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Blue light and NAA treatment significantly improve rooting on single leaf-bud cutting of Chrysanthemum via upregulated rooting-related genes

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ABSTRACT

Single leaf-bud cutting has been attempted in an effort to produce multiple individuals; however, these cuttings produce individuals of irregular quality with different rooting abilities based on the cutting positions of the mother stem. We evaluated whether light and 1-naphthaleneacetic acid (NAA) treatment can improve root formation from relatively poor stem cuttings on single leaf-bud cuttings and which factor is more effective for rapid root formation. Blue (BL), red (RL), and fluorescent (FL) with/without NAA were independently applied to single leaf-bud cuttings. High-intensity BL together with NAA treatment efficiently induced adventitious root formation and improved root formation from poor stems. We also evaluated relative expression levels of seven kinds of rooting-related genes on cutting processes to define the critical light factor leading to rapid adventitious rooting. *CmLBD1* was significantly upregulated after 5 day BL treatment and initiated rooting, whereas the expressions of other rooting-related genes were significantly increased by fluorescent light after 14 days cutting initiation.

1. Introduction

Chrysanthemum 'Baekma' is a standard cultivar used as a cut flower. This cultivar is popular in Korea and Japan because it has a large white flower with many ray florets. Because it is mainly used in funeral ceremonies, the plant is in high demand year round for large amounts of high-quality cut flowers, which can be obtained from healthy chrysanthemum cuttings (Yoo and Roh, 2012). A sufficient number of healthy cuttings can be produced in a short culturing period and multiple propagations; therefore, adequate environmental conditions during the culturing period are suggested in order to guarantee that healthy cuttings will be produced.

Vegetative propagation is a common method by which to preserve the superior quality of stock plants (Zhang et al., 2013). Taking cuttings from the stock plant is a simple and economical method by which to improve production of nursery individuals within a short period of time (Yoo and Kim, 1996). As propagation purpose and plant species, cutting methods can vary. For example, leaf cutting, stem cutting, root cutting, or leaf-bud cutting can be used to propagate new individuals. Among these methods, stem cutting is the most widely used for chrysanthemum propagation. Using terminal stems is general because the terminal stem has numerous intrinsic auxin, which induces adventitious root formation. Although terminal stem cuttings rapidly develop adventitious roots, they produce only a single individual plant per stem. Therefore, this method occasionally constrains mass production of cut flowers when cutting production from the mother plant is limited under adverse environmental conditions, such as severe heat or cold. Using single leaf-bud cuttings is a potential option that can produce multiple individuals. Although this method produces multiple cuttings from one stem, there are some problems with its use. Single leaf-bud cuttings consist of small amounts of intrinsic auxin; therefore, exogenous auxin such as NAA is applied to the cuttings for overcoming the disadvantages caused by contained little intrinsic auxin. Without NAA treatment, the optimum culturing period is extensive at >6 weeks (Singh and Chettri, 2013). This relatively long culturing period hinders the efficient use of energy and of the nurseries, thereby decreasing cost efficiency of this method. In addition, the rooting quality is differently influenced by where a cutting is taken on the stock plant, such as from the upper, middle, or bottom of the plant. Furthermore, as the culturing period for the cuttings continues under heavy moisture conditions, the contamination rate becomes higher; therefore, the development of approaches by which uniform and superior cuttings can be cultured within a shorter

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period of time has the potential to greatly improve the efficiency of cutting propagation.

Light conditions have been reported to affect morphological patterns, physiology, and development in plant growth cycle such as root formation, shoot growth, nutrient status, antioxidant capacity, and flower initiation (Akoyunoglou and Anni, 1984; Cosgrove, 1981; Schroeter-Zakrzewska and Kleiber, 2014; Schwartz and Zeiger, 1984; Xu et al., 2019). Previous studies have proved that blue and red lights (BL and RL, respectively) are critical wavelengths for plants. BL is important for chlorophyll formation, chloroplast maturity, stomatal opening, phototropism, inhibition of hypocotyl elongation, and improvement of rooting percentage (Christie, 2007; Schroeter-Zakrzewska and Kleiber, 2014; Schwartz and Zeiger, 1984; Senger, 1982; Spalding, 2000). In contrast, RL affects shoot elongation, photosynthesis regulation, and flower initiation based on periodic phytochrome metabolism (Schroeter-Zakrzewska and Kleiber, 2014; Schuerger et al., 1997). Moreover, high portion of RL mixed with other color lights improved root growth and antioxidant capacity in *Cunninghamia lanceolate* seedling (Xu et al., 2019). Although many studies have been conducted to evaluate the effects of light on several crops, less is known about the optimal light wavelength and intensity for propagating chrysanthemum cuttings.

Auxin is a key factor among various endogenous factors inducing adventitious roots. Auxin is transported to wounded portion of cutting, which induces the local concentration and accumulation of auxin leading adventitious rooting (Liu et al., 2013). A number of genes were reported as relating to auxin transport and accumulation. *AUXIN RESPONSIVE* (*AXR*) affected to enhance the auxin-related growth response such as cell division and elongation (Walker and Estelle, 1998). *AXR1* and *AXR6* mutants of *Arabidopsis* generated shorter and less number of lateral roots (Hobbie et al., 2000; Ruegger et al., 1998). *AXR2* mutants had shorter hypocotyl losing gravitropism than wild type in *Arabidopsis* (Nagpal et al., 2000). The functions of *TOLL/INTERLEUKIN RECEPTOR 1* (*TIR1*) were overlapped with *AXR* influences in auxin-regulated growth. The *TIR1* mutants of *Arabidopsis* generated insufficient length of lateral roots and hypocotyl (Ruegger et al., 1998). *TIR3* is included in *BIG* gene group with *DOC1*, *UMB1*, and *ASA1*. This group is known to encode a calossin-like protein which is required to polar auxin transport (Desgagné-Penix et al., 2005). *PIN-FORMED* (*PIN*) proteins are auxin efflux facilitators and regulate the auxin distribution, which contribute to cell division and expansion (Blilou et al., 2005). *LATERAL ORGAN BOUNDARIES DOMAIN* (*LBD*) plays an important role in the auxin signal cascades in the architecture of the root systems (Okushima et al., 2007; Sengupta and Reddy, 2018; Zhu et al., 2016). *LBD* is considered to be an acting transcription activator to promote adventitious and lateral root formation in plants regardless of the species. Previous studies have reported that *LBD* is expressed in the adventitious root primordia in rice (Liu et al., 2005) and *Arabidopsis* (Fan et al., 2012). Additionally, the number of lateral roots increased in soybeans (*Glycine max*) with overexpressed *LBD* (Yang et al., 2017). *CmLBD1* in chrysanthemum responds to auxin and triggers the process of adventitious root formation (Zhu et al., 2016). Although various research studies on auxin-induced genes have been conducted, the effects of light on gene expression have not to date been clearly identified.

Although the use of single leaf-bud cuttings has potentially an advantage for the mass production of individuals from cuttings, the method has been limited in nursery production of chrysanthemums because its use produces irregular root formation in dependent of cutting position in a stem (Fig. 1). In this experiment, we evaluated the properties of the single leaf-bud cutting method and developed conditions for more rapid establishment of roots from multiple positions on the stock chrysanthemum stems where cuttings were made using light and hormonal controls. Additionally, we evaluated relative expression levels of seven kinds of rooting-related genes on cutting processes to define the critical factor for upregulating the genes as a key environmental element, which induces rapid adventitious rooting.

2. Materials and methods

2.1. Plant material and controlled culturing conditions

Dendranthema × *grandiflorum* cv. Baekma in its juvenile stage was purchased from a private farm located in Jeonju, Korea (35°52′40.29″ N; 127°5′34.76″ E, elevation 13.0 m). Leaf and bud cuttings taken from same node position of chrysanthemum stems were generated from stock plants which were maintained in vegetative growth phase. Each cutting was measured 1.64 ± 0.46 g·FW^{-1}. Eight cuttings per treatment were planted in a 24-well plug tray (7 × 7 × 8 cm, Bumnong Co., Jeongeup, Korea) filled with mixed soil containing equal volumes of horticultural soil (Baroker, Seoulbio Co., Eumseong, Korea) and vermiculite (Greenfire Chemicals Co., Hongsung, Korea). The temperature was set at 25 °C ± 1 °C. The relative humidity of the culture room was maintained at 90 % ± 5 % throughout the cultivation period using siding covered with transparent plastic film. Daylight was restricted to 16 h to maintain the vegetative period. Cuttings were irrigated daily with distilled water for six weeks.

Fig. 1. Irregular rooting quality of terminal cuttings and single leaf-bud cuttings taken from different stem parts of stock plant. Every cutting was cultured under 50 μmol m^{-2}·s^{-1} white fluorescent lamp for 3 weeks.

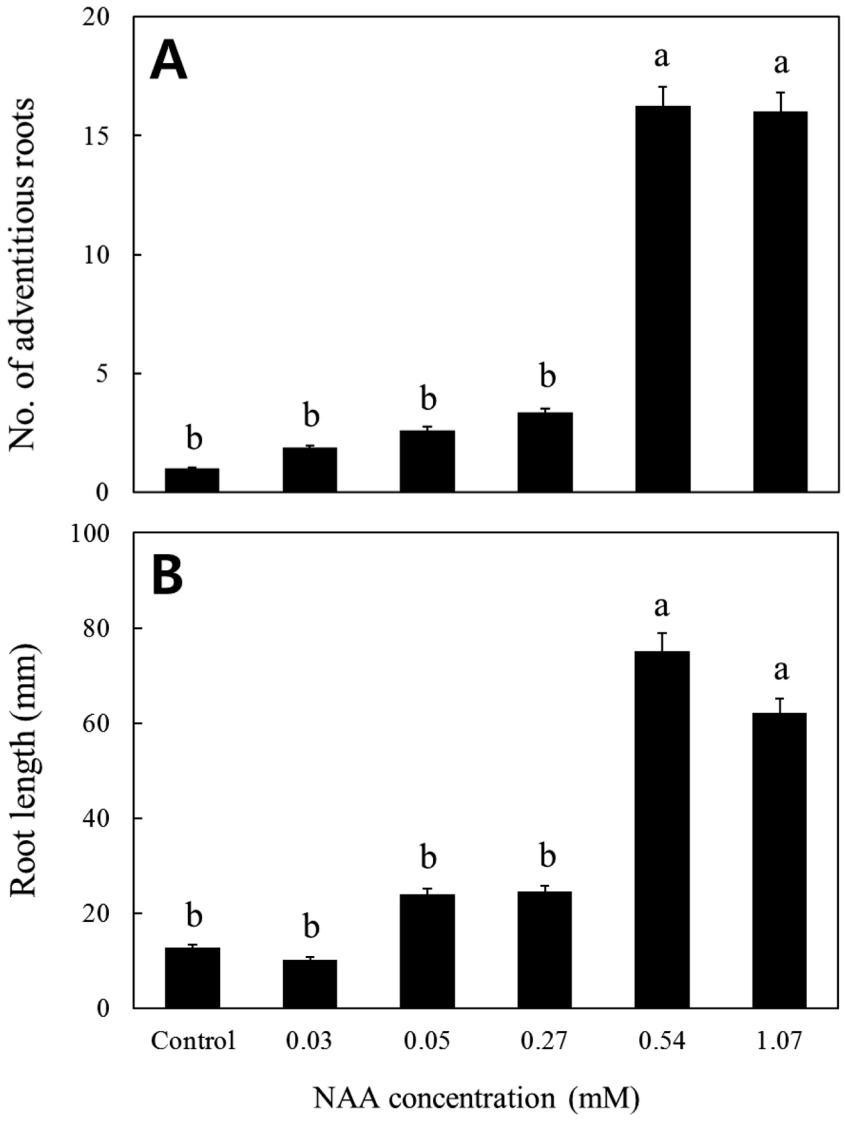

Fig. 2. Number of adventitious roots (A) and root length (B) from chrysanthemum cuttings treated with different concentrations of 1-naphthaleneacetic acid (NAA) for 2 weeks. Different letters mean statistical differences as assessed by the Fisher's least significant difference test at a 5 % level.

2.2. Treatment with 1-naphthaleneacetic acid

Serial concentrations of NAA were applied to chrysanthemum single leaf-bud cuttings before applying the light treatments. The basal portions of the cuttings were dipped into 0.03, 0.05, 0.27, 0.54, 1.07, and 2.68 mM NAA solution (MB Cell, Seoul, Korea) for 1 h. Eight individual cuttings were used for each treatment. The treated cuttings were planted in 24-well plug trays containing equal volumes of horticultural soil and vermiculite, which were placed in a controlled-growth chamber irradiated using a fluorescent lamp at 50 μmol m^{-2}•s^{-1}. Cuttings were propagated at 25 °C in an atmosphere of 90 % relative humidity. After treating for two weeks, the root formation was measured. These data were used to select 0.54 mM NAA as the optimal concentration for growing chrysanthemum cuttings. Chrysanthemum cuttings were subjected to various light treatments in either the presence or absence of NAA.

2.3. Light treatment

During the culturing period, cuttings were exposed to blue (460 nm) light-emitting diode (LED), red (625 nm) LED, or FL lights. The light intensity was measured using the HD2302.0 photoradiometer (DELTA

Table 1
Survival rates of chrysanthemum cuttings irradiated with different light wavelengths at 35 μmol m^{-2}•s^{-1} in the presence or absence of 1-naphthaleneacetic acid for 2, 4, and 6 weeks.

Hormonal treatment	Culture period (weeks)	Survival rate (%)		
		BL	RL	FL
non-NAA	2	100.0a[a]	100.0a	100.0a
	4	100.0a	75.0b	75.0b
	6	75.0a	37.5b	75.0a
NAA	2	100.0a	100.0a	100.0a
	4	100.0a	87.5a	100.0a
	6	87.5a	50.0b	75.0a
Significance[b]				
Culture period (C)		***	***	***
Hormonal treatment (H)		NS	NS	*
(C) × (H)		NS	NS	*

Abbreviations: BL blue light; RL red light; FL fluorescent light; NAA 1-naphthaleneacetic acid.

[a] Different letters within rows indicate significant differences as assessed by Tukey's honestly significant difference test with a 5 % cutoff.

[b] Nonsignificant (NS) or significance at $p < 0.05$ (*), 0.01 (**), or 0.001 (***).

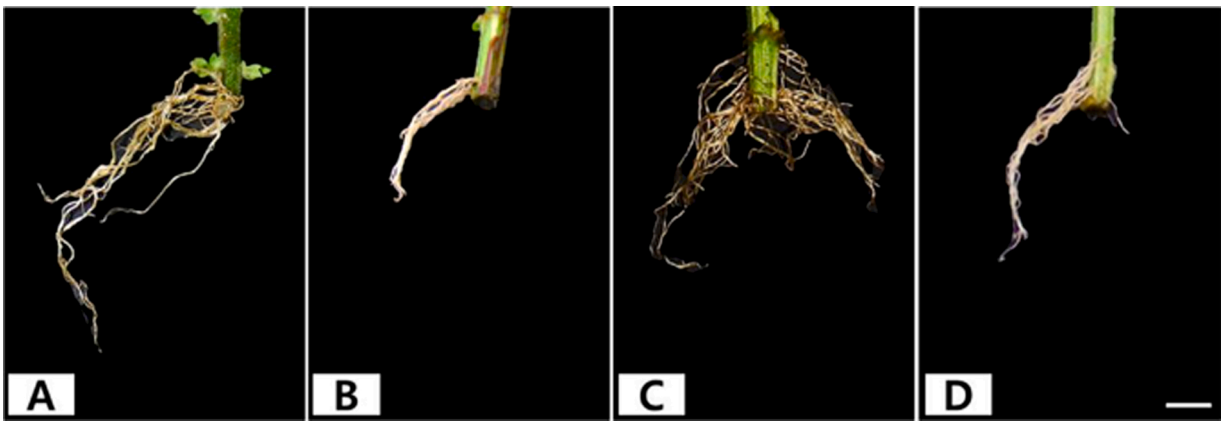

Fig. 3. Morphological differences of chrysanthemum cuttings treated with different light wavelengths and either with or without 1-naphthaleneacetic acid (NAA). (A) blue light without NAA, (B) red light without NAA, (C) blue light with NAA, (D) red light with NAA. White bar on pictures indicates 1 cm.

OHM SRL, Marconi, Italy) and was presented as the photosynthetic photon flux density (PPFD). Light intensities were differently applied to each experiment. The light intensity of 50 μmol $m^{-2}\cdot s^{-1}$ was mainly used to determine optimal NAA concentration and expression levels of rooting-related genes. To investigate the effect of varying light wavelengths and intensities on the cuttings, a light intensity of 35 μmol $m^{-2}\cdot s^{-1}$ was mainly used and 5 or 65 μmol $m^{-2}\cdot s^{-1}$ was applied.

2.4. Plant growth

The quality of the cuttings grown for two, four, or six weeks was evaluated by measuring survival rate, root dry weight, adventitious root formation, and root elongation. The longest root represented the treatment most competent for growth.

2.5. Analysis of quantitative real-time polymerase chain reaction (PCR)

Basal stem tissues containing cut sections were collected separately from chrysanthemum cuttings raised under BL, RL, and FL of 50 μmol $m^{-2}\cdot s^{-1}$ for either 1, 5, or 14 d. Tissues were stored at −70 °C before being ground in liquid nitrogen using a mortar and pestle treated with RNaseZap (Thermo Fisher, Waltham, MA, USA). Total RNA was extracted from the tissue using the Direct-zol RNA Miniprep Plus kit (Zymo Research, Irvine, CA, USA) and Trizol reagent (Thermo Fisher) and was treated at the same time with DNase to remove any genomic DNA contamination. The first strand of cDNA was synthesized using oligo (dT) primers with the ProtoScript M-MuLV Taq RT-PCR kit (New England Biolabs, Ipswich, MA, USA).

Quantitative PCR (qPCR) was conducted using the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with iTaq Universal SYBR Green Supermix (Bio-Rad). Each 20-μL quantitative reverse transcription (qRT)-PCR contained 10 μL SYBR Green Supermix, 5 μL cDNA template (1 ng·μL^{-1}), and 0.5 μL of each primer (10 μM). All reactions were conducted in triplicate and the chrysanthemum EF1α (KF305681) primer sequence was used as the internal control. Cycling conditions were as follows: 3 min at 95 °C; then 40 repeats of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C, followed by the melt-curve analysis. Primers used for PCR were the same as those used in the study by Zhu et al. (2016). The cycle threshold values were calculated using CFX manager (Bio-Rad). The relative expression values were calculated using the $2^{-\Delta\Delta Ct}$ method.

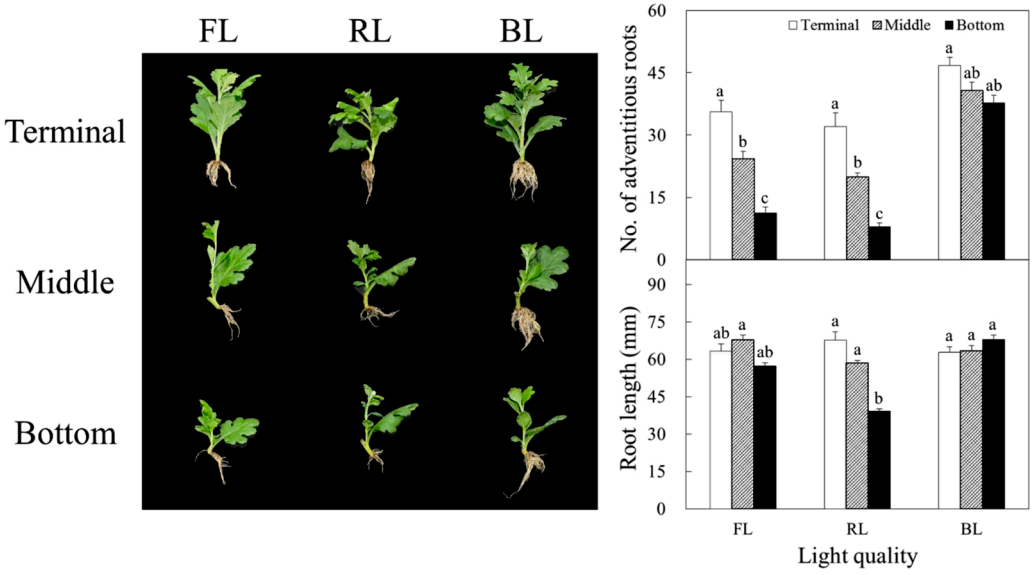

Fig. 4. Photomorphological analysis of single leaf-bud cuttings taken from terminal, middle, or bottom parts per stem. Each cutting was irradiated 50 μmol $m^{-2}\cdot s^{-1}$ blue light (BL), red light (RL), or fluorescent light (FL) for 3 weeks. Different letters over bars indicate significant differences of each light wavelength treatment as assessed by Tukey's honestly significant difference test with a 5 % cutoff.

2.6. Statistical analyses

The number and length of chrysanthemum cuttings are presented as mean values with standard errors. Analyses of variance for the experimental data was performed using SAS v. 9.3 (SAS Institute Inc., Cary, NC, USA). Significant differences were set at $p < 0.05$ using Tukey's honestly significant difference test or Fisher's least significant difference test.

3. Results and discussion

3.1. Determination of NAA concentration

The appearance of adventitious roots was slightly increased as NAA concentrations ranging 0 to 0.2d 7 mM (Fig. 2). The root appearance was dramatically increased at 0.54 mM NAA and kept until 1.07 mM NAA. Longer and vigorous roots were observed between 0.54 and 1.07 mM NAA with no significant difference each other, exhibiting up to 75.2 mm in root length. The wilted leaves of cuttings were observed at 2.68 mM NAA (data not shown). Our results are similar to those of Yoo and Roh (2012), which suggested that 100 ppm NAA was a suitable concentration for propagating chrysanthemum 'Baekma' terminal cuttings. Therefore, we used 0.54 mM NAA in all further experiments.

Table 2
Root formation of chrysanthemum cuttings irradiated with different light wavelengths at 35 μmol $m^{-2}·s^{-1}$ in the presence or absence of 1-naphthaleneacetic acid during the culturing period.

Hormonal treatment	Culture period (weeks)	Light wavelength	Root formation		
			No. of adventitious roots	Length (mm)	Dry weight (mg)
non-NAA	2	BL	1.8a[a]	16.0a	8.7a
		RL	0.4a	5.3b	3.3ab
		FL	0.0a	0.0b	0.0b
	4	BL	12.9a	72.0a	56.5a
		RL	5.7b	30.0b	21.1b
		FL	7.4b	55.7ab	35.5ab
	6	BL	8.5a	87.3a	59.4a
		RL	10.8a	33.2a	29.8b
		FL	8.0a	50.5a	38.0ab
NAA	2	BL	23.7a	28.6a	88.5a
		RL	2.0b	4.5b	16.3b
		FL	7.5b	13.6b	23.8b
	4	BL	23.1a	57.0a	126.7a
		RL	11.7ab	33.3a	63.2b
		FL	11.0b	51.3a	69.2b
	6	BL	24.7a	76.3a	137.5a
		RL	13.0b	28.4b	53.7b
		FL	19.5a	61.7ab	78.9ab
Significance[b]					
Hormonal treatment (H)			***	NS	***
Light wavelength (W)			***	***	***
(H) × (W)			***	NS	***

Abbreviations: BL, blue light; RL, red light; FL, fluorescent light; NAA, 1-naphthaleneacetic acid.
[a] Different letters within columns each same period indicate significant differences as assessed by Tukey's honestly significant difference test with a 5 % cutoff.
[b] Nonsignificant (NS) or Significance at $p < 0.05$ (*), 0.01 (**), or 0.001 (***).

3.2. Effect of light wavelength and NAA on cutting survival

The survival rates of chrysanthemum cuttings cultivated for two, four, and six weeks under FL, BL, or RL in the presence or absence of NAA are shown in Table 1. As the culture period continued, survival rates gradually decreased. The survival rate rapidly decreased under RL among light treatments. Most cuttings were able to survive long-term with BL and NAA treatments. Cuttings treated with NAA developed more adventitious roots compared to those without NAA treatment (Fig. 3). This difference was especially apparent on cuttings also treated with BL. Symptoms of tissue necrosis were mainly observed on cuttings treated with RL without NAA. This necrotic symptom was observed opposite the surface where the adventitious roots developed. BL-, FL-, or NAA-treated cuttings did not exhibit any symptoms of necrosis (Fig. 3). Kaur et al. (2002) in their study reported that adventitious root formation was an important factor for the survival of grapevine cuttings. In the propagation of yellow poinciana (*Peltophorum pterocarpum*) cuttings, auxin treatment prevented a decrease in survival rate because it improved root formation in the cuttings (Saifuddin et al., 2013).

It has been reported that BL contributes to root formation of nursery plants and is effective in generating lateral roots similar to those of tobacco seedlings treated with NAA (Meng et al., 2015). In the case of sweet basil cuttings, BL without NAA quickly established root formation compared to cuttings grown under RL or mixed-light treatment (Lim and Eom, 2013). Furthermore, additional BL irradiation enhanced rooting in the chrysanthemum cuttings (Christiaens et al., 2019). The rapid recovery of cut surface due to rapid adventitious root formation guarantees cutting survival. Therefore, it is recommended that BL is suitable for chrysanthemum propagation using single leaf-bud cuttings.

Table 3
Root formation of chrysanthemum cuttings treated with various light intensities at 4 weeks of cultivation.

Light wavelength	PPFD (μmol·$m^{-2}·s^{-1}$)	Root formation		
		No. of adventitious roots	Length (mm)	Dry weight (mg)
BL	5	8.7b[a]	55.2a	45.3b
	35	12.9b	72.0a	56.5b
	65	32.3a	71.5a	118.4a
RL	5	8.3b	39.2b	14.8b
	35	5.7b	30.0c	21.1b
	65	27.0a	67.4a	43.5a
FL	5	7.8b	53.2a	12.6b
	35	7.4b	55.7a	35.5ab
	65	35.0a	71.0a	42.5a
Significance[b]				
Light wavelength (W)		**	***	***
Light intensity (I)		***	***	***
(W) × (I)		*	***	***

Abbreviations: BL, blue light (BL); RL, red light (RL); FL, fluorescent light (FL); PPFD, photosynthetic photon flux density.
[a] Different letters within columns each light wavelength group indicate significant differences as assessed by Tukey's honestly significant difference test with a 5 % cutoff.
[b] Significance at $p < 0.05$ (*), 0.01 (**), or 0.001 (***).

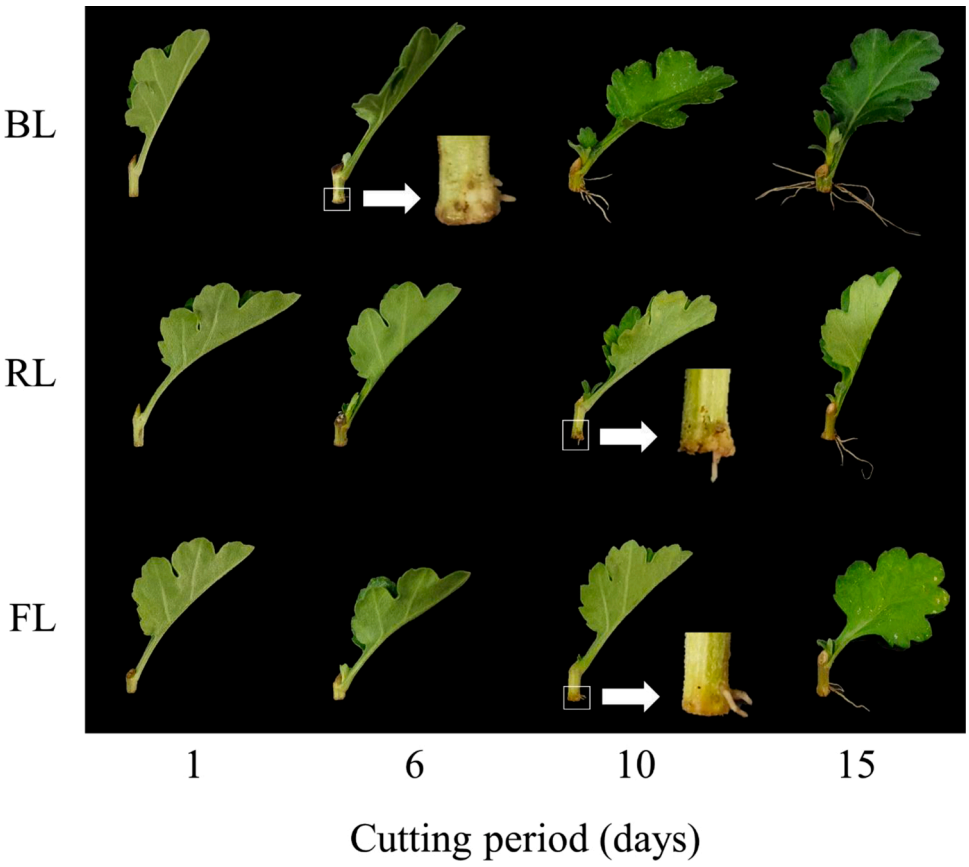

Fig. 5. Morphological differences of adventitious root formation in basal stem of chrysanthemum cuttings treated to blue (BL), red (RL), and fluorescent light (FL) during cutting period. Primary rooting position of stem base appears in zoomed image.

3.3. Root formation by controlling light and NAA

Although using single leaf-bud cuttings is effective on mass propagation of chrysanthemum, it is less preferred than using terminal cuttings caused by considering poor root formation. As shown in Fig. 1, the number of roots was irregular depending on which node position of stock plant was taken. The highest number of roots appeared in terminal cuttings. In single leaf-bud cuttings, the number of roots of basal position cuttings was less than that of middle position cuttings. This pattern was similar to reported results in node cutting propagation of *Eucalyptus grandis* (Abu-Abied et al., 2012) and *rosa hybrid* (Park et al., 2011). The rooting potential of each node cutting depends on the concentration of indole-3-acetic acid (IAA) as endogenous auxin (Al-Saqri and Alderson, 1996; Tchoundjeu and Leakey, 1996; Park et al., 2011). In our results, single leaf-bud cuttings which have lower concentration of IAA than terminal cuttings overcame their poor rooting capacity by BL irradiation without exogenous auxin treatment (Fig. 4). The number of roots was similar in both middle and bottom cuttings exposed to BL for 3 weeks. In addition, the number was higher than that of terminal cutting treated to FL or RL. This result suggests that BL treatment enhances the number of adventitious roots of single leaf-bud cuttings, so mass production of regular individuals is possible in chrysanthemum propagation.

Treatment with both light and NAA significantly improved adventitious root development in chrysanthemum cuttings when compared with treatments without NAA (Table 2). Treatment with both BL and NAA were the most successful for promotion of the development of adventitious roots, even within a relatively short period of time. FL and RL treatments, regardless of exogenous NAA treatment, led to slow root development up to six weeks. These cuttings included less adventitious roots than in those cuttings treated with BL together with NAA (Table 2). Although adventitious root development was delayed without NAA treatment, the BL-treated cuttings developed 12.9-times more abundant roots in four weeks than did RL- and FL-treated cuttings. Adventitious root formation is critical for the survival and growth of propagated cuttings and for the regenerated plantlets (Koroch et al., 2002). That is the reason why successful propagation of cuttings requires abundant adventitious root formation and development within a short period of time. Several studies have been conducted to date to determine how to improve adventitious root development and have concluded that light wavelength and hormone treatment are key factors in efficient rooting (Pinker et al., 1989; Tchoundjeu et al., 2002). In particular, BL has been shown to be effective for inducing adventitious roots in cherry rootstock (Iacona and Muleo, 2010) and sweet basil (Lim and Eom, 2013).

The effects of light intensity on adventitious root development in chrysanthemum single leaf-bud cuttings after culturing for four weeks are shown in Table 3. Among the three light intensities, 65 μmol $m^{-2} \cdot s^{-1}$ improved adventitious root formation, regardless of the wavelength of the light. The number of adventitious roots gradually increased in the BL-treated cuttings as the light intensity increased. The effect of RL or FL on improving adventitious root growth appeared to irradiation with the greatest intensity. High-intensity irradiation is known to improve the rate of photosynthesis (Zhong and Chen, 2008). By photosynthesis, carbohydrates that are synthesized from source leaves influence the root formation and development in sink stem of cuttings (Rapaka et al., 2005). This result supports the conclusion that high-intensity light improves photosynthesis in leaves attached cutting, which leads to abundant generation of adventitious roots.

The effect of NAA treatment on root elongation in chrysanthemum cuttings was insignificant; however, the effect of light wavelength on root elongation was significant (Table 2). The lengths of roots of all cuttings rapidly increased for four weeks and then the values were similarly maintained for up to six weeks. In particular, BL treatment

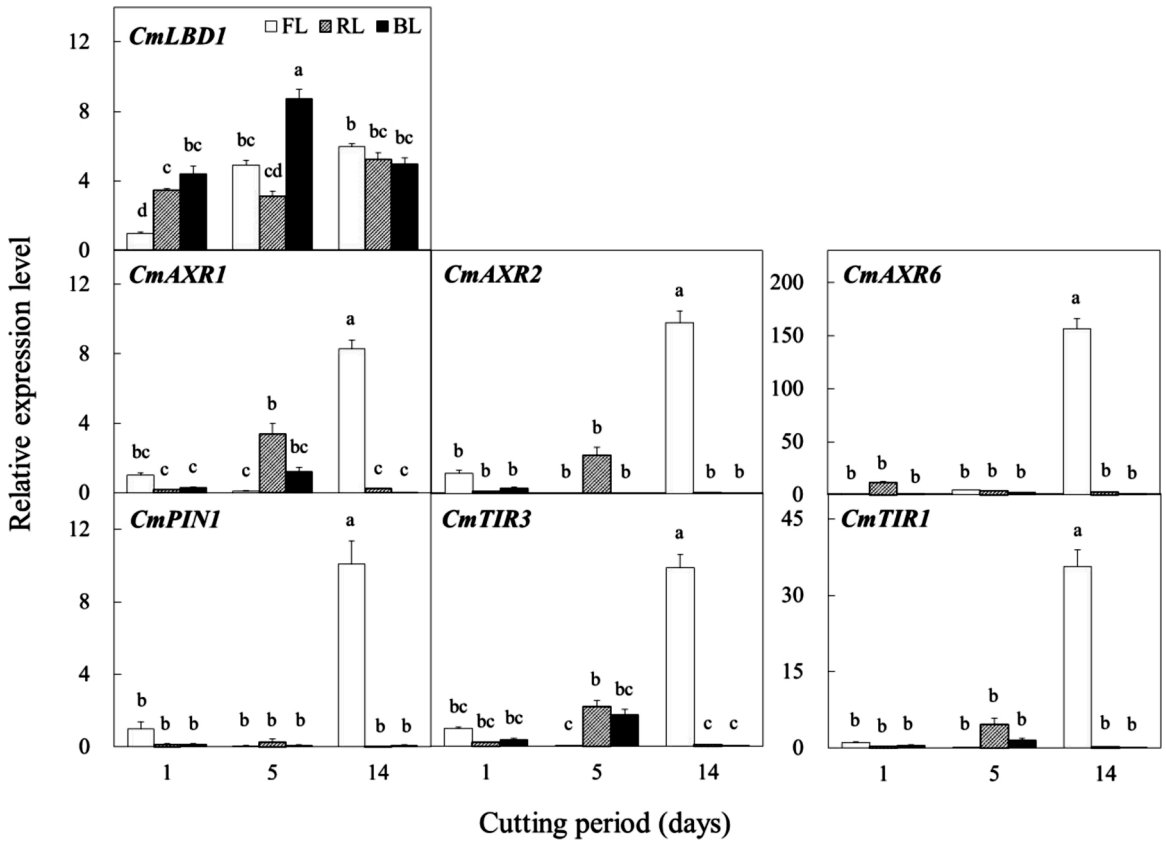

Fig. 6. Relative expression levels of 7 kinds of genes extracted from the stem base of chrysanthemum single leaf-bud cuttings irradiated with 50 μmol $m^{-2}·s^{-1}$ blue (BL), red (RL), and fluorescent (FL) light during cutting period. Different letters over bars indicate significant differences as assessed by Tukey's honestly significant difference test with a 5 % cutoff.

triggered roots that were six times longer than those treated with RL and twice as long as those treated with FL in their initial stages (Table 2). Of the light used, RL yielded the shortest roots. This result is consistent with findings of previous studies that demonstrated that RL irradiation inhibits the initiation of root growth (Correll and Kiss, 2005) and the growth of cuttings (Furuya and Torrey, 1964). The root elongation pattern was similar as the light intensity increased to adventitious root formation in cuttings treated with RL or FL (Table 3). Cuttings treated with BL developed the longest roots (72.0 mm) at 35 μmol $m^{-2}·s^{-1}$; whereas, cuttings treated RL and FL needed 65 μmol $m^{-2}·s^{-1}$ to develop a root length that was similar to that of the BL-treated cuttings. It has been reported that *A. thaliana* seedlings with a malfunctioning BL receptor show reduced root length, while seedlings containing an overexpressed BL receptor established longer roots compared to those in the control (Canamero et al., 2006); therefore, we suggest that BL has a positive effect on root length on successful cuttings.

NAA treatment significantly affected the dry weight of roots per cutting (Table 2). The root dry weight of the cuttings treated with BL together with NAA was 88.5 mg, a value 10 times higher than that for cuttings without NAA after two weeks. Compared with other light sources, BL treatment developed the heaviest root dry weight in the cuttings. BL-treated cuttings rapidly increased the root dry weight for four weeks and maintained that value until the end of the culturing period (Table 2). Root dry weight for each light source progressively increased as the light intensity increased (Table 3). Although irradiated at a low intensity, BL-treated cuttings accumulated more biomass in their roots than RL- or FL-treated cuttings. The root dry weight of cuttings irradiated with BL at the lowest intensity was 45.3 mg. This value was higher than that of cuttings irradiated with the highest intensity of RL (43.5 mg) or FL (42.5 mg) (Table 3). According to several studies, BL in either single or mixed with other wavelengths as light source acts to reduce the shoot/root growth ratio of the plant (Nhut et al., 2003; Poudel et al., 2008). The BL treatment on red-leaf lettuce promoted the growth of rhizospheres and led to the accumulation of biomass in the root that increased its weight (Johkan et al., 2010).

3.4. Relative RNA expression of rooting-related genes

Morphological analysis were performed to determine the critical time of initial adventitious root formation in chrysanthemum cuttings irradiated with 50 μmol $m^{-2}·s^{-1}$ BL, RL, and FL (Fig. 5). The 6-day-old cuttings irradiated with BL developed the initial roots, whereas cuttings irradiated with RL and FL maintained root-free until 9 d. Consequently, root length of BL-treated chrysanthemum cuttings was significantly longer at 10 d and 15 d compared to those of RL or FL-treated cuttings (Fig. 5). To understand molecular mechanism of BL-induced root formation of cuttings, six genes involved in auxin signaling pathway and one gene participated in the lateral root formation in chrysanthemum were selected and used for qRT-PCR analysis (Fig. 6). Two auxin receptor genes (*CmTIR1* and *TIR3*), three auxin responsive genes (*CmAXR1*, *AXR2*, and *AXR6*) and one auxin transport (*CmPIN*) were included. The expression level of *CmLBD1* was dramatically increased in BL-treated cutting up to 5 d whereas the highest expression of *CmLBD1* in RL- and FL-treated cuttings was observed at 14 d. Furthermore, much higher expression of *CmLBD1* was detected even in 1 d in BL-treated cutting compared to FL-treated cutting. The expression levels of *CmAXR1*, *CmTIR1*, and *CmTIR3* were gradually increased in BL-treated cutting and reached at the basal level at 14 d. The *CmPIN1* gene was not responded to the BL. Interestingly, all six auxin-related genes were dramatically expressed in FL-treated cuttings at 14 d, but not in RL-

treated samples. These findings indicate that BL treatment on cuttings rapidly induces *CmLBD1* transcript and elevated levels of *CmLBD1* might play a critical role to initiate adventitious root formation. Considering the fact that the expression levels of six auxin-related genes were not dramatically changed upon BL treatment, transcriptional activation of *CmLBD1* by BL treatment would be auxin-in dependent. Further study is required to reveal the molecular link between BL treatment and the transcriptional activation of *CmLBD1*. On 14 d after cutting, the highest expression of other 6 genes occurred in FL-treated cuttings. Because FL-treated cuttings already generated initial adventitious roots before 14 d, these genes are not likely to play a major function in primary root formation. In previous research, it was reported that *AXR* expression induced appearance and growth of lateral roots in *Arabidopsis* (Estelle and Somerville, 1987; Hobbie and Estelle, 1995; Reed et al., 1998). Also, TIR and PIN proteins have a role of auxin polarity, which results in cell expansion and division for lateral roots elongation (Ruegger et al., 1998; Friml et al., 2002; DiDonato et al., 2004). In addition, although *CmAXR1*, *CmAXR2*, *CmTIR1*, and *CmTIR3* were responded to RL irradiation on 5 d, RL-treated cuttings did not show rooting initiation on 5 d cuttings. It is demonstrated that these expression levels were not likely insufficient to induce significant results in RL-treated cuttings or not directly related to root initiation.

4. Conclusion

Our results indicate that BL treatment together with NAA treatment might accelerate adventitious root formation and establishment, which is critical for the successful growth of single leaf-bud chrysanthemum cuttings. Irradiation with high-intensity BL resulted in more abundant adventitious roots in both the presence and absence of NAA compared to treatments with RL and FL. In gene regulation study, the expression of *CmLBD1* sensitively responded to light irradiation, which induced rapid adventitious root formation. The abundance of *CmLBD1* transcripts was higher after BL irradiation than RL or FL irradiation; therefore, we suggest that the application of the single leaf-bud cutting method, coupled with irradiation with BL and NAA treatment, should guarantee the mass production of health cuttings and withdrawal cutting duration to successful the overall annual production of cuttings.

Credit author statement

Chan Saem Gil: Data curation, Formal analysis, Writing-original draft Ho Young , **Ho Young Jung**: Formal analysis, **Chanhui Lee**: Data curation, **Seok Hyun Eom**: Conceptualization, Writing-review & Editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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