Journal of Plant Biology, August 2006, 49(4) : 286-290

Influence of Mixed LED Radiation on the Growth of Annual Plants

Jeong Wook Heo[1], Chun Woo Lee[2], and Kee Yoeup Paek[2]*

[1]Division of Bio-Production Fundamental Engineering, National Institute of Agricultural Engineering, RDA, Suwon 441-100, Korea

[2]Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju 361-763, Korea

We investigated the effect of mixed radiation from light-emitting diodes (LEDs) on the growth and flowering of ageratum, marigold, and salvia bedding plants. Blue, red, and far-red lights were applied under controlled environmental conditions for 28 d. Both the combination of blue-plus-red radiation as well as fluorescent lighting treatment (control) caused increases in dry weights, but shoot lengths were shortest when plants were exposed to blue plus red light compared with either red or blue plus far-red treatments. The number of floral buds as well as the occurrence of flower opening for ageratum and salvia plants was also enhanced under the blue plus red mixture. Likewise, carbohydrate accumulation was stimulated by that combination compared with the other radiation treatments.

Keywords: chlorophyll, light-emitting diodes, mixture radiation, morphogenesis, starch

Environmental conditions, such as relative humidity, temperature, light intensity, and light quality, commonly influence the growth and development of plants. Among these various factors, light quality affects stem elongation, lateral branching, leaf extension and pigmentation, and photosynthetic activity (Moe and Heins, 1990; Heo et al., 2003; Jayakumar et al., 2004). Desirable morphological characteristics can be obtained when horticultural plants are grown under less light and low temperatures, thereby reducing heat energy costs with only a minimal delay in their blooming (Merritt and Kohl, 1991).

The objectives of this study were (1) to produce ageratum, marigold, and salvia bedding plants with compact growth, good branching, and a large number of flowers when treated with different mixtures of radiation sources, and (2) to evaluate the starch and sugar contents, as well as the optimum chlorophyll fluorescence, that would cause minimal or no delays in blooming when those plants were grown under various combinations of radiation provided by light-emitting diodes (LEDs).

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Bedding plants of ageratum (*Ageratum houstonianum* Mill. cv. Blue Field), marigold (*Tagetes erecta* L. cv. Orange Boy), and salvia (*Salvia splendens* F. Sello ex Ruem & Schult. cv. Red Vista) with two to four true leaves were used for our experimental materials. On Day 14 after sowing, all plants with two true leaves were transplanted to a plug tray (50 × 50 mm) filled with a soil mixture (BM1; Berger Horticulture, Canada) of 75 to 85% Canadian sphagnum peat moss, 15 to 20% perlite, and 4 to 10% vermiculite. Two types of liquid fertilizers, 20:10:20 and 14:0:14 (Plant Prod; Plant Products, Canada), were alternately applied at a rate of 100 ppm during the experimental period. We used an LED system (GF-320s; Good Feeling, Korea) comprising LED sticks, a panel, and a main controller to maintain a photosynthetic photon flux (PPF) of 90 ± 10 μmol m^{-2} s^{-1} over a 16-h photoperiod, as measured on an empty culture shelf. All plants were reared for 28 d in a growth chamber (Tasol Science, Korea) at 25 ± 2°C and 60 ± 10% relative humidity, and either under various 1:1 combinations of radiation (blue+ red, blue+far-red, or red+far-red) from the LEDs, or under cool-white fluorescent lamps as the control. Energy ratio (%) in spectral distributions of light-emitting diodes used in the experiment was 1:1 in all mixed radiations. The experimental plants were kept in a growth chamber (Tasol Science), at 25 ± 2°C and 60 ± 10% relative humidity.

Plant Growth Responses

On Day 21, 10 plants per treatment were harvested to determine their total dry weight, stem length, and the number of flowering buds and open flowers. Leaf area per plant also was measured, using the Skyeleaf leaf-area-analysis program (Skye, UK). The relative growth rate (RGR) of five plants was estimated, on a dry-weight basis, for the time periods of 0 to 7, 7 to 14, 14 to 21, and 21 to 28 d, according to the following equation: RGR (d^{-1}) = [ln(W_2) - ln(W_1)] / (T_2-T_1), where W_1 and W_2 were the dry weights measured at Day T_1 and T_2, respectively ($T_1 < T_2$) (Kozai and Sekimoto, 1988).

Analysis of Starch and Reducing Sugar Contents

Starch and reducing sugars were analyzed for five leaves at Day 0, 7, 14, 21, and 28 after treatment. Samples (1 g fresh weight) were homogenized in 20 mL of 80% ethanol, then centrifuged at 10,000g for 3 min. This supernatant was used to determine the soluble sugar content. The pellet was further treated for starch analysis by washing the residue three times with 20 mL of 40% ethanol. Starch was solubilized using a HCl-DMSO pretreatment, then hydrolyzed via incubation with amyloglucosidase at 60°C for 2 h. The formation of reducing sugars was measured spectrophotometrically at 570 nm, according to the dinitrosalicylic acid method (Chaplin, 1986).

Chlorophyll Fluorescence Measurement

The chlorophyll fluorescence for each sampled plant was

*Corresponding author; fax +82-43-272-5369
e-mail paekky@chungbuk.ac.kr

measured at the center of the third-from-the-top fully developed leaf, using a fluorometer (PAM-2000; Walz, Germany) under 90 ± 10 μmol m^{-2} s^{-1} for all treatments (van Huylenbroeck and Riek, 1995). Minimal fluorescence (F_0) was determined after 20 min of dark adaptation, while maximal fluorescence (F_m) was measured after a saturation pulse. The fluorescence ratio F_v/F_m (maximal PS II photochemical efficiency) was calculated as F_v being equal to F_m-F_0 (van Kooten and Snel, 1990). Photochemical quenching (qP) was measured by the saturation pulse method, with five replications.

Statistical Analysis

Statistical analysis was performed according to the SAS System (Version 6.21; SAS, USA). Mean and standard errors were used throughout, and significances between mean values were assessed by Duncan's multiple range tests. A probability of $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

We investigated the effect of various combinations of radiation sources -- blue plus red (BR), blue plus far-red (BFr), or red plus far-red (RFr) light, as provided by light-emitting diodes (LEDs). The growth and flowering response of ageratum, marigold, and salvia bedding plants were monitored for 28 d in a controlled environment. Dry weights were remarkably increased after plants of all three species were exposed to either BR or FL (Fig. 1). Although no significant difference was observed for this parameter at Day 7, at Day 21, the ageratum plants grown under BR and FL radiation were almost twice as heavy as those treated with BFr or RFr. Moreover, BR or FL radiation significantly stimulated dry weight accumulations in the marigold and salvia plants. However, for all three species, the addition of far-red radiation to the blue and red lights probably limited that accumulation of dry weight. Heo et al. (2002) reported that, when the proportion of far-red light provided by LEDs is increased to 96%, relative to the level of fluorescent lighting, the dry weights of salvia plants are higher than those exposed to monochromic blue or red light. Dry weights of red beet (*Beta vugaris* L.) hairy roots grown under 1:1 BFr or RFr mixtures also are significantly increased compared with their performance under monochromic LED lights (Shin et al., 2003). The effect of different light qualities, e.g., mixed LEDs, on these growth responses seems to vary according to plant species. Here, our conventional light sources, i.e., fluorescent lamps with a wide spectral distribution, probably influenced the accumulation of dry weight in the salvia plants compared with the role played by our mixtures of BFr or RFr, each having narrow band of wavelength.

Development was rapid between Days 21 and 28 for plants treated with FL, while the growth rate of marigold and salvia plants under BR was comparatively slower but steady (Table 1). The highest RGR was calculated for FL-treated plants from Day 14 to Day 21, but this subsequently decreased. The lower accumulation of dry mass under the BFr or RFr treatment was probably due to diminished growth rates over the period.

The effect of different light qualities on leaf area and stem elongation is shown in Table 2. Over the 28 d period, BR or FL treatment induced significant expansion in leaf area, i.e., with a higher number of unfolded leaves being produced in

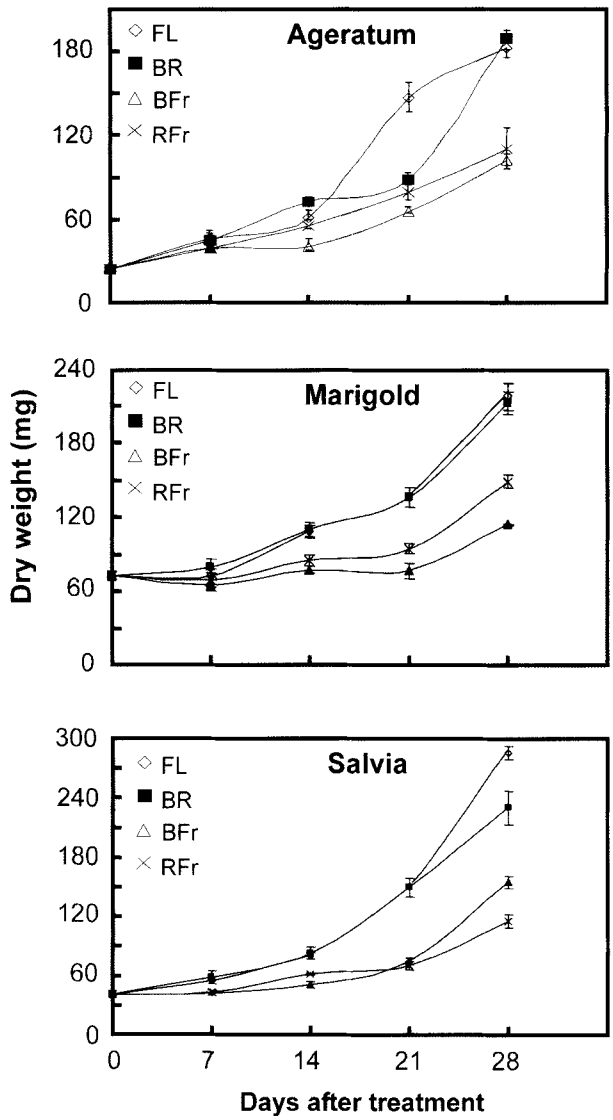

Figure 1. Time courses of dry weight for ageratum, marigold, and salvia plants grown for 28 d under different radiation mixtures of blue+red (BR), blue+far-red (BFr), and red+far-red (RFr), or under fluorescent lamps (FL) as the control. Vertical bars represent means ± standard errors. Initial mean dry weights of individual plants were 24.2 (BR), 72.6 (BFr), and 40.8 (RFr) mg.

all three species (data not shown). In contrast, BFr or RFr radiation inhibited leaf growth in ageratum and salvia plants whereas in the marigolds, leaf area was almost twice larger under those lights than when exposed to BFr. Although we found that far-red light inhibited leaf extension in ageratum and marigold, silver birch seedlings treated for 10 d with Fr radiation show expanded leaf areas and greater leaf dry weights compared with those grown under red lighting only (Tegelberg et al., 2004). However, *in vitro*-cultured chrysanthemums are significantly stimulated by 1:1 blue plus red in contrast to their performance under either blue or red plus far-red light (Kim et al., 2004). These mixed results suggest that, when some species are grown under a variety of light qualities, their leaves develop differently when exposed to only far-red light rather than in combination with changing ratios of Fr to blue or red light.

For all species, stems were shorter under BR and FL lights when compared with the BFr and RFr mixtures. Growth and

Table 1. Relative growth rates (RGR), on a dry-weight basis, for ageratum, marigold, and salvia plants grown under different radiation mixtures for 28 d.

Light source	Time period (d)	RGR (d^{-1})		
		Ageratum	Marigold	Salvia
Fluorescent lamp (FL)	0-7	0.09	0.00	0.04
	7-14	0.04	0.06	0.05
	14-21	0.13	0.05	0.01
	21-28	0.03	0.15	0.19
Blue+Red (BR)	0-7	0.09	0.01	0.05
	7-14	0.07	0.05	0.05
	14-21	0.03	0.03	0.09
	21-28	0.11	0.06	0.06
Blue+Far-red (BFr)	0-7	0.06	0.02	0.01
	7-14	0.05	0.03	0.03
	14-21	0.07	0.00	0.06
	21-28	0.06	0.06	0.10
Red+Far-red (RFr)	0-7	0.07	0.01	0.01
	7-14	0.05	0.03	0.05
	14-21	0.05	0.02	0.02
	21-28	0.05	0.06	0.07

development are enhanced at certain red to far-red ratios, but can be inhibited at the upper limit (Smith and Morgan, 1983). Devlin et al. (1998) have reported that, when Phytochromes A through E are monitored, internode elongation in *Arabidopsis* is more influenced by the level of phyE, such that its deficiency is typical in shade avoidance responses to a low red to far-red ratio. In two ecotypes of alpine and prairie *Stellaria*, stem elongation is affected by both low (0.9) and high (1.9) R:Fr ratios (Alokam et al., 2002), with the former situation stimulating this development more in the prairie ecotype than in the alpine ecotype. In our study, a 1:1 mixture of BFr and RFr promoted significant stem elongation compared with plants under either FL or BR, but lateral branching and leaf unfolding were inhibited in all species (data not shown). Enhanced elongation mediated by far-red rather than red light has been reported in Norway spruce (*Picea abies*) (Molmann et al., 2006), as well as in *Pelargonium* 'Penny Irene' (Appelgren, 1991). Conversely, Tsegay et al. (2005) have demonstrated that monochromic red or far-red light inhibits hypocotyl elongation in seedlings of *Betula pendula*, with this effect being significantly greater under continuous R+Fr radiation. However, we observed inhibited stem elongation with the BR treatment. Therefore, regardless of the R:Fr ratio, these negative or positive stimulations of growth in bedding plants and other species are probably mediated not only by phytochromes but also by the cryptochrome family-related red and blue lights.

Development of the first flowering node in our ageratum was achieved under a mixture of radiation combined with far-red light, but these conditions did not influence the setting of the first flowering node in the marigold and salvia plants (Table 3). Bud development in ageratum and marigold was especially dependent upon BFr or RFr mixtures. For salvia, the number of flowering buds was higher for plants treated with combinations of blue, red, or far-red light compared with performance by the control. The number of open flowers on the marigolds and salvia was lower under the mixtures of BR, BFr, or RFr because delayed flowering is mediated by photoreceptors of those light qualities. However, in ageratum, the delay in bud set and the lower number of flowering buds was significantly greater under BR treatment. This observation is supported by a report that low R/Fr light quality under a 20-h photoperiod delays flowering in *E7E7* soybean lines (Cober and Voldeng, 2001). In contrast, floral development on 'Mercedes' rose plants is unaffected by blue light, and an end-of-day treatment with red light, rather than far-red light, significantly increases the number of shoots with flowers (Maas and Bakx, 1995).

Despite the significantly enhanced formation of floral buds in all three species, the opening of those flowers was delayed when plants were grown under radiation mixtures of BFr or RFr. In addition, treatment with RFr promoted earlier flowering in marigold compared with the other mixtures (data not shown). These results suggest that flowering can be initiated by manipulating the ratio of red to far-red light rather than using red light alone. However, research on

Table 2. Influence of different radiation mixtures on per-plant leaf areas and stem lengths for ageratum, marigold, and salvia after 28 d.

Light source	Leaf area (cm^2)			Stem length (cm)		
	Ageratum	Marigold	Salvia	Ageratum	Marigold	Salvia
FL	43.0 a[z]	37.2 b	55.9 a	8.2 c	6.1 c	6.6 c
BR	47.9 a	44.6 a	47.3 a	9.2 c	6.6 c	5.8 c
BFr	21.5 b	24.6 c	27.9 b	14.2 b	15.1 a	13.5 b
RFr	23.9 b	44.4 a	33.2 b	29.3 a	11.8 b	15.9 a

[z]Values followed by the same letter within a column are not significantly different at the 5% level, based on a Duncan's multiple range test.

Table 3. Flowering responses (per plant) of ageratum (Ag), marigold (Ma), and salvia (Sa) grown for 28 d under different mixtures of radiation.

Light sources	Flowering node			No. flowering buds			No. open flowers		
	Ag	Ma	Sa	Ag	Ma	Sa	Ag	Ma	Sa
FL	7.2 b[z]	5.0 a	6.6 a	0.6 a	1.4 c	0.4 b	0.4 b	1.0 a	0.6 a
BR	8.0 a	6.2 a	6.8 a	0.4 b	2.0 b	0.8 a	0.8 a	0.8 b	0.2 b
BFr	7.4 b	6.2 a	6.6 a	1.0 a	2.0 b	1.0 a	0.0 b	0.0 d	0.0 b
RFr	7.2 b	5.0 a	6.8 a	0.0 b	3.0 a	1.0 a	0.0 b	0.2 c	0.0 b

[z]Values followed by the same letter within a column are not significantly different at the 5% level, based on a Duncan's multiple range test.

Table 4. Fluorescence parameters, qP, qN, and Fv/Fm, measured from leaves of ageratum (Ag), marigold (Ma), and salvia (Sa) plants grown for 28 d under different radiation mixtures.

Light source	qP			qN			Fv/Fm		
	Ag	Ma	Sa	Ag	Ma	Sa	Ag	Ma	Sa
FL	0.740 b[z]	0.711 c	0.687 d	0.739 b	0.711 c	0.687 c	0.621 c	0.681 c	0.541 c
BR	0.729 c	0.744 b	0.709 a	0.729 c	0.744 b	0.709 a	0.644 b	0.691 b	0.596 a
BFr	0.756 a	0.767 a	0.704 b	0.756 a	0.767 a	0.704 b	0.617 d	0.745 a	0.556 b
RFr	0.707 d	0.744 b	0.652 c	0.707 d	0.744 b	0.652 d	0.701 a	0.744 a	0.540 c

[z]Values followed by the same letter within a column are not significantly different at the 5% level, based on a Duncan's multiple range test.

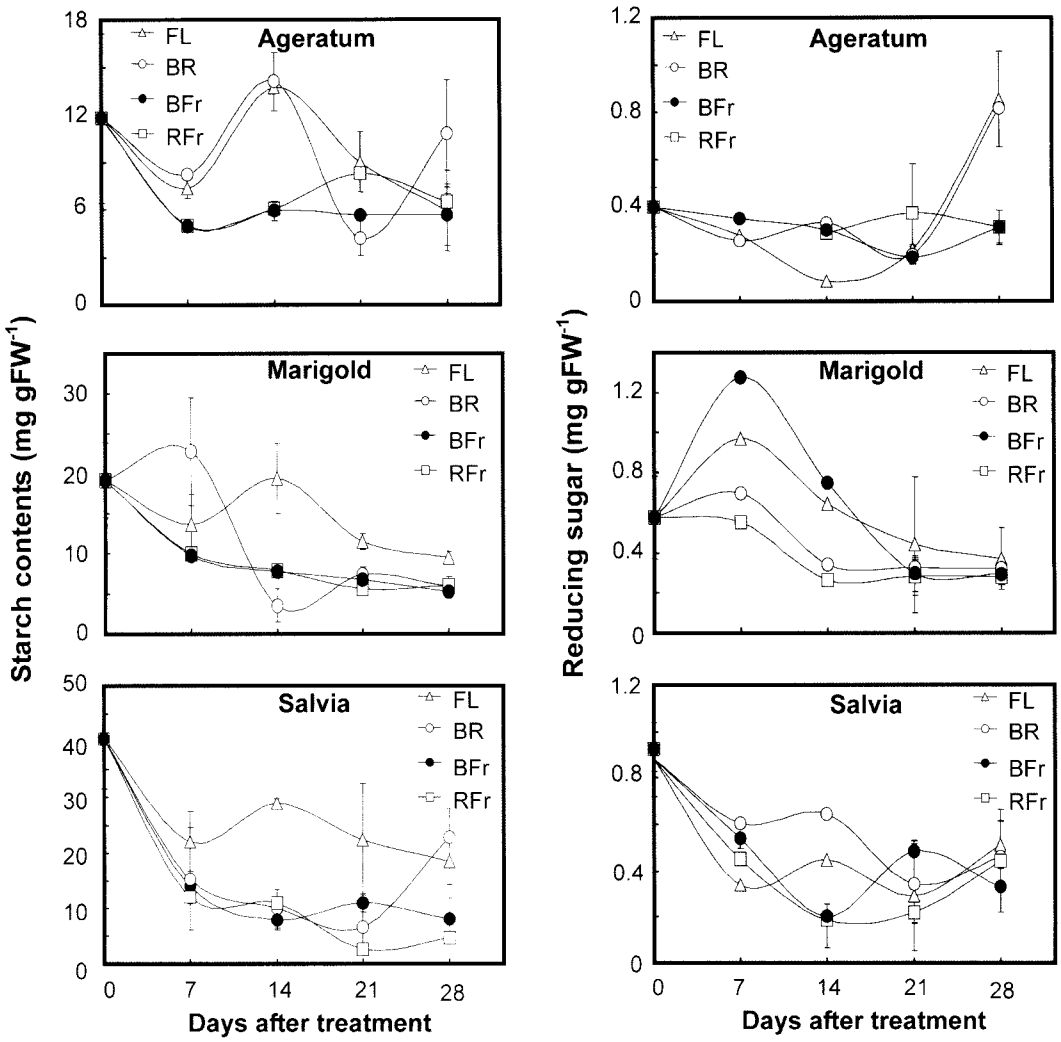

Figure 2. Changes in starch and reducing sugar contents in leaves of ageratum, marigold, and salvia plants grown under different mixtures of radiation for 28 d. Vertical bars represent means ± standard errors.

petunia seedlings has shown that, under red light, flowering is not delayed by red-rich treatment in a greenhouse (Kubota et al., 2000). Furthermore, red and far-red lights not only affect stem elongation but also promote the flowering of *Lolium* spp. (Casal et al., 1985). Therefore, these studies of flowering physiology suggest that the important role of phytochromes can be exploited to control vegetative growth and the flowering response, but this phytochrome factor depends upon the species being grown (e.g., Weinig, 2002; Mockler et al., 2003).

Photochemical quenching of qP and qN was greatest under BFr lighting compared with BR or FL, resulting in lower dry weights for both ageratum and marigold. However, the photochemical values calculated for salvia were higher under the mixture treatment of blue and red light (Table 4). Photochemical efficiency of PS-II (Fv/Fm) for ageratum and marigold plants also increased under BFr or RFr, but declined under FL. The maximum photochemical efficiency of Fv/Fm along with greater dry mass values were observed in salvia plants grown under mixed BR radiation. Photoinhibition in salvia was more remarkable as a result of FL or RFr treatment rather than with BR, thus promoting higher growth rates in terms of dry mass and leaf expansion. The photosynthetic apparatus of marigold and salvia under FL were more susceptible to photoinhibition

than under the other light qualities. Our determination of chlorophyll fluorescence provides valuable information concerning the interaction between different light qualities and fundamental photosynthesis processes, here implying that these varying photochemical responses were probably modified according to the species tested as well as the light intensities or mixture ratios selected.

Changes in starch and reducing sugar contents in all three species were sensitive to the different mixtures of radiation (Fig. 2). For all plants, BR promoted the accumulation of starch and sugar in the leaves while those levels decreased under BFr or RFr treatment. The amounts of reducing sugars as well as starch content for ageratum from Day 21 to 28 were significantly higher with the FL and BR treatments, whereas sugar contents in marigolds were not affected by these various light qualities. These increases in dry weight and leaf area, and the onset of floral initiation were probably due to the effect that these radiation mixtures had on carbohydrate accumulation compared with the results measured under BFr or RFr treatment. Although such metabolic responses to monochromic light quality have been observed in other higher plants and in green algae (Wild and Holzapfel, 1980), the action of spectral quality from the blue, red, or far-red combinations on flowering physiology is still unclear with regard to carbohydrate accumulation.

Based on our results, we can conclude that high-value ageratum, marigold, and salvia plants, i.e., those with improved vegetative growth and numerous open flowers, can be produced when grown for 28 d under a radiation mixture of blue, red, or far-red light, and within a controlled, closed system. These efforts translate into higher profits for the horticulture trade. Nevertheless, further works are needed to investigate the relationship between photosynthetic activity and flowering physiology when different mixtures of light quality are provided by light-emitting diodes.

ACKNOWLEDGEMENT

This work was supported by the Korea Science and Engineering Foundation through the Research Center for the Development of Advanced Horticultural Technology at Chungbuk National University.

Received March 21, 2006; accepted June 19, 2006.

LITERATURE CITED

Alokam S, Chinnappa CC, Reid DM (2002) Red/far-red light mediated stem elongation and anthocyanin accumulation in *Stellaria longipes*: Differential response of alpine and prairie ecotypes. Can J Bot 80: 72-81

Appelgren M (1991) Effects of light quality on stem elongation of *Pelargonium in vitro*. Sci Hort 45: 345-351

Casal JJ, Deregibus VA, Sanchez RA (1985) Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam.: Vegetative and reproductive plants as affected by differences in red/far-red irradiation. Ann Bot 56: 553-559

Chaplin MF (1986) Monosaccharides, *In* MF Chaplin, JF Kennedy, eds, Carbohydrate Analysis, A Practical Approach. IRL Press, Oxford, pp 1-36

Cober ER, Voldeng HD (2001) Low R:FR light quality delays flowering of *E7E7* soybean lines. Crop Sci 41: 1823-1826

Devlin PF, Patel SR, Whitelam GC (1998) Phytochrome E influences internode elongation and flowering time in *Arabidopsis*. Plant Cell 10: 1479-1487

Heo JW, Lee CW, Chakrabarty D, Paek KY (2002) Growth responses of marigold and salvia bedding plants as affected by monochromic or mixture radiation provided by a Light-Emitting Diode (LED). Plant Growth Reg 38: 225-230

Heo JW, Lee CW, Murthy HN, Paek KY (2003) Influence of light quality and photoperiod on flowering of *Cyclamen persicum* Mill. cv. 'Dixie White'. Plant Growth Reg 40: 7-10

Jayakumar M, Amudha P, Kulandaivelu G (2004) Effect of low doses of UV-A and UV-B radiation on photosynthetic activities in *Phaseolus mungo* L. J Plant Biol 47: 105-110

Kim SJ, Hahn EJ, Heo JW, Paek KY (2004) Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets *in vitro*. Sci Hort 101: 143-151

Kozai T, Sekimoto K (1988) Effects of the number of air exchanges per hour of the culture vessel and the photosynthetic photon flux on the carbon dioxide concentration inside the vessel and the growth of strawberry plantlets *in vitro*. Environ Control Biol 26: 21-29

Kubota S, Yamato T, Hisamatsu T, Esaki S, Oi R, Roh MS, Koshioka M (2000) Effects of red-and far-red rich spectral treatments and diurnal temperature alternation on the growth and development of *Petunia*. J Jpn Soc Hort Sci 69: 403-409

Maas FM, Bakx EJ (1995) Effects of light on growth and flowering of *Rosa hybrida* 'Mercedes'. J Amer Soc Hort Sci 120: 571-576

Merritt RH, Kohl HC (1991) Morphology of bedding plants in response to low night temperature and energy use implications. Sci Hort 45: 295-302

Mockler TM, Yang HY, Yu XH, Parikh D, Cheng YC, Dolan S, Lin C (2003) Regulation of photoperiodic flowering by *Arabidopsis* photoreceptors. Proc Natl Acad Sci USA 100: 2140-2145

Moe R, Heins RD (1990) Control of plant morphogenesis and flowering by light quality and temperature. Acta Hort 272: 81-89

Molmann JA, Junttila O, Johnsen O, Olsen JE (2006) Effects of red, far-red and blue light in maintaining growth in latitudinal populations of Norway spruce (*Picea abies*). Plant Cell Environ 29: 166-172

Shin KS, Murthy HN, Heo JW, Paek KY (2003) Induction of betalain pigmentation in hairy roots of red beet under different radiation sources. Biol Plantarum 47: 149-152

Smith H, Morgan DC (1983) The function of phytochrome in nature, *In* JW Shropshire, H Mohr, eds, Encyclopedia of Plant Physiology. New series, Springer, Berlin, 16(B), pp 491-517

Tegelberg R, Julkunen-Tiitto R, Aphalo PJ (2004) Red:far-red light ratio and UV-B radiation: Their effects on leaf phenolics and growth of silver birch seedlings. Plant Cell Environ 27: 1005-10130

Tsegay BA, Olsen JE, Junttila O (2005) Effect of red and far-red light on inhibition of hypocotyl elongation in ecotypes of *Betula pendula* Roth. Afr J Biotech 4: 50-56

van Huylenbroeck JM, Riek JD (1995) Sugar and starch metabolism during *ex vitro* rooting and acclimatization of micropropagated *Spathiphyllum* 'Petite' plantlets. Plant Sci 111: 19-25

van Kooten O, Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25: 147-150

Weinig C (2002) Phytochrome photoreceptors mediate plasticity to light in flowers of the Brassicaceae. Amer J Bot 89: 230-235

Wild A, Holzapfel A (1980) The effect of blue and red light on the content of chlorophyll, cytochrome f, soluble reducing sugars, soluble proteins and nitrate reductase activity during growth of the primary leaves of *Sinapsis alba*, *In* H Senger, ed, The Blue Light Syndrome. Springer-Verlag, Berlin, pp 444-451