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Selection of cut flower species affected promotion of flowering and stem elongation by far-red lighting or heating treatments on end of day under limited sunshine from autumn to winter

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Abstract: The effect of a brief exposure to treatments of end-of-day (EOD) far-red (FR) light and EOD-heating on flowering and stem elongation in certain species cultivated in areas with limited sunshine from autumn to winter has been investigated. Thirteen EOD-FR treated cultivars among all 24 cultivars tested in experiment 1, showed earlier flowering than control plants. Additionally, Kanzaki No.21, F_1 Winter cherry and Gypsy deep rose were earlier to flower under the all night-FR treatment than under the EOD-FR treatment. Further, 13 cultivars among all cultivars tested in experiment 1 showed greater stem length, whereas 16 cultivars of them showed greater mean internode length than controls when treated with EOD-FR. The days to flowering in 13 cultivars treated by EOD-Heating, flowering was earlier than in controls. Also, stem length and mean internode length were also promoted by the same treatment in 7 cultivars. Additionally, flowering and stem elongation of Stella rose, Arizona sun, Suzuhime and Extra carmine rose were promoted by a combination of both treatments, and productivity and quality were improved. These results suggest that the treatments tested can help the production of cut-flowers in areas with limited sunshine from autumn to winter, although the method of application of the treatments must be optimized for each species.

Keywords: EOD; FR; flowering days; stem length

Field production of cut flowers depends on natural conditions of light and temperature that promote flowering and stem elongation. Thus, low light intensity and low temperature in areas with limited sunshine from autumn to winter is a major issue when it comes to the production of photoperiod sensitive cut flower species. In such places, a large variety of plant growth regulators are widely used for promoting flowering and stem elongation of plants. However, many cultivars do not respond consistently to plant growth regulator application and, in many cases, species response may be highly regulator-specific. Therefore, the promotion of flowering and stem elongation by environmental control is very important for the production of cut flowers when many different cultivars are involved.

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Recently, the use of far-red (FR) light for illumination treatment has received a great deal of attention as an effective means to force flowering. The most well-known of light receptors present in leaves are phytochromes, which are soluble pigmented proteins that can exist in two spectrally distinct forms (phytochromes A [phyA] and B [phyB]) and can sense ambient light conditions by photo interconversion between red (R) and FR light-absorbing forms (Furuya 1993). Additionally, the R/FR ratio has been shown to effectively control short elongation in a number species during the entire light phase or over a short period at EOD, with FR and R enhancing and reducing elongation, respectively (Kasperbauer, Peaslee 1973; Gilbert et al. 1995). In addition, the acceleration of flowering by illumination with a low R/FR ratio has recently been described in a wide range of long-day plants, including *Arabidopsis* (Hori et al. 2011), *Eustoma grandiflorum* R. (Sato et al. 2009), and *Gypsophila paniculata* (Hori et al. 2011).

Additionally, a new night temperature regime known as EOD-heating has been used on growing flowers (Douzono et al. 2012a, b). Treatment by EOD-heating is effective to promote flowering under an energy-saving night temperature condition. In the spray-type chrysanthemum 'Sei-Roza' grown under a temperature regime of 16/8 °C (light/dark period), it was shown that EOD-heating treatment accelerated flower initiation and early flower bud development (Douzono et al. 2012a). Additionally, in the African marigold, it was reported that early growth and flower bud development after flower budding were accelerated by EOD-heat treatment (Douzono et al. 2012b). On the other hand, little is known about the promotion of flowering or stem elongation by this treatment in other cut flower plant species. Additionally, it is assumed that cut flower species have many different cultivars that may vary widely in their response to these treatments. Therefore, it is important to evaluate days to flowering and stem elongation in field plants to gain knowledge of the effect of these treatments.

In this study, we investigated the effect of brief exposure to EOD-FR and EOD-heating treatments on flowering and stem elongation in certain photoperiod sensitive cut flower species grown in areas with limited sunshine from autumn to winter. We focused on the *Brassicaceae* and *Asteraceae*, within which a large number of member species are cultivated as cut flowers.

MATERIAL AND METHODS

Effect of EOD- or all night- FR treatments on growth (Experiment 1). Twenty-four cut flower cultivars from 11 families as recommended cultivar during winter in Tottori prefecture (Tables 1 and 2) (ESM Table S1) were grown in the Tottori Horticultural Experiment Station (35.5°N, 133.7°E) for this experiment (ESM Table S2). Seeds of all cultivars were sown on August 25th, 2014 in plastic germination trays with 288 cells (21 mL/cell) filled with Metromix 350 (Sun Gro Horticulture Distribution Inc., USA). The seeded trays were kept in a dark cool-room at 18 °C. When seedlings reached leaf overlapping stage within cultivars they were transplanted into polyethylene pots (9 cm diameter, 7.6 cm deep) filled with chaff, Kanuma and Akadama soil mixture, peat moss, and perlite at a ratio of 1 : 1 : 1 : 1 (v/v). Afterwards, the pots were transferred to a greenhouse to grow under a constant temperature of 25 °C during the day (08:00–17:00) and 11 °C at night (17:00–08:00). Subsequently, the seedlings were treated with EOD or with all night FR light during sunset to after 3 hours, or sunrise, using emits pure far-red light fluorescent lamp with a peak at 740 nm (0.14 W/m^2) (Fuji Electric Co., Tottori, Japan) (ESM Figure S1). Control plants were grown under ambient light without FR treatment.

The date of flowering was recorded in 10 plants from each treatment; days to flowering were defined as the number of days from planting to flowering in each cultivar. Based on the definition of flowering in each cultivar, flowering time was defined visually as day observed open flower of fist flower in inflorescences or a single flower. At flowering, each plant was collected, and the following characteristics of the cut flowers were recorded: node number of flower bud differentiation, stem length, nude number, and plant width. Additionally, chlorophyll content of five randomly selected expanded leaves were measured using a chlorophyll meter (SPAD-502 plus; Konica Minolta, Inc., Tokyo, Japan). Statistical analysis was performed by Tukey–Kramer HSD tests using SPSS software (SPSS Statistics 23.0, Chicago, USA).

Effect of EOD- heating or -FR treatments on growth (Experiment 2). Eighteen cut flower cultivars from 11 families as recommended cultivar during winter in Tottori prefecture (Tables 3 and 4, ESM Table S1) were grown in the Tottori Horticultural

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Table 1. Effect of EOD- or all night- FR treatments on the growth of long-day plants

Family Genus Cultivar	Treatment	Days to flowering	Node number of flower bud differentiation	Stem length (cm)	Mean internode length (mm)	Plant width (cm)	Leaf colour (SPAD value)
Brassicaceae	Control		22^{a}	6.0^{c}	0.27^{c}	26.2^{a}	27.6^{a}
Brassica rapa	EOD-FR	88^{b}	16^{b}	41.0^{b}	2.56^{b}	26.5^{a}	31.6^{a}
Kanzaki No.21	All night-FR	84^{c}	16^{b}	54.7^{a}	3.42^{a}	23.0^{b}	29.2^{a}
Brassicaceae	Control	112^{a}	51^{a}	17.2^{b}	0.34^{b}	22.5^{a}	60.5^{a}
Brassica oleracea	EOD-FR	92^{b}	48^{b}	18.6^{b}	0.39^{ab}	23.0^{a}	46.4^{a}
F_1 Winter cherry	All night-FR	82^{c}	48^{b}	27.0^{a}	0.56^{a}	19.5^{b}	60.3^{a}
Brassicaceae	Control	107^{a}	20^{a}	19.2^{b}	0.96^{b}	24.0^{a}	53.3^{a}
Matthiola incana	EOD-FR	95^{b}	20^{a}	29.0^{a}	1.45^{a}	17.0^{b}	48.6^{c}
Baby white	All night-FR	95^{b}	20^{a}	30.2^{a}	1.51^{a}	18.5^{b}	50.9^{b}
Brassicaceae	Control	64^{a}	13^{c}	5.2^{b}	0.40^{a}	9.3^{b}	47.6^{a}
Lobularia maritima	EOD-FR	61^{ab}	15^{b}	5.6^{b}	0.37^{a}	10.4^{ab}	49.3^{a}
Easter bonnet white	All night-FR	59^{b}	17^{a}	6.7^{a}	0.39^{a}	11.8^{a}	41.8^{a}
Asteraceae	Control	115^{a}	28^{a}	9.8^{a}	0.35^{b}	34.2^{a}	46.8^{a}
Calendula officinalis	EOD-FR	115^{a}	25^{b}	9.9^{a}	0.40^{a}	33.9^{a}	45.0^{a}
Orange gem	All night-FR	115^{a}	24^{b}	10.5^{a}	0.44^{a}	34.0^{a}	45.8^{a}
Asteraceae	Control	121^{a}	17^{a}	9.4^{b}	0.55^{b}	20.0^{a}	46.6^{a}
Bellis perennis	EOD-FR	123^{a}	13^{b}	13.1^{a}	1.01^{a}	20.5^{a}	47.0^{a}
Early pomponette red	All night-FR	121^{a}	13^{b}	13.6^{a}	1.05^{a}	21.5^{a}	46.9^{a}
Plantaginaceae	Control	158^{a}	10^{a}	17.9^{a}	1.79^{b}	21.5^{a}	40.7^{a}
Antirrhinum majus	EOD-FR	151^{b}	9^{b}	19.6^{a}	2.18^{a}	20.5^{a}	42.3^{a}
Snapshot yellow	All night-FR	149^{b}	9^{b}	19.5^{a}	2.17^{a}	20.5^{a}	44.4^{a}
Scrophulariaceae	Control	124^{a}	10^{a}	13.3^{b}	1.33^{b}	26.0^{b}	25.1^{a}
Nemesia caerulea	EOD-FR	121^{a}	9^{b}	16.3^{a}	1.81^{a}	32.0^{a}	26.4^{a}
Rapin yellow	All night-FR	121^{a}	9^{b}	17.7^{a}	1.97^{a}	34.5^{a}	31.6^{a}
Violaceae Viola	Control	115^{a}	16^{a}	2.2^{c}	0.14^{b}	13.7^{b}	73.2^{a}
Viola tricolor	EOD-FR	109^{b}	16^{a}	2.4^{b}	0.15^{b}	16.7^{a}	62.1^{b}
Delta premium pure-goldenyellow	All night-FR	109^{b}	16^{a}	3.2^{a}	0.20^{a}	16.1^{a}	58.1^{c}
Solanaceae	Control	106^{a}	23^{a}	11.4^{b}	0.50^{b}	29.2^{a}	46.6^{a}
Petunia hybrida	EOD-FR	109^{a}	23^{a}	11.7^{b}	0.51^{b}	26.6^{a}	45.5^{a}
Baccarat rose morne	All night-FR	106^{a}	23^{a}	15.1^{a}	0.66^{a}	29.4^{a}	55.9^{a}
Caryophyllaceae	Control	146^{a}	12^{a}	9.4^{b}	0.78^{b}	20.8^{b}	–
Gypsophila muralis	EOD-FR	132^{b}	11^{b}	10.8^{ab}	0.98^{a}	24.5^{a}	–
Gypsy deep rose	All night-FR	122^{c}	11^{b}	11.4^{a}	1.04^{a}	25.0^{a}	–
Caryophyllaceae	Control	148^{a}	13^{a}	22.7^{c}	1.75^{c}	29.5^{a}	41.2^{a}
Dianthus chinensis	EOD-FR	127^{b}	12^{b}	26.0^{b}	2.17^{b}	26.5^{b}	51.6^{a}
Supra purple	All night-FR	127^{b}	12^{b}	27.6^{a}	2.30^{a}	26.5^{b}	40.1^{a}
Dipsacaceae	Control	162^{a}	7^{a}	1.3^{b}	0.19^{b}	12.0^{b}	59.9^{a}
Scabiosa japonica	EOD-FR	149^{b}	6^{b}	2.5^{a}	0.42^{a}	22.0^{a}	57.1^{a}
Ritz blue imp	All night-FR	146^{b}	6^{b}	2.3^{a}	0.38^{a}	21.0^{a}	51.1^{a}

[z]Different letters indicate significant differences T among species at $P < 0.05$ by Tukey-Kramer's HSD test ($n = 5$)

Experiment Station for this experiment. Seeds of all cultivars were sown on August 25, 2014, kept in the dark cool-room and then transplanted into the same pots as previously described in Experiment 1.

The pots were transferred to a greenhouse and grown under EOD-heating treatment under a constant temperature of 25 °C during the day (sunrise to sunset), at 20 °C at EOD (for 3 h starting from sunset) and at 11 °C at night (from the time at which EOD treatment ended to sunrise). Control plants were grown under a constant temperature of 25 °C during the day (sunrise to sunset) and 11 °C at night

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Table 2. Effect of EOD- or all night- FR treatments on the growth of day-neutral or short-day plants

Family Genus Cultivar	Treatment	Days to flowering	Node numb. of flower bud differentiation	Stem length (cm)	Mean inter-node length (mm)	Plant width (cm)	Leaf color (SPAD value)
Asteraceae	Control	162^{az}	17^{a}	4.9^{a}	0.29^{a}	24.0^{b}	62.2^{a}
Gazania rigens	EOD-FR	149^{b}	16^{b}	1.5^{b}	0.09^{b}	31.5^{a}	56.3^{a}
F_1 New day clear orange	All night-FR	145^{b}	16^{b}	1.1^{c}	0.07^{b}	30.0^{a}	60.7^{a}
Asteraceae	Control	135^{a}	23^{a}	5.4^{c}	0.23^{b}	21.5^{b}	54.4^{a}
senecio bicolor	EOD-FR	135^{a}	22^{a}	10.3^{b}	0.47^{a}	24.5^{a}	62.2^{a}
Silver dust	All night-FR	135^{a}	22^{a}	11.1^{a}	0.50^{a}	24.0^{a}	56.3^{a}
Asteraceae	Control	75^{a}	5^{a}	12.5^{b}	2.50^{b}	18.0^{a}	43.7^{a}
Cosmos bipinnatus	EOD-FR	72^{b}	5^{a}	14.0^{ab}	2.80^{ab}	13.0^{b}	43.0^{a}
Road orange	All night-FR	71^{b}	5^{a}	16.2^{a}	3.24^{a}	12.5^{b}	42.1^{a}
Asteraceae	Control	115^{b}	9^{b}	19.6^{a}	2.18^{a}	41.0^{a}	35.6^{a}
Dahlia hybrida	EOD-FR	133^{a}	12^{a}	19.2^{a}	1.60^{b}	30.5^{b}	40.0^{a}
Early bird	All night-FR	135^{a}	11^{a}	19.5^{a}	1.77^{b}	26.5^{c}	32.6^{a}
Asteraceae	Control	121^{a}	20^{b}	21.5^{b}	1.08^{a}	28.0^{a}	41.3^{a}
Helianthus annus	EOD-FR	123^{a}	23^{a}	22.5^{ab}	0.98^{a}	27.5^{a}	39.3^{a}
F_1 Ballade	All night-FR	123^{a}	24^{a}	26.6^{a}	1.11^{a}	25.0^{b}	38.1^{a}
Asteraceae	Control	88^{a}	5^{b}	14.6^{b}	2.92^{b}	13.2^{c}	30.0^{b}
Zinnia elegans	EOD-FR	88^{a}	6^{a}	24.2^{a}	4.03^{a}	16.7^{b}	39.5^{a}
Zinnita scarlet	All night-FR	88^{a}	6^{a}	25.0^{a}	4.17^{a}	18.4^{a}	37.6^{a}
Asteraceae	Control	150^{a}	9^{a}	33.7^{b}	3.74^{c}	25.0^{b}	45.0^{b}
Ageratum houstonianum	EOD-FR	130^{b}	8^{b}	38.6^{ab}	4.83^{b}	25.5^{b}	51.6^{a}
Top blue	All night-FR	127^{b}	8^{b}	40.3^{a}	5.04^{a}	27.5^{a}	48.8^{a}
Balsaminaceae	Control	92^{a}	16^{a}	5.2^{b}	0.33^{a}	12.0^{a}	49.5^{a}
Impatiens walleriana	EOD-FR	92^{a}	16^{a}	6.3^{a}	0.39^{a}	10.5^{b}	53.8^{a}
F_1 Impureza red	All night-FR	92^{a}	16^{a}	6.2^{a}	0.39^{a}	10.8^{b}	55.9^{a}
Lamiaceae	Control	102^{a}	8^{a}	10.2^{c}	1.28^{c}	13.0^{a}	45.1^{a}
Salvia splendens	EOD-FR	88^{b}	7^{b}	12.0^{b}	1.71^{b}	14.2^{a}	47.3^{a}
Red alert	All night-FR	88^{b}	7^{b}	14.5^{a}	2.07^{a}	14.4^{a}	43.3^{a}
Lamiaceae	Control	–	9^{a}	6.0^{c}	0.67^{c}	14.4^{b}	20.3^{a}
Solenostemon scutellari-oides	EOD-FR	121^{a}	9^{a}	6.8^{b}	0.76^{b}	16.0^{a}	22.5^{a}
Gorilla Jr. Watermelon	All night-FR	121^{a}	9^{a}	7.4^{a}	0.82^{a}	17.0^{a}	21.9^{a}
Amaranthaceae	Control	68^{a}	6^{a}	5.8^{b}	0.97^{c}	11.1^{a}	35.0^{a}
Celosia argentea	EOD-FR	65^{b}	6^{a}	7.7^{a}	1.28^{b}	11.6^{a}	34.6^{a}
Kimono scarlet	All night-FR	65^{b}	6^{a}	8.4^{a}	1.40^{a}	12.2^{a}	32.8^{a}

^{z}Different letters indicate significant differences among species at $P < 0.05$ by Tukey-Kramer's HSD test ($n = 5$)

(sunset to sunrise). Seedlings under EOD-FR treatments during sunset to after 3 hours or sunrise were treated using a far-red light fluorescent lamp.

The date of flowering, characteristics of the cut flowers, the node number of flower bud differentiation, stem length, nude number, and plant width was recorded on plants from each treatment as previously described in Experiment 1. Statistical analysis was performed by Tukey–Kramer HSD tests and two-way analysis of variance (ANOVA) using SPSS software (SPSS Statistics 23.0, Chicago, USA).

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Table 3. Effect of EOD-heating or -FR treatments on the growth of long-day plants

Family Genus Cultivar	Treatment		Days to flowering	Node numb. of flower bud differentiation	Stem length (cm)	Mean inter-node length (mm)	Plant width (cm)
	EOD-heating	EOD-FR					
Asteraceae *Callistephus chinensis* Stella rose	Control	Control	228^{az}	46^{a}	13.4^{b}	0.3^{b}	17.5^{a}
		EOD-FR	197^{b}	36^{b}	31.4^{a}	0.9^{a}	18.5^{a}
	EOD-heating	Control	212^{a}	45^{a}	15.7^{b}	0.3^{b}	19.0^{a}
		EOD-FR	186^{b}	36^{b}	35.9^{a}	1.0^{a}	17.0^{a}
	Two-way ANOVA	Heating	NS^{y}	NS	NS	NS	NS
		FR	**	**	**	*	NS
		Heating and FR	**	**	**	*	NS
Asteraceae *Gaillardia grandiflora* Arizona sun	Control	Control	213^{a}	24^{b}	11.6^{d}	0.5^{b}	25.5^{b}
		EOD-FR	199^{b}	22^{c}	19.6^{b}	0.9^{ab}	29.5^{a}
	EOD-heating	Control	197^{b}	27^{a}	15.4^{c}	0.6^{b}	25.5^{b}
		EOD-FR	191^{c}	19^{d}	29.7^{a}	1.6^{a}	30.0^{a}
	Two-way ANOVA	Heating	*	*	*	NS	NS
		FR	*	**	*	*	*
		Heating and FR	**	**	**	*	*
Asteraceae *Coleostephus myconis* Upright yellow	Control	Control	169^{a}	36^{a}	12.9^{b}	0.4^{a}	26.2^{b}
		EOD-FR	169^{a}	36^{a}	19.2^{a}	0.5^{a}	28.5^{a}
	EOD-heating	Control	165^{a}	33^{b}	15.2^{a}	0.5^{a}	29.0^{a}
		EOD-FR	165^{a}	33^{b}	14.6^{a}	0.4^{a}	31.0^{a}
	Two-way ANOVA	Heating	NS	*	*	NS	*
		FR	NS	NS	*	NS	*
		Heating and FR	NS	*	*	NS	*
Asteraceae *Pericallis hybrida* Tear blue	Control	Control	184^{a}	17^{a}	19.0^{b}	1.1^{b}	33.0^{a}
		EOD-FR	175^{b}	16^{ab}	21.0^{a}	1.3^{ab}	36.5^{a}
	EOD-heating	Control	171^{b}	15^{b}	19.6^{b}	1.3^{ab}	32.5^{a}
		EOD-FR	165^{c}	15^{b}	20.5^{a}	1.4^{a}	32.0^{a}
	Two-way ANOVA	Heating	*	*	NS	NS	NS
		FR	*	NS	*	NS	NS
		Heating and FR	*	*	*	*	NS
Asteraceae *Rudbeckia hirta* Tiger eye gold	Control	Control	289^{a}	28^{a}	13.5^{c}	0.5^{b}	26.5^{a}
		EOD-FR	286^{a}	27^{a}	19.3^{b}	0.7^{ab}	28.7^{a}
	EOD-heating	Control	274^{b}	25^{b}	19.5^{b}	0.8^{ab}	21.5^{b}
		EOD-FR	271^{b}	24^{b}	29.2^{a}	1.2^{a}	17.2^{c}
	Two-way ANOVA	Heating	*	*	**	NS	*
		FR	NS	NS	*	NS	*
		Heating and FR	*	*	**	*	*
Lamiaceae *Lavandula angustifolia* Elegance purple	Control	Control	253^{a}	64^{a}	11.3^{c}	0.2^{b}	15.5^{c}
		EOD-FR	234^{ab}	58^{b}	17.7^{b}	0.3^{ab}	19.5^{a}
	EOD-Heating	Control	244^{a}	66^{a}	15.6^{bc}	0.2^{b}	14.5^{c}
		EOD-FR	229^{b}	56^{b}	28.1^{a}	0.5^{a}	16.5^{b}
	Two-way ANOVA	Heating	NS	NS	*	NS	*
		FR	*	**	*	*	*
		Heating and FR	*	**	*	*	*

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Table 3 to be continued: Effect of EOD-heating or -FR treatments on the growth of long-day plants

Family Genus Cultivar	Treatment		Days to flowering	Node numb. of flower bud differentiation	Stem length (cm)	Mean internode length (mm)	Plant width (cm)
	EOD-heating	EOD-FR					
Campanulaceae *Campanula rapunculus* Suzuhime	Control	Control	212^{a}	25^{a}	60.9^{c}	2.4^{c}	31.5^{a}
		EOD-FR	203^{b}	23^{b}	83.4^{a}	3.6^{a}	24.5^{a}
	EOD-Heating	Control	193^{c}	20^{c}	59.4^{c}	3.0^{b}	23.0^{a}
		EOD-FR	188^{c}	20^{c}	78.5^{a}	3.9^{a}	32.0^{a}
	Two-way ANOVA	Heating	**	*	NS	*	NS
		FR	*	*	**	*	NS
		Heating and FR	**	*	**	*	NS
Plumbaginaceae *Limonium sinuatum* Extra carmine rose	Control	Control	200^{a}	9^{a}	52.1^{c}	5.8^{d}	29.5^{b}
		EOD-FR	193^{b}	9^{a}	55.7^{b}	6.2^{c}	31.0^{a}
	EOD-Heating	Control	188^{c}	7^{b}	51.7^{c}	7.4^{b}	27.0^{b}
		EOD-FR	188^{c}	7^{b}	66.7^{a}	9.5^{a}	31.0^{a}
	Two-way ANOVA	Heating	*	*	*	*	NS
		FR	*	NS	**	*	**
		Heating and FR	*	*	**	*	**
Apiaceae *Ammi majus* White lace flower	Control	Control	243^{a}	32^{a}	89.2^{c}	2.8^{b}	49.5^{a}
		EOD-FR	212^{c}	24^{c}	115.4^{a}	4.8^{a}	59.0^{a}
	EOD-Heating	Control	233^{b}	28^{b}	103.4^{b}	3.7^{ab}	50.0^{a}
		EOD-FR	199^{d}	23^{d}	115.0^{a}	5.0^{a}	51.5^{a}
	Two-way ANOVA	Heating	*	*	*	NS	NS
		FR	*	*	**	*	NS
		Heating and FR	*	*	*	*	NS
Febaceae *Lupinus polyphyllus* Gallery red	Control	Control	253^{a}	33^{a}	17.3^{b}	0.5^{b}	29.0^{c}
		EOD-FR	233^{c}	26^{b}	38.8^{a}	1.5^{a}	50.5^{a}
	EOD-Heating	Control	249^{b}	28^{ab}	19.6^{b}	0.7^{b}	33.5^{c}
		EOD-FR	236^{c}	26^{b}	30.1^{a}	1.2^{a}	38.0^{b}
	Two-way ANOVA	Heating	*	NS	NS	NS	*
		FR	**	*	**	**	**
		Heating and FR	*	*	**	**	**

^{z}Different letters indicate significant differences among species at $P < 0.05$ by Tukey-Kramer's HSD test ($n = 5$); ^{y} NS, * and ** indicate non-significant, significant at $P < 0.05$ and significant at $P < 0.01$, respectively by Two-way ANOVA ($n = 5$)

RESULTS AND DISCUSSION

In the field of horticultural science, EOD-FR light treatment has been applied to numerous plant species as an artificial method to promote stem elongation or flowering of ornamental cut flowers, including *Chrysanthemum morifolium* R. (Lund et al. 2007), *E. grandiflorum* R. (Yamada et al. 2008), *Antirrhinum majus* L. (Sumitomo et al. 2009), *Helianthus annuus* L. (Sumitomo et al. 2009), *Matthiola incana* L. (Sumitomo et al. 2009), and *Cucumis sativus* L. (Xiong et al. 2011). The present study was performed to estimate the effects of EOD- or all night- FR light treatment on the morphology of several cut flowers in Experiment 1. Table 1 shows the effect of both FR treatments on the growth of each long-day cultivar tested. Among cultivars tested as long-day plant, days to flowering in 8 EOD- FR treated cultivars ('Kanzaki No. 21', 'F_1 Winter cherry', 'Baby white', 'Snapshot yellow', Delta premium puregoldenyellow', 'Gypsy deep rose', 'Supra purple', 'Ritz blue imp') were less than in controls, i.e., each of these cultivars flowers earlier than untreated plants of the same cultivar (Table 1). Additionally, the flowering of 'Kanzaki No. 21', 'F_1 Winter cherry' and 'Gypsy deep rose' among those 8 cultivars was promoted more significantly by the all-night – FR treatment. These results indicate that the FR treatment duration also affected shortening of the days to flowering. Additionally, the node number of flower bud differentia-

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Table 4. Effect of EOD-heating or -FR treatments on the growth of day-neutral or short-day plants

Family Genus Cultivar	Treatment		Days to flowering	Node numb. of flower bud differentiation	Stem length (cm)	Mean internode length (mm)	Plant width (cm)
	EOD-Heating	EOD-FR					
Asteraceae *Gerbera jamesonii* Festival scarlet eye	Control	Control	209^{bz}	22^{a}	15.2^{b}	0.7^{b}	37.3^{a}
		EOD-FR	217^{a}	23^{a}	16.2^{b}	0.7^{b}	33.6^{a}
	EOD-Heating	Control	197^{b}	19^{b}	24.6^{a}	1.3^{a}	36.4^{a}
		EOD-FR	198^{b}	19^{b}	28.2^{a}	1.5^{a}	38.2^{a}
	Two-way ANOVA	Heating	*	*	*	*	NS
		FR	*	NS ^{y}	NS	NS	NS
		Heating and FR	*	*	*	*	NS
Asteraceae *Tagetes erecta* Discovery yellow	Control	Control	176^{a}	9^{a}	9.2^{a}	1.0^{a}	18.0^{a}
		EOD-FR	172^{a}	9^{a}	9.2^{a}	1.0^{a}	17.0^{a}
	EOD-Heating	Control	167^{b}	8^{b}	9.5^{a}	1.2^{a}	16.0^{b}
		EOD-FR	164^{b}	8^{b}	9.1^{a}	1.1^{a}	16.5^{b}
	Two-way ANOVA	Heating	**	*	NS	NS	*
		FR	NS	NS	NS	NS	NS
		Heating and FR	*	*	NS	NS	*
Campanulaceae *Isotoma axillaris* Avant-garde blue	Control	Control	205^{a}	18^{a}	25.9^{b}	1.4^{d}	35.5^{b}
		EOD-FR	200^{a}	18^{a}	30.7^{a}	1.7^{b}	35.5^{b}
	EOD-Heating	Control	195^{b}	16^{b}	25.6^{b}	1.6^{c}	39.0^{a}
		EOD-FR	191^{b}	17^{b}	37.3^{a}	2.2^{a}	41.0^{a}
	Two-way ANOVA	Heating	**	*	NS	*	*
		FR	NS	NS	**	*	NS
		Heating and FR	**	*	**	**	*
Geraniaceae *Pelargonium hortorum* F_1 Horizon lavender	Control	Control	203^{a}	10^{a}	29.9^{b}	3.0^{c}	33.5^{b}
		EOD-FR	197^{a}	10^{a}	32.7^{ab}	3.3^{b}	33.5^{b}
	EOD-Heating	Control	185^{b}	10^{a}	33.8^{ab}	3.4^{b}	36.0^{ab}
		EOD-FR	181^{b}	10^{a}	43.1^{a}	4.3^{a}	40.0^{a}
	Two-way ANOVA	Heating	**	NS	NS	*	*
		FR	NS	NS	NS	*	NS
		Heating and FR	**	NS	*	*	*
Solanaceae *Capsicum annuum* Salsa red	Control	Control	228^{a}	15^{a}	5.8^{c}	0.4^{b}	10.5^{b}
		EOD-FR	223^{a}	15^{a}	8.7^{b}	0.6^{a}	12.5^{a}
	EOD-Heating	Control	210^{b}	14^{b}	6.1^{c}	0.4^{b}	10.5^{b}
		EOD-FR	207^{b}	14^{b}	9.9^{a}	0.7^{a}	12.0^{a}
	Two-way ANOVA	Heating	*	*	*	NS	NS
		FR	NS	NS	*	*	*
		Heating and FR	*	*	*	*	*
Apocynaceae *Catharanthus roseus* F_1 Titan rose	Control	Control	235^{a}	5^{a}	12.0^{a}	2.4^{b}	17.5^{b}
		EOD-FR	235^{a}	5^{a}	11.3^{a}	2.3^{b}	19.1^{a}
	EOD-Heating	Control	225^{b}	5^{a}	12.4^{a}	2.5^{a}	16.0^{b}
		EOD-FR	223^{b}	5^{a}	12.8^{a}	2.6^{a}	20.0^{a}
	Two-way ANOVA	Heating	*	NS	NS	*	NS
		FR	NS	NS	NS	NS	*
		Heating and FR	*	NS	NS	*	*

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Table 4 to be continued. Effect of EOD-heating or -FR treatments on the growth of day-neutral or short-day plants

Family Genus Cultivar	Treatment EOD-Heating	EOD-FR	Days to flowering	Node numb. of flower bud differentiation	Stem length (cm)	Mean internode length (mm)	Plant width (cm)
Begoniaceae Begonia semperflorens F_1 Sprint red	Control	Control	169[a]	8[a]	5.2[d]	0.7[a]	11.5[d]
		EOD-FR	169[a]	8[a]	7.7[c]	1.0[a]	13.5[c]
	EOD-Heating	Control	169[a]	8[a]	8.8[b]	1.0[a]	14.0[b]
		EOD-FR	169[a]	8[a]	10.3[a]	1.1[a]	16.0[a]
	Two-way ANOVA	Heating	NS	NS	*	NS	*
		FR	NS	NS	*	NS	*
		Heating and FR	NS	NS	*	NS	*
Ranunculaceae Ranunculus asiaticus Mache purple	Control	Control	203[a]	14[a]	18.5[c]	1.3[b]	24.5[c]
		EOD-FR	199[ab]	12[b]	27.3[a]	2.3[a]	29.5[b]
	EOD-Heating	Control	191[b]	10[c]	25.2[b]	2.5[a]	24.0[c]
		EOD-FR	191[b]	10[c]	28.9[a]	2.9[a]	32.5[a]
	Two-way ANOVA	Heating	**	*	**	*	*
		FR	NS	*	*	*	**
		Heating and FR	**	*	**	*	**

[z]Different letters indicate significant differences among species at $P < 0.05$ by Tukey-Kramer's HSD test ($n = 5$); [y]NS, * and ** indicate non-significant, significant at $P < 0.05$ and significant at $P < 0.01$, respectively by Two-way ANOVA ($n = 5$)

tion in 6 cultivars among cultivars in which flowering was effectively promoted by the FR treatment was also significantly lower than in controls. The acceleration of flowering by the illumination of a low R/FR ratio has recently been described in a wide range of long-day plants, including *Arabidopsis* (Hori et al. 2011), *E. grandiflorum* R. (Sato et al. 2009), and *Gypsophila paniculata* (Hori et al. 2011). Furthermore, the flowering of all 4 cultivars belonging to *Brassicaceae* plants used in this study was promoted by EOD or all-night FR treatment.

Additionally, *Eustoma* plants known as long-day plants grown under FR light for only 3 h at the EOD during winter showed early flower budding (Takemura et al. 2014). On the other hand, 5 cultivars (F_1 New day clear orange, Road orange, Top blue, Red alert, Kimono scarlet) among cultivars tested as day-neutral or short-day species also showed earlier flowering under EOD- or all night- FR treatment than control plants of the same species (Table 2). Additionally, F_1 New day clear orange, Top blue, Red alert among those 5 cultivars showed a lower node number of flower bud differentiation in EOD- or all night-FR treatment than control plants. The promotive effect of FR on flowering is generally observed in long-day plants, but may also be found in short-day plants. Therefore, it is important to evaluate days to flowering in field-grown plants to gain knowledge on the effect by FR treatment. Additionally, the flowering of 'Kanzaki No. 21', 'F_1 Winter cherry', and 'Gypsy deep rose' under all night-FR treatment occurred earlier than under the EOD-FR treatment. These results indicate that it is suitable to prolong FR treatment duration for promoting more flowering of these cultivars.

Further, among all long-day cultivars treated with EOD-FR, 7 cultivars ('Kanzaki No. 21', 'Baby white', 'Early pomponette red', 'Rapin yellow', 'Delta premium puregoldenyellow', 'Supra purple', 'Ritz blue imp') showed greater stem length and mean internode length, compared with untreated plants. Also, 'Kanzaki No. 21', 'Delta premium puregoldenyellow' and 'Supra purple' in the all-night- FR treatment showed increased stem length and mean internode length over the EOD-FR- treatment. In day-neutral or short-day plants, among all cultivars, 6 cultivars ('Silver dust', 'Zinnita scarlet', 'F_1 Impureza red', 'Red alert, Gorilla Jr. Watermelon', 'Kimono scarlet') showed increased stem length when treated with EOD-FR, compared with untreated control plants. Stem length in 3 cultivars ('Silver dust', 'Red alert', 'Gorilla Jr. Watermelon') increased more under all night- FR treatment, than under EOD-FR treatment. This method of promoting stem elongation

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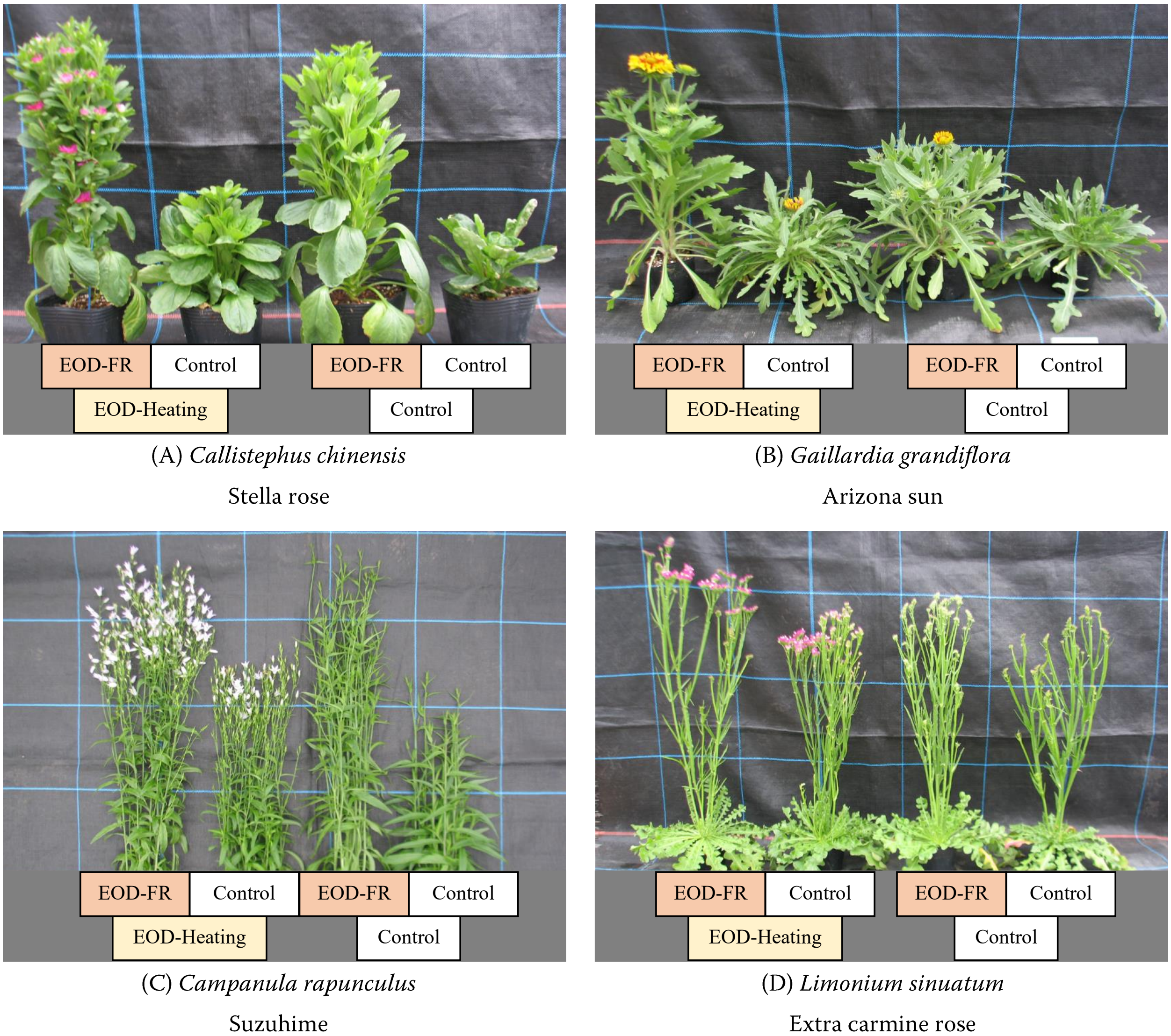

Fig. 1. Effect of a 3-h end-of-day (EOD) treatment with far red light (FR) or heating on the morphology of four cut flowers

by FR light treatment has been used for several plant species, including tobacco (Kasperbauer 1971), radish (Proctor 1973), and soybean (Kasperbauer et al. 1984). However, the effect of EOD-FR light treatment varied among cultivars of the same species; for example, EOD-FR light treatment of *Chrysanthemum* cultivars for 15 min promoted stem elongation in cultivars Dekmona, Sei-elza, and Tourmalin, but it had no effect on stem elongation in cultivar Jimba (Sumitomo et al. 2009). Similarly, our study showed cultivar-dependent sensitivity to FR treatment, although the treatment has been demonstrated effective as a method for promoting stem elongation in many plant species.

Previous studies have shown an increase of bioactive GA content in elongating epicotyls of cowpea by brief EOD-FR light treatment, as well as petiole, stem elongation, and increase in GA content in Arabidopsis, bean, and spinach (Beall et al. 1996; Wu et al. 1996). High expression levels of GA20ox related to GA synthesis is observed in the stem of EOD-FR treatment, 3 hours after treatment in Eustoma plants (Takemura et al. 2015). Based on these previous studies, we propose that the promotion of stem elongation by FR treatment observed in this study likely occurred by a similar reaction.

On the other hand, both FR treatments had differential effects on plant width, but little impact on SPAD values. In long-day plants, plant width increased and decreased by EOD-FR treatments in 4 and 2 cultivars, respectively. In addition, plant width increased and decreased by the same treat-

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ments in 4 day-neutral and 3 short-day cultivars, respectively. In plants showing higher values of plant width, it was presumed that the elongation of petiole length was promoted by this treatment. Additionally, the effect was different among cultivars. Petiole length of poinsettia was significantly higher under EOD-FR only in Christmas Spirit, although Christmas Eve showed similar statistically insignificant trends (Islam et al. 2014). In contrast, it was presumed that plants showed lesser plant width as a shade-avoidance response.

Secondly, Tables 3 and 4 summarize effects on growth by EOD-heating or -FR treatments in long-day plants and day-neutral or short-day plants, respectively. Among long-day cultivars, the days to flowering in 6 cultivars ('Arizona sun', 'Tear blue', 'Tiger eye gold', 'Suzuhime', 'Extra carmine rose', 'White lace flower') treated by EOD-Heating was lower than in control plants (Table 3). Additionally, the node number of flower bud differentiation of those 6 cultivars, was also significantly lower than in controls. Similarly, days to flowering and flower was also promoted in 6 cultivars ('Discovery yellow', 'Avant-garde blue', 'F_1 Horizon lavender', 'Salsa red', 'F_1 Titan rose', 'Mache purple') among day-neutral or short-day cultivars by the same treatment, respectively (Table 4). Additionally, the effect on flowering by this treatment was also positive when applied in combination with EOD-FR treatment. In the spray-type chrysanthemum 'Sei-Roza' with a controlled at 16/8 °C (light/dark period), it was shown that EOD-heating treatment of 24 °C for 3 h accelerated flower initiation and early flower bud development (Douzono et al. 2012a).

Additionally, in short-day African marigold (*Tagetes erecta* L.), it was reported that early growth and flower bud development after flower budding were accelerated by heat treatment at 30 °C for 3 hours in EOD (Douzono et al. 2012b). From these results, it was suggested that EOD-Heating was effective as a technique for promoting flower bud differentiation of many cut flower species. However, little is known about the effect on stem elongation by this treatment in cut flowers. In this study, 4 long-day cultivars and 3 day-neutral or short-day cultivars showed enhanced stem length by EOD-heating treatment, respectively. Additionally, a significantly higher value of mean internode length in EOD-heating treatment was shown in only 2 among these cultivars. Therefore, it is presumed that the effect on internode elongation by EOD-Heating in the plants tested in this study was the lowest. On the other hand, flowering and stem elongation of 'Stella rose', 'Arizona sun', 'Suzuhime' and 'Extra carmine rose' were promoted by a combination treatment, which improved productivity and quality (Figure 1). On the other hand, flowering potted plants suitable a compact stem as plant form. Based on these results, we conclude that these treatments can help the production of cut-flowers by reduction of heating cost associated with shortening of the growth period in areas with limited sunshine from autumn to winter, although the method for the application of these treatments must be optimized for each species.

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