The influences of monochromatic light irradiation as pre-rooting treatment on the rooting of cuttings in spray-type chrysanthemum cultivars ‘Sei Prince’ and ‘Remidas’ examined using light-emitting diodes. The unrooted cuttings were irradiated with red (660 nm), green (520 nm), and blue (450 nm) light and fluorescent light at around 60 μmol m<sup>–2</sup> s<sup>–1</sup> of photosynthetic photon flux density (PPFD) for 24 h continuously at 20°C for 7 days. In ‘Remidas,’ the rooting percentage of cuttings were high above 85% irrespective of light wavelength. In ‘Sei Prince,’ red light irradiation promoted the rooting of cuttings compared to the other light irradiation. When the cuttings were irradiated with red light at 59.4, 37.4, and 12.4 μmol m<sup>–2</sup> s<sup>–1</sup> of PPFD, a decrease in rooting percentage was not observed even at a low PPFD in ‘Remidas.’ On the other hand, in ‘Sei Prince,’ the rooting percentage of cuttings decreased at the lowest PPFD. Thus, it was shown that monochromatic light irradiation for 7 d before rooting could promote the rooting of spray-type chrysanthemum cuttings, however, not only light wavelength but also light intensity that could promote rooting differed between two cultivars.

Keywords : light-emitting diode, light intensity, light wavelength, root primordia

INTRODUCTION

Vegetative propagation through cuttings is a common way of reproduction in chrysanthemums. There are two main ways to grow chrysanthemums commercially in Japan. One is to transplant the unrooted cuttings in a propagation bed or soil block, to root the cuttings, and to transplant rooted cuttings in the field (rooted cutting cultivation). The other is to plant the unrooted cuttings directly in the field (direct cutting cultivation). Since the process of rooting cuttings can be omitted in the direct cutting cultivation, labor time can be greatly reduced compared to rooted cutting cultivation (Shigeki et al., 2006). To produce chrysanthemums stably around the year in Japan, it is necessary to transplant them even in hot summer. It was found that rooting of chrysanthemum cuttings initiated more quickly at a temperature of 30°C, but it required over 7 days to initiate rooting (Dykeman, 1976; Ooshi et al., 1978; Takahashi et al., 1981). Especially in direct cutting cultivation in summer with high temperature and high solar irradiation, leaf wilting and rot of cuttings are frequently observed from transplanting to establishment (Sasaki et al., 1996; Nishio and Fukuda, 1998). Therefore, in direct cutting cultivation, it is necessary to root the cuttings as quickly as possible after transplanting, and to establish those.

Pre-rooting treatments that promote the rooting of the cuttings had been studied to reduce the period from transplanting to establishment in direct cutting cultivation. Some studies showed the duration, temperature, and light conditions of pre-rooting treatments, as well as the concentration and soaking time of rooting promoters (Nishio and Fukuda, 1998; Yonekura et al., 1999). Nishio and Fukuda (1998) have reported that light as a pre-rooting treatment has little effect on the formation of root primordia. Meanwhile, Yamamura’s research group has improved the previous treatment conditions to a pre-rooting treatment that can be practiced by growers. Namely, the whole cutting is soaked in a 40 μg g<sup>–1</sup> of indole-3-butyric acid (IBA) solution for 10 s and then irradiated with light at 300 lx for 24 h at 20°C for 7 d. Additionally, Yamamura et al. (2006) have developed a system that can treat under these conditions. In this system, a fluorescent lamp has been used as a light source to irradiate more than 300 lx near the shoot apices of the cuttings. However, in the previous study, the mechanism of rooting promotion by light irradiation remains to be clarified.

Recently, light-emitting diodes (LEDs) have been practically developed as an alternative light source for horticultural lighting to fluorescent and high-pressure sodium lamps. LED lighting has several advantages compared to existing horticultural lighting, including the ability to reduce electrical energy consumption, the ability to produce high light levels with low radiant heat output when cooled properly, and the ability to maintain a useful light output for years without replacement (Morrow, 2008; Davis and Burns, 2016). Since LED lighting can also control the spectral composition and irradiate with narrow spectrum light, the effects of light quality on the growth
and development of several horticultural crops have been studied (Hoe et al., 2002; Hirai et al., 2006; Ishii et al., 2018). In chrysanthemums, it has been reported that the effect of light quality on the flower bud differentiation (Sumitomo et al., 2012; Hakuzan and Nagayoshi, 2013; Liao et al., 2014; Ochiai et al., 2015; Nissim-Levi et al., 2019), and that red light promotes stem elongation, while blue light inhibits it (Kim et al., 2004; Zhiyu et al., 2007; Asami and Kuroyanagi, 2014). The effect of light quality on the rooting of chrysanthemum cuttings has also been reported in some studies (Heins et al., 1980; Borowski and Kozlowska, 1986; Hong et al., 2015; Christiaens et al., 2019; Gil et al., 2020). However, these studies were conducted over several weeks of light irradiation treatment. There are few reports on the effects of light irradiation for 7 d before rooting, such as the pre-rooting treatment described above, on the rooting of cuttings. This study investigated the effect of monochromatic light irradiation for 7 d on the rooting of cuttings of spray-type chrysanthemums to determine the light quality suitable for rooting promotion treatment in direct cutting cultivation.

MATERIALS AND METHODS

Pretreatment of cuttings

Two cultivars of spray-type chrysanthemum, ‘Remi-das’ (Inochio Seikoen Inc., Hiroshima, Japan) and ‘Sei Prince’ (Inochio Seikoen Inc.,) were used in this experiment. These cultivars are autumn-flowering type, popular and commonly grown in Japan. Cuttings were harvested from stock plants grown in a greenhouse ventilated at 25°C during the day and maintained above 16°C during the night, with a 6-h night interruption from 23:00 to 5:00 by fluorescent lamp (Biotechlight 23 W, Biotech Co., Ltd., Shizuoka, Japan). The cuttings were adjusted to about 8 cm length and about 4 expanded leaves by removing the lower leaves. The cuttings were used for the experiment without refrigeration. The cuttings were stored in a 105-hole cell-tray with the shoot apex upward at a density of 24 cuttings per cell for each cultivar. The whole cutting was soaked in 40 μg g⁻¹ of IBA solution (OXYBERON 1.0 of each peak wavelength.

Light irradiation treatment

The cell-trays covered with a plastic wrap film were irradiated with light at 20°C in a benchtop plant growth chamber (LH-55FL3-DT, Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan). The space in the growth chamber was divided into upper and lower sections. The light from the upper section of the growth chamber was shaded with an aluminum foil to prevent it from reaching the lower section. In the upper section, a daylight fluorescent lamp (FL10D, NEC Lighting, Ltd., Tokyo, Japan), which was installed in the growth chamber, was used as the light source as a control. In the lower section, a LED irradiation device was used as the light source. The LED irradiation device consisted of a LED board (EKL10-0070, ECS Co., Ltd., Aichi, Japan) equipped with a full-color chip-type LED (SL3528TH04T-BRG, Showa Denko K.K., Tokyo, Japan) and a control unit (EKL10-0020, ECS Co., Ltd., Aichi, Japan) capable of independently irradiating red (660 nm), green (520 nm), and blue (450 nm) light. Spectral photon flux distributions of fluorescent light (white light) and red, blue, and green light measured using a spectroradiometer (MS-720; Eco Instruments Co., Ltd., Tokyo, Japan) are shown in Fig. 1.

In Experiment 1, the effects of red, green, and blue light on the rooting of cuttings were evaluated. The photosynthetic photon flux density (PPFD) near the shoot apex was measured using a light quantum sensor (LP471, Delta OHM S. r. l., Selvazzano Dentro, Italy), and the PPFD of each light color was adjusted to be similar to that of fluorescent light on the rooting of cuttings was evaluated. The red light PPFD near the shoot apex was adjusted to 59.4, 37.4, and 12.4 μmol m⁻² s⁻¹. In both experiments, 24-h continuous light irradiation was conducted for 7 d.

Determination of rooting state and statistical analysis

The rooting state of the cuttings was determined after light irradiation. The rooting state was classified into 3 stages: “No rooting,” “Root primordia formation,” in which tiny bumps were visible at the base of cuttings, and “Rooting,” in which roots of 1–2 mm in length were observed. The number of cuttings in each stage was counted.

The rooting state was measured for 10 cuttings per treatment per cultivar, with 10 replicates in both experiments. The effects of the two cultivars, light irradiation treatment (light color, and PPFD of red light), and their interaction on the rooting state of cuttings were analyzed by two-way analysis of variance. Means were separated with Tukey’s test (P < 0.05).
ROOTING OF CUTTINGS UNDER LED

RESULTS AND DISCUSSION

Effect of light quality on rooting of cuttings (Experiment 1)

In ‘Remidas,’ almost all cuttings rooted in the fluorescent light treatment (Table 1). Nearly 100% of the cuttings was rooted in the red and green light treatments as well as in the fluorescent light treatment. In the blue light treatment, the incidence of rooted cuttings (rooting percentage) tended to be slightly lower than in the fluorescent light treatment. On the other hand, in ‘Sei Prince,’ the rooting percentage in the fluorescent light treatment was less than 60%, which was significantly lower than that in ‘Remidas.’

Instead, the incidence of cuttings with root primordia formation increased, and cuttings without rooting occurred. The red light treatment increased rooting percentage in ‘Sei Prince’ compared to the fluorescent light treatment, and produced no cuttings without rooting. The green light treatment decreased the rooting percentage compared to the fluorescent light and red light treatments, although it increased the incidence of cuttings with the root primordia formation. In the blue light treatment, the rooting state was similar to that in the fluorescent light treatment.

In a previous study in which fluorescent lights of white, red, green, and blue were irradiated on the cuttings of chrysanthemum, it was reported that the rooting percentage of the cuttings in the early stage of rooting was higher in the cuttings irradiated with white and red light than blue or green light (Borowski and KozloWSka, 1986). In another previous study using LEDs, which can irradiate narrower spectral light than fluorescent lamps, it was reported that blue light (450 nm) inhibited the rooting of chrysanthemum seedlings, while the red light (650 nm) stimulated that compared to fluorescent light (Hong et al., 2015). On the other hand, Gil et al. (2020) reported that single leaf-bud cuttings of chrysanthemum cultivar ‘Backma’ irradiated with blue light (460 nm) formed the root more rapidly than those irradiated with red light (625 nm) or fluorescent light. In the present study, the effect of light quality on rooting of cuttings varied between the two cultivars (Table 1) and did not necessarily show the same trend as in these previous reports. These results considered resulting from differences in light intensity or the wavelength of the LED light. Because it has been demonstrated that both light intensity and specific wavelengths can affect the root formation on in vitro plants and in vitro cuttings, not only in chrysanthemum but also in different species (Christiaens et al., 2016). It has also been reported that the influence of light irradiation conditions on the rooting of cuttings differs between cultivars in pear (Bertazza et al., 1995).

Far-red light also affects the rooting of chrysanthemum cuttings (Heins et al., 1980; Christiaens et al., 2019). Compared to the monochromatic red light, lowering the R:FR ratio by additionally irradiating red light with far-red light significantly increased the rooting percentage at one week after light irradiation (Christiaens et al., 2019). The only light source that contained far-red light was the fluorescent lamp of the light sources used in this study (Fig. 1).

However, in ‘Sei Prince,’ only 60% of cuttings rooted after fluorescent light irradiation, which was lower than the rooting percentage after monochromatic red-light irradiation (Table 1). In ‘Remidas,’ almost all cuttings irradiated with fluorescent light rooted, although similar results were observed in the cuttings irradiated with monochromatic red or green light. Christiaens et al. (2019) also showed that specific wavelengths of light can improve the rooting of chrysanthemum cuttings by affecting auxin. In this study, the cuttings were soaked in IBA solution before light irradiation treatment, as proposed by Yamamura et al. (2006). This IBA treatment seemed to affect the stimulating effect of far-red light on the rooting of the cuttings (Morini et al., 1990; Bertazza et al., 1995). Future studies on the effects of monochromatic light irradiation on the rooting of cuttings without soaking in IBA solution may provide different results from this experiment.

Table 1 Effects of different light irradiation treatments on the rooting of cuttings in spray-type chrysanthemums ‘Remidas’ and ‘Sei Prince’ (Experiment 1).

| Light treatment (PPFD) | Cultivar | Rooting state of cuttings after treatment |
|------------------------|---------|----------------------------------------|
| Fluorescent light (61.3 μmol m⁻² s⁻¹) | Remidas | No rooting | Root primordia formation | Rooting |
|                        | 0 b     | 2 cd | 98 a |
|                        | 10 ab   | 34 b | 56 c |
| Red light (59.4 μmol m⁻² s⁻¹) | Remidas | 0 b | 1 d | 99 a |
|                        | 0 b     | 11 cd | 89 a |
|                        | 4 ab    | 68 a | 38 d |
| Blue light (68.7 μmol m⁻² s⁻¹) | Remidas | 3 ab | 12 cd | 85 ab |
|                        | 14 a    | 20 bc | 66 bc |
|                        | **      | *** | *** |

| Significance² | Light treatment | Cultivar |
|---------------|-----------------|---------|
|               | **              | ***     | ***    |
| Interaction   | *               | ***     | ***    |

²: Values within a column followed by different letters differ significantly at P < 0.05 by Tukey's test.

y: * *, ** and *** indicate significant difference at P < 0.05, 0.01 and 0.001, respectively by two-way ANOVA.
Effects of different PPFDs of red light by LED on rooting of cuttings (Experiment 2)

Red light, which resulted in higher rooting percentage than other colors in ‘Sei Prince’ in Experiment 1, was irradiated to cuttings with different PPFDs. In ‘Remidas,’ almost all cuttings rooted irrespective of the PPFD level (Table 2). In ‘Sei Prince,’ the rooting percentage hardly changed with a decrease in PPFD from 59.4 to 37.4 μmol m$^{-2}$ s$^{-1}$. A further decrease in PPFD to 12.4 μmol m$^{-2}$ s$^{-1}$ decreased the rooting percentage, resulting in more cuttings with formed root primordia.

High light-intensity irradiation (at levels up to 20 or 24 klx) enhances the rate of photosynthesis in chrysanthemum cuttings (Machida et al., 1977; Ooishi et al., 1984). Carbohydrates that are synthesized by photosynthesis of leaves on the cuttings influence the root formation and development of cuttings as well (Ooishi et al., 1984; Rapaka et al., 2005). The rooting percentage of cuttings irradiated with red light at low PPFD (12.4 μmol m$^{-2}$ s$^{-1}$) was lower in ‘Sei Prince’ than in ‘Remidas’ (Table 2). The ability to maintain photosynthetic activity under low-intensity light conditions varies between chrysanthemum cultivars (Han et al., 2015). Therefore, the photosynthetic capacity of the cuttings under low-intensity light conditions was inferred to be different between ‘Remidas’ and ‘Sei Prince,’ and to be lower photosynthetic rate of cuttings at low PPFD in ‘Sei Prince’ than in ‘Remidas.’

**CONCLUSION**

In this study, cuttings of two spray-type chrysanthemum cultivars were rooted by irradiating them with monochromatic LED light of red, green, and blue and fluorescent light (control) for 7 days. The results demonstrated that one cultivar ‘Remidas’ had a high percentage of rooting irrespective of light wavelength, while another cultivar ‘Sei Prince’ exhibited a higher rooting percentage of cuttings by red light. When the cuttings were irradiated with red light at different intensities, the cultivar, which always had a high percentage of rooting, did not observe a decrease in rooting percentage even at a low light intensity. The cultivar with high rooting percentages under red light decreased its rooting percentage at the lowest light intensity. Thus, it was shown that monochromatic light irradiation for 7 d could promote the rooting of spray chrysanthemum cuttings, however, not only the light wavelength but also the light intensity that could promote rooting differed between cultivars. It is necessary to be careful when applying light irradiation treatment of chrysanthemum cuttings for promoting rooting in actual cultivation situations.

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