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Photoreceptors Modulate the Flowering and Morphogenesis Responses of *Pelargonium* × *hortorum* to Night-Interruption Light Quality Shifting

Yoo Gyeong Park [1] and Byoung Ryong Jeong [1,2,3,*]

[1] Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea; ygpark615@gmail.com
[2] Department of Horticulture, Division of Applied Life Science (BK21 Four), Graduate School, Gyeongsang National University, Jinju 52828, Republic of Korea
[3] Research Institute of Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea
* Correspondence: brjeong@gmail.com; Tel.: +82-010-6751-5489

Abstract: This study examines how the day neutral plant (DNP) *Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet' is impacted by LED night-interruption light (NIL) quality shifting in terms of flowering, morphogenesis, and transcription of photoreceptor genes. A closed-type plant factory with white (W) LEDs providing 180 μmol·m^{-2}·s^{-1} PPFD light for long day (LD, 16 h light, 8 h dark), short day (SD, 10 h light, 14 h dark), or SD with 4 h night interruption (NI) with 10 μmol·m^{-2}·s^{-1} PPFD LEDs was used to grow the plants. Two NIL qualities were employed, where after the first two hours, the NIL quality was switched from one to another among white (W), far-red (Fr), red (R), and blue (B). A total of 12 SD treatments with NIL quality shifting were used, with the LD and SD serving as the control: NI-BR (from B to R), NI-RB (from R to B), NI-RFr (from R to Fr), NI-FrR (from Fr to R), NI-BFr (from B to Fr), NI-FrB (from Fr to B), NI-WB (from W to B), NI-BW (from B to W), NI-FrW (from Fr to W), NI-WFr (from W to Fr), NI-RW (from R to W), and NI-WR (from W to R). LD refers to a 16 h long-day treatment. Geranium plants were taller in NI treatments that included Fr light than those in other NI treatments and were the shortest in the NI-WB treatment. Flowering was seen in all treatments and was notably encouraged by NI with Fr light, regardless of the sequence of light quality applied. In NI-FrR and NI-RFr, high expressions of *phyA*, *phyB*, and *cry1* were observed. Flower formation and plant morphogenesis were both impacted by the photoperiod. Both morphogenesis and flowering were strongly impacted by the second NIL, but the first NIL had no effects on either. These findings indicate that NI-RFr and NI-FrR improve flowering, which may be used for commercial DNP production.

Keywords: anthesis; day neutral plant; light quality; lighting; night break

Citation: Park, Y.G.; Jeong, B.R. Photoreceptors Modulate the Flowering and Morphogenesis Responses of *Pelargonium* × *hortorum* to Night-Interruption Light Quality Shifting. *Agronomy* **2023**, *13*, 857. https://doi.org/10.3390/agronomy13030857

Academic Editor: Viktorija Vaštakaitė-Kairienė

Received: 18 February 2023
Revised: 12 March 2023
Accepted: 13 March 2023
Published: 15 March 2023

1. Introduction

Plants modify their biological cycles in response to ambient environmental information, such as the quality of light [1]. The biochemical, morphological, anatomical, and physiological characteristics of leaves are significantly affected by variations in the light quality, which are influenced by the spectrum qualities of tissue pigments [2,3]. Photoreceptors regulate plant growth and development over their entire life cycles; additionally, they keep track of the light environment and aid in timing the significant developmental transitions such as flowering commencement and germination [4]. Various photoreceptors sense environmental light signals and seasonal changes in the plant's leaves and relay those signals to the flowers [5]. Different photoreceptors differently control plants and development, so it is a photoperiodic perception process [6]. Cryptochromes and phytochromes are photoreceptors that primarily absorb blue (B) and ultraviolet-A (UV-A) lights, and red (R) and far-red (Fr) lights, respectively; both photoreceptors help control

flowering [7]. Depending on the species, there can be numerous phytochrome (*phyA, phyB, phyC, phyD,* and *phyE*) and cryptochrome (*cry1* and *cry2*) variants [8,9]. Photoperiodic flowering initiation is thought to be regulated by a systemic flowering inducer (florigen) and inhibitor (antflorigen) produced in the leaves [10]. Light plays important roles in controlling flowering in *A. thaliana* by regulating *CONSTANS* (*CO*) and *FT* [11]. Higuchi et al. [10] revealed the existence of an *Antiflorigenic FT/TFL*1 family protein (*AFT*) in *C. seticuspe* and clearly demonstrated that the *CsAFT* protein acted as a systemic floral inhibitor, an antiflorigenic signal produced in leaves under non-inductive conditions.

By speeding up production and enhancing overall crop quality, photoperiod manipulation can save agricultural costs [12]. To increase the quality of seedlings and rooted cuttings, light is frequently added; this additional light may lengthen the day or act as a complement to natural light [13]. To allow for earlier commercialization or seed production of long-day plants (LDPs), night interruption (NI) during short-day (SD) seasons significantly sped up the flowering, while during LD seasons, NI also delayed the blossoming of SD plants [14,15]. According to recent experiments, even low-intensity NI proved successful in regulating the flowering of plants [16–20]. The growth and development of LDPS during the SD season can effectively be encouraged by introducing NI, as observed in *Campanula carpatica* [21], *Coreopsis grandiflorum* [22], and *Cyclamen persicum* [23]. *Petunia hybrida*, an LDP, flowered when NI-treated with R or W light [24,25]. When *Pelargonium* × *hortorum*, a DNP, was NI-treated with Fr light, flowering was delayed [26]. A NI using either Fr, R, B, or W light promotes flowering in *A. thaliana*, with Fr light being the most effective [27]. NI treatment with a combination of B and R light promoted the flowering of *C. persicum* [28]. By keeping herbaceous SDPS in their vegetative growth stage, NI was also employed to prevent or delay flowering in *Dendranthema grandiflorum* [17,29] and *Kalanchoe blossfeldiana* [30]. NI treatment with B and R lights, as well as R light, delayed the flowering of *D. grandiflorum* [31]. NI treatment with B, R, and Fr light affected the flowering of chrysanthemums [32]. A NI with a very low (3–5 μmol·m^{-2}·s^{-1} PPF) light intensity promotes flowering induction and increases growth rates during the juvenile stage in *Cymbidium aloifolium* [16].

According to Park et al. [18], alterations in the NIL quality had a substantial impact, both positive and negative, on the flowering, expression of transcriptional factors, and morphogenesis of *D. grandiflorum* (SDP). Statistically, neither the flowering nor morphogenesis in *D. grandiflorum* was significantly impacted by the NIL quality of the first 2 h; however, the NIL quality during the last 2 h had a substantial impact on both [18]. However, only SDPs [18] and not DNPs were subjected to experiments with NI with LEDs of variable light qualities. We hypothesized that NIL shifting at a low intensity for 4 h would affect plant morphogenesis and blooming, either synergistically or antagonistically. The effects of NIL quality shifting on the blooming, transcription of photoreceptor genes, and morphogenesis in *Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet' (DNP) were, therefore, investigated in this work.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') seeds (Pan Seed Co., West Chicago, IL, USA) were planted in 288-cell plug trays with a commercial medium (Tosilee Medium, Shinan Grow Co., Jinju, Republic of Korea) from a glasshouse bench. Forty days after seeding, the seedlings were moved to 50-cell plug trays. On the day of transplanting, the rooted cuttings and seedlings were moved to a closed-type plant factory. After settling in for 24 days in the plant factory, the plants (at around 11.2 cm in height) were exposed to the photoperiodic light treatments. The plants were transferred to a closed-type plant factory after being grown propagated in a glasshouse, first to adapt to 20 ± 1 °C, 60 ± 10% RH, and 140 ± 20 μmol·m^{-2}·s^{-1} PPFD provided by fluorescent lamps (F48T12-CW-VHO, Philips Co Ltd., Eindhoven, The Netherlands) and subsequently for the photoperiodic treatments with LEDs 25 cm above the plant canopy. Through-

out the experiment, a greenhouse multipurpose nutrient solution (electrical conductivity 1.5 mS·cm^{-1} and pH 5.8) [15] was fertigated to plants once every day.

2.2. Photoperiodic Light Treatments

White (W) LEDs (MEF50120, More Electronics Co. Ltd., Changwon, Republic of Korea) at 180 µmol·m^{-2}·s^{-1} PPFD were used to cultivate the plants in this study under either long day (LD, 16 h light/8 h dark), short day (SD, 10 h light/14 h dark), or SD with a 4 h night interruption (NI, 23:00–03:00) with 10 µmol·m^{-2}·s^{-1} PPFD LEDs. After the first two hours of NI, the NIL quality was changed to another among blue (B, 450 nm), red (R, 660 nm), far-red (Fr, 730 nm), and white (W, 400–700 nm) [18]. The LD and SD were referenced as the control, and 12 SD treatments with the NIL quality shifting were employed as follows: from blue to red (NI-BR), from red to blue (NI-RB), from red to far-red (NI-RFr), from far-red to red (NI-FrR), from blue to far-red (NI-BFr), from far-red to blue (NI-FrB), from white to blue (NI-WB), from blue to white (NI-BW), from far-red to white (NI-FrW), from white to far-red (NI-WFr), from red to white (NI-RW), and from white to red (NI-WR) (Figure 1). A spectroradiometer (USB 2000 Fiber Optic Spectrometer, Ocean Optics Inc., Dunedin, FL, USA) 25 cm above the bench top was used to scan the spectral distribution of all lighting treatments in 1 nm intervals. At three locations within the plant-growing bench, the average maximum absolute irradiance and spectral distribution were measured.

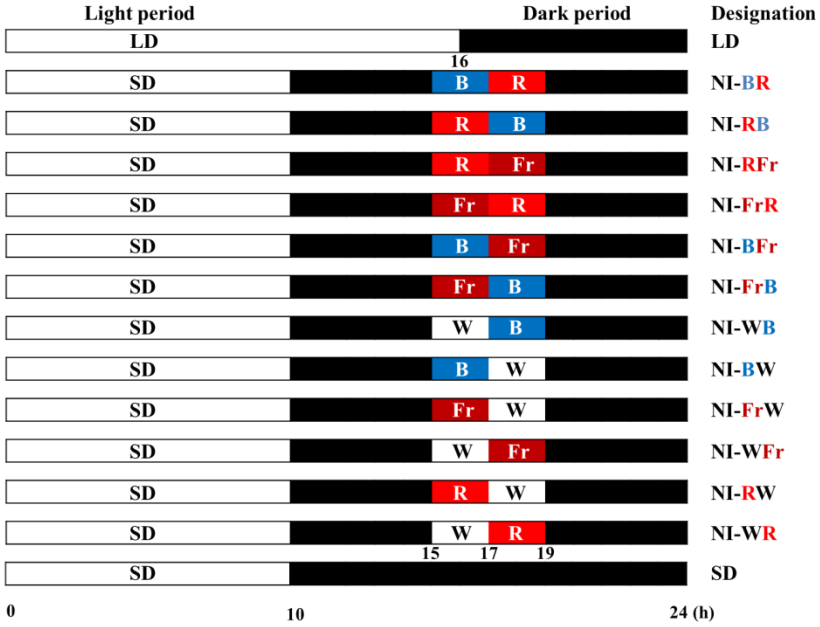

Figure 1. Light quality shifting with light-emitting diodes (LEDs) during the 4 h night interruption (NI) in the 10 h short-day (SD) treatments: NI-BR, blue to red; NI-RB, red to blue; NI-RFr, red to far-red; NI-FrR, far-red to red; NI-BFr, blue to far-red; NI-FrB, far-red to blue; NI-WB, white to blue; NI-BW, blue to white; NI-FrW, far-red to white; NI-WFr, white to far-red; NI-RW, red to white; and NI-WR, white to red. LD indicates the 16 h long-day treatment.

2.3. Data Collection and Analysis

The plant height, leaf width and length, petiole length, average number of nodes, bottom third internode length, chlorophyll content, relative growth rate, shoot/root fresh and dry weights, percent flowering, days from treatment initiation to visible flower bud or days to visible buds (DVB), average number of flowers, and photoreceptor gene expressions were measured after 40 days of starting the photoperiodic treatments. The leaf length to leaf width ratio was considered as the leaf expansion index, and the leaf length to petiole length ratio was considered as the overgrowth (stretchiness) index. The mean net increase in the plant dry biomass per unit of plant dry biomass over a given time interval was

considered as the relative growth rate. The total plant dry weight was determined before (W1) and after (W2) the photoperiodic treatments, and the relative growth rate across the time interval t2 − t1 was derived as:

$$\text{Relative growth rate} = (\ln W2 - \ln W1)/(t2 - t1)$$

To estimate the chlorophyll levels, 10 mg of fresh leaf samples was collected from young, completely developed leaves and extracted using 80% ice-cold acetone. A spectrophotometer (Biochrom Libra S22, Biochrom Co. Ltd., Holliston, MA, USA) was used to determine the absorbance of the supernatant at 663 and 645 nm, following a 3000 rpm centrifugation. Dere et al. [33] were referred to for the calculation methods. After drying for three days at 75 °C in an oven (Model FO-450M, Jeio Technology Co. Ltd., Seoul, Republic of Korea), the dry weights of the shoot and root were measured.

Furthermore, the effects of the photoperiodic treatments were separately assessed for the first NI and second NI, where the same light quality treatments during the same NI stage were considered as the same treatment; for example, NI-BR, NI-BFr, and NI-BW were grouped together.

This study used a randomized complete block design with 3 replications, with each replication containing 2 plants. To minimize the effects of positioning, the treatment sites in a controlled setting were randomly mixed between replications. SAS (Statistical Analysis System, V. 9.1, Cary, NC, USA) was used to determine the statistical significance of the acquired data. Duncan's multiple range test and an analysis of variance (ANOVA) were applied to the results. SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA, USA) was used to graph the results.

2.4. Isolation of Total RNA Isolation and Semi-Quantitative RT-PCR (Reverse Transcriptase–Polymerase Chain Reaction) Analysis of Selected Genes

Following the manufacturer's instructions, the total RNA was isolated from the shoot tip of plants after 33 days of exposure to the NI treatments using an RNA isolation kit (Promega, Madison, WI, USA). Using a reverse transcriptase kit from Promega (Madison, WI, USA), 1 µg of DNase-treated RNA was reverse-transcribed to create first-strand cDNA, which was then utilized as a template for polymerase chain reaction (PCR). *Cryptochrome 1* (*cry1*), *FLOWERING LOCUS T* (*FTL*), *Anti-florigenic FT/TFL1* family protein (*AFT*), *phytochrome A* (*phyA*), and *phytochrome B* (*phyB*) of the sequence from *Arabidopsis thaliana* were used as primers in separate PCRs with an equal amount of cDNA (Table 1). Since *Actin* is frequently employed to normalize molecular expression studies thanks to its high conservation as an endogenous housekeeping gene, it was used as the control in this study. The following PCR conditions were employed: 5 min initial denaturation at 95 °C; 35 cycles of 20 s at 95 °C, 30 s at 57 °C, and 30 s at 72 °C; and a 10 min final extension at 72 °C. After 35 cycles, the PCR results were tested on a 1% agarose gel to determine whether the transcripts were differently expressed.

Table 1. Primers for quantifying gene expression levels.

Gene	Accession No.	Forward Primer	Reverse Primer
phyA	EU915082	5′-GACAGTGTCAGGCTTCAACAAG-3′	5′-ACCACCAGTGTGTGTTATCCTG-3′
phyB	NM_127435	5′-GTGCTAGGGAGATTACGCTTTC-3′	5′-CCAGCTTCTGAGACTGAACAGA-3′
cry1	NM_116961	5′-CGTAAGGGATCACCGAGTAAAG-3′	5′-CTTTTAGGTGGGAGTTGTGGAG-3′
AFT	AB839766	5′-AGAACACCTCCATTGGATCG-3′	5′-CTGGAACTAGGTGGCCTCAC-3′
FTL	AB839767	5′-ACAACGGACTCCTCATTTGG-3′	5′-CGCGAAACTACGAGTGTTGA-3′
Actin	AB205087	5′-CGTTTGGATCTTGCTGGTCG-3′	5′-CAGGACATCTGAAACGCTCA-3′

3. Results

3.1. Morphogenesis

NI-FrB significantly led to the tallest plants, followed in order by NI-WFr (22.0 cm) and NI-FrW (20.3 cm) (Figure 2). Regardless of the order of NILs given, plants were taller with NI with Fr light than with others (Figure 2). In treatments with Fr light, there was an increase in plant heights (Figure 2). Among all NI treatments studied, NI-WB (17.7 cm) resulted in the shortest plants (Figure 2).

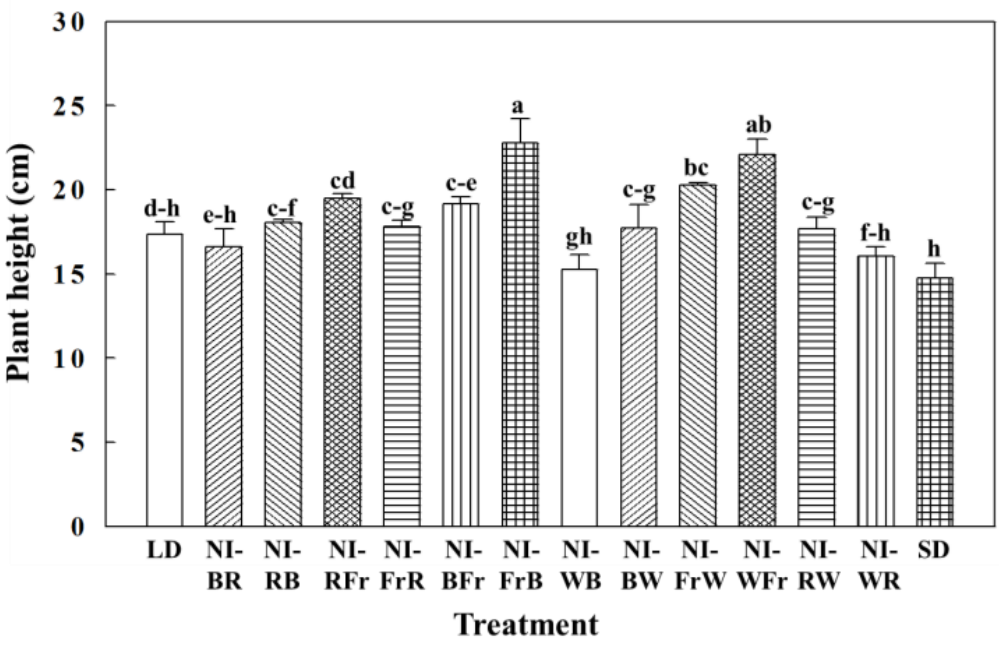

Figure 2. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on the plant height of geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') measured 40 days after treatment. Please refer to Figure 1 for detailed NIL qualities. Means accompanied by different letters are significantly different ($p < 0.05$) according to the Duncan's multiple range test at 5% significance level. Vertical bars are means ± S.E. (n = 3).

The ratio of the leaf length to leaf width was the greatest in NI-RB (Figure 3A). The ratio of the leaf length to leaf width was significantly greater in the combination of R, B, and W treatments (NI-BR, NI-RB, NI-WB, NI-BW, NI-RW, and NI-WR) in other NI treatments (Figure 3A). NI-BFr resulted in the lowest leaf length to width ratio, followed by NI-WFR and other Fr-containing treatments (Figure 3A). The ratio of the leaf length to petiole length was the greatest in NI-RB and the lowest in NI-BFr (Figure 3B). Regardless of the light sequence, the leaf length to petiole length ratio was lower (<1) when NI with Fr light was used than it was with other light quality treatments (Figure 3B). The average number of leaves was the highest in NI-WR (20) and lowest in NI-FrR (14.3) (Figure 3C). Combinations of B and R (NI-BR and NI-RB), W and B (NI-WB and NI-BW), and R and W (NI-RW and NI-WR) lights resulted in an increased leaf area (Figure 3D).

The relative growth rate was the highest in LD (Figure 4); however, due to the early flowering, the relative growth rate was lower in NI treatments with Fr light than in others. NI-RFr resulted in the highest chlorophyll content, followed by SD, and the lowest in LD (Figure 5). The shoot and root fresh/dry weights were the highest in LD (Table 2). Fresh and dry weights were higher in NI that combined R, B, and W (NI-RB, NI-WB, NI-BW, and NI-RW) light treatments compared with the other NI treatments (Table 2).

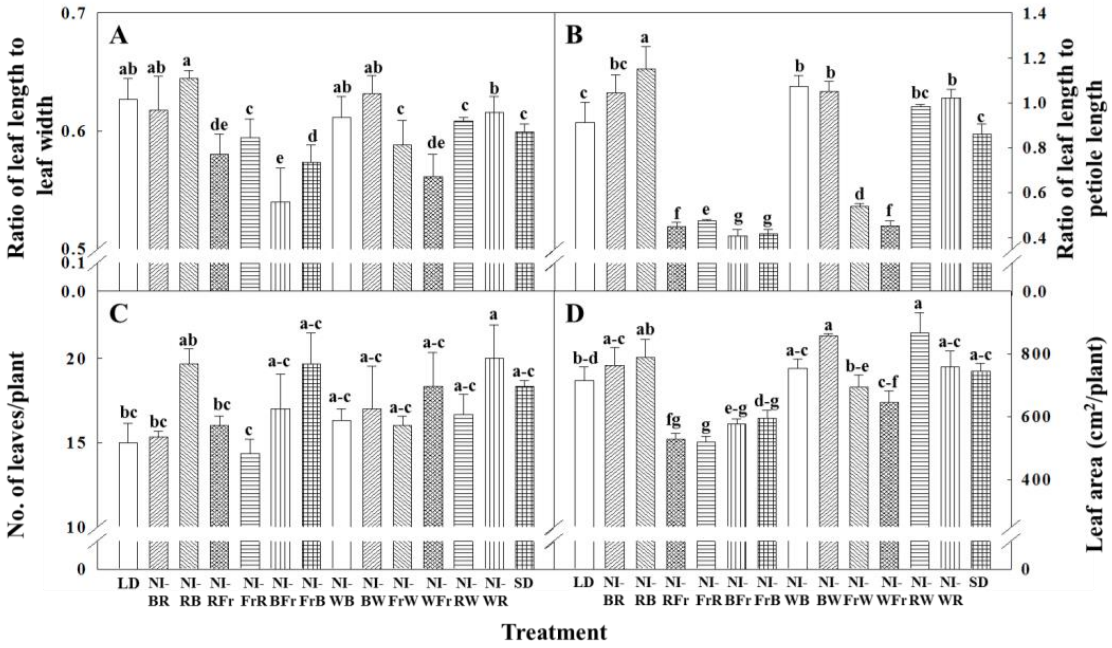

Figure 3. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on the leaf length to width ratio (**A**), leaf length to petiole length ratio (**B**), number of leaves per plant (**C**), and leaf area (**D**) of geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') measured 40 days after treatment. Please refer to Figure 1 for detailed NIL qualities. Vertical bars are means ± S.E. (n = 3). Means accompanied by different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test at 5% significance level.

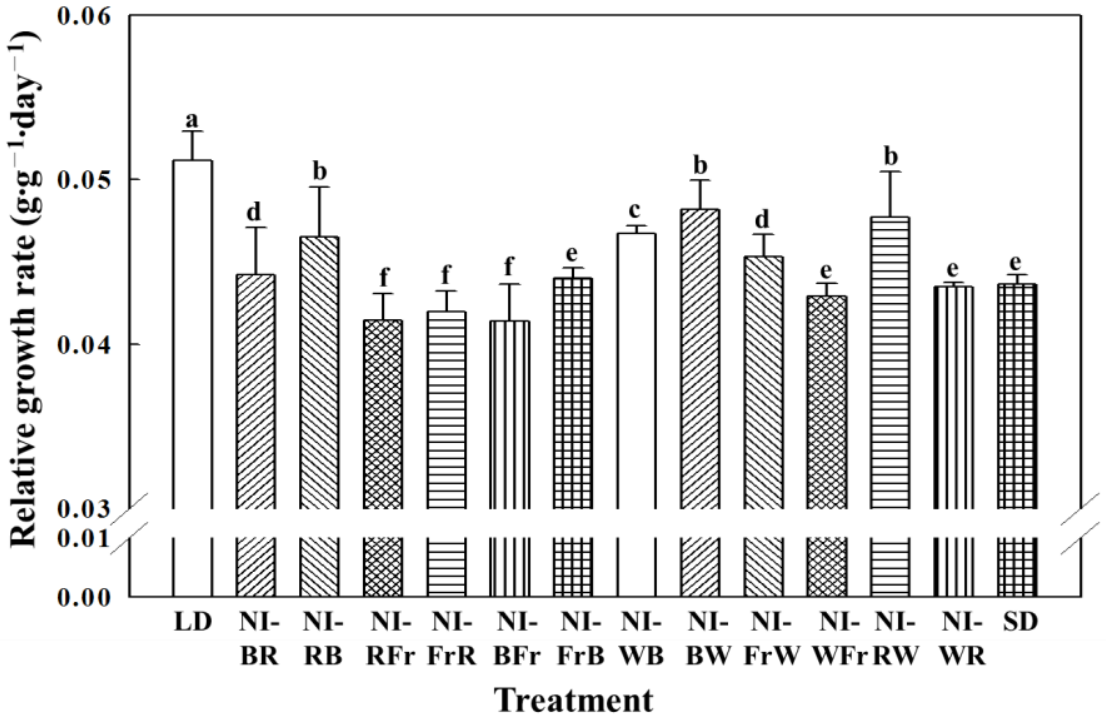

Figure 4. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on the relative growth rate of geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') measured 40 days after treatment. Please refer to Figure 1 for detailed NIL qualities. Vertical bars are means ± S.E. (n = 3). Means accompanied by different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test at 5% significance level.

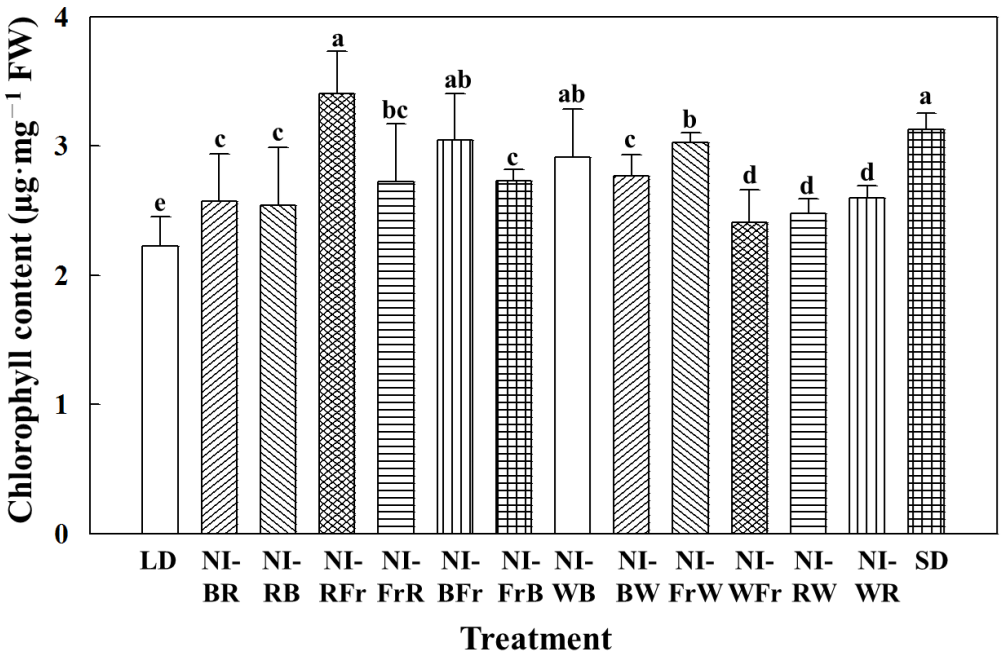

Figure 5. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on the chlorophyll content of geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') leaves measured 40 days after treatment. Please refer to Figure 1 for detailed NIL qualities. Vertical bars are means ± S.E. (n = 3). Means accompanied by different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test at 5% significance level.

Table 2. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on the shoot/root fresh and dry weights of geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') measured 40 days after treatment.

Treatment [z]	Fresh Weight (g)			Dry Weight (g)		
	Shoot	Root	Total	Shoot	Root	Total
LD	40.4 ab [y]	3.06 a	43.4 ab	4.61 a	0.51 a	5.13 a
NI-BR	36.0 a–c	2.94 ab	38.9 a–c	3.35 bc	0.38 bc	3.74 b–d
NI-RB	36.9 a–c	2.70 a–c	39.6 a-c	3.77 a–c	0.39 b	4.17 a–c
NI-RFr	23.2 d	1.19 g	24.4 d	3.02 c	0.22 e	3.24 d
NI-FrR	29.5 cd	1.56 d–f	31.1 cd	3.07 bc	0.24 de	3.31 cd
NI-BFr	29.6 cd	1.43 fg	31.1 cd	2.99 c	0.26 c–e	3.25 d
NI-FrB	35.7 a–c	1.39 fg	37.1 bc	3.38 bc	0.25 c–e	3.64 b–d
NI-WB	36.6 a–c	2.99 a	39.6 a–c	3.67 a–c	0.45 ab	4.13 a–d
NI-BW	44.3 a	2.37 b–d	46.6 a	4.09 ab	0.36 b–d	4.45 ab
NI-FrW	34.4 bc	2.07 de	36.5 bc	3.53 bc	0.34 b–e	3.88 b–d
NI-WFr	32.9 bc	1.42 fg	34.4 bc	3.21 bc	0.24 de	3.46 b–d
NI-RW	39.9 ab	1.97 d–f	41.8 ab	4.07 ab	0.33 b–e	4.40 a–c
NI-WR	36.4 a–c	2.08 de	38.5 a–c	3.18 bc	0.37 b–d	3.55 b–d
SD	32.5 bc	2.15 d–e	34.6 bc	3.22 bc	0.36 b–d	3.58 b–d
F-test	**	***	***	*	***	*

[z] Please refer to Figure 1 for detailed NIL qualities. [y] Mean separation within columns by Duncan's multiple range test at 5% level. *, **, ***: Significant at $p \leq 0.05$, 0.01, or 0.001, respectively. Means accompanied by different letters are significantly different ($p < 0.05$).

3.2. Flowering

All treatments caused flowering (Table 3 and Figure 6A,B). In NI-FrR (18.4), NI-RFr (20.0), NI-FrB (21.8), NI-BFr (21.8), and NI-WFr (22.2), the DVB was shortened (Table 3 and Figure 6A,B). NIL quality shifting did not significantly alter the average number of flowers or flower stalk length (Table 3). Flower stalk length was the tallest in NI-FrR

(14.4 cm) followed by NI-WFr (6.3 cm) (Table 3). NI-FrR with the fastest flowering times (18.4) also had the longest flower stalk length (14.4 cm) (Table 3). All treatments were seen to increase the expression of photoreceptor genes; NI-RFr and NI-FrR clearly increased such expressions the most (Figure 6). All photoreceptors had lower expression in LD and SD compared with the other NI treatments (Figure 6). Other photoreceptor genes such as *FTL* and *AFT* showed similar results as observed for *phyA*, *phyB*, and *cry1* (Figure 7).

Table 3. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on flowering characteristics of geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') measured 40 days after treatment.

Treatment [z]	Flowering (%)	DVB [y] (Day)	No. of Flowers/Plant	Flower Stalk Length (cm)
LD	100	23.8	1.0	0.7 b [x]
NI-BR	100	29.8	1.0	0.6 b
NI-RB	100	29.0	1.0	0.7 b
NI-RFr	100	20.0	1.3	5.1 ab
NI-FrR	100	18.4	1.0	14.4 a
NI-BFr	100	21.8	1.3	5.5 ab
NI-FrB	100	21.8	1.3	8.5 ab
NI-WB	100	29.0	1.0	0.7 b
NI-BW	100	29.4	1.0	1.0 b
NI-FrW	100	28.6	1.0	2.0 b
NI-WFr	100	22.2	1.3	6.3 ab
NI-RW	100	28.0	1.0	1.3 b
NI-WR	100	29.0	1.0	1.0 b
SD	100	29.8	1.3	1.7 b
F-test			NS	*

[z] Please refer to Figure 1 for detailed NIL qualities. [y] Days after treatment initiation to visible flower bud or days to visible buds. [x] Mean separation within columns by Duncan's multiple range test at 5% level. NS, *: Nonsignificant or significant at $p \leq 0.05$. Means accompanied by different letters are significantly different ($p < 0.05$).

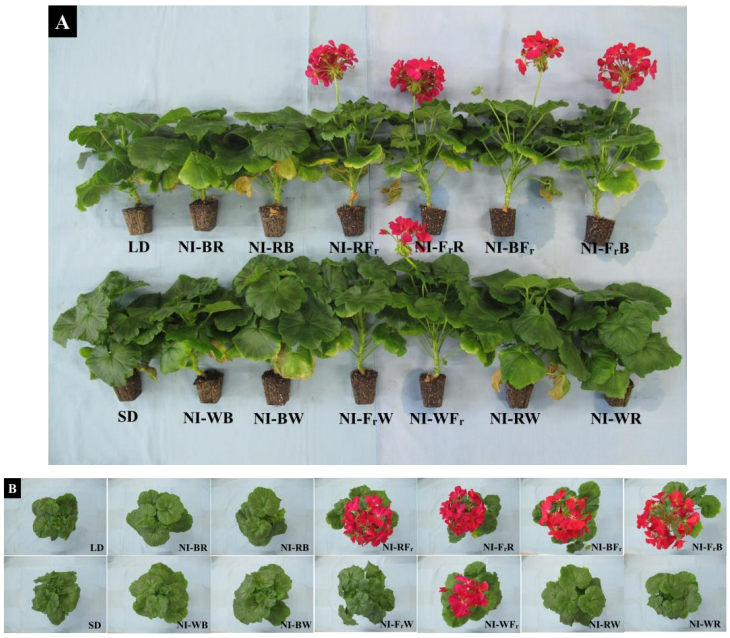

Figure 6. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on flowering of geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') measured 40 days after treatment: side view (**A**) and top view (**B**). Please refer to Figure 1 for detailed NIL qualities.

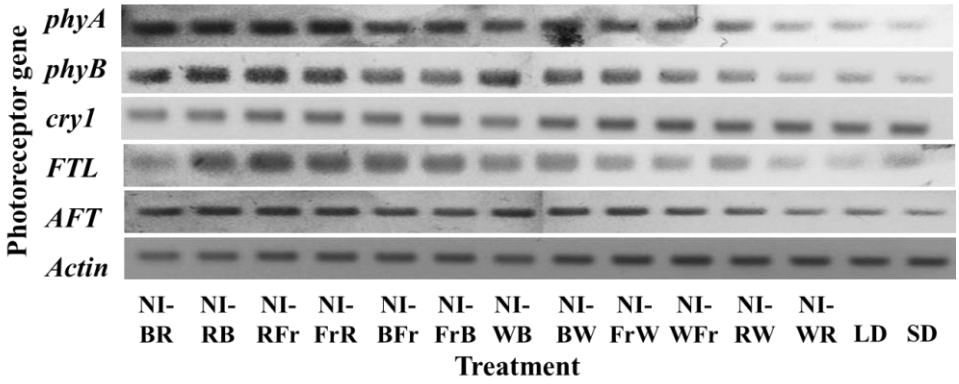

Figure 7. Effects of 10 μmol·m^{-2}·s^{-1} PPFD night-interruption light (NIL) quality shifting on the expression of photoreceptor genes in geranium (*Pelargonium* × *hortorum* L.H. Bailey 'Ringo 2000 Violet') measured at 40 days after treatment. Please refer to Figure 1 for detailed NIL qualities. Please refer to Table 1 for details of photoreceptor genes and *Actin*.

4. Discussion

The taller plants seen in response to NI with Fr light in this study may be a result of shade avoidance response. NI-FrB produced the tallest plants, followed in order by NI-WFr and NI-FrW. Regardless of the light sequence, plants grew taller when NI included Fr light than when they contained other lights. According to Devlin et al. [34], internode elongation in *Arabidopsis thaliana* was affected more by the *phyE* level, when monitoring phytochromes A to E, such that *phyE* deficiency was characteristic in the shade avoidance response to a low R-to-Fr ratio light. Increased elongation caused by Fr light, rather than R light, has also been seen in Norway spruce (*Picea abies*) [35] and *Pelargonium* × *hortorum* 'Penny Irene' [36]. In this study, NI-WB resulted in the shortest plants of all, perhaps as a result of B light. Folta and Spalding [37] found that the involvement of cryptochromes and phototropins was connected to the stem elongation inhibition response and showed that the inhibition process was a de-etiolated reaction. Earlier research in *A. thaliana* [37] and lettuce seedlings [38] demonstrated that exposure to B light reduced hypocotyl elongation, indicating that photoreceptors may play a role in regulating the plant height.

NI-BFr resulted in the lowest leaf length to leaf width ratio, followed in order by NI-BFr and other NI with Fr light. This suggests that Fr light during NI was involved in inhibiting leaf extension growth and encouraging leaf expansion growth. In this study, NI treatments that combined B and R lights (NI-BR and NI-RB), W and B lights (NI-WB and NI-BW), and R and W lights (NI-RW and NI-WR) all resulted in an increased leaf area. This supported the claim that the light quality shifting during the 4 h NI affected the growth of geranium leaves, evidenced by the enhanced leaf expansion in NI-RW and NI-WR, and reduced leaf expansion in NI-BFr and NI-FrB. These findings were consistent with the finding that geranium leaves expanded more with NI-B and NI-R treatments than with NI-Fr treatments [26]. The majority of phytochrome receptors, according to Weining [39], facilitated the plant flexibility in response to the light quality. During the photoperiod, photosynthetic pigments mostly absorb photons from B and R light bands of the visible light spectrum [40]. A decrease in the R:Fr light ratio has a number of remarkable impacts on plant growth and development in shade-intolerant plants *A. thaliana* [41]. This study's findings on the reduced leaf expansion with NI-BFr and NI-FrB are comparable with the shade avoidance response. The relative growth rate in this study was the highest in the LD, and lower with NI treatments with Fr light than in other treatments, because NI with Fr light caused earlier flowering.

In this study, NI-RFr resulted to the greatest chlorophyll concentration, followed by SD. In NI-RFr, the overall quantum yield of photosynthesis rises because R wavelengths are absorbed, but Fr wavelengths are transmitted due to the selective filtering by chlorophyll [42,43]. The total accumulated light energy of LD resulted in the greatest fresh and dry weights of the shoot and root in this study. The higher the daily light exposure plants

receive within the proper range of light intensity, the stronger they grow and provide a better yield [44]. All LD conditions generally enhanced plant growth parameters better than SD conditions, including stem diameter, plant height, plant dry weight, total chlorophyll content, starch content, and soluble protein content, according to Yang et al. [45].

In this study, the DVB was shortened in NI-RFr, NI-FrR, NI-BFr, NI-FrB, and NI-WFr. These results suggested that NI with Fr light, regardless of the NIL sequence, promoted flowering. A rise in the P_{fr} (phytochrome that absorbs Fr light) concentration may have contributed to the enhanced flowering in response to Fr light. However, according to Park et al. [46], the flower diameter, number of flowers, DVB, and days to flowering are unaffected by NI. The average number of flowers was the lowest with NI-Fr, the length of the flower stalk was the greatest with NI-B, and the SD and NI-W reduced the length of the flower stalk in comparison with other treatments, according to Park et al. [26]. The opposing results observed in this study are thought to be due to the complex effects of NIL quality shifting after the first 2 h of NI. The findings of this study demonstrate that flowering is complex, which involves interactions between light, endogenous biological clock, photoreceptors, and a number of genes related to flowering [47].

Flowering is thought to be promoted when *phyA* and/or *cry1*—two flowering promotor genes—are strongly expressed [48]. In contrast, *phyB* mediates the inhibition of flowering by R light in a largely redundant manner with *phyD* and *phyE* [32,46–48]. However, *phyB*'s role in floral initiation may be more nuanced than that of a sole floral inhibitor. *Cry1* and *cry2* mediate the enhancement of flowering by B light in a redundant manner [49,50]. A systemic flowering inhibitor (antiflorigen) and inducer (florigen), such as the *AFT* and *FTL* genes, respectively, are generated in the leaves and control the photoperiodic floral initiation [10]. It has been well-documented that phytochrome photoreceptors regulate changes in the gene expression in response to the R/Fr light signals by constitutively interacting with nucleus-localized basic helix–loop–helix transcription factors [51]. The results of this study showed that photoreceptor genes were strongly sensitive to all types of light quality. *PhyA*, *phyB*, and *cry1* play significant roles in regulating the flowering in NI-FrR and NI-RFr. Although the expression of these photoreceptor genes was observed to be enhanced in all treatments, these genes were expressed evidently more in NI-FrR and NI-RFr than in other treatments. Other photoreceptor genes such as *FTL* and *AFT* showed similar results as observed for *phyA*, *phyB*, and *cry1*, which indicates that flowering can be enhanced with NI-RFr and NI-FrR for potential applications in the commercial production of DNPs.

5. Conclusions

Plant morphogenesis and flowering were both affected by the photoperiod. Both morphogenesis and flowering were strongly impacted by the second NIL, but the first NIL had no effects on either. Geranium plants (DNP) were taller in NI treatments that included Fr light than those in other NI treatments. Flowering was seen in all treatments and was notably encouraged by NI with Fr light regardless of the sequence of light quality applied. These findings indicate that NI treatments that included Fr light can promote flowering, which may be used for commercial DNP production.

Author Contributions: Conceptualization, Y.G.P. and B.R.J.; methodology, Y.G.P. and B.R.J.; formal analysis, Y.G.P.; data curation, Y.G.P.; writing—original draft preparation, Y.G.P.; writing—review and editing, Y.G.P. and B.R.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. Yoo Gyeong Park was supported by the BK21 Program, Ministry of Education, Republic of Korea.

Data Availability Statement: Data sharing is not applicable to this article.

Acknowledgments: The authors express their gratitude to Sowbiya Muneer, Prabhakaran Soundararajan, and Young Don Chin for their assistance.

Conflicts of Interest: The authors declare no conflict of interest.

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