Light-Induced Anthocyanin Pigmentation in Transgenic *Lc* Petunia

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Abstract

Introduction of *Leaf colour* (*Lc*), a bHLH transcription factor from maize, under the control of the CaMV35S promoter into petunia (cv. Mitchell) plants resulted in enhanced anthocyanin pigmentation in vegetative tissues. Anthocyanin biosynthesis was observed to be dependent on the level of light the plants were grown under: plants grown in a plastic greenhouse remained green, while plants exposed to high-light were dark purple. The nature of this response to light and the associated molecular mechanisms were the focus of this investigation.

Molecular analysis of gene expression in Mitchell petunia showed that light induced the expression of the early flavonoid structural genes, as well as flavonol synthase (*FLS*) required for flavonol production. Light induced both the early and late structural genes required for anthocyanin biosynthesis in the transgenic *Lc* Mitchell petunia plants, but reduced the expression of *FLS*. Light-induced flavonoid gene expression was examined under three light treatments: shade (50 - 350 μmol m^{-2} sec^{-1}); ambient-greenhouse (300 - 750 μmol m^{-2} sec^{-1}) and high-light (750 μmol m^{-2} sec^{-1}). The level of flavonoid gene expression was dependent upon light intensity. High-light was required to maximally activate anthocyanin pigmentation in *Lc* petunia. Expression of the *Lc* transgene remained unchanged irrespective of light intensity, indicating that the light-induced changes in anthocyanin synthesis were not due to variable expression of the transgene.

Anthocyanin regulation occurs primarily at the transcriptional level, and two classes of transcription factors, Myb and bHLH, are generally involved. Transient expression studies using several exogenous Myb transcription were carried out using shade-grown (non-induced) *Lc* petunia material. The induction of coloured cells in the treated tissue supports the idea that the bHLH transgene (LC) is interacting with an endogenous Myb under high-light conditions, resulting in the activation of the flavonoid biosynthetic pathway and accumulation of anthocyanin pigments. A partial sequence of a candidate endogenous Myb transcription factor from petunia was cloned. It was light-induced and shares structural features with other anthocyanin-regulating Myb transcription factors, particularly *An2* from petunia. This Myb in combination with LC may be responsible for the light-induced anthocyanin pigmentation observed in *Lc* petunia.

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CaMV35S	35S promoter, from the cauliflower mosaic virus (CaMV)
4CL	4-coumarate:CoA ligase
A_{XXX}	absorbance, at the wavelength indicated by the numerical value $_{(XXX)}$
Amp	ampicillin
ANS	anthocyanidin synthase
ARE	anthocyanin regulatory element
bHLH	basic helix-loop-helix; class of transcription factor
bp	base pairs
bZIP	basic region leucine zipper; class of transcription factor
C4H	cinnamate 4-hydroxylase
cDNA	complementary deoxyribonucleic acid
CHI	chalcone isomerase. Syn. Chalcone flavanone isomerase (CFI)
CHS	chalcone synthase
cm	centimetre
cpm	counts per minute
CTAB	cetyltrimethyl ammonium bromide
°C	degrees Celsius
d	days
DFR	dihydroflavonol 4-reductase
DNA	deoxyribonucleic acid

dNTP	deoxy-nucleotide-triphosphate
DW	dry-weight
EDTA	ethylenediaminetetraacetate
F3H	flavanone 3-hydroxylase
F3′ H	flavonoid 3′-hydroxylase
F3′5′H	flavonoid 3′5′-hydroxylase
FNS	flavone synthase
FLS	flavanol synthase
FW	fresh-weight
g	gram
g	gravity, or g-force
GFP	green fluorescent protein, originally from *Aequorea victoria*
GMO	genetically modified organism
GT	glucosyl-transferase
h	hours
HPLC	high performance liquid chromatography
Kan	kanamycin
kb	kilobases
kPa	kiloPascal
LC	LEAF COLOUR; bHLH transcription factor from *Zea mays*
LB	Luria-Bertani; bacterial growth media
LRU	light regulatory unit. Syn. Light regulatory element (LRE)
M	molar; moles per litre
min	minute
milliQ	water which has been purified using Milli-Q Ultrapure system
µg	microgram
µM	micromolar
µL	microlitre
mg	milligram
mJ	millijoules
mL	millilitre
mM	millimolar
MOPS	3-(N-morpholino) propanesulfonic acid
MRE	myb recognition element

ms	millisecond
MS	Murashige and Skoog: tissue culture media
N	normal
NaB	sodium borate buffer
NaAc	sodium acetate
ng	nanograms
nm	nanometre
ORF	open reading frame
PAL	phenylalanine ammonia lyase
PCR	polymerase chain reaction
psi	pounds per square inch
PVP	polyvinyl pyrrolidone
RACE	rapid amplification of cDNA ends
RNA	ribonucleic acid
RNase	ribose nuclease
rpm	revolutions per minute
RT	reverse-transcription
RRE	'R' response element
rRNA	ribosomal ribonucleic acid
SDS	sodium dodecyl sulphate
sec	second
SSC	sodium chloride-sodium citrate buffer
Spec	spectinomycin
Strep	streptomycin
TAE	tris acetate EDTA buffer
TBE	tris borate EDTA buffer
tDNA	transfer DNA; DNA transferred from *Agrobacterium tumefaciens* into the host genome
Tris	tris(hydroxymethyl) aminomethane
U	enzyme units
UV	ultra violet
V	volt
v/v	volume/volume
w/v	weight/volume

WT	wild type
YM	yeast-mannitol; bacterial growth media

- Gene names (and loci) are italicised e.g. *Leaf colour,* e.g. *CHS.*

- Proteins are capitalised e.g. LEAF COLOUR, e.g. CHS.

- 'Light' refers to photosynthetically active radiation (400 - 700 nm). Photosynthetic photon flux density was measured in μmol m^{-2} sec^{-1}.