Journal of Food Measurement and Characterization (2022) 16:269–280
https://doi.org/10.1007/s11694-021-01150-y

ORIGINAL PAPER

External green light as a new tool to change colors and nutritional components of inner leaves of head cabbages

Yumiko Amagai[1,2] · Na Lu[2] · Eri Hayashi[1,3] · Michiko Takagaki[2] · Masao Kikuchi[2] · Yasuomi Ibaraki[4] · Toyoki Kozai[1]

Received: 2 June 2021 / Accepted: 3 September 2021 / Published online: 13 September 2021

Abstract

The color and nutritional quality of vegetables directly affect the choices of consumers and thus affect the commercial value of the vegetable products. Green light can penetrate the outer leaves and reach the inner leaves to promote photochemical reaction of the overlapping leaves of head vegetables. However, whether this promotion can increase the nutritional components and change the color of the inner leaves of head cabbages, which is one of the major head vegetables largely produced worldwide, remains unclear. Therefore, we investigated the changes in the colors and the concentrations of chlorophyll (Chl) and carotenoid of the inner leaves of two types of cabbages by externally irradiating the cabbage with green light. The results showed that a short-term (48 h) irradiation with low light intensity (50 µmol m^{-2} s^{-1}) of green light enhanced the Chl concentration and colors of the inner leaves of cabbages, and the positive changes of these indicators increased as the leaf layers approached the head center of the cabbage. Simultaneously, we also establish a method to effectively estimate the Chl concentration using luminosity (L^*) and greenness ($-a^*$) when the Chl concentration is so low that it is difficult or not possible to be measured by SPAD meter. Our findings demonstrated that green light, as a new tool, can be used to control the colors and nutritional components of the inner leaves of cabbages. The discoveries will help produce head vegetables with the preferred phenotype desired by consumers using a plant factory with artificial lighting.

Keywords Head vegetables · Green LED · Cabbage · Low SPAD · CIE $L^*a^*b^*$ color space

Introduction

Characteristics of head vegetables

The outermost leaves of head vegetables (such as cabbage, Chinese cabbage, and head lettuce) are usually green and the leaves closer to the head center are more yellowish or whitish. In principle, the tones and intensities of green, yellow, and white color of the vegetables have many impacts on the various usages of vegetables.

The color of vegetables and fruits represents nutritional value

Regarding vegetables and fruits, consumers first evaluate the visual appearance, then the flavor (taste and aroma), texture, and nutritional value [1]. When consumers buy vegetables in Japan, the color and size are more important than the shape and presence or absence of scratches [2]. In particular, the color is a criterion for judging not only deliciousness and freshness but also nutritional components [3–5]. For example, chlorophyll (Chl) concentration, which reflects leaf greenness, has been reported to have anticancer and anticholesterolemic effects as well as a preventive effect on lifestyle-related diseases owing to its antioxidant properties [6, 7]. Carotenoid (Crt) concentration, which naturally exhibit red, orange and yellow colors in plants [8], has been reported to reduce the incidence of certain cancers in humans [9]. About 60% of vitamin A is considered to come from carotenoids

Yumiko Amagai and Na Lu: share first authorship.

✉ Yumiko Amagai
amagai@npoplantfactory.org

1 Japan Plant Factory Association, Kashiwa, Chiba, Japan

2 Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Chiba, Japan

3 Graduate School of Horticulture, Chiba University, Matsudo, Chiba, Japan

4 Faculty of Agriculture, Yamaguchi University, Yoshida, Yamaguchi, Japan

[10], and it is a crucial nutrient for healing tissues and regenerating human epithelial tissues such as skin [11]. Therefore, improving the color of vegetables is an important means of increasing the commercial value of vegetables and fruits.

Light environment control in the plant factory with artificial lighting

Plant factory with artificial lighting (PFAL) is a closed plant production system with a nearly airtight and well-insulated structure, which allows increased resource utilization efficiency under a precisely controlled environment, aiming to produce high quantities of high-quality plants throughout the year, entirely independent of outdoor conditions [12–14]. Particularly the advances in lighting technology, including light-emitting diodes (LEDs) and others, could increase controllability of the light environment of plants, thereby improved the yield and functional components of vegetables and fruits [15–18].

Head vegetables, especially cabbage, are in great demand all over the world

Many PFALs have so far focused on growing non-head leafy vegetables, such as leaf lettuce. The main reasons are that non-head leafy vegetables have a short cultivation period, fast cultivation rotation, and high yield per unit time while densely planted in a limited space of PFAL. However, most demand in the lettuce market is for head lettuce that is usually grown outdoors. This occurs because it is easy to mass produce, easy to pack for transportation, and a high salad volume can be made from a small head [19]. The production of head lettuce is 3.3 times higher than that for leaf lettuce, 1.5 times higher than that for romaine lettuce in the United States [20], and 75 times higher than that for general non-head lettuce in Japan [21].

Indeed, it is cabbage that is produced on a larger scale and in greater demand worldwide than head lettuce (fifth in the leafy vegetables category). The production of cabbage is increasing year by year, mainly in China and developing countries [22]. Therefore, in this study, we used cabbage, instead of head lettuce, among the head vegetables with overlapping leaves for the following reasons: (1) cabbage can be more effectively measured because it has less unevenness of leaves, and (2) cabbage leaves are more in close contact with each other, compared with head lettuce.

Green light penetrates the outer leaves and may increase the photosynthetic rate of the inner layer leaves

Green light is considered to have a lower absorption rate by chlorophylls, compared with blue and red lights. However, it can be more effective in producing high density plants due to its higher penetration rate [23–25]. Sun et al. [26] showed that green light drives $^{14}CO_2$ fixation deeper within spinach leaves compared with red and blue light. Terashima et al. [27] revealed that green light with high light intensities may reach the underside of the leaf and enhance the photosynthetic rate of chloroplasts that have not yet achieved light saturation. Kim et al. [28] reported that the growth of non-head lettuce was promoted by adding green light to blue and red light. Saengtharatip et al. [29] showed that the ascorbic acid and Chl concentrations of the inner leaves of head lettuce were increased by the irradiation of a strong green light (400 μmol m^{-2} s^{-1}) compared with blue and red lights. Thus, the importance of green light in photosynthesis and photochemical reaction has been reevaluated. To the best of our knowledge, however, no study has addressed the effects of green light irradiation on the color and nutritional components of overlapping leaves of post-harvest cabbages.

Charles et al. [30] underlined the potential of using the cool white fluorescent lamps with a PPFD of 50 μmol m^{-2} s^{-1} for a short time treatment (2 days) to maintain the quality of fresh-cut products. Using a low light intensity for a short-term treatment is not only cost-effective, but also easy to apply during the PFALs production line and storage period after harvest. Therefore, one of the objectives of this paper is to investigate whether irradiation with a low light intensity (50 μmol m^{-2} s^{-1}) of green light for a short term (up to 48 h) could enhance the color and increase the nutritional component of the inner leaves of cabbages.

SPAD meter problems and solutions

The leaf color is affected by the Chl and the Chl concentration has a high correlation with value measured by the Soil Plant Analysis Development (SPAD) meter [31]. The SPAD meter can estimate the Chl concentration in a leaf nondestructively. However, the SPAD value of the pale green leaves in the inner of cabbage is unmeasurable regardless of leaf thickness or unevenness [32], and there have been no reports about elucidated solutions to this point. The $L^*a^*b^*$ color space is a common way to quantify leaf color, which can describe all colors visible to the human eyes in three coordinates [33]. To date, the $L^*a^*b^*$ system has been employed for the examination of cabbage freshness and minor color changes in wine and tomato [34–36]. It was reported for wheat and pepper leaves that the $L^*a^*b^*$ system has a significant relationship with the chlorophyll content [37, 38]. These studies compared the measurements obtained by SPAD meter and those obtained by colorimeter to examine how the Chl concentration was correlated with the parameters of the $L^*a^*b^*$ system. A common problem in these studies is that the leaves they examined, pepper, wheat, tobacco, and grape leaves, were all dark green, with

relatively high Chl concentration. The Chl concentration of head cabbage leaves, particularly the inner ones, could be too low to be measured by a SPAD meter. Therefore, in the present study, in addition to using the SPAD meter to estimate Chl concentration, we were also prepared to measure the Chl concentration of leaves by a destructive method, anticipating that there should be an alternative way to measure the leaf Chl concentration by measuring the *L*a*b** for cabbage leaves, especially for the inner leaves, for which a SPAD meter might fail to measure. The development of non-destructive, easy-to-apply method to measure Chl concentration would help plant factory operators to improve the quality of their vegetable products in a great way.

Objectives and approaches

The present study aimed to elucidate the effects of short-term weak green light irradiation on colors and nutritional components (Chl and Crt) of inner leaves of cabbages. Specifically, we (1) investigated in detail the effect of green light irradiation on colors (*L*a*b**), SPAD, and Chl and Crt concentrations at each leaf layer; (2) identified how the leaf color was related to the concentrations of Chl and Crt of leaves; and (3) proposed an alternative way to measure leaf Chl in case its concentration was too low to be measured by SPAD.

Materials and methods

Materials and environment

Plant materials

We used two types of cabbage (*Brassica oleracea* L. var *capitata*) with different colors of inner leaves, yellow (cv. Miharu, grown in spring season) and white (cv. Okina, grown in summer season). The yellow cabbages (YE) were purchased from a supermarket (Kashiwa city, Chiba, Japan) on April 6, 2020. The white cabbages (WT) were from those grown at the experimental fields at the Chiba University (Kashiwa city, Chiba, Japan) in a conventional agricultural system. The white cabbages were harvested on July 2, 2020. These cabbages were of the well-controlled standard quality in the Japanese market, with clear traceability.

For the experiment, four leaf discs (50 mm) that do not contain thick veins were punched from each of the 15 outside leaves (L1 to L15) of WT and YE, respectively. Leaves were numbered from 1 (L1), the outermost leaf of head consecutively to 15 (L15), the most inner leaf (Fig. 1). The leaf sets of L1–L15 were consecutively stacked together (with L1 on the top) and wrapped in aluminum foil to prevent additional light from entering the sides. To prevent the leaves from drying out during the observation period of 48 h, each leaf set was placed in a petri dish filled with filter paper soaked with 5 mL of distilled water; thereafter, the petri dishes were covered with a cling film (Fig. 2a, b).

Environmental conditions and treatments

The experiment was conducted in a small walk-in PFAL (floor area, 5.9 m^2, height, 2.3 m) with high airtightness and high heat insulation capable of air conditioning and CO_2 concentration control.

Green light (peak: 525 ± 16.6 nm) from LED lamps (ISL-150X150-series, CCS Inc., Burlington, MA, USA) was used for the treatments (Fig. 2b). The upper surface of the leaf set and the surface of a photosynthetic photon flux densities (PPFD) sensor (LI-190R, LI-COR) were adjusted to the same height (40 mm from the floor), and the light intensity was adjusted to 50 µmol m^{-2} s^{-1} at that surface. The air temperature and CO_2 concentration around the leaf set were kept at 20 °C and 1500 ppm respectively. The leaves of WT and YE were irradiated by green light for 0 h (control), 24 h, and 48 h, respectively.

Fig. 1 Schemes of the yellow cabbage (YE) and white cabbage (WT) with the annotation of leaf numbers taken for nutritional components and color analyses. We took 15 leaf discs (L1–L15) from a sample cabbage

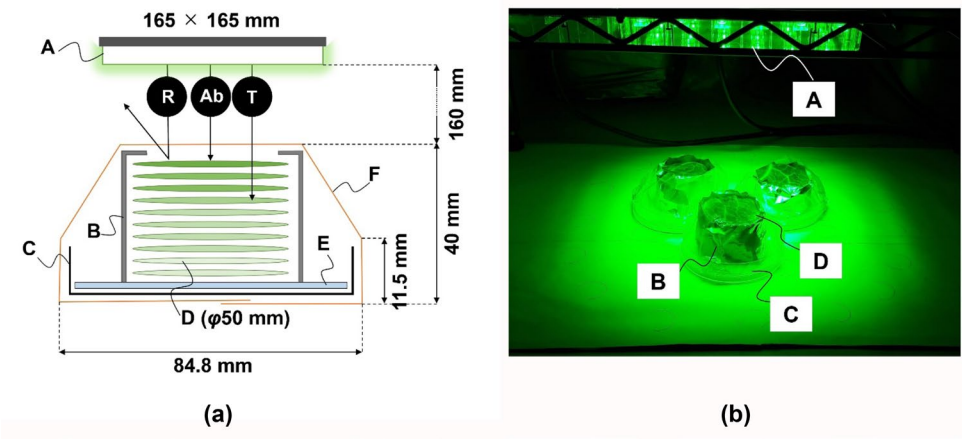

Fig. 2 The schematic diagram (**a**) and photo (**b**) showing the set-up of green LEDs (A), aluminum foil (B) for covering the leaf sets (15 leaf discs), Petri dish (C), leaf discs (D), wet paper (E), and cling film (F). The stacked leaf sets were exposed to the green light with PPFD of 50 µmol m^{-2} s^{-1}. Arrows indicate the path of light reflected (R), absorbed (Ab), and transmitted (T)

Measurement

Colors

The leaf discs were placed on a black top plate and the center of the leaf pieces was measured one time. Colors were obtained using CIE1976 $L^*a^*b^*$ color system according to CIE [33].

Three variables—L^*, a^*, and b^*—were measured using the colorimeter (CR-20, Konica Minolta, Inc.). In this meter, the standard deviation when the white calibration plate was measured 30 times at 10-s intervals was within ΔE^*ab of 0.1, according to the instruction manual, and ± 0.05 according to the measurer of this experiment. The CIE 1976 $L^*a^*b^*$ color space was adopted to quantify the minute differences in color of inner leaves of the cabbage for the experiments. L^*, a vector representing luminosity, is orthogonal to the a^*–b^* plane to make a color space and a^* and b^* are two axes in a Cartesian coordinate, the former being the red-green axis, and the latter being the yellow-blue axis (Fig. 3). Since we are interested in the greenness of cabbage leaves, we use, throughout this paper, the ($-a^*$) axis, the green–red axis, which is inverted from the a^* axis by multiplying (-1).

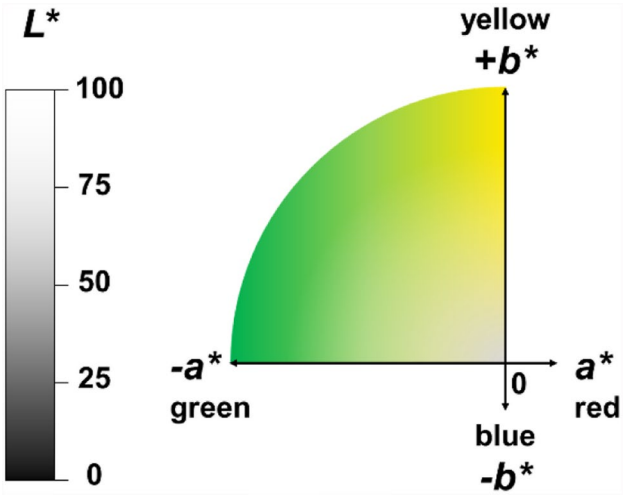

Fig. 3 The CIE 1976 $L^*a^*b^*$ color system

SPAD

SPAD was recorded using a chlorophyll meter (SPAD-502Plus, Konica Minolta, Inc., Tokyo, Japan). The mean value of 5 SPAD readings obtained around the center of leaf disc was determined. The repeatability of the chlorophyll meter was ± 0.3 SPAD according to the manufacturer's instruction and ± 0.25 SPAD according to the measurer of this experiment.

Chlorophyll and carotenoid concentrations

Two smaller leaf discs (12 mm) were sampled from each larger leaf disc (50 mm) and measured. The protocol described by Porra et al. [39] was used for Chl concentration measurements. According to the protocol, *N*,*N*-dimethylformamide was used, and the absorbance was obtained at wavelengths of 645, 663, and 480 nm using a spectrophotometer (SH-1300Lab, Corona Electric Co., Ltd., Ibaraki, Japan) for the measurement of Chl a, Chl b, and Crt concentrations.

Leaf thickness

Leaf thickness was measured for the lamina from the margin to the center of the leaf disc using a digital caliper (DT200, Niigata Seiki Co., Ltd. Niigata, Japan) before treatment.

PPFD

For adjustment of the lighting environments, PPFDs were measured using a photon flux density (quantum) sensor (LI-COR Li-190SA, LI-COR, Inc., Lincoln, NE, USA) with a light meter (LI-250A, LI-COR, Inc.). The accuracy of the

photon flux density sensor and light meter was ± 5% and ± 0.4%, respectively (room temperature, 25 °C).

Measurement timing, repetition and numbers of observations by treatment

The color parameters and SPAD of the sampled leaves were measured, nondestructively, at 0, 24, and 48 h after treatment, and the Chl and Crt concentrations were measured, destructively, at 0 and 48 h after treatment (Table 1). Under the nondestructive measurement, for each leaf set, five samples (ϕ50 mm disc) were obtained for YE and four samples (ditto) for WT. For both YE and WT, after measuring SPAD and color parameters nondestructively, two small discs (ϕ12 mm) were hollowed out from one of the ϕ50 mm discs as samples used to measure Chl and Crt concentrations destructively. The remaining ϕ50 mm samples, three for YE and two for WT, were used for the measurements at 24 and 48 h after treatment. Of the fifteen layers of leaves sampled, L15 was excluded from the statistical analyses, because its conditions, as being facing the floor (Fig. 2a), were different from the other leaves.

Statistical analyses

The results of the experiments were first presented as bar-charts with standard-error bars. When two means were subject to the t-test, we adopted $p < 0.05$ as the critical level. The multiple regression analysis was adopted first to ascertain if the irradiation of green light gave significant effects on the two target nutritional components, i.e., Chl and Crt concentrations in the leaves, and second to identify how Chl and Crt concentrations affected the luminosity and colors of cabbage leaves. The former problem, whether the irradiation gave significant effects on Chl and Crt concentrations in leaves, can be answered by adopting the variance analysis. We adopted the regression analysis, since we were interested in knowing the magnitudes of the effects. In the regression analyses conducted, the probability to accept the null hypothesis that the regression coefficient was nil was presented for each coefficient estimated.

Table 1 Experimental factors that affected the concentrations of chlorophyll (Chl) and carotenoid (Crt) of cabbage leaves: the results of regression analyses regressing Chl and Crt, both in logarithm, on factors in the experiment (N = 48)[a]

Explanatory variables[b]	Dependent variable			
	Chlorophyll [Ln (Chl)]		Carotenoid [Ln (Crt)]	
	Regression coefficient	Prob.[c]	Regression coefficient	Prob.[c]
Green light irradiation dummy				
After 48 h	0.702	**4.6×10^{-5}**	0.091	0.494
Whiter cabbage (WT) dummy	− 0.702	**4.6×10^{-5}**	− 2.068	**3.8×10^{-19}**
Cross term				
(After 48 h) × (WT)	− 0.275	0.215	− 0.360	0.061
Leaf position dummy				
Middle (6th–10th)	− 0.714	**6.1×10^{-6}**	− 0.171	0.154
Deep inner (11th–14th)	− 1.066	**4.2×10^{-9}**	− 0.371	**0.004**
Intercept	− 2.998	**1.1×10^{-23}**	− 4.253	**2.1×10^{-32}**
R^2	0.773		0.935	

The probability of $p < 0.05$ is written in bold letter

[a]The data used for the regression analyses are obtained by (1) pooling the YE and WT data used for measuring nutritional components (Chl and Crt) by applying a destructive method, (2) averaging over the two replications, and 3) excluding the data on the two surface leaves. The total number of observations is N = 48 (2 × 2 × 12)

[b]The experiment conducted is represented by the following dummy variables: (1) Green light irradiation dummy for 'after 48 h' takes 1 if the observation is of 'after 48 h' and 0 if it is of 0 h (before irradiation). (2) White cabbage (WT) dummy takes 1 if the observation is of WT and 0 if the observation is of yellow cabbage (YE). (3) Cross term dummy takes 1 if the observation is of 'after 48 h' and WT and 0 otherwise. (4) Leaf position dummies: (i) Middle position dummy takes 1 if the leaf is from 6th (L6) to 10th (L10) and 0 otherwise, (ii) Deep inner dummy takes 1 if the leaf is from 11th (L11) to 14th (L14).

[c]The probability that the null hypothesis that the estimated regression coefficient is nil is accepted

Results

Changes in colors and nutritional components for each leaf position of cabbage before and after green light irradiation treatment

Figure 4a shows the colors, nutritional components, and leaf thickness of the two types of cabbages (YE and WT) before the green light treatment. The levels of SPAD, Chl and Crt concentrations, $-a^*$ (greenness), and b^* (yellowness) tended to increase towards the outer leaves, although YE's $-a^*$ and b^* were lower for the outermost two leaves, L1 and L2, than for L3, and YE's b^* was nearly constant after L3. L^* (luminosity) increased from L1 to L6 but remained nearly constant thereafter. YE had distinctly higher SPAD and Chl concentration than WT, while WT was characterized by distinctly lower b^* and Crt concentration than YE. The WT's leaf thickness was higher than YE's, and WT had leaves that tended to be thicker toward the inside. For both YE and WT, SPAD and Chl concentration shared a very similar downward trend from L1 to L7. It should be noted, however, that the SPAD meter failed to give any measured value for the inner leaves beyond L8 for both YE and WT, whereas the measurement by a destructive method revealed positive, though low, levels of Chl concentration for all the leaves at these intermediate and deep positions.

Figure 4b shows the changes in colors and nutritional components for the two types of cabbages (YE and WT) after treatment with green light. As for SPAD, as seen in Fig. 4a, the measurement of SPAD failed for the inner leaves beyond L8, for which no change was shown in this figure. At a glance, it is clear that WT showed smaller changes than YE, so let us observe changes for YE first. The irradiation of greenlight had an effect to decrease L^*: the inner leaves decreased more, and the degree of decrease increased from 24 to 48 h. The irradiation, conversely, induced increases in $-a^*$ and b^*: the rate of increase was greater for the inner leaves, and the longer the irradiation time, the greater the positive change tended to be. For these three color-related variables, the change from 0 to 24 h or 48 h was statistically significant for many leaves. In the case of Chl concentration, the change in L3 and the inner leaves was an increase, and the degree of increase tended to be larger for more inner leaves, showing a statistically significant increase in L11 and L14. The trend of change in SPAD from L1 to L7 was similar to that in Chl concentration, but no significant change was found for SPAD.

Next, looking at WT, it was observed that L^*, $-a^*$, and b^* tended to show almost the same trend as for YE, though with much smaller degrees of change. There were many cases in which the general movements were reversed, but none of these changes in the opposite direction were statistically significant. The patterns of change in Chl concentration and SPAD were similar to those for YE, but the tendency for Chl concentration to increase was not seen in the inner leaves beyond L10. The change in Crt concentration was also small, and no systematic trend was observed.

Figure 5 shows the images of the leaf discs of cabbages (YE and WT) obtained before (0 h) and after (48 h) green light treatment. YE showed higher greening at 48 h in stronger yellowish central leaf discs. Conversely, WT was pale green and almost white at 0 h and a little darker green at 48 h.

Statistical confirmation of the effects of greenlight irradiation on leaf nutritional components and relationship between leaf nutritional components and leaf colors

Our interest in this paper was to know if green light irradiation could change the color of post-harvest head cabbage leaves in favorable directions, and if so, what nutritional components in the leaves contributed to the changes. Figure 4a and b revealed that the irradiation tended to increase the greenness and yellowness of leaves, particularly for those in the middle and closer to the center of the cabbages. It was also shown in these figures that the irradiation appeared to increase the concentrations of Chl and Crt in the leaves of middle and deep inner positions. The small sample number due to the destructive measuring of Chl and Crt concentrations, however, the statistical supports for these were not sufficient. We expected that the use of the SPAD meter, a non-destructive method of measuring Chl concentration in leaves, was amenable to produce as many samples as we need. However, as shown in Fig. 4a, the SPAD meter failed to give SPAD estimates for inner leaves below L8 for both YE and WT. In fact, we found that the SPAD meter had difficulties supplying reliable SPAD estimates for leaves with very low concentration of Chl (typically if SPAD < 10 or Chl < 0.05 g kg^{-1} in the current case). This was a perplexing situation because we were interested in changing the color of inner leaves, the color of which was very light green or even whitish, with very low concentration of Chl. Here, we try to find the relationship between nutritional components and the color parameters, using the data obtained by destructive method with a smaller number of observations.

First, we confirm statistically if the findings in the previous subsection that the green light irradiation appeared to increase the concentrations of Chl and Crt in the inner leaves. For this purpose, we estimated two regression equations, in which Chl concentration in the first regression and Crt concentration in the second regression, both in logarithms, were regressed, respectively, to the dummy variables

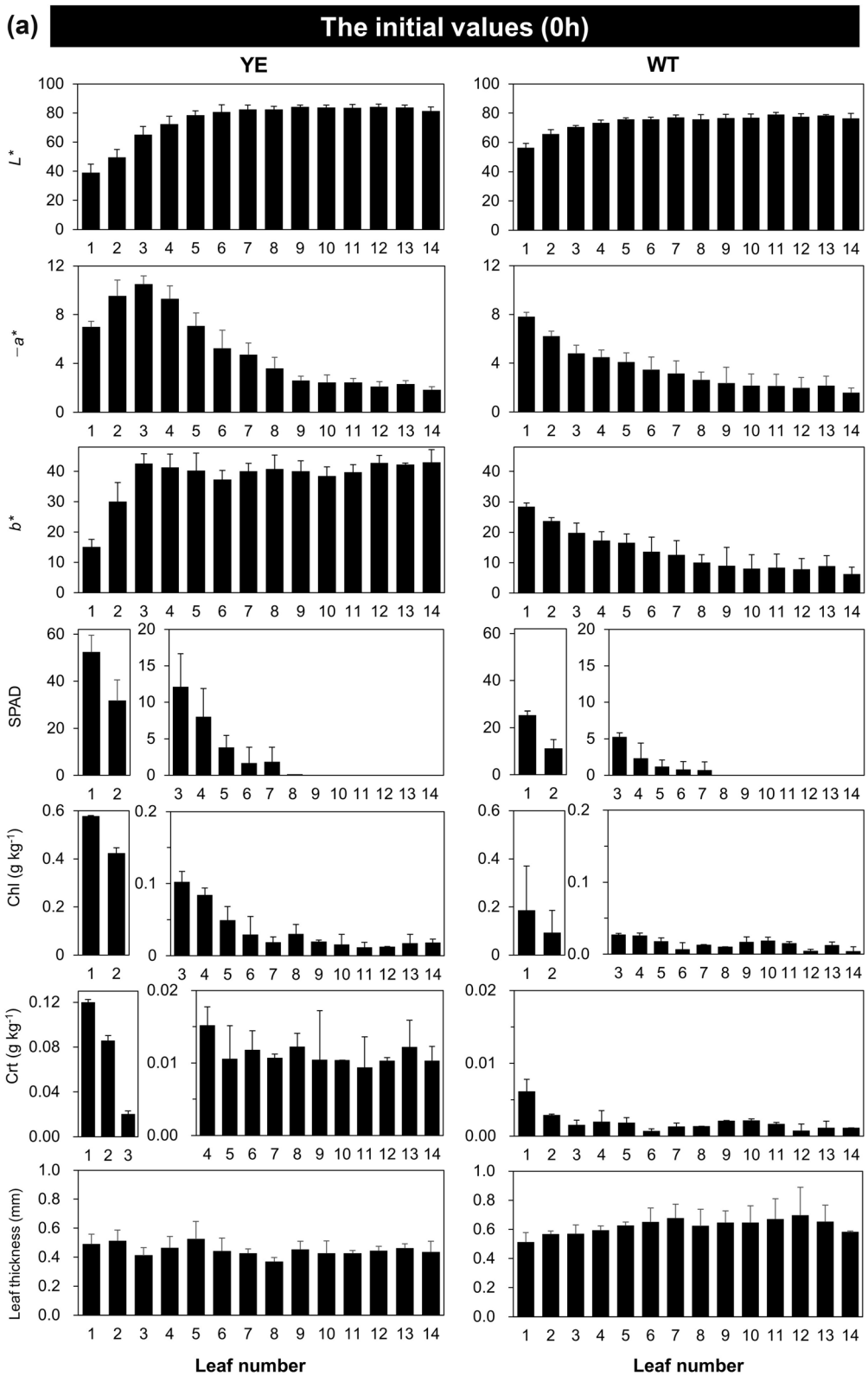

Fig. 4 The initial values of L^*, $-a^*$, b^*, SPAD, chlorophyll (Chl) concentration, carotenoid (Crt) concentration, and leaf thickness at 0 h (4a) and the changes of the values at 24 h and 48 h compared with 0 h (4b) for the 1st–14th leaves in yellow cabbage (YE) and white cabbage (WT). The initial value at 0 h is shown as black bars, the changes at 24 h as blue bars, and the changes at 48 h as pink bars. Chl and Crt concentrations were measured only at 0 h and 48 h. Error bars represent standard deviations (n = 2 to 3). Bars labeled "*" indicate that the values at 24 h or 48 h were significantly different from the initial values (0 h) (Student's *t* test, $P < 0.05$)

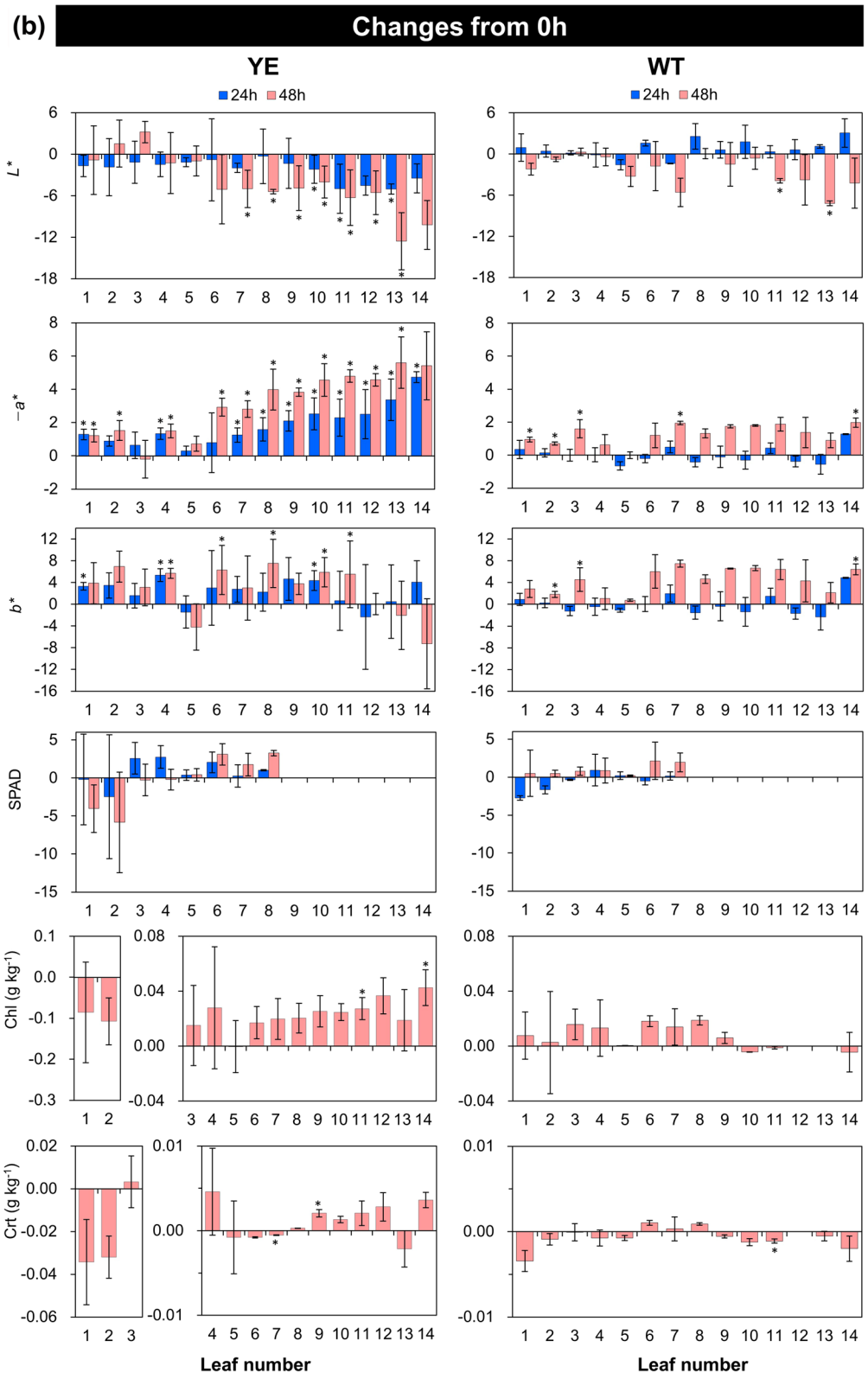

Fig. 4 (continued)

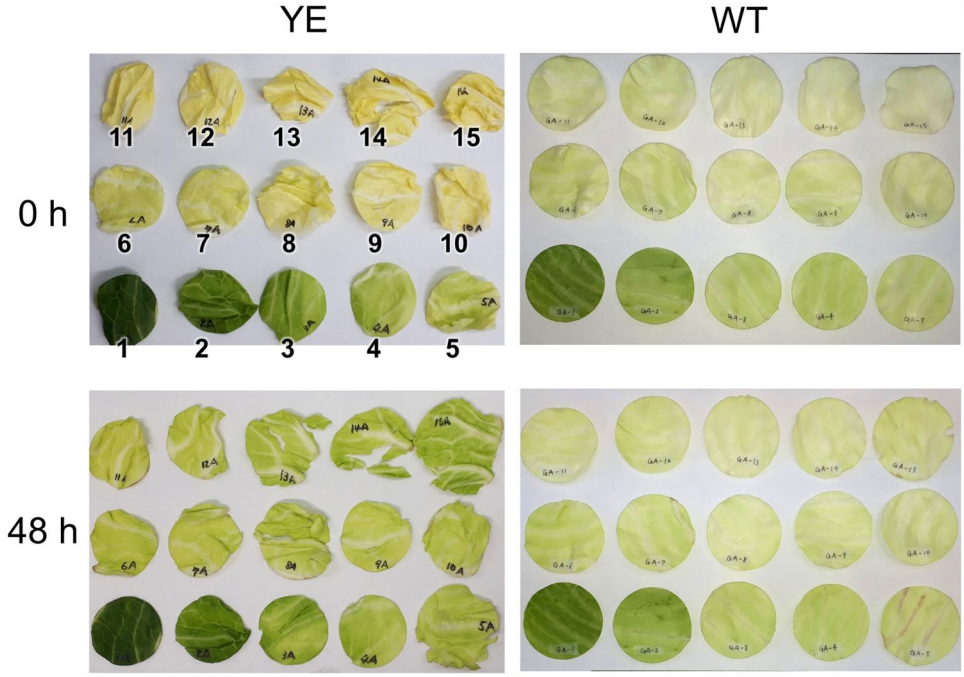

Fig. 5 Colors of leaf discs by type of cabbage and the timing of sampling. The discs are arranged in order, from the surface leaf (L1) to the innermost leaf (L15)

Table 2 The relationships between the chlorophyll (Chl) and carotenoid (Crt) concentrations of cabbage leaves and their luminosity (L^*), greenness ($-a^*$) and yellowness (b^*): the results of regression analyses regressing L^*, $-a^*$, and b^*, respectively, on Chl and Crt, both in logarithm (N = 48)[a]

Explanatory variables	Dependent variable					
	Luminosity (L^*)		Greenness ($-a^*$)		Yellowness (b^*)	
	Regression coefficient	Prob.[b]	Regression coefficient	Prob.[b]	Regression coefficient	Prob.[b]
Ln (Chl)	− 6.133	$\mathbf{8.1\times10^{-11}}$	3.308	$\mathbf{3.4\times10^{-12}}$	1.609	0.358
Ln (Crt)	4.324	$\mathbf{3.1\times10^{-12}}$	− 0.143	0.522	11.151	$\mathbf{2.6\times10^{-13}}$
Intercept	78.007	$\mathbf{2.3\times10^{-35}}$	16.389	$\mathbf{2.7\times10^{-20}}$	95.291	$\mathbf{3.9\times10^{-23}}$
R^2	0.681		0.774		0.834	

The probability of $p < 0.05$ is written in bold letter

[a]See the footnote a of the previous table

[b]The probability that the null hypothesis that the estimated regression coefficient is nil is accepted

that represent the treatments in our experiment. It should be noted that the first and second leaves of the sample cabbages, which were distinctly in dark green (Fig. 5) with distinctly high values for SPAD and concentrations of Chl and Crt, were excluded from the dataset. The results clearly showed that, after controlling the differences in the types of cabbage (YE or WT) and in the leaf position (near surface, middle, and deep inner), the green light irradiation significantly increased the concentration of Chl concentration in the leaves (Table 1). For Crt concentration, on the contrary, the irradiation gave no significant impact on the concentration of Crt in the leaves, the variation in Ln (Crt) being explained largely by the difference between YE and WT.

With the positive effect of the green light irradiation on Chl concentration confirmed, we examine the relationship between the nutritional components and the color parameters, by regressing L^*, $-a^*$, and b^*, respectively, on Chl and Crt concentrations, both in logarithms. The results are clear (Table 2). The luminosity of leaves decreased as leaf Chl concentration increased but increased as leaf Crt concentration increased, the relationships of which were statistically highly significant. The greenness of leaves increased significantly as Chl concentration increased but was not affected by Crt concentration, whereas the yellowness of leaves increased significantly as Crt concentration increased but was not affected by Chl concentration. For all these significant relationships, the significance levels were very high.

Discussion

Green light can easily penetrate to the middle layer of the plant community [24], and irradiation with a high light intensity of green light (400 µmol m^{-2} s^{-1}) could increase

the Chl concentration in the inner leaves of head lettuce due to its higher light transmission than red and blue light [29]. In the present study, the inner yellow or white leaves of cabbages had higher Chl concentration and darker green color after irradiation by green light and the changes in Chl concentration and color were more significant in the leaf layers closer to the head center (Figs. 4b, 5), even though we used much lower light intensity (50 μmol m^{-2} s^{-1}) and shorter time for treatments. These results demonstrated that green light can be used as a tool to change colors and nutritional components of inner leaves of head cabbages, effectively, even with a low light intensity and a short treatment time.

Changes in Chl concentration, and Crt concentration at L9–L13 in WT were smaller compared with YE. For leaf thickness of the L1–L14, the average of YE was 0.45 ± 0.07, but the average of WT was significantly thicker at 0.62 ± 0.09 ($p \leq 0.01$) (Fig. 4a). The small change in colors and nutritional components of WT cabbage was possibly caused by the thickness of the leaves reducing the light transmission and suppressing the effects of green light.

The colors of leaves, measured by the $L^*a^*b^*$ color system, were well explained by the concentrations of Chl and Crt in the leaves: L^*, the luminosity, was determined negatively by Chl concentration and positively by Crt concentration, $-a^*$, the greenness, positively by Chl concentration, and b^*, the yellowness, positively by Crt concentration. These results are consistent with previous studies that the $L^*a^*b^*$ color system have a significant correlation with chlorophyll and carotenoid contents [37, 40]. Since the green light irradiation significantly increases leaf Chl concentration, the irradiation is a legitimate method to be adopted in an attempt to improve the greenness of leaves of post-harvest head cabbages.

Yellow and white leaf discs (Fig. 5, L8–L15 at 0 h), despite Chl of > 0, were difficult to measure their SPAD (our SPAD meter failed to give SPAD measures for 56% of sample leaves for which the concentration of Chl was less than 0.05 g kg^{-1}, and for 93% if Chl < 0.01.). All samples in the present study had the thickness of 1.0–1.2 mm (Fig. 4a) that are within the specified range by the SPAD meter instruction manual (Konica Minolta, Inc., Tokyo, Japan), and the manual states that "measurement is possible within the range of −9.9 to 199.9 SPAD units for wheat, rice, corn, cotton, and others". The manual does not specify if "others" include cabbage. If it were to be included, it is necessary to improve the accuracy of the instrument to be able to read the SPAD values for samples with low concentration of Chl (≤ 0.05 g kg^{-1}). On the other hand, the present study found highly statistically significant relationships between the nutritional components and the parameters in the $L^*a^*b^*$ color system. It was confirmed that Chl concentration had a significant relationship with L^* and with $-a^*$. This means that the L^* and $-a^*$ equations in Table 2 could be used as a tool to estimate the concentration of Chl of a leaf, without resorting to the destructive measuring method. If we have data on L^* or $-a^*$ of leaves, we can estimate the Chl concentration of the leaves by inserting these data into the appropriate equation and solving it for Chl concentration. The use of the colorimeter, a non-destructive method, to measure L^* or $-a^*$, therefore, a feasible method to estimate the Chl concentration of those leaves containing low chlorophylls.

Except for the Chl concentration in WT, the SPAD and Chl concentration of the cabbages tended to decrease only on the first and second outer leaves (Fig. 4b) after green light irradiation. One of the factors related to this can be the leaf senescence. Before harvesting, since light barely reaches the inner leaves of cabbage, the growth of the inner leaves mainly rely on the outer leaves, which intercept light for photosynthesis and supply photosynthate to the inner leaves [41]. However, after harvest, the metabolism typically switches from vegetative growth to aging process, leading to a rapid degradation of biopolymers such as chlorophyll (chlorophyll loss) [42]. Moreover, it is known that nitrogen utilization in photosynthesis immediately reaches a peak after leaf development is complete and thereafter decreases with leaf aging [43, 44]. Therefore, even though the outer leaves of cabbages were exposed to light after harvest, the Chl concentration was still rapidly degraded as the progress of leaf senescence.

The production of head vegetables in PFALs will be commercialized in the near future. In the case of head vegetables grown in open fields, the very first layer leaves of the head vegetables (leaves further outside the "outer leaves" described in this study) are discarded as plant residue. In the head-vegetable production in PFALs, however, these leaves could be made edible by fine-tuning the harvest time [45]. Furthermore, it is possible to promote the growth of heads by externally irradiating the outer leaves because it could improve the leaf photosynthesis and thus enhance the translocation of photo-assimilates from these outer leaves to inner leaves of the head [46, 47]. These perspectives, coupled with our research results, suggest that it is important, for realizing head-vegetable production in PFALs in the future, to develop an environmental control system that focuses on promoting photosynthesis of the inner leaves directly to enhance head formation.

The typical YE and WT cabbages were used for the experiments in the present study and the samples of YE were purchased from a local market with marketable quality but unknown age. It should be pointed out that the maturity and age of the raw materials may affect the response of samples to irradiation treatments. The present study confirmed the positive effect of greenlight irradiation on the leaf Chl concentration, but not the effect on the Crt concentration. Whether these results can be generalized must be followed

up in the future, for different cabbage varieties, plant ages, directions and timing of light irradiation, and wavelengths and photosynthetic photon flux densities of light. The use of different instruments for measurements and the application of artificial intelligence algorithms for data analysis, should also be considered.

Conclusion

The color of vegetables is an indicator for consumers to determine whether the vegetables are fresh and tasty, and therefore whether they are of economic value. This study showed that the colors of inner yellow and white leaves of post-harvest head cabbages can be altered by increasing the concentration of Chl in the leaves by irradiating the heads with low intensity (50 µmol m^{-2} s^{-1}) green light for a short-term (48 h). This result demonstrated a potential new approach to maintain or improve the quality of the head vegetables during their cultivation and/or postharvest storage in PFAL. The concentration of Chl of yellow to white leaves of cabbage is often too low to be measured with a SPAD meter, which could create difficulty in conducting experiments in this research theme. However, this study established that each of the first two parameters of the $L^*a^*b^*$ color space has a clear regression relationship determined by the Chl concentration in the leaves. Using these regression equations, the low concentration of Chl can be estimated from L^* or a^* measured non-destructively by the colorimeter. These discoveries would also help to produce head vegetables with the preferred phenotype desired by consumers, using PFAL that can effectively control the light environments. More research, such as using different light intensities and different cabbage varieties, with more sophisticated instruments for measurements, artificial intelligence algorithms for data analysis, etc., need to be sought in the future.

Acknowledgements We would like to thank Yoshiko Komaba and Tomomi Nozaki for supporting the experiment, Toshitaka Yamaguchi for his valuable advice, and Hideo Enomoto for providing the cabbages. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions TK and YA conceived and designed the experiments. YA and NL performed the experiments. EH, MT, YI and TK advised on the analytical framework and analysis methods. MK advised and interpreted the statistical analyses. YA, NL, MK and TK prepared the manuscript. All the authors discussed the results and implications and commented on the manuscript.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. D.M. Barrett, J.C. Beaulieu, R. Shewfelt, Crit. Rev. Food Sci. Nutr. (2010). https://doi.org/10.1080/10408391003626322
2. MAFF, Ministry of Agriculture, Forestry and Fisheries. (2019), https://www.maff.go.jp/e/index.html Accessed 5 Feb 2021
3. E.D. Cömert, B.A. Mogol, V. Gökmen, Curr. Res. Food. Sci. (2020). https://doi.org/10.1016/j.crfs.2019.11.001
4. I. Filella, L. Serrano, J. Serra, J. Penuelas, Crop Sci. (1995). https://doi.org/10.2135/cropsci1995.0011183X003500050023x
5. J.A.T. Pennington, R.A. Fisher, J. Food Compost. Anal. (2009). https://doi.org/10.1016/j.jfca.2008.11.012
6. J. De Vogel, D.S. Jonker-Termont, E.M. Van Lieshout, M.B. Katan, R. van der Meer, Carcinogenesis (2005). https://doi.org/10.1093/carcin/bgh331
7. K. Yamashita, Y. Nohara, M. Kumagai, M. Namiki, J. Home Econ. Jpn. (1991). https://doi.org/10.11428/jhej1987.42.589
8. L. Ngamwonglumlert, S. Devahastin, N. Chiewchan, V. Raghavan, Compr. Rev. Food Sci. Food Saf. (2020). https://doi.org/10.1111/1541-4337.12564
9. H. Gerster, Int. J. Vitam. Nutr. Res. **63**, 93–93 (1993)
10. D.B. Rodriguez-Amaya, *John Snow Incorporated/OMNI Project* (Arlington, 1997), pp. 1–93
11. A.M. Kligman, O.H. Mills Jr., J.J. Leyden, P.R. Gross, H.B. Allen, R.I. Rudolph, Int. J. Dermatol. (1981). https://doi.org/10.1111/j.1365-4362.1981.tb04338.x
12. E. Hayashi, Y. Amagai, T. Maruo, T. Kozai, Agronomy (2020). https://doi.org/10.3390/agronomy10111680
13. T. Kozai, Y. Amagai, E. Hayashi, in *Achieving sustainable greenhouse cultivation*, ed. By L. Marcelis, E. Heuvelink, (Burleigh Dodds Science Publishing, Cambridge, 2019), pp. 177–204. https://doi.org/10.19103/AS.2019.0052.06
14. T. Kozai, E. Hayashi, Y. Amagai, Acta Hortic. (2020). https://doi.org/10.17660/ActaHortic.2020.1273.34
15. S.D. Carvalho, M.L. Schwieterman, C.E. Abrahan, T.A. Colquhoun, K.M. Folta, Front Plant Sci. (2016). https://doi.org/10.3389/fpls.2016.01328
16. Y. Ibaraki, in *Light Emitting Diodes for Agriculture*, ed. By S.D. Gupta, (Springer, Singapore, 2017), pp. 27–36
17. T. Shiga, K. Shoji, H. Shimada, S. Hashida, F. Goto, T. Yoshihara, Plant Biotechnol. (2009). https://doi.org/10.5511/plantbiotechnology.26.255
18. G. Zhang, S. Shen, M. Takagaki, T. Kozai, W. Yamori, Front Plant Sci. (2015). https://doi.org/10.3389/fpls.2015.01110
19. O.R. Zandvakili, A.V. Barker, M. Hashemi, F. Etemadi, W.R. Autio, J. Plant Nutr. (2019). https://doi.org/10.1080/01904167.2019.1589505
20. USDA, United States Department of Agriculture. (2020), https://www.nass.usda.gov/Publications/Todays_Reports/reports/vegean20.pdf. Accessed 5 Feb 2021

21. e-Stat, Statistics of Japan. (2019), https://www.e-stat.go.jp/en Accessed 5 February 2021
22. FAO, Food and agriculture organization of the United Nations. (2020), http://www.fao.org/faostat/en/?#data/QC Accessed 5 February 2021
23. N. Lu, S. Shimamura, in Smart Plant Factory, ed. By T. Kozai, (Springer, Singapore, 2018), pp. 31–49
24. M.C. Snowden, K.R. Cope, B. Bugbee, PLoS ONE (2016). https://doi.org/10.1371/journal.pone.0163121
25. Y. Wang, K.M. Folta, Am. J. Bot. (2013). https://doi.org/10.3732/ajb.1200354
26. J. Sun, J.N. Nishio, T.C. Vogelmann, Plant Cell Physiol. (1998). https://doi.org/10.1093/oxfordjournals.pcp.a029298
27. I. Terashima, T. Fujita, T. Inoue, W.S. Chow, R. Oguchi, Plant Cell Physiol. (2009). https://doi.org/10.1093/pcp/pcp034
28. H.H. Kim, R.M. Wheeler, J.C. Sager, G. Gains, J. Naikane, Acta Hortic. (2006). https://doi.org/10.17660/ActaHortic.2006.711.11
29. S. Saengtharatip, N. Goto, T. Kozai, W. Yamori, Acta Hortic. (2020). https://doi.org/10.17660/ActaHortic.2020.1273.35
30. F. Charles, P. Nilprapruck, D. Roux, H. Sallanon, Postharvest Biol Technol. (2018). https://doi.org/10.1016/j.postharvbio.2017.08.024
31. J. Uddling, J. Gelang-Alfredsson, K. Piikki, H. Pleijel, Photosynth Res. (2007). https://doi.org/10.1007/s11120-006-9077-5
32. L. Limantara, M. Dettling, R. Indrawati, T.H.P. Brotosudarmo, Proc. Chem. (2015). https://doi.org/10.1016/j.proche.2015.03.032
33. J. Schanda, *Colorimetry* (Wiley, Hoboken, 2007), pp. 61–63
34. L. Almela, S. Javaloy, J. Fernández-López, J. Lôpez-Roca, Food Chem. (1995). https://doi.org/10.1016/0308-8146(95)93940-S
35. E. Manolopoulou, T. Varzakas, Food. Nutr. Sci. (2011). https://doi.org/10.4236/fns.2011.29130
36. R. Dhakal, K.-H. Baek, Postharvest Biol. Technol. (2014). https://doi.org/10.1016/j.postharvbio.2013.12.007
37. H. Hu, H.Q. Liu, H. Zhang, J.H. Zhu, X.G. Yao, X.B. Zhang, K.F. Zheng, *ICIECS* (IEEE, Wuhan, 2010), pp. 1–3 https://doi.org/10.1109/ICIECS.2010.5678413
38. A.C. Madeira, A. Ferreira, A. de Varennes, M.I. Vieira, Commun. Soil Sci. Plant Anal. (2003). https://doi.org/10.1081/css-120024779
39. R.J. Porra, W.A. Thompson, P.E. Kriedemann, Biochim Acta Biophys (1989). https://doi.org/10.1016/S0005-2728(89)80347-0
40. R.A. Itle, E.A. Kabelka, HortScience **10**, 20 (2009). https://doi.org/10.21273/HORTSCI.44.3.633
41. H.C. Wien, D.C.E. Wurr, in *The Physiology of Vegetable Crops*. ed. by H.C. Wien (CAB International, Wallingford, 1997), pp. 345–386
42. M. Yano, A. Hayami, Bulletin of the Vegetable and Ornamental Crops Research Station. (Series A) 4, 89–101 (1978)
43. S. Oikawa, N. Osada, Jpn. J. Ecol. (2013). https://doi.org/10.18960/seitai.63.1_59
44. K. Ono, S. Nagano, Jpn. J. Ecol. (2013). https://doi.org/10.18960/seitai.63.1_49
45. T. Kozai, Y. Amagai, N. Lu, E. Hayashi, Y. Ibaraki, M. Takagaki, Y. Shinohara, T. Maruo, in *Plant Factory: Basics, Applications and Advanced Research*, (Elsevier press) (to be published), ch. 23. https://doi.org/10.1016/B978-0-323-85152-7.00019-7
46. T. Hara, A. Nakagawa, Y. Sonoda, J. Jpn. Soc. Hortic. Sci. (1982). https://doi.org/10.2503/JJSHS.50.481
47. T. Kato, A. Sooen, J. Japan. Soc. Hort. Sci. (1978). https://doi.org/10.2503/jjshs.47.351