Regulation of the carotenoid biosynthetic pathway in petals of California poppy (*Eschscholzia californica*)

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Abstract

Carotenoids are essential plant pigments. They function in a wide range of processes including light harvesting in the photosynthetic apparatus, photoprotection against light damage, and pigmentation in flowers and fruits to attract pollinators and seed-dispersal herbivores. Carotenogenesis has been studied extensively in the last century in both photosynthetic and non-photosynthetic tissues of many plant species. Although most of the enzymes and their metabolites of the pathway have been identified, little is still known about how carotenoid production is regulated.

Previous studies have proposed that regulation of the carotenoid pathway is through metabolite feedback occurring at both transcriptional and post transcriptional levels. This thesis examines the evidence for carotenogenesis gene transcription being feedback regulated by changes in carotenoid metabolites in petals of California poppy (*Eschscholzia californica*), and if so, by which metabolite(s).

Virus-induced gene silencing (VIGS) was used to silence carotenoid biosynthetic genes in the petals of orange California poppy. High efficacy of silencing was achieved by first infiltrating and then drenching the California poppy seedlings with the *Agrobacterium tumefaciens* strain GV3101 containing the VIGS vectors. The VIGS vectors included portions of carotenoid gene fragments isolated from California poppy. qRT-PCR confirmed that transcript abundance of the targeted carotenogenesis genes *EcaPDS*, *EcaZDS*, *EcaLCYb*, *EcaCHYb* and *EcaZEP* was significantly reduced in the flower petals. Reduced transcript abundance of all genes apart from *EcaLCYb* altered flower colour. HPLC analyses revealed that the colour altered flower petals with knocked-down expression of each targeted gene resulted in a reduction of total carotenoid content and an altered profile of carotenoids. This manifested as an accumulation of higher amounts of intermediates including phytofluene, ζ-carotene, β-carotene and zeaxanthin, some of which are not usually seen in the flowers, and a reduction of the end products such as *retro*-carotene-triol and eschscholtzxanthin. However, these alterations in carotenoid profiles were not associated with any dramatic changes in transcript abundance of the non-TRV-targeted endogenous genes in the pathway. Therefore, little evidence was found for metabolite feedback regulation of transcriptional activity in the carotenoid biosynthetic pathway from this study. Other possible mechanisms for controlling carotenogenesis are discussed.

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List of abbreviations

h:	hours
sec:	seconds
min:	minutes
dpi:	days post inoculation
˚C:	degrees Celsius
U:	enzyme unit
V:	voltage
g:	gram
g:	*g*-force or gravity
rpm:	revolution per minute
mL:	millilitre
M:	molar; moles per litre
mM:	millimolar; millimole per litre
μM:	micromolar; micromole per litre
mg:	milligram
ng:	nanogram
amu:	atomic mass unit
w/v:	weight-to-volume ratio
v/v:	volume-to-volume ratio
m/z:	mass-to-charge ratio
DW:	dry-weight
bp:	base pair
Kb:	kilo base pair
DNA:	deoxyribonucleic acid
cDNA:	complementary deoxyribonucleic acid
RNA:	ribonucleic acid
rRNA:	ribosomal RNAs
siRNA:	small interfering RNA
miRNA:	microRNA
dNTP:	deoxy-nucleotide- triphosphate

TM:	melting temperature
LB:	Luria-Bertani broth
DMSO:	dimethyl sulfoxide
EDTA:	ethylenediaminetetraacetic acid
SDS:	sodium dodecyl sulfate
MES:	2-(N-morpholino)ethanesulfonic acid
TBE:	Tris/Borate/EDTA
BHT:	butylated hydroxytoluene
PVP:	polyvinylpyrrolidone
MOPS:	3-(N-morpholino)propanesulfonic acid
CPTA	2-(4-chlorophenylthio)triethylamine hydrochloride
HPLC:	high performance liquid chromatography
LC-MS:	liquid chromatography–mass spectrometry
GMO:	genetically modified organism
PC2:	physical containment level 2
UV:	ultra violet
OD:	optical density
milliQ:	purified water using Milli-Q ultrapure system
CaMV 35S:	35S promoter, from cauliflower mosaic virus
STDEV:	standard deviation