Europ.J.Hort.Sci., **71** (6). S. 240–244, 2006, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

The Effects of Paclobutrazol, Light Emitting Diodes (LEDs) and Sucrose on Flowering of *Euphorbia millii* Plantlets *in Vitro*

Y. H. Dewir[1,2)], D. Chakrabarty[1,3)], E. J. Hahn[1)] and K.Y. Paek[1)]
([1)]Research Center for The Development of Advanced Horticultural Technology; Chungbuk National University, Cheong-ju, Korea, [2)]Department of Horticulture, Faculty of Agriculture, Kafr El-Sheikh University, Egypt and [3)]Floriculture Section, National Botanical Research Institute, Lucknow, India)

Summary

The effects of paclobutrazol, light emitting diodes (LEDs) and sucrose on inflorescence development *in vitro* were investigated in photoperiodically day-neutral *Euphorbia millii*. Paclobutrazol, a growth retardant, was mandatory to the shift from the vegetative to reproductive stage. Inflorescence development in the apical bud was observed after 21–30 days of culture on MS media containing paclobutrazol. Different light qualities also influenced *in vitro* flowering of *E. millii*. Highest flowering percentage (90 %), number of inflorescences per plantlet, percentage of mature inflorescences and early flowering (days to first bloom) were observed in fluorescent light. Among the LEDs treatments, red light reduced the flowering percentage, whereas blue, red plus far-red and blue plus far-red LEDs stimulated flowering *in vitro*. Sucrose concentration also influenced the growth and morphogenic patterns of *in vitro* plantlets. Sucrose at 5 % enhanced inflorescence differentiation. The results indicate that inflorescence development of *E. millii* plantlets in tissue culture can serve as a potential model to study the role of paclobutrazol, LEDs and sucrose in the flowering process of a day-neutral plant that does not require external signals for flower induction. Also the technique described here can be used as an efficient method for production of early, synchronized and profuse flowering of *E. millii* pot plants.

Key words. *in vitro* flowering – paclobutrazol – light emitting diodes – sucrose

Introduction

Euphorbia millii or "Crown of Thorns" is a succulent ornamental used as an edging plant for decades, whereas the interest for growing it as a pot plant is more recent. For pot plant production to be successful an efficient method for early, synchronized and profuse flowering, together with a compact and homogeneous plant size is thus required.

Flowering transition is a major event in the plant life cycle that has to be precisely timed for reproductive success. Both physiological and genetical studies have revealed the complexity of the mechanisms that tightly control the apical meristem switch from vegetative to reproductive growth (Silva and Tan Nhut 2003). The ability of explants to form flowers *in vitro* depends on numerous factors, internal and external, chemical and physical, and virtually all of these factors interact in various complex and unpredictable ways (Tran Than Van 1973; Compton and Vielleux 1992; Silva and Tan Nhut 2003). Although *in vitro* flower formation has been observed in several plants, its occurrence is relatively rare and poorly understood. The effects of sucrose, GA_3, photoperiod and temperature on apical meristem development and the transition to reproductive growth have been studied extensively (Silva and Tan Nhut 2003). Further studies on light quality and growth retardant involvement in the induction of flowering and further flower development in plantlets cultivated *in vitro* are rare.

Previous studies indicate that GA_3 acts additive to photoperiodic induction (Naor et al. 2004). *E. millii* is a day-neutral plant and flowers irrespective of short or long day length. Sugars are necessary for *in vitro* vegetative development and flowering, presumably as an energy source. The mechanisms by which sugars act to influence plant development and gene expression are just beginning to be deciphered. Understanding of sugar response is complicated by the fact that plants have multiple sugar response pathways and that the molecules actually being perceived are not known in all cases. Although glucose and sucrose appear to be sensed directly, in some cases, different sugars or sugar metabolites might act as the actual signal molecules (Gibson 2005). It is also known that during the change from the vegetative to the flowering state, the growth correlations within the apical meristem of a shoot are changed, which leads to loss of apical dominance (Pidkowich et al. 1999). Therefore, the influence of paclobutrazol (a growth retardant), light quality (LEDs) and sucrose, applied in succession, on the formation of flower buds in isolated shoot apices of *E. millii* was investigated.

Materials and Methods

Plant material

In vitro regenerated plantlets derived from inflorescences of *Euphorbia millii,* maintained onto MS basal medium (Murashige and Skoog 1962) with 3 % sucrose (w/v), 1.0 mg l^{-1} BA and 0.3 mg l^{-1} IBA solidified with 0.8 % agar (w/v), were used for this study (Dewir et al. 2005).

Effect of paclobutrazol

Shoot tips of 2.5–3 cm in length (6 per culture vessel) were inoculated to cylindrical culture vessels (700 ml capacity) containing 150 ml MS medium with 3 % sucrose (w/v) and different concentration of paclobutrazol (0, 2, 4, 6, 8 mg l^{-1}) solidified with 0.8 % agar. All culture vessels were maintained at 25 °C under 50 μmol m^{-2} s^{-1} PPF (florescent light) and 16 h photoperiod per day.

Effect of LEDs

The effect of different light qualities [fluorescent (FL), red (R), blue (B), blue + red (BR; 1:1), red + far red (RFR; 1:1) and blue + far red (BFR; 1:1), provided by a LED system; GF-320, Good Feeling Co. Ltd., Sungnum, Korea] on *in vitro* flowering was also studied. Spectral distributions in relative energy of the improved blue (peak at 440 nm; GaAsAIP, Nichia, Japan), red (peak at 650 nm; GaAsAl, Sharp, Japan), or far-red (peak at 720 nm; GaAsAl, Showa Denko, Japan) regions, as determined with a spectroradiometer (Li-1800, LI-COR, Lincoln, Nebraska, USA) in the range of 300–1,100 nm, are shown by Heo et al. (2002). Totally radiated PPF in all the treatments was set at 50 μmol m^{-2} s^{-1} on average. The photoperiod was 16 h for all the treatments. The basic medium consisted of MS constituents with 2 mg l^{-1} paclobutrazol solidified with 0.8 % agar (w/v). The pH of the medium was adjusted to 5.8 before autoclaving (at 121 °C and 1.2 kg cm^{-2} pressure for 15 min). All culture vessels were maintained at 25 °C.

Effect of sucrose

Shoot tips (6 per culture vessel) were inoculated to cylindrical culture vessels (700 ml capacity) containing 150 ml MS medium with different concentration of sucrose [0, 1, 3, 5, 7, 9 % (w/v)] and 2 mg l^{-1} paclobutrazol solidified with 0.8 % agar. All culture vessels were maintained at 25 °C under 50 μmol m^{-2} s^{-1} PPF (white fluorescent light) and 16 h photoperiod per day.

Acclimatization

Flowering plantlets (6–9 cm) at the seven-to-ten leaf stage were harvested from the culture vessels and transplanted into small pots (10 cm×5.1 cm) filled with soil and leaf compost (1:1). The pots were kept in a growth chamber under a 25±2 °C air temperature, 40–50 % relative humidity and a PPFD of 100 μmol m^{-2} s^{-1} with a 16 h photoperiod using halide lamps and high pressure sodium lamps for 15 days before their transfer to a greenhouse.

Experimental design

Experiments were set up in completely randomized design and repeated three times with 4 replicates. Observation on the flowering percentage, days to first bloom, number of inflorescences per plantlet, plantlet height and fresh weight were recorded after 8 weeks of culture and dry weight was determined after drying the shoots for 48 h at 60 °C. Chlorophyll and carotenoid contents were measured according to Lichtenthaler (1987). Young leaves weighing 0.5 g were extracted with 80 % cold acetone for 48 h and the absorbance was measured at 663.2, 646.8 and 470 nm. Calculations were made according to Lichtenthaler (1987). Data were subjected to Duncan's multiple range test using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

Results and Discussion

The effect of paclobutrazol on *in vitro* flowering of *E. millii* is presented in Table 1. The control plantlets remained in the vegetative stage (Fig. 1A). However, the plantlets treated with paclobutrazol formed floral buds. The response of flowering depended on the concentration of paclobutrazol in the culture medium. Flowering percentage increased with increasing paclobutrazol content. High concentration (6 mg l^{-1} paclobutrazol) induced 100 % flowering, however the quality of inflorescences in terms of flower diameter, peduncle length and portion of mature inflorescences (first stage and second stage inflorescence development). The best response was observed in medium with 2 mg l^{-1} paclobutrazol. Paclobutrazol also inhibited the vegetative development of the plantlets (Fig. 1A). The fresh weight, dry weight and plant height was significantly lower in paclobutrazol treated plantlets as compared to the control. However, chlorophyll and carotenoid content was higher in paclobutrazol treated plants except for the highest concentration. Paclobutrazol, which is widely used as a plant growth retardant in potted ornamental crops, provides height control in several floriculture crops (Prusakova et al. 2004). In the present study, the growth retardant stimulated the reproductive production at the expense of vegetative plant growth. It is also reported that the mature state of the plantlets is usually a prerequisite for *in vitro* flowering and juvenility does not allow for flower production due to an inability to produce a flowering factor or the inability of active meristems to respond to a flowering factor (Hackett 1985).

Different light qualities also influenced the *in vitro* flowering of *E. millii* (Table 1). Significant differences were found between FL and LEDs treatments (Fig. 1B). The highest flowering percentage (90 %), number of inflorescences per plantlet, percentage of mature inflorescences and early flowering (days to first bloom) were observed in FL treatment. Among the LEDs treatment, it is clear that R reduced the flowering percentage, whereas B, RFR and BFR LEDs stimulated flowering *in vitro*. Furthermore flowering was delayed in R, BFR and B LEDs. The flower diameter and peduncle length was highest in R and RFR treatments. Vegetative growth was also affected by light qualities. Fresh weight, dry weight and leaf per plantlets were greater in R and BR LEDs. Plantlet height was higher in R treatment as compared to other

Table 1. Effect of paclobutrazol, LEDs, and sucrose on *in vitro* growth and flowering of *Euphorbia millii* after 8 weeks of culture.

Growth and flowering characteristics	Paclobutrazol (mg l^{-1})					
	0	2	4	6	8	
Fresh weight (mg plantlet^{-1})	1434 a[z]	1192 ab	977 bc	846 c	425 d	
Dry weight (mg plantlet^{-1})	119 ab	123 a	99 ab	95 b	53 c	
Plant height (cm)	8.6 a	6.4 b	5.9 c	5.6 c	4.5 d	
Leaf no. plantlet^{-1}	8.3 a	8.5 a	7.9 a	5.4 b	4.0 b	
Total chlorophyll (mg g^{-1} fw)	1.755 bc	2.292 a	2.025 ab	2.075 ab	1.479	
Carotenoids (mg g^{-1} fw)	0.367 b	0.420 a	0.425 a	0.462 a	0.366 b	
Days to first bloom	–	21	28	30	–	
Flowering (%)	–	89	89	100	–	
Immature inflorescences (%)	–	27	33	52	–	
1st stage inflorescence (%)	–	39	46	48	–	
2nd stage inflorescence (%)	–	34	21	–	–	
No. inflorescences plantlet^{-1}	–	1.5 a	1.5 a	1.3 a	–	
Peduncle length (cm)	–	1.36 a	1.13 b	0.83 c	–	
Flower diameter (cm)	–	1.06 a	1.00 a	0.73 b	–	
	Fluorescent (FL)	Blue (B)	Red (R)	Blue + Red (BR) (1:1)	Red + Far red (RFR) (1:1)	Blue + Far red (BFR) (1:1)
Fresh weight (mg plantlet^{-1})	1396 b[z]	1652 a	1615 a	1672 a	1465 b	1199 c
Dry weight (mg plantlet^{-1})	139 b	148 b	174 a	176 a	133 b	111 c
Plant height (cm)	6.9 c	7.5 bc	8.9 a	7.8 b	7.7 b	6.7 c
Leaf no. plantlet^{-1}	9.5 b	11.3 a	12.1 a	12.4 a	9.9 b	8.0 c
Total chlorophyll (mg g^{-1} fw)	2.69 a	2.81 a	2.03 b	2.58 a	2.27 b	2.04 b
Carotenoids (mg g^{-1} fw)	0.50 ab	0.54 a	0.39 c	0.48 b	0.43 c	0.40 c
Days to first bloom	21	30	49	27	29	42
Flowering (%)	90	67	17	25	58	60
Immature inflorescences (%)	30	57	–	40	40	60
1st stage inflorescence (%)	35	29	50	60	30	30
2nd stage inflorescence (%)	35	14	50	–	30	10
No. inflorescences plantlet^{-1}	1.6 a	1.1 b	0.2 d	0.4 c	0.8 bc	0.8 bc
Peduncle length (cm)	1.4 cd	1.8 c	2.9 a	1.8 c	2.4 b	1.1 d
Flower diameter (cm)	1.1 b	1.1 b	1.2 ab	1.0 b	1.4 a	1.0 b
	Sucrose % (w/v)					
	0	1	3	5	7	9
Fresh weight (mg plantlet^{-1})	966 c[z]	1586 b	1232 bc	2527 a	2656 a	1663 b
Dry weight (mg plantlet^{-1})	74 c	123 c	131 c	283 b	302 b	387 a
Plant height (cm)	7.4 b	8.8 a	6.9 b	8.4 a	7.4 b	6.4 c
Leaf no./plantlet	8.9 b	10.9 a	8.5 b	9.3 b	8.6 b	8.1 b
Total chlorophyll (mg g^{-1} fw)	2.44 ab	2.52 a	2.37 ab	2.25 bc	2.08 c	1.56 d
Carotenoids (mg g^{-1} fw)	0.51 a	0.52 a	0.43 b	0.42 b	0.40 b	0.32 c
Days to first bloom	51	47	21	21	30	30
Flowering (%)	10	22	90	90	72	50
Immature inflorescences (%)	50	50	27	20	9	27
1st stage inflorescence (%)	50	50	33	20	73	55
2nd stage inflorescence (%)	–	–	40	60	18	18
No. inflorescences plantlet^{-1}	1.0 c	1.0 c	1.5 b	2.0 a	1.4 b	1.4 b
Peduncle length (cm)	0.5 d	0.6 d	1.4 b	2.0 a	1.2 c	0.5 d
Flower diameter (cm)	0.6 c	0.6 c	1.1 b	1.5 a	1.4 a	1.0 b

[z] Mean separation within rows by Duncan's multiple range test at 5 % level.

treatments. However, chlorophyll and carotenoid contents was highest in fluorescent light as well as B and lowest in R treatments. In tissue culture, light quality, duration and intensity affect shoot growth and morphogenesis and have a role in photosynthesis (HUGHES 1981). Red light is important for the development of the photosynthetic apparatus and accumulation of starch (SAEBO et al. 1995), whereas blue light is important for the formation of chlorophyll, chloroplast development, stomatal opening and photomorphogenesis (SENGER 1982; AKOYUNOGLOU and ANNI 1984). As a developmental stage, flowering can be defined as the transition from vegetative to reproductive development. This transition is manifested as a change in properties of the shoot apical meristem, which stops producing leaves and instead starts producing the floral meristems that give rise to flowers. Both developmental cues and environmental signals control the timing of flowering. One important signal that regulates flowering time is light (BERNIER et al. 1993). Based on the responses to LEDs, it is clear that flowering of *E. millii* is promoted by blue light. While induction of flowering is rather inhibited by red light. However, such photobiological studies alone do not elucidate which plant photoreceptor is involved. Phytochrome(s) can be activated in the blue, albeit weakly, as well as being strongly activated in the red and far-red. A specific blue light receptor could be involved alone or independently in addition to phytochrome or it could also act synergistically with phytochrome (BRIGGS and HUALA 1999).

Sucrose concentration also influenced the growth and morphogenic patterns of *in vitro* shoots (Fig. 1C). Sucrose allowed flowering from *in vitro* shoots of *E. millii* at concentration from 0–9 % (Table 1). Sucrose at 5 % was the most effective in terms of flowering percent (90 %), number of inflorescences per plantlet (2), peduncle length (2 cm), flower diameter (1.5 cm), mature inflorescence (first stage and second stage inflorescence development, Fig. 1D), early flowering (21 days to first bloom), fresh weight (2.5 g), dry weight (0.28 g) and shoot length (8.4 cm). The effect of sucrose concentration was also reflected in photosynthetic pigment content. Chlorophyll and carotenoid contents were lower in plantlets grown on higher sucrose levels. Many studies have reported the effects of sucrose concentration on *in vitro* flower induction, for example, in *Kalanchoe blossfeldians* (DICKENS and VAN STADEN 1988), *Tornenia* (TANIMOTO and HARADA 1981), *Murraya paniculata* (JUMIN and NITO 1996), *Leptinella nana* L. (CARSON and LEUNG 1994), and bamboos (JOHN and NADGAUDA 1999). Sugar levels have been postulated to play an important role in determining floral initiation time, and flower-inducing treatments may result in an increased transport of carbohydrates from leaves to shoot apical meristems (SAMs) (CORBESIER et al. 1998). This increase in sugar flow to SAMs prior to

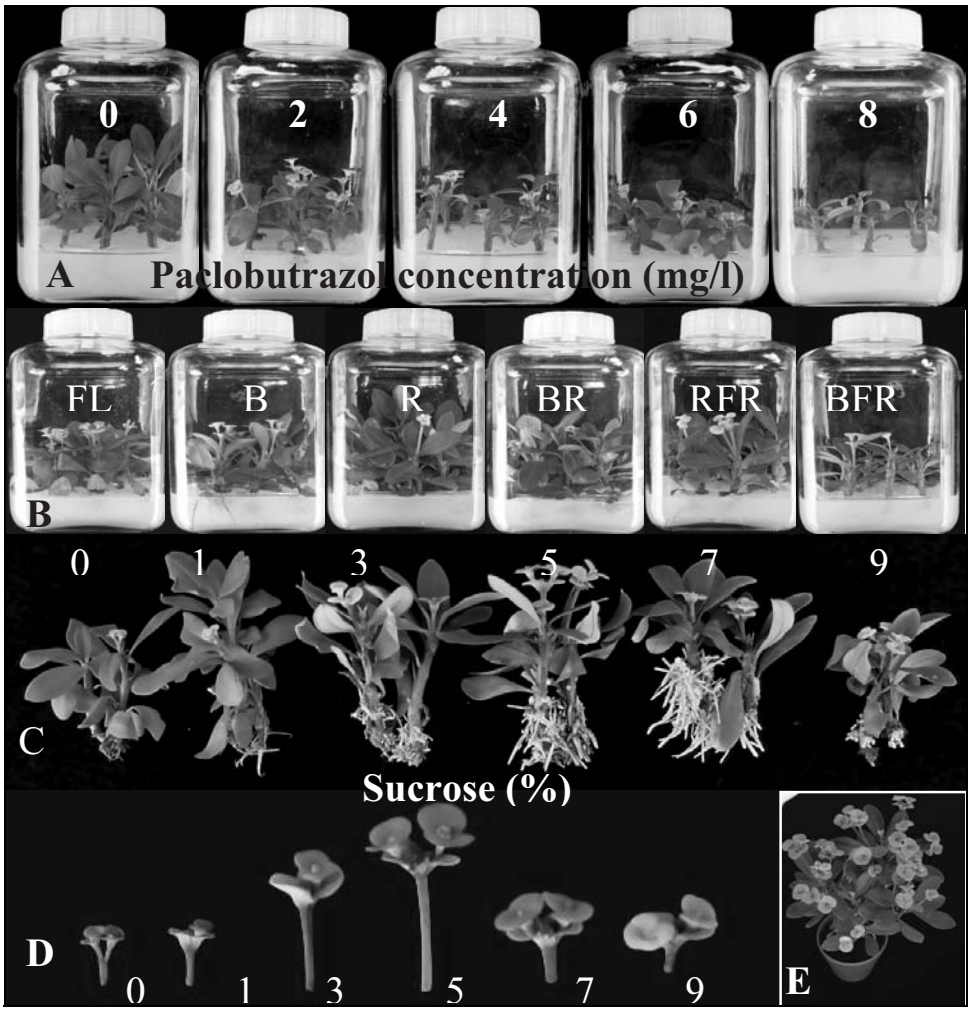


Fig. 1. *In vitro* flowering of *Euphorbia millii*. A. Effect of paclobutrazol, B. Effect of LEDs (FL: Fluorescent, B: blue, R: Red, BR: Blue plus Red, RFR: Red plus Far-red, BFR: Blue plus Far-red), C. Effect of sucrose on *in vitro* flowering in *E. millii* after 8-weeks of culture. D. Effect of sucrose on inflorescence development. E. Acclimatized plantlet in greenhouse.

flowering suggests that sugar levels may play role in regulating floral induction, and that their movement to the SAM is not simply a response to increasing metabolic activity (BERNIER et al. 1993). Recent findings show that *Arabidopsis* can be induced to flower in the dark simply by manipulating the sucrose levels (ROLDAN et al. 1999). The mechanisms by which sugars act to influence plant development and gene expression are just beginning to be deciphered. Understanding of sugar response is complicated by the fact that plants have multiple sugar response pathways and that the molecules actually being sensed are not known in all cases. In addition, many sugar responses may actually be regulated by alterations in sugar flux or in C:N ratios rather than by absolute sugar or sugar-metabolite levels (CORUZZI and BUSH 2001; CORUZZI and ZHOU 2001). Sugar response pathways also 'interact' or exhibit 'cross-talk' with numerous other pathways, including those for phytohormone and light responses (LEON and SHEEN 2003; ELLIS et al. 2002).

Flowering plantlets after being harvested from the culture vessels were acclimatized successfully (100 %) and transferred to greenhouse (Fig. 1E).

In general, our results indicate that growth retardants, light quality and sucrose significantly affected the growth and *in vitro* flowering. Further experiments could lead to a better understanding of the reproductive biology of *E. millii*. Furthermore, the technique described here can be used as an efficient method for production of early, synchronized and profuse flowering *E. millii* pot plants.

Acknowledgement

This work was supported by grants from Korea Science and Engineering Foundation (KOSEF) to Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Korea. One of the authors (DC) also wishes to acknowledge the KOSEF for providing financial assistance in the form of "Long-term Foreign Scientist Fellowship".

References

AKOYUNOGLOU, G. and H. ANNI 1984: Blue light effect on chloroplast development in higher plants. In: SENGER, H. (ed.): Blue light effects in biological systems. Berlin, Springer-Verlag, 397–406.

BERNIER, G., A. HAVELANGE, C. HOUSSA, A. PETITJEAN and P. LEJEUNE 1993: Physiological signals that induce flowering. Plant Cell **5**, 1147–1155.

BRIGGS, W.R. and E. HUALA 1999: Blue-light photoreceptors in higher plants. Annu. Rev. Cell Dev. Biol. **15**, 33–62.

CARSON, J.A. and D.W.M. LEUNG 1994: *In vitro* flowering and propagation of *Leptinella nana* L., an endangered plant. New Zealand J. of Botany **32**, 79–83.

COMPTON, M.E. and R.E. VIELLEUX 1992: Thin cell layer morphogenesis. Hort. Rev. **14**, 239–264.

CORBESIER, L., P. LEJEUNE and G. BERNIER 1998: The role of carbohydrates in the induction of flowering in *Arabidopsis thaliana*: comparison between the wild-type and a starchless mutant. Planta **206**, 131–137.

CORUZZI, G. and D.R. BUSH 2001: Nitrogen and carbon nutrient and metabolite signaling in plants. Plant Physiol. **125**, 61–64.

CORUZZI, G.M. and L. ZHOU 2001: Carbon and nitrogen sensing and signaling in plants: emerging 'matrix effects'. Curr. Opin. Plant Biol. **4**, 247–253.

DEWIR, Y.H., D. CHAKRABARTY, E.J. HAHN and K.Y. PAEK 2005: Reversion of inflorescence development in *Euphorbia millii* and its application to large scale micropropagation in an air-lift bioreactor. J. Hort. Sci. & Biotech. **80**, 581-587.

DICKENS, C.W.S. and J. VAN STADEN 1988: The *in vitro* flowering of *Kalanchoe blossfeldiana* Poellniz. (1) Role of culture conditions and nutrients. J. Exp. Bot. **39**, 461–471.

ELLIS, C., J.G. TURNER and A. DEVOTO 2002: Protein complexes mediate signalling in plant responses to hormones, light, sucrose and pathogens. Plant Mol. Biol. **50**, 971–980.

GIBSON, S.I. 2005: Control of plant development and gene expression by sugar signalling. Curr. Opi. Plant Biol. **8**, 93–102.

HACKETT, W.P. 1985: Juvenility, maturation, and rejuvenation in woody plants. Hort. Rev. **7**, 109–155.

HEO, J.W., C.W. LEE, D. CHAKRABARTY and K.Y. PAEK 2002: Growth response of marigold and salvia bedding plants as affected by monochromic or mixture radiation provided by a light emitting diode (LEDs). Plant Growth Regul. **38**, 225–230.

HUGHES, K.W. 1981: *In vitro* ecology: exogenous factors effecting growth and morphogenesis in plant culture system. Environ. Exp. Bot. **21**, 281–288.

JOHN, C.K. and R.S. NADGAUDA 1999: *In vitro*-induced flowering in bamboos. *In vitro* Cell. & Dev. Bio. Plant. **35**, 309–315.

JUMIN, H.B. and N. NITO 1996: *In vitro* flowering of orange jessamine, (*Murraya paniculata* (L.) Jack). Experientia **52**, 268–272.

PRUSAKOVA, L.D., S.I. CHIZHOVA and V.V. PAVLOVA 2004: Assessment of triazole growth-retarding activity in an α-amylase bioassay using spring barley endosperm. Russ. J. Plant Physiol. **51**, 563–567.

LEON, P. and J. SHEEN 2003: Sugar and hormone connections. Trends Plant Sci. **8**, 110–116.

LICHTENTHALER, H.K. 1987: Chlorophyll and carotenoides: Pigments of photosynthetic biomembranes. Methods in Enzymology **148**, 350–382.

MURASHIGE, T. and F. SKOOG 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473–497.

NAOR, V., J. KIGEL and M. ZIV 2004: Hormonal control of inflorescence development in plantlets of calla lily (*Zantedeschia* spp.) grown *in vitro*. Plant Growth Regulation **42**, 7–14.

PIDKOWICH, M.S., J.E. KLENZ and G.W. HAUGHN 1999: The making of flower: control of floral meristem identity in Arabidopsis. Trends in Plant Sci. **4**, 64–70.

ROLDAN, M., C. GOMEZ-MENA, L. RUIZ-GARCIA, J. SALINAS and J.M. MARTINEZ-ZAPATER 1999: Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering in *Arabidopsis* in the dark, Plant J. **20**, 581–590.

SAEBO, A., T. KREKLING and M. APPELGREN 1995: Light quality affects photosynthesis and leaf anatomy of brich plantlets *in vitro*. Plant Cell, Tissue and Organ Culture **41**, 177–185.

SENGER, H. 1982: The effect of blue light on plants and microorganisms. Phytochem. Photobiol. **35**, 911–920.

SILVA, J.A.T. and D. TAN NHUT 2003: Thin cell layers and floral morphogenesis, floral genetics and *in vitro* flowering. In: TAN NHUT, D., B. VAN LE, K. TRAN THANH VAN and T. THORPE (eds): Thin cell layer culture system: Regeneration and transformation application. Kluwer Academic Publishers, Dordrecht, Boston, London, 285–342.

TANIMOTO, S. and H. HARADA 1981: Chemical factors controlling floral bud formation of *Torenia* stem segments cultured *in vitro*, (1) Effects of minerals, nutrients and sugars. Plant Cell Physiol. **22**, 533–541.

TRAN THAN VAN, M. 1973: Direct flower neoformation from superficial tissue or small explants of *Nicotiana tabacum* L.. Planta **115**, 87–92.

Received March 04, 2005 / Accepted January 04, 2006

Addresses of authors: Y. H. Dewir[1 2)], D. Chakrabarty[1 3)], E. J. Hahn[1)] and K.Y. Paek[1)],[1)]Research Center for The Development of Advanced Horticultural Technology; Chungbuk National University, Cheong-ju 361-763, Korea, e-mail: paekky@chungbuk.ac.kr, [2)]Department of Horticulture, Faculty of Agriculture, Tanta University, Kafr El-Sheikh 33516, Egypt, and [3)]Floriculture Section, National Botanical Research Institute, Lucknow, India, e-mail: chakrabartyd@hotmail.com.