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GROWTH DIFFERENCES AMONG EIGHT LEAF LETTUCES CULTIVATED UNDER LED LIGHT AND COMPARISON OF TWO LEAF LETTUCES GROWN IN 2016 AND IN 2018

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Abstract. *Effects of five kinds of LED irradiation in a growth chamber were compared. This study examined the growth and morphology of eight leaf lettuce (Lactuca sativa L.) cultivars. Plant growth under mixed red and blue, and under white with added red LED seemed best, respectively, for the growth of 'Red wave', 'Banchu sun bright', 'Sun bright' and 'Fancy green', and for 'Banchu sun bright', and 'Fancy green'. The other two cultivars, 'Red fire' and 'Frill lettuce', grew better under red LED than under other light. Furthermore, this study compared plant growth differences between 2016 and 2018 for two cultivars. Results show that plants grown in 2016 had significantly lower fresh weight, leaf weight, and dry weight. They were one-sixth to one-eighth of those achieved in 2018.*

Keywords: Difference of wavelengths, Eight cultivars, Growth analysis, LED, Light quality

1. **Introduction.** Lettuce (*Lactuca sativa* L.) is often used as a model crop to grow in plant factories with artificial lighting, especially light-emitting diodes (LEDs), because of its fast growth, short production period, low energy demand, and high nutritional value [1,2]. As an important environmental factor affecting lettuce production in plant factories, light not only provides a source of energy but also acts as an ambient light signal inducing various physiological responses [3]. Fluorescent lamps have been applied mainly in commercial plant factories with artificial lighting until recent years [4]. However, LEDs are being used increasingly in newly built plant factories because LEDs provide tremendous potential attributable to their long operating life, low radiant heat output, and flexibility of spectral configuration [5].

An earlier paper described plant growth and morphological differences under light irradiation by LEDs that are red, blue, mixed red and blue, green, white, and white with added red, for four cultivars of leaf lettuce in growth cabinets to maintain environments in terms of temperature, humidity, and concentrations of CO_2 and nutrient solutions [6]. In addition, plants have different morphological and physiological responses according to a specific spectrum [7]. It is different for the most suitable LED depending on cultivars and how to grow lettuce depending on kind of LEDs. Before starting to cultivate in commercial plant factories, it is one of the most important to determine optimal LED.

This study investigated differences of plant growth and morphology under light irradiation by LEDs (red, blue, mixed red and blue, white, and white with added red) for eight leaf lettuces grown in a growth cabinet to maintain the same environment for temperature, humidity, and concentrations of CO_2 and nutrient solutions. Dry and fresh masses of plants were compared for eight leaf lettuce cultivars grown under LEDs of five kinds. In

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addition, we compared growth differences for plants cultivated in the same environment in a growth cabinet in 2016 and in 2018.

2. Materials and Methods.

2.1. **Cultivation methods.** Seeds for 'Red wave (Sakata Seed Co., Japan)', 'Red fire (Takii Seed Co., Japan)', 'Sanmarino (Takii Seed Co., Japan)', 'Banchu Sun Bright (Nakahara Seed Co., Ltd., Japan)', 'Sun Bright (Nakahara Seed Co., Ltd., Japan)' as red leaf lettuce, and 'Leaf Lettuce Green (Nakahara Seed Co. Ltd., Japan)', 'Fancy Green (Sakata Seed Co., Japan)', and 'Frill Lettuce (Sakata Seed Co., Japan)' were sown on urethane cubes (M Hydroponic Research Co., Ltd., Japan) with distilled water and were then germinated in a growth chamber (TGE-5-2L; Espec Corp., Japan) at 25°C, at 70% relative humidity, 600 ppm CO_2, for 16 hr under continuous illumination at 100 μmol/m^2/s^1 cool white fluorescent lamps (FHF32EX-D-HX-S; NEC Corp., Japan) for 1 week. Subsequently, the germinated seeds were transferred onto nutrient solution to grow for an additional week.

Groups of eight plantlets in a urethane cube were transferred to other containers (293 × 211 × 106 mm) with 6 liters of commercial nutrient solution (A treatment: OAT Agrio Co., Ltd., Japan) to observe growth under LEDs of five kinds (Union Electronics Industrial Co., Ltd.): red (660 mm max. wavelength), blue (450 nm max. wavelength), mixed red and blue (450 and 660 nm max. wavelength), white, and white with added red for 3 weeks. The wavelengths for all LEDs are shown in Figure 1 of an earlier paper [6]. During cultivation, their roots were given sufficient air by air pumping (Kotobuki Kougei Co., Ltd., Japan) to avoid root rot. Then all solutions were exchanged for new ones once a week, adjusted to 1.2 dSm^{-1} for electric conductivity (EC) value. All LED treatments had intensity of 100 μmol/m^2/s^1.

After 3 weeks, all plants were harvested. We randomly selected five plants and measured their fresh weight (g; FW), leaf weight (g; LW), stem weight (g; SW), root weight (g; RW), maximum leaf length (cm; ML), main stem length (cm; MS), number of leaves (LN), SPAD value (SPAD-502; Konica Minolta Holdings Inc., Japan) and dry weight (g; DW) after they were kept in a dryer at 70°C for more than 3 days. Data were analyzed using Tukey's multiple test (Statcel; OMS Publishing Inc., Japan) and principal component analysis (Mulcel; OMS Publishing Inc., Japan).

2.2. **Comparison of growth differences for two cultivars grown under the same environment in 2016 and in 2018.** All conditions, measurements, and analysis grown lettuce cultivar, 'Red wave' and 'Leaf lettuce green', are the same in 2.1. All data from 2016 are referred from our other paper [8] for comparisons of growth differences between 2016 and 2018.

3. **Results and Discussion.** Morphological and physiological changes of plants show adaptation to light environments [9]. Nowadays, leaf lettuce is grown in controlled environments such as plant factories under red and/or blue LED light. However, it is more expensive and not easy to obtain compared to white LEDs because we are using white LEDs in our own life and can buy them easily [6]. Furthermore, a broad spectrum might be more practical and sufficient for plant growth under a controlled environment. Broad spectrum white LEDs were used for lettuce production in recent research [10].

Results of biomass measurements of eight leaf lettuce cultivars influenced by the light spectrum treatments are presented in Table 1. Plants showed distinct growth response to different light quality treatments. For 'Red wave' and 'Leaf lettuce green', plants under red LED had significantly lower FW, LW, SW, and RW than those under other LEDs, although completely opposite results were obtained for 'Red fire', 'San Marino', 'Banchu sun bright', 'Sun bright' and 'Frill lettuce'. Only for 'Fancy green' did differences not

TABLE 1. Growth differences by LED wave length for leaf lettuce cultivars

Cultivars	Light source	Fresh weight (g)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Length of maximum leaf (cm)	Length of main stem (cm)	Number of leaves	SPAD value	Dry weight (cm)
Red wave	1 Red	8.55±1.61a[z]	7.11±1.40a	1.44±0.23a	1.32±0.28a	15.32±0.75ab	10.34±0.81a	11.20±0.58a	17.06±0.94	2.62±0.10a
	2 Blue	15.10±0.67bc	12.99±0.53ab	2.11±0.14ab	3.21±0.38b	16.86±0.64b	5.58±0.22b	8.80±0.20b	15.96±0.94	3.05±0.06a
	3 Red and blue	19.61±2.21b	16.91±1.87b	2.69±0.35b	3.55±0.55b	17.60±0.72b	5.54±0.41b	10.80±0.58ab	18.52±0.51	3.45±0.19a
	4 White	6.29±1.13a	5.05±0.77a	1.24±0.46a	1.21±0.44a	14.40±0.80ab	4.56±1.00b	9.60±0.60ab	17.52±0.80	0.35±0.06b
	5 White added red	11.67±0.83ac	8.87±0.72a	2.80±0.11b	3.00±0.11b	13.32±0.48a	6.14±0.37b	9.20±0.37ab	17.60±0.81	0.58±0.06b
Red fire	6 Red	16.31±3.81a	14.56±3.45a	1.75±0.41a	2.73±0.98	21.88±0.74a	7.60±1.00a	13.60±1.21a	15.46±2.03	0.61±0.16a
	7 Blue	9.20±0.67ab	8.81±0.63ab	0.40±0.04b	1.91±0.29	15.46±0.68b	2.16±0.22b	9.20±0.37b	15.98±0.95	0.43±0.04a
	8 Red and blue	2.54±0.67b	2.44±0.30b	0.10±0.02b	0.47±0.05	8.52±0.33c	1.00±0.04b	10.40±0.93ab	17.00±1.41	0.21±0.02b
	9 White	9.66±1.67ab	9.14±1.53ab	0.57±0.15b	2.05±1.06	17.67±1.01b	2.14±0.49b	11.20±0.73ab	13.54±1.49	0.36±0.06ab
	10 White added red	9.51±1.52ab	9.12±1.46ab	0.40±0.06b	2.04±0.32	17.26±0.50b	1.76±0.12b	10.20±0.37b	19.30±3.31	0.42±0.08ab
Sanmarino	11 Red	15.58±1.37a	15.27±1.35a	0.31±0.02a	2.82±0.67	16.44±0.81a	1.48±0.19a	10.80±0.58a	16.78±2.86	0.88±0.07a
	12 Blue	4.62±0.43b	4.49±0.41b	0.13±0.02b	1.38±0.14	9.36±0.20b	0.86±0.07b	8.40±0.24b	29.74±4.60	0.40±0.03b
	13 Red and blue	7.79±0.59bc	7.63±0.58bc	0.16±0.01b	1.90±0.24	10.94±0.46bc	0.98±0.07b	10.20±0.20a	25.28±3.75	0.67±0.07a
	14 White	10.45±0.89c	10.15±0.88c	0.30±0.01a	6.06±4.52	13.16±0.10c	1.20±0.07ab	9.80±0.20ab	20.52±1.81	0.65±0.04a
	15 White added red	8.91±1.09c	8.74±1.07c	0.17±0.02b	1.75±0.20	12.04±0.24c	1.08±0.04ab	10.60±0.40a	19.72±2.42	0.64±0.07ab
Banchu sun bright	16 Red	23.56±2.48a	22.55±2.37	1.01±0.14	3.36±0.58	18.20±0.79a	2.76±0.11	10.00±0.32	15.46±0.81	1.12±0.16
	17 Blue	14.34±0.85b	13.52±0.83	0.82±0.02	2.86±0.63	15.32±0.73b	2.40±0.16	9.40±0.40	17.44±0.80	0.71±0.05
	18 Red and blue	24.14±1.87a	23.04±1.83	1.14±0.15	4.50±0.78	14.50±0.53b	2.40±0.09	11.00±0.55	16.04±0.70	1.37±0.18
	19 White	22.91±2.10a	19.13±3.29	0.86±0.20	3.02±0.77	16.72±0.77ab	2.46±0.16	10.00±0.45	16.22±0.53	0.84±0.15
	20 White added red	21.95±3.81a	20.80±3.54	1.15±0.30	3.93±1.05	14.52±0.56b	2.22±0.12	10.40±0.40	17.60±0.99	1.08±0.21
Sun bright	21 Red	33.15±3.46a	30.90±3.21a	2.22±0.28a	4.48±0.38	16.30±0.75	3.84±0.34a	12.40±1.00	16.50±1.72a	2.24±0.34a
	22 Blue	18.58±1.25b	17.30±1.24b	1.24±0.07b	2.30±0.44	17.30±0.94	3.10±0.26ab	10.60±0.60	19.00±1.73a	0.80±0.10b
	23 Red and blue	29.24±2.54a	27.50±2.40a	1.75±0.15ab	4.36±0.69	16.40±0.37	3.00±0.10ab	13.40±0.40	26.30±1.01b	1.91±0.17ac
	24 White	27.20±2.97a	22.72±1.75ab	1.67±0.11ab	4.15±0.22	16.40±0.72	3.00±0.21ab	11.80±0.70	15.10±0.85a	1.19±0.14bc
	25 White added red	19.00±1.55b	17.82±1.40b	1.15±0.16b	3.30±0.45	16.00±0.20	2.80±0.10b	11.60±0.68	16.30±1.78a	1.00±0.12b
Leaf lettuce green	26 Red	6.68±0.30a	5.39±0.48a	1.30±0.25a	1.16±0.11a	11.38±0.16a	7.44±1.52a	7.80±0.37	19.14±2.27	0.85±0.07ac
	27 Blue	7.32±1.12a	6.51±0.94a	0.81±0.19a	2.04±0.42a	15.16±0.60b	3.26±0.39b	7.80±0.58	20.72±1.88	0.61±0.10c
	28 Red and blue	15.41±2.55b	11.79±1.88bc	3.62±0.68b	4.83±1.27b	15.76±0.61b	8.44±0.88a	10.00±1.10	17.42±0.53	1.58±0.37a
	29 White	8.13±1.33a	7.22±1.12ac	0.91±0.22a	2.04±0.42a	15.16±0.60b	3.26±0.39b	7.80±0.58	20.72±1.88	0.61±0.10c
	30 White added red	4.50±0.44a	3.97±0.40a	0.53±0.09a	0.81±0.06a	12.42±0.66a	6.10±1.15ab	8.40±0.87	16.62±1.53	2.44±0.10b
Fancy green	31 Red	21.87±0.61	20.19±0.52	1.68±0.18a	2.55±0.35	25.06±0.47a	5.04±0.51a	10.00±0.32	27.00±1.19ab	0.96±0.11ab
	32 Blue	19.50±3.05	18.42±2.92	1.08±0.14b	2.91±1.13	22.12±0.43b	2.74±0.19b	10.00±0.55	30.04±2.06ab	0.75±0.08b
	33 Red and blue	23.91±1.46	22.97±1.34	0.94±0.13b	3.79±1.44	21.40±0.60b	1.72±0.18b	10.80±0.58	32.24±1.76a	1.34±0.08a
	34 White	20.70±1.52	19.76±1.43	0.94±0.09b	3.09±0.60	21.72±0.63b	1.84±0.10b	10.60±0.51	25.48±0.78b	0.95±0.13ab
	35 White added red	23.04±1.84	21.86±1.78	1.18±0.10ab	3.27±0.58	22.26±0.24b	2.44±0.18b	10.60±0.40	28.66±1.36ab	0.97±0.05ab
Frill lettuce	36 Red	29.74±3.40a	25.31±2.57a	4.43±0.88a	3.90±1.44	16.92±0.94a	8.00±0.50a	19.20±1.98	25.58±1.78	1.74±0.21a
	37 Blue	14.71±1.13b	12.96±0.94b	1.74±0.20b	3.19±0.70	14.12±0.88ac	5.14±0.31b	15.80±1.36	22.80±1.19	0.81±0.06b
	38 Red and blue	14.13±1.19b	12.57±1.11b	1.56±0.10b	2.93±0.51	12.88±0.38bc	4.40±0.44b	17.00±0.95	23.16±0.89	0.85±0.07b
	39 White	20.95±2.40ab	18.41±2.05ab	2.53±0.39b	2.46±0.37	14.14±0.85ac	4.76±0.45b	16.00±1.38	26.52±1.48	0.92±0.08b
	40 White added red	16.87±1.55b	16.16±1.51b	0.72±0.04b	3.18±0.38	16.30±0.84a	2.34±0.12c	17.20±1.39	29.12±2.54	0.82±0.09b

[z]Each value represents the mean±standard error. Different letters denote significant differences by Tukey's multiple test with a significance level of 0.05.

depend significantly on LED treatments for FW, LW, RW, and NL. For six cultivars, the main stem length was affected strongly by red LED treatment: half of them (FW, LW, and DW) were significantly increased under red LED. Nevertheless, two of them were significantly decreased. The mixed red and blue LED treatment had increased FW, LW, and DW for in 'Red wave', 'Banchu sun bright', 'Sun bright' and 'Leaf lettuce green'. These results suggest that growth under red or mixed red and blue LED light might be useful, depending on the cultivar.

Furthermore, principal component analysis (PCA) was conducted to ascertain general trends related to the effects on plant growth of LEDs. Plants showed distinct growth response to different light treatments. As first components found through principal component analysis, FW, LW, SW, RW, ML, MS, LN, and DW were found to have positive factor loadings: only SPAD was negative. As second components, SW, ML, and MS were positive; the others were negative. The respective contributions of first and second components were 55.60% and 16.18% (Table 2).

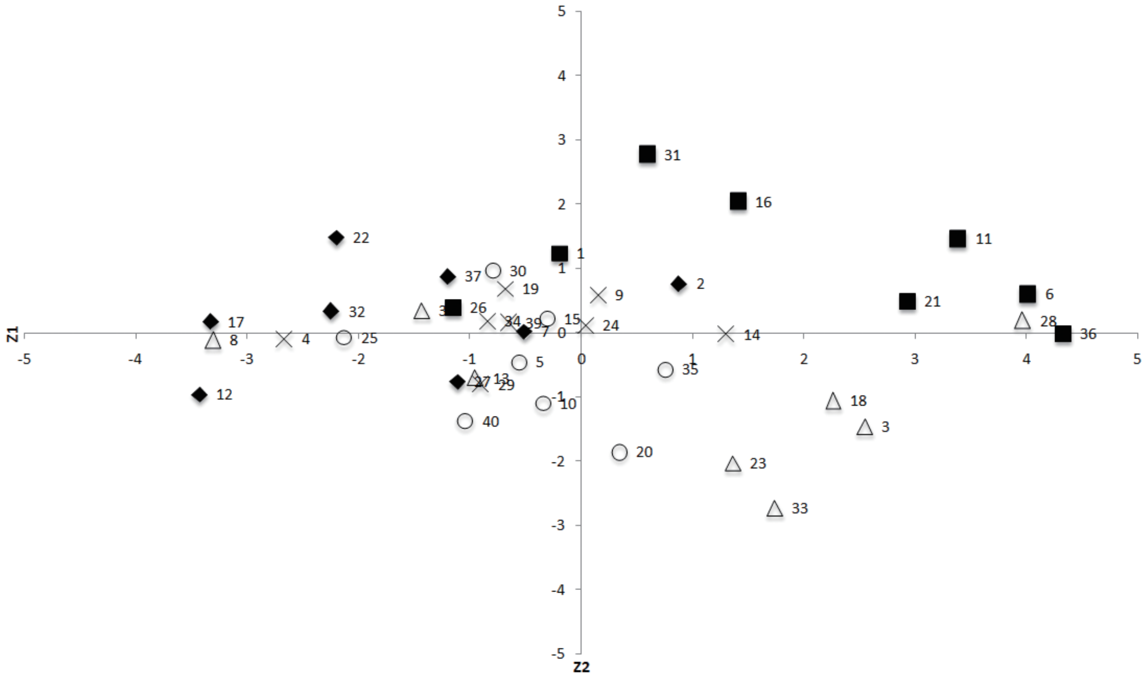
Table 2. Eigen value, contribution and factor loading of 1st, 2nd and 3rd principal components in leaf lettuce cultivars

Characteristic	Component No.		
	1	2	3
Fresh weight (g)	0.425	−0.100	0.127
Leaf weight (g)	0.424	−0.148	0.133
Stem weight (g)	0.366	0.103	0.096
Root weight (g)	0.342	−0.355	−0.085
Maximum leaf length (cm)	0.224	0.321	0.717
Length of main stem (cm)	0.268	0.549	−0.048
No. of leaves	0.333	−0.217	−0.357
SPAD value	−0.104	−0.613	0.495
Dry weight (g)	0.380	−0.057	−0.247
Eigen value	4.106	1.195	0.749
Contribution	55.60%	16.18%	10.14%
Cumulative contribution	55.60%	71.78%	88.12%

Figure 1 presents results of the PCA and the score plots obtained for each treatment along the first and second components, Z1 and Z2. Each LED treatment was well separated along Z1, except for two plants under red or white with added red, one plant under blue, and three plants under mixed red and blue, and under white LED that fall near scores corresponding to LED treatment. Additionally, in general, plants grown under red or mixed red and blue LED light are apparently separated along Z2 and to increase tendency compared plants under other LEDs. In addition, plants grown under mixed red and blue and white with added red LED are apparently separated along Z1 and to decrease tendency compared plants grown under other ones.

As a conclusion obtained from PCA, considering separation along Z1 observed in Figure 1, it can be inferred that the main affected characteristics attributable to LED treatment were FW, LW, SW, RW, ML, MS, LN, and DW. However, SW and MS were not good characteristics for growth in leaf lettuce because they would be decreased in terms of quality, taste, and appearance. However, LED treatment by mixed red and blue, and white with added red LED seem to be the best, respectively, to grow 'Red wave', 'Banchu sun bright', 'Sun bright' and 'Fancy green', and to grow 'Banchu sun bright', and 'Fancy green'.

When people grow plants in a growth chamber (plant factory), they can control environmental factors such as temperature, humidity, light intensity, kinds of light, and



■: Red LED, ◆: Blue LED, △: Mixed red and blue LED, ×: White LED,
○: White added with red LED.
* Number indicates in Table 1.

Figure 1. Scatter diagram in Z1-Z2 plane nine characteristics in lettuce arranged by the principal component analysis in leaf lettuce cultivars

CO_2 concentration. However, it would be impossible to keep from changing the personnel growing plants in the laboratory of university. Even for the present study, different research team members conducted these experiments in 2016 and in 2018. When scientists and researchers conduct experiments for cultivation in field and greenhouse, they might obtain different data depending on the season and year especially [11]. By contrast, a growing factory facilitates control of the environment to support plant growth in a growth chamber as desired. They then might be able to obtain similar data from any experiment. Therefore, we confirmed growth differences in plants grown under identical environments in 2016 and in 2018.

Results of biomass measurements of two cultivars in leaf lettuce influenced by light spectra treatments between in 2018 and in 2016 are presented in Table 3. For both cultivars, plants grown in 2016 had significantly lower FW, LW, and DW: one-sixth to one-eighth of those compared in 2018.

Furthermore, to ascertain general trends of plant growth and the year during which plants were grown, principal component analysis (PCA) was conducted. Plants showed distinct growth response to different light quality treatments. As first components found through principal component analysis, FW, LW, SW, ML, MS, LN, and DW were found to have positive factor loadings: only RW and SPAD were negative. As second components, ML and LN were positive. The others were negative. The respective contributions of first and second components were 55.74% and 18.26% (Table 4), especially lettuce under red LED.

Figure 2 presents results of the PCA and the score plots obtained respectively for each treatment along the first and second components, Z1 and Z2. Red LED treatments were separated completely along Z1 between 2016 and 2018. This result indicates that plants grown in 2016 had significantly lower FW, LW, and DW compared to plants grown in 2018.

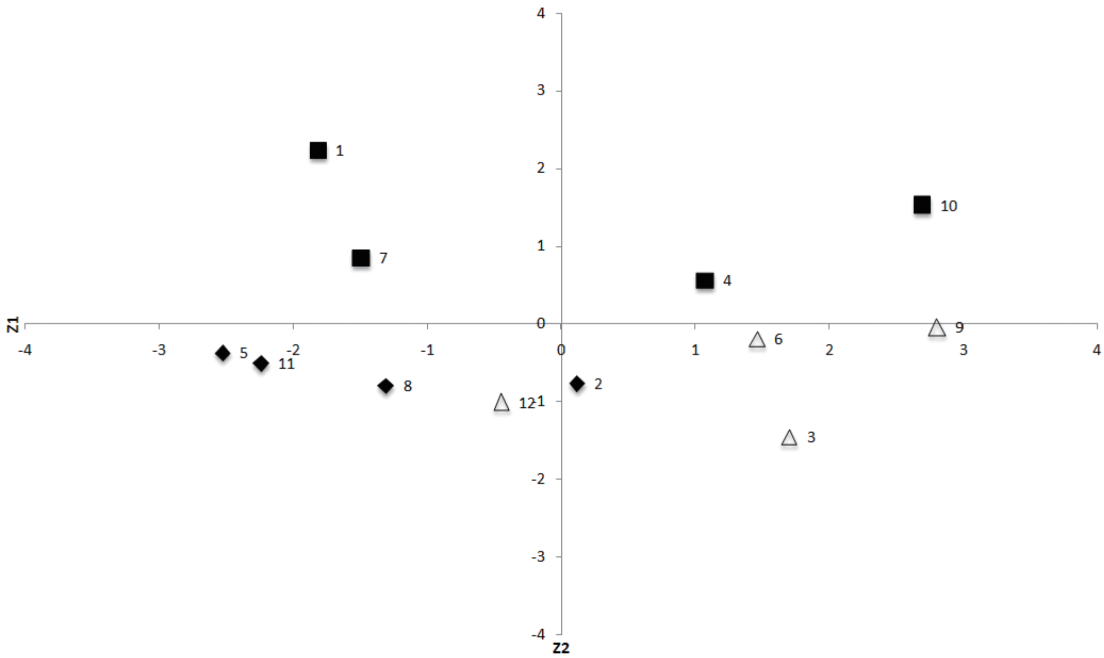
Table 3. Growth differences and comparison by LED wave length and year of cultivation for red wave and leaf lettuce green in 2016 and 2018

Cultivars		Light source	Fresh weight (g)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Length of maximum leaf (cm)	Length of main stem (cm)	Number of leaves	SPAD value	Dry weight (cm)
Red wave	2018[z]	1 Red	8.55±1.61a[x]	7.11±1.40a	1.44±0.23a	1.32±0.28a	15.32±0.75a	10.34±0.81a	11.20±0.58a	17.06±0.94a	2.62±0.10a
		2 Blue	15.10±0.67b	12.99±0.53ab	2.11±0.14ab	3.21±0.38b	16.86±0.64a	5.58±0.22b	8.80±0.20b	15.96±0.94a	3.05±0.06a
		3 Red and blue	19.61±2.21b	16.91±1.87b	2.69±0.35b	3.55±0.55b	17.60±0.72a	5.54±0.41b	10.80±0.58ab	18.52±0.51a	3.45±0.19a
	2016[y]	4 Red	2.99±0.43c	2.25±0.43c	0.39±0.03ac	0.35±0.02a	11.58±0.27b	5.46±0.41b	5.38±0.26c	19.86±0.86a	0.29±0.03b
		5 Blue	2.93±1.37c	0.60±0.14d	0.19±0.01c	2.33±1.43ab	7.55±0.33c	7.45±0.71b	4.38±0.26c	27.56±1.97b	0.13±0.01c
		6 Red and blue	3.11±0.73c	2.48±0.63c	0.16±0.04c	0.47±0.10b	10.29±0.60b	1.70±0.47c	5.75±0.31c	17.73±0.81a	0.44±0.23b
Leaf lettuce green	2018	7 Red	6.68±0.30a	5.39±0.48a	1.30±0.25a	1.16±0.11a	11.38±0.16a	7.44±1.52a	7.80±0.37a	19.14±2.27a	0.85±0.07ab
		8 Blue	7.32±1.12a	6.51±0.94a	0.81±0.19a	2.04±0.42a	15.16±0.60b	3.26±0.39b	7.80±0.58a	20.72±1.88a	0.61±0.10b
		9 Red and blue	15.41±2.55b	11.79±1.88b	3.62±0.68b	4.83±1.27b	15.76±0.61b	8.44±0.88a	10.00±1.10a	17.42±0.53ab	1.58±0.37a
	2016	10 Red	2.90±0.48c	1.91±0.31c	0.63±0.11a	0.35±0.06c	11.20±0.48a	14.39±1.31c	8.38±0.78a	11.86±1.11c	0.26±0.04c
		11 Blue	1.75±0.4c	1.29±0.3c	0.07±0.04c	0.39±0.10c	7.31±0.54c	1.38±0.69d	6.13±0.48b	19.88±0.65a	0.19±0.04c
		12 Red and blue	2.15±0.54c	1.56±0.16c	0.17±0.06c	0.41±0.12c	7.65±0.73c	3.06±0.82bc	5.88±0.48b	15.71±1.41b	0.26±0.02c

[z]Data is from this paper in Table 1.
[y]Data are from Ishii et al. [8] in Tables 2 and 3.
[x]Each value represents the mean±standard error. Different letters denote significant differences by Tukey's multiple test with a significance level of 0.05.

Table 4. Eigen value, contribution and factor loading of 1st, 2nd and 3rd principal components in lettuces cultivated in 2016 and 2018

Characteristic	Component No.		
	1	2	3
Fresh weight (g)	0.422	−0.129	−0.064
Leaf weight (g)	0.441	−0.111	−0.044
Stem weight (g)	0.362	−0.007	0.407
Root weight (g)	−0.002	−0.587	0.594
Maximum leaf length (cm)	0.384	−0.137	−0.144
Length of main stem (cm)	0.027	0.580	0.661
No. of leaves	0.322	0.374	−0.095
SPAD value	−0.290	−0.317	0.097
Dry weight (g)	0.401	−0.177	−0.011
Eigen value	3.648	1.195	0.718
Contribution	55.74%	18.26%	10.97%
Cumulative contribution	55.74%	74.00%	84.97%



■: Red LED, ◆: Blue LED, △: Mixed red and blue LED.
* Number indicates in Table 3.

Figure 2. Scatter diagram in Z1-Z2 plane nine characteristics in lettuce arranged by the principal component analysis in lettuce cultivated in 2016 and 2018

This result suggests that growing plants during the same period with the same personnel, even in plant factories, might be important to control the environment. For this experiment, we sowed all seeds into urethane cubes and made all nutrient solutions by hand. Light quality signals captured by plants during the seedling stage affected subsequent growth and quality remarkably [12]. However, the environment during the seedling stage influenced subsequent plant growth and quality. When people have a habit of sowing seeds into urethane cubes, depth and direction might be different for growth from seedlings to mature plants depending on the personnel. Of course, one must also consider human error when people mix a nutrient solution. Consequently, when growing plants in a commercial plant factory, it might be necessary to use an automatic system for all of them. Furthermore, when conducting experiments even in a growth cabinet and chamber, one must grow plants during the same period and with the same people conducting experiments. The same experiments should be repeated even after changing the personnel conducting the experiments.

4. **Conclusion.** We used eight leaf lettuce cultivars for this experiment. Their response differed depending on the kind of LED. LED treatment by mixed red and blue, and by white with added red LED seem best, respectively, to grow 'Red wave', Banchu sun bright', 'Sun bright', and 'Fancy green', and to grow 'Banchu sun bright' and 'Fancy green'. The remaining two cultivars, 'Red fire' and 'Frill lettuce', grew better under red LED than under other lights.

Furthermore, we compared the differences of plant growth in 2016 and in 2018 for two cultivars. Results show that plants grown in 2016 had significantly lower FW, LW, and DW: one-sixth to one-eighth of those in 2018. That finding suggests that it might be important to grow plants during the same period with the same personnel, even in plant factories with a controlled environment. However, it is difficult that the same personnel continue to sow and cultivate in plant factories, so it might be the most important to find a stable cultivation system without automation or it might be necessary to induce automation system for all cultivation period. Our results suggested that it might have to repeat the same experiments even under the same controlled environment and condition when the researchers changed.

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