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Effect of far-red light applied at the end of the day in red and green leaf lettuce cultivars grown under two types of white LED

H. Kamimaeda [1], Y. Yoshida [1], K. Tamaki [1], M. Ichihara [1], M. Akutsu [1, 2 (*)]

[1] *Department of Electrical Engineering and Computer Science, Tokai University, 9-1-1 Toroku, Kumamoto-City, Kumamoto 862-8652, Japan.*

[2] *Current address: Faculty of Agriculture, Shinshu University 8304, Minamiminowa-Village, Kamiina-County Nagano, 399-4598 Japan.*

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Abstract: The growth and yield of 13 red and green leaf lettuce (*Lactuca sativa* L.) cultivars were evaluated under two types of white LED irradiation. There was a difference in growth under the two types white LEDs, specifically in the fresh total weight, fresh leaf weight and dry weight in all cultivars. In addition, the main stem elongation was confirmed for red and green lettuce cultivars under all treatments, but some cultivars promoted the growth of the main stem and the others were inhibited by treatment with far-red light applied at the end of the day (EOD-FR). Furthermore, the EOD-FR treatment affected the characteristic reactions due to white LED light quality in each of the cultivars. These results showed that it is necessary to investigate the selection of white LEDs with and without EOD-FR treatment for each lettuce cultivar.

(*) **Corresponding author:**
akutsu@shinshu-u.ac.jp

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All relevant data are within the paper and its Supporting Information files.

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1. Introduction

Lettuce (*Lactuca sativa* L.), which is often cultivated not only in greenhouses but also in indoor plant factories, has become a model plant for studying the response of plants grown under LEDs (Lin *et al*., 2013; Yan *et al*., 2019). Among them, there are many reports of growth differences by irradiating red and blue LEDs with a monochromatic color, mixing red and blue LEDs, and irradiating them in combination, such as changing the irradiation time. In particular, depending on the quality of the LED light used for irradiating the plants, differences in morphological growth such as fresh and dry weight and leaf area, and in the amount of substances such as anthocyanins and vitamins in plants have been reported (Bleiss and Smith, 1985; Jieun *et al*., 2012; Jishi *et al*., 2016; Bian *et al*., 2018; Ishii *et al*., 2018). It is also known that light quality and quantity not only promotes plant photosynthesis, but also regulates plant growth (Chen *et al*., 2016). However, it is reported that blue lights, which affect photomorphogenesis and red, which affects photosynthesis, are

highly efficient for plant growth (Goto, 2005).

Moreover, far-red (FR) light, along with red light, is known to have a significant effect on plant growth and has been shown to affect seed germination, plant growth, and flowering (Hisamatsu *et al.*, 2002). It has been reported that FR LED treatment after sunset (end of day-far red: EOD-FR) promotes shoot elongation in poinsettia (*Euphorbia pulcherrima*) and chrysanthemum (*Chrysanthemum sp.*) (Lund *et al*., 2007; Islam *et al*., 2014), and also extends the hypocotyl axis in the rootstock of pumpkin (*Cucurbita maxima*) seedlings (Yang *et al*., 2012). In komatsuna (*Brassica rapa* var. *perviridis*) and pak choi (*Brassica rapa* var. *chinensis*), EOD-FR treatment has been found to increase fresh weight, dry matter weight, and leaf area (Akutsu *et al*., 2017). It has been reported that the fresh and dry weights and leaf length of baby leaf lettuce grown under white light irradiation with FR light increased by more than 10% compared to those grown without the FR light irradiation (Li and Kubota, 2009). In Japan, most indoor plant factories growing lettuce use red or red-blue mixed LEDs. However, white LEDs are used extensively in our daily life and are easily available. If white LEDs can be used for lettuce production in an indoor plant factory, it may reduce the unit price spent on LEDs and consequently increase cost effectiveness. In addition, white LEDs have begun to be used for growing leafy vegetables such as leaf lettuce in plant factories, but it might be difficult to optimize the light wavelength and intensity for plant cultivation as they are originally used for indoor lighting in households. Compared to using white LED irradiation alone for plant production, it is possible to optimize the light intensity for each wavelength by combining monochromatic LED lighting (Watanabe *et al*., 2016), but we confirmed a difference in the growth using white LED irradiation and whether changes were made by FR LED irradiation. In this study, we investigated the effects of EOD-FR treatment on lettuce using 13 leaf lettuce cultivars under irradiation with two types of white LEDs. Plants were grown hydroponically in growth chambers to maintain environmental conditions for air temperature, humidity and concentration of CO_2 other than light conditions.

2. Materials and Methods

The 13 cultivars of leaf lettuce used in this study are listed in Table 1. The seeds of each cultivar were sown on urethane cubes (M Hydroponic Research Co. Ltd., Aichi, Japan) with distilled water and then germinated for one week in a growth chamber (TGE-5-2L; Espec Corp., Osaka, Japan) at 25°C, 70% relative humidity, and 600 ppm CO_2 for 16 h under continuous illumination at 100 µmol/m^2/s cool white fluorescent lamps (FHF32EX-D-HX-S; NEC Corp., Tokyo, Japan). Subsequently, the germinated seeds were transferred into a commercial A treatment nutrient solution suitable for lettuce cultivation (OAT Agrio Co., Ltd., Tokyo, Japan) and grown for an additional week. After that, eight seedlings were transferred to containers (293 mm × 211 mm × 106 mm) with 6 L of commercial A treatment nutrient solution (OAT Agrio Co., Ltd., Japan). The growth of the seedlings was observed for three weeks under the following four

Table 1 - Lettuce cultivars used in this experiment

Kinds of lettuce	Cultivar name	Name of seedling company
Red leaf lettuce	Leaf lettuce red	Sakata Seed Corp., Japan
	Red wave	Sakata Seed Corp., Japan
	Bancyu sun bright	Nakahara Seed Co. Ltd.,Japan
	Sun bright	Nakahara Seed Co. Ltd.,Japan
	Fancy red	Nakahara Seed Co. Ltd.,Japan
	Red fire	Takii Seed Co. Ltd., Japan
	Bancyu red fire	Takii Seed Co. Ltd., Japan
	Sun marino	Takii Seed Co. Ltd., Japan
	Calbee red	Nakahara Seed Co. Ltd.,Japan
Green leaf lettuce	Summer green	Sakata Seed Corp., Japan
	Fancy green	Nakahara Seed Co. Ltd.,Japan
	Green wave	Takii Seed Co. Ltd., Japan
	Yakiniku lettuce	Sakata Seed Corp., Japan

irradiation treatments: white LED (White A; 16 h white photoperiod, Fluorescent lamp-type LED for growing plants, Espec Corp., Osaka, Japan), FR LED irradiation for 3 h after irradiation with white A (White A + FR; 16 h white + 3 h FR photoperiod, FR LED: Valore Corp., Kyoto, Japan), another white LED (White B; 16 h white photoperiod, Fluorescent lamp-type LED for growing plants, Espec Corp., Osaka, Japan), and FR LED irradiation for 3 h after irradiation with white B (White B + FR; 16 h white + 3 h FR photoperiod). The intensity of irradiation in white LED treatments was 100 µmol/m^2/s and FR LED treatment was 13.2 µmol/m^2/s. The wavelengths for all LEDs are shown in figure 1. During cultivation, to their roots were given sufficient air using an air pump (Kotobuki Kougei Co., Ltd., Japan) to avoid root rot. Once a week, all solutions were replaced with fresh ones, and the electric conductivity (EC) value adjusted to 1.2 dS/m^1. Twenty-one days after the start of cultivation, all plants were harvested and their fresh weight, root weight, maximum leaf length, number of leaves, and SPAD value (SPAD-502; Konica Minolta Holdings Inc., Tokyo, Japan) were measured. After drying for more than three days at 70°C, the dry leaf weight and dry root weight were also measured. The plants on day 14 were alternately harvested, and all cultivation experiments were repeated twice. All data were evaluated by one-way ANOVA (analysis of variance) using the Statcel add-in (OMS Publishing Inc., Saitama, Japan) in Excel (Microsoft Corp., Redmond, WA), followed by Tukey's multiple post-hoc comparison test.

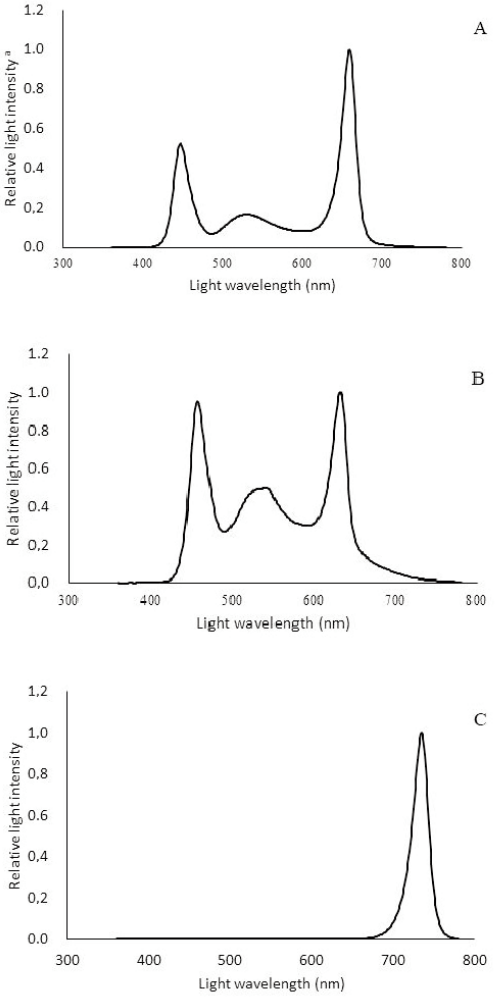

Fig. 1 - Wavelength distribution characteristics of various LED light sources used in this experiment. A= White A LED, B= White B LED, C= FR LED. [(a)] It was indicated relative value with the maximum peak taken as 1 against the measured light intensity.

3. Results

Among the red leaf lettuce cultivars, the effect of white LED irradiation was observed on the fresh total weight, fresh leaf weight, root weight, and dry weight of 'Leaf lettuce red' (Table 2). Fresh and dry weights of 'Red wave', 'Fancy red', and 'Calbee red' cultivated under White A and White A + FR irradiation were significantly greater than those cultivated under White B and White B + FR. The fresh total weight, fresh leaf weight, stem weight, and maximum leaf length in 'Bancyu sun bright' and 'Sun bright' grown under White A + FR and White B + FR tended to be more, and the fresh leaf weight and dry total weight in 'Sun bright' and the fresh total weight, fresh leaf weight, and stem weight in 'Sun bright' were significantly greater than those cultivated under White A and White B. The fresh total weight, fresh leaf weight, and dry total weight of 'Red fire' and 'Sun marino' cultivated under White A + FR tended to be more, and the fresh leaf weight in 'Red fire' and the fresh total weight in 'Sun marino' were significantly greater than those cultivated under the other LEDs. All investigated items except the number of leaves and SPAD in 'Bancyu red fire' grown under White A and White B showed a higher value, especially for the dry total weight, which was significantly greater than that grown under White A + FR and White B + FR.

Among the green leaf lettuce cultivars, in 'Summer green', the effect of white LED irradiation was observed on the fresh total weight, fresh leaf weight, and dry total weight (Table 3). In 'Fancy green', the fresh total weight, fresh leaf weight, and

Table 2 - The growth difference in red lettuce cultivars by two kinds of white LED and FR light treatment

Cultivars		Kinds of LED	Fresh total weight (g)	Fresh leaf weight (g)	Stem weight (g)	Root weight (g)	Maximum leaf length (cm)	Main stem length (cm)	Number of leaf	SPAD	Dry weight (g)
Leaf lettuce red	1	White A	5.7±1.0 ab	3.7±0.3 b	1.5±0.3 b	0.4±0.0 b	13.3±0.6	18.6±0.4b	8.1±0.5	22.6±2.6a	0.31±0.05 b
	2	White A+FR	4.3±0.6 b	3.0±0.4 b	1.3±0.2 b	0.3±0.1 b	13.0±0.4	19.4±0.3b	7.4±0.6	16.8±1.5b	0.23±0.03 b
	3	White B	8.7±0.7 a	6.3±0.5 a	2.4±0.2 a	1.0±0.2 a	13.2±0.6	21.2±0.3ab	8.8±0.4	16.9±0.8b	0.50±0.07 a
	4	White B+FR	4.3±0.2 b	2.8±0.8 b	2.0±0.5 ab	0.4±0.1 b	13.6±0.8	21.7±0.3a	7.4±0.5	18.2±1.3ab	0.38±0.07 a
Red wave	5	White A	9.1±1.5 a	8.5±1.4 a	0.6±0.2	1.7±0.3 a	16.7±0.6	3.5±0.7b	6.7±0.3	17.2±2.0	0.50±0.08 a
	6	White A+FR	9.0±1.5 a	8.1±1.3 a	0.8±0.2	1.2±0.2 a	17.3±0.7	4.7±0.9b	6.0±0.3	15.1±1.7	0.50±0.08 a
	7	White B	7.0±1.3 ab	6.1±1.1 ab	0.9±0.3	0.9±0.1 ab	17.4±0.4	6.8±0.5a	6.5±0.5	17.8±3.1	0.36±0.06 ab
	8	White B+FR	3.5±0.7 b	3.1±0.7 b	0.5±0.1	0.4±0.1 b	15.7±0.6	7.4±0.2a	6.1±0.4	12.8±1.2	0.20±0.04 b
Bancyu sun	9	White A	6.8±1.1 b	6.2±1.0 b	0.6±0.1 b	0.7±0.2 b	16.6±1.2b	8.3±0.3ab	9.1±0.4	17.5±1.7	0.35±0.06 b
	10	White A+FR	13.3±2.0 a	12.3±0.9 a	1.0±0.2 a	1.8±0.2 a	20.1±0.4a	6.4±0.3b	9.9±0.5	16.0±1.2	0.51±0.10 a
	11	White B	6.5±1.2 b	5.8±1.2 b	0.8±0.1 ab	0.9±0.2 b	19.0±0.5ab	9.5±0.6a	9.5±0.6	14.4±1.7	0.30±0.04 b
	12	White B+FR	11.3±0.9 a	10.5±0.8 a	0.9±0.1 a	1.6±0.1 a	20.8±0.7a	7.3±0.7b	10.4±0.5	16.9±1.5	0.54±0.05 a
Sun bright	13	White A	10.1±0.7 b	8.9±0.6 b	0.9±0.0 b	1.2±0.2 b	15.6±0.5c	7.4±0.4b	9.6±0.3	15.2±1.3b	0.47±0.03 b
	14	White A+FR	23.5±1.3 a	18.4±1.1 a	2.0±0.1 a	2.0±0.3 a	20.0±0.6b	9.5±0.7ab	9.6±0.3	17.0±2.4ab	0.62±0.03 a
	15	White B	11.3±0.9 b	11.6±0.3 b	1.0±0.1 b	1.1±0.1 b	17.7±0.5 bc	8.7±0.8 b	9.9±0.4	15.9±2.0 b	0.47±0.07 b
	16	White B+FR	22.7±3.7 a	15.7±0.9 a	2.6±0.2 a	1.1±0.2 b	26.1±0.9 a	11.5±0.6 a	9.1±0.4	24.0±1.4 a	0.59±0.05 a
Fancy red	17	White A	9.1±0.6 a	9.3±0.4 ac	0.6±0.2	1.7±0.3 a	16.7±0.6	3.5±0.7 b	8.2±0.3	17.2±1.0 a	0.49±0.10 ab
	18	White A+FR	8.9±0.6 a	9.5±0.3 a	0.8±0.2	1.2±0.2 a	17.3±0.7	4.7±0.9 b	8.3±0.5	15.1±0.7 ab	0.52±0.07 a
	19	White B	3.5±0.7 b	3.1±0.7 b	0.5±0.1	0.4±0.1 b	15.7±0.6	7.4±0.2 a	8.0±0.4	12.8±1.2 b	0.20±0.03 c
	20	White B+FR	6.6±1.1 c	5.3±0.8 c	0.6±0.1	0.9±0.2 a	17.4±0.4	4.9±0.6 b	8.2±0.3	17.8±1.1 a	0.36±0.06 b
Red fire	21	White A	10.9±0.9 c	9.4±1.0 b	1.5±0.1 b	1.4±0.1	19.2±0.3 b	6.9±0.6 c	6.5±0.3 b	14.4±1.7	0.71±0.08 b
	22	White A+FR	20.7±1.3 a	15.1±1.7 a	5.6±0.6 a	2.0±0.6	22.5±0.5 a	19.9±0.8 b	8.3±0.4 a	13.2±0.9	1.17±0.15 a
	23	White B	17.9±0.8 bc	13.0±1.2 ab	4.9±0.6 a	2.0±0.4	22.1±0.6 a	22.3±1.3 ab	7.4±0.3 ab	14.9±1.1	0.91±0.11 ab
	24	White B+FR	16.7±0.6 b	11.6±1.1 b	5.1±0.6 a	1.3±0.2	21.4± 0.6 a	24.7±1.6 a	8.5±0.3 a	14.3±0.7	0.93±0.10 ab
Bancyu red fire	25	White A	9.5±0.6 a	8.6±0.4 a	1.0±0.3 a	1.5±0.3	19.1±0.8 ab	7.0±0.7 a	8.4±0.5	16.5±2.3	0.48±0.08
	26	White A+FR	5.6±0.7 b	5.4±0.7 b	0.3±0.1 b	0.9±0.1	16.3±0.8 b	3.4±0.6 c	8.1±0.3	19.8±2.4	0.34±0.05
	27	White B	8.5±0.6 a	8.0±0.5 a	0.6±0.1 ab	1.2±0.2	20.7±0.8 a	5.3±0.4 bc	8.5±0.7	15.5±2.0	0.47±0.03
	28	White B+FR	6.0±0.9 ab	6.0±0.9 ab	0.2±0.0 b	1.0±0.1	15.3±0.8 b	3.1±0.4 c	8.6±0.4	18.8±2.6	0.38±0.06
Sun marino	29	White A	3.7±0.3 b	3.6±0.3 ab	0.1±0.0 b	1.1±0.2	10.0±0.3 b	2.5±0.6	8.1±0.3	14.1±1.4 b	0.20±0.02 b
	30	White A+FR	6.3±0.8 a	5.5±0.9 b	0.3±0.1 a	0.8±0.2	11.8±0.7 ab	2.8±0.5	8.3±0.5	17.0±1.0 a	0.34±0.03 a
	31	White B	4.2±0.6 b	3.6±0.7 ab	0.2±0.0 a	0.8±0.2	12.1±0.3 a	2.5±0.3	7.8±0.3	17.9±1.1 a	0.33±0.05 a
	32	White B+FR	2.7±0.2 b	2.7±0.3 a	0.4±0.1 a	0.7±0.1	10.8±0.7 ab	3.5±1.3	7.5±0.3	12.5±1.4 b	0.24±0.06 ab
Calbee red	33	White A	13.2±1.5 a	8.5±0.9 a	1.0±0.4 b	1.3±0.2 ab	16.5±0.4 b	5.9±1.2 c	7.7±0.2	19.8±0.9 a	0.73±0.10 a
	34	White A+FR	13.1±0.9 a	8.7±0.5 a	2.9±0.2 a	1.6±0.2 a	20.3±0.6 a	16.2±0.6 a	7.8±0.3	18.5±0.5 ab	0.64±0.04 a
	35	White B	4.6±0.7 b	4.0±0.6 b	1.7±0.3 b	0.9±0.1 ab	17.6±0.4 b	13.8±0.5 b	6.7±0.5	19.4±1.2 ab	0.26±0.03 b
	36	White B+FR	7.5±0.6 b	4.6±0.5 b	1.7±0.3 b	0.6±0.1 b	18.0±1.4 ab	17.7±0.7 a	6.6±0.3	16.0±1.14 b	0.38±0.06 b

Each value was indicated by mean±standard error (n=8). Different letters indicate significant differences by Tukey's multiple test with a significance level of 0.05.

maximum leaf length tended to be more when grown under White A + FR and under White B. The main stem was significantly greater than that grown under White A, but the SPAD was lower than that grown under the other LEDs. In 'Green wave', there was no difference in fresh total weight grown under White A, White B, and White B + FR, and the fresh leaf weight and dry total weight tended to be more when grown under White A. In 'Yakiniku lettuce', there was no difference in fresh total weight, stem weight, maximum leaf length, and dry total weight when grown under White A + FR, White B, and White B + FR. The root weight, stem weight, number of leaves, and dry total weight of plants grown under White A tended to

Table 3 - The growth difference in green lettuce cultivars by two kinds of white LED and FR light treatment

Cultivars		Kinds of LED	Fresh total weight (g)	Fresh leaf weight (g)	Stem weight (g)	Root weight g)	Maximum leaf length (cm)	Main stem length (cm)	Number of leaf	SPAD	Dry weight (g)
Summer green	37	White A	14.4±0.8 a	12.2±0.6 a	2.1±0.4 ab	1.7±0.2	18.1±0.6	9.5±0.8 b	14.8±1.3	32.2±3.2	0.42±0.09 ab
	38	White A+FR	12.7±1.1 b	8.6±1.0 b	2.3±0.3 ab	1.6±0.2	18.3±0.6	10.6±0.5 b	15.3±0.9	39.2±3.0	0.31±0.05 b
	39	White B	12.4±0.6 b	10.4±0.4 b	2.0±0.2 b	1.6±0.4	17.7±0.8	11.6±0.5 b	16.1±1.1	36.3±1.9	0.33±0.08 ab
	40	White B+FR	13.8±1.1 ab	12.1±0.5 a	3.1±0.4 a	1.6±0.5	19.3±0.3	16.3±1.1 a	14.1±1.0	28.4±4.5	0.63±0.10 a
Fancy green	41	White A	14.1±0.4 b	11.8±0.7 b	1.6±0.1 ab	1.0±0.1 b	19.3±0.5 b	10.4±0.4 a	10.0±0.4	16.4±1.5 b	0.49±0.04 b
	42	White A+FR	17.5±1.3 a	15.2±0.4 a	1.3±0.2 bc	1.5±0.3 ab	23.5±0.7 a	6.4±0.4 b	9.9±0.6	24.9±1.3 a	0.57±0.06 a
	43	White B	19.5±0.7 a	16.6±0.7 a	1.9±0.1 ab	1.8±0.2 ab	24.7±0.7 a	7.3±0.5 b	9.1±0.7	27.8±2.7 a	0.63±0.07 a
	44	White B+FR	10.4±0.7 c	9.2±0.7 b	0.7±0.1 c	2.1±0.5 a	19.7±0.6 b	5.7±0.6 b	9.8±0.3	30.3±1.4 a	0.49±0.05 b
Green wave	45	White A	6.2±0.4 a	3.9±0.9	2.3 ±0.5	0.6±0.1 a	21.7±0.8 b	13.5±0.5	7.0±0.5	24.3±2.8	0.57±0.11
	46	White A+FR	4.4±0.5 b	2.7±0.3	1.7 ±0.2	0.4±0.1 b	21.5±1.3 b	13.4±0.5	6.7±0.6	22.9±0.9	0.47±0.06
	47	White B	5.1 ±1.1 ab	2.8±0.7	2.3±0.5	0.6 ±0.1 a	25.9±1.4 a	13.8±0.6	7.0±0.3	22.3±1.1	0.50±0.09
	48	White B+FR	5.1 ±0.6 ab	3.0±0.4	2.1± 0.3	0.4±0.1 b	26.8±1.6 a	14.3±0.5	6.6±0.3	22.3±1.1	0.53±0.03
Yakiniku lettuce	49	White A	2.5±0.4 b	1.8±0.3 b	0.3±0.1 b	0.4±0.1 b	10.3±0.5 b	7.4±0.6 b	6.3±0.5	18.6±1.3 a	0.16±0.02 b
	50	White A+FR	4.1±0.3 a	3.4±0.3 a	0.7±0.1 a	0.7±0.1 a	12.0±0.4 ab	9.1±0.5 ab	6.5±0.4	20.8±1.9 a	0.39±0.04 a
	51	White B	3.4±0.6 ab	2.0±0.3 b	0.7±0.1 a	0.3±0.1 b	13.7±0.8 a	11.1±0.9 a	6.6±0.6	14.8±1.5 b	0.31±0.01 a
	52	White B+FR	4.0±0.2 a	3.3±0.2 a	0.7±0.1 a	0.5±0.1 ab	12.9±0.3 a	10.3±0.5 a	6.8±0.6	15.7±1.2 ab	0.32±0.04 a

Each value was indicated by mean±standard error (n=8). Different letters indicate significant differences by Tukey's multiple test with a significance level of 0.05.

decrease, and a significant difference was observed in the stem weight and dry total weight. Although the total light intensity was higher in White A + FR and White B + FR than in White A and White B, White B + FR showed no significant decrease in fresh leaf weight and dry weight only in 'Fancy green' grown under white B, suggesting that FR LED had little effect on the total light intensity in this experiment. However, it would be necessary to investigate the effects of similar total light intensity with and without FR irradiation in the future.

Principal component analysis was conducted to divide the cultivars into red lettuce cultivars and green lettuce cultivars to make it easier to understand the tendency. The average values of various traits for each LED treatments for each cultivar were used, and the parameters are shown in Tables 4 and 5. The contribution rates of the first principal components of the red lettuce cultivars and green lettuce cultivars were 47.34% and 38.23%, respectively. The first principal component in red lettuce cultivars showed a positive factor loading for all traits except the main stem length, and a negative factor loading for only the main stem length. The first principal component in green lettuce cultivars showed a positive factor loading for fresh total weight, fresh leaf weight, stem weight, maximum leaf length, and main stem length, and a negative factor loading for root weight, number of leaves, SPAD, and dry total weight. Furthermore, the second main component in red lettuce cultivars showed a positive factor loading for stem weight and main stem length, and a negative factor loading for the others. The second main component for green lettuce cultivars showed a positive factor loading for fresh total weight, and fresh

Table 4 - Eigen value, contribution and factor loading of 1st, 2nd and 3rd principal components in red leaf lettuce cultivars

Characteristics	Component No.		
	1	2	3
Fresh total weight (g)	0.460	-0.011	0.049
Fresh leaf weight (g)	0.458	-0.027	-0.028
Stem weight (g)	0.327	0..541	0.179
Root weight (g)	0.411	-0.154	-0.234
Maximum leaf length	0.333	0.281	0.425
Lengh of main stem (cm)	-0.038	0.648	-0.124
No. of leaves	0.266	-0.347	-0.332
SPAD	0.071	-0.385	0.738
Dry weight (g)	0.337	-0.051	-0.244
Eigen value	3.287	1.423	0.868
Contribution	47.34%	20.49%	12.51%
Cumulative contribution	47.34%	67.84%	80.34%

Table 5 - Eigen value, contribution and factor loading of 1st, 2nd and 3rd principal components in green leaf lettuce cultivars

Characteristics	Component No.		
	1	2	3
Fresh total weight (g)	0.354	0.418	0.059
Fresh leaf weight (g)	0.285	0.442	-0.015
Stem weight (g)	0.473	0.188	0.143
Root weight (g)	-0.101	0.462	0.330
Maximum leaf length	0.449	0.004	-0.227
Lengh of main stem (cm)	0.427	-0.256	0.196
No. of leaves	-0.030	-0.189	0.875
SPAD	-0.357	0.380	-0.041
Dry weight (g)	-0.216	0.368	0.095
Eigen value	2.752	2.297	0.890
Contribution	38.23%	31.93%	12.37%
Cumulative contribution	38.23%	70.13%	82.5%

leaf weight, stem weight, root weight, maximum leaf length, SPAD, and dry total weight, and a negative factor loading for the others. The cumulative contribution rates of the first and second principal components were 67.84% and 70.13% in the red and green lettuce cultivars, respectively. Principal component analysis was performed because the values were considered to be effective.

For red lettuce cultivars, the scatter diagram of the types of LEDs and each cultivar for the first (Z1) and second main components (Z2) showed that the fresh total weight, fresh leaf weight, stem weight, root weight, and dry total weight in 'Red wave', 'Bancyu sun bright', 'Sun bright', 'Fancy red', 'Red fire', 'Sun Marino' and 'Calbee red' grown under White A + FR was higher (Fig. 2). In addition, it showed an opposite tend for the fresh total weight, fresh leaf weight, and dry weight in 'Leaf lettuce red' and 'Bancyu red fire' grown under white B. Also, for 'Bancyu sun bright', 'Sun bright', 'Fancy red', and 'Calbee red' grown under White B, the fresh total weight, fresh leaf weight, and dry weight tended to be less. While the maximum leaf length for these cultivars grown under White B tended to be less, the main stem length tended to be more. On the other hand, for green lettuce cultivars, 'Summer green' grown under White A + FR and White B, 'Yakiniku lettuce' grown under White A, and 'Green wave' grown under White A + FR showed a tendency for the fresh weight, fresh leaf weight, stem weight, and dry weight and the maximum leaf length to be lower as shown in the scatter diagram for the first and second main components (Fig. 3).

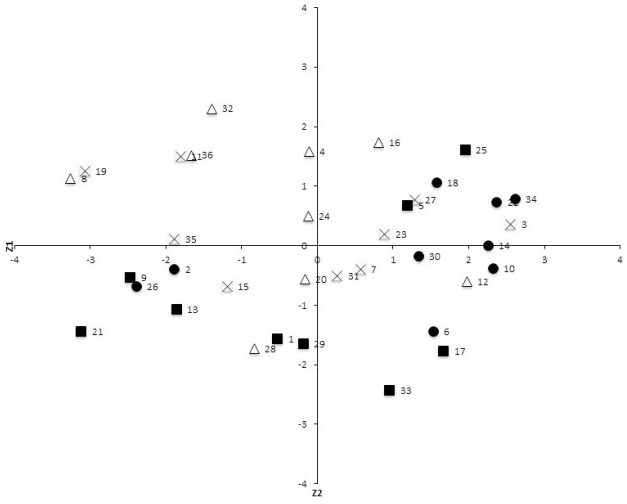

Fig. 2 - The scatter diagram in Z1-Z2 plane nine characteristics in red lettuce cultivars arranged by the principal component analysis. ■: White A irradiation, ●: White A + FR irradiation, ×: White B irradiation, △: White B + FR irradiation *Numbers were shown in Table 2.

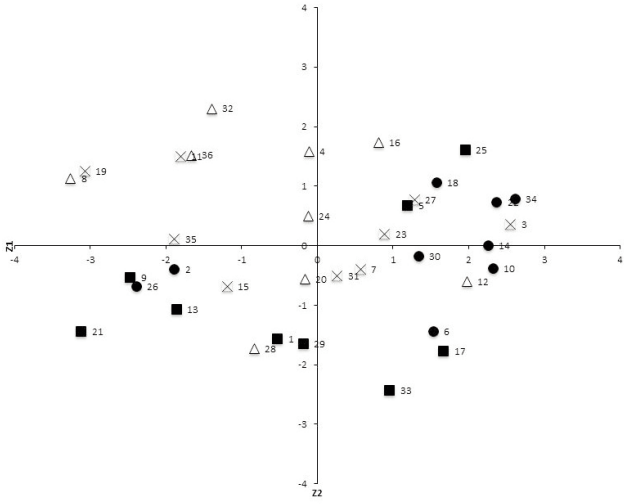

Fig. 3 - The scatter diagram in Z1-Z2 plane nine characteristics in red lettuce cultivars arranged by the principal component analysis. ■: White A irradiation, ●: White A + FR irradiation, ×: White B irradiation, △: White B + FR irradiation. * Numbers were shown in Table 3.

4. Discussion and Conclusions

The fresh total weight, fresh leaf weights, and dry total weight cultivated under White A and B with or without FR LED in lettuce cultivars tested in this study increased or decreased. It was considered that the

type of LED irradiated in the photoperiod was deeply related to the effect of the FR irradiated in the dark period, as this phenomenon was similar to the elongation of the main stem (Tables 2 and 3). For the cultivars 'Leaf lettuce red', 'Bancyu red fire', 'Calbee red', and 'Green wave', it was considered better to use only white LEDs without FR treatment because white LED irradiation with FR LED did not increase leaf weight and the running cost was more. However, white LEDs that are generally sold have different wavelengths depending on the manufacturer, and hence this inference may not apply to all white LEDs.

In general, it has been reported that FR light treatment promotes the elongation of the main stem or hypocotyl in cucumber (*Cucumis sativus* L.), lily *(Lilium longiforum* Thumb.), and chrysanthemum (*Chrysanthemum moriforlium* Ramat.) (Blom *et al*., 1995; Xiong *et al*., 2002; Hisamatsu *et al*., 2008). However, in Brassicaceae, Akutsu *et al*. (2016) reported that komatsuna and red mustard (*B. juncea*) were unable to extend the main stem, while pak choi and 'shimana' (a cultivation of *B. juncea*) easily extended the main stem regardless of the cultivation period and the light intensity of FR LEDs; thus, the effect differed depending on the plant species and cultivars being treated with the FR LED. Furthermore, the effect of FR treatment on leaf area and main stem in komatsuna and pak choi differed depending on the variety (Akutsu *et al*., 2017). The elongation of the main stem causes quality deterioration in terms of appearance and taste in leafy vegetables such as lettuce. The main stem was elongated regardless of the FR treatment for all lettuce cultivars tested in this experiment. However, it was found that the relationship between the main stem elongation and FR treatment can be divided into several patterns regardless of whether they were green or red lettuce cultivars. Under White A irradiation, FR treatment did not promote the main stem elongation in 'Leaf lettuce red', 'Red wave', 'Fancy red', 'Sun marino', 'Summer green', 'Green wave', and 'Yakiniku lettuce', while it promoted the main stem elongation in 'Sun bright', 'Red fire', and 'Calbee red'. In addition, FR treatment suppressed the main stem elongation in 'Bancyu sun bright', 'Bancyu red fire', and 'Fancy green'. On the other hand, under White B irradiation, FR treatment did not have an effect on the main stem elongation in 'Leaf lettuce red', 'Red wave', 'Bancyu sun bright', 'Red fire', 'Sun marino', 'Fancy green', 'Green wave', and 'Yakiniku lettuce', while it promoted the main stem elongation in 'Sun bright', 'Calbee red' and 'Summer green'. In addition, FR treatment suppressed the main stem elongation in 'Fancy Green' and 'Bancyu red fire'. Thus, it was considered that the relationship between FR irradiation and main stem elongation depends on the variety and cultivar. In addition, some cultivars such as 'Fancy red', 'Red fire', 'Summer green' and 'Fancy green', which showed an increase or a decrease in the main stem elongation after FR treatment, depending on if they were cultivated under White A or White B irradiation. There are also reports that FR treatment did not have any effect on main stem elongation in lettuce (Mickens *et al*., 2018; Lin *et al.*, 2020). Therefore, it was found that the effect of FR treatment on the main stem elongation differs depending on the wavelength of the LED irradiated in the photoperiod and on the cultivars. On the other hand, some cultivars such as 'Sun bright', 'Sun marino', and 'Fancy green', which an increase in SPAD after FR treatment depending on if they were cultivated under White A or White B irradiation, there was no effect of FR irradiation on SPAD in the other cultivars investigated in this experiment (Table 2 and 3). Thus, it was considered that no relationship between FR irradiation and SPAD depends on the variety and cultivar.

Furthermore, based on the results of the main component analysis for fresh and dry weights, the fresh total weight and the fresh leaf weight increased as the Z1 axis (the first principal component) became positive in all lettuce cultivars tested in this experiment, and the dry weight increased as the Z2 axis (the second principal component) became positive in red lettuce cultivars and as the Z2 axis became negative in green lettuce cultivars. Under White A LED irradiation, fresh total weight and fresh leaf weight were increased in all red lettuce cultivars except 'Leaf lettuce red' and 'Bancyu red fire' on treatment with FR LED, while the fresh total weight and fresh leaf weight were increased in only 'Fancy green' in green lettuce cultivars. This suggests that the effect of FR treatment cultivated under White A irradiation may be higher in red lettuce cultivars than in green lettuce cultivars. On the other hand, under White B irradiation, fresh total weight and fresh leaf weight were increased in only 'Bancyu sun bright' and 'Sun bright' of red lettuce cultivars and in 'Summer green' and 'Yakiniku lettuce' of green lettuce cultivars on the treatment FR LED. This suggests that the effect of FR treatment when cultivated under White A may be higher than when cultivated under White B in red let-

tuce cultivars. Therefore, it is necessary to select lettuce cultivars that match the type of white LED used as a source of light in the photoperiod, and depending on the lettuce cultivar used, select whether to treat with FR LED.

The main stem, which is unsuitable for sale was remarkably elongated under the two kinds of white LEDs either with or without FR treatment for the 13 leaf lettuce cultivars tested in this experiment. In general, white LEDs have different intensity, spectrum, and shade by adjusting the monochromatic emission of red, yellow, green, and blue (Chang *et al*., 2012), as shown in white A and B, respectively, used in this experiment. Also, In lettuce, the effect of FR light is clearly intensity-dependent, and the intensity required for maximum response depends on the trait (Zou *et al*., 2021). Furthermore, the effect of FR light has been found to depend on the type of photosynthetic photon flux density (PPFD, 400-700 nm) radiation and light intensity (Meng and Runkle, 2019). This suggests that it might be possible to obtain the effect of FR light by changing the type and intensity of the white LEDs used in this experiment, or by changing the intensity of FR light, even in lettuce cultivars that were investigated in this study and did not show the FR light effect. Therefore, it would be necessary to investigate the characteristics of the White LEDs used and the plants before growing plants. Furthermore, as used in a previous report (Ishii *et al*., 2018), it would be necessary to investigate the effect of FR light on lettuce cultivars that did not elongate the main stem when they were cultivated under monoclonal red or blue, or mixed red-blue LEDs.

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