

Effect of Light Combination and Timing of Supplemental Lighting on Growth Characteristics and Flowering of Pansy (*Viola × wittrockiana* Rose)

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The goal of this study was to examine how different light combinations and timing of supplemental lighting affect the vegetative and reproductive responses of pansy (*Viola × wittrockiana* Rose). The present study was designed as a factorial experiment based on a completely randomized block design with three replications and two factors of timing of supplemental lighting (night-interruption and day-extension) and light combination treatments consisting of different proportion of blue (B, 467 nm) and red (R, 625 nm) light with high pressure sodium lamp (HPS) serving as control. The light combination treatments ($65 \mu\text{mol m}^{-2} \text{s}^{-1}$) included 100% R, 85% R: 15% B, 70% R: 30% B, and HPS. Our results showed that the plants exposed to 85% R: 15% B had the highest foliage fresh and dry weights (2.06 g and 0.23 g), stem diameter (1.42 mm), leaf area (44.72 cm²), and leaf number (16.5), and plants exposed to 100% R had the highest root fresh and dry weights (1.63 g and 0.39 g) and height (6.17 cm), respectively. The results showed a significant interaction between light combination and the timing of supplemental lighting on Chl a, Chl b, Chl total, and carotenoids contents and flowering time. Night-interruption supplemental lighting in exposure to 85% R: 15% B led to the highest Chl a (0.86 mg g⁻¹ FW), Chl b (0.52 mg g⁻¹ FW) and Chl total (1.51 mg g⁻¹ FW) contents. The highest carotenoids content (0.69 mg g⁻¹ FW) was obtained from night-interruption supplemental lighting under 70% R: 30% B. Night-interruption supplemental lighting was related to the shortest time to flowering (56 days after seed sowing), but it did not differ significantly from day-extension supplemental lighting under 70% R: 30% B.

Abstract

Keywords: Day-extension, LEDs, Light quality, Night-interruption, Photoperiod.

INTRODUCTION

Light is one of the important environmental factors so that day length, light intensity, and spectral combinations affect the quality and quantity of vegetative and reproductive growth of plants (Currey and Lopez, 2013). Plants respond to light combinations via their photosynthetic pigments like chlorophylls, carotenoids, and phycobilin and photoreceptor pigments like phytochromes, cryptochromes, and phototropins (Fukuda *et al.*, 2016). These pigments react to various light combinations differently (Currey and Lopez, 2013). Photosynthetic pigments such as chlorophylls (Chl) and carotenoids have high light absorption and activity when exposed to blue (B) and red (R) lights, and it is suggested that B and R light related-photoreceptors play promoting role in the flowering process (Jeong *et al.*, 2014). Due to the high absorption rate of B and R lights by plant leaves and because of their positive effects on the performance of photosynthetic and photoreceptor pigments, it can be expected that plant development and reproductive growth are strongly influenced when certain light spectra are applied (Nissim-Levi *et al.*, 2008). However, plant species and varieties, growth phase, light intensity and combinations, and other environmental conditions could affect plant physiological, morphological and anatomical reactions against different combinations of B and R spectra (Islam *et al.*, 2012).

Plants use light as a source of energy for photosynthesis and in case of light deficiency, their growth is stunted (Dole and Wilkins, 2005). Failure to meet photoperiodic light requirements of plants prevents them from achieving maximum flowering capacity (Mattson and Erwin, 2005). In commercial greenhouses, supplemental lighting is used during short winter days or very overcast days.

The most important photoperiodic lighting methods are day continuation lighting, also called day-extension lighting, and night-interruption lighting or the so-called night-break lighting (Craig and Runkle, 2012). In both methods, lights are commonly turned on for four hours when the days are short (Shin *et al.*, 2010), and 300 to 600 fc (60 to 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$) is a general recommendation for minimum supplemental irradiance for different stages of growth in various species (Dole and Wilkins, 2005).

Fluorescent, metal-halid and high-pressure sodium lamps are conventional and commonly used sources of artificial light, but because of such problems as a high rate of electricity use, heat generation, and emission of some unnecessary wavelengths, attempts have focused on finding alternative light sources such as LED lamps (Blanchard and Runkle, 2012). The production of specific spectra of light and the possibility of spectral combinations are two advantages of LED lamps (Terfa *et al.*, 2013; Wojciechowska *et al.*, 2016). Other remarkable features of LED lamps are their small size, long operation lifetime, durability, diverse light intensity, minimum heating, and relatively good efficiency of electricity conversion to light (Korner and van Straten, 2008). There is a possibility to increase the amount of B and R lights with the use of LED lamps, thereby promoting the activity of some photosynthetic and photoreceptor pigments. But, spectral light changes evoke different responses among different plant species (Brown *et al.*, 1995; Folta and Spalding, 2001). The aim of this study was to investigate the effect of two methods of supplemental lighting, including night-interruption and day-extension, with different light combinations on growth characteristics and flowering timing of pansy plants.

MATERIALS AND METHODS

Plant materials and pre-culture

Seeds of *Viola × wittrockiana* Rose were planted in 10 cm pots (containing a mixture of 50% composted leaves, 40% cocopeat, and 10% perlite). The temperature was set at $20 \pm 1^\circ\text{C}$, the average daily relative air humidity (RH) was set at $60 \pm 5\%$, and there was indirect natural light with $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ intensity. After germination and emergence of the second pair of leaves, treatments were carried out.

Experiment setup

In order to investigate the effect of light combinations and the timing of supplemental lighting treatments on the growth characteristics and flowering time of *Viola × wittrockiana* Rose, the present factorial experiment was performed on the basis of a randomized complete block design with three replications and two factors. The first factor was the timing of supplemental lighting including day-extension lighting (from 06.00 p.m. to 10.00 p.m.) and night-interruption supplemental lighting (from 10.00 p.m. to 02.00 a.m.). Second factor was the light combinations including L₁ (100% R), L₂ (85% R: 15% B), L₃ (70% R: 30% B) and L₄ (HPS). Therefore, eight treatments in three replicates were applied in this study. The experiment was conducted from January 20 to April 8, 2016. The pots were placed in a greenhouse from 06.00 a.m. to 06.00 p.m. and the greenhouse temperature was set at $24 \pm 1^\circ\text{C}$. After 06.00 p.m., the plants were exposed to supplemental lighting in two groups in a growing chamber: day-extension lighting and night-interruption supplemental lighting. Light combinations used for supplemental lighting treatments included 100% R, 85% R: 15% B, 70% R: 30% B, and HPS lamp. The temperature inside the growing chamber was set at $18 \pm 1^\circ\text{C}$, and average daily relative air humidity (RH) was set at $55 \pm 5\%$.

Light sources and irradiation system

In order to set spectra combinations, LED lamps (SENYANG LIGHT company) emitting red (R625nm with 1.2 fc intensity) and blue (B_{476nm} with 1fc intensity) were used. The required spectra ratios and low irradiance ($65 \mu\text{mol m}^{-2} \text{s}^{-1}$) were set by 400 LED lamps installed on a Plexiglass plate. The 85% R: 15% B ratio was obtained by 340 R lamps plus 60 B lamps and the 70% R: 30% B ratio was obtained by 280 R lamps plus 120 B lamps on a separate Plexiglass plate. Four custom-designed closed growth chambers without natural light were used. The size of three LED-equipped growth chambers were $70 \times 60 \times 60 \text{ cm}^3$ and the size of the HPS lamp-equipped growth chamber was $120 \times 60 \times 60 \text{ cm}^3$ (Fig. 1). Increasing the height of the HPS chamber prevented the heat stress on plants because of the heat generation of HPS lamp. During the experiment, the light intensity was measured and set by an LI-COR® light meter (model LI-250A).



Fig. 1. The setup of the growth chambers that hosted the experiment.

Plant growth measurements and flower bud observation

After growing for 80 days, all the plants were removed from pots. The height was measured from the edge of the pot to the top of the plant with a ruler. The number of total leaves and visible buds were counted, and stem diameter was measured at the point just below the first true leaf by a micrometer (ACCUD digital outside micrometer series 311). Fresh weights of foliage and root

were measured by an accuracy readability 0.001 g electronic scale and dry weights were measured after 48 hours drying on 70°C. Leaves longer than 1 cm were scanned using an hp scanner (model G3110) and the leaf area was measured using the imaging software Image J-Win32 (<http://rsbweb.nih.gov/ij>). The growth assessment was repeated three times with five plants in each treatment. Chlorophylls (Chl) and carotenoids were extracted from the leaves of 12 plants at the similar position on the leaf in each treatment. Chl a, Chl b and Chl total and carotenoids contents were measured by Arnon method (Arnon, 1949; Saini, 2006).

Statistical analysis

All measurements were evaluated for significant differences using analysis of variance (ANOVA) and the differences between the means were tested using the Least Significant Difference (LSD) test at the $P < 0.05$ level. All statistical analyses were conducted using Minitab 16 and the diagrams were drawn in MS-Excel.

RESULTS AND DISCUSSION

The analysis of variance showed significant interactions between light combinations and timing of supplemental lighting for Chl a, Chl b, Chl total, and flowering time ($P < 0.01$) and for carotenoids contents ($P < 0.05$). The effect of light combination on all studied traits was significant ($P < 0.01$). The effect of timing of supplemental lighting was significant on root fresh weight, Chl total, Chl a, carotenoids contents, flowering time ($P < 0.01$), and leaf area ($P < 0.05$) (Table 1).

Light combination effects on biomass accumulation

The foliage fresh and dry weights and stem diameter of plants exposed to 85% R: 15% B were 44%, 91.6%, and 52.6% greater than those of control, respectively. They were significantly higher than those of other light combinations. Root fresh and dry weights of plants exposed to R light were 150.7% and 129.4% greater than those of control, respectively. Again, they were significantly greater than those of other light combinations (Table 2). In this study, the foliage fresh and dry weights were increased by 19% and 43.7%, respectively and stem diameter was increased by 1.4% when comparing R light and 85% R: 15% B combination, respectively. This indicates that the combined spectrum of B and R lights had stimulating effects on the above-mentioned characteristics. The positive effect of B and R combined spectrum on the increase in plant weight like petunia (Randall and Lopez, 2014), Norwegian and Scottish pine (Riikonen *et al.*, 2016), lettuce, radishes and spinach (Yorio *et al.*, 2001; Pinho *et al.*, 2004) have been reported. However, the results of some previous studies have pointed out that B light had no effect on increasing the weight of plants like radish, soybean, and wheat (Cope and Bugbee, 2013) or even had negative effects on the dry weight of plants like viola and marigold (Heo *et al.*, 2002; Randall and Lopez, 2014). We found that the extent of B light level (70% R: 30% B compared to 85% R: 15% B) caused a significant reduction in the foliage fresh weight (8.4%), foliage dry weight (27.7%) and stem diameter (0.7%) (Table 2).

The results of root fresh and dry weights revealed that root fresh and dry weights decreased by 53.7% and 105.2% under exposure to 85% R: 15% B compared to exposure to R light, respectively. This represents a significant reduction of root fresh and dry weights in plants exposed to B light. However, as the level of B light (70% R: 30% B) was increased, 47.16% and 36.84% increases occurred in root fresh and dry weights when compared to 85% R: 15% B, respectively. So, it is likely that the growth of root depends on not only the presence but also the intensity of B light. There are some reports about the positive effects of B light on increasing cytokinin synthesis (Kohler *et al.*, 1980), and cytokinin is effective in plants' root growth (Arteca, 1995). However, the increase in cytokinin synthesis does not always lead to root development because the impact of cytokinin on root development could be stimulating or inhibiting depending on its concentration

and plant species (Arteca, 1995).

Light combination effects on leaf number and leaf area

In this study, plants grown under 85% R: 15% B had significantly the highest leaf area (44.72 cm²) and leaf number (16.5), which were 90% and 50% more than those of control, respectively. The combined spectrum of B and R lights increased leaf area and leaf number significantly. Leaf number and leaf area were increased by 83.3% and 6.14% when compared to 85% R: 15% B combination and R light, respectively. The results showed that although B light had stimulatory effects on leaf number and leaf area, these two traits were suppressed by extension of B light level so that leaf area reduced by 2.4% and leaf number reduced by 17.8% by exposure to 70% R: 30% B versus 85% R: 15%B (Table 2).

Leaves absorb B and R lights, so when a combined spectrum of B and R lights is used, effective energy for photosynthesis is provided (Klein, 1992). The expansion of leaf area increases light absorption and as a consequence, the photosynthesis rate and plant growth are increased (Adams *et al.*, 2008). It has been reported that B light has had various influences on leaf area in different plants. For example, it had negative effects on leaf area of salvia, petunia and lettuces (Ohashi-Kaneko *et al.*, 2007; Wollaeger and Runkle, 2014) and positive effects on leaf area of roses and bell peppers (Brown *et al.*, 1995; Terfa *et al.*, 2013), but it had been ineffective in tomatoes and spinaches (Ohashi-Kaneko *et al.*, 2007; Liu *et al.*, 2012).

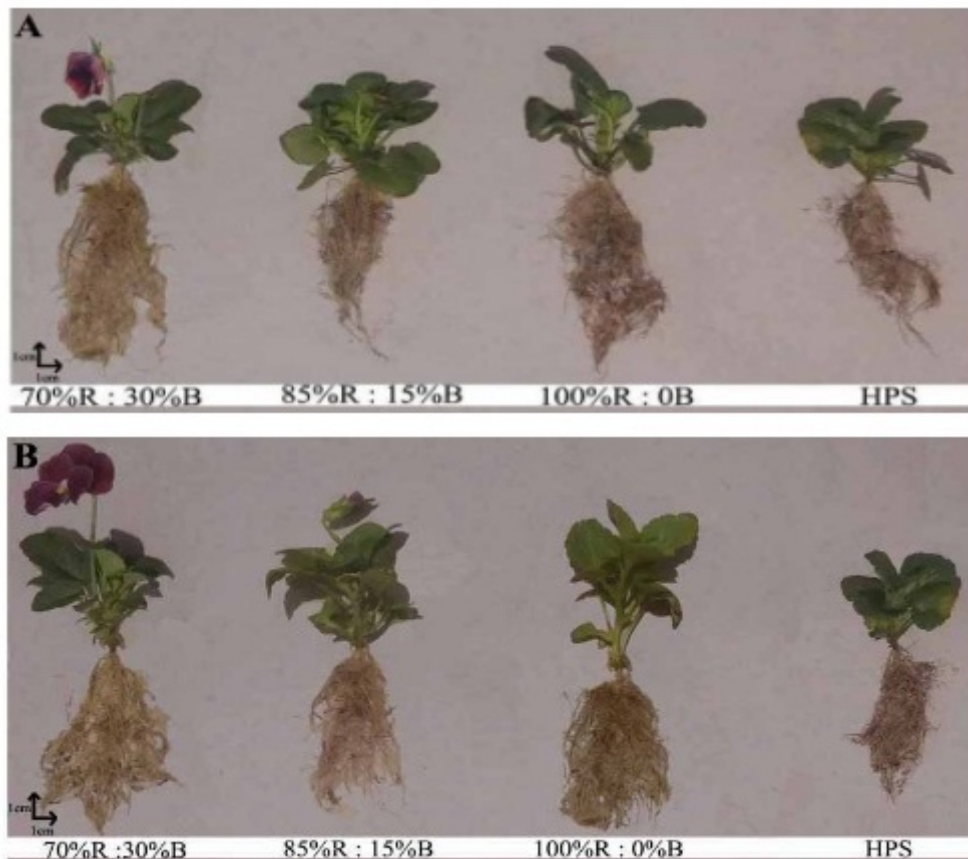


Fig. 2. Plants exposed to supplemental lighting from 06.00 p.m. to 10.00 p.m. (A) and from 10.00 p.m. to 02.00 a.m. (B) after growing for 80 days.

Table 1. Analysis of variance of light combination and timing of supplemental lighting on the studied characteristics.

Sov	df	MS												
		Foliage fresh weight	Foliage dry weight	Root fresh weight	Root dry weight	Stem diameter	Height	Leaf number	Leaf area	Flowering time	Chl. a	Chl. b	Chl. total	Carotenoid
Block	2	0.001**	0.00001**	0.00178**	0.000003 ^{ns}	0.0000014 ^{ns}	0.00017 ^{ns}	3.375**	0.0012**	0.0008 ^{ns}	0.000006 ^{ns}	0.000002 ^{ns}	0.00001 ^{ns}	0.000007 ^{ns}
Timing of lighting (T)	1	0.00004 ^{ns}	0.000003 ^{ns}	0.00067**	0.0000004 ^{ns}	0.0000002 ^{ns}	0.00028 ^{ns}	0.0004 ^{ns}	0.00047*	37.5**	0.00831**	0.003142**	0.08994**	0.001367**
Light combination (L)	3	0.433**	0.012**	1.358**	0.0637**	0.347**	2.989**	65.37**	604.74**	5794.5**	0.0905**	0.0333**	0.3568**	0.131**
T x L	3	0.00002 ^{ns}	0.0000005 ^{ns}	0.00002 ^{ns}	0.000002 ^{ns}	0.0000003 ^{ns}	0.00011 ^{ns}	0.0004 ^{ns}	0.000044 ^{ns}	6.5**	0.000347**	0.000095**	0.00662**	0.000072*
Error	14	0.000089	0.000001	0.00003	0.000003	0.000001	0.0002	0.4464	0.0001	0.86	0.000016	0.000003	0.000062	0.000016
CV (%)		0.52	0.58	0.45	0.69	0.08	0.27	5.29	0.03	2.01	0.54	0.41	0.69	0.7

^{ns}, * and ** means insignificant and significant differences at the P < 0.05 and P < 0.01 levels, respectively.

Table 2. Means comparison of light combinations on the studied characteristics.

Light combinations	Foliage fresh weight (g)	Foliage dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Stem diameter (mm)	Height (cm)	Leaf number	Leaf area (cm2)
Red	1.73 ^c	0.16 ^c	1.63 ^a	0.39 ^a	1.40 ^c	6.17 ^a	9 ^d	42.13 ^c
85% R: 15%B	2.06 ^a	0.23 ^a	1.06 ^c	0.19 ^c	1.42 ^a	5.13 ^c	16.5 ^a	44.72 ^a
70% R: 30%B	1.90 ^b	0.18 ^b	1.56 ^b	0.26 ^b	1.41 ^b	5.48 ^b	14 ^b	43.64 ^b
1.43d HPS	1.43 ^d	0.12 ^d	0.65 ^d	0.17 ^d	0.93 ^d	4.47 ^d	11 ^c	23.53 ^d

Means with similar letter(s) in each column show insignificant differences according to the LSD test (P < 0.05).

Light combination effects on plant height

The results show that the plants had the greatest height in R light treatment (6.17 cm) so that they were 38.03% taller than the control plants. The height was reduced by 20.27% in 85% R: 15% B exposure compared to R light. Thus, the use of blue light inhibited the elongation of plants. However, increasing the proportion of blue light alleviated the inhibitory role of B light because the height was increased by 6.82 % in plants exposed to 70 % R: 30 % B compared to 85 % R: 15 % B. It has been reported that B light has various influences on plant height. For example, B light increased shoot length of petunia (Fukuda and Olsen, 2011), chrysanthemum (Jeong *et al.*, 2014), marigold (Heo *et al.*, 2002), and eggplant (Hirai *et al.*, 2006), but it decreased shoot length of Arabidopsis (Folta and Spalding, 2001), chrysanthemum (Shimizu *et al.*, 2006), and poinsettia (Islam *et al.*, 2012).

B light can act via photoreceptors like cryptochrome and can change their performance; this affects plant height (Fukuda *et al.*, 2016). Cryptochrome activity has an inhibitory effect on hypocotyl growth (Ahmad and Cashmore, 1997). B light promotes cryptochrome activity and produces signals that affect the synthesis of gibberellins (Fukuda and Olsen, 2011). Some researchers argue that there might be interactions between the activity of cryptochromes and phytochromes (Ahmad *et al.*, 2002). Red light has an additive effect on phytochrome activity and because phytochromes produce signals that affect gibberellins production, therefore the presence of R light could affect gibberellins level and plant height (Folta and Spalding, 2001; Neff, 2012).

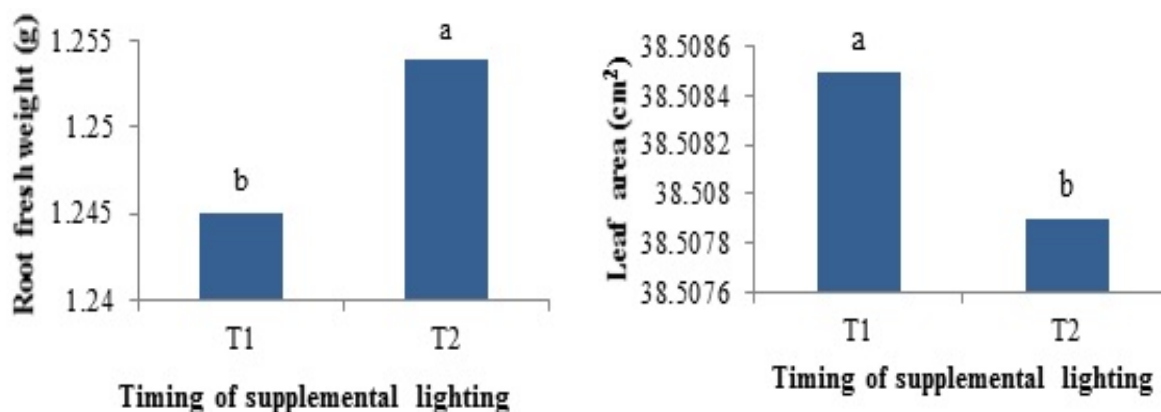


Fig. 3. The effect of timing of supplemental lighting on root fresh weight and leaf area; T1: day-extension supplemental lighting, T2: night-interruption supplemental lighting.

Timing of supplemental lighting effect on growth characteristics

The results showed that the effect of timing of supplemental lighting was significant on root fresh weight ($P < 0.01$) and leaf area ($P < 0.05$). Root fresh weight and leaf area of plants exposed to night-interruption supplemental lighting were 0.87% and 0.02% higher than those of plants exposed to day-extension supplemental lighting (Fig. 3). The vegetative activity of plants can be stimulated by proper usage of supplemental lighting (Hopkins and Huner, 2004). It has been reported that night-interruption supplemental lighting is more effective than day-extension supplemental lighting in improving plant growth (Thomas and Vince-Prue, 1996; Anderson, 2007).

Light combination and the timing of supplemental lighting effects on flowering time, chl. a, chl. b, total chl. and carotenoids contents

Analysis of variance showed a significant interaction between light combination and timing of supplemental lighting for Chl a, Chl b, total Chl and flowering time ($P < 0.01$) and carotenoids contents ($P < 0.05$). The highest Chl a ($0.86 \text{ mg g}^{-1} \text{ FW}$), Chl b ($0.57 \text{ mg g}^{-1} \text{ FW}$) and Chl total

(1.51 mg g⁻¹ FW) contents were obtained from night-interruption supplemental lighting in 85% R: 15% B exposure. Only carotenoid content (0.69 mg g⁻¹ FW) was the highest in night-interruption supplemental lighting under 70% R: 30% B (Fig. 4; Table 1).

Among the light-absorbing pigments, chlorophyll and carotenoids play an important role in the absorption of light energy. In chloroplast photosystem, Chl b and carotenoids help to absorb and transfer light energy to Chl a (Agarwal and Pandey, 2004). Carotenoids absorb B light whose energy is used in photosynthesis (Franklin and Whitelam, 2005). B and R lights have a positive effect in improving the performance of photosynthesis and consequently plant weight due to their impact on the formation and function of photosynthetic pigments like Chls (Randall and Lopez, 2015). The results of the present study showed that the simultaneous application of R and B lights increased Chl a, Chl b, total Chl and carotenoids contents of plants as compared to exposure to R light or HPS light (Fig. 4), and these plants had greater foliage fresh and dry weights, stem diameter, leaf number, and leaf area versus those that were exposed to R light or HPS light (Table 2).

Carotenoids are pigments that affect photosynthesis efficiency and suitable growth (Zhu *et al.*, 2010). The lighting conditions that are effective in Chls pigments production stimulate the synthesis of carotenoids (Bohne and Linden, 2002), and B light is effective in increasing carotenoid content (Johkan *et al.*, 2010). R light exposure could also help to make carotenoids (Ma *et al.*, 2015). The results of this experiment show that the biosynthesis of carotenoids is possible in the absence of B light (Fig. 4). It has been documented that the reaction of phytochrome to environmental factors such as light or temperature can lead to gene expression adjustment related to the synthesis of carotenoids (Liorente *et al.*, 2015) and the combined spectrum of B and R lights is more effective in the formation of carotenoids than the red light (Fu *et al.*, 2013). Our results confirmed this claim (Fig. 4). On the other hand, there is limited information about the effect of narrow spectra on the synthesis of carotenoids (Fu *et al.*, 2013), and the results of this research show that red light narrow spectrum (R625nm) was effective in the formation of carotenoids. For example, the application of R light during night-interruption led to 68.75% more carotenoid contents than control in the same period of supplemental lighting (Fig. 4).

It has been reported that night-interruption is more effective than day-extension in controlling plant growth (Thomas and Vince-Prue, 1996; Anderson, 2007). The results revealed that under all tested light combinations, night-interruption led to higher amounts of Chl a, Chl b and total Chls than day-extension (Fig. 4). With respect to carotenoids, the portion of B light in light combination affected the action of timing of lighting and there were no significant differences between timing of supplemental lighting when R light or 85% R: 15% B were used but when the higher amount of B light was used (70% R: 30% B), there were 2.38% more carotenoids during night-interruption than day-extension (Fig. 4).

The results showed a significant interaction between light combination and timing of supplemental lighting for flowering time ($P < 0.01$) (Fig. 4; Table 1) and after 80 days of cultivation, the shortest flowering time (56 days after sowing) happened when plants were exposed to 70% R: 30% B from 10.00 p.m. to 02.00 a.m. (night-interruption supplemental lighting) with no significant difference with 70% R: 30% B application from 06.00 p.m. to 10.00 p.m. (day-extension supplemental lighting). The flowering time was the longest significantly (69 days after sowing) when plants were exposed to R light from 06.00 p.m. to 10.00 p.m. No flower buds were obtained from exposure to HPS lamps after 80 days (Fig. 4).

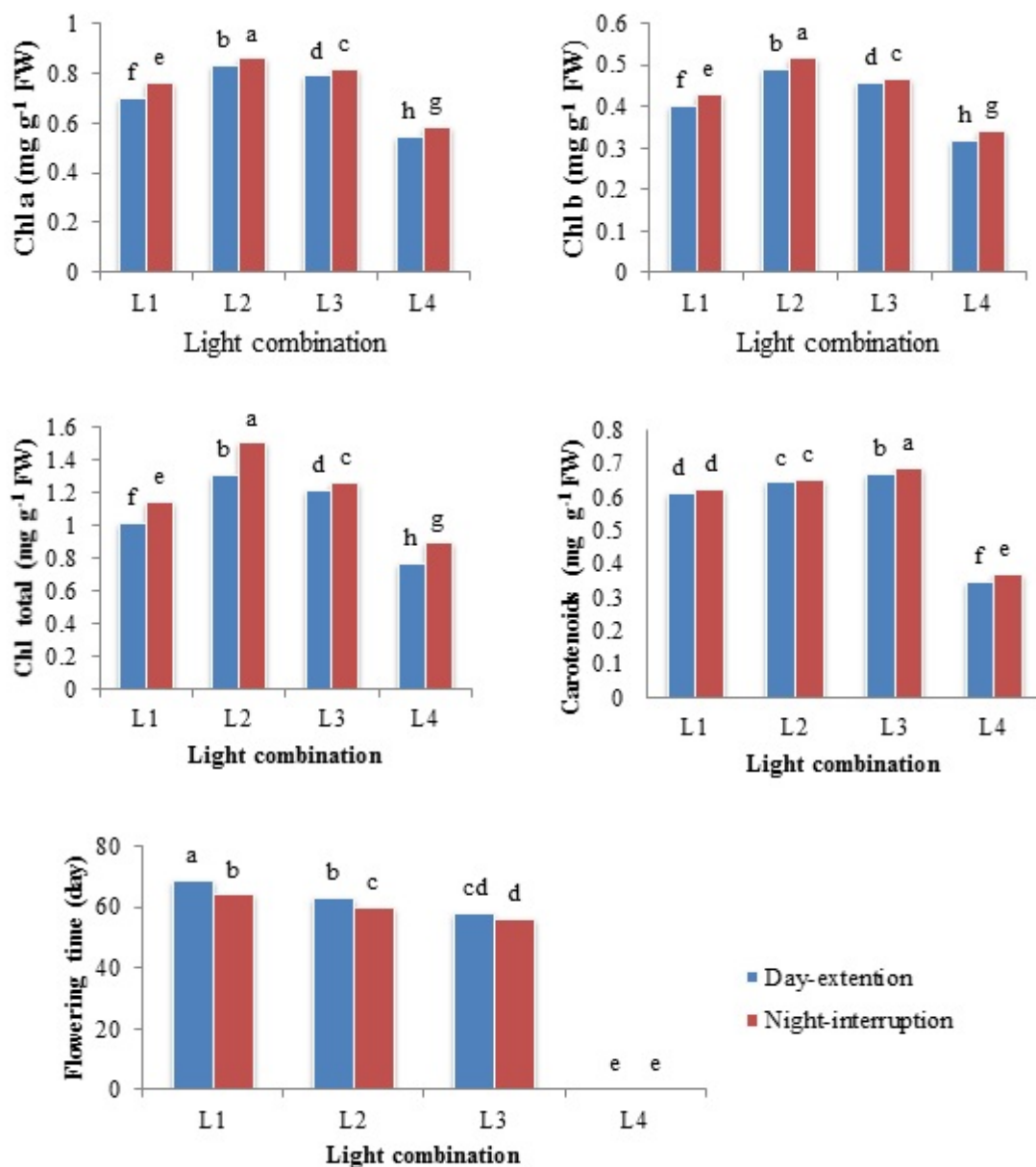


Fig. 4. Interaction of light combination and timing of supplemental lighting for Chl a, Chl b, total Chl, and carotenoid contents and flowering time. L1: 100%R, L2: 85% R: 15%B, L3: 70%R: 30%B, L4: HPS.

The duration of light exposure and spectral combination influence flowering process (Heo *et al.*, 2002; Anderson, 2007; Ho *et al.*, 2012). Pansy is one of the plants whose growth and flowering are influenced by the exposure duration, intensity and combinations of light (Izhaki *et al.*, 2001, Oh *et al.*, 2009). The results of this experiment showed that the application of B light reduced the time required to produce flower buds (Fig. 4). Photosynthetic and photoreceptor pigments activity influence vegetative and reproductive growth, and the reactions of these pigments depend on the quantity and quality of light (Hopkins and Huner, 2004). Among the photoreceptor pigments, the effect of phytochrome (absorbing red and far-red light) and cryptochrome (absorbing blue light) and among light spectrum, the effect of red and far-red (FR) light on physiology of flowering have been studied to a greater extent than other pigments (Smith, 2000; Chen *et al.*, 2014). Also, the effect of the combined spectrum of B, R and FR lights on crop production has not well known yet (Meng and Runkle, 2015).

Since B light promotes cryptochrome activity, so it is considered effective in stimulating

flowering (Bagnall *et al.*, 1996) in which case its role is almost similar to FR light (Esashi, 1966; Kenneth, 1992). It seems that the presence of B light stimulates the expression of genes involved in shoot apical meristem differentiation to develop flowers (Izhaki *et al.*, 2001). As already mentioned, B light is one of the most important factors that promote the synthesis and operation of cryptochromes and especially under low-intensity conditions, these kinds of photoreceptors have a key role to play in the control of flowering (Giliberto *et al.*, 2005). On the other hand, R light converts phytochrome Pr to Pfr (an active form of phytochrome) and it is supposed to be effective in the flowering process (Baba-kasai *et al.*, 2014). The outcomes of all these experiments suggest that phytochromes and cryptochromes are responsible for the flower induction (Takemiya *et al.*, 2005). But, R light can lead to delay or prevent flowering in some long-day plants (Kim *et al.*, 2002).

Stimulation of some kinds of phytochrome (phytochrome B) by R light and B light was supposed to be responsible for these conflicting results because R light and B light could inhibit Hd3a gene expression and the declined activity of this gene prevents flowering (Fukuda and Olsen, 2011). Our results confirmed that R light delayed flowering because day-extension lighting by R light led to the latest flower bud formation (Fig. 4). On the other hand, the results showed that the timing of supplemental lighting affected light spectrum mode of action on flowering time. For example, flower buds emerged 5 days earlier when R light used as day extension lighting as compared to the application of R light as night-interruption supplemental lighting with no significant difference with the application of 85% R: 15% B as day extension supplemental lighting (Fig. 4).

Data analysis of the interaction of factors showed that the effect of B light on flowering time depended on its proportion combination with R light and on the timing of supplemental lighting treatments. For example, exposure to 85% R: 15% B as night-interruption supplemental lighting led to a faster flowering rate with a significant difference from the application of this light combination as day-extension lighting. It has been reported that night-interruption is more effective than day-extension in controlling vegetative and reproductive growth (Thomas and Vince-Prue, 1996; Anderson, 2007). Our results revealed that between the timing of supplemental lighting treatments under specific light combinations, night-interruption supplemental lighting led to faster flowering than day-extension lighting. However, the use of 70% R: 30% B or 85% R: 15% B as day-extension (06.00 p.m. to 10.00 p.m.) versus R light as night-interruption reduced the time required for flower bud emergence by 5-10 days.

CONCLUSION

The results indicated positive effects of combined spectra of B and R lights on pansy vegetative growth characteristics and flowering time. Light combinations influenced studied traits differently. The best results for foliage fresh and dry weights, stem diameter, leaf area, and leaf number were obtained from exposure to 85% R: 15% B. The highest root fresh and dry weights and height were obtained from R light exposure. The results showed a significant interaction between light combination and the timing of supplemental lighting treatments for Chl a, Chl b, Chl total and flowering time. The highest Chl a, Chl b, and Chl total contents were obtained from 85% R: 15% B exposure as night-interruption supplemental lighting, The highest carotenoid content was obtained from 70% R: 30% B exposure as night-interruption supplemental lighting and the application of 70% R: 30% B as night-interruption supplemental lighting led to the shortest flowering time; however, it did not differ from day-extension supplemental lighting significantly. So, it seems that the application of combining light spectra as supplemental lighting at proper time could lead to improving the vegetative and reproductive traits of the studied plants.

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