The Regulation of Anthocyanin Biosynthesis in *Eustoma grandiflorum* under Low Light Conditions

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Summary

We investigated the influence of low light intensity on the pigmentation of petals in potted lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.]. When whole plants were placed under 1,000 lx, the petals at anthesis had lighter petal than control flowers exposed to 10,000–15,000 lx. Shading flower buds during the opening process had no effect on petal color intensity. However, shading the stem and leaf parts significantly reduced the intensity of petal color. When flower buds were detached with peduncles and placed in sucrose solutions, their petal color became more intense as the sucrose concentration was increased. There was a positive correlation between anthocyanin and soluble sugar content in the petals, regardless of light conditions. In detached flowers, the transcript levels of chalcone synthase, chalcone isomerase, and dihydroflavonol 4-reductase were enhanced as the sucrose concentration increased. These results suggest that low light intensity affects petal pigmentation through reduced photosynthesis in the leaves or stems, which, in turn, reduces the soluble sugar content of petals and leads to a repression of the genes that encode enzymes of anthocyanin biosynthetic pathway.

Key Words: anthocyanin biosynthesis, light, sugar, *Eustoma grandiflorum*.

Introduction

Light intensity is an important environmental factor, which affects the pigmentation of petals in many flowers. In lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.], low light conditions during the flowering process markedly reduced the color intensity of the petals (Griesbach, 1992; Halevy and Kofranek, 1984). A 75 % decrease in light intensity was related to a 40 % reduction in color intensity (Griesbach, 1992). The HPLC analysis showed that the total amount of flavonols and the composition of anthocyanins were not affected by light conditions, but the total anthocyanins were smaller in the flowers that opened under low light conditions (Griesbach, 1992).

Cut lisianthus flowers are usually marketed when an inflorescence consists of both open flowers and buds. While open flowers at harvest will keep their quality for about one week, buds will not develop well under room conditions. Some of these buds may open in a few days, but their petals are usually very thin and faded in color, making them unattractive.

The effect of light on anthocyanin biosynthesis has been studied in many plants. In general, the effect of light was attributed to a requirement for a photomorphogenic signal, mediated by photoreceptors (Mancinelli, 1983, 1985) or to a shortage of carbohydrates

caused by restricted photosynthesis under low light conditions (Biran and Halevy, 1974). The requirement of carbohydrates was demonstrated in cut flowers of petunia (Weiss and Halevy, 1989, 1991) and *Antirrhinum* (Sang et al., 1991), by exogenously supplying sucrose to promote anthocyanin production. Carbohydrates can play two roles in anthocyanin biosynthesis: firstly, as a substrate for flavonoid biosynthesis via shikimic acid and phenylpropanoid pathways, and secondly as an inducer of genes involved in anthocyanin biosynthesis (Murray et al., 1994). Anthocyanin biosynthesis involves several enzymes, including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), and dihydroflavonol 4-reductase (DFR). Exogenous sucrose promoted the expression of CHS in petunia (Moalem-Beno et al., 1997), tea (Takeuchi et al., 1994), and CHS and DFR in ivy (Murray et al., 1994).

The objective of this study was to determine whether the effect of light on anthocyanin production was attributable to a direct effect on flower petals through photoreceptors or to an effect on leaves or stems through altered photosynthesis. In addition, we investigated the accumulation of transcripts for CHS, CHI, and DFR to examine whether the anthocyanin production is controlled by light conditions at the transcription level.

Materials and Methods

1. Plant materials

Seeds of *Eustoma grandiflorum* 'Royal Purple' (Takii Seed Co, Kyoto) were germinated in peat moss at 20 °C under natural light. After 2 and 4 months, plants were transplanted to 5 cm and 10 cm pots, respectively, and grown at 25/20 °C (day/night). Potting medium was a 4:6 mixture of peatmoss and 'Kureha Engei Baido' (Kureha), a heat-processed granulated soil with N : P : K = 0.4 : 1.4 : 0.4 g · kg⁻¹.

Developmental stages of *lisianthus* flowers are defined as follows: stage 1, the growth rate of flower petals is slow; stage 2, (3–5 days before anthesis), flowers enlarge but without pigmentation; stage 3, (2–1 day before anthesis), the growth of the petals accelerates and pigmentation starts from the edge of the petals; and stage 4, anthesis, when the petals are fully expanded and pigmented. Before the experiments, all flowers at stages 3 and 4 on the inflorescence were removed and only those in stage 2 were used.

2. Experiment 1: Effects of light intensity on petal pigmentation

Potted plants were transferred to a growth room at 23 °C and a 14-hr photoperiod; light (15,000 lx) was supplied by metal halide lamps (Yoko lamp, Toshiba). Six plants were placed in a frame, the sides of which were covered with 2 layers of black cheese cloth and topped with aluminum foil. The light intensity inside the frame was set at 1,000 lx by adjusting the distance from the lamps to the plants. Another six plants used for control were not shaded. At anthesis, the fresh weight of flowers, color intensity, and anthocyanin content of the petals were measured.

3. Experiment 2: Effects of shading plant parts on pigmentation

Potted plants were transferred to a growth room kept at 25 °C, 14-h photoperiod, 10,000 lx, and divided into three groups. In group 1, whole plants were shaded as in Experiment 1. In group 2, flowers at the end of stage 2 were selected and their parts were enclosed in a box covered with aluminum foil on top and 2 layers of black cheese cloth on the sides and bottom. In group 3, the stems and leaves were shaded with 2 layers of black cheese cloth along the entire stem length, and with aluminum foil just below the calyx. For each group, a comparable group of unshaded plants was prepared as control. The light intensity around shaded parts of the plants in each treatment was 1,000 lx. At anthesis, fresh weight of the flowers and color intensity of the petals from 6 to 13 plants were measured.

4. Experiment 3: Effects of light and sucrose on petal pigmentation in detached flowers

Flowers at the end of stage 2 with peduncles of about 5 cm long were detached from the plants using a sharp blade. Groups of 4 to 7 detached flowers were placed in vials containing 0, 0.125, 0.25, or 0.50 M sucrose solutions and shaded (700~1,000 lx); a comparable group of flowers was placed in sucrose solutions and exposed to 15,000 lx in the growth room as in Experiment 1. After the flowers were sampled at anthesis and their fresh weight of the flowers and color intensity of the petals were measured, the petals were frozen in liquid nitrogen, and stored at -80 °C until used for northern blot analysis. Another group of detached flowers was placed in vials containing 0.25 M sucrose solution and shaded (700~1,000 lx) or kept in sucrose solutions but under 15,000 lx. At anthesis, 11 and 6 flowers were sampled and analyzed, respectively, for anthocyanin and soluble sugar contents.

5. Measurements of color intensity

Pigmentation of flower petals was evaluated using scores between 0 and 255. Graphical gray scale images of petals were scanned with a scanner (Epson GT6000), and the average color intensity of 0.5 to 2 cm² petal was calculated using 'NIH Image'.

For anthocyanin analysis, 1 cm² sections were excised from petals and steeped in 1 ml of acidic methanol containing 1% HCl at 4 °C for 12 to 24 hours. After diluting the solution to a known volume with the acidic methanol, its absorbance at 510 nm was measured. The optical density of the original 1 ml solution was calculated as the relative anthocyanin content.

6. Carbohydrate analysis

The samples were freeze-dried, ground into a fine powder in a mortar and pestle and extracted with 80 % ethanol at 80 °C for 1.5 hour. The extract was filtered through a Whatmann GF/F filter, dried in a rotary evaporator at 45 °C; the residue was dissolved in 2 ml distilled water and the solution was centrifuged at 15,000 x g for 10 min. The supernatant was loaded on a Cosmosil 5NH₂ (Nakarai) column and eluted with 80 % acetonitrile-water (80 : 20) at the rate of 1 ml · min⁻¹ at 33 °C. The eluates were analyzed for carbohydrates using a Shimadzu 10A HPLC system equipped with a refractive index detector (RI-2, Japan Analytical Industry).

7. Preparation of total RNA

Petal tissue was ground into a fine powder under liquid nitrogen and suspended in an extraction buffer (100mM Tris-HCl, pH 8.5, 200 mM NaCl, 10 mM EDTA, 1 % w/v SDS). The suspension was extracted twice with an equal volume of phenol-chloroform (1 : 1 v/v) and once with chloroform, followed by propanol

Table 1. Nucleotide sequences of the specific primers used for amplifying chalcone synthase (CHS), chalcone isomerase (CHI), and dihydroflavonol 4-reductase (DFR) genes.

Gene	Fragment length (bp)	Sequences of primers
CHS	164	5'-AARGARTGGGGNCARCCNAA-3' 5'-GTNCCNCCNGCRAARCANCC-3'
CHI	194	5'-TTYCTYGNYYGGKGCNCG-3' 5'-GTRAAAYTTYTCRAANGGNCC-3'
DFR	284	5'-ACMGTNTGYGTACACNCG-3' 5'-TTDATNAYTTCRTTCTCAGG-3'

N: A, C, G, T; D: A, G, T; R: A, G; Y: C, T; M: A, C; K: G, T.

precipitation. The sample was subjected to selective LiCl precipitation. After ethanol precipitation, the pellet containing RNAs was dissolved in sterile water.

8. Isolation of partial cDNAs for CHS, CHI, and DFR

For the isolation of cDNAs for CHS, CHI, and DFR assays, RNA was extracted from partially pigmented petals of lisianthus. The total RNA was subjected to reverse transcription followed by polymerase chain reaction (PCR), using RNA PCR kit (Perkin Elmer Cetus). The degenerate primers used are listed in Table 1. The amplified fragments were separated on agarose gels by electrophoresis and recovered from the gels by using Gene Clean II (Bio101). The purified fragments were cloned into pCRII (Invitrogen) or pBlue-scriptIISK+ (Stratagene) for sequence analysis and preparation of hybridization probes. The sequences of the clones were determined on A.L.F. DNA sequencer (Pharmacia) by a dye primer method using T7 DNA polymerase according to the manufacturer's instruction.

9. Northern blot analysis

mRNA was purified from the total RNA by using Oligotex-dT30 Super (Takara). One μ g of mRNA was separated on 1.2% agarose gels, containing formaldehyde (Maniatis et al., 1982). Northern blot analyses were performed on nylon membrane (MagnaGraph, Funakoshi) with probes labeled with digoxigenin (Boehringer Mannheim) by PCR labeling. Hybridizations and signal detections were performed by using DIG Nucleic Acid Detection kit (Boehringer Mannheim), according to the manufacturer's instructions.

Results

1. Experiment 1: Effects of light intensity on petal pigmentation

Pigmentation of lisianthus flowers starts 2 to 3 days before anthesis and is completed at anthesis. While investigating the effects of light on pigmentation, we found that plants with flowers at stage 2 exposed to 15,000 lx or 1,000 lx took 2 to 3 days and 3 to 6 days for the flowers to open, respectively. The pigmentation, whether measured by color intensity or anthocyanin

Table 2. Effects of light intensity on flower fresh weight and petal pigmentation in *E. grandiflorum*.

Condition	Fresh weight (g)	Color intensity ^z	Anthocyanin content ($A_{510} \cdot \text{cm}^{-2}$ petal)
Control	2.14	143	1.15
Shaded	1.90 ^{n.s.}	97***	0.47**

Potted plants with flowers at stage 2 (3–5 days before anthesis; without pigmentation) were placed at 15,000 lx (control) or at 1,000 lx (shaded) until the flowers opened.

^z Images converted to gray scale, 0: white, 255: black.

^{n.s.}, **, *** Nonsignificant or significant at $P = 0.01$ or 0.001 by t -test, respectively. $n=6$.

Fig. 1. A scatter plot showing a correlation between anthocyanin content and petal color intensity measured with a scanner in petals of lisianthus flowers which opened under 1,000 lx (●) or 15,000 lx (○). Graphical gray scale images were scanned and the average tone of the images was expressed as scores between 0 (white) and 255 (black). ** Significant at $P = 0.01$ by F -test.

content, was lower in shaded flowers than it was in unshaded ones (Table 2).

In this study, color intensity was measured with a scanner because it gave a good estimate (gray scale scores) of the petal color as unpigmented petals were very pale yellow to greenish white. Hence, the color intensity measured by a scanner was positively corre-

Table 3. Effects of shading whole plants, flowers, or stem and leaf parts on flower fresh weight and petal pigmentation in *E. grandiflorum*.

Shaded part of the plants	Fresh weight (g)		Color intensity ^z	
	Control	Shaded	Control	Shaded
Whole plants	1.70	1.41*	136	96***
Flowers	1.55	1.45 ^{n.s.}	133	129 ^{n.s.}
Stems and leaves	1.55	1.35**	132	87***

Potted plants with flowers at stage 2 were placed at 10,000 lx with or without shading until the flowers opened. Light intensity at shaded parts was 1,000 lx.

^z Images converted to gray scale, 0: white, 255: black.

^{n.s.}, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 by t -test, respectively. $n = 6$ to 13 .

lated ($r=0.753$) with anthocyanin content (Fig.1).

2. Experiment 2: Effects of shading plant parts on pigmentation

When flower, leaf and stem parts, or whole plants were shaded to see how plants respond to low light intensity with respect to pigmentation of petals, the results varied. When only flower parts were shaded, the flowers showed the same degree of growth and petal pigmentation as did unshaded flowers (Table 3), whereas when stems and leaves were shaded, growth and petal pigmentation of flowers were reduced. These results indicate that low light intensity reduced the petal pigmentation through an effect on stems or leaves but not through that on flowers.

3. Experiment 3: Effects of light and sucrose on petal pigmentation in detached flowers

Experiment with potted plants suggested that under low light intensity, an insufficient supply of photosynthates to flowers was responsible for the reduced level of anthocyanins.

To test this hypothesis, unopened flowers with their peduncles at the end of stage 2 were detached and immediately placed in various concentrations of sucrose and kept under 15,000 or 1,000 lx. These detached flowers opened 3 to 6 days after the beginning of the

incubation. As sucrose concentration was increased from 0 to 0.5 M, the flower color became darker at both 15,000 lx and 1,000 lx (Table 4). Analysis of variance showed that the effects of sucrose and shading on color intensity were significant at 0.1 % and 1 % level, respec-

Fig. 2. A scatter plot showing a correlation between anthocyanin and soluble sugar contents in petals of lisianthus flowers with peduncles placed in 0.25 M sucrose solution at 1,000 lx (●) or 15,000 lx (○) for 4 to 6 days.

*** Significant at $P = 0.001$ by F -test.

Table 4. Effects of exogenous sucrose on flower fresh weight and petal pigmentation in *E. grandiflorum*.

Sucrose Concentration (M)	Fresh weight (g)		Color intensity ^z	
	Control	Shaded	Control	Shaded
0	1.46a	1.46a	73c	69c ^y
0.125	1.38a	1.39a	104b	79c
0.25	1.53a	1.53a	120ab	108b
0.5	1.67a	1.65a	131a	120ab

Flowers at stage 2 were detached from plants and placed in sucrose solutions of various concentrations at 15,000 lx (control) or 1,000 lx (shaded) until the flowers opened.

^z Images converted to gray scale, 0: white, 255: black.

^y Means followed by the same letters within control and shaded columns are not significantly different at $P = 0.05$ by Duncan's new multiple range test. $n = 4$ to 7 .

CHS

	10	20	30	40	50	60
<i>E. grandiflorum</i>	AAGGAGTGGG	GCCAACCCAA	GTCTAAGATC	ACCCATCTAG	TCTTCTGCAC	CACTAGTGGT
<i>A. majus</i>	..A.....	....G....	A..C..A..	.....CT.G.	....T....	...C..C...
<i>P. hybrida</i>	.....A....	....G....	...C..A..T	.....T.GT	....T....	A.....

	70	80	90	100	110	120
<i>E. grandiflorum</i>	GTGGACATGC	CTGGTGTGA	CTATCAGCTC	ACCAAGCTCC	TGGGCCTTCG	GTCCTCGGTT
<i>A. majus</i>	..C.....	.C..G..A..	T.....	....A....	.C....A..	CC.T..C..C
<i>P. hybrida</i>	.....	....GTG..	.....A...	..T....A.	.T..G....	TC.A....C

	130	140	150	160	164
<i>E. grandiflorum</i>	AAGCGGTTCA	TGATGTACCA	ACAGGGTTGC	TTTGCGGGTG	GGAC
<i>A. majus</i>	..A..T....	.....	.....	....T....	.T..
<i>P. hybrida</i>	...A..C...	.....	...A.....	.....T....	.C..

CHI

	10	20	30	40	50	60
<i>E. grandiflorum</i>	TTCCTCGGTG	GCGCTGGGGT	GAGGGGACTG	GAAATCGGAG	GAAATTTCTG	GAAGTTCACG
<i>A. majus</i>	.....T..A.....	...A..GG.T	..G..CA..	.G..G...A.	C.....T	
<i>P. hybrida</i>	.....T.C..	.T.....CA	T..A..T...	..G..A.A..	.G..G..T..	T....T...

	70	80	90	100	110	120
<i>E. grandiflorum</i>	GCGATCGGTA	TTTACTTGGA	CGAGACTGCA	ATTCCGTGGC	TCGCCGGCAA	GTGGAAAGGC
<i>A. majus</i>	..C..T.C.G	.C...A.A..	A..GAC..C	G.CT.T.C..	.T...TT..	.....G...
<i>P. hybrida</i>	.....A..G	.G..TC.A..	A...G...T	.....T.TT.	.G....AA..	A.....

	130	140	150	160	170	180
<i>E. grandiflorum</i>	AAGACGGCCG	AGGAGTTGGC	TGATTCCGTT	GACTTCTTCG	CCGATATCAT	CACTGGTCCA
<i>A. majus</i>	.....C....	.....C.CA.	.....T..C	.....A	AA..C..TG.	C
<i>P. hybrida</i>	..A..CC..C	.....A.	...C..G..C	..G....TA	GG...G.TG.	T..A.....

	190	194
<i>E. grandiflorum</i>	TTTGAGAAGT	TCAC
<i>A. majus</i>	.....	....
<i>P. hybrida</i>	.....A.	.T..

DFR

	10	20	30	40	50	60
<i>E. grandiflorum</i>	ACCGTGTGTG	TCACAGGAGC	TGCAGGATAC	ATTGGATCAT	GGCTAGTCAT	GAGACTGCTT
<i>A. majus</i>	..A..T..C.	....T....	...T....TT	.....C..T.	....T....	.....C...
<i>P. hybrida</i>	.....A....	.....	...C..C.T.	..A..C....	....T....	...G..T..C

	70	80	90	100	110	120
<i>E. grandiflorum</i>	GAACGCGGCT	ACACTGTTTCG	TGCCACTGTC	CGTGATCCTG	GTGATGTAAA	GAAGGTGAAG
<i>A. majus</i>	.....T..	...A....A	C..T....T	.....	AGA.CAAG..	.....A
<i>P. hybrida</i>	.....T....	....C....	...A....T	.....	..A..A.G..	.....A..A

	130	140	150	160	170	180
<i>E. grandiflorum</i>	CATCTATTTG	AGTTGCCAAA	GGCCAGCACA	AATTTGACAG	TGTTAAAGC	AGACTTGATT
<i>A. MAJUS</i>	....GC.G.	.AC.....	...TGAT..G	..C..A...C	....G....	G.....CA
<i>P. hybrida</i>	..C...A.A.	.AC.....	A..AGA...G	..C.....T	..GG..G..	....A...CA

	190	200	210	220	230	240
<i>E. grandiflorum</i>	GAAGAAGGAA	GCTTTGATGA	AGCCATTCAA	GGCTGTCATG	GAGTGTTTCA	TATGGCTACA
<i>A. majus</i>	.T.....	.....C..	G.....	.....A.	....A....	.G.A..A...
<i>P. hybrida</i>	.T.....	....C..C..	...A.....	..T..CG.A.	.....	CT...C..G

	250	260	270	280	284
<i>E. grandiflorum</i>	CCTATGGAAT	TCGAATCCAA	AGACCCTGAG	AATGAAATGA	TCAA
<i>A. majus</i>	.....T.	....G....	.....	....G.A.	....
<i>P. hybrida</i>	T.....	.T..T...GT	G..T.....	....G...	....

Fig. 3. Nucleotide sequences of CHS, CHI, and DFR cDNA fragments isolated from partially pigmented petals of *E. grandiflorum*. The sequences are compared with *Antirrhinum majus* (CHS: Sommer and Saedler, 1986; CHI: Martin et al., 1991; DFR: Beld et al., 1989) and *Petunia hybrida* (CHS: Koes et al., 1989b; CHI: van Tunen et al., 1988; DFR: Beld et al., 1989). Dots denote identity to the *E. grandiflorum* sequences.

Fig. 4. Gel plates (northern-blot analysis) of CHS, CHI, and DFR mRNAs derived from detached flowers of *E. grandiflorum*. Flowers with peduncles were placed in 0, 0.125, 0.25, or 0.5 M sucrose solution (Suc) under 1,000 lx(D) or 15,000 lx(L) for 3 to 6 days until the anthesis. The cDNA clone used as a probe in hybridization is indicated at the left.

tively. When the detached flowers were incubated in 0.5 M sucrose solutions at high light intensity, the petal color was as dark as that of the intact flowers that opened under high light intensity.

Unlike attached flowers, low light intensity significantly decreased flower color in detached flowers (Table 4). However, carbohydrate analysis showed that contents of total soluble sugars in the petals were lower in unshaded flowers; hence, a positive correlation was found between the sucrose and anthocyanins contents (Fig. 2), suggesting that the effect of light on petal pigmentation in detached flowers was through reduced sucrose uptake.

4. Transcriptional control of anthocyanin synthesis

Using degenerate primers listed in Table 1, partial cDNAs for CHS, CHI, and DFR were successfully amplified. The amplified fragments were cloned into plasmids and identified by determining the nucleotide sequences (Fig. 3). Using the isolated clones as probes, northern blot analysis was performed for detached flowers in Experiment 3.

At both 15,000 lx and 1,000 lx, the levels of the transcripts of CHS, CHI, and DFR increased as the concentration of sucrose was elevated in the culture solution (Fig. 4); they were also higher in plants exposed to high light intensity.

Discussion

The light condition affects anthocyanin content in petals through photoreceptor mediated reaction or photosynthesis which provides necessary carbohydrates for anthocyanin biosynthesis. In studies on 'Masquerade' roses, the effects of light on pigmentation were attributed to the effects on petals (Shisa and Takano, 1964), but on leaves on 'Baccara' roses (Biran and Halvey, 1974). In petunia (Moscovici et al., 1996), pigmentation was reduced by shading flowers or a leaf, suggesting that photoreceptors in the petals as well as the carbohydrate supply participated in the control of pigmentation. In our study of lisianthus (Table 3), shading entire plants had no effects on pigmentation of petals, whereas shading leaves and stems markedly reduced it. The results indicate that reduced pigmentation under low light intensity occurs through an effect on the leaves or stems but not through that on petals.

The experiments on detached flowers demonstrated a promotive effect of sucrose and high light intensity on pigmentation. The light effect was attributable to the higher content of soluble sugars in the petals rather than to the light intensity. The degree of pigmentation was positively correlated with the soluble sugar content independent of light conditions. Therefore, the degree of pigmentation seems mainly dependent on the soluble sugar content in the petals, and that restricted export of photosynthates from the leaves or stems under low light conditions is most responsible for the reduced pigmentation in lisianthus.

With detached flowers, irradiation with high light intensity significantly increased the soluble sugar content in the petals (Fig. 2). That the uptake of sucrose by detached corolla of petunia was enhanced by high light intensity was reported by Moscovici et al. (1996). In *Antirrhinum*, optimum sucrose concentration in the vase solution for improving the pigmentation was lower for flowers placed at high light intensity (Sang et al., 1991), indicating that the uptake of sucrose was more efficient at high light intensity. Regarding these results, it appears that the sink activity of rose flowers was promoted by irradiation of shoots (Mor and Halevy, 1980). Lang and Thorpe (1986) and Pickard et al. (1979) proposed that carbohydrate translocation depends on water potential gradient between the source and the sink organs. Similarly, Lee (1989) and Swanson et al. (1976) postulated that transpiration in the sink organ was the driving force behind carbohydrate mass flow into the sink. Because high light intensity probably enhances the transpiration from the irradiated tissues (Swanson et al., 1976), while simultaneously increasing soluble sugar content, both processes enhanced transpiration-driven sucrose accumulation by the flowers.

Moscovici et al. (1996) reported that corolla growth and anthocyanin biosynthesis in petunia were in response to light on green leaves or sepals. They proposed

that an unknown metabolite produced in leaves or stems exposed to light was transmitted to flowers and participated in the regulation of anthocyanin biosynthesis. In our study, however, flowers separated from leaves and stems became equally pigmented as attached flowers under high light conditions as long as the flowers were supplied with 0.5M sucrose. Therefore, the carbohydrate supply seems sufficient for pigmentation and that other metabolites may not be involved in pigment formation in *lisianthus*. Moreover, shading of intact flowers with sepals had no effect on pigmentation, suggesting that light perceived by the sepals was not involved in this process.

CHS, CHI, and DFR cDNA clones which we isolated to investigate the expression patterns in relation to anthocyanin production revealed that the genes were all dependent on the concentration of exogenous sucrose. The transcript levels for these enzymes were also correlated with anthocyanin accumulation in *petunia* (van Tunen et al., 1988; Beld et al., 1989; Koes et al., 1989a) and *Antirrhinum* (Martin et al., 1991; Jackson et al., 1992).

Of these three enzymes, CHS and CHI are involved in biosynthesis of both anthocyanin and flavonol, whereas DFR is specifically involved in anthocyanin biosynthesis. CHS and CHI provide dihydroflavonol, the common substrate for both DFR and flavanol synthase (FLS), which catalyze the production of flavonol. Griesbach (1992) showed that low light conditions affected anthocyanin accumulation without any influence on flavonol accumulation; this indicates that the production of dihydroflavonol through CHS and CHI under low light conditions was sufficient for FLS activity. In *lisianthus*, anthocyanin accumulation in relation to flower development was correlated with the transcript level of DFR, but not with PAL, CHS, and CHI transcripts (Davies et al., 1993). In this regard, the activity of DFR or other enzymes in the later steps of the pathway, instead of CHS or CHI, may play a key role in the control of anthocyanin biosynthesis in *lisianthus*.

In conclusion, our results suggest that the pale color of petals in flowers under low light conditions is caused by the restricted supply of carbohydrates from leaves or stems, which in turn lowers soluble sugar content in the petals. This reduced soluble sugar content caused the repression of genes in the anthocyanin biosynthetic pathway. Hence, petal pigmentation of *lisianthus* flowers under low light conditions can be improved either by increasing carbohydrate content in the leaves or stems before harvest, or by genetically transforming the plants so that genes responsible for anthocyanin biosynthesis become independent of soluble sugar content in the petals.

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低照度環境におけるトルコギキョウのアントシアニン合成の調節

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摘 要

低照度条件が鉢植えトルコギキョウの花弁着色に及ぼす影響について調査した。植物体全体を遮光すると(1,000 lx), 遮光環境下で開花した花の花色が薄くなった。開花時に花のみを遮光した場合には花色に影響は見られなかったが、茎葉部のみを遮光すると、植物体全体の遮光時と同じく花色が薄くなった。花を花柄部で切り離しスクロース溶液に浸漬させた状態で開花させると、スクロース濃度の上昇とともに花弁の色は濃くなった。さらに、花弁の糖濃度とアントシアニン濃

度との間には、光条件に関係なく正の相関がみられた。これらの花では、スクロース濃度の上昇にともなってカルコン合成酵素、カルコン異性化酵素、ジヒドロフラボノール4-還元酵素の遺伝子の発現量が促進された。以上の結果より、低照度環境下でトルコギキョウ花弁の着色が低下するのは、茎葉部における光合成の低下にともなう花弁中の可溶性糖含量の低下によって、アントシアニン合成経路上の酵素の発現が抑制されるためであることが示唆された。