

千葉大学学位申請論文

Prospect of LED light sources
to enhance lettuce quality and
productivity in plant factory

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LIST OF ABBREVIATION

θ	Curvature factor
B/C ratio	Benefit-cost ratio
CPPS	Closed plant production system
FL	Fluorescent lamp
FW	Fresh weight (gram)
HPS	High sodium pressure lights
LED	Light emitting diode
F_v/F_m	Maximal photochemical efficiency of PSII
NIR	Near-infrared
PAR	Photosynthetically active radiation composition
PFAL	Plant factory with artificial light
PPFD	Photosynthetic Photon Flux Density
RUE	Resources use efficiency
UV	Ultraviolet

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Chapter I

Introduction

Plant factory with artificial light (PFAL), city farm, or vertical farming refers to a new modern style of agriculture, introduced to the world decades ago. In brief, the PFAL is a warehouse-like structure with well-insulated wall, nearly airtight system, and controlled environment (Kozai, 2013). Moreover, it is a type of closed plant production system (CPPS) for à-la-mode plant growing system. This cultivation is utilized to produce vegetables commercially throughout the world, e.g., England, Japan, Russia, South Korea, Taiwan, The Netherlands, and The U.S. for protected horticulture (Hayashi, 2016). Raising vegetable principally needs light as an energy source and water or nutrient solution simultaneously. Quality of product is a major priority while resources use efficiency (RUE) is another critical factor, especially electricity cost.

The technology of PFAL cultivation lighting system has been pursued endlessly to seek out ideal ratio and/or spectrum for optimum productivity. Replacement of a traditional light source, fluorescence (FL) with a modern light emitting diode illuminant (LED) provides tremendous benefit to both vegetable and producer. The plant will receive a specific light quality while electric usage of LED is far less than FL. Growing plants under multi spectrum for desirable traits might be necessary for further economic value. This could be discovered under this lighting technology, perhaps. On the other hand, production quality is determined by: affordable price, attractive appearance, delightful taste, greater vitamins content, insecticide and fungicide free, and etc. But, none of aforementioned, quality is of high interested. Hence, lesser LED electric input greater vegetable fresh weight output. Furthermore, declining waste is doubted after having had enormous production fresh weight including senescent leaves and roots. For its declination to fit the RUE concept is needed to be more investigated.

Redesign strategies on discovering novel cultivation in this scrutinize dissertation will illustrate ideas for enhancing vegetable quality production with artificial lighting, i.e., LED. Four experiments were conducted by using some lettuce cultivars (*Lactuca sativa* L.) with the LED lighting system under PFAL condition to determine any possibility for enhancing vegetable crop quality. Dawn of the twenty-first century after obscurity, the expansion of PFAL will alleviate food famine in very limited urban area; boost vegetable nourishment for society consumption; and, above all, be environmentally friendly to make the world a better place to next generations of humanity.

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Chapter II

Literatures review

PLANT FACTORY WITH ARTIFICIAL LIGHTING

General characteristics

Plant factory with artificial light (PFAL), which also known as vertical farming, is a closed plant production system which can obtain annual productivity of leaf vegetable per unit area above 100 fold, compared with conventional system (Kozai, n.d.). In Japan, plant factory with artificial light has been increasing since 2009 due to the modern trend of urban agriculture. However, profitability could be obtained approximately 25% of plant factory with artificial light while 50 and 25% are breakeven and lost in business, respectively (Kozai, 2014).

In the production system, many resources are used to maintain plant cultivation, i.e., water, CO₂, light, electric, and fertilizer, which are essential resources for growing plant in the system. Utilization of these resources per unit time interval has been clarified to 'the use efficiency' (Kozai, 2013). The resources use efficiency, for instance, is a ratio between amount of resource fixed or held in production to amount of resource input. The resources use efficiency is significantly greater in the plant factory with artificial light than in the solar light greenhouse (Table 2.1; Kozai, 2014).

Several light sources have been irradiated in plant production, e.g., fluorescent lamp (FL), high sodium pressure lights (HPS), and recently light emitting diodes (LED). Each light sources has its own advantages or disadvantages. The light source for closed plant production system is of high interest since the light use efficiency can be increased more than 70%. Increasing light use efficiency will be an important factor to achieve the profitability in this area. Energy use efficiency, on the other hand, can be improved by using a light source with a high percentage of electric-light energy conversion, optimizing light source with photoperiod and intensity, or pointing light direction to leaves with reflectors. Nowadays, energy conversion in plant production is higher in LED than fluorescent lamp which is approximately 40 to 25%, respectively (Table 2.2; Kozai, 2014).

Thus, this dissertation will mainly focus on a theme of light-use to improve plant production.

Economic aspect

Production cost of vegetable in plant factory with artificial light is clarified (Table 2.3). The major costs are combined between depreciation and electricity. Using minimum electricity to maximize production is a major concern by the system. Productions from plant factory with artificial light is designed and operated to produce a maximum amount of high quality plants with minimum input variation. Using minimum amounts and types of resources with minimum emission of environmental pollutants by providing the optimal amount of each resources at optimal timing for human welfare and global sustainability is the final goal of the system (Kozai, 2014).

Many techniques have been issued in order to maintain the system operation. Principally, light is a major controlling factor. Cost reduction could be obtained by, for example, spacing plant within transplanting periods to maintain leaf area index between 3-4; maximize net photosynthetic rate under a given lighting condition; maintain light source temperature to get a maximum light energy output; leveling the daily electricity consumption; or increasing a portion of salable part of plants (Kozai, 2014).

Light-emitting diodes (LED)

LED is a new light source integrating by injecting electrons into the semiconductors to generate light. The semiconductors or diodes are comprised of different inorganic materials each of them emits different light quality (Pimputkar et al., 2009). For instance, aluminum (Al) and gallium (Ga) have been used as semiconductors for early LED production, which emit red color (Kish et al., 1996). However, AlGa-base wavelength could not be shifted to lower band until Nakamura et al. (1994), whom was awarded the Nobel Prize with his colleagues in Physics in 2014 for the invention of the blue LED, by doubling heterostructure of semiconductors between indium (In) and Al to Ga. With the InGa-base semiconductors, UV, blue, green, or red color are obtained to fabricate white LED type efficiently (Mukai et al., 1999). In addition, semiconductor materials, other than Al, Ga, or In, use for light regions were shown in Table 2.4.

White LED type has been concerned on its impact to energy consumption since replacing FL by LED could reduce worldwide energy consumption approximately 1,000 TWh year⁻¹ (Krames et al., 2007). Three type-base LED have been used to generate white LED type which are; 1) blue LED with yellow phosphor, 2) ultraviolet LED with mixture phosphor, and 3) combined blue, green, and red LED device (Pimputkar et al., 2009). So, one white LED type from those could be an ideal light source for the closed plant production system (CPPS) since white light is a representative of action spectra (McCree, 1971).

Light and their action spectra

Light is a major environmental factor that influences plant growth and development. It is the basic driving force of photosynthesis where crop biomass, mainly, will be derived from increment of photosynthesis (Terashima et al. 2006). Theoretically, blue and red light is strongly absorbed by chlorophyll in leaves, indicating strong effects on plant development and physiology (Moss and Loomis, 1951). However, white and green light could be a new inevitable light color in agricultural production because of photosynthetically active radiation composition (PAR).

Different light sources, i.e., fluorescent lamp (FL), HPS, or LED, are used to irradiate light as a direct light source or supplemental light in the production (Table 2.5). Downward lighting in the controlled environment production has initiated for more than 2 decades famously in the Dutch greenhouse by using HPS (Stanghellini, 2017). Higher production is obtained by extending photoperiod in several plant species which is not only in vegetable, e.g., sweet pepper (Hovi-Pekkanen et al., 2005), or tomato (Hernández and Kubota, 2012), but also in ornamental plants such as rose (Paradiso and Marcelis, 2012) or petunia (Graper

and Healy, 1991). Certainly, downward lighting could improve plant growth and production, however, plants are suffered from shading of leaves in between plant canopy resulting in plant production, in other word, insufficient light within the canopy.

Supplemental lighting is an alternative technique which appears to be advantageous. Inter and/or upward lighting from different direction of plant canopy is introduced to the mass-plant production system. Obviously, more light intensity plant receives from different direction, higher plant biomass is obtained. For example, cucumber and tomato yield was increasing 15 and 27% by using HPS and; blue and red LED inter lighting, respectively (Hovi et al., 2004; Tewolde et al., 2016).

Several light region is used as the supplemental lighting sources to induce and grow desirable traits in plant production from UV to far red region (Table 2.6). UV-A (370-400 nm) increased anthocyanin in red lettuce (Li and Kubota, 2009) and pigments in *Fuchsia hybrid* (Helsper et al., 2003) while UV-B (20-320 nm) increased the carotenoid and chlorophyll concentration of 8 green leaf lettuce varieties (Caldwell and Britz, 2006). But, generally, the UV region showed negative effect on plant biomass, i.e., fresh and dry mass (Krizek et al., 1998).

Apparently, blue light is used to promote stomatal opening (Taiz and Zeiger, 2006). Blue light has been confirmed to accumulate and increase antioxidant content and capacity, e.g., carotene (Lefsrud et al., 2008; Samuolienė et al., 2017), chlorophyll (Mizuno et al., 2009), Chlorogenic acid (Johkan et al., 2010), and total phenolic content and antioxidant capacity (Son and Oh, 2013). In addition, supplemental blue light under red light elevated photosynthetic rates per unit leaf area indicating to dry matter production which contributes from stomatal opening vice versa (Matsuda et al., 2004).

Green light has been believed not to be used efficiently in plant cultivation because of low absorptance for several decades (Gates et al., 1965). It tends to resist the effect of blue and red light in plant physiology and morphology (Wang and Folta, 2013). Yet, it should not be ignored to gain crop yield recently (Smith et al., 2017). Nowadays, green region has been concerned and proven that photosynthetic rate (Johkan et al., 2012), plant biomass (Kim et al., 2004), ascorbic acid (Samuolienė et al., 2012), and α carotene (Samuolienė et al., 2013) can be increased under this region.

Red region is well known to increase photosynthesis (Taiz and Zeiger, 2006) and stem elongation (Okamoto et al., 1996). Especially, photosynthetic rate that can increases plant growth (Wojciechowska et al., 2015). Still, red region also be able to improve, such as, vitamin B9 (Długosz-Grochowska et al., 2016) and C (Zukauskas et al., 2009), or dry biomass (Yanagi et al., 1996).

Supplemental far red (735 nm) to red LED (660 nm) is not only induced stem elongation 100% deep down into its anatomical features compared to metal halide in pepper (Brown et al., 1995; Schuerger et al., 1997) but, in contrast, plant biomass was proven to be increased with supplemental far red in lettuce (Stutte et al., 2009; Kubota et al., 2011)

Tremendous research had been determined effective of light quality, especially blue and/or red light with downward lighting on plant production, extensively (Table 2.5 and 2.6). Nonetheless, using white and green LED as a major or a supplemental light source; and

supplemental upward lighting are rarely investigated compared to the blue or red light; or inter lighting. Green light can potentially penetrate into the plant canopy greater since blue or red light are mostly absorbed more than 95% from the top canopy (Gates et al., 1965). Recent researches showed that green light potentially plays an important role in photosynthesis (Terashima et al., 2009; Johkan et al., 2012). White light, in other word, consists of all spectra including green light region (Moss and Loomis, 1951).

Thus, the author believes that including green region, under white LED condition, could be an important factor in plant production.

LETTUCE (*Lactuca sativa* L.)

Morphology

Genus *Lactuca* L. has been cultivated regularly in the northern hemisphere, i.e., temperate and mediterranean region. More than 100 species is membered in this genus. Cultivated lettuce is related in specie *sativa*. *L. sativa* consists of 2 varieties, i.e., *capitata* and *crispa*, or 6 cultivar groups, i.e., 1) butterhead, 2) crisp head, 3) Latin, 4) cutting or loose leaf, 5) cos, and 6) stalk lettuce (Figure 2.1; De Vries and Van Raamsdonk, 1994).

In this study, 3 types of lettuce from 3 cultivar groups are studied the effect of growth under different light quality (Table 2.7), which are 1) crisp head (cultivar group 1), 2) frill (cultivar group 4), and 3) romaine (cultivar group 6).

Production and major nutrition facts

A conventional technique for lettuce production has been changed from soil to soilless culture. A trend of lettuce consumption becomes more interesting in tropical area even more because of this technique can decrease barrier of growth condition, for example, controlling growing environment (Jie and Kong, 1998; Thompson et al., 1998).

Two major components of lettuce consumption have been concerned to be improved (Table 2.7). The first nutrition is vitamin C or ascorbic acid, considered as its precursor. Under the conventional cultivation, the ascorbic acid content is low (<10 mg 100 g⁻¹ FW) compared to lettuce cultivated under the closed system (>50 mg 100 g⁻¹ FW; Zhang et al., 2015). Besides, nitrate is the second nutrition which becomes more concern in health. Negative effect appears with exceeding in nitrate consumption and harmful to human (Katan, 2009). Mechanism begins from nitrite, a reduced form of nitrate, converted to nitrosamines and nitrosamides and reacted to amines and amides, respectively. These reaction led to carcinogen accumulation in human's body (Anjana and Iqbal, 2007). Moreover, nitrite can oxidate with ferrous iron (Fe²⁺) in haemoglobin to the ferric valence state (Fe³⁺) which producing methaemoglobin that harmful especially in infant (Hegesh and Shiloah, 1982). The symptoms are mainly bluish gray or brownish gray skin color, irritability, and excessive crying in children; some the worst case, infant mortality increasing (Fewtrell, 2004). One of the many goals of the close system cultivation is to include there nutritional values which is needed to be improved.

MAJOR COMPONENTS FOR FOOD CULTIVATION

General ideas and concepts

Producing plants based on desirable traits require several environmental controlling factors in plant cultivation. Since plant produces food from photosynthetic process by changing physical energy (light) to chemical energy (sugar), light is, thus, considered to be the most essential factor in this study. Light is composed from 3 major group which are PAR (47%), near-infrared (NIR; 50%), and so on (UV, etc.; 3%; Stanghellini, 2017). Plants use photoreceptors to receive different PAR and transfer signal for further process on plant growth and development (Franklin and Quail, 2009). Hence, using specific light to cultivate desirable species are major interest for the future of urban plant production.

First of all, visible light consists of the spectra between 400 to 700 nm. The spectra can be utilized by any plants and using light can be referred to many technique. For instance, blue and red light were proven to increase net photosynthetic rate resulting in higher plant biomass (Kim et al., 2004). Or, blue light could induce vitamin A and carotenoid (Ruiz-Hidalgo et al., 1997). Besides the spectra, direction of light irradiation is another approach to improve plant growth and quality. Such as, supplying interlighting could improve production of year-round cucumber (Hovi et al., 2004; Jiang et al., 2017). Thus, the direction of lighting does not come from the top of the canopy, but all the angle.

Secondly, plant species that can be cultivated and suitable inside the closed plant production system. Several aspects are required, e.g., short and mall size in depth (<30 cm), shot cycle to be harvested, approximately 30 to 40 days, can be grown under relative low light intensity and high plant density, or enrich in nutrition. Mainly, these refer to leafy vegetable, i.e., lettuce family, green mustard, sweet basil, or spinach (Kozai, 2014). Under shorter plant production cycle, faster increment plant production.

Lastly, producing specific plant quality can be extended by controlling nutrient solution. Antioxidant, minerals, taste, and/or vitamins content should be manipulated under several light regions condition, i.e., using specific light spectrum. Moreover, chronic kidney disease is a major public health issue recently (Hoerger et al., 2015). It is affected by potassium (Musso, 2004). Reducing potassium in plant composition by controlling nutrient solution can be one of the ideas to support dietary program to patients. Nonetheless, this study will not focus on the nutrient solution and its composition in plant production.

In conclusion, growing plants is not only to cultivate desirable plants, but also to grown plants that almost all part of plant is edible and salable with least residue (Kozai, n.d.). Integrating techniques between lighting condition (quality and direction) and plants species must be the alternative method for the next agricultural generation.

OBJECTIVE

Aforementioned, taking aspect between the lighting system and the plant production will lead to 2 types of objective within this study. Five cases from each chapter were performed according to objectives and described from chapter III to VI which stated below;

General objective

1. To create an idea of cultivating superior vegetables to fulfill multi desirable traits in PFAL condition under LED, the newest modern lighting technology.
2. To improve lettuce quality and productivity under closed plant production system
3. To evaluate if LED efficiency is applicable and feasible for commercial production.

Specific objectives

1. Chapter III: assess and rapidly screen plant growth and antioxidant contents of frill lettuce cultivated under various white LED type spectrum for quality production which could fulfill and meet customer multipurpose satisfaction.;
2. Chapter IV: analyze the effective of blue, green, red, and white LED types on plant growth and quality in crisp head lettuce under plant factory with artificial light condition;
3. Chapter V: investigate the effect of supplemental upward lighting under different light color and intensity on the retardation of outer leaf senescence, photosynthesis, growth, and yield in romaine lettuce;
4. Chapter VI: determine the productivity in plant production, biological as well as economic productivity, differed among the different LED types; and
5. Chapter VII: evaluate economic feasibility of supplemental upward lighting in growing romaine lettuce in a closed plant factory system

Table 2.1 Comparison of the resources use efficiency between plant factory with artificial light and greenhouse. Source: Kozai, 2014.

Use efficiency	PFAL	Greenhouse closed ventilation	Greenhouse opened ventilation	Maximum value
Water	0.960	N/A	0.02	1.00
CO ₂	0.880	0.5	N/A	1.00
Light	0.027	0.01	0.01	0.10
Electric	0.007	N/A	N/A	0.04

Table 2.2 Energy conversion percentage in plant production. Source: Kozai, 2014.

Percentage (%)	Fluorescent lamp	LED
Light energy from electric energy	25	40
Light energy received by leaves	50	90
Chemical energy fixed by plants	2	10
Salable parts	70	90

Table 2.3 Production cost and components in Japan. Source: Kozai (2014).

Cost type	Percentage (%)	Details
Depreciation	35	Infrastructure and facilities
Electricity	25	Lighting, temperature controlling units, and nutrient solution supply units
Labor	25	Man-labor
Others	15	Seeds, growing media, packaging, and logistic

Table 2.4 Spectra and wavelength from different semiconductor materials

Spectrum	Wavelength (nm)	Semiconductor materials	References
UV	315-399	Aluminium gallium indium nitride (AlGaInN)	McKendry et al. (2009)
		Indium gallium nitride (InGaN)	Wang et al. (2002) Mukai et al. (1999)
Blue	400-499	Indium gallium nitride (InGaN)	Nakamura et al. (1994)
		Silicon carbide (SiC)	Morkoc et al. (1994)
		Zinc selenide (ZnSe)	
Green	500-599	Gallium phosphide (GaP)	Hart (1973)
		Indium gallium nitride (InGaN)	Mukai et al. (1999)
Red	600-699	Aluminium gallium arsenide (AlGaAs)	Kish et al. (1996)
		Indium gallium nitride (InGaN)	Mukai et al. (1999)
Far red	700-799	Aluminium gallium arsenide (AlGaAs)	Kato et al. (1991)
		Gallium arsenide (GaAs)	

Table 2.5 Supplemental lighting direction and condition on plant production under greenhouse condition.

Lighting direction	Lighting sources and condition	Plants species	Effectiveness	References
Downward	HPS 167 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 24h	<i>Petunia x hybrids</i> Villm. 'Red Flash'	45% seedling relative growth rate increasing	Graper and Healy (1991)
	Red LED (661 nm) 55 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 18h	Tomato (<i>Solanum lycopersicum</i> 'Komeett')	55% shoot dry mass increasing	Hernández and Kubota (2012)
	HPS 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 16h	<i>Salvia splendens</i> 'Vista Red'	57% flowering time decreasing	Moccaldi and Runkle (2007)
	Combined blue (460 nm) and red (660 nm) LED 258 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 12 h	<i>Fragaria x ananassa</i> 'Everest' and 'Rondo'	3 and 25% yield and total soluble solid increasing	Nestby and Trandem (2013)
	HPS 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 16h	Rose (<i>Rosa hybrida</i> 'Akito')	15% net photosynthesis increasing	Paradiso and Marcelis (2012)
Inter	HPS 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 20h	Cucumber (<i>Cucumis sativus</i> 'Cumuli')	15% yield increasing	Hovi et al. (2004)
	HPS 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 16h	Sweet pepper (<i>Capsicum annuum</i> L.)	22% yield increasing	Hovi-Pekkanen et al. (2005)
	Combined blue (450 nm) and red (680 nm) LED 165 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 12 h	Tomato (<i>S. lycopersicum</i> 'Sanbi')	27% yield increasing	Tewolde et al. (2016)
Upward and inter	Combined blue (450 nm) and red (680 nm) LED 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 16 h	Tomato (<i>S. lycopersicum</i> 'Momotaro York')	19 and 13% yield; and 11 and 15% total soluble solid increasing by upward and inter lighting, respectively	Jiang et al. (2017)

Table 2.6 Main and supplemental lighting on leafy and vegetable plant production.

Spectrum	Lighting Sources and condition			Plants species	Effectiveness	References	
	Main	Supplemental	Time (hour)				
UV (315-399 nm)	FL 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	UV-A (373 nm) 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	16	Red lettuce (<i>Lactuca sativa</i> 'Red Cross')	11% anthocyanin concentration increasing	Li and Kubota (2009)	
	HPS 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	Blue LED (470 nm) 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	16	Green leaf lettuce (<i>L. sativa</i> 'Multigreen 3')	76% sucrose, 23% specific leaf area, and 20% net assimilation rate increasing	Sirtautas et al. (2014)	
	Red LED (660 nm) 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	Blue LED (447 nm) 50 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	16	Parsley (<i>Petroselinum crispum</i>)	59% β carotene at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 17% tocopherol at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD increasing	Samuolienė et al. (2017)	
	Blue (400-499 nm)	Blue LED (470 nm) 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	N/A	16	Cabbage (<i>Brassica oleracea</i> var. <i>capitata</i> 'Kinshun')	20% chlorophyll content higher than red LED	Mizuno et al. (2009)
	Natural sunlight	Blue LED (440 nm) 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	16h for 7 days before harvest		Kale (<i>B. oleracea</i> 'Winterbor')	8% β carotene higher than red LED	Lefsrud et al. (2008)
	Red LED (655 nm) 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	Blue LED (456 nm) 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	12	Red leaf lettuce (<i>L. sativa</i> 'Sunmang')	150% total phenolic content and 290% antioxidant capacity higher than red LED	Son and Oh (2013)	

Table 2.6 (cont). Main and supplemental lighting on leafy and vegetable plant production.

Spectrum	Lighting Sources and condition			Plants species	Effectiveness	References
	Main	Supplemental	Time (hour)			
Blue (400-499 nm)	Natural sunlight	Blue (468 nm) 100 $\mu\text{mol m}^{-2}$ s^{-1} PPFD	14h for 7 days at seedling stage before transplanting	Red leaf lettuce (<i>L. sativa</i> 'Banchu Red Fire')	700% Chlorogenic acid higher than red LED	Johkan et al. (2010)
	HPS 90 μmol $\text{m}^{-2} \text{s}^{-1}$ PPFD	Green LED (505 nm) 30 μmol $\text{m}^{-2} \text{s}^{-1}$ PPFD	16	Romaine lettuce (<i>L. sativa</i> 'Thumper')	33% α carotene increasing compared with no supplemental lighting	Samuolienė et al. (2013)
Green (500-599 nm)	HPS 170 μmol $\text{m}^{-2} \text{s}^{-1}$ PPFD	Green LED (505 nm) 30 μmol $\text{m}^{-2} \text{s}^{-1}$ PPFD	16	Green leaf lettuce (<i>L. sativa</i> 'Multi green 3')	26% ascorbic acid higher than blue LED supplemental	Samuolienė et al. (2012)
	Green (510 nm) 300 $\mu\text{mol m}^{-2}$ s^{-1} PPFD	N/A	24	Red leaf lettuce (<i>L. sativa</i> 'Banchu Red Fire')	18% photosynthetic rate increasing compared to FL	Johkan et al. (2012)
	Blue and red LED 150 μmol $\text{m}^{-2} \text{s}^{-1}$ PPFD	additional of 24% green fluorescent lamp (500-600 nm)	18	Lettuce (<i>L.</i> <i>sativa</i> 'Waldmann's green')	31% leaf area increasing by adding green light supplemental compared to blue and red solely	Kim et al. (2004)

Table 2.6 (cont). Main and supplemental lighting on leafy and vegetable plant production.

Spectrum	Lighting Sources and condition			Plants species	Effectiveness	References
	Main	Supplemental	Time (hour)			
Red (600-699 nm)	Red (660 nm) 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	N/A	1 day after harvest	Broccoli (B. <i>oleracea</i> var. <i>italica</i>)	37% ascorbic acid higher than blue LED	Ma et al. (2014)
	Natural sunlight	Red LED (638 nm) 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	16h for 3 days before harvest	Red leaf lettuce (<i>L. sativa</i> 'Multired 4')	50% total phenolic and vitamin C content increasing compared with no supplemental lighting	Samuolienė et al. (2011)
	Red LED 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	N/A	N/A	Lettuce (<i>L. sativa</i> 'Okayama- saradana')	20% dry biomass higher than blue LED	Yanagi et al. (1996)
	Red FL 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	N/A	12	Leaf lettuce (<i>L.</i> <i>sativa</i> 'Redfire')	50% chlorophyll and soluble sugar higher than blue LED	Ohashi-Kaneko et al. (2007)
	Natural sunlight	Red LED (660 nm) 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	16	Lamb's lettuce (<i>Valerianella</i> <i>locusta</i>)	8% folate increasing compared with HPS supplemental	Długosz- Grochowska et al. (2016)
	Natural sunlight	Red (660 nm) 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	4	Lamb's lettuce (<i>V. locusta</i> 'Nordhollandse')	25% yield and soluble sugar; and 13% ascorbic acid increasing compared with HPS supplemental	Wojciechowska et al. (2015)

Table 2.6 (cont). Main and supplemental lighting on leafy and vegetable plant production.

Spectrum	Lighting Sources and condition			Plants species	Effectiveness	References
	Main	Supplemental	Time (hour)			
Red (600-699 nm)	HPS 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 13 h	Red LED (638 nm) 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 12h	Combination	Green leaf lettuce (<i>L. sativa</i> 'Grand Rapids')	73% total phenolic content and 2% vitamin C increasing compared with no supplemental lighting	Zukauskas et al. (2009)
Far red (700-799 nm)	FL 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	Far red (700-800 nm) 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	16	<i>L. sativa</i>	28% fresh leaf production enhancing	Kubota et al. (2011)
	Red (640 nm) 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	Far red (730 nm) 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	18	Red lettuce (<i>L. sativa</i> 'Outredgeous')	20% dry mass production increasing	Stutte et al. (2009)

Table 2.7 The cultivation group and nutrition facts of 3 lettuces, crisp head, cutting leaf, and cos, growing under conventional cultivation which is subjected in this study.

	Crisp head	Frill	Romaine
Cultivar group	Crisp head	Cutting leaf	Cos
Synonym	Iceberg	Frilly	Cos
Vitamin C (mg 100 g ⁻¹ FW)	5.5 (Sørensen et al., 1994)	3.5 (Ogawa et al., 2014)	2.8 (Llorach et al., 2008)
Nitrate regulation (mg 100 g ⁻¹ FW; Alexander et al., 2008)	220	360	360

Packaging and
price



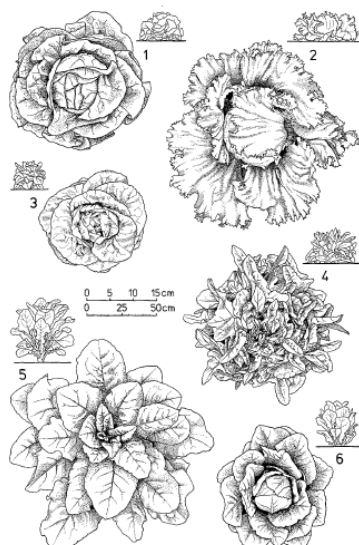


Figure 2.1 Drawing of six representatives of the six *Lactuca* cultivar groups. 1 'Hilde' (Butterhead), 2 'Great Lakes' (Crisp head), 3 'Bella' (Latin), 4 'Oakleaf' (Cutting), 5 'Celtuce' (Stalk), 6 'Verte Maralch6res' (Cos). Source: De Vries and Van Raamsdonk (1994).

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Chapter III

Frill lettuce quality assessment under various white LED spectra

ABSTRACT

Producing vegetables in a closed plant production system is a new modern agricultural practice because of efficient resources use efficiency. Safe and high-quality vegetable production is needed for urban areas. At the present, white LED type is new establishment of light source which uses to cultivate lettuce in so-called plant factory instead of blue and red colors. Many white LED types are utilized to obtain high production. Plant quality is a parallel factor to be considered for plant production. Six different white LED types are used for frill lettuce cultivation at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ in this study to determine its effectiveness on plant growth and quality. LED-A significantly increases shoot biomass and total soluble solid. LED-E obtains high compression and ascorbic acid content. All of the LED show high total phenolic content, but not LED-C. LED-E and LED-A exhibit the highest overall ranking among all the treatments. These results indicate that plant quality can be varied under different light quality to obtain any desirable traits for customers.

Key words: antioxidant, mixing spectra, plant growth and quality, taste, white LED

INTRODUCTION

Plant factory with artificial light (PFAL) is an up-to-date trend of agricultural production, especially leafy vegetable, alongside of intention to stabilize premium vegetable production with high quality and taste, e.g., higher in fresh weight, minerals, or antioxidant (Kozai et al., 2016). For decades, a fluorescent lamp had been used for the crop production in commercial business, however, its spectrum is not necessarily used for plant growth (Ikeda et al., 1991; Watanabe, 2009). Using light-emitting diode (LED) instead of the fluorescence for plant cultivation becomes common nowadays with many advantages such as true spectrum utilization, controlling intensity, or low radiant heat (Morrow, 2008; Shimizu et al., 2011). Principally, blue and red regions of the visible spectrum are strongly absorbed by a photosynthetic pigment for photosynthesis potential while little absorption spectrum will occur in green region spectrum (Gates et al., 1965; Taiz and Zeiger, 2006; Moss and Loomis, 1951). Plant growth and development are efficiency impacted by these spectra (Poudel et al., 2008; Yorio et al., 2001). The visible spectrum ratio manipulation could be a successive key to produce premium vegetable with high quality and taste regarding on weight and minerals or antioxidant contents.

Effect of monochromic light (blue and red) on plant growth has been studied extensively (Heo et al., 2002; Li and Kubota, 2009; Son and Ho, 2013). Furthermore, advantages of specific spectrum are varied. For example, supplemented monochromic blue LED light (468 nm) before transplanting promoted plant growth of red leaf lettuce (Johkan et al., 2010). Sole green LED light (510 nm) had the greatest effect to enhance lettuce growth (Johkan et al., 2012). Plant biomass was increased under the combination of far red (730 nm) and red (640 nm) in lettuce (Stutte et al., 2009). Total phenolic, sugar,

and free radical scavenging activity increased under supplemental red LED light (638 nm) for natural illumination (Samuoliene et al., 2012; Zukauskas et al., 2011). This alternative approach could be done under artificial lighting plant cultivation for customers need. In commercial business, on the other hand, LED mixing spectrum is of high interest (De Almeida et al., 2014). Many LED companies indicate their products to be the most suitable light source in plant factory with artificial light cultivation. This unrevealed circumstance awaits for determination. Plant growth and quality were determined under various LED light sources.

In vegetable production, secondary metabolite is considered to be an additional parameter to be considered beside plant growth which is usually classified into three major classes: terpenoid, alkaloid, and phenolic (Bourgau et al., 2001). Among them, phenolic compounds from plants are one of largest group which could act as oxidative stress protection or antioxidant (Kabera et al., 2014; Vinson et al., 1998). Moreover, this stress causes by free radicals and reactive oxygen species (ROS) which has deleterious effects on the human body (Lobo et al., 2010). Several screening assays for antioxidant activities and contents are well determined throughout many plants (Dudonné et al., 2009). Briefly, total phenolic content is widely used to detect polyphenol while the activities are detected from synthetic chemical, i.e., free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl; DPPH) or ferric reducing antioxidant potential (FRAP assay). Hence, different light spectrum of LED could affect lettuce biomass and quality. The aim of this study is to assess and rapidly screen plant growth and antioxidant contents of frill lettuce cultivated under various white LED type spectrum for quality production which could fulfill and meet customer multipurpose satisfaction.

MATERIALS AND METHODS

Plant materials and growth conditions

Frill lettuce seeds (*Lactuca sativa* L.; Takii Seed Co., Kyoto, Japan) were used in this experiment. A coated seed was sown in an urethane cube (W 2.3 cm × D 2.3 cm × H 2.7 cm) and placed under dark condition for 48 h. Seedlings were raised in an environment-controlled growth chamber under cool white fluorescent lamps at under light intensity $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD for 16 h (Panasonic FHF32EX-N-H). At 12 days after sowing, uniformed-size with 4-leaf plantlets were transplanted into a polystyrene panel (84 plant m^{-2}) and cultivated under different whit LED types. At 23 days after sowing, the plantlets were transplanted (42 plants m^{-2}) and cultivated at the similar light condition until harvest at 35 days after sowing.

All the treatments were cultivated in a commercial scale PFAL with cultivated floor area 335 m^{-2} (10 layers × 9 rows) and hydroponically grown with nutrient film technique using modified 0.75-strength Enshi solution. The nutrient solution was renewed weekly where pH and electrical conductivity were properly adjusted to 7.0 ± 0.2 and $2.0 \pm 0.2 \text{ mS.cm}^{-1}$. The environment was controlled throughout the experiment, i.e., the average air temperature, relative humidity, and CO_2 concentration, 25/20 °C (day and night), 45/65% (day and night), and $1,200 \mu\text{mol mol}^{-1}$, respectively.

Light treatments and sampling method

Treatments, consisted of 9 replicates, were cultivated with $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD under 7 different LED spectrum. Photon flux density was adjusted by covering with aluminum paper and measured approximately 0.25 m from the light sources with a light

sensor (Licor 250A; Li-cor Inc., Lincoln, NE, USA). The spectra were measured by a spectroradiometer (Lighting Passport, AsenseTek, Taiwan; Figure 3.1). The photoperiod of the treatments were maintained at 16/8 h light/dark condition. Seven types LED with different spectra, of which six were white LED type and one was a blue+red LED type, were used

At the harvest stage, 9 uniformed-size plants were selected for plant growth parameters measurement while another 9 uniformed-size plants were chosen and stored at -30 °C for plant quality measurement. An expanded leaf number 4, 8, and 12 were used for the quality measurement which represented each sample to an outermost, middle, and innermost leaf.

Plant growth and quality measurement

Frill lettuces were harvested at 35 days after sowing. Shoot fresh weight was measured immediately after harvested from the cultivation area.

Separated samples from the shoot fresh weight were used to measured compression, total soluble solid, ascorbic acid content, and total phenolic content. Three leaves were selected from each sample as a representative of each leaf layer and used throughout the quality measurement, i.e., leaf no. 4 or 5 (an outermost leaf layer), leaf no. 9 or 10 (an intermediate leaf layer), leaf no. 14 or 15 (an innermost leaf layer).

Compression

A sclerometer was used with 3 mm tip to measure a leaf compression (Lutron FR-5120, Taiwan). Average compression of 3 different spots on midrib was carried out in a newton unit (N) from the 3 selected leaves.

Ascorbic acid content

The 1-gram mixture of the selected leaves was, then, grinded homogeneously in 5 ml 5% metaphosphoric acid solution and filtrated with a filter paper (Mapson and Mawson, 1943). A test strip of AsA was dipped into the sap and recorded by RQflex 10 (Merck KGaA; Darmstadt, Germany). The values were expressed as mg ascorbic acid 100 g⁻¹ fresh weight (modified from Pantelidis et al., 2007).

Total soluble solid

The 2-gram mixture of the selected leaves was grinded homogeneously in 4 ml distilled water. The 1-ml sap was poured onto a refractometer (Pal-1, Atago, Japan). The values were expressed as mg ascorbic acid 100 g⁻¹ fresh weight (modified from MacGillivray and Graham, 1969).

Total phenolic content

A 2-gram mixture of the selected leaves was 0.45-min grinded homogeneously in 18 ml 80% methanol (Wako Pure Chemical, Japan; modified from Thaipong et al., 2006). The mixture was centrifuged at a constant 4 °C 15,000 rpm for 20 minutes. The sap sample was prepared and stored at -80 °C. Total phenolic content was measured according to ISO14502-1 (2005). Briefly, gallic acid (Wako Pure Chemical, Japan) was used to prepare a standard curve. Then, the 1-ml of the sap was mixed with 5 ml 10% Folin-Ciocalteu phenol reagent (Sigma-Aldrich, USA) and 4 ml 7.5% Na₂CO₃ (Kanto

chemical, Japan). A mixed solution was incubated 60 minutes at room temperature then irradiated with a broad absorption at 765 nm with a spectrophotometer (Hitachi U2910, Japan).

Product comparison

The values of plant growth and quality were calculated into percentage and illustrated in windrose diagram to compare its scores. The scoring was divided to high quality (>90%), moderate (81-89%), and low quality product (<80%).

Statistical analysis

Measurements were analyzed for the significance by analysis of variance (ANOVA) with Tukey's honest significant difference test (HSD) at the $p<0.05$ and $p<0.01$ level in SPSS statistical software v. 16.0 (SPSS, Chicago, IL, USA).

RESULTS

Plant growth

All the white LED type significantly improved frill lettuce plant growth compared with the blue+red LED type except the LED-F. LED-A exhibited the highest shoot fresh weight followed by the LED-B (Figure 3.2 and 3.5).

Plant quality

LED-E was solely significant difference compared to the other LED in compression of frill lettuce. LED-F was also higher than the blue+red LED type, but insignificant (Figure 3.3A). LED-A and LED-C showed the highest total soluble solid, but insignificant to the blue+red LED type. LED-B had the lowest total soluble solid (Figure 3.3B). Ascorbic acid content was significantly high in LED-E compared to the blue+red LED type. LED-B also showed higher ascorbic acid content even insignificant difference to the blue+red LED type (Figure 3.3C). All the treatments including blue+red LED type illustrated high total phenolic content which was significantly different compared to LED-C (Figure 3.3D). Furthermore, LED-C showed the lowest value in both ascorbic acid and total phenolic content (Figure 3.3 C and D).

Plant scoring and comparison

The shoot fresh weight was lowest under the blue+red LED type and LED-F, however, frill lettuce cultivate under the blue+red LED type had high values of total soluble solid and total phenolic content although LED-A and LED-C; and LED-F and LED-D had the highest values, respectively. LED-E presented highest value in compression and ascorbic acid content (Figure 3.4A).

The overall scoring of frill lettuce cultivated under various white LED type indicated that LED-E and LED-A were the best quality product (>90%), while the blue+red LED type, LED-F, and LED-C were, possibly, not satisfied (<70%). LED-B and LED-D were the moderate product (~80%).

DISCUSSION

White LED type is an ideal light source to replace the conventional fluorescent lamps inside plant factory with artificial light due to suited brightness to human's eye.

Moreover, a fluorescent lamp, in the condition, has been neglected because of light intensity could not be reached at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. It is decreased approximately 30% after having used for 10,000 h (Kozai, 2016). Whilst, frill lettuce was chosen because of its extreme reputation in PFAL model above another varieties around Tokyo area (Hagiya and Watanabe, 2016).

It is clear that the white LED types can prosperously use to cultivate frill lettuce. The plant biomass of the white LED types showed positive effect compared to the lettuce grew under solely blue and red region LED (Figure 3.2). The white LED types consisted of full photosynthetically active radiation, blue, green, and red region (400-700 nm). Each color region individually affects plant growth and development. Frill lettuce growth and quality under various white LED were improved compared to the lettuce grew under blue+red LED (LED-G). Apparently, the lettuce biomass was high when the red and farred region percentage was higher than 50 and 10%, respectively, while the low biomass occurred when the green region was removed (less than 1%) or more than 40% (Table 3.1). Red region is absorbed almost 9% by chlorophyll in leaves, indicating strong effects on plant development and physiology which could promotes photosynthesis, induces hypocotyl elongation and leaf area expansion (Muneer et al., 2014). Green region penetrates into plant canopy greater than other regions (Gates et al., 1965), but this characteristic is, yet, insufficient for plant growth (Wang and Folta, 2013).

Considerately, plant quality and taste are essential for consumers who purchasing plant productions for their good health (Veenhoven, 2013). This investment requires high expectation of return similarly to the producer. The compression was insignificant among the treatments except the blue+red LED type was the lowest while total soluble solid was extremely low, approximately 1 °brix, which cannot be detected the taste of sweet by human tongue (Figure 3.3 A and B;). Interestingly, LED-B and LED-E nearly exhibited the similar spectral distribution where ascorbic acid content was higher than the other LED (Figure 3.3C). Total phenolic content was insignificance among the treatments except LED-C, which also had the lowest ascorbic acid content. Ascorbic acid total phenolic content are considered to be an antioxidant which play an important role in human health (Sies, 1993). It is a stable molecule that has ability to pair a free radical electron and reactive oxygen species (Lobo et al., 2010). As a consequence regarding to frill lettuce growth and quality, LED-E revealed the highest overall scoring even though LED-A was the runner-up (Figure 3.4B). The lettuce cultivated under LED-B and LED-D, perhaps, are suitable for specific consumers who avoid sweet taste, especially LED-B that contained more ascorbic acid content (Figure 3.4A).

Nowadays, a price of white LED has been decreasing as the LED becomes more common as a household light source (Humphreys, 2008). Besides, conversion of electric to photon energy has been improved more than 50% in blue and red LED which the colors are consisted in the white LED type (Mitchell et al., 2012; Pinho et al., 2012). All the PAR region is essential similarly to the sun spectrum which has been used to cultivate plant in the history of agriculture, none of which is in-negligible. Hence, using the white LED type as the new light source for commercial closed plant production system is feasible.

CONCLUSION

The white LED types can be utilized in the closed system to produce high quantity and quality frill lettuce although obtaining high plant biomass in parallel with high plant

quality under one light condition is not yet to be achieved up-to-date. Combining different white LED type in one cultivation or changing different white LED type in different transplanting period may be a further solution to obtain the super-plant under the controlled environment plant production.

Table 3.1 Spectral distribution ratio for white LED types with ultraviolet, blue, green, red, and farred region. Spectral scan was recorded at 20 cm from the light sources with a spectroradiometer (Lighting Passport, AsenseTek, Taiwan).

	LED-A	LED-B	LED-C	LED-D	LED-E	LED-F	LED-G
Ultraviolet (380 - 399 nm)	0.04	0.31	0.02	0.05	0.04	0.03	0.04
Blue (400 - 499 nm)	20.74	12.98	7.52	26.27	13.22	15.76	12.20
Green (500 - 599 nm)	0.52	24.17	28.41	40.65	34.25	10.89	21.68
Red (600 - 699 nm)	77.46	52.28	51.51	29.82	43.75	63.88	58.55
Farred (700 - 780 nm)	1.24	10.27	12.54	3.20	8.74	9.45	7.53

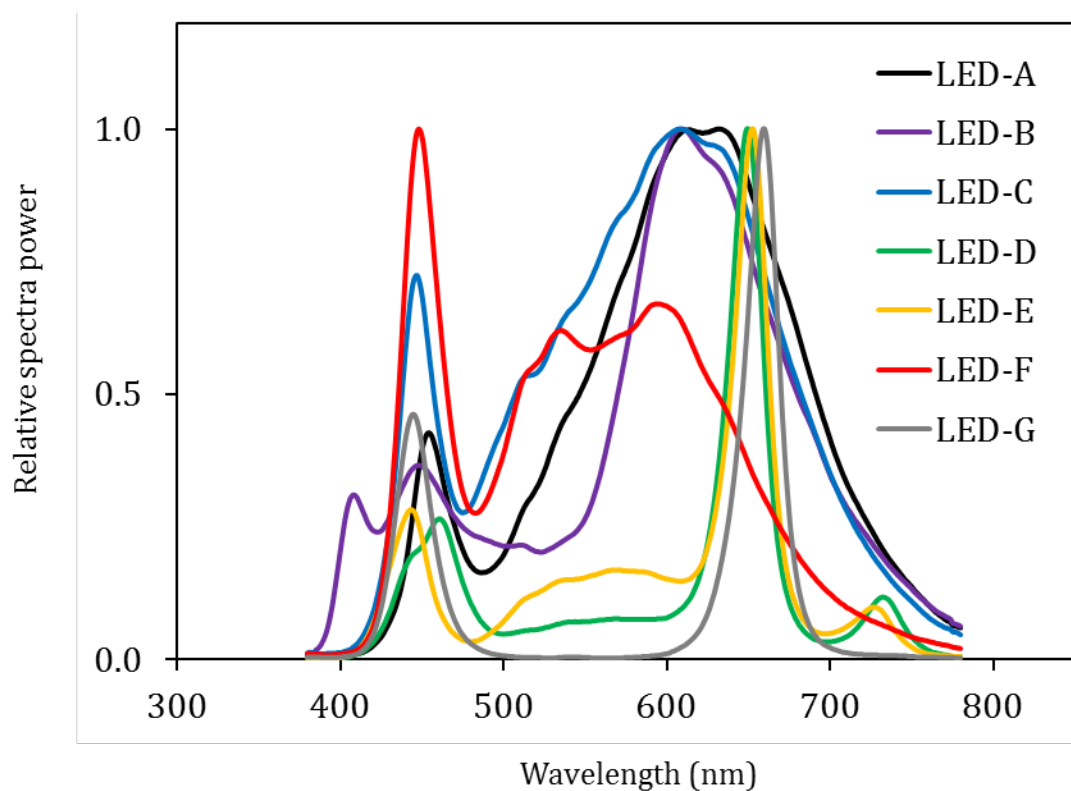


Figure 3.1 The spectral profiles of white LED types used in this study. The wavelengths of LED types were recorded at 380-780 nm with a spectroradiometer (Lighting Passport, AsenseTek, Taiwan).

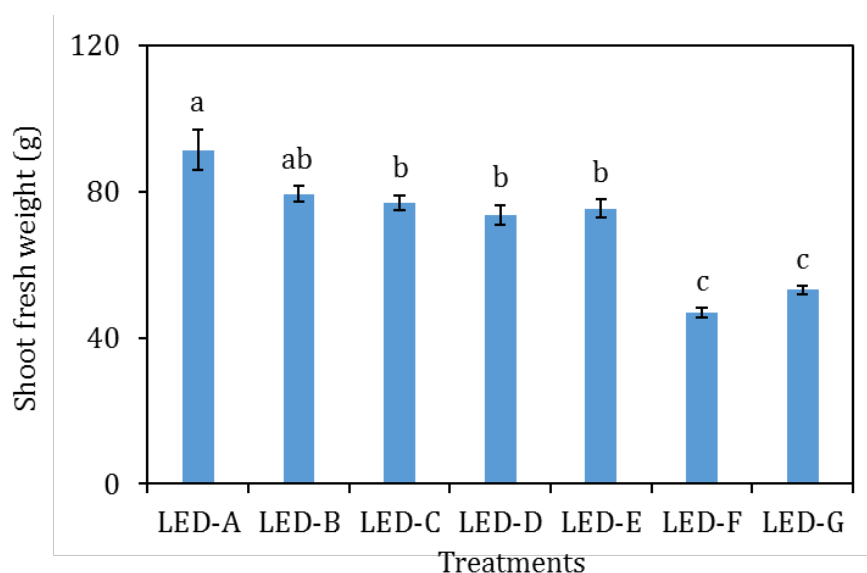


Figure 3.2 Shoot fresh weight of frill lettuce cultivated under different white LED types. The plants were grown under each LED types for 14 hours at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and harvested at 35 days after sowing. Data are means \pm SE ($n = 9$). Different letters indicate statistically significant differences among the treatments (Tukey's HSD at $P < 0.05$).

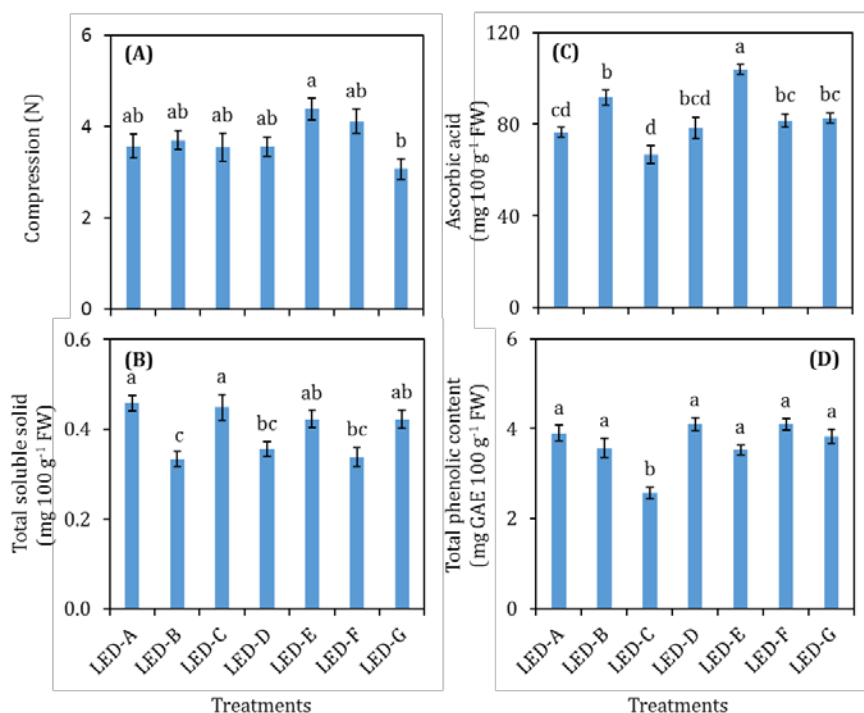


Figure 3.3 Plant quality of frill lettuce cultivated under different white LED types; (A) compression, (B) total soluble solid, (C) ascorbic acid content, and (D) total phenolic content. The plants were grown under each LED types for 14 hours at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and harvested at 35 days after sowing. Total phenolic content values were expressed as gallic acid equivalents (GAE) mg 100 g⁻¹ fresh weight. Data are means \pm SE ($n = 9$). Different letters indicate statistically significant differences among the treatments (Tukey's HSD at $P < 0.05$).

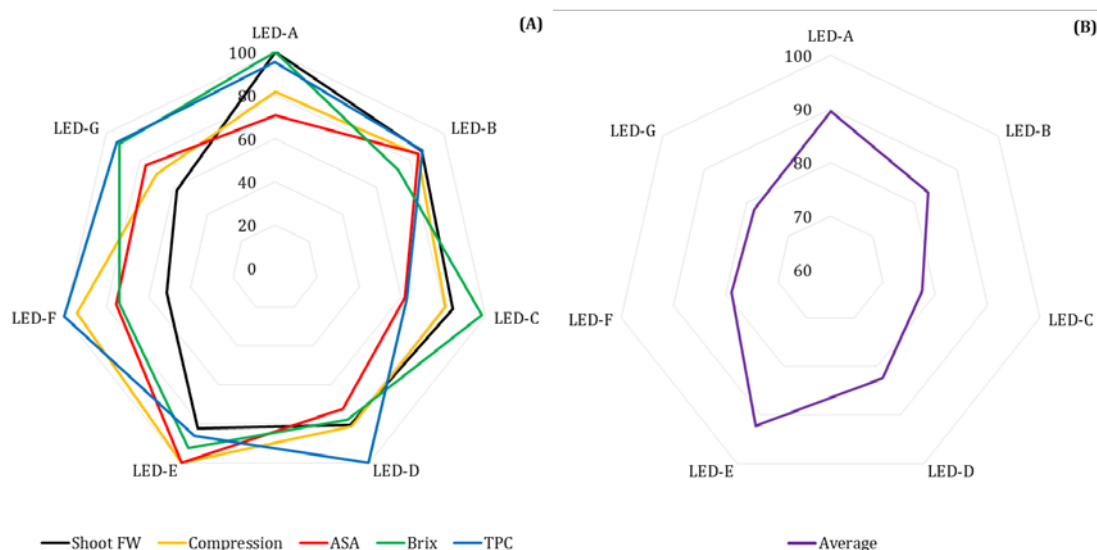


Figure 3.4 Plant growth and quality parameter of frill lettuce cultivated under different white LED types; (A) radar comparison and (B) overall scoring. All the score is calculated in percentage from each parameter.

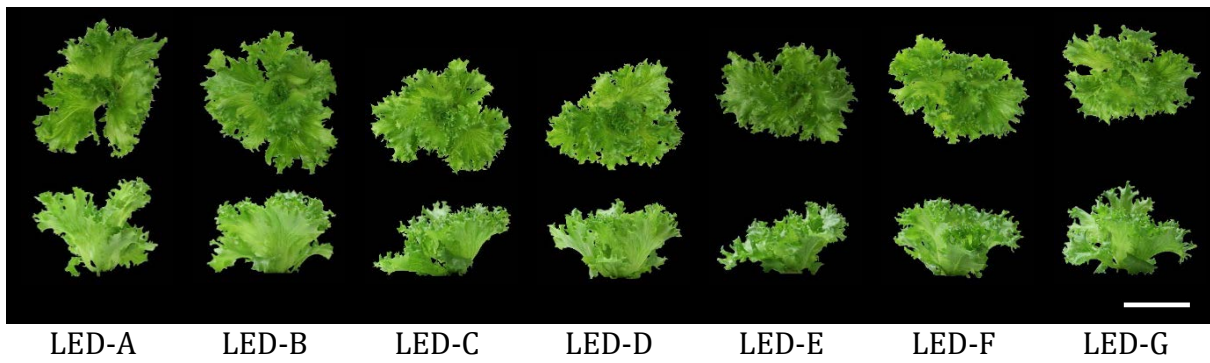


Figure 3.5 Appearance of frill lettuce cultivated under different white LED types. A bar indicated 5 centimeter.

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Chapter IV

Green light penetrates into inside of crisp head lettuce leading to enhancement of chlorophyll and ascorbic acid contents

ABSTRACT

Increasing safe and steady food production has been approached under plant factory production. Plants are often cultivated under LED which emit blue and red light regions because photosynthetic pigments strongly absorb these regions while green light region has been considered to be less effective due to its less absorptance. Crisp head lettuce is an economic crop which outer leaves have to be removed when shipping for round-shape display in a market, in spite that these removed leaves contain higher nutritional values. The present study shows that effect of photosynthetic photon flux density (PPFD) of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ green light on chlorophyll and ascorbic acid content; and shoot biomass have been examined in crisp head lettuce by using a characteristic of lower light absorptance, but greater light transmittance in leaves. The contents are high inside the crisp head lettuce especially under green light irradiation. The present study proposes an availability and integration of green light to improve plant quality even inside of the crisp head lettuce on its cultivation for a further novel cultivation system in plant factory.

Keyword: plant factory, green light, ascorbic acid, crisp head lettuce, cultivation system

INTRODUCTION

Leaf vegetables are increasingly produced under the controlled environment by using closed plant production system, so-called plant factory, to maximize the plant growth with the minimum inputs of light, energy, water, CO_2 , and inorganic fertilizer (Kozai, 2013). This system can produce high quality plants under irregular plant cultivation condition, including in desert or ice field, because of the controlled environmental system inside which flavor for plant production (Kozai et al., 2015). Nowadays, tremendous researches on leafy vegetable families, mainly LED, have been achieved on plant factory with artificial light to improve its production and quality because of the specific spectrum emission (Massa et al., 2008; Morrow, 2008). The blue and red regions of the visible spectrum are strongly absorbed by a photosynthetic pigment while little absorptance spectrum will occur in green region spectrum (Gates et al., 1965; Taiz and Zeiger, 2006; Moss and Loomis, 1951). Plant growth and development are potentially impacted by monochromic blue or red light and/or the combination of blue and red light (Poudel et al., 2008; Kami et al., 2010). For example in lettuce, raising seedlings treated with blue LED type promoted the growth of lettuce plants after transplanting (Johkan et al., 2010). Highest growth rate in height was obtained under red LED type (Chen et al., 2014). Combination of blue fluorescent lamp and red LED type promoted plant biomass (Yorio et al., 2001). Blue fluorescent lamp had also been reported to increase ascorbic acid (Ohasi-Kaneko et al., 2007).

On the other hand, green light had been believed not to use efficiency on plant cultivation because of low absorptance (Gates et al., 1965). It tends to oppose the effect of blue and red light in plant physiology and morphology (Wang and Folta, 2013). For example, shade avoidance syndrome was induced by green LED type (Zhang et al., 2011). Blue light-stimulated stomatal conductance could be reversed by green light pulse (Frechilla et al., 2000). Supplemental of green light to blue and red light also decreased anthocyanin (Zhang and Folta, 2012). These may affect to plant growth and quality. However, green light has several benefit that can be served as a signal for inducing phototropic responses (500-550 nm) in some further cases (Steinitz et al., 1985). For example, short-wavelength green LED type 510 nm peak with high photosynthetic photon flux density (PPFD) $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ was the most effective to promote leaf lettuce comparing to different short-wavelength green LED type at 520 or 530 nm peak (Johkan et al., 2012). The additional of 24% green fluorescent lamp (500-600 nm) to blue and red LED types enhanced lettuce growth compared to plants grew under cool white fluorescent lamps (Kim et al., 2004). Supplemental green LED type to romaine baby leaf lettuce grown under natural sunlight enhanced α -carotene and anthocyanin (Samuolienė et al., 2013). Principally, green light can penetrate into the plant canopy better than blue or red light (Klein, 1992). Although the blue and red light were depleted from the top canopy, plants below are suffused in an environment dominated by far-red light, along with a disproportionate amount of green wavebands. Continued deep into the leaf, sufficient green light penetrated and was absorbed by the chloroplasts in the abaxial side (Terashima et al., 2009). Hence, the merit of green light penetration could be advantages in plant factory because the light transmittance would penetrate deeper into high plant density production.

Lettuce is an economic vegetable which consumption grew steadily from 40 to 60 g of lettuce per person per week (Hospido et al., 2009). It is rich in antioxidant, i.e. ascorbic acid that lowered risk of cancer and cardiovascular diseases (Nicolle et al., 2004). In shipment, outer leaves of crisp head lettuce were removed. These removing leaves contain higher ascorbic acid than inner leaves (Poulsen et al., 1995). Thus, increasing nutrition inside crisp head lettuce canopy is desirable for plant factory with artificial light production. Using potential of green light to penetrate into plant canopy can be benefitted. Based on this hypothesis, green light could enhance nutrition in inner leaves inside crisp head lettuce. The present study analyzed the effective of blue, green, red, and white LED types on plant growth and quality in crisp head lettuce under plant factory with artificial light condition.

MATERIALS AND METHODS

Preparation of plant materials

The seeds of crisp head lettuce (*Lactuca sativa* L. var. *Capitata*; Takii Seed Co., Kyoto, Japan) were germinated in urethane cubes (Width 2.3 cm × Depth 2.3 cm × Height 2.7 cm) in dark for 48 hours and raised for 15 days under fluorescent lamps (FL; Mitsubishi Osram FHF32EN-H3) at photosynthetic photon flux density (PPFD; 400-700 nm) of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. The average air temperature, relative humidity, and CO₂ concentration throughout the preparation were 23/18 °C (light/dark), 65/85% (light/dark), and $400 \mu\text{mol mol}^{-1}$,

respectively. These seedling 17 days after sowing (including 2 days for germination) were used in Exp. 1. The plants 56 days after sowing were used in Exp. 2.

Experimental conditions common to Exp. 1 and 2**1) Characteristic of LED**

In Exp. 1 and 2, the plants were grown at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with LED types of either white, blue, green, or red (ISL-150X150 Series, CCS Inc., Burlington, MA, U.S.A.). Peak wavelengths of blue, green, and red LED were 480, 520, and 630 nm, while peak wavelengths of white LED were 455 and 570 nm (Macam SR9910, Lothian, UK; Figure 4.1B). The white LED was emitted 24, 46, and 30% of blue, green and red, respectively.

2) Growing conditions

Uniform-sized seedlings were transplanted and grown with deep flow technique (DFT) under LED at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (9 plants m^{-2} ; Figure 4.1C), supplied with Otsuka solution (electrical conductivity: $1.5 \pm 0.2 \text{ dS m}^{-1}$ and pH 6.0 ± 0.5). The photo-/dark periods were 14/10 h (light/dark). Inside surface of cultivation space was covered with reflective white plastic films to maintain the uniformity of PPFD (Wako and Taikisha, Japan; Figure 4.1C). The average air temperature, relative humidity, and CO₂ concentration throughout the experiment were 23/18 °C (light/dark), 65/85% (light/dark), and $1,500 \mu\text{mol mol}^{-1}$, respectively.

Experiment (Exp.) 1

The plants were raised under the LED 17 days after sowing, and harvested 60 days after sowing. Unfolded leaves were removed at the harvest stage. The outermost leaf from its head was counted as leaf no. 1 (Figure 4.1D). This experiment was conducted 1 time contained 3 replications.

Experiment (Exp.) 2

Unfolded leaves were removed 56 days after sowing. All the plants with fresh weight excluding unfolded leaves of about 440 g were transplanted and raised under the LED. The plants were harvested 60 days after sowing. This experiment was conducted 2 times contained 6 replications.

Leaf light absorptance characteristic

Leaf light absorptance characteristics were evaluated from the 56 days after sowing plants cultivated under FL before irradiated with the LED from the first leaf to the tenth leaf. A single leaf disc was punched out at the middle of each leaf by a cock borer (9.7 cm^2). Then, the leaf disc was arranged and placed orderly from the outermost leaf to the tenth (leaf no. 1 to no. 10; Figure 4.1D) under different light wavelength $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. An integrating light hemisphere was used to determine leaf light absorptance, reflectance, and transmittance (modified from Stambler and Dubinsky (2005)).

Plant growth and leaf characteristic

60 days after sowing, the plants were harvested and measured its fresh and dry weight either shoot or root. The leaf no. 1, 4, 6, 7, 8, and 10 were sampled (Figure 4.1D) for measuring chlorophyll content by spectrometer (Porra et al. 1989) and ascorbic acid content by RQflex 10 (Merck KGaA, Darmstadt, Germany; Pantelidis et al., 2007).

Statistical analysis

The statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's test in SPSS statistical software (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS**Characteristics of leaf absorptance from different light wavelength**

Leaf light absorptance in crisp head lettuce's leaves were determined (Table 4.1). Blue LED was absorbed the highest rate approximately 89% followed by red and white LED, 82 and 74%, respectively. Green LED was absorbed the lowest rate at 65% compared to other light wavelength. On the contrary, the deducted value from leaf absorptance was carried out onto leaf transmittance and reflectance. The highest leaf transmittance and reflectance were orderly prevailed on green (13 and 22%, respectively), white (8 and 18%, respectively), red (4 and 14%, respectively), and blue (2 and 9%, respectively) LED from the leaf (Table 4.1).

Light transmittance inside of crisp head lettuce was measured from leaf no. 1 (the outermost leaf) to leaf no.10 (the inner leaf; Figure 4.1D). A single leaf disc was measured and placed orderly. The light transmittance of blue and red LED decreased drastically from the leaf no. 2. The PPFD of blue and red LED illustrated approximately 1% PPFD transmitted at leaf no. 2 and 3, respectively. However, the transmittance of green LED remained the highest transmittance percentage from the leaf no. 1 (31%) to the leaf no. 10 (0.5%; Figure 4.2). In addition, white LED which consisted of mixed spectrum also disclosed greater reflectance and transmittance compared to the blue and red LED by its greater transmittance percentage (Figure 4.2). Emphatically, green LED transmittance traveled to afar or deeper leaf number than another wavelength based from its lowest absorptance.

Plant growth under different light wavelength

Shape and size of crisp head lettuce appeared similarly among different light wavelength (Figure 4.3). In the first experiment (I.), shoot and root fresh weight showed no statistical difference among treatments, although leaf number was increased significantly under green and white LED (Table 4.2). On the other hand, in the second experiment (II.), supplemented LED on 56 days after sowing, exhibited different in plant growth. Shoot fresh and dry weight of the crisp head lettuce cultivated under green and red LED were greater than blue LED. Plant growth of crisp head lettuce cultivated under white LED showed no difference in shoot fresh weight, but dry weight was lower than green and red LED. Moreover,

leaf number of crisp head lettuce cultivated under white, green and red LED were greater than blue LED (Table 4.3).

Effective of different light wavelength on greenness and ascorbic acid

Chlorophyll and ascorbic acid were quantified separately on each leaf number in the first experiment (I.). On the first leaf, chlorophyll and ascorbic acid was significantly increased by blue and red LED, whereas green LED showed the lowest values. However, chlorophyll and ascorbic acid accumulated from the 4th leaf to inside under green LED compared to blue and red LED. Furthermore, white LED (consisted of mixed spectrum) showed higher chlorophyll and ascorbic acid from the 4th leaf greater than blue and red LED. This order tended to be continued from the leaf no. 4 until the leaf no. 10 (Figure 4.4 A and B, 4.6). The greenness of inside crisp head lettuce was substantially presented under green and white LED while blue and red LED showed pale green in the inside (Figure 4.3).

On the second experiment (II.), the LED supplemental period was reduced to 4 days, from 56 days after sowing and harvested at 60 days after sowing. The data of chlorophyll and ascorbic acid showed the similar trend that the contents were accumulated and increased from the 4th to the 10th leaf (Figure 4.5). In other word, plant quality inside of crisp head lettuce can be increased even the supplement period was only 4 days.

DISCUSSION

Green light absorptance in plants

Many lettuce varieties are classified in *Lactuca sativa* which mainly is a leafy-type vegetable except crisp head lettuce (De Vries and Van Raamsdonk, 1994). Plant growth and/or metabolisms of the leafy-type lettuce could be intervened from external lighting environment. Thus, using a benefit from the round-shape type of the crisp head lettuce has been used to avoid any external lighting environment.

Plant growth of the crisp head lettuce showed no statistical difference (Table 4.2). Even it could be affected under different light wavelength (Olle and Virsile, 2013; Yanagi et al., 1996). However, green light transmittance penetrated into the crisp head lettuce canopy greater than blue, red, and white light. Even the small amount of green light transmittance inside crisp head lettuce, the improvement of chlorophyll and ascorbic acid had occurred for the crisp head lettuce cultivated under green LED type from the leaf no. 4 indicating that green light was effective for plant quality enhancement (Figure 4.7).

Principally, green light has been believed not to be efficient for plant cultivation because of low absorptance by chlorophyll (Moss and Loomis, 1951; Folta and Carvalho, 2015). However, Terashima et al. (2009) proposed that penetrating green light has benefited to increase photosynthesis by exciting chloroplasts located deep in the leaves, in this case, cultivation of inside crisp head lettuce and/or high density plant production. In plant canopy, lower leaves increased chlorophyll synthesis for capacity to absorb and use the green light (Murchie and Horton, 1998; Sun et al., 1998). But, high PPFD should be needed to get such advantage because photosynthetic rate of green light is lower at low PPFD (Wang et al., 2011). Moreover, weak absorptance of green light would have larger light scattering

diffusion (detour effect; Terashima and Saeki, 1983). The green light that passes through plant tissue could be absorbed by chlorophyll that lead to increase light absorptance (Vogelmann 1993). The light that penetrate deeper within leaves drives photosynthesis than blue or red light (Gould et al., 2002). Hence, chlorophyll concentration increases with the relative absorptance of green light compared to blue or red light (Sun et al., 1998). Apparently, the higher green PPF, i.e., 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Johkan et al., 2012) or 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (the present research), could have more ability to be absorbed in the crisp head lettuce canopy. As a consequence, the evidence of green light absorptance with high PPF lead to the increment of chlorophyll and ascorbic acid in this study.

Green light new challenging for cultivating plants in plant factory

The blue and red regions of the visible spectrum are the major light source for plant factory with artificial light because of its photosynthetic pigments absorptance (Tazawa, 1999), while green light has been believed not to be used efficiently for plant growth (Folta and Maruhnich, 2007). However, green light was transmitted through the canopy and leaves more than blue and red light (Kasperbauer, 1971). The light provided signals for acclimation of photosynthesis on plant to available irradiance (Walters, 2005). The present results clearly illustrated that supplemented green LED type at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF either 4 days or 43 days enhanced chlorophyll and ascorbic acid inside the crisp head lettuce (Figure 4.4 A and B; Figure 4.5). The outstanding transmittance of green light will improve plant factory with artificial light cultivation. For example, leaves which are not formed a round shape of crisp head lettuce must be removed for packing and transportation. This cause nutrition losing on waste of the removed leaves. Besides throwing the leaves, ascorbic acid in crisp head lettuce was regularly reduced from outer leaves to inner leaves (Poulsen et al., 1995). This nutrition could be increased because of the transmittance characteristic of green LED type inside the canopy. Light is necessary for chlorophyll formation. Lacking of light may cause chlorophyll development (Wann, 1930). Leaves beneath canopy could receive more light for its growth and synthetic nutrition (Bian et al., 2016). Supplement of green light may cause less initial cost and work compared to supplemental upward lighting (Zhang et al., 2015). Increasing green LED type PPF further than 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$; integrated LED types (green and white); or prolonged supplemental period will be subjected to be adjusted with suitability to plants production in plant factory with artificial light. Optimizing green light use efficiency on plant canopy is likely contributing to gain crop yield (Smith et al., 2017). These new techniques of green light could be undeniable to realize in the future of plant factory with artificial light cultivation.

CONCLUSION

The benefit of green light from low light absorptance can be extended in crisp head lettuce because of its transmittance characteristic. Chlorophyll, ascorbic acid content, and shoot biomass are increased by using 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ green LED type for at least 4 days before harvest. This supplemental lighting could be used on the pre-harvest stage to improve crisp head lettuce quality on plant factory with artificial light condition.

Table 4.1 Characteristics of leaf absorptance in *Lactuca sativa* L. var. *Capitata*. The absorptance percentage was calculated from absorptance = 100 - (transmittance + reflectance).

LED type	Transmittance (%)	Reflectance (%)	Absorptance (%)
White	7.6 ± 1.0 b	18.2 ± 1.5 ab	74.2 ± 1.8 b
Blue	1.6 ± 0.4 c	9.4 ± 1.1 c	89.1 ± 1.3 a
Green	12.6 ± 1.2 a	22.2 ± 2.3 a	65.2 ± 3.1 c
Red	3.7 ± 0.7 c	14.6 ± 1.0 bc	81.8 ± 1.1 ab

The measurement was measured on the 1st leaf of crisp head lettuce grown under different LED types at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and harvested at 60 days after sowing. Data are means \pm SE ($n = 6$). Different letters indicated significant difference within the column (Tukey's HSD test, $P < 0.05$).

Table 4.2 Plant growth and leaf number in *Lactuca sativa* L. var. *Capitata* of the first experiment (I.).

LED type	Fresh weight (g)		Dry weight (g)		Number of leaves
	Shoot	Root	Shoot	Root	
White	990 ± 61 a	49.0 ± 0.9 a	20.2 ± 1.7 a	1.81 ± 0.05 a	38.7 ± 1.2 a
Blue	871 ± 52 a	52.2 ± 4.5 a	24.0 ± 1.9 a	1.88 ± 0.14 a	32.0 ± 1.2 b
Green	828 ± 66 a	39.3 ± 3.8 a	20.9 ± 0.6 a	1.51 ± 0.09 ab	38.7 ± 0.9 a
Red	868 ± 55 a	41.5 ± 3.0 a	18.0 ± 0.9 a	1.23 ± 0.13 b	35.7 ± 1.2 ab

The measurement of crisp head lettuce cultivated under different LED types at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD from 17 days after sowing and harvested at 60 days after sowing. Data are means \pm SE ($n = 3$). Different letters indicated significant difference within a column (Tukey's HSD test, $P < 0.05$).

Table 4.3 Plant growth and leaf number in *Lactuca sativa* L. var. *Capitata* of the second experiment (II.)

LED type	Fresh weight (g)		Dry weight (g)		Number of leaves
	Shoot	Root	Shoot	Root	
White	491 ± 6 ab ¹	34.2 ± 0.1 a	11.2 ± 0.2 b	1.76 ± 0.07 a	28.0 ± 1.0 a
Blue	475 ± 4 b	30.0 ± 1.1 b	11.7 ± 0.2 b	1.41 ± 0.02 c	19.7 ± 0.9 b
Green	514 ± 5 a	34.0 ± 0.4 a	12.8 ± 0.1 a	1.68 ± 0.09 ab	26.7 ± 0.9 a
Red	503 ± 7 a	30.4 ± 0.4 b	12.8 ± 0.3 a	1.43 ± 0.04 bc	25.3 ± 1.5 a

The measurement of crisp head lettuce cultivated under different LED types at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD 56 days after sowing and harvested at 60 days after sowing. Data are means \pm SE ($n = 6$). Different letters indicated significant difference within a column (Tukey's HSD test, $P < 0.05$).

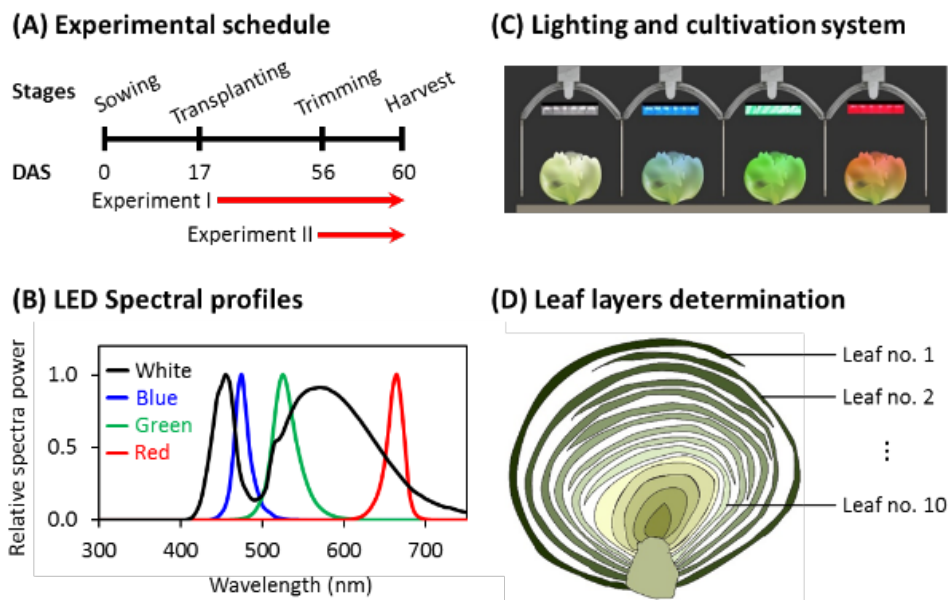


Figure 4.1 Experimental schematic diagram illustrated (A) experimental schedule where LED lighting was initiated either at 17 (I.) or 56 (II.) days after sowing until its harvest; (B) the spectral profiles of LED types used in this study; white, blue, green, and red; (C) imitative growing bench with LED lighting. The samples were surrounded with a reflectance white plastic film (Wako and Taikisha, Japan). The wavelengths of LED types were recorded at 240-800 nm with a spectroradiometer (SR9910-v7, Irradiant Ltd., Tranent, UK); and (D) leaf number determination which the outermost leaf of its head was considered to be the first leaf. The determination was utilized throughout the experiment.

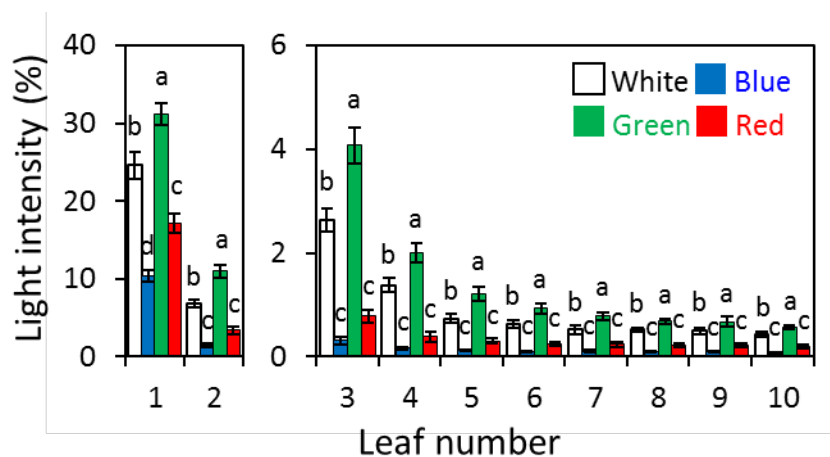


Figure 4.2 Leaf light transmittance of crisp head lettuce supplied with different LED types. The photosynthetic photon flux density was $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ measured by Licor light meter (Licor 250A, Li-cor Bioscience, Lincoln, NE, USA). The measurement was performed under integrating light hemisphere. Data are means \pm SE ($n = 6$). Different letters indicate statistically significant differences among the leaf number (Tukey's HSD at $P < 0.05$).

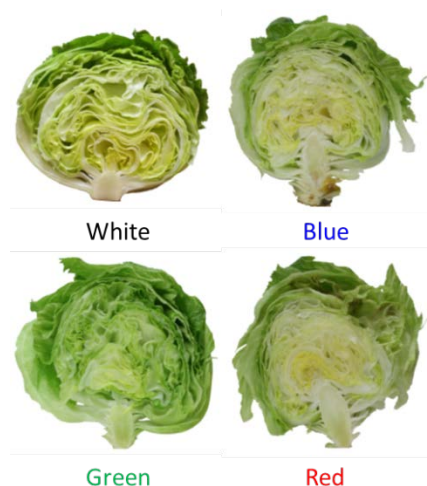


Figure 4.3 Crisp head lettuce from the first experiment (I.) cross section cultivated under different LED types at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD from 18 days after sowing and harvested at 60 days after sowing.

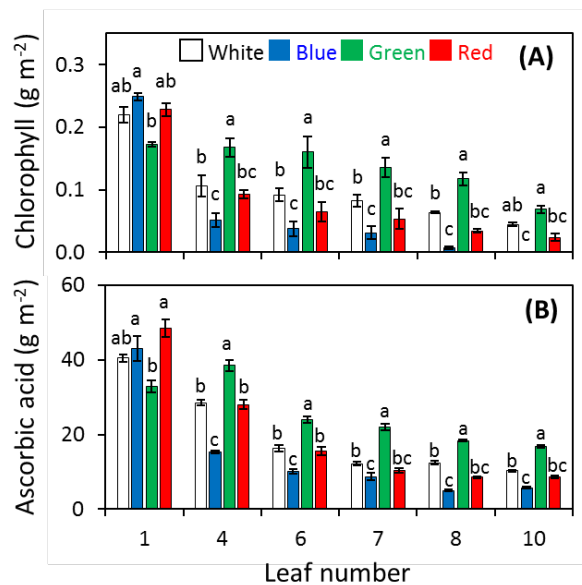


Figure 4.4 Leaf chlorophyll (A) and ascorbic acid content (B) per area from the first experiment (I.) of crisp head lettuce cultivated under different LED types at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD from 17 days after sowing and harvested at 60 days after sowing. Data are means \pm SE ($n = 3$). Different letters indicate statistically significant differences among the leaf number (Tukey's HSD at $P < 0.05$).

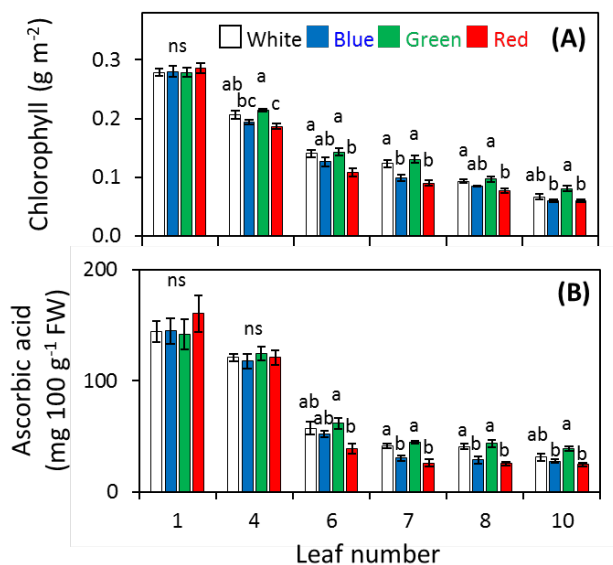


Figure 4.5 Leaf chlorophyll (A) and ascorbic acid content (B) from the second experiment (II.) of crisp head lettuce cultivated under different LED types at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD from 56 days after sowing and harvested at 60 days after sowing. Data are means \pm SE ($n = 6$). Different letters indicate statistically significant differences among the leaf number (Tukey's HSD at $P < 0.05$).

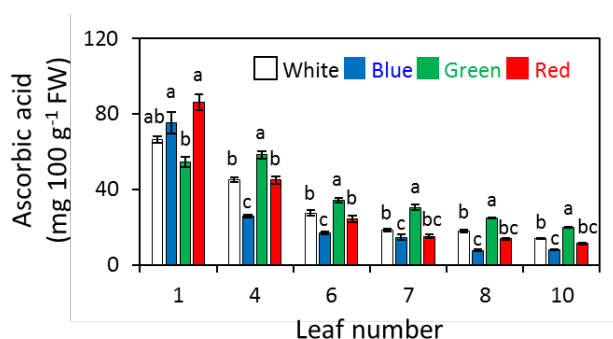


Figure 4.6 Ascorbic acid content in milligram unit from the first experiment (I.) of crisp head lettuce cultivated under different LED types at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD from 17 days after sowing and harvested at 60 days after sowing. Data are means \pm SE ($n = 6$). Different letters indicate statistically significant differences among the leaf number (Tukey's HSD at $P < 0.05$).

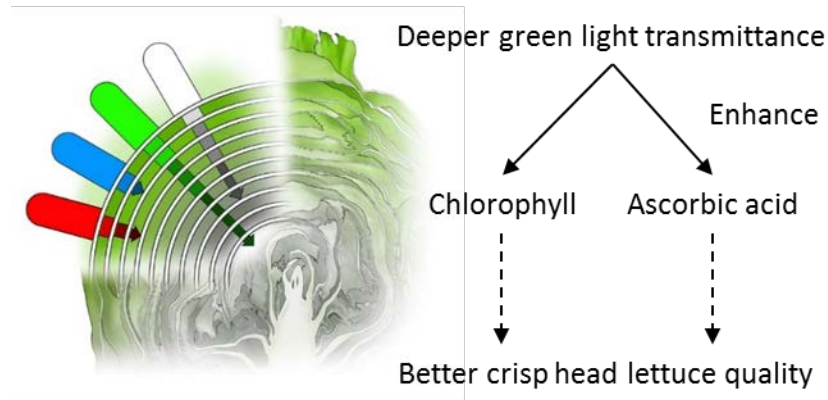


Figure 4.7 Light transmittance schematic diagram of different LED types illustrated green light transmitted inside crisp head lettuce canopy deeper than blue and red light. The greenness inside the crisp head lettuce cultivated under green light enhanced in its canopy resulting in higher ascorbic acid content. Beside, white light, which consisted of blue and green spectrum, showed greater transmittance compared to blue and red light. This cultivation strategy could be integrated to improve high quality of crisp head lettuce in plant factory with artificial light.

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Chapter V

Supplemental upward lighting with blue, red, or white LED increases yield and ascorbic acid content of romaine lettuce

ABSTRACT

A plant factory with artificial lighting is a closed plant production system designed to produce high yield with good quality. Outer leaves beneath the dense canopy get insufficient light and senesce fast, resulted in decreasing yield, under high-density cultivation in plant factories with artificial lighting. Thus, the establishment of novel cultivation system to retard senescence of outer leaves would be needed to improve yield and profitability in ant factories. Here, effect of supplemental upward lighting with different light wavelength (blue, green, red, and white) and intensities on the retardation of outer leaf senescence, photosynthesis, and plant growth have been examined in lettuce. Supplemental upward lighting of any light wavelength retards the senescence of outer leaves and decreases waste, leading to improvement of the marketable shoot biomass. Especially, the supplemental upward lighting with blue and red LED are most effective as both lights are efficiently absorbed by photosynthetic pigments (i.e., chlorophyll). Moreover, supplemental upward lighting with white, blue, or red LED, but not green LED, significantly increase the ascorbic acid content in the outer leaves. These results indicate that this adapted cultivation system for supplemental upward lighting with blue, red, or white LED can achieve high yield of lettuce with low waste and high ascorbic acid content.

Keyword: LED, light color, plant factory, photosynthesis, supplemental upward lighting

INTRODUCTION

The term “plant factory with artificial lighting” refers to an environment-controlled plant production facility that can produce pesticide-free plants with high yield and quality using water, nutrients and labor efficiently within a small area (Merrill et al., 2016). Year-round production is permitted by air conditioners, electric lamps, air circulation fans, CO₂, nutrient solution supply units, and an environmental control unit with artificial lighting to provide a relative optimal growth condition (Yamori et al., 2014). In many Asian, European and North American countries, such plant factory with artificial lighting is used for commercial production of leafy green, herb, and seedling (Hayashi, 2016). However, the technology is still in its infancy, and improvement needs to be made in construction and labor costs, electricity use, yield and quality, and efficient use of light-emitting diodes (LED) (Kozai, 2013). Currently, the production of leafy greens is managed through the use of multiple culture shelves and an intensive cultivation pattern, but shading of outer leaves by the canopy reduces photosynthetic rate and accelerates leaf senescence. The yellow senescent leaves have to be trimmed before packaging and shipment, resulting in yield losses of over 10% (Zhang et al., 2015; Kozai and Niu, 2016).

Photosynthesis, a chemical process in which green plants use light energy to transform water, carbon dioxide, and minerals into oxygen and organic compounds, is the basic process underlying plant growth and productivity (Yamori, 2016; Yamori and Shikanai, 2016). Light is one of the most important environmental factors that influences plant growth, which is not only the basic driving force of photosynthesis, but also an important regulator of plant growth and development (Terashima et al. 2006). Shape of photosynthetic gradient or curvature of light-response curve can be defined by curvature factor (θ), where the values determine photosynthetic efficiency under given light intensities (Evans et al., 1993; Ögren, 1993). Light is also a contributing factor to the curvature factor (θ) which high light saturation could estimate the bending of photosynthetic light-response curves (Evans, 1989; Pury and Farquhar, 1997). Deficient light intensity restricts the photosynthetic rate and a negative carbon balance would occur once the light intensity falls below the compensation point (i.e., the PPFD at which photosynthetic rate is zero). Low light triggers leaf senescence, resulting in yield loss (Frantz et al., 2000). More than 90% of crop biomass is derived from photosynthetic products, so the enhancement of leaf photosynthesis should increase yield (Long et al., 2006; Yamori et al., 2011, 2016).

As well as light intensity, light wavelength greatly affects photosynthesis and plant growth (McCree 1971, Inada 1976, Lin et al. 2013). As much as 90% of blue and red light is absorbed by chlorophyll in leaves, indicating strong effects on plant development and physiology (Terashima et al., 2009; Muneer et al., 2014). Especially, red light promotes photosynthesis, induces hypocotyl elongation and leaf area expansion; blue light regulates chlorophyll biosynthesis and suppresses hypocotyl elongation (McNellis and Deng, 1995; Han et al., 2017). Leaf senescence could be suppressed by the application of red light in soybean (Guiamet et al., 1989) and sunflower (Rousseaux et al., 1996), and of blue light in wheat (Causin et al., 2006). In contrast, green light is considered not to be used efficiently for plant growth since it is absorbed weakly by chlorophyll (Sun et al., 1998). However, recent researches showed that green light potentially plays an important role in photosynthesis (Terashima et al., 2009; Johkan et al., 2012), since it can penetrate the plant canopy better than blue or red light (Klein, 1992), and leaf photosynthesis and plant biomass are higher in plants grown under cool white fluorescent lamps, which emit light consisting of blue, green, and red wavelengths, than under red LED alone or red LED with blue light from fluorescent lamps (Yorio et al., 2001). Thus, the effect of different light color on plant growth and development is still unclear. It has been shown that supplemental upward lighting with white LED from underneath plants could retard senescence process in outer leaves of leafy vegetable (Joshi et al., 2017), but the most efficient color has not been elucidated. Therefore, here, the effect of supplemental upward lighting under different light color and intensity on the retardation of outer leaf senescence, photosynthesis, growth, and yield in romaine lettuce were investigated.

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of romaine lettuce (*Lactuca sativa* L. var. romana; Takii Seed Co., Kyoto, Japan) were sown in urethane cubes (W 2.3 cm × D 2.3 cm × H 2.7 cm). Seedlings were raised in an environment-controlled growth chamber under cool white fluorescent lamps at 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD for 12 h. At 28 days after sowing, uniformed size seedlings at five leaf stage were transplanted to a growth room and treated with supplemental upward lighting with a 14 h photoperiod and 25/20°C photoperiod/dark period (modified from Zhang et al., 2015). All plants were grown in a deep-flow hydroponic system (33 plants m^{-2}) using Enshi formula nutrient solution (EC: 2.0 ± 0.2 , pH: 7.0 ± 0.5 ; modified from Asao et al., 2013).

Supplemental upward lighting treatment

Cool white fluorescent lamps with 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD were used as the light source for downward lighting (plants grown solely under downward lighting as the control, Figure 5.1A-I). Four types of LED (blue, green, red, and white) provided supplemental upward lighting (PPFD of either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 3 cm from the light sources; Figure 5.1A-II) with the downward lighting. The LED was installed on aluminum plates to dissipate the heat and ensure that the leaf temperature was not significantly affected by the supplemental upward lighting. Each treatment had 5 replicates.

Leaf gas exchange measurements

The plants were divided into six layers of leaves, in which the 1st to 3rd layers were considered outer leaves and the 4th to 6th were considered inner leaves. Photosynthetic rate of the outer leaves (on the 3rd layer) in plants grown for 56 days after sowing was measured under each growth-light condition with a portable photosynthesis system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) as described by Yamori et al. (2009, 2010). In the present study, the portable photosynthesis system was fitted with a modified standard leaf chamber that the chamber equipped with a Propafilm window (6400-08 Clear Chamber) to measure photosynthetic rate. Since this clear chamber bottom transmits irradiation from the bottom, it is available to analyze effect of supplemental upward lighting from underneath plants on the photosynthetic rate in the outer leaves. The conditions in the measurement chambers were controlled with leaf temperature, CO_2 concentration, and relative humidity at 25 °C, 400 $\mu\text{mol m}^{-2}$, and 60%, respectively.

Photosynthetic light-response curves in the similar 3rd layer were evaluated. Downward lighting was provided by a Li-6400–18A RGB light (0, 50, 100, 200, 300, 400, 600, and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; LI-COR Inc., Lincoln, NE, USA) with the supplemental upward lighting from underneath the plants. The response of photosynthetic rate to the light was used to evaluate curvature factor (θ) according to Thornley (1998) and June (2005):

Chlorophyll fluorescence and chlorophyll content

Leaf discs (1.3 cm in diameter) taken from each leaf layer in plants grown for 64 days after sowing was placed into 24 well-plates filling with 1.5 ml 0.005% Triton-100 by facing

abaxial up and imbibed gas from its leaves at -0.1 MPa for 10 minutes in a vacuum chamber. Maximal photochemical efficiency of PSII (F_v/F_m) in the dark-adapted leaf discs were measured by Imaging PAM (M-series; Heinz Walz GmbH, Effeltrich, Germany). The measurement was performed after 30 minutes of incubation in darkness. The pulsed lower intensity-measuring beam to measure initial chlorophyll fluorescence (F_0) was irradiated after placing the well-plates' cover with weak light pulses ($<1 \mu\text{mol m}^{-2} \text{s}^{-1}$) at a low frequency (1Hz). Maximum chlorophyll fluorescence (F_m) was assessed by 0.8 s of saturated pulse ($2,700 \mu\text{mol m}^{-2} \text{s}^{-1}$). The variable fluorescence yield (F_v) was determined by $F_m - F_0$. The efficiency of excitation energy captured by open PSII reaction centers in dark-adapted leaf disc samples was estimated by F_v/F_m ratio (Shao et al., 2013; Zhang et al., 2015). Total chlorophyll content was determined using *N, N*-dimethylformamide in the same leaf with F_v/F_m measurements (Porra et al., 1989).

Plant growth analysis

Lettuces were harvested at 66 days after sowing. Marketable leaf fresh weight, waste percentage, and total leaf fresh weight were measured.

Determinations of leaf chlorophyll, ascorbic acid and nitrate contents

Total chlorophyll content was determined using *N,N*-dimethylformamide in the same leaf with F_v/F_m measurements (Porra et al., 1989). Ascorbic acid and nitrate content of outer and inner leaves was measured separately using an RQFlex plus reflectometer (Merck, Darmstadt, Germany) as described in (Zhang et al., 2015).

Statistical analysis

The statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's test in SPSS statistical software (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Leaf characteristics

Without supplemental upward lighting, the total chlorophyll content (Figure 5.2A-F, Table 5.1) and F_v/F_m values (Figure 5.2G-L, Table 5.2) in control plants maintained high in the inner leaves (4th to 6th layers), but decreased steeply from the 3rd to 1st layers (the outer leaves). With supplemental upward lighting, irrespective of color, both remained significantly higher total chlorophyll content and F_v/F_m values in the outer leaves than in control plants, and $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD was more effective than of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (Figure 5.2A-D, G-J, Table 5.1, 5.2). Supplemental upward lighting with red LED exhibited the highest chlorophyll content and F_v/F_m values in the outer leaves (Figure 5.2E, F, K, L, Table 5.1, 5.2).

Leaf gas exchange

The outer leaves of the control plants, grown solely under downward lighting, showed an obvious negative net photosynthetic rate (Figure 5.3). Under supplemental upward lighting, however, the photosynthesis was improved to a positive photosynthetic rate; that at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD were approximately 40% higher than the treatments of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD comparing in the identical light color (Figure 5.3). Among the supplemental upward lighting treatments with different light colors, the net photosynthetic rate was highest in the $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD of red and blue LED, respectively (Figure 5.3). The light-saturated photosynthetic rate under each grown light condition was similar in photosynthetic light-response curve (Figure 5.4, Table 5.4). The curvature factor of the photosynthetic light-response curve significantly tended to increase with the supplemental upward lighting compared with the control (Table 5.3). The supplemental upward lighting altered curvature of the light response of photosynthetic rate (bending shaped of the photosynthetic light-response curve) and significantly reduced the light compensation point as well as increased the photosynthetic rate at lower PPFD (Figure 5.4). In addition, supplemental upward lighting with red LED achieved the highest curvature factor value (Table 5.3).

Plant growth

Supplemental upward lighting at all light colors greatly improved plant growth compared with the control (Figure 5.5A, C). It significantly reduced the waste of outer senescent leaves, achieving high marketable yields, especially in the treatments of $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (Figure 5.5B). Supplemental upward lighting with red or blue LED exhibited the lowest waste of outer senescent leaf and the highest marketable leaf fresh weight, respectively.

Ascorbic acid content

Supplemental upward lighting increased the ascorbic acid content in the outer leaves (Figure 5.6A). The treatments of $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD white, blue, and red LED, but not green significantly increased the ascorbic acid content comparing in the identical light color to the treatments of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, and the highest value was found in red LED (Figure 5.6A). No significant difference was observed in inner leaves among all the treatments (Figure 5.6B).

DISCUSSION

Since the leafy greens production in plant factory with artificial lighting uses high plant density, outer leaves are shaded by upper leaves and by neighboring plants and then senesced fast. The outer senesced leaves which appear yellow have to be trimmed before packaging and shipment, resulting in a decline in plant yield up to 10% or more (Zhang et al., 2015; Kozai and Niu, 2016). Recent work has made progress in demonstrating that supplemental upward lighting using white LED could retard outer leaf senescence and also increase the photosynthetic rate, resulting in enhancement in the marketable lettuce yield

(Joshi et al., 2017). However, it remains unclear what light wavelength and what light intensity would be efficiently able to retard the senescence in outer leaves, and improve the leaf photosynthesis and thus plant growth. Our results clearly showed that (1) supplemental upward lighting retarded outer leaf senescence and improved the leaf photosynthesis as the light intensity of supplemental lighting was increased irrespective of wavelengths, (2) supplemental upward lighting with blue, red, or white LED effectively retarded outer leaf senescence, and (3) blue, red, or white LED, but not green LED promoted a high content of ascorbic acid.

Red, Blue, or White LED as a source of supplemental upward lighting effectively retards outer leaf senescence and improve ascorbic acid content of outer leaves

The degradation of chlorophyll and proteins is one of the most characteristic features in leaf senescence (Richmond and Lang, 1957; Brouwer et al., 2012). Chlorophyll is essential for photosynthesis, and its content directly affects the leaf photosynthetic ability (Evans, 1989; Evans and Poorter, 2001). Shading by the canopy reduced the chlorophyll content and *Fv/Fm* values in outer leaves, but supplemental upward lighting maintained both (Figure 5.2), thus retarding senescence. The degree of retardation of senescence in the outer leaves differed among light wavelengths. Red, blue or white (consisting of blue, green, and red spectrum; Figure 5.1B) LED irradiation delayed outer leaf senescence effectively to the same extent (Figure 5.2). It has been reported that the effect of light wavelength was related to changes in oxidative metabolism, whereby blue light efficiently retarded senescence in wheat (Causin et al., 2006). On the other hand, it also has been reported that irradiation with red LED suppressed ethylene production resulting in delayed senescence more effective than blue LED in postharvest broccoli (Ma et al., 2014). Taken together, both red and blue irradiation would have potential to retard senescence efficiently, although there could be a difference in its mechanism for the retardation of leaf senescence.

It has been reported that antioxidant contents, including ascorbic acid, were greatly affected by light wavelength, especially red (Moss and Loomis, 1952; Valpuesta and Botella, 2004; Zhou and Singh, 2002; Sirtautas et al., 2012; Bliznikas et al., 2012; Deng et al., 2017) and blue (Ohashi-Kaneko et al., 2007; Li, et al., 2012) light which is 90% absorbed by chlorophyll red (Moss and Loomis, 1952; Valpuesta and Botella, 2004; Zhou and Singh, 2002; Sirtautas et al., 2012; Bliznikas et al., 2012; Deng et al., 2017; Ohashi-Kaneko et al., 2007; Li, et al., 2012). Our data clearly showed that ascorbic acid content of romaine lettuce cultivated with supplemental upward lighting with blue, red, or white LED was increased more than 10% (Figure 5.6), compared to the conventional cultivation (Llorach et al., 2008; Zhan et al., 2013). In addition, nitrate content, which is concerned to human's health, is remained under European Commission levels for nitrate, 450 mg 100 g⁻¹ FW, even with all the treatments of supplemental upward lighting (Figure 5.7; Alexander et al., 2008). We conclude that red, blue or white LED as a source of supplemental upward lighting would be effective for both retarding leaf senescence and improving ascorbic acid content (Figure 5.8). As white LED becomes more common as a household light source, the price is decreasing (Humphreys,

2008). Thus, white LED could be an ideal light source for commercial plant productions in plant factory with artificial lighting, allowing high yields at low cost (Figure 5.8).

New light cultivation system for sun-type species by the application of downward lighting with supplemental upward lighting from underneath

Generally, one percent yield reduction was caused by decreasing one percent in cumulative daily light under natural sunlight greenhouse (Cockshull et al., 1992). Recent interest has focused on inter-lighting of the natural sunlight greenhouse (Tewolde et al., 2016). The supplemental inter-lighting under natural sunlight greenhouse is proved to increase yield, for example, in cucumber, sweet pepper, and tomato (Tewolde et al., 2016; Demer et al., 1998; Hovi and Tahvonen, 2008; Hao et al., 2012). The idea of light distribution through plant cultivation has extended below its canopy (Frantz et al., 2000), in this case, supplemental upward lighting under closed plant production systems with artificial light (Zhang et al., 2015) This light altered curvature of the light response of photosynthesis (Figure 4, Table 1), which also lowered the light saturation point of photosynthesis. The curvature factor of the light response of photosynthesis tended to increase with increasing light intensity of supplemental upward lighting (Figure 4, Table 1). These results would offer a feasibility of cultivating sun-type species (i.e., high light compensation point and light saturation point) under relatively low downward lighting with supplemental upward lighting. The present study clearly showed that the use of supplemental upward lighting in plant factory with artificial lighting not only reduce the waste by delaying the outer leaf senescence, but also enable to cultivate sun-type species and increase crop production with high ascorbic acid content.

Table 5.1 Total chlorophyll content in leaves of each layer in lettuce plant grown under downward lighting from above by 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD cool white fluorescent lamps without or with supplemental upward LED lighting either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD at 66 days after sowing. Data are means \pm SE ($n = 5$). Different letters indicated significant difference within the column (Tukey's HSD test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White30", "White60", "Blue30", "Blue60", "Green30", "Green60", "Red30", or "Red60" denotes plants grown under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

Treatments	Total chlorophyll content (g m^{-2})					
	1st layer	2nd layer	3rd layer	4th layer	5th layer	6th layer
Control	0.04 \pm 0.01 b	0.18 \pm 0.01 b	0.22 \pm 0.01 b	0.25 \pm 0.01 b	0.26 \pm 0.00 c	0.29 \pm 0.01
White30	0.21 \pm 0.02 a	0.23 \pm 0.01 a	0.26 \pm 0.01 ab	0.27 \pm 0.01 b	0.28 \pm 0.01 ab	0.30 \pm 0.01
White60	0.21 \pm 0.01 a	0.23 \pm 0.01 a	0.26 \pm 0.00 ab	0.28 \pm 0.00 ab	0.29 \pm 0.01 ab	0.30 \pm 0.01
Blue30	0.22 \pm 0.01 a	0.23 \pm 0.01 a	0.26 \pm 0.01 ab	0.26 \pm 0.01 b	0.28 \pm 0.01 bc	0.29 \pm 0.01
Blue60	0.22 \pm 0.00 a	0.24 \pm 0.01 a	0.26 \pm 0.00 ab	0.27 \pm 0.01 ab	0.29 \pm 0.00 ab	0.31 \pm 0.01
Green30	0.20 \pm 0.01 a	0.23 \pm 0.00 a	0.25 \pm 0.01 ab	0.27 \pm 0.00 ab	0.28 \pm 0.01 ab	0.30 \pm 0.00
Green60	0.20 \pm 0.01 a	0.24 \pm 0.01 a	0.27 \pm 0.00 a	0.28 \pm 0.01 ab	0.30 \pm 0.01 ab	0.31 \pm 0.01
Red30	0.20 \pm 0.02 a	0.25 \pm 0.01 a	0.28 \pm 0.01 a	0.28 \pm 0.01 ab	0.31 \pm 0.00 a	0.31 \pm 0.01
Red60	0.22 \pm 0.02 a	0.26 \pm 0.01 a	0.28 \pm 0.01 a	0.30 \pm 0.00 a	0.31 \pm 0.01 a	0.32 \pm 0.01

Table 5.2 Maximal photochemical efficiency of PSII (F_v/F_m) in leaves of each layer in lettuce plant grown under downward lighting from above by $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD cool white fluorescent lamps without or with supplemental upward LED lighting either 30 or $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD at 66 days after sowing. Data are means \pm SE ($n = 5$). Different letters indicated significant difference within the column (Tukey's HSD test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White30", "White60", "Blue30", "Blue60", "Green30", "Green60", "Red30", or "Red60" denotes plants grown under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

Treatments	Maximum potential photochemical efficiency (F_v/F_m)					
	1st layer	2nd layer	3rd layer	4th layer	5th layer	6th layer
Control	0.04 ± 0.01 c	0.14 ± 0.01 b	0.49 ± 0.09 b	0.69 ± 0.04 b	0.77 ± 0.00 bc	0.78 ± 0.00 c
White30	0.69 ± 0.03 ab	0.70 ± 0.04 a	0.77 ± 0.01 a	0.78 ± 0.01 a	0.79 ± 0.01 abc	0.80 ± 0.00 ab
White60	0.75 ± 0.01 ab	0.76 ± 0.00 a	0.77 ± 0.00 a	0.79 ± 0.00 a	0.80 ± 0.00 a	0.80 ± 0.00 a
Blue30	0.64 ± 0.03 b	0.70 ± 0.03 a	0.77 ± 0.01 a	0.77 ± 0.01 a	0.77 ± 0.01 c	0.79 ± 0.00 abc
Blue60	0.75 ± 0.01 ab	0.75 ± 0.01 a	0.77 ± 0.01 a	0.78 ± 0.01 a	0.80 ± 0.01 ab	0.79 ± 0.01 abc
Green30	0.68 ± 0.03 ab	0.73 ± 0.01 a	0.76 ± 0.00 a	0.77 ± 0.00 a	0.78 ± 0.00 abc	0.78 ± 0.01 bc
Green60	0.72 ± 0.02 ab	0.76 ± 0.01 a	0.76 ± 0.00 a	0.77 ± 0.01 a	0.79 ± 0.00 abc	0.80 ± 0.00 abc
Red30	0.67 ± 0.04 ab	0.73 ± 0.02 a	0.77 ± 0.00 a	0.78 ± 0.00 a	0.78 ± 0.01 abc	0.80 ± 0.01 abc
Red60	0.76 ± 0.01 a	0.77 ± 0.01 a	0.77 ± 0.01 a	0.79 ± 0.01 a	0.79 ± 0.00 abc	0.80 ± 0.00 abc

Table 5.3 Modeled parameters for the photosynthetic light-response curves derived from the non-rectangular hyperbolic response.

Treatments	Maximum rate of photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Curvature factor (θ)
Control	10.6 \pm 0.8	0.79 \pm 0.01 b
White30	10.7 \pm 0.8	0.85 \pm 0.01 a
White60	11.1 \pm 0.8	0.86 \pm 0.01 a
Blue30	11.0 \pm 0.9	0.85 \pm 0.01 a
Blue60	11.2 \pm 0.7	0.86 \pm 0.01 a
Green30	10.7 \pm 0.8	0.87 \pm 0.02 a
Green60	11.0 \pm 0.9	0.87 \pm 0.02 a
Red30	11.2 \pm 0.8	0.89 \pm 0.01 a
Red60	11.4 \pm 0.6	0.90 \pm 0.01 a

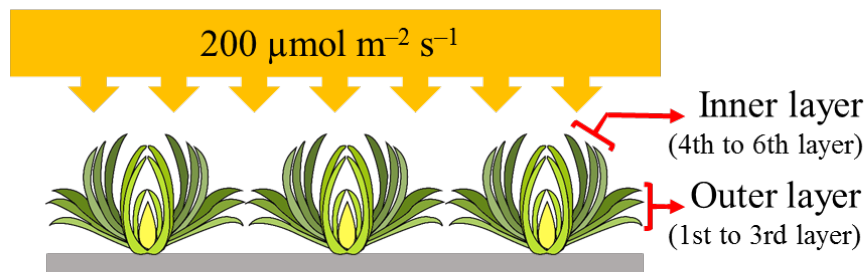
The measurement was measured on the 3rd layer in lettuce plant grown without or with supplemental upward LED lighting either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD at 56 days after sowing. The curvature factor was calculated by the equation (1). Data are means \pm SE ($n = 5$). Different letters indicated significant difference within the column (Tukey's HSD test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White30", "White60", "Blue30", "Blue60", "Green30", "Green60", "Red30", or "Red60" denotes plants grown under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

Table 5.4 Photosynthetic light-response curves of the 3rd layer in lettuce plant grown under different levels of downward lighting (0, 50, 100, 200, 300, 400, 600, and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) without or with supplemental upward LED lighting either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD at 56 days after sowing. Data are means \pm SE ($n = 5$). Different letters indicated significant difference within the column (Tukey's HSD test, $P < 0.05$). "*" differs significantly within the same light color (Student's t-test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White30", "White60", "Blue30", "Blue60", "Green30", "Green60", "Red30", or "Red60" denotes plants grown under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

Treatments	Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)							
	0	50	100	200	300	400	600	800
Control	-0.81 \pm 0.05 d	1.03 \pm 0.06 e	2.51 \pm 0.15 e	4.98 \pm 0.32 b	6.87 \pm 0.45	8.08 \pm 0.54	8.91 \pm 0.56	9.65 \pm 0.69
White30	0.18 \pm 0.05 c	1.74 \pm 0.08 cd	3.13 \pm 0.12 de	5.68 \pm 0.29 ab	7.34 \pm 0.40	8.41 \pm 0.51	9.34 \pm 0.63	9.74 \pm 0.69
White60	0.96 \pm 0.01 ab*	2.54 \pm 0.06 ab*	3.87 \pm 0.10 abc*	6.12 \pm 0.23 ab	7.75 \pm 0.40	8.81 \pm 0.52	9.75 \pm 0.67	10.09 \pm 0.73
Blue30	0.24 \pm 0.08 c	1.87 \pm 0.08 cd	3.26 \pm 0.13 cd	5.69 \pm 0.29 ab	7.49 \pm 0.48	8.57 \pm 0.61	9.63 \pm 0.80	10.00 \pm 0.84
Blue60	0.99 \pm 0.04 a*	2.55 \pm 0.06 a*	3.94 \pm 0.11 ab*	6.19 \pm 0.29 ab	7.87 \pm 0.39	8.88 \pm 0.48	9.77 \pm 0.61	10.15 \pm 0.65
Green30	0.04 \pm 0.05 c	1.58 \pm 0.11 d	2.98 \pm 0.15 de	5.34 \pm 0.29 ab	7.20 \pm 0.42	8.31 \pm 0.54	8.97 \pm 0.49	9.74 \pm 0.72
Green60	0.68 \pm 0.09 b*	2.11 \pm 0.14 bc*	3.43 \pm 0.20 bcd	5.73 \pm 0.38 ab	7.45 \pm 0.54	8.56 \pm 0.67	9.62 \pm 0.80	9.96 \pm 0.83
Red30	0.28 \pm 0.04 c	1.89 \pm 0.08 cd	3.36 \pm 0.12 bcd	5.91 \pm 0.20 ab	7.76 \pm 0.36	8.85 \pm 0.49	9.87 \pm 0.66	10.17 \pm 0.71
Red60	1.20 \pm 0.10 a*	2.73 \pm 0.12 a*	4.21 \pm 0.16 a*	6.56 \pm 0.25 a	8.29 \pm 0.36	9.20 \pm 0.42	10.04 \pm 0.55	10.33 \pm 0.57

A. Cultivation system and leaf layer

I. Control



II. Supplemental upward lighting with LEDs



B. Relative spectral photon flux of light sources

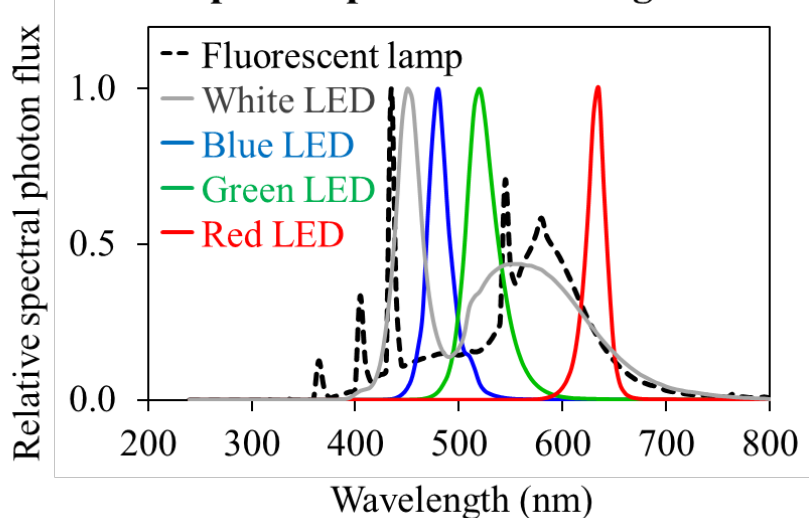


Figure 5.1 Experimental schematic diagram and relative spectral photon flux of LED of the supplemental upward lighting in the present study. (A-I) Imitative growing condition under cool white fluorescent lamps without supplemental upward lighting, and (A-II) with supplemental upward lighting. (A-I) Leaf layer were determined from the outer layer (1st to 3rd layer) and the inner layer (4th to 6th layer). (B) The wavelengths of cool white fluorescent lamps and white, blue, green, or red LED were recorded at 240-800 nm with a spectrometer (SR9910-v7, irradiant Ltd., Tranent, UK).

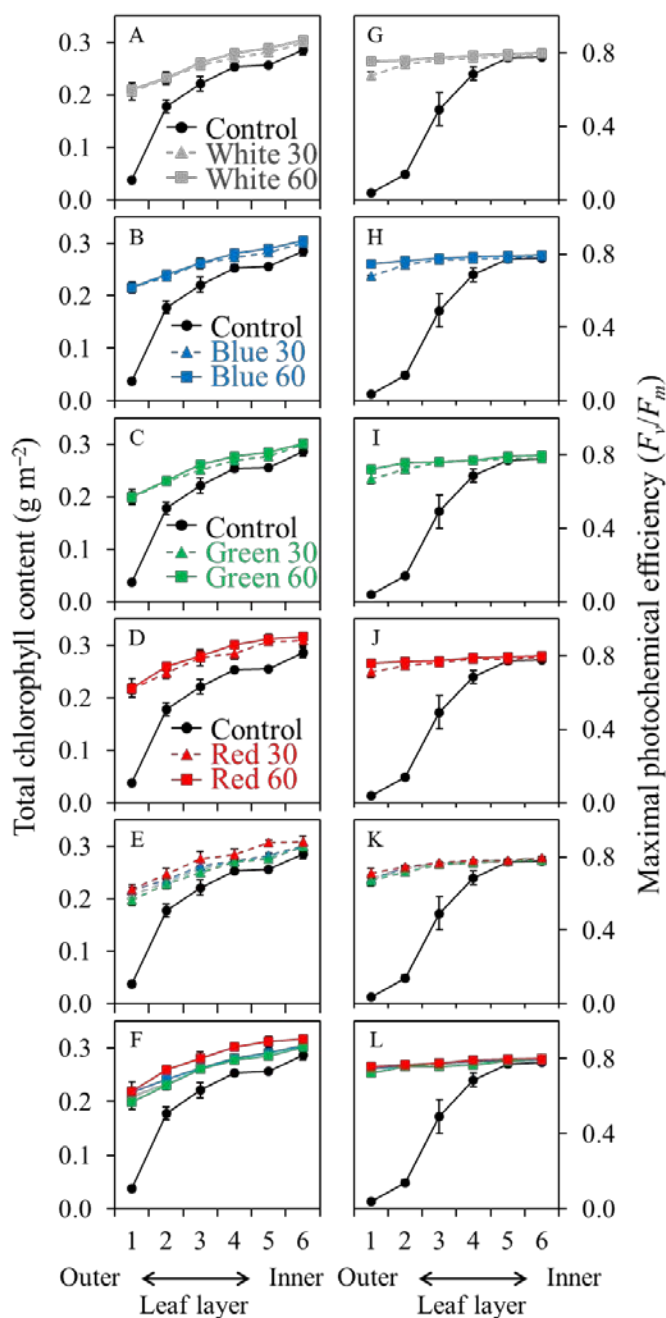


Figure 5.2 (A-F) Total chlorophyll content, (G-L) maximum photochemical efficiency of PSII (F_v/F_m), (E, K) $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, and (F, L) $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD in leaves of each layer in lettuce plant grown under different growth light conditions. Data are means \pm SE ($n = 5$). “Control” denotes plants grown solely under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; “White30”, “White60”, “Blue30”, “Blue60”, “Green30”, “Green60”, “Red30”, or “Red60” denotes plants grown under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

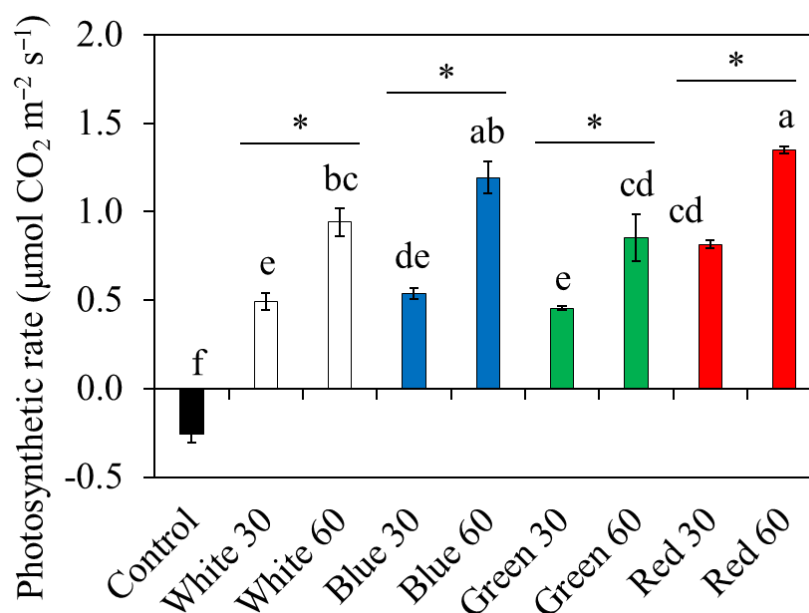


Figure 5.3 Photosynthetic rate of plants grown under different growth light conditions. Data are means \pm SE ($n = 5$). Photosynthetic rates were measured in the outer leaves (3rd layer). Different letters indicate statistically significant differences among all the treatments (Tukey's HSD at $P < 0.05$). "*" differs significantly within the same light color (Student's t-test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White30", "White60", "Blue30", "Blue60", "Green30", "Green60", "Red30", or "Red60" denotes plants grown under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

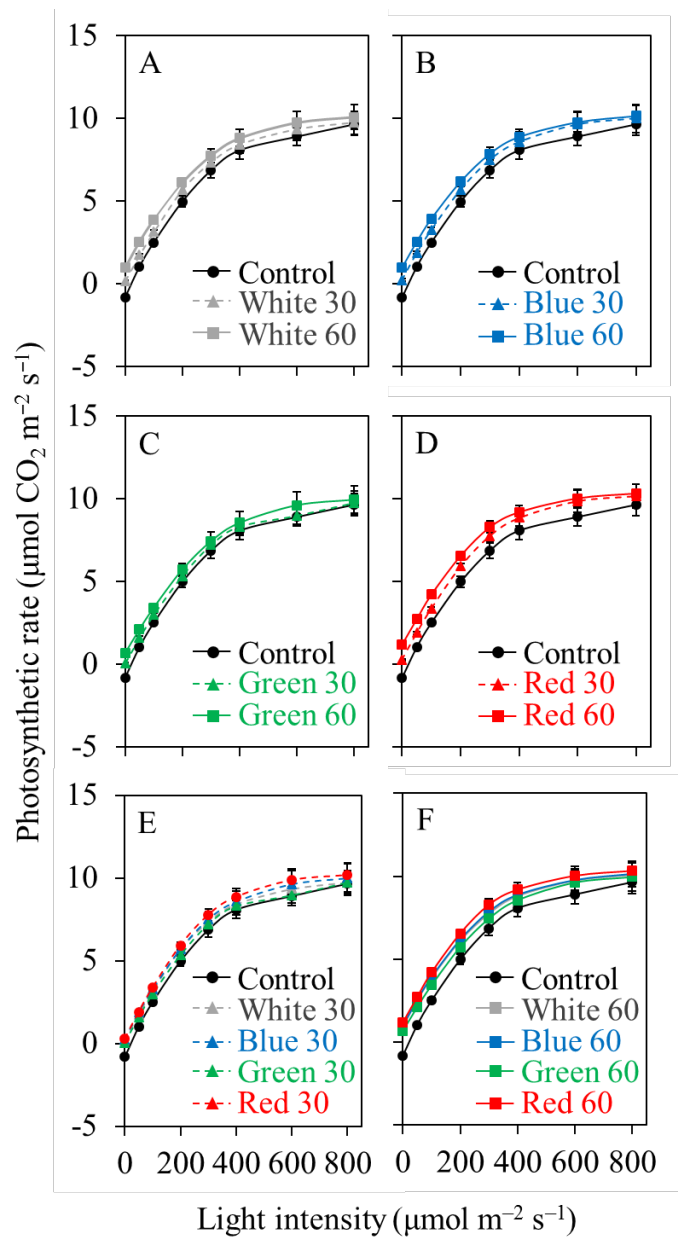


Figure 5.4 Photosynthetic light-response curves in (A) white LED, (B) blue LED, (C) green LED, (D) red LED, (E) 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, and (F) 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD on the outer leaves (in the 3rd layer) of plants grown under different growth light conditions. Data are means \pm SE ($n = 5$). “Control” denotes plants grown solely under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; “White30”, “White60”, “Blue30”, “Blue60”, “Green30”, “Green60”, “Red30”, or “Red60” denotes plants grown under downward lighting at a 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

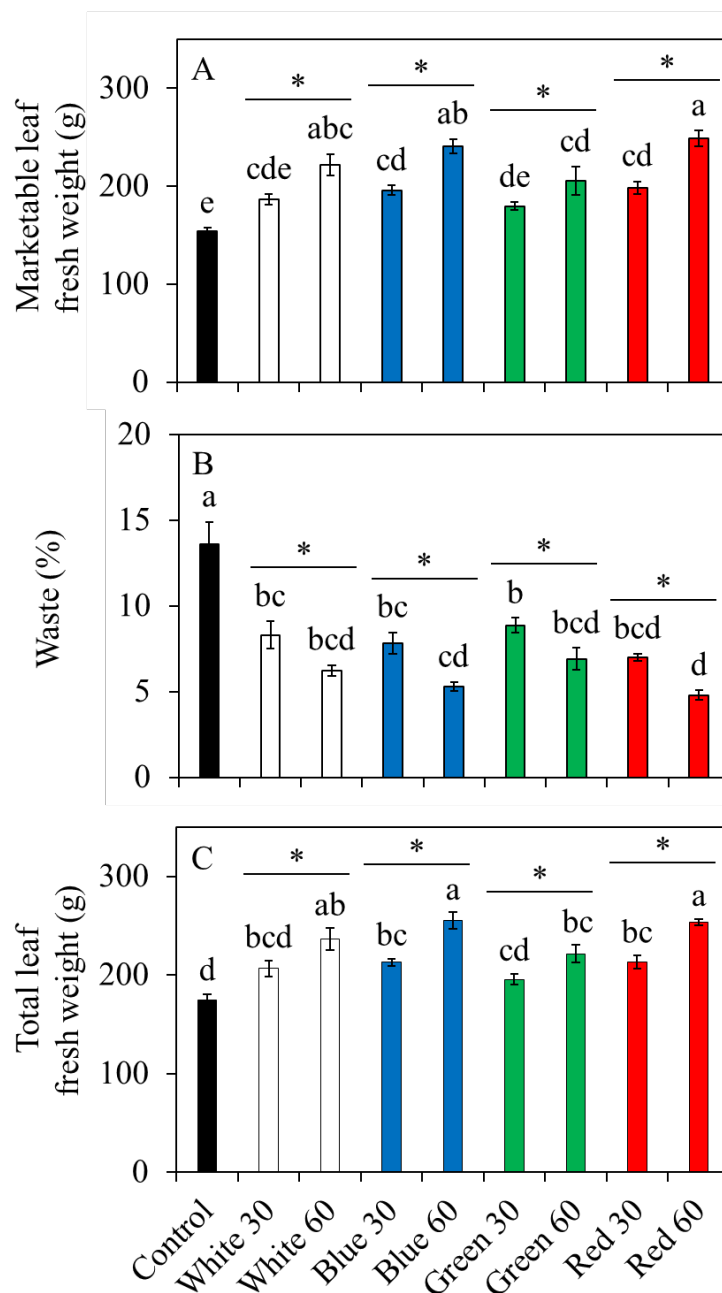


Figure 5.5 (A) Marketable leaf fresh weight, (B) waste of the outer senesced leaves, and (C) total leaf fresh weight of plants grown under different growth light conditions at 66 days after sowing. Data are means \pm SE ($n = 5$). Different letters indicate statistically significant differences among all the treatments (Tukey's HSD at $P < 0.05$). "*" differs significantly within the same light color (Student's t-test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White30", "White60", "Blue30", "Blue60", "Green30", "Green60", "Red30", or "Red60" denotes plants grown under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

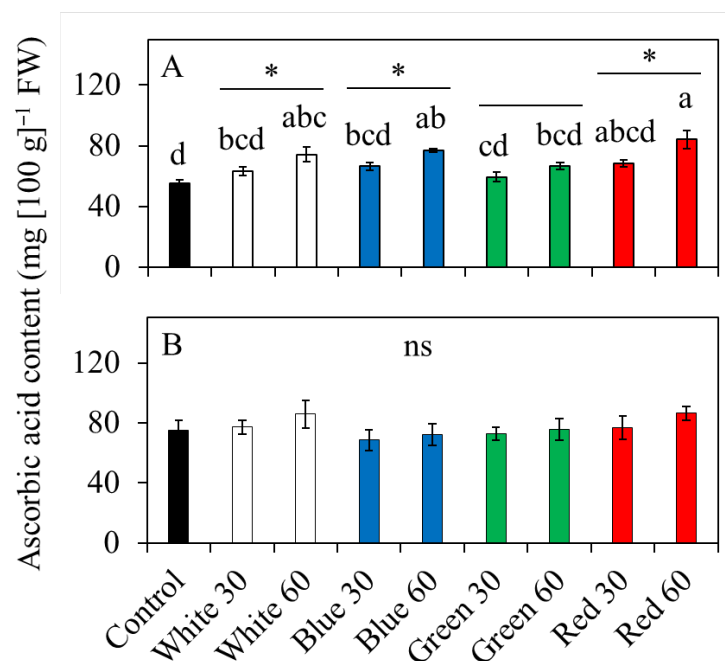


Figure 5.6 Ascorbic acid content in (A) the outer leaves and (B) the inner leaves of plants grown under different growth light conditions. Data are means \pm SE ($n = 5$). Different letters indicate statistically significant differences among all the treatments (Tukey's HSD at $P < 0.05$). "*" differs significantly within the same light color (Student's t-test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White30", "White60", "Blue30", "Blue60", "Green30", "Green60", "Red30", or "Red60" denotes plants grown under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

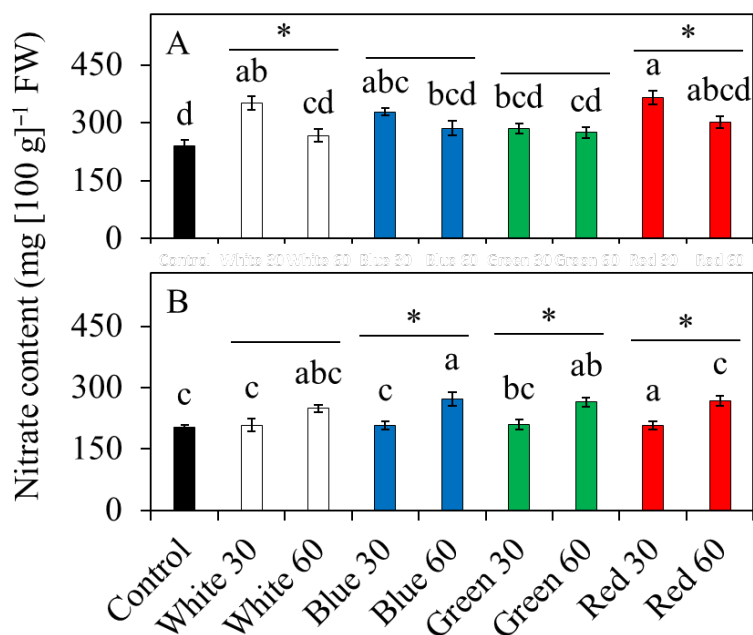


Figure 5.7 Nitrate content in (A) the outer leaves and (B) the inner leaves of plants grown under different growth light conditions. Data are means \pm SE ($n = 5$). Different letters indicate statistically significant differences among all the treatments (Tukey's HSD at $P < 0.05$). "*" differs significantly within the same light wavelength (Student's t-test, $P < 0.05$). "Control" denotes plants grown solely under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; "White 30", "White 60", "Blue 30", "Blue 60", "Green 30", "Green 60", "Red 30", or "Red 60" denotes plants grown under downward lighting at a $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD with supplemental white, blue, green, or red upward lighting at either 30 or $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

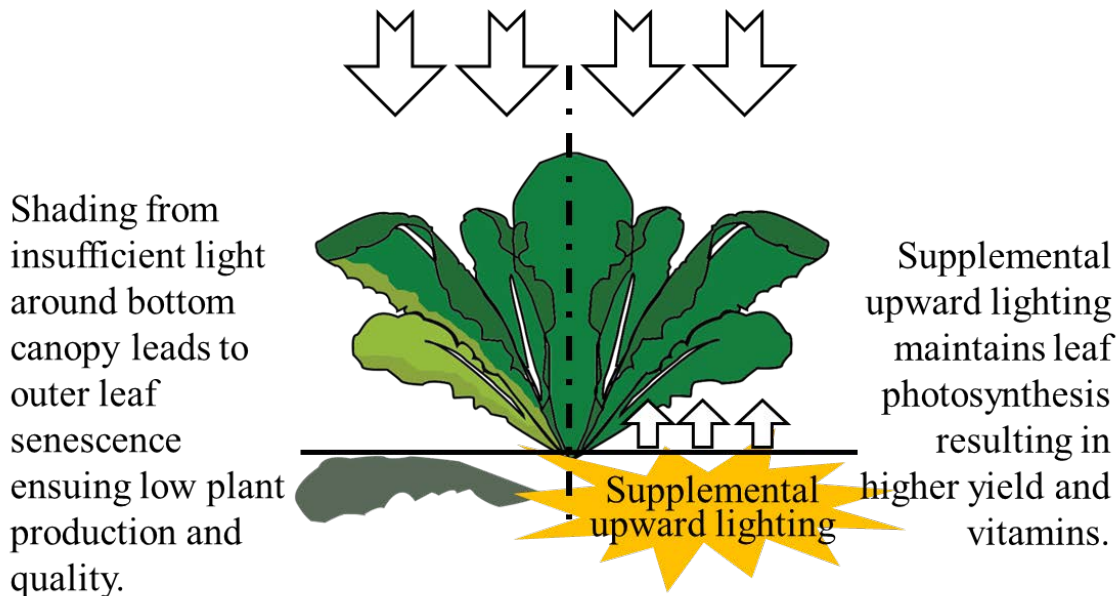


Figure 5.8 Lighting environment underneath plant canopy with supplemental upward lighting techniques increased light intensity beneath leaf canopy, more light intensity plant receives more quality plant produces. Without the supplemental upward lighting, lighting environment around bottom plant canopy is insufficient leading to low plant production.

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Chapter VI

Productivity and cost performance of lettuce production in plant factory using various LED lamps with different spectra

Abstract

Increasing attention has been paid on R&D in plant factory technologies not only in East but also in Southeast Asia. The lighting technology using LED lamps of proper light spectrum improves plant production in plant factory. What type of LED lamp is to be used for certain vegetables and whether it is economically feasible are two questions to be answered for a wide diffusion of the technology. This study intends to answer these questions by conducting an experiment in which lettuce is grown in a plant factory using seven types of LED lamp with different spectra. The results show that whitish LED could attain the shoot-fresh-weight productivity, comparable to the level attained by fluorescent lamp. However, the productivity varies greatly across different LED types. High productivity LED lamps are characterized by the spectrum pattern in which blue, green, red, and far-red lights are in a good balance, with red color taking about 50% of the entire PPFD. At the market prices prevailing in Japan of lettuce, electricity, and LED lamp, the cost-performance of all the seven LED lamps tested is above the break-even level, although there exists large differences in the performance between high and low productivity LED lamps.

Keywords: Benefit-cost ratio, fresh-weight productivity, light spectrum, percentage shares of PPFD

INTRODUCTION

Plant factory with artificial light located in urban areas is a trend of modern agricultural business. Increasing attention has been paid on the research and development in plant factory technologies in many countries including Southeast Asian countries, such as Thailand and Vietnam. Many projects are encouraged and supported by the government in these countries. This cultivation mode can control environmental factors, e.g., lighting, temperature, humidity, and CO₂ concentration (Kozai, 2013). The fluorescent lamp had been used as a major light source to produce indoor leafy vegetables for a few decades until the advent of LED (Massa et al., 2008). Many advantages of the new light source, such as low heat dissipation, specific photosynthetically active radiation (PAR) irradiation, and low electric consumption, have made the replacement of the conventional lamp by LED a priority issue (Morrow, 2008).

Plant production can be improved by LED lamps with a proper light spectrum. Since plants were considered to absorb blue and red lights more efficiently than other colors of light in the visible spectrum (Moss and Loomis, 1952), the LED lamps with blue and red lights were developed for the use in plant factory. Although the electric use efficiency of LED that converts electrical to photon energy has increased by more than 50% (Mitchell et al., 2012; Pinho et al., 2012), significantly higher investment costs for LED lamps aimed at the use in

plant factory, compared to the conventional light sources, have been a major barrier for the adoption of the LED system. In contrast, whitish LED lamps with wider spectra used widely for general lighting in offices and domestic domains have become cheaper and will be even cheaper in the near future because the demand for such LED has been and will be rapidly increasing. The use of whitish LED lamps for plant production, if possible, would help the plant factory system diffuse widely for effective vegetable production.

The number of available whitish LED lamps has been increasing rapidly. Although the color of the light of these LED lamps looks similar, the composition of the spectrum varies greatly across them. Little is known as to what type of whitish LED lamps is suited, or not suited, for plant production. Information concerning the plant-growth performance of different whitish LED lamps when used in plant factory as well as their cost performance is of substantial importance for growers to make decision. Using data obtained from an experiment in which seven types of LED lamps, including six types of whitish LED lamps, were used to grow lettuce in a plant factory, this study looks into how the productivity in plant production, biological as well as economic productivity, differed among the different LED types.

MATERIALS AND METHODS

Plant materials and growth conditions

Lettuce seeds (*Lactuca sativa* L., cv. Frill Ice; Yukijirushi Seed Co., Hokkaido, Japan) were sown in urethane cubes (W 2.3 cm × D 2.3 cm × H 2.7 cm) and placed under dark condition at 20 °C for 48 h. Seedlings were raised in an environment-controlled growth chamber under cool white fluorescent lamps (Panasonic FHF32EX-N-H) at a photosynthetic photon flux density (PPFD) of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 16 h d⁻¹. At 12 days after sowing, uniformed-size plantlets were transplanted into polystyrene panels (144.4 plant m⁻²) and cultivated under seven different types of LED lamps at the PPFD of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 14 h d⁻¹. At 23 days after sowing, the plantlets were spaced (33.3 plants m⁻²) and cultivated at the same light condition until harvest at 35 days after sowing. The plants from all the treatments were hydroponically grown under deep flow technique. The growth conditions throughout the experiment were summarized in Table 6.1.

Light treatments, sampling method, and measurements

Seven types of LED lamps with different spectra, of which six were whitish LED lamps and one was a blue+red LED lamp, were used (Table 6.2). PPFD was adjusted to be 200 ± 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the surface of the cultivation panel with a light sensor (Licor 250A; Li-cor Inc., Lincoln, NE, USA) for all treatments (the LED types). The spectra were measured by a spectroradiometer (Lighting Passport, AsenseTek, Taiwan). Lettuces were harvested at 35 days after sowing. Shoot fresh weight was measured immediately after harvested from the cultivation area. The experiment was repeated twice and nine and six plants from each experiment were sampled for measurements.

Estimation of cost performance

The cost performance of the seven types of LED lamp was evaluated in terms of the benefit-cost ratio of growing lettuce with the LED lamps, which was defined as follows:

$$(B/C)_i = (1-\alpha)P_oY_i[P_e(kWh)_i + I_i/LS + rI_i] \quad [1]$$

where $(B/C)_i$ = benefit-cost ratio of LED lamp type i ($i = A, B, C, \dots, G$), α = the percentage share of the total current production cost of lettuce, excluding electric power cost used for LED and investment cost on LED lamps, P_o = price of output (lettuce) (US\$ kg⁻¹), Y_i = lettuce yield = total shoot fresh weight (kg m⁻²) of LED lamp type i , P_e = price of electricity (US\$ kWh⁻¹), $(kWh)_i$ = electric power used for LED lamp of type i (kWh m⁻²), I_i = investment cost of LED lamp of type i (US\$ m⁻²), LS = life-span of LED lamps (number of crops), and r = interest rate. By definition, if $(B/C)_i > 1$, lettuce production with LED lamp of type i is economically feasible.

The data on the total shoot fresh weight produced by the LED lamp type i , Y_i , were given by the experiment as the mean total shoot fresh weight of lettuce. The rate of LED lamps' electricity consumption to produce Y_i , $(kWh)_i$, was calculated using the energy of photons emitted by LED during the cultivation period divided by the electro-optical conversion rate of LED. The energy of photons was calculated according to Planck's equation based on the light spectrum and the conversion rate from LED electrical energy to photosynthetically active radiation energy was assumed to be 0.5 (Kozai and Niu, 2016a). The percentage share of the total current production cost, not including costs for electric power for LED, LED-lamps' depreciation, and interests, was assumed to be one-third of the total value of the fresh weight produced (Tsuchiya, 2012). The cost items included in this current cost were seeds, fertilizers, chemicals, labor, electricity cost for fluorescent lamps (for 12 days), and other consumable goods. The prices prevailing in the respective markets in Tokyo, Japan, at the time of writing this paper were used for the prices of lettuce ($P_o = \text{US\$ } 10 \text{ kg}^{-1}$) and of electricity ($P_e = \text{US\$ } 0.3 \text{ kWh}^{-1}$). Since not all the LED lamps used in the experiment were sold in the market yet, the investment cost of LED lamps was assumed to be $I_i = I = \text{US\$ } 273 \text{ m}^{-2}$, based on the median price of LED lamps sold in the market at the time of the experiment, which was US\$ 54.5 per lamp (6,000 yen; US\$ 1.0 = 110 yen), and the number of LED lamps per m² used in the experiment, which was 5. The life time of the LED lamps (LS) was assumed to be 78 crops for all the LED lamp types, which was obtained by dividing the company-stated life span of LED (25,000 hours) by the lighting hours per crop (322 hours) in the experiment. The interest rate was assumed to be 12% per year or 1.09% per 35 days ($= 1 - 1.12^{35/365}$).

To examine how sensitive the cost performance was with respect to changes in the market prices and the depreciation cost of LED lamps, the B/C ratio was estimated by changing a price parameter (P_o , P_e , or I_i in Equation [1]) while fixing the other prices at their market levels.

RESULTS AND DISCUSSION

Fresh-weight productivity

The quantity of shoot fresh weight of lettuce was, on average over the seven LED lamp types used, 2.55 kg m^{-2} , or 2.66 kg m^{-2} if averaged over the six whitish LED lamps excluding the blue+red LED lamp (LED-G) (Table 6.3, Column [1]). This level of yield, or the productivity, per unit of planted area of lettuce in plant factory with whitish LED lamps was favorably compared to that of non-headed lettuce produced in plant factories with conventional fluorescent lamps. Tsuchiya (2012) reported that the yield of non-headed lettuce of a commercially operated plant factory with fluorescent lamps was 2.5 kg m^{-2} , just the same level as the yield performance with the LED lamps tested. Reviewing a large number of experiments, Kozai and Niu (2016b) stated that the yield of leaf lettuce in closed plant factory systems ranged between 2 kg m^{-2} and 3 kg m^{-2} . As far as the yield per unit area of 'one layer' was concerned, the productivity of non-headed lettuce production in plant factory was comparable to that of headed lettuce production in ordinary outdoor cultivation; the average yield of headed lettuce produced by farmers in Japan for 2010-2014 was 2.3 kg m^{-2} (2.3 t ha^{-1}) in winter crop, 2.6 kg m^{-2} in spring crop, and 3.0 kg m^{-2} in summer-autumn crop (MAFF, 2017). The cultivation with as many as 10 layers on a given ground area, coupled with shorter growing periods, brings about 100 times higher productivity in plant factory production compared to the ordinary outdoor cultivation.

However, the yield varied significantly across the treatments (LED lamp types) (Table 6.3, Column [1]). The LED used in the experiment were classified into three groups in terms of fresh-weight productivity: high, medium, and low productivity groups. It should be noted that the blue+red LED lamp (LED-G) was least productive, and that there was a whitish LED lamp (LED-F) the productivity of which was as low as that of LED-G.

LED lamps' electricity consumption also varied across treatments, but the degree of variation was much less than that of fresh weight (Table 6.3, Column [2]). As a result, the pattern of variation in fresh-weight productivity per kWh followed nearly the same pattern as of fresh-weight per m^{-2} (Table 6.3, Column [3]).

Simple correlation analysis conducted between fresh weight and LED lamps' spectrum characteristics showed that the percentage share in the entire PPFD of far-red had the highest positive correlation with fresh weight (Table 6.4). The correlation coefficient for blue was negative and significant and that of red was not significant at $p = 0.05$, whereas the ratio of red to blue correlated positively with fresh weight. The ratios of red to green and to far-red were also significantly and negatively correlated with fresh weight. Since it was so designed, the least productive LED-G was scarcely endowed with green and far-red lights (Table 6.2).

Such results implied that the balanced distribution of red and blue lights in the spectrum, with the relative share of red in the entire PPFD of about 50%, rather than the absolute levels of PPFD, was important in determining the shoot fresh weight (Tables 6.2 and 6.4). It was suggested that far-red, not its absolute level but its balance with lights in other wavelength bands, played certain roles in increasing fresh weight (Kubota, et al., 2012; Meng & Runkle, 2017). It was also suggested that green light, which is considered as playing

little role in plant growth, might be working well in balance with other lights, especially under a dense planting cultivation (Lu et al., 2012; Massa et al., 2008). Research is needed on what type of whitish LED lamp with what specific spectrum would fit better for the growth of specific vegetables.

Cost performance by LED type

At the price levels prevailing in the markets in Tokyo, Japan ($P_e=0.3$, $P_o=10$, and $I=273$), the B/C ratio of the lettuce production was more than unity for all the seven types of LED lamp (Table 6.3, Column [4]). This indicated that the cost performance of lettuce production in plant factory with whitish LED lamps was above the break-even level, and that the shift of lighting source in plant factory from fluorescent to whitish LED lamps was economically feasible under the present technological and economic conditions. However, the performance varied considerably among the whitish LED types. The B/C ratio of the LED lamp of the highest productivity was higher than that of the lowest-productivity by as much as 70%.

The results of sensitivity analysis revealed that the cost performance was sensitive to the changes in the lettuce price (Fig. 1, Panel A). A decline in the output price by 10-20%, with the other two prices remaining at the same market levels, would deprive the LED lamps in the low-productivity group of economic feasibility. The LED lamps in the high-productivity group would remain economically viable in areas and countries where the lettuce price is lower than the Japanese level by 53%. Countries in Southeast Asia, such as Thailand, Malaysia, and Indonesia, are examples of such countries where the level of lettuce price is lower than that in Japan by about 50% (NUMBEO, 2017).

With respect to the electricity price, the sensitivity test found that the price of electricity would not be a serious barrier for the introduction of whitish LED (Fig. 1, Panel B). The electricity price varies within and across countries; higher in some areas and countries (Mitchell et al., 2006; Lipp, 2007) and lower in some others (Poonpun and Jewell, 2008; Suramaythangkoor and Gheewala, 2010; Chua et al., 2011) than 0.3 US\$ kWh⁻¹. A lower electricity price would simply bring up the cost performance from the present level that was economically viable. On the other hand, the LED lamps in the high-productivity group would remain economically viable even in countries where the electricity price is nine times as high as the Japanese level.

Similarly, the investment cost, i.e., the cost of LED lamps, would not create any barrier to switch the lighting source to whitish LED lamps in the lettuce production in plant factory (Fig. 1, Panel C). Any downward change in this cost would increase the profitability of the lettuce production from the present level. A strong perspective that the price of whitish LED lamps would decrease as the production increases to satisfy rapidly increasing demand for it would facilitate the use of LED lamps in vegetable production in plant factory.

The profitability would be increased further by mixed use of different types of LED in different growth stages. For instance, using LED-B from 12 days after sowing for high plant biomass then LED-C for low tip-burn occurrence, can be alternative lighting recipe to attain a higher productivity. Another possibility to increase the cost performance is to improve

plant quality as well as quantity. Monochromic lighting had been proven to increase plant quality. Blue LED increased chlorogenic acid greater than red LED (Johkan et al., 2010), green LED as a supplemental light increased anthocyanin in lettuce leaves (Samuolienė et al., 2013), or blue LED increased ascorbic acid greater than blue+red light (Amoozgar et al., 2017). The use of these different types of LED lamp is another successive key to the closed plant production system. Finally, it should be remarked that other resources used in the closed production system, e.g., water, CO₂, and fertilizer, not considered in this study, need to be examined for attaining the overall resource-use efficiency (Kozai, 2013).

CONCLUSION

Replacement of light sources from the fluorescent lamps to the whitish LED lamps in the closed plant production system is feasible, economically as well as technologically. The use of whitish LED lamps for plant production in a plant factory could produce as much biomass as we could produce with fluorescent lamps.

The fresh-weight productivity per m² varies considerably across LED lamps tested, depending on the composition of their spectra. Since there will be a wide variety of whitish LED lamps available for the use in plant factory production, research on what type of whitish LED lamps with what specific spectrum would fit better for the growth of specific vegetables is urgently needed.

Lettuce production using whitish LED lamps is economically feasible under present technological and economic conditions. Not only in Japan but also in other countries where the lettuce price is lower and the electricity price and the LED lamp price are higher than in Japan, the use of whitish LED lamp in vegetable production in plant factory could be an economically feasible production option.

Table 6.1 Growth conditions of lettuce harvested at 35 days after sowing.

Condition	Seedling (12 days)		After transplanted (23 days)	
	Light	Dark	Light	Dark
Air temperature (°C)	25	20	25	20
Relative humidity (%)	45	65	45	65
CO ₂ concentration (μmol mol ⁻¹)		1,200		
Light source	Fluorescent lamps		LED lamp	-
Photoperiod (h)	16	8	14	10
PPFD (μmol m ⁻² s ⁻¹)	150	-	200	-
Nutrient solution (EC; dS m ⁻¹)*	1.8		2.4	

*EC represents Electrical Conductivity. The Enshi formula was used throughout the plant growth duration of 35 days (the full strength Enshi formula is as described by Asao et al., 2013).

Table 6.2 The percentage shares of photosynthetic photon flux density (PPFD) in the entire PPFD (380-780nm), by wavelength band, of seven LED lamps used in the experiment.

	PPFD Ultra violet (380-399nm)	PPFD Blue (400-499nm)	PPFD Green (500-599nm)	PPFD Red (600-699nm)	PPFD Far-red (700-780nm)
%.....				
LED-A	0.0	7.5	28.4	51.5	12.5
LED-B	0.3	13.0	24.2	52.3	10.3
LED-C	0.0	13.2	34.3	43.7	8.7
LED-D	0.0	15.8	10.9	63.9	9.4
LED-E	0.0	12.2	21.7	58.5	7.5
LED-F	0.0	26.3	40.7	29.8	3.2
LED-G ^a	0.0	20.7	0.5	77.5	1.2

^aNon-whitish type LED.

Table 6.3 Shoot fresh weight of lettuce grown for 35 d with LED, LED's electricity consumption, fresh-weight productivity per kWh, and B/C ratio evaluated at the market prices, by LED type

	Fresh weight [1] (kg m ⁻²)	Electricity consumption [2] (kWh m ⁻²)	Productivity [3]=[1]/[2] (g kWh ⁻¹)	B/C ratio [4]
LED-A	3.26 a	16.5	198	1.90
LED-B	3.21 a	16.7	192	1.86
LED-C	2.62 b	16.4	160	1.53
LED-D	2.40 b	17.8	135	1.35
LED-E	2.35 b	17.0	139	1.35
LED-F	2.12 c	16.4	129	1.23
LED-G	1.92 c	16.4	117	1.12
Mean	2.55	16.7	153	1.48

[1] Means of 15 replications. Figures followed by the same alphabet are not statistically different at $P < 0.01$ (multiple comparison by Tukey).

[2] Obtained by dividing the energy of photons emitted by LED during the cultivation period by the electro-optical conversion rate of LED. The energy of photons was calculated according to Planck's equation based on the light spectrum data of each LED lamp and the conversion rate from LED electrical energy to photosynthetically active radiation energy was assumed to be 0.5.

[4] Estimated by Equation [1] at the prevailing market prices for lettuce, electricity, and LED.

Table 6.4 Simple correlation coefficient ($n = 105$) between shoot fresh weight m⁻² and the percentage share of PPFD of the wavelength band of blue, green, red, and far-red, and between shoot fresh weight m⁻² and the ratios of red to blue, green, or far-red.

	PPFD percentage share				Ratio between the shares		
	Blue	Green	Red	Far-red	Red/ Blue	Red/ Green	Red/ Far-red
Correlation coefficient	-0.665	0.283	-0.188	0.760	0.526	-0.465	-0.514
Significance level ^a	**	**		**	**	**	**

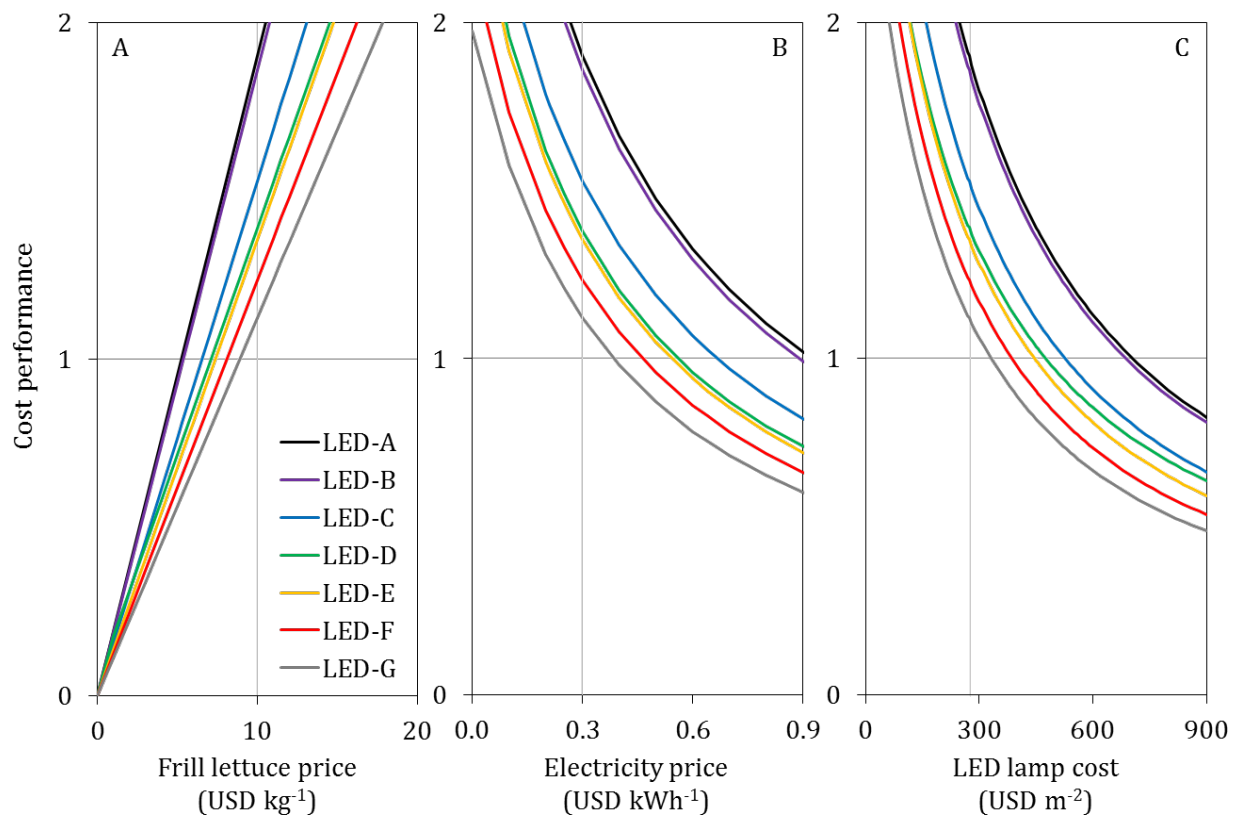


Figure 6.1 Sensitivity analysis: Changes in cost performance of different LED lamps used for lettuce production at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, measured by the Benefit-Cost ratio defined as the Equation [1], for different levels of lettuce price (P_o), electricity price (P_e), and LED cost (I_i), with the prevailing market prices for the non-varying prices ($P_o = \text{US\$ } 10 \text{ kg}^{-1}$; $P_e = \text{US\$ } 0.3 \text{ kWh}^{-1}$; and $I_i = \text{US\$ } 273 \text{ m}^{-2}$).

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Chapter VII

Supplemental upward LED lighting for growing romaine lettuce (*Lactuca sativa*) in plant factory: Cost performance by light intensity and different light spectra

Abstract

Numerous researches have established that closed plant production system is a powerful and effective mean to supply safe and high-quality vegetables in urban areas. Few researches have focused, however, on reducing plant organic residue in the closed plant production system, i.e., senescent leaves and roots that are nonsalable parts of the produced plants. Supplying upward lighting is considered as a method to improve the light environment beneath plant canopy, enhance plant production, and reduce the residues. This study compares the electricity usage and energy use efficiency in growing romaine lettuce with and without supplemental upward LED lighting. Four light spectra (white, blue, green, and red) and two light intensities (30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) of upward lighting are tested. The red LED shows the largest increment in saleable yield both m^{-2} and kWh^{-1} under supplemental upward lighting, followed by blue, white, and green, in that order. Under the market conditions prevailing in Tokyo, Japan, the supplemental upward lighting is highly economically viable, particularly so for higher levels of light intensity and for red and blue LED. With the light intensity of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, the break-even price of electricity per kWh is estimated to be US\$ 1.0, US\$ 0.7, US\$ 0.55, and US\$ 0.35, respectively, for red, blue, white, and green LED.

Keyword: bottom LED lighting, energy use efficiency, electricity usage, saleable leaf, vertical farming, waste reduction

INTRODUCTION

The mass leafy vegetable production system, popularly called “plant factory with artificial light,” located in urban areas, becomes a trend of modern agricultural business. This system is a closed plant production system that controls environmental factors for plant growth, such as lighting, temperature, humidity, and CO₂ concentration (Kozai, 2013). Plant factory with artificial light is able to attain high productivity because of high plant density, but shaded leaves lead to light insufficiency resulting in leaf senescence, which is a nonsalable part (Frantz et al., 2000). Recently, an alternative technique to improve this problem has been proposed, in which light by white LED was supplied upward from the bottom of plant canopy to maintain photosynthetic capacity in chlorophyll (Zhang et al., 2015). This effect is further enhanced if LED color, supplying spectra other than white, are used (Saengtharapip, et al. 2017). Provided that additional light spectra and excessive energy usage are not costly relative to increase in saleable leaf productivity, this system could provide plant-factory entrepreneurs with a better business chance.

Lighting is one of the major energy inputs which needs to be optimized for cost reduction (Mitchell et al., 2012). An advantage of LED lighting for vegetable production in the closed plant production system is possibility to compose spectra that are essential for plant growth while minimizing unnecessary spectra (Morrow, 2008). However, replacing conventional light sources with LED is still limited due to high capital cost (Olle and Viršile,

2013). Integration of conventional light sources with LED could be economically efficient lighting system (Li and Kubota, 2009; Samuolienė et al., 2009; Lin et al., 2013). Recently, the electric use efficiency of LED that converts electrical to photon energy has increased by more than 50% in blue and red LEDs since the past decade (Mitchell et al., 2012; Pinho et al., 2012). These colors of light are absorbed by plants more efficiency compared to green light (Nishio, 2000; Morrow, 2008).

The purpose of this study is to evaluate economic feasibility of supplemental upward lighting in growing romaine lettuce in a closed plant factory system, based on the data reported by Saengtharatip et al. (2017), which studied the effects on plant growth in a closed system of the upward LED lighting for different spectra and different energy levels. The growth conditions of the experiment were summarized in Table 7.1.

MATERIALS AND METHODS

Data Used

To evaluate the economic performance of the upward LED lighting system in a closed-system plant factory, five groups of data are necessary: 1) the increment rate due to the upward lighting in saleable fresh weight of romaine lettuce per a unit area, 2) the necessary quantity of electricity to generate the increment in the product, 3) the installation (investment) cost of the LED module for the upward lighting, 4) the life time of the LED module, and 5) related market prices to value the benefit and costs, i.e., the prices of romaine lettuce and electricity, and the interest rate for the initial investment.

Necessary data for 1) were obtained from Figure 7.2 of Saengtharatip et al. (2017), which showed the change due to the upward lighting in the total fresh weight, the nonsalable fresh weight, and the increment of saleable fresh weight by LED color and the level of PPFD. Data for 2) were calculated using the energy of photons emitted by LEDs during cultivation period divided by the electro-optical conversion rate of LED. The energy of photons was calculated according to Planck's equation based on the light spectrum (Figure 7.1B of Saengtharatip et al. (2017)) and the conversion rate from LED electrical energy to photosynthetically active radiation energy was assumed to be 0.5 (Kozai and Niu, 2016). Data for 3) were obtained by identifying the sales price of the LED module and necessary accessories, which totaled US\$ 100 m⁻². Data for 4) were obtained by dividing the company-stated life span of LEDs (25,000 hours) by the lighting hours per crop (532 hours) of the experiment (Table 7.1). Data for 5) were obtained as the prices prevailing in the respective markets in Tokyo, Japan at the time of writing this paper; the price of romaine lettuce = US\$ 10 kg⁻¹, the price of electricity = US\$ 0.3 kWh⁻¹, and the interest rate = 1.3% per 1.3 month (1% per month). The interest rate per 1.3 month was used because a crop cycle with the supplemental LED upward lighting was 38 days (Table 7.1).

Estimation of cost performance

The cost performance of the additional upward-lighting system was evaluated in terms of the benefit-cost ratio of installing the LED module, which was defined as follows:

$$(B/C)_{jk} = (P_o * \Delta FW_{jk}) / (P_e * kWh_{jk} + I/LS + i), \quad (j = 1 \text{ or } 2, k = 1, 2, 3, \text{ or } 4) \quad (1)$$

where $(B/C)_{jk}$ = benefit-cost ratio of the upward lighting using LED of k-color when light intensity = j, P_o = price of output (romaine lettuce) (US\$ kg⁻¹), ΔFW_{jk} = increase in saleable fresh weight (kg m⁻²) for LED of k-color when light intensity = j, P_e = price of electricity (US\$ kWh⁻¹), kWh_{jk} = electric power used for LED of k-color (kWh m⁻²) when light intensity = j, I = installment (investment) cost of the LED module (US\$ m⁻²), LS = life-span of the LED module (= 47 crops), i = interest rate per crop, j stands for light intensity (30 or 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD), and k stands for LED color (white, blue, green, or red). By definition, if $B/C > 1$, the supplemental upward lighting system is economically feasible.

To examine how sensitive the cost performance was with respect to changes in the market prices and the cost of LED module, the B/C ratio was estimated by changing a price parameter (P_o , P_e , or I in Equation 1) while fixing the other prices at their market levels.

RESULTS

Effect of supplemental upward LED lighting on plant growth and production

As the light intensity increase, the supplemental upward lighting was not only significantly increased the fresh weight of total leaf of romaine lettuce, but also significantly reduced the non-saleable leaf, both in terms of g m⁻² for all the types of LED (Figure 7.2 A and B). Red LED showed the highest performance in plant growth and production, followed by blue, white, and green LED in that order. In terms of the increment of saleable leaf per electric power used in the additional upward lighting (kgWh⁻¹), the performance significantly increased from 0 to 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, but stayed at around the same level with a higher light intensity of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (Fig. 2 C). This means the difference in the physical productivity per kWh was insignificant between the two levels of light intensity. The order of the performance was invariant in this conversion; the red LED was clearly the most effective LED type, followed by the blue and white LED; and the green LED was apparently the least effective.

Cost performance of supplemental upward LED lighting

In spite of the similar levels of physical productivity between the two levels of light intensity, the cost performance differed substantially among them (Figure 7.3 A, B, and C vs Figure 7.3 A', B', and C'). At the price levels prevailing in markets in Tokyo, Japan ($P_e=0.3$, $P_o=10$, and $I=100$), the B/C ratio was greater than unity for all LED when light intensity = 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (Figure 7.3 A', B', and C'), whereas $B/C > 1$ only for red and blue LED when light intensity = 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (Figure 7.3 A, B, and C). Such difference in the economic productivity emerged because the contribution to the B/C ratio of the output price (P_o) was greater than that of the price of electricity (P_e). As long as the light intensity of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD is used, the supplemental upward LED lighting is an economically profitable option at the prevailing market prices regardless of the colors of LED; and it is so for the red and blue LED, even if with a lower light intensity of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

The degree of the profitability differed substantially among the four colors for both levels of light intensity. With respect to the higher intensity level of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, the B/C ratio of green LED with the lowest efficiency in increasing saleable leaf was barely above unity, whereas the red LED was as high as more than 2.0 (Figure 7.3 A', B', and C'). With respect to the price of lettuce, only a 10% decline in the market price from the prevailing price level would bring the B/C ratio of green LED down to the break-even, while white, blue,

and red LEDs would be tolerant to decline in the output price by 30%, 40%, and 60%, respectively (Figure 7.3A'). With respect to the price of electricity, the positive profitability would be lost at US\$ 0.35 kWh⁻¹ for the green LED, US\$ 0.55 kWh⁻¹ for the white LED, US\$ 0.70 kWh⁻¹ for the blue LED, and US\$ 1.0 kWh⁻¹ for the red LED (Figure 7.3B'). With respect to the investment cost, the break-even cost of the LED module was US\$ 120 for the green LED, but the white, blue, and red LED would be tolerant to the cost escalation of the LED module by as much as 2, 2.8, and 3.5 times, respectively (Figure 7.3C').

DISCUSSION

Increasing plant production in a closed plant factory system is desirable for society as long as it is economically viable. This study examined whether the supplemental upward lighting by LED is economically viable. The nonsalable leaf as well as the total leaf were improved by the upward lighting, resulting in significant yield increase in the saleable leaf. The efficiency to increase saleable leaf was highest for the red LED, followed by the blue LED, both of which are essential on plant growth and yield (Goins et al., 1997). On the other hand, the nonsalable leaf is to be removed before packing and selling, causing a reduction in plant yield up to 10% or even more. Even with the same amount of total leaf production, a higher yield of the romaine lettuce could be made possibly by reducing the rate of non-saleable leaf (Kozai and Niu, 2016). This could be achieved by the supplemental upward lighting technique (Zhang et al., 2015). Among four LED types, the yield increase in saleable leaf was significantly higher for the red LED.

The price of LED modules has been declining at least by 10 per lumen in the last decade (Pimputkar et al., 2009). The price of electricity varies across countries; it could be higher than 0.3 USD kWh⁻¹ in some countries, e.g. in Denmark and Germany (Mitchell et al., 2006; Lipp, 2007); but it could be lower than that level in many countries, e.g. in Malaysia, Thailand, or USA (Poonpun and Jewell, 2008; Suramaythangkoor and Gheewala, 2010; Chua et al., 2011). These imply that the technique is worthwhile to invest in many countries. The profitability could be further enhanced, because the plant density and cultivation period reported in the experiment by Saengtharatip et al. (2017) could be substantially improved (Kozai and Niu, 2016). Moreover, this study did not take into account on the possibilities that the value of output could be increased by changing the combination and/or proportion of light recipe in order to increase specific nutrients to satisfy customers' specific needs. For example, the supplemental lighting of blue LED increased antioxidant (Johkan et al., 2010; Li et al., 2012), or the sweetness of lettuce was enhanced by mixing white, blue, and red LED (Lin et al., 2013).

It should also be remarked that other resources used in the production system, e.g., water, CO₂, and electric appearances, which were not considered in this study, is needed to be examined for attaining the overall resource-use efficiency (Kozai, 2013).

CONCLUSIONS

The supplemental upward LED lighting for romaine lettuce in a closed plant factory system was an economically viable technique at a high light intensity, i.e., 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, for all the LED types used in this study. The profitability was particularly high when

red LED was used, followed by blue, white, and green LED in that order. However, the impact of the upward lighting on plant quality, e.g., antioxidant, minerals, and vitamins, need to be examined in parallel to plant production, to produce vegetables of higher qualification. Further study on combining and mixing different light spectra with the supplemental upward LED lighting technique is of high priority.

Table 7.1 Growth conditions of romaine lettuce (*Lactuca sativa* L. var. *romana*) harvested at 66 days after sowing. The seedling and the experimental plants grew under downward cool white fluorescent lamp using modified 0.75-strength Enshi formula nutrient solution (modified from Asao et al., 2013). Source: Saengtharatip et al. (2017)

Condition	Seedling (28 days)		Experimental (38 days)	
	Light	Dark	Light	Dark
Air temperature (°C)	25	20	25	20
Relative humidity (%)	45	65	45	65
CO ₂ concentration (μmol mol ⁻¹)		1,200		480
Photoperiod (h)	12	12	14	10
PPFD (μmol m ⁻² s ⁻¹)	250	-	200	-

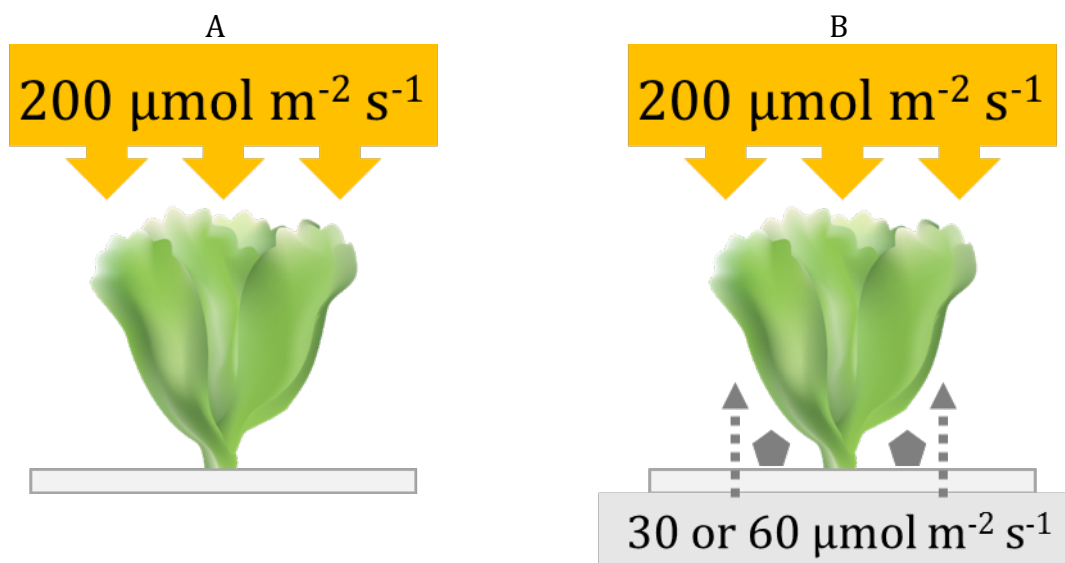


Figure 7.1 Romaine lettuce cultivation experiment by Saengtharatip et al. (2017): Diagram A for an imitative sample growing solely under $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD cool white fluorescent lamps without supplemental upward lighting and diagram B for an imitative sample growing under $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD cool white fluorescent lamps with either 30 or $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD supplemental upward LED lighting. Supplemental upward LED lighting was initiated at 28 days after sowing and stopped at 66 days after sowing.

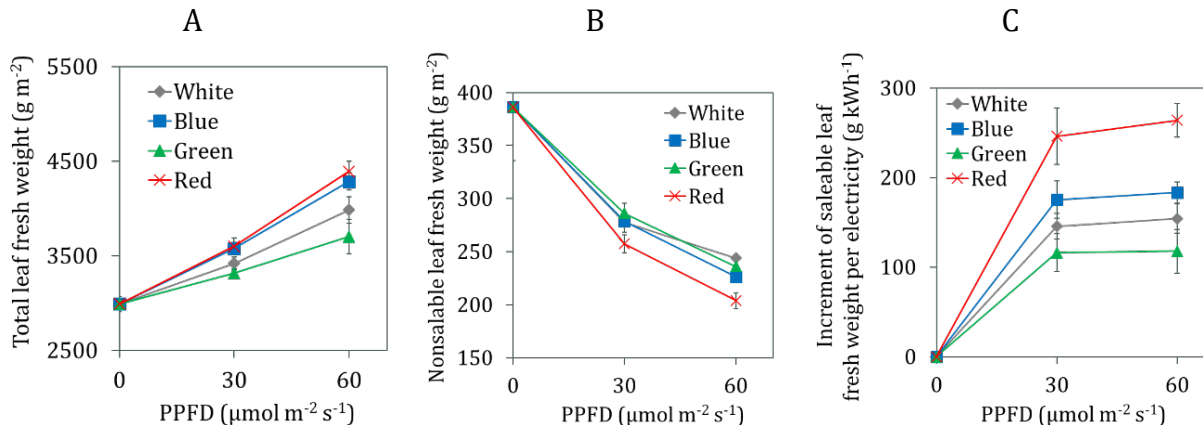


Figure 7.2 Effect of supplemental upward LED lighting on (A) fresh weight (FW) of total leaf, (B) FW of nonsalable leaf, and (C) FW increment of saleable leaf by supplemental upward LED lighting of romaine lettuce harvested at 66 days after sowing. Data are means \pm SE (Tukey's HSD at $P < 0.05$; $n = 5$). Source: Saengtharatip et al. (2017)

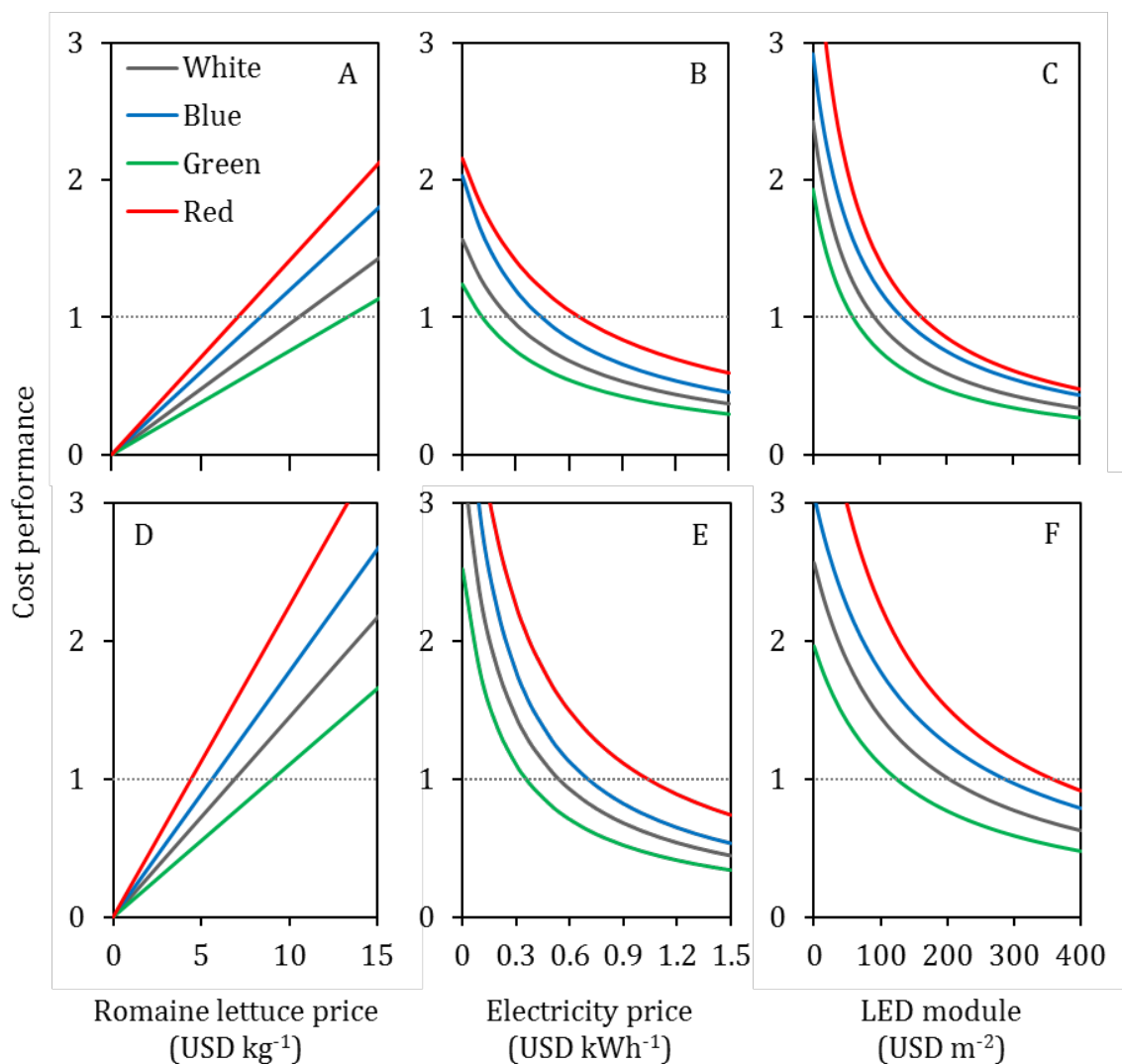


Figure 7.3 Changes in cost performance of supplemental upward LED lighting at $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (A, B, and C) and $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (A', B', and C') for romaine lettuce, measured by Benefit/Cost ratio defined by the Equation (1), for different levels of lettuce price (A and A'), electricity price (B and B'), and LED module costs (C and C').

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Chapter VIII

Conclusion, perspective, and remark

RESEARCH CONCLUSION

All the lettuce cultivar groups have been improved throughout the experiments under different light quality in this study. Plant growth is not only one factor increasing, but also plant quality. Compare to the conventional cultivation, the ascorbic acid content is improved under artificial lighting from all experiments. On the other hand, nitrate content is remained below the regulation (Table 8.1). These imply that the closed system is eligible and trustworthy to produce leafy vegetable with no harmful effect for consumption.

Red LED type (635 nm) is the most effective color for supplemental upward lighting, particularly when the light intensity is $60 \mu\text{mol m}^{-2} \text{s}^{-1}$. Cultivating romaine lettuce with this LED is also profitable under the market prices prevailing around in Tokyo area, Japan.

Green LED type (530 nm) demonstrates an outstanding characteristic of green light which penetrates into deeper layer of crisp head lettuce resulting to increase ascorbic acid content inside the lettuce. This remarkable result will completely bring the brighter future of plant production in the closed system. However, more minerals and vitamins are needed to be determined with specific methodology, e.g., high performance liquid or gas chromatography (HPLC or GC), to confirm whether the results is reliable.

White LED type is the up-to-date trend of light source for agricultural business. The use of white LED is represented the PAR region and emulated the spectrum of the sun. Nevertheless, one LED could increase plant growth while another LED could improve plant quality. To tie these factors together is the next challenging task for the up-coming research topic which will be occur, soon enough.

In conclusion of this study, the bright and shine future of the closed plant production regarding to plant growth and quality is reachable by the end of next decade. Applying optimal lighting condition of both quality and quantity is a key factor to implement inside the close cultivation system. Not long enough, one lettuce may contains minerals, vitamins, or even vaccines to the better health in life.

PERSPECTIVE AND REMARK

Land reformation from forest to town to community space

Several physiological of human needs, shelters, nutriments, medications, and clothes, are extremely needed (Maslow, 1943). Among them, land is the most essence. Land is reformed from wild forests to cultivation spaces, residential areas, and roads (Lindley, 1987). The land becomes extremely scarcity. Many areas have been converted from forests to cropland (Pielke et al., 2002). Deforestation occurs when population rising to agricultural area and its production, such as, Costa Rica (Lambin and Meyfroidt, 2011). Limited cultivation area is being concerned for substance production (Haaland and Van den Bosch, 2015). Meanwhile, an inflation land cost for living is also facing agricultural area for making the food. Temperature evidence increased with urban areas compared to rural areas (Kalnay

and Cai, 2003). This implies that human resembles into places of hope for better well-being especially for income (Forster and Pellizzari, 2000; Tan et al., 2005).

Arable land may be rarely be scarcity because of urbanization within a few decades, land-use efficiency will overwhelmingly be following concern on soilless growing technique, e.g., aeroponic, hydroponic, and drip irrigation (Döös, 2002; Despommier, 2009). For example, using a small space sunlight greenhouse, i.e., 100 m² to produce 11,000 plants nearby a living area can maintain both of the vegetables cost and quality (Baylay et al., 2010). An integration hydroponic technique between vegetable and fish known as aquaponic was commonly applied. The benefit occurred with both production simultaneously (Bailey et al., 1997).

Self-cultivation system, especially hydroponic, in undesignated area would be able to feed their living area (Eigenbrod and Gruda, 2015). In other word, this idea could be fitted within a town where a bunch of people is living to decrease a transportation cost and avoid handle deficiency. A new growing system is established and introduced to agriculturalists which is vertical farming or plant factory. This system is able to produce high yield and quality of vegetables as well as using resources efficiency, e.g., light, water, and nutrients, within a small area. Plant factory improved a high potential in environmental, social, economic aspects, and reduced transportation (Specht et al., 2014).

Urban congestion is another subject to consider beside overpopulated. Using a public space efficiency for socialization must be taken into account for building a healthy community. It must be carry out since a childhood stage (Bixler et al., 2002). An idea which could be used for social integration and relation could be applied from a reputed philosophy on sufficient economy is to divide any given area (rooftop, public space, or backyard) to cultivate; a carbohydrate source, vegetable or fruits, and water reservoir. Initially, these spaces could be established and shared as a common space to neighborhood which gather residents to have activities together (Kweon et al., 1998). Alternatively, using abandoned spaces, i.e., rooftop for greenhouse for vegetable production had been proven to supply yearly tomato demand with community engagement. This is not only assemblage, but also productivity (Sanyé-Mengual et al., 2015). Enormous benefits from the community gardens have been reveal not only for food production, but also spaces for parties and events (Saldivar-Tanaka and Krasny, 2004). Hence, better socialization stronger community.

The author is aspiring with hope that the closed-type cultivation area could be reshaped for everyone in a given community to mitigate food accessible and recall lively community to the world with better place.

Table 8.1 The comparison of major nutrition fact from each cultivar groups between conventional and closed system.

	Crisp head		Frill		Romaine	
	Conventional	PFAL	Conventional	PFAL	Conventional	PFAL
Ascorbic acid (mg 100 g ⁻¹ FW)	5.5 (Sørensen et al., 1994)	110	3.5 (Ogawa et al., 2014)	80	2.8 (Llorach et al., 2008)	70
Nitrate regulation (mg 100 g ⁻¹ FW; Alexander et al., 2008)	220	180	360	291	360	275

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