

Effects of Different Air Temperature and Light Quality / Intensity on Growth and Folate Biosynthesis in Leaf Lettuce

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Abstract

The paper aimed to explore the effects of different air temperature and light irradiation conditions on growth and folate biosynthesis in leaf lettuce using a plant growth cabinet with LEDs. Leaf lettuce was cultivated under combination conditions with 3 different air temperatures (20°C, 25°C and 28°C) and 4 different light irradiations (photosynthetic photon flux density [PPFD] of 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [P150]/RB, P150/RGB, P200/RB and P200/RGB) for 21 days. At the end of the cultivation, growth endpoints including edible portion weight, and folate contents were examined. The experiment results demonstrated significant effects of air temperature and light irradiation conditions on growth and folate biosynthesis in leaf lettuce. We identified the optimal combination condition, 25°C and P200/RGB, significantly promoting growth and folate biosynthesis in leaf lettuce. The edible portion weight and folate contents in leaf lettuce cultivated under our optimal combination were 15%-62% and 83%-137% greater, respectively, compared to any light irradiation conditions at 20°C, an optimal temperature for conventionally growing leaf lettuce. Addition of green LED to red and blue LEDs maximized leaf lettuce growth and folate biosynthesis. Plant factory with optimal temperature and light irradiation conditions utilizing RGB independent dimming type LED lights is a promising novel agriculture concept maximizing plant growth and critical nutrients such as folate.

Keywords

Light quality; Intensity; Air temperature; Leaf lettuce.

Introduction

Closed-type plant factories with artificial light enable us to grow various vegetables, fruits and leafy greens under controlled environments to maximize their yield and nutritional contents. When we plan to develop specific nutrient-rich plants in plant factories, it is important to select critical nutrients that are highly involved in human health; therefore, we have been focusing on one of the most critical nutrients, folate. Insufficient folate consumption is known to cause severe developmental disorders in humans including spina bifida and anencephaly. Since mammals lack *de novo* folate biosynthetic pathway, animals including humans entirely depend on their dietary sources for the folate supply. However, most of staple crops such as rice, potato and maize are poor sources of folate, and rich sources include green leaf vegetables and some meat, especially liver and kidneys, eggs, and some seafood (Castellano et al., 2016; El-Mahrouk et al., 2016; Foyo-Moreno et al., 2017).

Previous investigations for the mechanism of folate biosynthesis suggest that manipulation of photosynthetic photon flux density (PPFD) and growing air temperature could increase folate contents in plants. Rébeillé et al. reported that folate synthesis and accumulation were elevated in leaves upon exposure to light (Bruns, 2016; Leite et al., 2017; Lu et al., 2017).

Although fluorescent lights have been mainly used for closed-type plant factories, light-emitting diodes (LEDs) have attracted attention for cultivation of functional vegetables. LEDs enable us to combine lights with specific wavelengths suitable for light absorption property of photosynthetic pigments and photoreceptors in plants; therefore, LEDs have been used for the investigation about the mechanism of plant light response. Cultivation under the condition of combination with red and blue lights was studied in various vegetables including spinach, radish, lettuce, komatsuna. It has been reported that light is highly involved in the production of various high-value secondary metabolites. As far as we searched, there are no studies evaluating effects of light quality and intensity on growth and folate biosynthesis in leaf lettuce. Thus, we evaluated effects of different air temperature and light irradiation conditions on growth and folate biosynthesis in leaf lettuce using a plant growth cabinet with LEDs.

Materials and Methods

Plant Materials and Growth Conditions

A cultivation experiment was performed at the laboratory in the LED artificial light-type plant factory of Keystone Technology Inc. (Yokohama, Japan). Leaf

lettuce (*Lactuca sativa* var. *crispa*) (Fancy Green[®], Nakahara Seed Product Co., Fukuoka, Japan) was cultivated in a 4-story growth cabinet (Plant Cellar TAPS 6T, Espec Mic Corp., Osaka, Japan; Figure 1) in which air temperature and humidity were controllable. Each of the 4 trays had 15 holes for the deep flow technique (DFT) enabling a 24-hour continuous circulation of the nutrient solution (Vege-solution[™], Keystone Technology Inc.) with pH of 6.0 and electric conductivity of 2.0 dSm⁻¹. RGB independent dimming type LED lights specialized for plant cultivation (Keystone Technology Inc.) including 660 nm red, 525 nm green, and 460 nm blue LEDs were set to the growth cabinets (Figure 2).



Figure 1: A 4-story growth cabinet (Plant Cellar TAPS-6T).

Experimental Design

To assess the effects of different air temperature and light intensity / quality conditions on the growth and folate biosynthesis, the experiment included 12 different combination conditions with 3 different air temperatures (20°C, 25°C and 28°C) and 4 different light irradiations (P150/RB, P150/RGB, P200/RB and P200/RGB). Lettuce seeds were placed on the sponge

medium containing sufficient water in the polystyrene white tray for a 3-week propagation. At the end of the propagation, uniformly sized lettuce seedlings were selected, and randomly planted on urethan foam placed in the holes of the trays in the 4-story growth cabinet (5 plants for each temperature / light irradiation condition). Light irradiation was controlled with the LED controller (HAC-M801A, Keystone Technology Inc.) with 16-hour light / 8-hour dark cycle. Sensing and measurement of PPFD on the growth urethan foam were performed with LI-190 quantum sensor and LI250A light meter (Li-Cor Biosciences, Lincoln, NE, USA), respectively. Four different light irradiation conditions were set by story in the 4-story growth cabinet. Detailed light quality characteristics and irradiation condition are shown in Table 1. The difference in B/R ratio due to different PPFD and addition with green light was minimized (0.29 to 0.34).

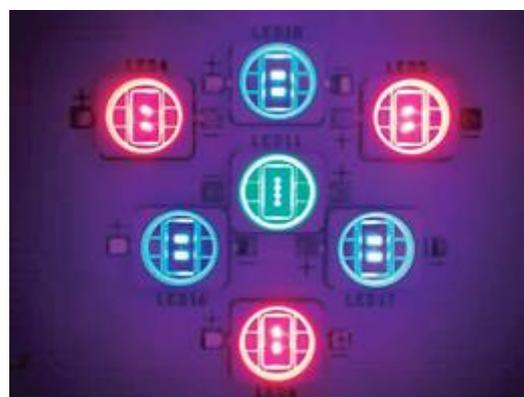


Figure 2: RGB Independent dimming type LED light specialized for plant cultivation. A patent-registered LEDs having a green LED at the center surrounded by three blue LEDs and three red LEDs.

Relative humidity was maintained at 60±5% across groups throughout the cultivation, but carbon dioxide concentration within the growth cabinet was not controlled. The leaf lettuce was cultivated in the growth cabinet throughout the 21-day cultivation period from planting to harvest (Matsuda et al., 2016; Murakami et al., 2016; Velumani et al., 2017).

Table 1: Light intensity and quality characteristics of the plant growing LED lights.

Characteristic	P150		P200	
Color (Wavelength) (nm)	Photon flux density (mmol·m ⁻² ·s ⁻¹)			
Red (660 nm)	112	98	150	139
Green (525 nm)	-	22	-	21
Blue (460 nm)	38	30	50	40
PPFD	150	150	200	200
B/R ratio	0.34	0.31	0.33	0.29

PPFD: photosynthetic photon flux density.

B/R ratio: blue/red ratio.

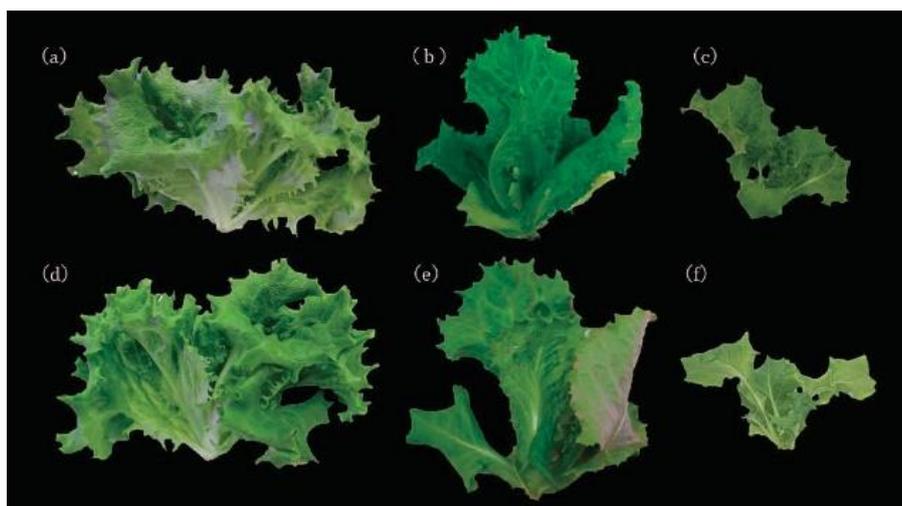


Figure 3: The morphological characteristics of the harvested leaf lettuce. Leaf lettuce was cultivated in the growth cabinet for 21 days under the following air temperature and light irradiation conditions: (a) 20°C/P150/RGB, (b) 25°C/P150/RGB, (c) 28°C/P150/RGB, (d) 20°C/P200/RGB, (e) 25°C/P200/RGB, (f) 28°C/P200/RGB. The photograph includes representative plant for each air temperature and light irradiation condition.

Endpoints

At the end of the 21-day cultivation, leaf lettuce was harvested for the assessment of growth and folate contents. Growth endpoints included plant height (the length from the base to the top of the leaves), the number of leaves, chlorophyll content, and edible portion weight. Chlorophyll contents were measured with chlorophyll meter (SPAD-502; Konica Minolta Sensing Inc., Osaka, Japan) as SPAD score ranging from -9.9 to 199.9, and a mean value was obtained from 3 different measurement points on each plant. Edible portion weight was obtained from each plant after removing roots, cotyledons, and, discolored and dead leaves. In addition to the quantitative growth endpoints, morphological characteristics were macroscopically compared among different conditions at the harvest. Folate contents in the edible portion (pooled plants / combination condition \times 3 experiments) were measured with microbiological assay at the Kanagawa Institute of Industrial Science and Technology (former name: Kanagawa Prefectural Industrial Technology Center, Ebina, Japan).

Statistical Analysis

The cultivation experiments were repeated three times. Average and standard error were obtained from numerical data for each combination condition. The data were subjected to analysis of variance, followed by TukeyKramer multiple comparison across combination conditions using statistical software (JMP[®] 8, SAS, Cary, NC, USA) with a significance level of $p \leq 0.05$. In addition, potential correlations between each of 4 growth endpoints and folate contents were examined with logistic regression analysis.

Results and Discussion

Effects of Air Temperature and Light Irradiation Conditions on Growth

Under combination cultivation conditions with 3 different air temperatures (20°C, 25°C and 28°C) and 4 different light environments (P150/RB, P150/RGB, P200/RB and P200/RGB) in the growth cabinet for 21 days, the leaf lettuce in general extended horizontally at 20°C while vertically at 25°C (Figure 3). The plants tended to grow spindly accompanied with more leaves at 28°C compared to the conditions at 20°C and 25°C. Although lettuce tended to grow slightly better with P200 compared to P150, there was no apparent effect of different light intensities on morphological characteristics at each temperature. Plant heights were significantly greater at 25°C than those at 20°C irrespective of light irradiation conditions (Figure 4). There was a trend to increased number of leaves as increased air temperature. Chlorophyll contents (evaluated with SPAD scores) tended to be higher at 20°C and 28°C compared to 25°C, especially with P200/RB and P200/RGB at 20°C and P150/RGB, P200/RB and P200/RGB at 28°C. Edible portion weight was the highest at 25°C followed by 20°C, and the lowest at 28°C. Addition of green light to red and blue lights increased edible portion weight (Figure 4). Namely the edible portion was significantly greater in P150/RGB compared to P150/RB at both 20°C and 25°C. Although statistically significant difference was not observed, the edible portion was always numerically greater with RGB compared to RB when it was compared within the same air temperature and light intensity. Especially the edible portion weight under the combination condition at 25°C with P200/RGB was approximately 15% to 62% greater compared to any light irradiation conditions at 20°C, which was optimal temperature for natural growth of leaf lettuce.

The increased plant height at 25°C compared to 20°C might partially contribute to the increased edible portion

at 25°C; however, pattern in the number of leaves and chlorophyll contents were not consistent with the

change in edible portion across different air temperatures, warranting further detailed assessment.

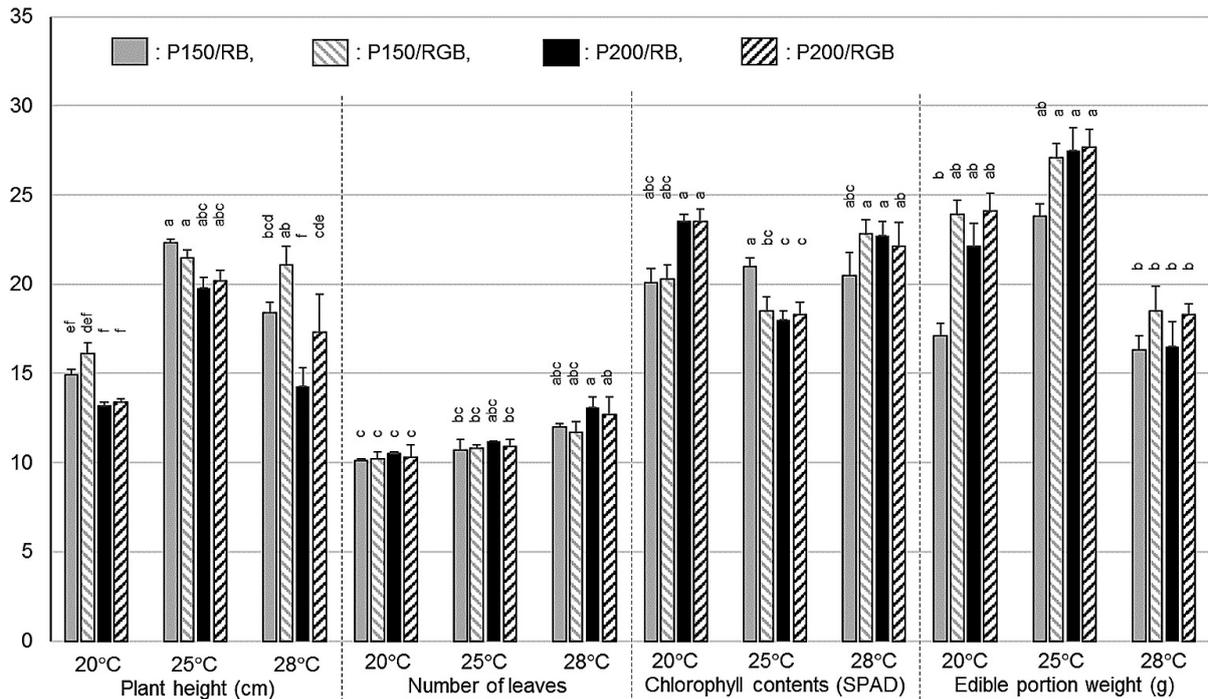


Figure 4: Effects of different air temperature and light irradiation conditions on growth. Leaf lettuce was cultivated in the growth cabinet for 21 days under 3 different air temperature conditions (20°C, 25°C and 28°C) and 4 different light conditions (P150/RB, P150/RGB, P200/RB and P200/RGB). The data are shown as group mean ± standard error. a to f: Different alphabets indicate significant differences ($p \leq 0.05\%$) based on multiple comparison test. $n=5$.

Effects of Air Temperature and Light Conditions on Folate Contents

Folate contents were the highest at 25°C and the lowest at 28°C under all of the light irradiation conditions (Figure 5). Lettuce is categorized into highland vegetables favoring cool climate, and the optimal temperature for natural growth is considered 20°C. Photorespiration is highly involved in folate biosynthesis; therefore, we hypothesized that increased photorespiration induced by higher temperature would enhance folate biosynthesis. Our experiment

successfully demonstrated greater folate contents at higher temperature (25°C) compared to the optimal temperature (20°C) for naturally growing lettuce.

Folate contents were the highest under the P200/RGB condition at all of the 3 temperatures; especially the condition with P200/RGB at 25°C successfully demonstrated more than 2-fold greater folate contents compared to the other temperatures and 1.8-fold compared to the P150/RB at the same temperature (Figure 5). These results indicate that not only temperature-dependent photorespiration but also PPFD plays an important role for folate biosynthesis, which is consistent with the previous reports.

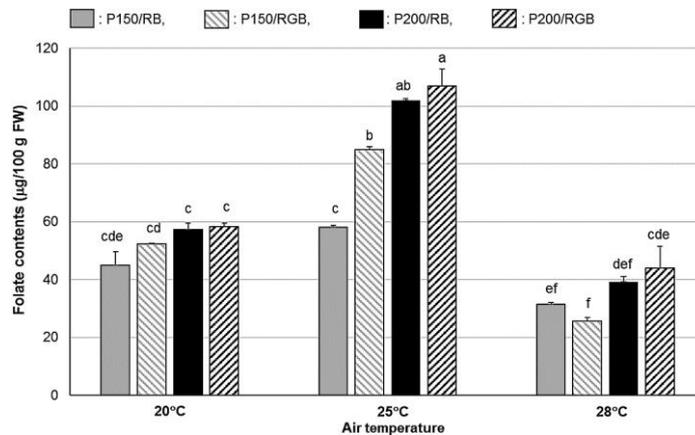


Figure 5: Effects of different air temperature and light irradiation conditions on folate contents. Leaf lettuce was cultivated in the growth cabinet for 21 days under 3 different air temperature conditions (20°C, 25°C and

28°C) and 4 different light conditions (P150 / RB, P150 / RGB, P200 / RB and P200 / RGB). The data are shown as group mean \pm standard error. a to f: Different alphabets indicate significant differences ($p \leq 0.05$) based on multiple comparison test. $n=3$.

Addition of green light to red and blue lights increased folate contents (Figure 5); however, the degree of increased folate contents was various depending on air temperature and light intensity. The folate contents with P200/RGB were numerically slightly greater than those with P200/RB; however, there was no statistically significant difference between RGB and RB, indicating that the addition of green light has an only modest effect on folate biosynthesis under P200 condition. In contrast, under the P150 condition, the RGB demonstrated significantly higher folate contents compared to the RB at 20°C and 25°C, indicating a large impact of green light on folate biosynthesis. Increased folate contents may be due to green light-induced increase in photorespiration

mediated with supplemental light-harvesting action and xanthophyll cycle of carotenoid.

Correlation between Edible Portion Weight and Folate Contents

The regression analysis revealed strong correlation between folate contents and edible portion weight (Figure 6). The combination condition at 25°C and P200/RGB demonstrated the highest values for both edible portion weight and folate contents. There was no correlation between folate contents and the other growth endpoints including plant height, number of leaves and chlorophyll contents.

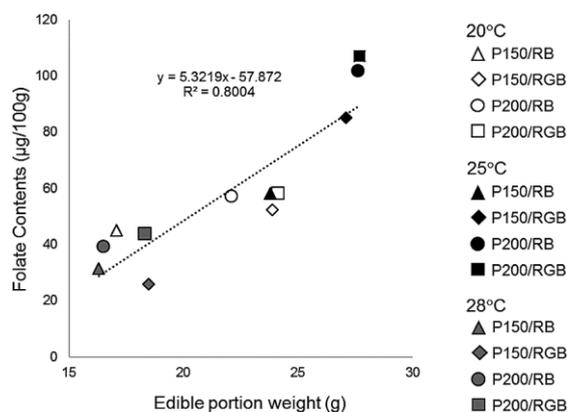


Figure 6: Correlation between edible portion weight and folate contents. Leaf lettuce was cultivated in the growth cabinet for 21 days under 3 different air temperature conditions (20°C, 25°C and 28°C) and 4 different light conditions (P150/RB, P150/RGB, P200/RB and P200/RGB). Each dot represents mean values of edible portion weight and folate content from each different combination condition.

Scott et al. expected that folate contents would be increased when the weight gain of lettuce was enhanced based on the mechanism of folate biosynthesis, which was consistent with our results. Although our results were based on the data after the 21-day cultivation, a longer-term trial would provide further confirmatory data to support strong correlation based on the logistic growth curve.

Compared to red and blue lights, green light had been considered inefficient for photosynthesis in green leaves. Several studies, however, indicated that green leaves of land plants absorb a substantial fraction of green light, and that green light, once absorbed by the leaves, drives photosynthesis with high efficiency.

Higher plants utilize chlorophylls a and b and variety of carotenoids to capture light for photosynthesis. Blue and red lights with high absorption rate (90%) have a large impact on plant growth and physiology. For instance, blue light inhibits hypocotyl elongation of dark-grown *Sinapis alba* seedlings to induce biomass production while red light enhances hypocotyl elongation and extension. Green light with absorption rate of about 70%-80% also has an important

role on plant morphology and physiology, including leaf growth, stomatal conductance and early stem elongation. Kitazaki et al. reported that the energy transmitted by green light was effective in creating a balance between biomass production and the production of secondary metabolites involved in plant defense in leaf lettuce grown under different light quality and intensity conditions using narrow-band LEDs. In addition, Kim et al. reported that addition of green light to red and blue LEDs enhanced lettuce growth. Since plants reflect green light, many researchers believed that lettuce plants would have grown abnormally; however, Johkan et al. confirmed that high-intensity green LED light (short-wave length in particular) was effective to promote plant growth. Consistent with the above-mentioned previous reports, our experiment also demonstrated that addition of green light to red and blue LEDs maximized leaf lettuce growth and folate biosynthesis. Plant factory with optimal temperature and light irradiation conditions utilizing RGB independent dimming type LED lights is a promising novel agriculture concept maximizing plant growth and critical nutrients such as folate.

Conclusion

Our experiment results demonstrated significant effects of air temperature and light irradiation conditions on growth and folate biosynthesis in leaf lettuce. We identified the optimal combination condition, 25°C and P200/RGB, significantly promoting growth and folate biosynthesis in leaf lettuce. The edible portion weight and folate contents in leaf lettuce cultivated under our optimal combination were 15%-62% and 83%-137% greater, respectively, compared to any light irradiation conditions at 20°C, an optimal temperature for conventionally growing leaf lettuce. Addition of green LED to red and blue LEDs maximized leaf lettuce growth and folate biosynthesis. Plant factory with optimal temperature and light irradiation conditions utilizing RGB independent dimming type LED lights is a promising novel agriculture concept maximizing plant growth and critical nutrients such as folate.

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