affordable to consumers (Falleh et al., 2011).

Mesembryanthemum species (of the Aizoaceae family) are halophytes widely found in semiarid zones of Tunisia (Adams et al., 1998; Bohnert and Cushman, 2000). *Mesembryanthemum crystallinum* is well known for its enzymatic antioxidant activity by betacyanin and other flavonoids, which can detoxify reactive oxygen species (Agarie et al., 2009; Hanen et al., 2009; Ibdah et al., 2002; Ślesak et al., 2008; Vogt et al., 1999). In addition, this plant possesses the ability to rapidly accumulate phytochemicals and secondary metabolites, such as beta-carotene, pinitol, betacyanin, phenolic compounds, and flavonol conjugates, in a cell-specific manner. Moreover, *M. crystallinum* is used to medically treat ocular infections and has become a good candidate for pharmaceutical and cosmetic applications (Agarie et al., 2009; Falleh et al., 2011; Ibdah et al., 2002).

Light is an important environmental factor that affects plant survival, growth, and reproduction and also influences the metabolism of phytochemicals in plants (Li and Kubota, 2009). The light spectrum, quantity, and duration that plants receive all affect photosynthesis, plant growth, and development (Folta and Childers, 2008; Liao et al., 2006; Samuolienė et al., 2011; von Arnim and Deng, 1996). Natural light received by a plant is visible light in the range of wavelengths of 400–700 nm, and this range of light is called photosynthetic active radiation (*PAR*). Fluctuation of natural light is the major limiting factor for open-field and greenhouse plant production. Therefore, a supplementation with artificial lighting is essential to maintaining stable year-round vegetable production, which is mostly used in greenhouses of boreal and temperate climatic zones such as in Scandinavia, Estonia, Russia, and Canada (Vänninen et al., 2010). Light acts as a plant signal and is known to affect germination, seedling development, flowering time, leaf movements, phytochemical development, and other aspects of plant biology (Spalding and Folta, 2005). Recently, most studies used B, green (G), R, and Fr spectra of LED lighting as a potential light source for plant growth and to determine if the requirements for plant photosynthesis and photomorphogenesis were met (Samuolienė et al., 2011).

To evaluate the antioxidant activity of plant tissues, the commonly used in vitro free radical method is the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Shalaby and Shanab, 2013). The DPPH method is used to determine the activity of antiradical/antioxidant of plant extracts (Fukumoto and Mazza, 2000; Shalaby and Shanab, 2013). In addition to antioxidative properties, the major functional compound that accumulates in *M. crystallinum* plants is pinitol, which has an insulin-like function and may be helpful in regulating human metabolism and lowering blood glucose levels (Chen et al., 2014; Chiera et al., 2006). Lee et al. (2014) evaluated the effect of D-pinitol and *M. crystallinum* water extracts on blood glucose levels in a rat model. Results showed that *M. crystallinum* extracts significantly suppressed blood glucose, similar to the effect of D-pinitol.

Previous studies focused on physiological responses of plants under environmental stresses. For the mass-scale hydroponic production of high yields of high-quality *M. crystallinum* plants as edible vegetables in a controlled environment, advanced studies on LED light formulas are necessary. Therefore, the aim of this study was to investigate the effects of different combinations and light intensities of R, G, B, and Fr light spectra on the phytochemicals and secondary metabolites of edible *M. crystallinum*.

Materials and Methods

Experimental setup and growth conditions. *Mesembryanthemum crystallinum* seeds were

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[1]Corresponding author. E-mail: ysho@mail.npust.edu.tw.

sown in a 406-cell tray filled with medium sand (with a particle size of 0.42–2.0 mm) and germinated under a *PPFD* of 140–190 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot d^{-1}$ provided by fluorescent T5 lamps under 23 °C day/18 °C night air temperatures in a growth chamber. The seedlings were watered twice a day and supplemented with 1/2 strength (S) of modified Taichung District Agricultural and Extension Station Nutrient Solution for Leafy Vegetables (TDAES, NO_3^- 84 $mg \cdot L^{-1}$, NH_4^+ 7 $mg \cdot L^{-1}$, P 16 $mg \cdot L^{-1}$, K 146 $mg \cdot L^{-1}$, Ca 40 $mg \cdot L^{-1}$, Mg 12.4 $mg \cdot L^{-1}$, S 16.3 $mg \cdot L^{-1}$, Fe 0.05 $mg \cdot L^{-1}$, Mn 0.50 $mg \cdot L^{-1}$, Zn 0.02 $mg \cdot L^{-1}$, Cu 0.01 $mg \cdot L^{-1}$, B 0.21 $mg \cdot L^{-1}$, Mo 0.05 $mg \cdot L^{-1}$, and an electrical conductivity of 1.2 $mS \cdot cm^{-1}$) every 7 d. Thirty days after sowing (DAS), seedlings with four pairs of true leaves were transplanted to a vertical circulating hydroponic system. According to results of previous studies (data not shown), 1 S of TDAES supplemented with 100 mM NaCl was used as the nutrient solution. Different light spectra with two light intensities were applied.

Light treatments. In Expt. 1, to determine the correlation between light spectra and plant growth, five combinations of R, B, Fr, and W LED lights (Philips GreenPower LED; Philips Lighting Holding, Amsterdam, The Netherlands) were set up. Light spectrum treatments included RW, RWFr, RWFrD, RB, and RBFr. The spectral distribution of each treatment was measured with a spectrometer (HR-350; Hipoint, Kaohsiung, Taiwan) at 15 cm under the LED lamps as shown in Fig. 1. To find the optimal light intensity of each spectrum, two treatments were set up under each light spectrum by regulating the distance of the planting boards under the LED lamps to 30 cm (high *PPFD*) and 45 cm (low *PPFD*). The *PPFD* of each light spectrum was measured by *PAR* Smart Sensor (Onset Computer, Bourne, MA) (Table 1). To compare the effects of different light spectra, the light intensity of a selected treatment was maintained at a mean *PPFD* of 215 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which nonsignificantly differed among the light spectrum treatments.

Expt. 2 repeated the five light spectrum treatments as in Expt. 1, but only the low *PPFD* of each light spectrum was used. Additional light treatments with and without Fr were tested. The RW–Fr treatment was applied during 0–75 DAS in the early growth stage and RW+Fr afterward until the final harvest (at 105 DAS). RW–Fr was configured by covering the Fr lamps. The RW+Fr treatment was configured by removing the covers to provide the Fr spectrum. The RW–Fr and RW+Fr spectra are shown in Fig. 1. The light intensity was maintained at a *PPFD* of 165 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 1).

Experimental layout. Expt. 1 was conducted in May to Aug. 2014 in an air-conditioned room (28 °C day/23 °C night air temperature and 65% to 75% relative humidity). The photoperiod was set to 12 h of light (0800 to 2000 HR) and 12 h of dark (2000 to 0800 HR). Ten light treatments were set up in two three-layered racks (with one light spectrum per layer). There was one circulating hydroponic system in each rack that used troughs as growing containers. Each treatment included three troughs as replications. There were 18 plants in each trough. The experimental layout followed a completely randomized design.

Expt. 2 was conducted in Feb. to May 2015 at 25 °C day/20 °C night air temperature. The relative humidity and photoperiod were similar to those in Expt. 1. Each treatment had six troughs as replications.

Measurements of plant growth and morphology. Morphological measurements in Expt. 1 were performed twice at 60 and 90 DAS. Nine plants of each treatment were harvested, and the shoot fresh weight (FW), stem and internode lengths, number of branches, number of flowers, number of leaves, and total leaf area were determined. Plant tissues were dried at 80 °C in a hot air oven for 72–96 h before determining the dry weight (DW). However, because of the slower rate of root recovery after transplantation in Expt. 2, morphological measurements were performed twice at 75 and 105 DAS. Each time, 18 plants from each light spectrum were harvested, and the measurement and data analytical procedures of Expt. 1 were followed.

DPPH radical scavenging. The scavenging effect on the DPPH radical was measured following the method described by Shimada et al. (1992). The absorbance of the extract was read at 517 nm using an ultraviolet-VIS 911 Spectrophotometer (GBC Scientific Equipment, Victoria, Australia), with methanol used as a blank. The scavenging effect on the DPPH radical was calculated by the following equation:

$$\text{DPPH radical} - \text{scavenging effect } (\%) = [(A_c - A_s)/A_c] \times 100, \quad [1]$$

where A_c and A_s are the absorbances of the control and the sample, respectively, at 30 min.

Total phenolic compounds. The samples were prepared using the method described by Falleh et al. (2011). The filtrate was analyzed using a Purospher® STAR RP-18 endcapped (5 µm) Hibar® RT 250-4.6 column (Merck, Darmstadt, Germany) with an L-7420 ultraviolet-VIS Detector (Hitachi, Tokyo, Japan) to separate the sample components. Each 10 µL sample was chromatographed at a flow rate of 1 $mL \cdot min^{-1}$. The mobile phase was 10% acetonitrile and 90% of 2.5% acetic acid. The absorption was read at 280 nm. The total amount of phenolic compound was calculated by an absolute calibration curve method, reported as milligrams of gallic acid equivalent (mg GAE/kg).

Pinitol accumulation. Pinitol was measured according to a procedure described by Agarie et al. (2009) with slight modification in high-performance liquid chromatography procedure. The filtered sample was analyzed using a Mightysil NH_2 250-4.6 (5 µm) column (Kanto Chemical, Tokyo, Japan) with an L-7420 ultraviolet-VIS Detector. The eluent was MilliQ water, and each 50 µL sample was eluted at a flow rate of 1 $mL \cdot min^{-1}$. The concentration was calculated by an absolute calibration curve method using commercial D-pinitol (Sigma-Aldrich, St. Louis, MO) as a standard.

Betacyanins. The analytical method was described by Vogt et al. (1999). A Hypersil ODS C18 reverse-phase column with a particle size of 5 µm (Thermo Scientific, Waltham, MA) with an L-7420 ultraviolet-VIS Detector was used. Each 10 µL sample was eluted at a flow rate of 1 $mL \cdot min^{-1}$. The eluents were 1.5% H_3PO_4 (A) and 80% acetonitrile (B), which were programmed to a linear gradient of 30 min from 10% B/90% A to 45% B/55% A. The absorbance of betacyanin was read at 540 nm. Betanin isolated from red beet extract diluted with dextrin (Sigma-Aldrich) was used as the standard.

Statistical analysis. Data analyses of all measurements in Expt. 1 were based on a completely randomized design, and the mean separation among light spectrum treatments was performed by the least significant

Table 1. Photosynthetic photon flux density (*PPFD*) of each light spectrum treatment under light-emitting diode (LED) lamps in the two experiments. In each spectrum, two light intensity treatments were created by setting planting boards at 30 (high *PPFD*) and 45 cm (low *PPFD*) under the lights. The target light intensity for installing LED lamps was measured at 15 cm under lamps for each light spectrum.

	Light spectrum[z]						
	RW	RWFr	RWFrD	RB	RBFr	RW–Fr	RW+Fr
Light intensity	*PPFD* ($\mu mol \cdot m^{-2} \cdot s^{-1}$)						
45 cm (low *PPFD*)	227.7 b[y]A[x]	168.0 bB	225.0 bA	162.4 bB	225.6 bA	162.0 bB	168.0 bB
30 cm (high *PPFD*)	243.7 aA	197.3 aB	244.7 aA	205.3 aB	247.6 aA	—	—
15 cm	277.7 a[w]	251.5 a	300.8 a	270.5 a	295.2 a	245.0 a	251.5 a

[z]Light spectrum treatments included combinations of red (R), blue (B), white (W), far red (Fr), and high-intensity far red (FrD) LED lights.
[y]Different lowercase letters in a column indicate a significant difference among light intensity treatments ($P < 0.05$, $n = 10$).
[x]Different capital letters in a row indicate a significant difference among light spectrum treatments ($P < 0.05$, $n = 10$).
[w]Different lowercase letters in a row indicate a significant difference between the target light intensity at 15 cm under the lamps ($P < 0.05$, $n = 10$).

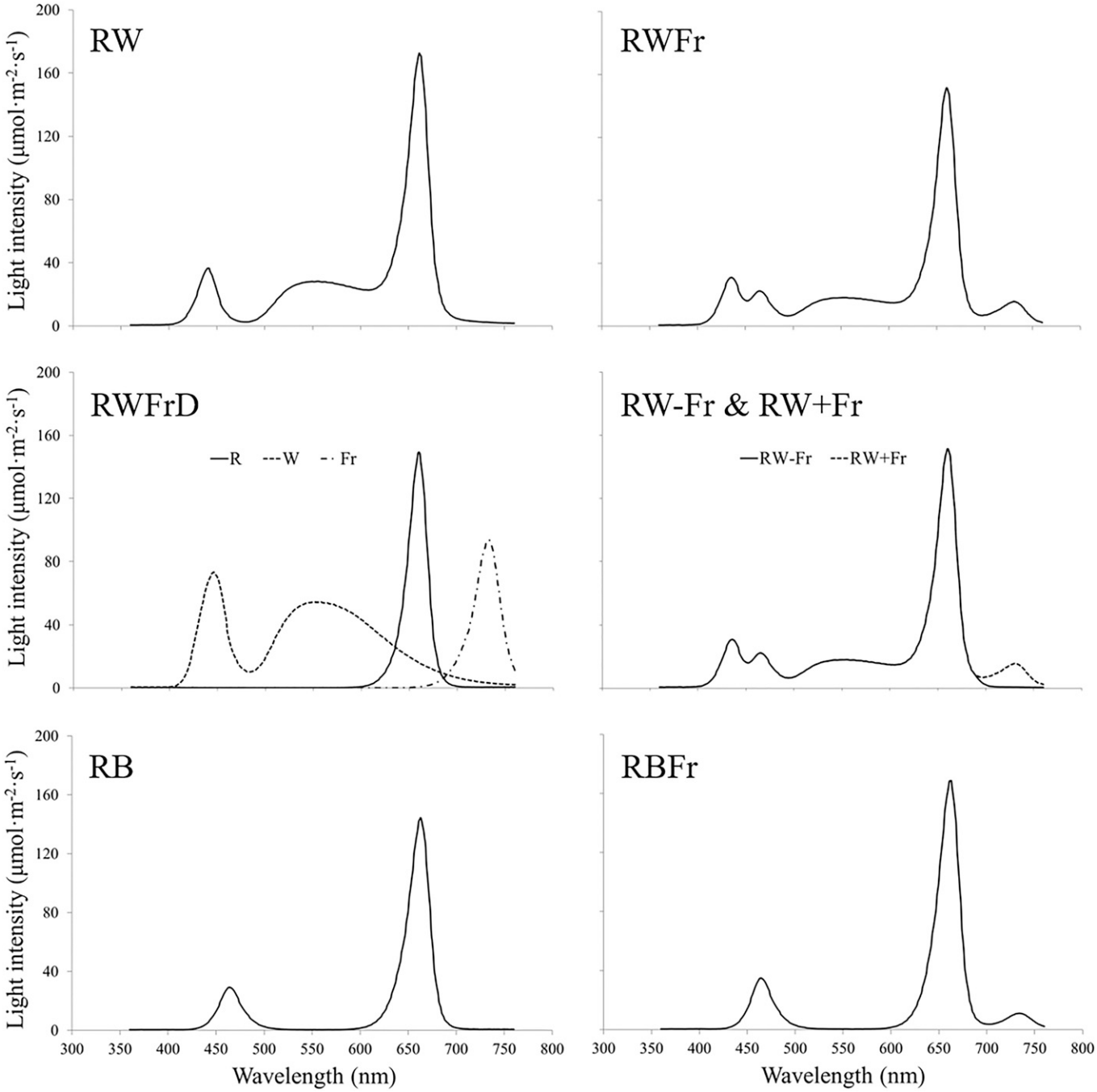

Fig. 1. Spectral distribution of light-emitting diode (LED) light treatments in two experiments: red/white (RW), red/white/far red (RWFr), red/white/high-intensity far red (RWFrD), red/blue (RB), and red/blue/far red (RBFr). In Expt. 2, an extra layer of RWFr light was modified by covering the far red lamps red/white without far red (RW–Fr) in the early growth stage and later removing the covers red/white with far red (RW+Fr). Light spectra were measured at 15 cm under the LED lamps.

difference test ($\alpha = 0.05$). An independent two-sample *t* test was used to compare the difference between two different light intensity treatments of individual light spectra. The effects of the RW–Fr/RW+Fr and RWFr in Expt. 2 were compared by an independent two-sample *t* test. SAS software version 9.4 (SAS Institute, Cary, NC) was used for all analyses.

Results and Discussion

Expt. 1. Light intensities of each treatment are shown in Table 1. The *PPFDs* of all light treatments did not significantly differ at 15 cm under the LED lamps. However, increasing the distance between the plants and LED lamps caused the *PPFD* to differ because of the optical design of different LED lamp modules. This reason caused different *PPFDs* at 30 and 45 cm under LED lamps to differ among the five light spectra. Morphologies of *M. crystallinum* plants grown under different light treatments are shown in Fig. 2.

Shoot FW and DW. Shoot FWs of plants subjected to low *PPFD* under RW (36.81 ± 3.74 g), RWFrD (30.99 ± 1.23 g), and RB (37.49 ± 1.49 g) were significantly heavier than that of high *PPFD* plants at 60 DAS. Plants of the RWFr and RBFr treatments had the same trend although they were not significant (Table 2). In 90-DAS plants, the trend was similar under the two light intensities except for the RWFr spectrum for which the FW of low *PPFD* was lower than that of high *PPFD* (72.23 ± 11.16 vs. 77.75 ± 9.28 g, respectively). In the second sampling, the FW (64.88 ± 6.33 g) of the RWFrD treatment with low *PPFD* was still significantly heavier (48.18 ± 4.68 g) than that of

high *PPFD*. DWs were similarly affected by the two light intensity treatments in the plants of the two samplings. However, only the DW of RWFrD treatment in 60-DAS plants significantly differed (1.39 ± 0.14 g at low *PPFD* and 0.97 ± 0.08 g at high *PPFD*). The higher light intensity did not increase the biomass of the plant tissues.

Comparing FWs of five spectrum treatments at a *PPFD* of 215 ± 15 μmol·m^{-2}·s^{-1}, RW treatment (36.81 ± 3.74 g) produced the highest weight and was significantly heavier than RB treatment (28.37 ± 2.04 g) in 60-DAS plants (Table 3). In 90-DAS plants, RW treatment (96.77 ± 10.56 g) produced heavier plants than did RWFr, RWFrD, RB, and RBFr treatments (77.75, 64.88, 67.77, and 66.83 g, respectively). Light spectrum treatments did not affect the DWs of plants of either sampling. However, RW plants had a heavier weight, which were lighter under RB treatment.

Plant growth and development were strongly influenced by B or R light because chlorophyll molecules that more efficiently absorb these two spectra (Hogewoning et al., 2010; Kim et al., 2004a; Terashima et al., 2009; Vogelmann and Han, 2000). Kim et al. (2004b) used R light to promote the leaf biomass of chrysanthemum plantlets. The FW of *Lactuca sativa* plants was greatly promoted under a combination of fluorescent lamps and R LED lights (Kim et al., 2004a). On the other hand, Olle and Viršilė (2013) reviewed that using only R light supplementation was ineffective at increasing the biomass of *Lycopersicon esculentum*, *Cucumis sativus*, and *Capsicum annuum* plants, whereas the growth and yield of these species were accelerated by a combination of R and Fr light. An increase in the end-of-day (EOD) Fr light intensity (10 μmol·m^{-2}·s^{-1} for 10 min) in *L. esculentum* seedlings resulted in a 15% increase in the stem FW (Cao et al., 2016), which indicated that a combination of R and Fr lights influenced biomass production in certain species, and the impact also varied by species. Therefore, *M. crystallinum* plants exposed to 90% R and 10% B resulted in higher shoot and root biomass than R or B alone (He et al., 2017). The previous finding was similar to that of our study, and light treatments incorporating Fr or high-intensity Fr did not perform better than only RW light.

Stem length. Stem length was unaffected by different *PPFDs* in all light spectra at 60 DAS. However, high *PPFD* inhibited stem elongation of RB (3.33 ± 0.18 cm) and RBFr (3.98 ± 0.28 cm) treatments at 90 DAS (Table 2). The different light spectrum treatments at a *PPFD* of 215 ± 15 μmol·m^{-2}·s^{-1} showed that stem length of RWFrD was significantly the longest in both samplings (3.17 ± 0.52 and 7.57 ± 0.52 cm, respectively). Among Fr treatments, RWFrD had the lowest R:Fr ratio (1.9) compared with RBFr (15.1) and RWFr (9.6) (Table 4) and, thus, it promoted stem and internode elongation

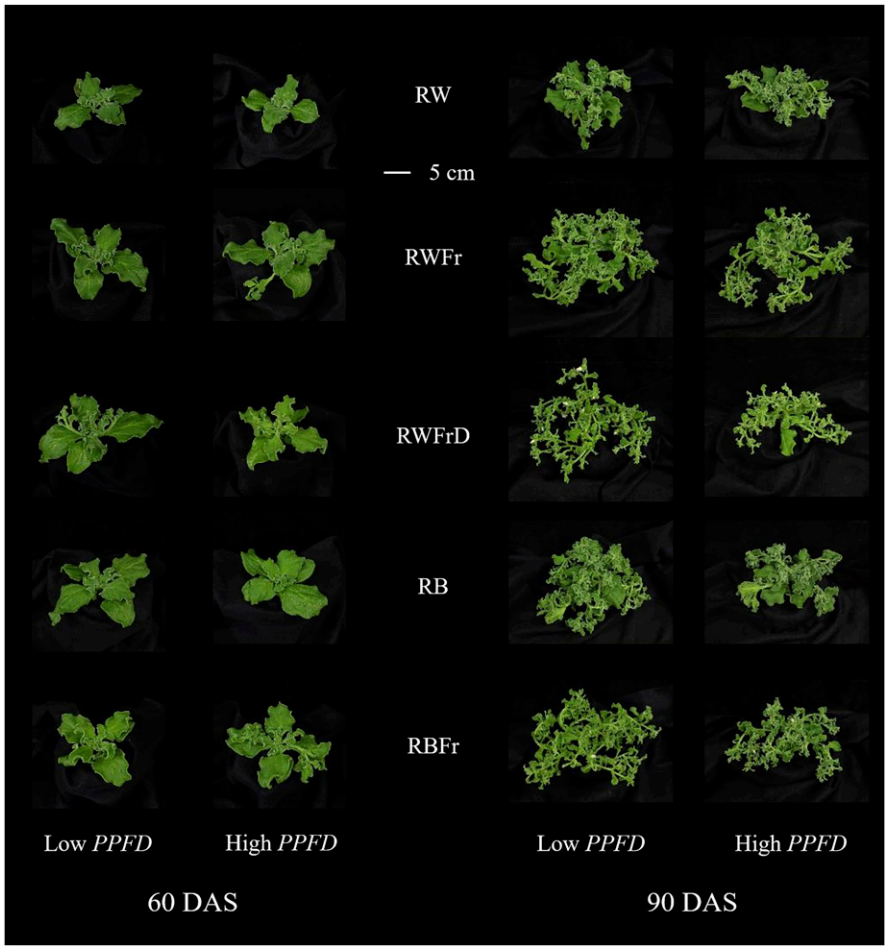

Fig. 2. Morphology of *Mesembryanthemum crystallinum* plants grown under different light spectra and light intensities at 60 and 90 d after sowing (DAS) in Expt. 1. Treatments included red/white (RW), red/white/far red (RWFr), red/white/high-intensity far red (RWFrD), red/blue (RB), and red/blue/far red (RBFr). Expt. 1 was conducted at 28 °C day/23 °C night air temperatures. Bar indicates 5 cm. *PPFD* = photosynthetic photon flux densities.

Table 2. Shoot fresh weight (FW) and dry weight (DW) and stem length of *Mesembryanthemum crystallinum* plants grown under different light treatments in two growth periods.

		FW (g)		DW (g)		Stem length (cm)	
Light spectrum[z]	Light intensity	60 DAS[y]	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS
RW	Low	36.81 ± 3.74 a[x]	96.77 ± 10.56 a	1.48 ± 0.15 a	4.03 ± 0.43 a	1.59 ± 0.14 a	4.19 ± 0.17 a
	High	25.72 ± 1.24 b	78.15 ± 4.84 a	1.31 ± 0.11 a	4.04 ± 0.42 a	1.56 ± 0.19 a	3.74 ± 0.26 a
RWFr	Low	32.38 ± 2.00 a	72.23 ± 11.16 a	1.42 ± 0.13 a	3.71 ± 0.50 a	1.71 ± 0.19 a	6.06 ± 0.38 a
	High	30.94 ± 2.41 a	77.75 ± 9.28 a	1.41 ± 0.16 a	3.87 ± 0.36 a	2.19 ± 0.20 a	4.62 ± 0.60 a
RWFrD	Low	30.99 ± 1.23 a	64.88 ± 6.33 a	1.39 ± 0.14 a	3.35 ± 0.25 a	3.17 ± 0.52 a	7.57 ± 0.52 a
	High	24.33 ± 1.79 b	48.18 ± 4.68 b	0.97 ± 0.08 b	2.75 ± 0.24 a	2.98 ± 0.24 a	6.79 ± 0.30 a
RB	Low	37.49 ± 1.49 a	79.79 ± 4.40 a	1.35 ± 0.10 a	3.36 ± 0.18 a	1.74 ± 0.19 a	5.56 ± 0.60 a
	High	28.31 ± 2.04 b	67.77 ± 6.34 a	1.13 ± 0.08 a	3.18 ± 0.30 a	1.80 ± 0.22 a	3.33 ± 0.18 b
RBFr	Low	32.58 ± 2.81 a	66.83 ± 9.09 a	1.42 ± 0.11 a	3.67 ± 0.24 a	1.72 ± 0.16 a	5.58 ± 0.68 a
	High	31.17 ± 2.87 a	53.98 ± 5.23 a	1.48 ± 0.14 a	3.14 ± 0.33 a	1.62 ± 0.17 a	3.98 ± 0.28 b

[z]Light spectrum included combinations of red (R), blue (B), white (W), far red (Fr), and high-intensity far red (FrD) light-emitting diode lights.
[y]DAS = days after sowing.
[x]Means ± SE with different letters indicate significant difference between low photosynthetic photon flux density (*PPFD*) (Low) and high *PPFD* (High) in each light spectrum by an independent two-sample *t* test ($P < 0.05$, $n = 9$).

in test plants. These results revealed an important effect of phytochrome mediation under R and Fr light on the growth and development of higher plants. The active form of phytochrome (P_{fr}) responds to R, whereas it switches to an inactive form (P_r) under Fr exposure (Demotes-Mainard et al., 2016; Nagatani, 2010). Previous studies in some plant species such as *L. sativa* and *Oryza sativa* were reported by Behringer et al. (1990), which indicated that phytochrome signaling under a low R:Fr ratio induced gibberellin synthesis and promoted stem elongation in plants. Under an R light–deficient environment, the height of *Campanula carpatica* increased 65% and that of *Pisum sativum* 23% (Runkle and Heins, 2001). Cerny et al. (2003) used a photoselective film to reduce the R:Fr ratio from 1.51 to 0.77, which resulted in 35% elongation in *Zinnia elegans*, 17% in *Dendranthema ×grandiflorum*, 14% in both *Cosmos bipinnatus* and *Petunia ×hybrida*, and 10% in *Antirrhinum majus*. Similar to *Eustoma grandiflorum* plants, a longer internode length was observed under R:Fr ratio of smaller than 1.0–2.0. Stem elongation also increased by increasing the Fr light ratio (Yamada et al., 2009). Chia and Kubota (2010) studied the effect of different ratios of Fr light at the EOD on *L. esculentum* seedlings. They discovered that hypocotyl elongation increased by 20% under low R:Fr ratio (0.47), and even further elongated (44%) under an R: Fr ratio of 0.05.

Number of branches. Branch development in RW light was promoted by low *PPFD* (3.1 ± 0.6 branches per plant) at 60 DAS. Different *PPFD*s did not affect branch development at 90 DAS in any light spectrum treatments (Table 5). At a *PPFD* of 215 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the number of branches in 60-DAS plants under five spectrum treatments ranged 2.4–4.5 branches per plant. The number of branches in 90-DAS plants was higher under RW (8.8 ± 0.6 branches) and RWFr (8.3 ± 0.4 branches) treatments than the 6.9 ± 0.5 branches per plant with RWFrD treatment (Table 6). From Table 4, the portion of G spectrum in RWFrD treatment was 14.4%, which was higher than the portion of G in RW (11.9%) and RWFr (7.6%). This phenomenon suggests that either a lower R:Fr ratio (1.9) in RWFrD treatment or a higher G portion would inhibit the development of branches. The low R: Fr ratio and G portion contributed to a shade-avoidance response in plants, which was characterized by inhibition of axillary bud development. These two factors resulted in enhanced stem elongation but reduced branching (Ballaré and Casal, 2000; Leduc et al., 2014; Zhang and Folta, 2012).

Number of leaves. In 60-day-old plants, low *PPFD* treatment produced more leaves with RW (29.0 ± 5.8 leaves) and RWFrD treatment (23.3 ± 3.7 leaves), whereas there was less leaf production with RWFr treatment (20.6 ± 2.3 leaves). RB and RBFr treatments were unaffected by different *PPFD* levels in the early growth stage. At 90 DAS, although low *PPFD* did not significantly promote leaf development, results showed a trend of enhancement (Table 5). Low *PPFD* of RWFr treatment had a light intensity of 168 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 1), which was lower than the low *PPFD* of RW and RWFrD treatments (225 and 228 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively), suggesting that a *PPFD* of 168 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of RWFr treatment might be lower than the optimum required for leaf development of *M. crystallinum* plants in the early growth stage. Light spectrum treatments at a *PPFD* of 215 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ resulted in no significant differences in leaf number (19.0–31.4 leaves) at 60 DAS. In 90-DAS plants, a higher number of leaves was observed with RW treatment (127.9 ± 20.7 leaves), which was more than that with RB treatment (83.8 ± 9.7 leaves). Other spectra with Fr (79.2–104.4 leaves) exhibited no differences in total leaf numbers (Table 6).

Total leaf area. Low *PPFD* of RW (238.7 ± 31.3 cm^2) and RB treatments (223.2 ± 10.4 cm^2) produced significantly larger total leaf areas at 60 DAS. In the same spectrum, although there were not significant differences between light intensities, low *PPFD* produced a larger leaf area during the early growth stage. At 90 DAS, only low *PPFD* of RW treatment showed a significantly larger leaf area (141.1 ± 7.2 cm^2) than high *PPFD* (110.2 ± 12.4 cm^2) (Table 5). Among light spectra at a *PPFD* of 215 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the total leaf area was significantly larger under RW treatment (238.7 ± 31.3 cm^2) at 60 DAS. The total leaf area decreased from 60 to 90 DAS. However, 90-DAS plants grown under RW (141.1 ± 7.2 cm^2) and RB treatments (118.9 ± 19.6 cm^2) had significantly larger leaf areas than those combined with Fr light treatments (74.1–100.4 cm^2) at a similar light intensity (Table 6). The leaf area decreased when developing to the later growth stage in all treatments. It was reported that new leaves of *M. crystallinum* become smaller when developing from the juvenile to the mature stage and then, all leaves drop during the reproductive stage (Adams et al., 1998; Bohnert and Cushman, 2000).

Number of flowers. Flower development was only observed on 90-DAS plants. Low *PPFD* produced more flowers with RW (11.3 ± 6.0 flowers) and RWFrD (28.1 ± 4.0 flowers) treatments, whereas less flower development was seen under low *PPFD* with RWFr treatment (11.0 ± 3.5). The number of flowers with RB and RBFr treatments were not significantly affected by a difference in the *PPFD* (Table 5). Test plants grown under RWFrD and RWFr treatments at a *PPFD* of 215 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had more flowers (28.1 ± 2.8 and 21.9 ± 2.8 flowers, respectively) than the other treatments (10.0–11.4 flowers) (Table 6). Results revealed that the light spectrum markedly affected the flowering of *M. crystallinum*. Treatment with the Fr spectrum produced a greater number of flowers with RW, regardless of the light intensity. This suggests that *M. crystallinum* plants grown under a combination with Fr more quickly developed to the reproductive stage. A similar effect of the Fr spectrum on flowering was also reported. A deficiency in the Fr spectrum delayed initiation of flowering in *C. carpatica* and *Conradina ×grandiflora* and also the development of flowers in *Viola ×wittrockiana* (Runkle and Heins, 2001). *Antirrhinum majus* plants grown under a lower R:Fr ratio (0.77) flowered 9 d earlier than under a higher R:Fr ratio (1.51), whereas *P. ×hybrida* plants flowered 1–12 d earlier, depending on the photoperiod (Cerny et al., 2003). In *E. grandiflorum* plants, initiation of flowering was promoted by an R:Fr ratio of <1.0 (Yamada et al., 2009). On the other hand, a lower R:B ratio with RWFr (2.9) and RWFrD treatments (2.3) promoted greater flower development in *M. crystallinum* plants than other spectra that had higher R:B ratios (4.7–5.0) as shown

Table 3. Shoot fresh weight (FW) and dry weight (DW) and stem length of *Mesembryanthemum crystallinum* plants grown under different light treatments at a photosynthetic photon flux density of 215 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in two growth periods.

Light spectrum[z]	FW (g)		DW (g)		Stem length (cm)	
	60 DAS[y]	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS
RW	36.81 ± 3.74 a[x]	96.77 ± 10.56 a	1.48 ± 0.15 a	4.03 ± 0.43 a	1.59 ± 0.14 b	4.19 ± 0.17 c
RWFr	30.94 ± 2.41 ab	77.75 ± 9.28 ab	1.41 ± 0.16 a	3.87 ± 0.36 a	2.19 ± 0.20 b	4.62 ± 0.60 bc
RWFrD	30.99 ± 1.23 ab	64.88 ± 6.33 b	1.39 ± 0.14 a	3.35 ± 0.25 a	3.17 ± 0.52 a	7.57 ± 0.52 a
RB	28.31 ± 2.04 b	67.77 ± 6.34 b	1.13 ± 0.08 a	3.18 ± 0.30 a	1.80 ± 0.22 b	3.33 ± 0.18 c
RBFr	32.58 ± 2.81 ab	66.83 ± 9.09 b	1.42 ± 0.11 a	3.41 ± 0.24 a	1.72 ± 0.16 b	5.58 ± 0.68 b

[z]Light spectrum included combinations of red (R), blue (B), white (W), far red (Fr), and high-intensity far red (FrD) light-emitting diode lights.
[y]DAS = days after sowing.
[x]Means ± SE with different letters in the same column indicate significant difference by the least significant difference multiple comparison procedure ($P < 0.05$, $n = 9$).

Table 4. Percentages of blue (B), green (G), red (R), and far red (Fr) spectra and their ratios of different light spectrum treatments in the two experiments.

	Percentage (%)					
Light spectrum[z]	B	G	R	Fr	R:B	R:Fr
Expt. 1						
RW	15.4	11.9	72.7	—	4.7	—
RWFr	22.3	7.6	63.5	6.6	2.9	9.6
RWFrD	18.7	14.4	43.4	23.5	2.3	1.9
RB	16.8	—	83.2	—	5.0	—
RBFr	16.3	—	78.5	5.2	4.8	15.1
Expt. 2						
RW–Fr	23.9	8.1	68.0	—	2.9	—
RW+Fr	22.3	7.6	63.5	6.6	2.9	9.6

[z]Light spectrum treatments included combinations of R, B, white (W), Fr, and high-intensity far red (FrD) light-emitting diode lights.

in Table 4. The effect of the R:B ratio on plant flowering was reported. *Solanum lycopersicum* plants that received a lower R:B ratio (1.0) developed more flower trusses compared with those that received a higher R:B ratio (10.0) (Nanya et al., 2012).

DPPH radical scavenging effect. The DPPH radical scavenging effect of light spectra was unaffected by different *PPFD* levels in any samplings (Table 7). However, a scavenging effect was observed among the five light spectrum treatments at a *PPFD* of 215 ± 15 μmol·m^{-2}·s^{-1}. In 60-DAS plants, the effects were higher under RWFrD, RW, and RBFr treatments (60.27% ± 1.55%, 54.49% ± 2.83%, and 54.02% ± 2.83%, respectively). A difference was seen at 90 DAS, and RWFr and RWFrD treatments of 90-DAS plants produced the highest scavenging effects (66.95% ± 4.84% and 73.19% ± 4.63%, respectively) (Table 8). The scavenging effect of RW-treated plants decreased during the 90-d period but increased when combined with the Fr spectrum. Contrary to the induced effect of Fr with RW, combining Fr with RB did not improve the DPPH scavenging capability of plants.

Total phenolic compounds. Phenolic compounds of both samplings were significantly higher under low *PPFD* of RW (1.27 ± 0.03 and 1.21 ± 0.01 mg GAE/kg) and RWFrD treatments (1.13 ± 0.01 and 1.67 ± 0.01 mg GAE/kg). The amounts of phenolic compounds with RB treatments significantly differed only in 90-d-old plants. However, the phenolic compounds were not correlated with the light intensity (Table 7). Among light spectrum treatments at 215 ± 15 μmol·m^{-2}·s^{-1} of *PPFD*, RW markedly promoted total phenolic compounds at 60 DAS with the highest value of 1.27 ± 0.03 mg GAE/kg. RW containing 11.9% of G spectrum (Table 4) induced higher amounts of phenolic compounds, compared with an additional Fr spectrum in RWFr and RWFrD treatments. RB and RBFr treatments, which contained no G spectrum, also resulted in lower total phenolic compounds at 60 DAS (Table 8). Graham (1998) suggested that changes in the light intensity affected the production of phenolic compounds in *Arabidopsis*. Li and Kubota (2009) reported that total

Table 5. Number of branches, number of leaves, total leaf area, and number of flowers of *Mesembryanthemum crystallinum* plants grown under different light treatments in two growth periods.

		No. branches		No. leaves		Total leaf area (cm^2)		No. flowers
Light spectrum[z]	Light intensity	60 DAS[y]	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS	90 DAS
RW	Low	3.1 ± 0.6 a[x]	8.8 ± 0.6 a	29.0 ± 5.8 a	127.9 ± 20.7 a	238.7 ± 31.3 a	141.1 ± 7.2 a	11.3 ± 6.0 a
	High	1.7 ± 0.3 b	9.1 ± 0.2 a	15.8 ± 1.4 b	100.4 ± 8.4 a	135.0 ± 7.2 b	110.2 ± 12.4 b	2.0 ± 0.7 b
RWFr	Low	2.7 ± 0.6 a	8.2 ± 0.5 a	20.6 ± 2.3 b	109.4 ± 18.1 a	168.5 ± 15.3 a	90.9 ± 8.7 a	11.0 ± 3.5 b
	High	4.1 ± 0.1 a	8.3 ± 0.4 a	31.4 ± 6.2 a	80.5 ± 7.6 a	154.5 ± 21.4 a	82.2 ± 12.2 a	21.9 ± 2.8 a
RWFrD	Low	2.9 ± 0.5 a	6.9 ± 0.5 a	23.3 ± 3.7 a	79.2 ± 7.7 a	160.0 ± 17.1 a	74.1 ± 4.0 a	28.1 ± 4.0 a
	High	2.3 ± 0.3 a	6.4 ± 0.2 a	12.9 ± 0.9 b	59.9 ± 7.0 a	125.6 ± 11.4 a	81.8 ± 5.6 a	12.2 ± 2.0 b
RB	Low	3.6 ± 0.4 a	8.7 ± 0.6 a	21.3 ± 2.2 a	108.8 ± 9.0 a	223.2 ± 10.4 a	130.0 ± 15.1 a	5.4 ± 2.2 a
	High	2.4 ± 0.7 a	7.6 ± 0.4 a	19.0 ± 1.7 a	83.8 ± 9.7 a	167.0 ± 14.3 a	118.9 ± 19.6 a	10.0 ± 4.5 a
RBFr	Low	4.5 ± 0.8 a	7.8 ± 0.2 a	23.3 ± 4.6 a	100.4 ± 14.0 a	174.8 ± 21.3 a	73.5 ± 13.1 a	11.4 ± 2.9 a
	High	3.8 ± 0.7 a	8.2 ± 0.5 a	22.0 ± 2.8 a	75.2 ± 8.3 a	141.5 ± 11.0 b	72.0 ± 7.7 a	10.9 ± 2.6 a

[z]Light spectrum included combinations of red (R), blue (B), white (W), far red (Fr) and high-intensity far red (FrD) light-emitting diode lights.
[y]DAS = days after sowing.
[x]Means ± SE with different letters indicate significant difference between low photosynthetic photon flux density (*PPFD*) (Low) and high *PPFD* (High) in each light spectrum by an independent two-sample *t* test ($P < 0.05$, $n = 9$).

Table 6. Number of branches, number of leaves, total leaf area, and number of flowers of *Mesembryanthemum crystallinum* plants grown under different light treatments at a photosynthetic photon flux density of 215 ± 15 μmol·m^{-2}·s^{-1} in two growth periods.

	No. branches		No. leaves		Total leaf area (cm^2)		No. flowers
Light spectrum[z]	60 DAS[y]	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS	90 DAS
RW	3.1 ± 0.6 a[x]	8.8 ± 0.6 a	29.0 ± 5.8 a	127.9 ± 20.7 a	238.7 ± 31.3 a	141.1 ± 7.2 a	11.6 ± 6.0 b
RWFr	4.2 ± 1.1 a	8.3 ± 0.4 a	31.4 ± 6.2 a	80.5 ± 7.6 ab	154.5 ± 21.4 b	82.2 ± 12.2 bc	21.9 ± 2.8 a
RWFrD	2.9 ± 0.5 a	6.9 ± 0.5 b	23.3 ± 3.7 a	79.2 ± 7.7 b	160.0 ± 17.1 b	74.1 ± 4.0 c	28.1 ± 4.0 a
RB	2.4 ± 0.7 a	7.6 ± 0.4 ab	19.0 ± 1.7 a	83.8 ± 9.7 ab	167.0 ± 14.3 b	118.9 ± 19.6 ab	10.0 ± 4.5 b
RBFr	4.5 ± 0.8 a	7.8 ± 0.2 ab	23.3 ± 4.6 a	100.4 ± 14.0 ab	174.8 ± 21.3 b	100.4 ± 14.0 b	11.4 ± 2.9 b

[z]Light spectrum included combinations of red (R), blue (B), white (W), far red (Fr), and high-intensity far red (FrD) light-emitting diode lights.
[y]DAS = days after sowing.
[x]Means ± SE with different letters in the same column indicate significant difference by the least significant difference multiple comparison procedure ($P < 0.05$, $n = 9$).

Table 7. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect, total phenolic compounds, pinitol accumulation, and betacyanins of *Mesembryanthemum crystallinum* plants grown under different light treatments in two growth periods.

		DPPH (%)		Phenolic (mg GAE/kg)[y]		Pinitol (mg·g^{-1})		Betacyanin (mg·g^{-1})	
Light spectrum[z]	Light intensity	60 DAS[x]	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS
RW	Low	54.5 ± 3.2 a[w]	45.2 ± 2.2 a	1.27 ± 0.03 a	1.21 ± 0.01 a	3.54 ± 0.22 b	1.24 ± 0.03 b	0.43 ± 0.02 a	2.56 ± 0.12 a
	High	49.3 ± 3.5 a	38.6 ± 4.4 a	1.13 ± 0.01 b	1.11 ± 0.00 b	5.22 ± 0.16 a	1.88 ± 0.01 a	0.42 ± 0.02 a	1.17 ± 0.12 b
RWFr	Low	53.7 ± 2.1 a	70.7 ± 4.1 a	1.06 ± 0.00 a	1.13 ± 0.01 a	3.84 ± 0.32 a	2.08 ± 0.12 a	0.69 ± 0.03 a	2.80 ± 0.17 a
	High	48.4 ± 4.7 a	67.0 ± 4.8 a	1.04 ± 0.01 a	1.12 ± 0.01 a	5.01 ± 0.48 a	0.72 ± 0.06 b	0.72 ± 0.05 a	2.69 ± 0.13 a
RWFrD	Low	60.3 ± 1.6 a	73.2 ± 4.6 a	1.13 ± 0.01 a	1.67 ± 0.01 a	3.33 ± 0.14 b	1.08 ± 0.09 b	0.70 ± 0.02 a	2.05 ± 0.15 a
	High	56.5 ± 3.1 a	66.2 ± 9.1 a	1.09 ± 0.01 b	1.14 ± 0.00 b	5.85 ± 0.26 a	13.60 ± 0.26 a	0.62 ± 0.03 a	1.97 ± 0.19 a
RB	Low	52.8 ± 3.5 a	54.4 ± 3.6 a	1.07 ± 0.01 a	1.09 ± 0.00 b	4.90 ± 0.40 a	4.62 ± 0.18 a	0.66 ± 0.03 b	2.31 ± 0.18 a
	High	48.1 ± 3.2 a	51.5 ± 4.9 a	1.06 ± 0.01 a	1.14 ± 0.00 a	3.99 ± 0.19 a	1.80 ± 0.10 b	0.79 ± 0.03 a	2.19 ± 0.09 a
RBFr	Low	54.0 ± 2.8 a	48.4 ± 3.8 a	1.06 ± 0.00 a	1.19 ± 0.01 a	5.74 ± 0.11 b	5.49 ± 0.49 a	0.93 ± 0.05 a	2.04 ± 0.14 a
	High	55.0 ± 0.5 a	59.9 ± 2.5 a	1.06 ± 0.00 a	1.15 ± 0.01 b	7.48 ± 0.48 a	4.88 ± 0.16 a	0.58 ± 0.01 b	2.21 ± 0.13 a

[z]Light spectrum included combinations of red (R), blue (B), white (W), far red (Fr), and high-intensity far red (FrD) light-emitting diode lights.
[y]GAE = gallic acid equivalent.
[x]DAS = days after sowing.
[w]Means ± SE with different letters indicate significant difference between low photosynthetic photon flux density (*PPFD*) (Low) and high *PPFD* (High) in each light spectrum by an independent two-sample *t* test ($P < 0.05$, $n = 9$).

Table 8. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect, total phenolic compounds, pinitol accumulation, and betacyanins of *Mesembryanthemum crystallinum* plants grown under different light treatments at a photosynthetic photon flux density of 215 ± 15 μmol·m^{-2}·s^{-1} in two growth periods.

	DPPH (%)		Phenolic (mg GAE/kg)[y]		Pinitol (mg·g^{-1})		Betacyanin (mg·g^{-1})	
Light spectrum[z]	60 DAS[x]	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS
RW	54.49 ± 2.83 ab[w]	45.22 ± 2.19 b	1.27 ± 0.03 a	1.21 ± 0.01 a	3.54 ± 0.22 b	1.24 ± 0.03 bc	0.43 ± 0.02 c	2.56 ± 0.12 ab
RWFr	48.42 ± 4.69 b	66.95 ± 4.84 a	1.04 ± 0.01 c	1.12 ± 0.01 d	5.01 ± 0.48 a	0.72 ± 0.06 c	0.72 ± 0.05 b	2.69 ± 0.13 a
RWFrD	60.27 ± 1.55 a	73.19 ± 4.63 a	1.13 ± 0.01 b	1.17 ± 0.01 c	3.33 ± 0.14 b	1.08 ± 0.09 c	0.70 ± 0.02 b	2.05 ± 0.15 c
RB	48.14 ± 3.15 b	51.45 ± 4.88 b	1.06 ± 0.01 c	1.14 ± 0.00 d	3.99 ± 0.19 b	1.80 ± 0.10 b	0.79 ± 0.03 b	2.19 ± 0.09 bc
RBFr	54.02 ± 2.83 ab	48.41 ± 3.83 b	1.06 ± 0.00 c	1.19 ± 0.01 b	5.74 ± 0.11 a	5.49 ± 0.49 a	0.93 ± 0.05 a	2.04 ± 0.14 c

[z]Light spectrum included combinations of red (R), blue (B), white (W), far red (Fr), and high-intensity far red (FrD) light-emitting diode lights.
[y]GAE = gallic acid equivalent.
[x]DAS = days after sowing.
[w]Means ± SE with different letters in the same column indicate significant difference by the least significant difference multiple comparison procedure ($P < 0.05$, $n = 9$).

phenolic compounds in *L. sativa* under W light with supplemental G light did not significantly differ from W light alone or that supplemented with B light. An increasing light intensity had a positive effect on total phenolic compounds in leaves and stems of *Zingiber officinale* (Ghasemzadeh et al., 2010). However, phenolic compounds of *Ocimum basilicum* increased under additional B light (Bantis et al., 2016). It was suggested that secondary metabolites under different light spectra and intensities are species dependent.

Pinitol accumulation. Pinitols were significantly higher under high *PPFD* of RW (5.22 ± 0.16 mg·g^{-1}), RWFrD (5.85 ± 0.26 mg·g^{-1}), and RBFr treatments (7.48 ± 0.48 mg·g^{-1}) at 60 DAS. In 90-DAS plants, except for high *PPFD* of the RWFrD treatment (13.60 ± 0.26 mg·g^{-1}), pinitols decreased in other light treatments. The effect of light intensity in a spectrum was inconsistent (Table 7). At a *PPFD* of 215 ± 15 μmol·m^{-2}·s^{-1}, the pinitols were significantly higher under RWFr (5.01 ± 0.48 mg·g^{-1}) and RBFr treatments (5.74 ± 0.11 mg·g^{-1}) at 60 DAS and decreased in later growth stages. However, the pinitols with RBFr treatment of 90-DAS plants remained at 5.49 ± 0.49 mg·g^{-1}, whereas it decreased under other light spectra (Table 8). Cockburn et al. (1996) investigated pinitol accumulation of *M. crystallinum* plants under different *PPFD* ratios of R and Fr lights using fluorescent and tungsten lamps. They showed that plants grown under a lower R:Fr ratio (0.07) with salt stress (400 mM NaCl) had five times higher pinitol accumulation than plants that received a higher R:Fr ratio (6.8). Similar results were observed in this study; a decrease in the R:Fr ratio increased pinitol accumulation in both the early and later growth stages. It was speculated that the extremely high pinitols of plants that received RWFrD with high *PPFD* might have been due to the light stress that induced a strong antioxidative reaction as a photoprotective response. Previous studies reported that the pinitols in plants were induced by environmental stresses such as temperature, water deficit, and salinity (Guo and Oosterhuis, 1997; Keshtehgar et al., 2013; Palma et al., 2014; Parida and Das, 2005; Williamson et al., 2002). However, information on the relationship between light stress and pinitol synthesis in plants is lacking. The result of an increasing pinitol accumulation observed in this study indicated that the high intensity of the Fr spectrum might have induced photooxidative stress in plants.

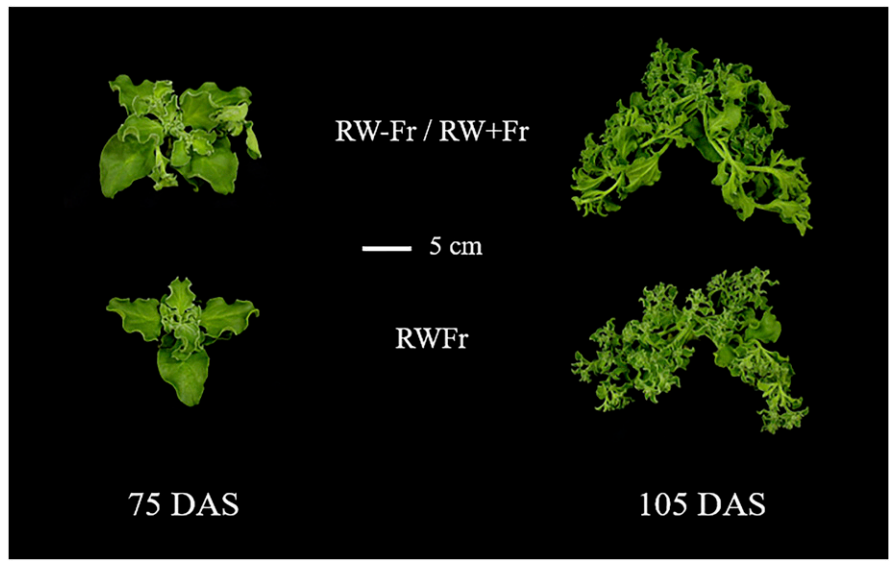

Fig. 3. Morphology of *Mesembryanthemum crystallinum* plants grown under modified red/white spectra at 75 and 105 d after sowing (DAS) in Expt. 2. Treatments included red/white without far red (RW–Fr) and then red/white with far red (RW+Fr). Expt. 2 was conducted at 25 °C day/20 °C night air temperatures. Bar indicates 5 cm. RBFr = red/blue/far red.

Betacyanins. Different *PPFDs* did not affect betacyanin synthesis with RW, RWFr, and RWFrD treatments. The betacyanins were significantly higher under high *PPFD* with RB and low *PPFD* with RBFr treatments at 60 DAS. The betacyanins increased from 60 to 90 d. In 90-DAS plants, except for RW light which had higher betacyanins at a low *PPFD*, other treatments were unaffected by different *PPFDs* at the later growth stage (Table 7). At a *PPFD* of 215 ± 15 μmol·m^{-2}·s^{-1}, RBFr treatment produced the significantly highest betacyanins (0.93 ± 0.05 mg·g^{-1}) at 60 DAS. However, RWFr and RW treatments produced significantly higher levels at 90 DAS (2.69 ± 0.13 and 2.56 ± 0.12 mg·g^{-1}, respectively) (Table 8). The phytochrome control of betacyanin production was reported by Wagner and Cumming (1970), and monochromatic R or Fr irradiation induced betacyanin production in *Chenopodium rubrum* plants after 1 h of exposure. However, they found that the R spectrum was more effective than the Fr one, which was similar to our results in 60-DAS plants. The portion of R in RBFr treatment was 78.5% higher than in RW (72.7%) and RWFr treatments (63.5%) (Table 4). Vogt et al. (1999) investigated the spectral dependence on the accumulation of betalains, which replaces anthocyanin pigments in the family Caryophyllales. High *PPFD* of ultraviolet light spectra (280, 295, and 305 nm; 1200–1500 μmol·m^{-2}·s^{-1}) induced the level of betacyanins in *M. crystallinum* plants up to 300 nM·g^{-1} FW (equal to 168 mg·g^{-1}) after 5 d of treatment (Vogt et al., 1999). The betacyanins of RW (15.4% B light portion) that were increased more than other treatments from 60 to 90 DAS might be related to the activity of the cryptochrome 2 photoreceptor. The accumulation of betacyanin was suppressed after exposure to B light observed in the halophyte *Suaeda salsa* (Wang and Liu, 2006). The light intensity was positively correlated with betacyanin accumulation in plants and betacyanin formation in epidermal bladder cells of *M. crystallinum* leaves. Results of this experiment showed that the betacyanins in *M. crystallinum* plants not

only increased with a high light intensity but also depended on the light spectrum.

Results of Expt. 1 showed that regardless of the light intensity, RW promoted plant biomass growth. A decrease in the R:Fr ratio had a negative effect on vegetative growth while promoting the developmental process in *M. crystallinum*. An increase in the light intensity was nonbeneficial for promoting plant growth. RW and RB combined with Fr had different effects on secondary metabolisms. Pinitol was a major compound promoted by high *PPFD* treatments of RBFr in 60-DAS and RWFrD in 90-DAS plants. RWFrD treatments might have caused light stress or photooxidative damage to plants because of Fr or the high *PPFD*. The results led to a proposal that the addition of Fr during later growth stage could avoid the adverse effect of Fr to plant growth in the early stage and maximize the vegetative growth and then induce secondary metabolites in the later growth stage. Moreover, modification of RWFr light at a similar light intensity was tested in Expt. 2 to confirm the effects of additional Fr in different growth stages.

Expt. 2. Morphologies of *M. crystallinum* plants grown under different light spectra in Expt. 2 are shown in Fig. 3. The growth of test plants under RW, RWFrD, RB, and RBFr treatments showed similar patterns to those in Expt. 1 (data not shown), according to results of the statistical analysis. The discussion in Expt. 2 focuses on the effects of Fr on growth and antioxidative properties from transplantation to 75 d and the 76 to 105-d period.

Growth and morphology. Shoot FW and DW in the RW–Fr treatment were significantly higher at 75 DAS. Although there were not significant differences at the end of 105 d, the addition of Fr (RW+Fr) during this stage did not reduce biomass accumulation in test plants (Table 9). Shoot biomass was promoted by RW–Fr treatment, especially in the early growth stage (31–75 DAS). The FW of RW–Fr was 1.30 times that of RWFr at 75 DAS. After adding Fr to RW, the FW at 105 DAS was 1.21 times that of RWFr, which indicated that adding Fr to RW at the late growth stage did not stress growth as did RWFr. The number of branches was affected under RW+Fr treatment at 105 DAS as more branches developed. The number of leaves did not significantly differ in the two samplings. Although it was not significant, more leaves developed in the later growth stage as a higher leaf number and leaf area were observed under RW+Fr treatments (Table 9).

Flowers only developed under RWFr treatment at 105 DAS, whereas no flowers were observed under RW+Fr treatment, which did not receive the Fr spectrum until the later growth stage (from transplantation to 75 DAS). This confirms that Fr light provided in the early growth stage induced plants to more rapidly develop to the reproductive stage. Therefore, during the later growth period (76–105 DAS), the addition of the Fr spectrum in the RW+Fr treatment did not lead to plants developing to the reproductive stage compared with RWFr treatment (Table 9). The total leaf area was significantly larger with RW–Fr treatment during the early growth stage. The addition of Fr during the later growth stage also produced a larger leaf area but did not differ from that of RWFr treatment. Similar to Expt. 1, RW without Fr produced a larger leaf area (Tables 5 and 6), which suggested that the addition of Fr in the later growth stage could maintain test plants in the vegetative stage. Consumer opinion is firmly in favor of mature leaves, which is characterized by secondary leaves grown from branches. As a result, RW and RW–Fr treatments produced the larger size of mature leaves, which suited the consumer demand.

Antioxidative properties. The DPPH radical scavenging effect was unaffected by combining Fr light treatment. Overall, scavenging activities observed in Expt. 2 (22.5%–25.1% at 75 DAS and 34.2%–34.5% at 105 DAS) were lower than those in Expt. 1 (Tables 7 and 8). This might be a response of plants under different air temperature ranges (28 °C day/25 °C night in Expt. 1 and 25 °C day/20 °C night air temperatures in Expt. 2). The DPPH antioxidant activity showed a negative correlation with the temperature increases, which was reported in *L. sativa*, *Z. officinale*, and *Ipomoea batatas* (Boo et al., 2011; Chua et al., 2015; Ghasemzadeh et al., 2010; Islam et al., 2003; Li et al., 2010).

Phenolic compounds were unaffected by Fr during the early growth stage, whereas they increased in the period from 75 to 105 d. The addition of Fr during the late growth stage (RW+Fr) produced greater amounts of phenolic compounds (1.17 ± 0.00 mg GAE/kg) in test plants than the 1.12 ± 0.01 mg GAE/kg of the RWFr treatment (Table 9). This suggests that Fr can promote phenolic compound production.

In 75-d-old plants, pinitol accumulation (4.28 ± 0.69 mg·g^{-1}) was significantly higher under RW–Fr treatment. The pinitols further increased in the later growth stage. After adding Fr, pinitol accumulation was significantly higher under RW+Fr treatment (8.84 ± 1.53 mg·g^{-1}), which was 2.72 times higher than that with RWFr treatment (3.25 ± 0.32 mg·g^{-1}) at 105 DAS (Table 9). Light regulation from RW–Fr to RW+Fr can be applied to induce secondary compounds in *M. crystallinum* along with a growth optimization strategy.

The betacyanins did not significantly differ between the two light treatments in the early growth stage, whereas it increased in 105-d-old plants of RWFr treatment (Table 9). The betacyanins were significantly higher with RWFr treatment (0.50 ± 0.04 mg·g^{-1}) during the late growth stage, but it was 10-fold lower than that found in Expt. 1 (Table 8). This might have been due to the air temperature being lower in the Expt. 2. As a result, light programming of the RW–Fr and RW+Fr treatments did not improve the betacyanins.

RW–Fr treatment had an R:B ratio of 2.9 (23.9% B and 68.0% R) which was lower than the 4.7 R:B ratio (15.4% B and 72.7% R)

Table 9. Plant growth responses and antioxidative properties of *Mesembryanthemum crystallinum* plants grown under different light spectra in Expt. 2.

Light spectrum[z]	Shoot wt (g)		No.			Leaf area (cm^2)	2, 2-diphenyl-1-picrylhydrazyl (%)	Phenolic (mg GAE/kg)[y]	Pinitol	Betacyanin
	Fresh	Dry	Branches	Leaves	Flowers				(mg·g^{-1})	
75 DAS[x]										
RW–Fr	40.8 ± 3.0 a[w]	1.81 ± 0.11 a	3.2 ± 0.4 a	23.2 ± 2.4 a	ND	249.3 ± 15.5 a	22.5 ± 3.2 a	1.00 ± 0.00 a	4.28 ± 0.69 a	0.05 ± 0.01 a
RWFr	31.4 ± 1.7 b	1.47 ± 0.09 b	3.1 ± 0.3 a	24.3 ± 1.3 a	ND	191.3 ± 7.5 b	25.1 ± 2.6 a	1.03 ± 0.02 a	2.98 ± 0.22 b	0.06 ± 0.02 a
P value	0.0099*	0.0203*	0.8826	0.6643		0.0019*	0.5457	0.2443	0.0335*	0.4006
105 DAS										
RW+Fr	105.0 ± 10.9 a	5.37 ± 0.41 a	8.2 ± 0.5 a	135.3 ± 16.7 a	ND	491.8 ± 64.1 a	34.2 ± 2.0 a	1.17 ± 0.00 a	8.84 ± 1.53 a	0.14 ± 0.04 b
RWFr	86.9 ± 8.2 a	4.66 ± 0.42 a	7.1 ± 0.3 b	124.6 ± 13.1 a	4.0	365.1 ± 52.3 a	34.5 ± 2.0 a	1.12 ± 0.01 b	3.25 ± 0.32 b	0.50 ± 0.07 a
P value	0.1917	0.2303	0.0327*	0.6143		0.1351	0.9180	0.0001*	0.0049*	0.0034*

[z]Light spectrum included combinations of red (R), blue (B), white (W), far red (Fr), and high-intensity far red (FrD) light-emitting diode lights.
[y]GAE = gallic acid equivalent.
[x]DAS = days after sowing.
[w]Different letters in a column of a factor indicate significant difference between treatments by an independent two-sample *t* test ($P < 0.05$, $n = 9$).
*Significant difference at 95% confidence level.

of RW treatment (Table 4) and a significantly lower light intensity (168.0 vs. 227.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively) (Table 1). Results of Expt. 2 showed that RW–Fr treatment promoted plant biomass during the early growth stage (Table 9). This was higher than with RW treatment in Expt. 1 (Table 3). The addition of the Fr light spectrum (RW+Fr) in the late growth stage also more greatly promoted plant biomass than did RWFr treatment. Treatment with a lower R:Fr ratio promoted stem elongation in *M. crystallinum* plants. The number of leaves and the leaf area were promoted by RW+Fr treatment, which had a larger leaf area compared with RW and RB treatments in Expt. 1 (Tables 5 and 6). Late RW+Fr treatment can especially be used to induce secondary compounds in *M. crystallinum* plants without sacrificing plant growth. Late RW+Fr treatment dramatically promoted pinitol accumulation, which was even higher than the results of RBFr treatment with a low *PPFD* in Expt. 1 (Table 7).

Conclusions

RW (227 $\mu mol \cdot m^{-2} \cdot s^{-1}$), RW–Fr (162 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and RB (162 $\mu mol \cdot m^{-2} \cdot s^{-1}$) treatments were better light combinations for promoting plant vegetative growth, and the RW combined with Fr in the later growth stage is suggested to induce secondary metabolism in *M. crystallinum* plants, particularly when the target compound is pinitol. In conclusion, using RW light to promote vegetative growth in the early growth stage and then adding Fr light to induce secondary metabolism are recommended for edible *M. crystallinum* production. The optimum light intensity of RW, RB, and Fr treatments and the timing of adding Fr in the growth period still need further study in the future for the customized commercial application.

Literature Cited

Adams, P., D.E. Nelson, S. Yamada, W. Chmara, R.G. Jensen, H.J. Bohnert, and H. Griffiths. 1998. Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). New Phytol. 138:171–190.

Agarie, S., A. Kawaguchi, A. Kodera, H. Sunagawa, H. Kojima, A. Nose, and T. Nakahara. 2009. Potential of the common ice plant, *Mesembryanthemum crystallinum* as a new high-functional food as evaluated by polyol accumulation. Plant Prod. Sci. 12(1):37–46.

Albright, L.D. and R.W. Langhans. 1996. Controlled environment agriculture scoping study. Cornell Univ., Ithaca, NY.

Ballaré, C.L. and J.J. Casal. 2000. Light signals perceived by crop and weed plants. F. Crop. Res. 67:149–160.

Bantis, F., T. Ouzounis, and K. Radoglou. 2016. Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success. Scientia Hort. 198:277–283.

Behringer, F.J., P.J. Davies, and J.B. Reid. 1990. Genetic analysis of the role of gibberellin in the red light inhibition of stem elongation in etiolated seedlings. Plant Physiol. 94:432–439.

Bohnert, H.J. and J.C. Cushman. 2000. The ice plant cometh: Lessons in abiotic stress tolerance. J. Plant Growth Regul. 19:334–346.

Boo, H.O., B.G. Heo, S. Gorinstein, and S.U. Chon. 2011. Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. Plant Sci. 181:479–484.

Cao, K., J. Yu, L. Ye, H. Zhao, and Z. Zou. 2016. Optimal LED far-red light intensity in end-of-day promoting tomato growth and development in greenhouse. Trans. Chinese Soc. Agr. Eng. 32(8):171–176.

Cerny, T.A., J.E. Faust, D.R. Layne, and N.C. Rajapakse. 2003. Influence of photoselective films and growing season on stem growth and flowering of six plant species. J. Amer. Soc. Hort. Sci. 128:486–491.

Chen, J., D. Fernandez, D.D. Wang, Y.J. Chen, and G.H. Dai. 2014. Biological control mechanisms of D-pinitol against powdery mildew in cucumber. Physiol. Mol. Plant Pathol. 88:52–60.

Chia, P.L. and C. Kubota. 2010. End-of-day far-red light quality and dose requirements for tomato rootstock hypocotyl elongation. HortScience 45:1501–1506.

Chiera, J.M., J.G. Streeter, and J.J. Finer. 2006. Ononitol and pinitol production in transgenic soybean containing the inositol methyl transferase gene from *Mesembryanthemum crystallinum*. Plant Sci. 171:647–654.

Chua, I.Y.P., P.J.H. King, K.H. Ong, S.R. Sarbini, and P.H. Yiu. 2015. Influence of light intensity and temperature on antioxidant activity in *Premna serratifolia* L. J. Soil Sci. Plant Nutr. 15(3):605–614.

Cockburn, W., G.C. Whitelam, A. Broad, and J. Smith. 1996. The participation of phytochrome in the signal transduction pathway of salt stress responses in *Mesembryanthemum crystallinum* L. J. Expt. Bot. 47(298):647–653.

Demotes-Mainard, S., T. Péron, A. Corot, J. Bertheloot, J. Le Gourrierec, S. Pelleschi-Travier, L. Crespel, P. Morel, L. Huché-Thélier, R. Boumaza, A. Vian, V. Guérin, N. Leduc, and S. Sakr. 2016. Plant responses to red and far-red lights, applications in horticulture. Environ. Expt. Bot. 121:4–21.

Falleh, H., R. Ksouri, F. Medini, S. Guyot, C. Abdelly, and C. Magné. 2011. Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. Ind. Crops Prod. 34:1066–1071.

Folta, K.M. and K.S. Childers. 2008. Light as a growth regulator: Controlling plant biology with narrow-bandwidth solid-state lighting systems. HortScience 43:1957–1964.

Fukumoto, L.R. and G. Mazza. 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. J. Agr. Food Chem. 48:3597–3604.

Ghasemzadeh, A., H.Z.E. Jaafar, A. Rahmat, P.E.M. Wahab, and M.R.A. Halim. 2010. Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). Intl. J. Mol. Sci. 11:3885–3897.

Graham, T.L. 1998. Flavonoid and flavonol glycoside metabolism in *Arabidopsis*. Plant Physiol. Biochem. 36(1–2):135–144.

Guo, C. and D.M. Oosterhuis. 1997. Effect of water-deficit stress and genotypes on pinitol occurrence in soybean plants. Environ. Expt. Bot. 37:147–152.

Hanen, F., K. Riadh, O. Samia, G. Sylvain, M. Christian, and A. Chedly. 2009. Interspecific variability of antioxidant activities and phenolic composition in *Mesembryanthemum* genus. Food Chem. Toxicol. 47:2308–2313.

He, J., L. Qin, E.L.C. Chong, T.W. Choong, and S.K. Lee. 2017. Plant growth and photosynthetic characteristics of *Mesembryanthemum crystallinum* grown aeroponically under different blue- and red-LEDs. Front. Plant Sci. 8:1–13.

Hogewoning, S.W., G. Trouwborst, H. Maljaars, H. Poorter, W. van Ieperen, and J. Harbinson. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Expt. Bot. 61(11):3107–3117.

Ibdah, M., A. Krins, H.K. Seidlitz, W. Heller, D. Strack, and T. Vogt. 2002. Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. Plant Cell Environ. 25:1145–1154.

Islam, M.S., M. Yoshimoto, K. Ishiguro, S. Okuno, and O. Yamakawa. 2003. Effect of artificial shading and temperature on radical scavenging activity and polyphenolic composition in sweetpotato (*Ipomoea batatas* L.) leaves. J. Amer. Soc. Hort. Sci. 128:182–187.

Keshtehgar, A., K. Rigi, and M. Vazirimehr. 2013. Effects of salt stress in crop plants. Intl. J. Agr. Crop Sci. 5(23):2863–2867.

Kim, H.H., G.D. Goins, R.M. Wheeler, and J.C. Sager. 2004a. Green-light supplementation for enhanced lettuce growth under red-and blue-light-emitting diodes. HortScience 39:1617–1622.

Kim, S.J., E.J. Hahn, J.W. Heo, and K.Y. Paek. 2004b. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. Scientia Hort. 101:143–151.

Leduc, N., H. Roman, F. Barbier, T. Péron, L. Huché-Thélier, J. Lothier, S. Demotes-Mainard, and S. Sakr. 2014. Light signaling in bud outgrowth and branching in plants. Plants 3:223–250.

Lee, S.W., J.M. Seo, M.K. Lee, J.H. Chun, P. Antonisamy, M.V. Arasu, T. Suzuki, N.A. Al-Dhabi, and S.J. Kim. 2014. Influence of different LED lamps on the production of phenolic compounds in common and Tartary buckwheat sprouts. Ind. Crops Prod. 54:320–326.

Li, Q. and C. Kubota. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Expt. Bot. 67:59–64.

Li, Z., X. Zhao, A.K. Sandhu, and L. Gu. 2010. Effects of exogenous abscisic acid on yield, antioxidant capacities, and phytochemical contents of greenhouse grown lettuces. J. Agr. Food Chem. 58:6503–6509.

Liao, J.X., X.Y. Zou, Y. Ge, and J. Chang. 2006. Effects of light intensity on growth of four Mosla species. Bot. Stud. 47:403–408.

Nagatani, A. 2010. Phytochrome: Structural basis for its functions. Curr. Opin. Plant Biol. 13:565–570.

Nanya, K., Y. Ishigami, S. Hikosaka, and E. Goto. 2012. Effects of blue and red light on stem elongation and flowering of tomato seedlings. Acta Hort. 956:261–266.

Olle, M. and A. Viršilė. 2013. The effect of light-emitting diode lighting on greenhouse plant growth and quality. Agr. Food Sci. 22:223–234.

Palma, F., F. Carvajal, C. Lluch, M. Jamilena, and D. Garrido. 2014. Changes in carbohydrate content in zucchini fruit (*Cucurbita pepo* L.) under low temperature stress. Plant Sci. 217–218:78–86.

Parida, A.K. and A.B. Das. 2005. Salt tolerance and salinity effects on plants: A review. Ecotoxicol. Environ. Saf. 60:324–349.

Runkle, E.S. and R.D. Heins. 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. J. Amer. Soc. Hort. Sci. 126:275–282.
Samuolienė, G., R. Sirtautas, A. Brazaityte, J. Sakalauskaite, S. Sakalauskiene, and P. Duchovskis. 2011. The impact of red and blue light-emitting diode illumination on radish physiological indices. Cent. Eur. J. Biol. 6(5):821–828.
Shalaby, E.A. and S.M.M. Shanab. 2013. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. Indian J. March Sci. 42(5):556–564.
Shimada, K., K. Fujikawa, K. Yahara, and T. Nakamura. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agr. Food Chem. 40:945–948.
Ślesak, I., H. Ślesak, M. Libik, and Z. Miszalski. 2008. Antioxidant response system in the short-term post-wounding effect in *Mesembryanthemum crystallinum* leaves. J. Plant Physiol. 165:127–137.
Spalding, E.P. and K.M. Folta. 2005. Illuminating topics in plant photobiology. Plant Cell Environ. 28:39–53.
Terashima, I., T. Fujita, T. Inoue, W.S. Chow, and R. Oguchi. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. Plant Cell Physiol. 50(4):684–697.
Vänninen, I., D.M. Pinto, A.I. Nissinen, N.S. Johansen, and L. Shipp. 2010. In the light of new greenhouse technologies: 1. Plant-mediated effects of artificial lighting on arthropods and tritrophic interactions. Ann. Appl. Biol. 157:393–414.
Vogelmann, T.C. and T. Han. 2000. Measurement of gradients of absorbed light in spinach leaves from chlorophyll fluorescence profiles. Plant Cell Environ. 23:1303–1311.
Vogt, T., M. Ibdah, J. Schmidt, V. Wray, M. Nimtz, and D. Strack. 1999. Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. Phytochemistry 52:583–592.
von Arnim, A. and X.W. Deng. 1996. Light control of seedling development. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:215–243.
Wagner, E. and B.G. Cumming. 1970. Betacyanin accumulation, chlorophyll content, and flawer initiation in *Chenopodium rubrum* as related to endogenous rhythmicity and phytochrome action. Can. J. Bot. 48(1):1–18.
Wang, C.Q. and T. Liu. 2006. Cryptochrome 2 is involved in betacyanin decomposition induced by blue light in *Suaeda salsa*. Funct. Plant Biol. 33:697–702.
Williamson, J.D., D.B. Jennings, W.W. Guo, and D.M. Pharr. 2002. Sugar alcohols, salt stress, and fungal resistance: Polyols—multifunctional plant protection? J. Amer. Soc. Hort. Sci. 127:467–473.
Yamada, A., T. Tanigawa, T. Suyama, T. Matsuno, and T. Kunitake. 2009. Red:far-red light ratio and far-red light integral promote or retard growth and flowering of *Eustoma grandiflorum* (Raf.). Shinn. Acta Hort. 120:101–106.
Zhang, T. and K.M. Folta. 2012. Green light signaling and adaptive response. Plant Signal. Behav. 7(1):1–4.