

Supplementary Blue and Red Radiation at Sunrise and Sunset Influences Growth of Ageratum, African Marigold, and Salvia Plants

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Abstract

BACKGROUND: Light-emitting diodes (LEDs) with lower electric cost and the specific wavelength have been considering as a novel light source for plant production in greenhouse conditions as well as in a closed culture system. Supplementary lighting for day-length extension was considered as light intensity, light quality, and/or photoperiod control on plant growth and development. Effects of supplementary blue or red LED radiation with lower light intensity on growth of Ageratum (*Ageratum houstonianum* Mill., cv. Blue Field), African marigold (*Tagetes erecta* L., cv. Orange Boy), and Salvia (*Salvia splendens* F. Sello ex Ruem & Schult., cv. Red Vista) were discussed during sunrise and sunset twilight in the experiment.

METHODS AND RESULTS: Supplementary lighting by blue and red LEDs for 30 (Treatment B30; R30) or 60 (Treatment B60; R60) min. per day were established in greenhouse conditions. Photosynthetic photon flux for supplementary radiation was kept at $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ on the culture bed. Natural condition without supplementary light was considered as a control. The highest shoot and root dry weights were shown in African marigold exposed by red

light for 60 min. per day. Supplementary blue and red lighting regardless of the radiation time significantly stimulated development of lateral branches in African marigold. Stem growth in Ageratum and Salvia seedlings was significantly promoted by red radiation as well as natural light.

CONCLUSIONS: Extending of the radiation time at sunrise and sunset twilight using LEDs stimulated reproductive growth of flowering plant species. Different characteristics on growth under supplementary blue or red lighting conditions were also observed in the seedlings during supplementary radiation.

Key Words: Bedding plants, Development, Flowering, Light-emitting diode

INTRODUCTION

Light-emitting diodes (LEDs) have been considered as a novel radiation source for the growth and development in plant species because of several characteristic roles in practical use for plant production. Special bands of short waves in spectral energy can be influenced on growth and development *in vitro* and/or *ex vitro* plant species (Tanaka *et al.*, 1998; Barreiro *et al.*, 1992; Jayakumar *et al.*, 2004;

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Amudha *et al.*, 2005; Heo *et al.*, 2006; Heo *et al.*, 2010a; 2010b). It was reported that red light in short waves is important for the development of photosynthetic apparatus in plants (Saebo *et al.*, 1995). LEDs have been studied on photobiological area such as chlorophyll synthesis (Tripathy *et al.*, 1995), photosynthesis (Tenessen *et al.*, 1994) or morphogenesis (Hoenecke and Tibbitts, 1992) in plants.

In plant production, plant growth and development under greenhouse conditions commonly depend on the exogenous environment such as an air temperature, relative humidity, light intensity or quality. Light quality or intensity among the environments was occasionally not optimum for establishment in photosynthesis and morphogenesis. Light environment in a greenhouse has been controlled for the limited solar availability i.e., use of supplemental lighting during a dark day (Albright, 1997). Various types of artificial lamps such as a high-intensity discharge lamps, fluorescent lamps, incandescent lamps, high-pressure sodium lamps, or metal-halide lamps have been used for greenhouse-plant production. The supplementary lighting has been conventionally considered in terms of light intensity, light quality, and/or photoperiod control in the meaning of seasonal variation. From the attractive characteristics, the LEDs may be suitable for plant production in greenhouse conditions as well as in a closed culture system in terms of lower electric cost and the specific wavelength for plant growth and development.

Objective of this study was to investigate the effects of supplemental lighting at sunrise and sunset controlled by blue and red LEDs on growth and development of *Ageratum*, African marigold, and *Salvia* seedlings grown under greenhouse conditions.

MATERIALS AND METHODS

Plant materials and growth conditions

Ageratum (*Ageratum houstonianum* Mill., cv. Blue Field), African marigold (*Tagetes erecta* L., cv. Orange Boy), and *Salvia* (*Salvia splendens* F. Sello ex Ruem & Schult., cv. Red Vista) seedlings with two true leaves were used for plant materials. All of the seedlings were transplanted to the plug tray (50 mm length × 50 mm width) filled with soil mixture. The soil mixture (BM1, Berger Horticulture, Quebec, Canada) consisted of Canadian sphagnum peat moss (75-85%), perlite (15-20%), and vermiculite (5-10%). Two kinds

of liquid fertilizers (Plant Prod, Plant Products Co. Ltd., Quebec, Canada) were used as a nutrient solution. Liquid fertilizers controlled at 100 mg/L were alternatively supplied to the seedlings during the experimental period. All the seedling were grown for 70 days in a greenhouse, which maintained at 25/15°C (day/night) and 40% relative humidity. The experiment was carried out from January to March with shorter day length of 9 hrs in average compared to other seasons at local time during the period.

Lighting conditions

LED system (GF-320S, Good Feeling Co. Ltd., Sungnam, Korea) with blue or red lights was used for supplementary light sources. The LED system was consisted of LED sticks and a main controller (27.5 cm length × 33.5 cm width × 10 cm height) for maintaining photoperiod and light intensity. LED sticks were mounted on a polycarbonate board (28 cm length × 1 cm width) in an array of 20 lamps. The LED sticks of blue and red were set on the upper part of each culture bed inside a greenhouse. Supplementary lighting with a photosynthetic photon flux of 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on the tray was examined by exposing plants to blue and red lights for 30 and 60 minute per day at sunrise and sunset twilight. Natural condition without supplementary radiation was considered as a control. The day length in natural condition was 9 hrs per day in average during the experimental period.

Measurement of growth and development

Growth of the seedlings was determined by measuring fresh weight, dry weight, plant height, number of internodes, and number of lateral branches on day 70 after light treatment. Flowering characteristics such as number of flowering buds, number of open flowers, days to flowering, and blooming period were also recorded. The dry weight was measured after drying the samples in a hot air oven at 60°C for 3 days.

Stomata observation

The seedlings stomata were observed by confocal system on day 70. A laser scanning confocal system (MRC1024es, Bio-Rad Microscience Ltd., Hemel Hempstead, Herts, U.K.) with a krypton-argon mixed gas laser attached to a Nikon Diaphot-300/200 inverted microscope was used to observe stomata. Central leaf

disk of the 4th leaf from the top of the seedlings grown under the different light qualities for 70 days were used as a sample for the observation.

Statistical analysis

Statistical analysis was performed according to the SAS System (Version 6.21, SAS Institute Inc. Cary, NC, USA). Means and standard errors were used throughout and statistical significance between mean values was assessed using ANOVA and Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Light exposing by supplementary blue and red for 30 or 60 minute during sunrise and sunset twilight comparing with the natural lighting influenced on growth and development of *Ageratum*, African marigold, and *Salvia* seedlings for 70 days. In African marigold, red radiation of 60 minute per day was effective on increasing of fresh weight rather than under blue light regardless of radiation time (data not shown). No difference in fresh weight of *Ageratum* and *Salvia* seedlings was observed under supplementary red or blue lighting. Total dry weight of shoot and root in African marigold was greater in the supplementary blue and red lighting compared to natural light (Fig. 1). Under R60 treatment, the dry weight was approximately 60 % higher than under control without light treatment. In African marigold seedlings, the higher dry weight under R60 treatment of the supplementary red light with longer radiation time is probably attributed to increase of reproductive growth such as shorter days to flowering or longer blooming period compared to blue light quality (Table 3). However, increasing of dry weight in *Ageratum* and *Salvia* plants did not affected by the supplementary light quality and radiation time during the experimental period. Growth of African marigold stimulated by the supplementary red radiation is most probably related to vigorous photosynthesis rather than under blue light as shown in grape plantlets *in vitro* (Heo *et al.*, 2006). Plant growth such as a dry weight is resulted from red or blue plus red light quality compared to blue in several *in vitro* or *ex vitro* plants according to the report of Tanaka *et al.* (1998), Heo *et al.* (2002), and Kim *et al.* (2004). Contrarily, it is suggested that red plus far-red light with different mixture ratios did not influenced on shoot and root growth of potted

Chrysanthemum under growth chamber conditions (Lund *et al.*, 2007). However, Sung *et al.* (1998) reported that supplementary blue radiation for 5 minute at morning twilight promoted photosynthesis with a higher stomatal conductance, leaf extension and increased dry mass in cucumber seedlings. When low light intensity effects of blue light on promotion of plant growth were described by phototropins as a photoreceptor in the natural environment (Takemiya *et al.*, 2005). These conflicted results show the action characteristics of the blue or red light quality on plant growth can be changed by various environmental factors such as a growth stage, light intensity, radiation time, or plant species.

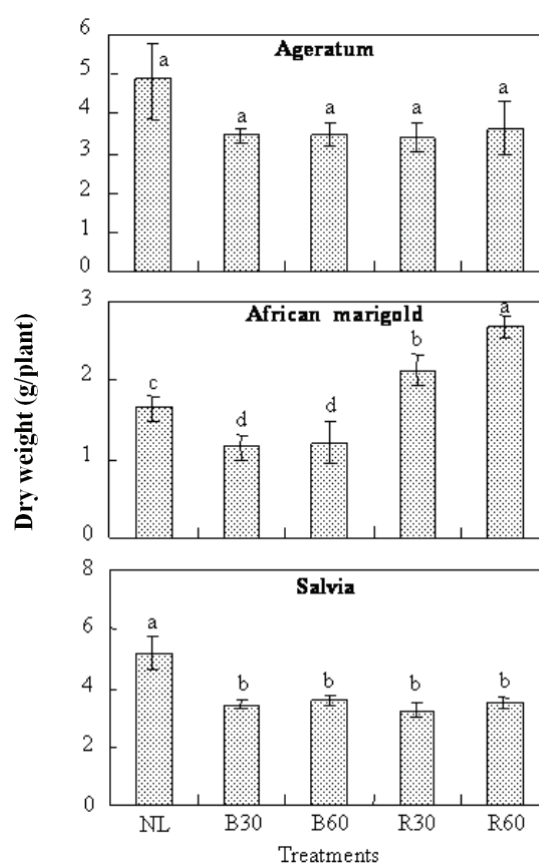


Fig. 1. Dry weights of *Ageratum*, African marigold, and *Salvia* seedlings grown under supplementary blue and red lighting for 30 or 60 minute per day for 70 days. Vertical bars represent means \pm standard errors. Natural light without supplementary lighting (NL); Supplementary blue or red lighting for 30 minute per day (B30, R30); Supplementary blue or red lighting for 60 minute per day (B60, R60).

Effect of blue light quality on stem elongation of African marigold and Salvia seedlings under closed chamber system was mentioned by Heo *et al.* (2002). According to the report, stem growth of the seedlings exposed by monochromic blue light of $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity was significantly stimulated regardless of dry weight increment. Under present experimental conditions, stem elongation in Ageratum and Salvia plants was stimulated by blue light radiation regardless of lighting time (Fig. 2). In African marigold, higher stem growth was established under B60, R30, or R60 treatment showing higher number of internodes, however, it was inhibited under natural treatment without light supplementation. These results suggest that our consideration in light-quality effects can be focused on photosynthetic ability and/or morphogenesis mediated by light receptors as mentioned by Schuerger *et al.* (1997). In Ageratum and Salvia seedlings, no differences in number of internodes mean that blue light influenced node elongation compared to red light in both seedlings (Table 1). Otherwise, the supplementary light qualities regardless of radiation time increased stem growth with increasing on the number of internodes in African marigold.

Lateral branching in Ageratum was inhibited by red light (Table 2) comparing to the previous result on tobacco branching under field condition (Kasperbauer, 1971). The number of lateral branches in the seedling increased in the treatment of NL and B regardless of radiation time. The supplementary light qualities or radiation times at sunrise and sunset did not influence on the lateral branching in Salvia plants. Formation of lateral branches in African marigold was stimulated by the supplementary lighting regardless of light quality or radiation time. Blue-light quality inducing the higher shoot growth in Ageratum or Salvia plant probably enhances lateral branching by stimulating a light perception by axillary buds. However, shoot growth and formation of lateral branches in African marigold were affected by the supplementary blue and red light compared to the plants grown under sunlight condition. Muir and Zhu (1983) reported that shoot growth and lateral branching are affected by changes in the endogenous concentration of plant growth regulators which is affected by light quality. Physiological researches are necessary to validate the various relations between light qualities and endogenous hormones on lateral-branch development in the near future.

Table 1. Influence of supplementary blue and red lighting on number of nodes per plant of Ageratum, African marigold, and Salvia seedlings

| Treatments | Ageratum | African marigold | Salvia |
|----------------------|---------------------|------------------|--------|
| NL | 11.0 a ^z | 5.0 c | 8.0 bc |
| B30 | 11.6 a | 6.0 b | 10.0 a |
| B60 | 10.8 a | 7.8 a | 9.2 ab |
| R30 | 10.8 a | 8.0 a | 8.8 b |
| R60 | 11.0 a | 7.8 a | 8.0 bc |
| Light quality (a) | ns | ** | ** |
| Radiation time (b) | ns | ns | ns |
| (a)×(b) ^y | ns | ns | ns |

^zMean value (in columns) followed by a different letter are significantly different at the $P < 0.05\%$ level by Duncan's multiple range test.

^yRepresent interaction between light quality and radiation time.

Table 2. Number of lateral branches per plant of Ageratum, African marigold, and Salvia seedlings grown under supplementary blue and red lighting for 70 days

| Treatments | Ageratum | African marigold | Salvia |
|------------------------|---------------------|------------------|--------|
| NL | 20.4 a ^z | 5.4 c | 7.6 a |
| B30 | 19.2 ab | 8.2 a | 6.6 a |
| B60 | 18.4 ab | 9.2 a | 8.0 a |
| R30 | 17.0 c | 9.0 a | 6.6 a |
| R60 | 17.8 c | 9.0 a | 7.0 a |
| Light quality (a) | * | ** | ns |
| Radiation time (b) | ns | ns | ns |
| (a) × (b) ^y | ns | ns | ns |

^zMeans followed by a same letter are not significantly different at at the $P < 0.05\%$ level by Duncan's multiple range test.

^yRepresent interaction between light quality and radiation time.

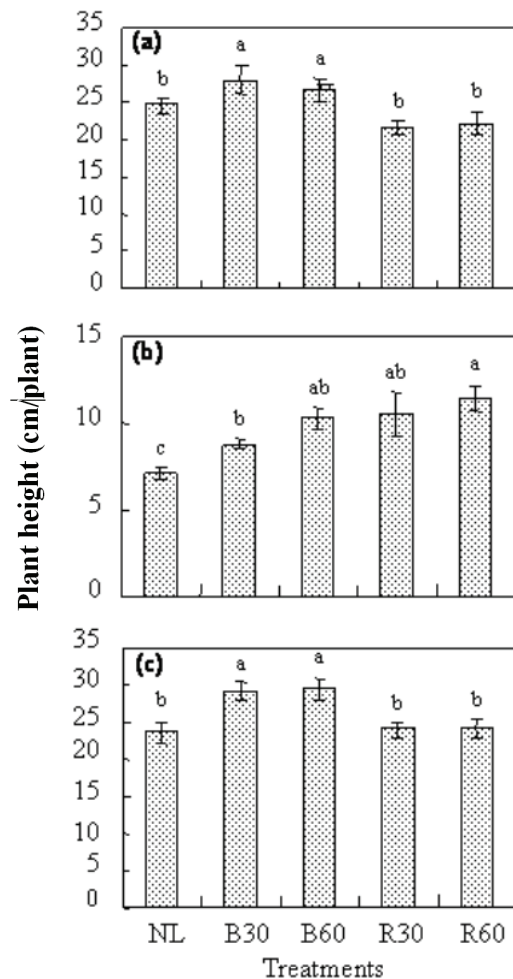


Fig. 2. Plant heights of *Ageratum* (a), African marigold (b), and *Salvia* (c) seedlings grown under supplementary blue and red lighting for 30 or 60 minute per day for 70 days. Vertical bars represent means \pm standard errors. Natural light without supplementary lighting (NL); Supplementary blue or red lighting for 30 minute per day (B30, R30); Supplementary blue or red lighting for 60 minute per day (B60, R60).

Number of flowering buds in African marigold seedlings increased under R60 treatment (data not shown). No significant differences in number of flowering buds were observed in *Ageratum* and *Salvia* seedlings. Significant interaction between supplementary light quality and radiation time at sunrise and sunset was shown in flowering physiology of the seedlings (Table 3). First flowering after the light treatments was stimulated by blue or red light in *Ageratum* and African marigold seedlings. Days to flowering of *Ageratum* plants were

shortened in the R30 or R60 treatment, and B30 treatment caused the flowering retardation. The R60 treatment prolonged the days to flowering in African marigold seedlings. Contrarily, the days to flowering in *Salvia* seedlings were not affected by light quality or radiation time, however blooming period was prolonged by R60 treatment. Blooming period in African marigold and *Salvia* seedlings was effectively improved by the red radiation for 60 minute and shorter under natural light condition than under other light treatments. Blooming period of the seedlings was significantly prolonged more than 10 days compared to the NL treatment. Mockler *et al.* (1999) reported that monochromic blue light delayed flowering in *Arabidopsis* as noted in African marigold of the present experiment. On the contrary, opposite effect of the blue light on days to flowering was shown in African marigold plants in this experiment. Blooming period in African marigold and *Salvia* seedlings was above 10 days extended in the R60 treatment for 12 days as compared to the control without supplementary lighting.

Transition to reproductive growth in flowering plants depends on plant's sensitivity to light quality. It has been suggested that extending the radiation time at sunrise and sunset twilight could stimulate reproductive growth of the seedlings grown under supplementary blue or red lighting. The blue light exposure regardless of radiation time significantly influenced the extension of the blooming period in both seedlings compared to the control. Generally, action spectra for flowering in long day plants is promoted by red light and reversed by far-red light. Phytochrome action related with light environment on flowering physiology of plants had been suggested by Hendricks and Borthwick (1963). Similarity of the spectra for the flowering promotion in long day plants and the inhibition of flowering in short day plants led to the concept of phytochrome as a primary photoreceptor mediating photoperiodic responses in plants. In *Ageratum* and African marigold seedlings, it was suggested that phytochrome activity could be maintained by supplementary red radiation longer than 30 minute at sunrise and sunset twilight, and thus prolonged blooming period as compared to natural sunlight without supplementary radiation.

Table 3. Influence of supplemental blue and red lighting on days to flowering and blooming period per plant of Ageratum, African marigold, and Salvia seedlings grown under supplementary blue and red lighting for 30 or 60 minute per day for 70 days

| Treatments | Days to flowering (days) | | | Blooming period (days) | | |
|------------|--------------------------|----------|--------|------------------------|----------|--------|
| | Ageratum | Marigold | Salvia | Ageratum | Marigold | Salvia |
| NL | 52.0 b ^z | 32.3 c | 35.3 a | 70 ^y | 29.3 c | 23.7 c |
| B30 | 60.0 a | 32.0 c | 35.3 a | 70 | 32.3 b | 28.0 b |
| B60 | 50.0 b | 33.0 c | 35.3 a | 70 | 34.0 b | 29.7 b |
| R30 | 38.7 c | 35.0 b | 36.0 a | 70 | 36.0 b | 29.7 b |
| R60 | 39.7 c | 38.3 a | 36.3 a | 70 | 41.3 a | 35.3 a |

| | Days to flowering (days) | | | Blooming period (days) | | |
|------------------------|--------------------------|----------|--------|------------------------|----------|--------|
| | Ageratum | Marigold | Salvia | Ageratum | Marigold | Salvia |
| Light quality (a) | ** | ** | ns | ** | ** | ** |
| Radiation time (b) | ** | ** | ns | * | * | ** |
| (a) × (b) ^w | * | * | ns | * | * | * |

^zMean value (in columns) followed by a different letter are significantly different at the $P < 0.05\%$ level by Duncan's multiple range test.

^yStill blooming after experiment in all the treatment.

^wRepresent interaction between light quality and radiation time.

Stomata formation and development of Ageratum, African marigold, and Salvia seedlings was significantly affected by the supplementary light quality at sunrise and sunset compared to NL of natural lighting condition (Fig. 3). Number of stomata per mm² leaf of Ageratum and African marigold significantly increased in R60 treatment compared with B or NL treatment. Stomatal opening in Ageratum and African marigold seedlings grown under the R60 treatment were obvious and morphologically shown different forms compared to B30 or B60 treatment. Morphological difference was clear in Salvia seedlings exposed by red light. Day-length supplementation by red light also increased the number of stomata in Salvia seedlings. The supplementary red light with longer radiation time induced bigger stoma size compared with blue light of shorter radiation time and natural light treatments. Cosgrove (1981) reported that blue light quality is important for the formation of chlorophyll, stomatal opening, or photomorphogenesis. Effects of light quality provided by LEDs on stomata formation were also discussed *in vitro* Chrysanthemum plantlets (Kim *et al.*, 2004). The present research shown the different patterns in stoma formation of the flowering seedlings compared to the previous reports. The stomatal opening and number in all the seedlings were enhanced by the supplementary red light with longer radiation time compared to the blue

or natural light. Characteristic differences in stoma size and number of several plant leaves including their different photosynthetic abilities can be induced by light qualities in Ageratum, African marigold, and Salvia seedlings.

From the present results, we can conclude that extending of day length by using red light with a lower light intensity at sunrise and sunset twilight has an impact on the vegetative and reproductive growth such as a stem elongation, stomata opening, or flower formation in Ageratum, African marigold, or Salvia seedlings. It is suggested that supplementary light quality and radiation time at sunrise and sunset twilight influence dry weight increasing, reproductive growth of the seedlings used as plant materials in the present or morphological responses in plant species grown under greenhouse conditions. Detailed researches are necessary for demonstration the co-relation between light qualities and various growth responses in physiological and molecular biological points.

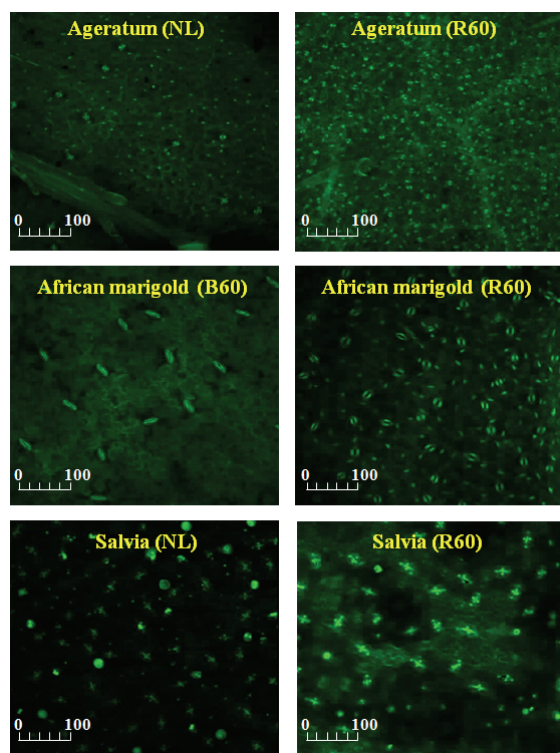


Fig. 3. Stomata formation and development of *Ageratum*, African marigold, and *Salvia* seedlings grown under supplementary blue and red lighting for 30 or 60 minute per day for 70 days. Natural light without supplementary lighting (NL); Supplementary blue or red lighting for 30 minute per day (B30, R30); Supplementary blue or red lighting for 60 minute per day (B60, R60).

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