Red and blue light ratio affects the growth and quality of edible cactus (*Nopalea cochenillifera*)

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ABSTRACT

This study investigated the effects of light of different wavelengths on the growth and quality of daughter cladodes in the edible cactus *Nopalea cochenillifera*. Mother cladodes were cultivated under a deep-flow hydroponic culture system and irradiated with red or blue light alone or in combination (in the ratio 1:3 or 3:1) in an enclosed-type plant factory. Daughter cladodes developed from mother cladodes in all treatments. The rate of elongation of first cladodes was lower under blue light and the combination of red and blue light (in the ratio 1:3) compared with other treatments. The number of daughter cladodes was also low under blue light. Thus, compared with red light, blue light appears to suppress daughter cladode development. However, the width of daughter cladodes was higher under blue light and the combination of red and blue light (in the ratio 1:3) compared with other treatments. Total fresh weight (FW) of daughter cladodes emerging from one mother cladode was lowest under blue light and high under combined red and blue light (1:3 and 3:1). The number of spines, an undesirable characteristic of edible cacti, was significantly higher under both combinations of red and blue light (1:3 and 3:1) compared to other treatments. On the other hand, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of extracts of the daughter cladodes was highest under blue light or under combined red and blue light (1:3). Our results show that light wavelength strongly affects daughter cladode growth and development. Thus, controlling the lighting environment is effective for improving growth and quality in edible cacti.

Key words: Edible cacti, hydroponics culture, LED, spines, plant factory.

INTRODUCTION

Edible cacti of the genera *Opuntia* and *Nopalea* (subfamily Opuntioideae, family Cactaceae), commonly called nopal cactus or prickly pear, are characterized by their remarkable adaptation to arid and semi-arid climates. Their flat stems have been used by human beings for hundreds of years in various ways: for animal feed, in the production of natural dyes such as cochineal red, as a vegetable for human consumption and as a source of nutraceutical compounds (Guzmán-Maldonado and Paredes-López 1999; Silos-Espino *et al.*, 2006). For example, the stems of prickly-pear cacti are widely consumed as a vegetable in Mexico, Latin America, South Africa, and Mediterranean countries (Stintzing and Carle 2005; Cruz-Hernández and Paredes-López 2010; El-Mostafa *et al.*, 2014). Furthermore, these plants are used in some countries as a remedy for a variety of health problems including edema and indigestion (El-Mostafa *et al.*, 2014). Edible cacti are also produced in Japan, mainly in Kasugai City, Aichi Prefecture, although only on a small scale.

Edible cacti are commonly grown in soil or pot culture. Major problems commonly encountered in growing vegetables in soil include soil-borne disease, salt accumulation, and difficulty in fertilizer management (Lakkireddy *et al.*, 2012). Hydroponics is a method of growing plants using nutrient solution (water and fertilizer) with or without the use of an artificial medium, and it can be used for the production of several crops with added advantages. For example, this system can avoid the costly and time-consuming task of soil sterilization to prevent soil-borne disease and enable precise fertilizer management (Wahome *et al.*, 2011; Lakkireddy *et al.*, 2012). In hydroponics, several methods have been established to reduce the nitrate content of vegetables (Wang *et al.*, 2007; Stefanelli *et al.*, 2011). In addition, precise salinity control in hydroponics for tomato cultivation has been reported to improve fruit quality (Sakamoto *et al.*, 1999). Thus, there are many advantages associated with the hydroponic culture of edible cacti, and although this method is not yet commercially practiced, we have previously shown that they can be grown using a simple hydroponic culture system based on commercially available materials (Horibe and Yamada 2016; Horibe 2017).

Edible cacti exhibit crassulacean acid metabolism (CAM), a CO_2-concentrating mechanism that potentially leads to higher optimal temperatures for photosynthesis (Monson, 1989). CAM plants also have certain anatomical modifications that enable them to survive droughts,

such as thick cuticles and low stomatal frequency, together with nighttime CO_2 uptake (Drennan and Nobel 2000; Pimienta-Barrios *et al*., 2005). Daughter cladodes show C3 photosynthesis with daytime stomatal opening during the early stages of development (Osmond, 1978; Acevedo *et al*., 1983) and they import water from the mother cladode (Pimienta-Barrios *et al*., 2005). Growth responses of *Opuntia* plants to temperature and CO_2 concentration are well known (Gulmon and Bloom 1979; North *et al*., 1995). Elevated CO_2 concentrations increase the daily net CO_2 uptake of cladodes and lead to increased biomass production (Cui *et al*., 1993; Nobel and Israel, 1994).

Light is essential for plant growth, with both wavelength and intensity affecting plant growth and morphogenesis (Yanagi *et al*., 2006; Mortensen and Stromme, 1987). Studies have shown that light intensity affects the elongation of the cactus stem and the malate content of cladodes (North *et al*., 1995; Littlejohn *et al*., 1985). However, to our knowledge, few studies have investigated the relationship between the light environment and growth of edible cacti (North *et al*., 1995). Understanding the relationship between environmental conditions and cladode growth is important for improving the production and quality of edible cacti. Here we cultivated the edible cactus *Nopalea cochenillifera* using hydroponic culture in an enclosed-type plant factory and investigated the effects of different light conditions on growth and quality.

MATERIALS AND METHODS

Plant materials

In April and October 2018, edible cactus cladodes (*N. cochenillifera*) averaging 13 cm in length, 6 cm in width, and 0.8 cm in thickness were harvested at a commercial cactus farm (Goto saboten) in Aichi Prefecture, Japan. Cladodes were transported under dry conditions to our laboratory within 1 h and were then trimmed to a length of 12 cm.

Treatments

The cladodes were cultivated using a two-layer hydroponic system (Churitsu Electric Co., Japan) with the deep-flow technique in an enclosed-type plant factory (Fig. 1). OAT House solution A (Otsuka AgriTechno Co., Ltd., Japan; electrical conductivity = 2.6 $mS \cdot cm^{-1}$) was used as the hydroponic nutrient solution, which was prepared by dissolving 150 g of Otsuka House 1 and 100 g of Otsuka House 2 in 100 L of water. The cladodes were transplanted

into cultivation panels (88 cm long × 57 cm wide × 4 cm high) with an inter-plant spacing of 4.5 cm and an inter-row spacing of 4 cm, which were then floated on the nutrient solution in cultivation beds (90 cm long × 60 cm wide × 8 cm high).

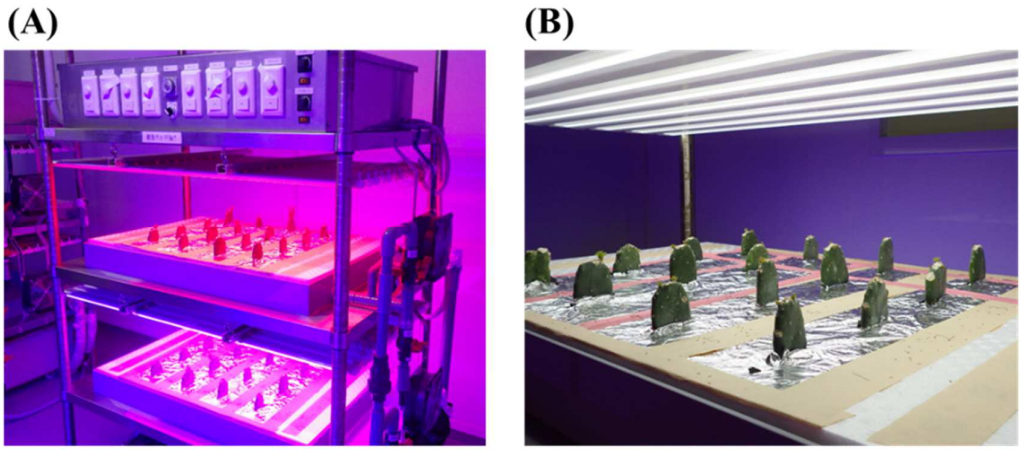

Figure 1. Photographs of (A) the hydroponic culture system used to grow the edible cactus *Nopalea cochenillifera* under artificial light, and (B) the cladodes planted on a cultivation panel.

The cladodes were separated into four treatment groups (n = 16 mother cladodes per group), which were placed under different-colored LEDs (Churitsu Electric Co., Japan): (1) red light, peak emission = 660 nm (Red); (2) blue light, peak emission = 440 nm (Blue); (3) simultaneous irradiation with red and blue light at a ratio of 1:3 (R+B [1:3]); (4) simultaneous irradiation with red and blue light at a ratio of 3:1 (R+B [3:1]). The relative spectral photon flux distribution of the LEDs used in this experiment was measured using a light analyzer (Nippon Medical & Chemical Instruments Co., Japan) (Fig. 2). The cladodes were grown under a 14-h light period (photosynthetic photon flux density = 150 μmol·m^{-2}·s^{-1}) and a 10-h dark period, and the cultivation room inside the enclosed-type plant factory was maintained at a temperature of 27 °C and a relative humidity of 60%–80%.

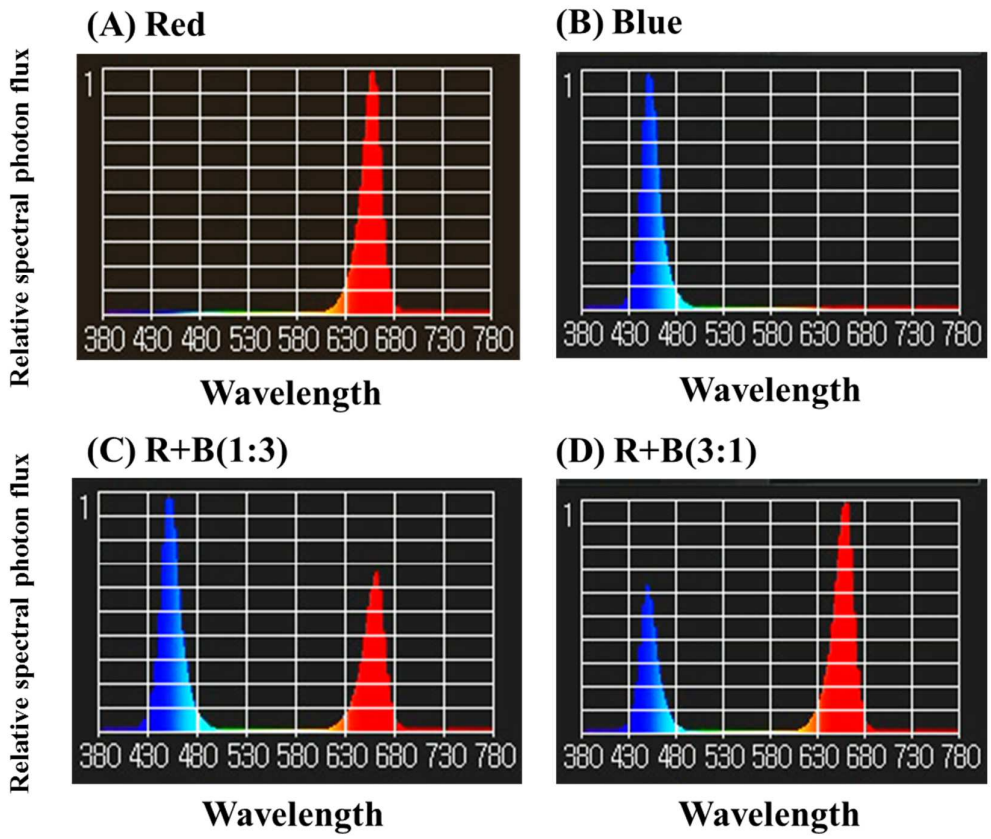

Figure 2. Relative spectral photon flux distributions of the light-emitting diodes (LEDs) used in this experiment. (A) red light, (B) blue light, (C) R+B (1:3), (D) R+B (3:1).

The numbers of daughter cladodes produced and the length of the first daughter cladode were measured weekly. The daughter cladodes were then harvested and weighed when they reached a height of 17 cm (by which point they had reached the LED panel). The width of 30 daughter cladodes was then counted in each treatment group. In addition, the number of areoles having spines was counted on 15 daughter cladodes from each treatment group and spine occurrence was then calculated as spine occurrence = (total number of areoles with spines longer than 1 mm)/(total number of areoles). When a mother cladode had multiple daughter cladodes, the mean spine occurrence was calculated.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant scavenging activities of five daughter cladodes in each treatment group were determined using the DPPH method. A 1-g sample of each daughter cladode was

homogenized in 5 mL of methanol:water (6:4 v/v), and the resulting homogenate was centrifuged at 6,000 × g for 10 min at room temperature. The capacity to scavenge the "stable" free radical DPPH was monitored following the method of Du Toit *et al*. (2001). The DPPH assay was initiated by adding 23 µL of the homogenate to a microplate well containing 210 µL of 0.6 mM DPPH methanolic solution (Tinyane *et al*., 2013). The reaction mixture was then left at 25°C for 60 min, following which the decrease in absorbance at 515 nm was measured using a microplate reader. In addition, a solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, an antioxidant) was prepared immediately before the analysis and used as a positive control, and the same reagents were used without the addition of cactus extract as a negative control. The antioxidant scavenging activities were expressed in µg of Trolox·g FW^{-1}.

Experimental design and statistical analysis

Sixteen cladodes were used for each treatment (Red, Blue, R+B [1:3], R+B [3:1]). All experiments were repeated twice. The first experiment was started in April 2018, and the second experiment was started in October 2018. The data were subjected to analysis of variance, and differences across means were determined using Tukey's test, with significance defined as $p < 0.05$.

RESULTS

Changes in length of first daughter cladode and number of daughter cladodes

In all treatments, daughter cladodes developed from mother cladodes during the first 2 weeks and continued to elongate until harvesting (Fig. 3A). The rate of cladode elongation was highest in Red and lowest in Blue or R+B (1:3). Growth rate was intermediate in cladodes in R+B (3:1).

The number of daughter cladodes continued to increase during the experimental periods in all treatments (Fig. 3B). In all treatments, the number of daughter cladodes rapidly increased over the first 2 weeks, became almost stable until 6 weeks, and then increased more rapidly again until the end of experiment. The number of daughter cladodes produced in Red was significantly higher than in Blue and R+B (1:3) beyond week 6 (Fig. 3B). There were no significant differences between Blue and R+B (1:3) beyond week 8. The width of daughter cladodes was highest in Blue and R+B (1:3), shortest in Red, and intermediate in

R+B (3:1) (Fig. 4).

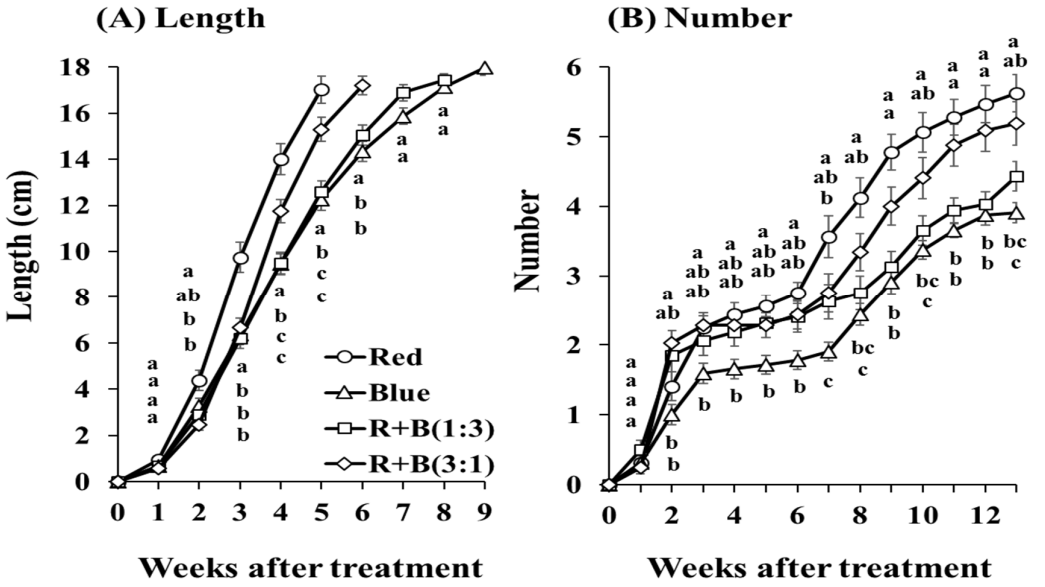

Figure 3. Length of the first daughter cladode (A) and number of daughter cladodes produced (B) in each treatment group. Means followed by a different letter within each week are significantly different ($p < 0.05$). Values are mean ± SE (n = 32).

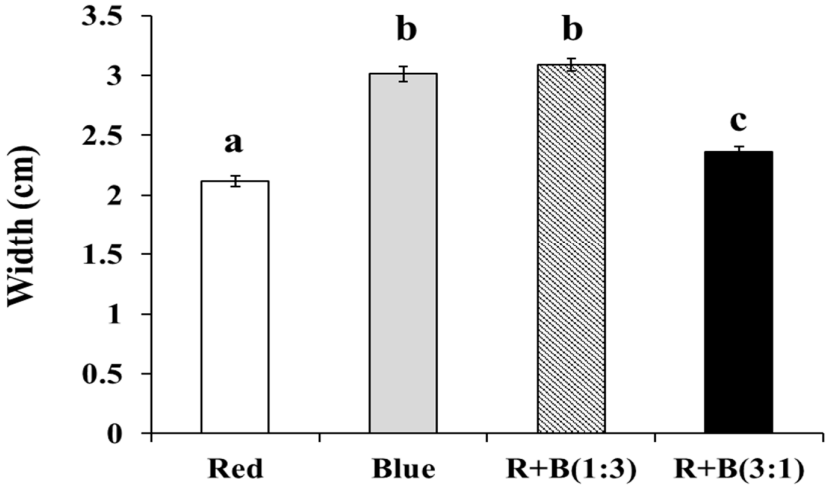

Figure 4. Width of the daughter cladodes in each treatment group. Means followed by a different letter within each week are significantly different ($p < 0.05$). Values are mean ± SE (n = 60).

Average and total fresh weight of daughter cladodes

The average FW of daughter cladodes was highest in Blue and R+B (1:3), followed by R+B (3:1), and lowest in Red (Fig. 5). The total FW of daughter cladodes harvested from one mother cladode was similar and highest in R+B (1:3) and R+B (3:1), somewhat lower in Red, and significantly lower in Blue. (Fig. 6).

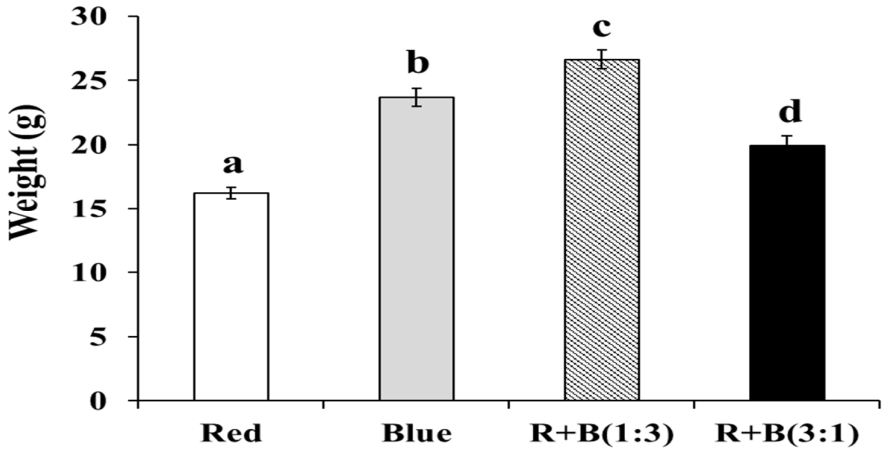

Figure 5. Average fresh weight (FW) of the daughter cladodes in each treatment group. Means followed by different letters are significantly different according to the least significant difference ($p < 0.05$). Values are mean ± SE (n = 60).

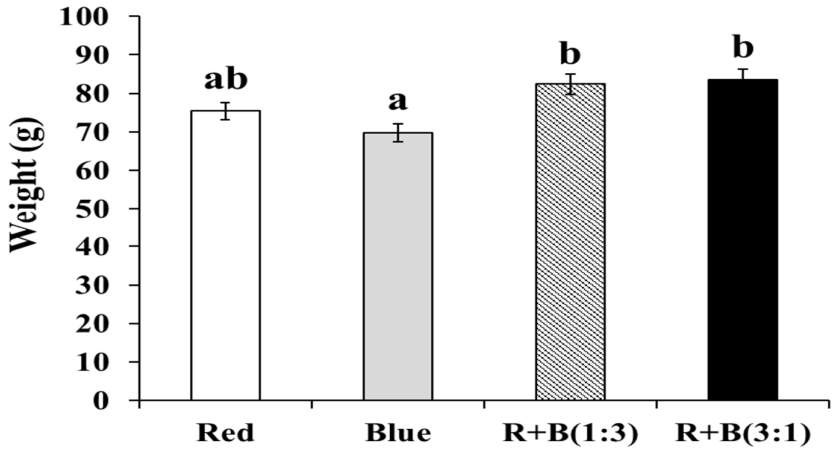

Figure 6. Total FW of the daughter cladodes harvested from a single mother cladode in each treatment group. Means followed by different letters are significantly different according to the least significant difference ($p < 0.05$). Values are mean ± SE (n = 32).

Spine occurrence

The occurrence of spines on daughter cladodes was similar and highest under the two treatments providing simultaneous red and blue light irradiation, somewhat lower in Blue, and significantly lower in Red (with no significant difference between Red and Blue) (Fig. 7).

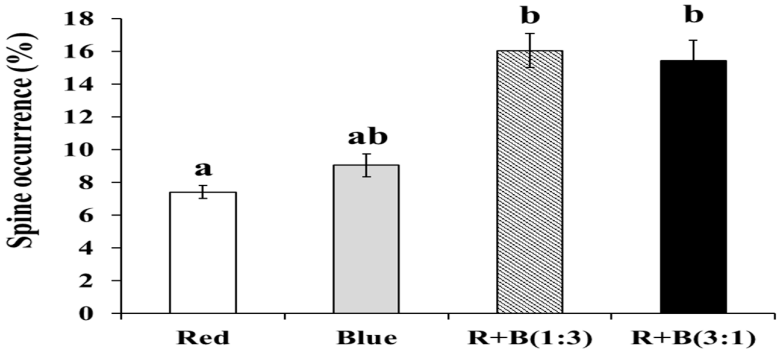

Figure 7. Occurrence of spines on the daughter cladodes in each treatment group. Means followed by different letters are significantly different according to the least significant difference ($p < 0.05$). Values are mean ± SE (n = 30).

DPPH radical scavenging activity

The DPPH radical scavenging activity values were similar and significantly higher under blue and R+B (1:3) than in red or R+B (3:1) (Fig. 8). The difference in values between Red and R+B (3:1) was not significantly different.

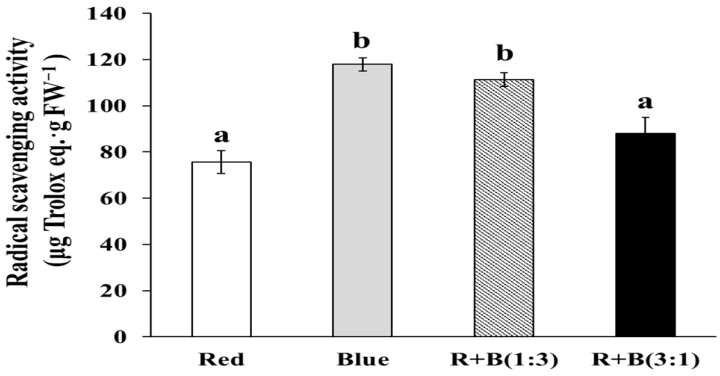

Figure 8. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the daughter cladodes extracts. Means followed by different letters are significantly different according to the least significant difference ($p < 0.05$). Values are mean ± SE (n = 10).

Hydroponic culture is the main cultivation system used in enclosed plant factories, whereas both soil and hydroponic technologies are used in natural sunlight systems. Hydroponic culture can circumvent the expensive and time-consuming task of soil sterilization to prevent soil-borne diseases and enables precise fertilizer management (Wahome *et al*., 2011; Lakkireddy *et al*., 2012).

With respect to the growth behavior of the edible cactus *N. cochenillifera*, daughter cladodes develop from the areoles of the mother cladode and this process is repeated (Pimienta-Barrios *et al*., 2005). In this study, daughter cladodes developed from mother cladodes and continued to grow under all treatments (Fig. 3). Although it is not clear whether mother cladodes also undergo C3 photosynthesis or CAM, this result indicates that *N. cochenillifera* can be successfully grown by hydroponic culture using the deep-flow technique under different-colored LED lights.

Under blue light, the speed of elongation of the first daughter cladodes was slowest, and the number of daughter cladodes was lowest compared with red light (Fig. 3). Under simultaneous irradiation with red and blue light, the ratio of red and blue light significantly affected the speed of elongation and number of daughter cladodes, with slower growth under R+B (1:3) than under R+B (3:1) (Fig. 3). These results indicate that blue light appears to suppress development of daughter cladodes.

Blue light has been reported to suppress plant growth in several studies (Kigel and Cosgrove 1991; Maas *et al*., 1995). For instance, hypocotyl growth of *Arabidopsis* is suppressed by blue light through cryptochrome-mediated signal transduction (Zhao *et al*., 2007). It has been shown that the amount of hypocotyl GA_4 decreases after irradiation with blue light resulting in the suppression of elongation (Zhao *et al*., 2007). Thus, changes in the concentrations of plant hormones, such as gibberellin, in response to blue light might be involved in the observed suppression of daughter cladode growth in the Blue and R+B (1:3) treatments. The final width of daughter cladodes was smallest under red light followed by the R+B (3:1) treatment (Fig. 4), resulting in the low average FW of daughter cladodes under these treatments (Fig. 5). Considering that the width of daughter cladodes under R+B (3:1) was smaller than under R+B (1:3), the higher rate of elongation under red light seems to result in narrower daughter cladodes. Since daughter cladodes were harvested when

they reached a height of 17 cm (at the point they reached the LED panel), cladodes grown under red light had a shorter time until harvest than those grown under blue light and in the R+B (1:3) treatment.

Although average FW of daughter cladodes under Red and R+B (3:1) treatments was small, the total FW of daughter cladodes harvested from one mother cladode was not low compared with the other treatments due to the large number of cladodes under these treatments (Figs. 3B, 5 and 6). The total FW of daughter cladodes under blue light was smaller than simultaneous irradiation with red and blue light because the number of daughter cladodes was less, even though the average of FW of daughter cladodes was relatively high (Figs. 3B and 6). These results suggest that the use of red light is effective in increasing cladode number and harvest.

Comparison of spine occurrence on daughter cladodes revealed that the number of spines on the cladodes under simultaneous irradiation with red and blue light was higher than that under red or blue light alone, while there was no significant difference among other treatments (Fig. 7). These findings suggest that red and blue light might induce spine development via different signal transduction pathways as simultaneous irradiation with red and blue irradiation had the strongest effect on spine development. It has been reported that phytochrome and phototropin interact with each other (Devlin and Kay, 2000; Hughes *et al.*, 2012); such interaction might also affect cladode growth and spine development. Although a number of beneficial functions have been attributed to the spines on cacti, including mechanical protection from herbivores (Norman and Martin, 1986), shading of the stem (Nobel *et al.*, 1986), and collection of condensation from fog to absorb water (Ju *et al.*, 2012), spines on edible cacti are one of the most undesirable characteristics for consumers. They are usually burnt off or removed using a knife before the cactus is put on sale. Therefore, cultivation techniques that reduce the number of spines could contribute to improving the commercial value of edible cacti; we think that control of the light environment could be useful for this purpose.

We also measured the DPPH radical scavenging activity of daughter cladode extract to assess the effect of the wavelength of the illumination light on the nutritional quality of edible cacti (Fig. 8). DPPH activity was highest under blue light or under the R+B (1:3) (Fig. 8),

indicating that the strength of the blue light is positively correlated with DPPH radical scavenging activity. Several studies in green vegetables have shown that blue light stimulates antioxidant status, such as increased polyphenol (Johkan *et al*., 2010), vitamin C (Li *et al*., 2012) and anthocyanin content (Stutte *et al*., 2009). Similarly, our results suggest that blue light is effective in increasing the antioxidant status of *N. cochenillifera*.

Plant factories are horticulture greenhouses or automated facilities where vegetables and other crops can be produced throughout the year by controlling environmental conditions such as light, temperature, humidity, CO_2, and nutrient availability (Yanata and Takata, 2014; Hirama, 2015). There are two main types of plant factory, fully enclosed artificial lighting-based systems, and natural sunlight-based systems. In an artificial lighting-based fully enclosed plant factory, high quality pesticide-free vegetables can be produced throughout the year due to the controlled, optimal cultivation environment (Yamori *et al*., 2014).

However, plant factories also have some disadvantages. For instance, the initial investment and running costs (especially personnel, fuel, and lighting expenses) are very high (Yanata and Takata, 2014). Thus, most enclosed-type plant factories grow leafy vegetables, herbs, root crops, medicinal plants, and other crops which are short in height and which command high prices (Kozai, 2013). Edible cacti have many suitable features for production in plant factories. For instance, they grow rapidly and can be vegetatively propagated using stems (in the short term until harvest), and they can be planted on cultivation panels at high density (effective use of available space) and cultivated under relatively low light intensity using artificial light (Horibe and Yamada, 2016). Therefore, we consider that plant factories are powerful tools to cultivate and improve the quality of edible cacti.

CONCLUSION

In this research, we evaluated the effects of light wavelength on the growth and the quality of edible cacti, demonstrating that lighting conditions strongly affect the growth and quality of daughter cladodes. Manipulating the light environment to promote daughter cladode growth and suppress spine development could improve the quality and production level of edible cacti. However, it is still not clear how other aspects of the lighting conditions, including photoperiod and wavelength, nor the physiological mechanisms underlying the

light wavelength effects seen in this study affect cladode growth. Therefore, additional studies are needed to further understand the relationship between the light environment and edible cactus development.

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