16[TH] INTERNATIONAL SYMPOSIUM ON CAROTENOIDS
17-22 JULY 2011, KRAKOW

האוניברסיטה העברית בירושלים
The Hebrew University of Jerusalem

A JOURNEY ALONG THE PATHWAY OF CAROTENOID BIOSYNTHESIS: MORE ENZYMES AND NEW ROUTES OF INTERACTIONS WITH PLANT METABOLISM

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The carotenoid pathway (ca. 1990)

BIOSYNTHESIS OF CAROTENOIDS

MVA

Early stages ↓

IPP

Phytoene formation ↓

Phytoene

Desaturation ↓

Lycopene

Cyclization ↓

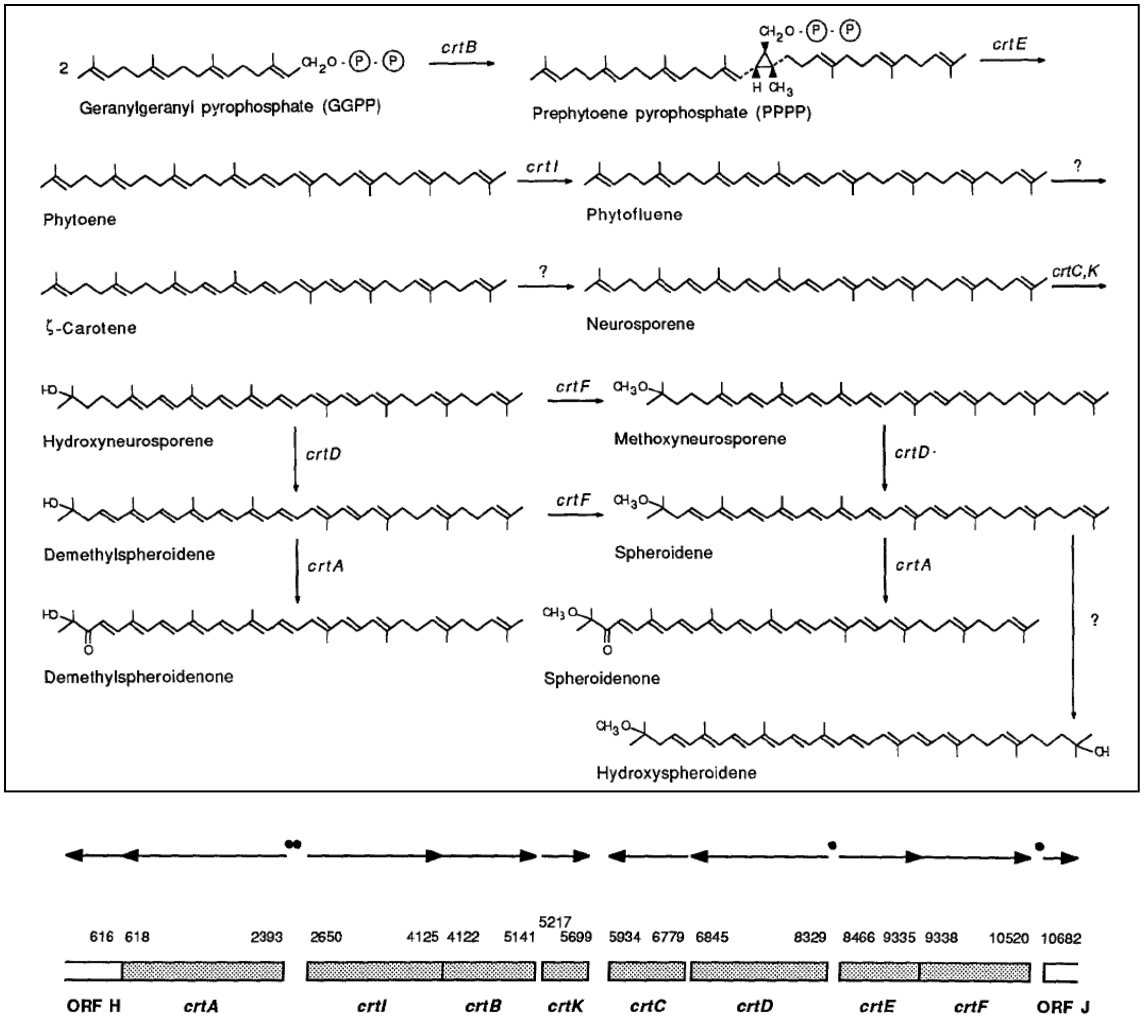
β-Carotene

Hydroxylation etc. ↓

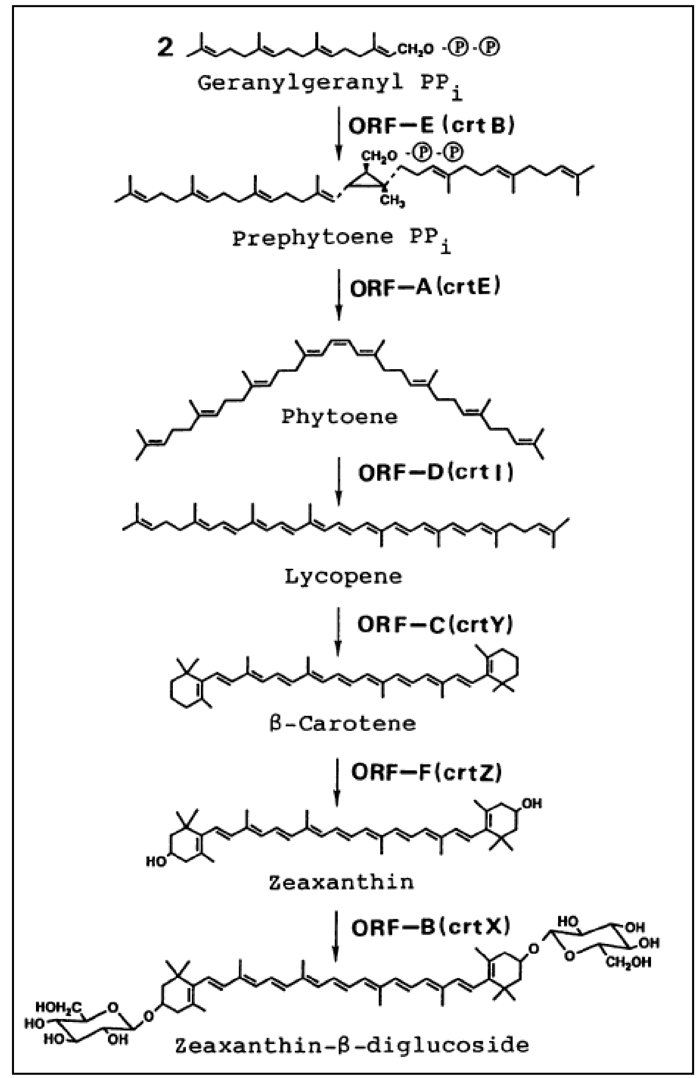
Xanthophylls

Discovery of carotenoid pathways in bacteria

Rhodobacter capsulatus
Armstrong et al. (1989)

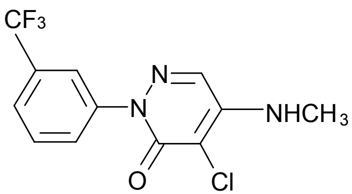


Erwinia uredovora
Misawa, et al. (1990)

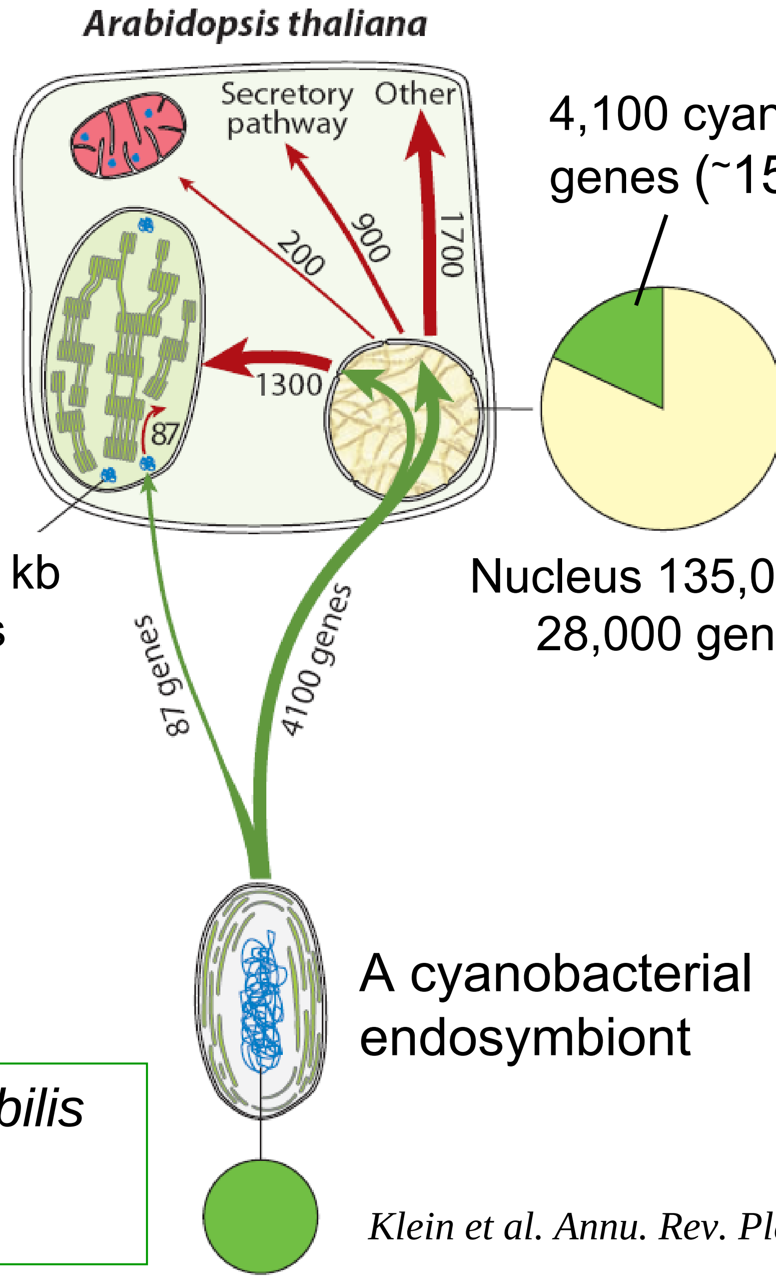


Cyanobacterial origin of carotenoid genes in plants

Gerhard Sandmann



Norflurazon



4,100 cyanobacterial genes (~15%)

Plastid 154 kb
87 ORFs

Nucleus 135,000 kb
28,000 genes

A cyanobacterial endosymbiont

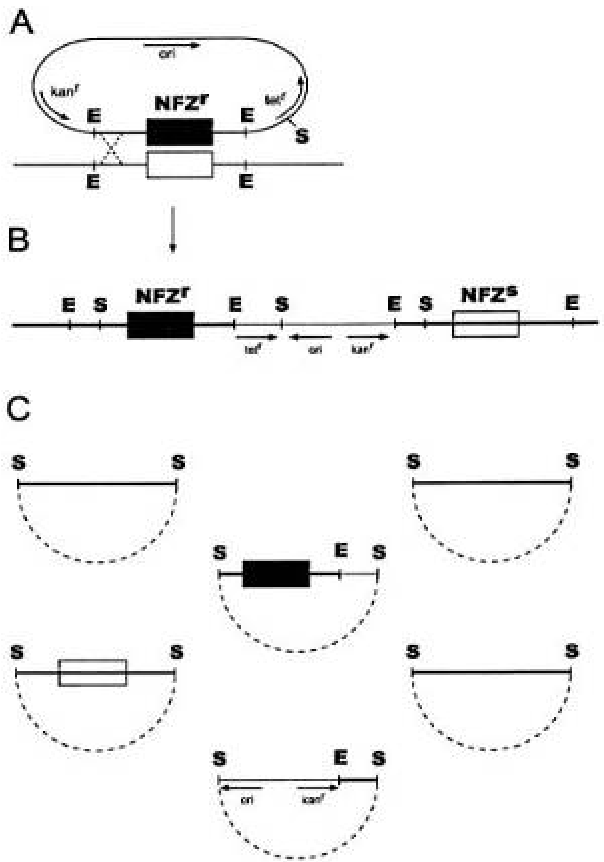
Nostoc sp. PCC7120 6,130 genes

Anabaena variabilis ATCC 29413 5,661 genes

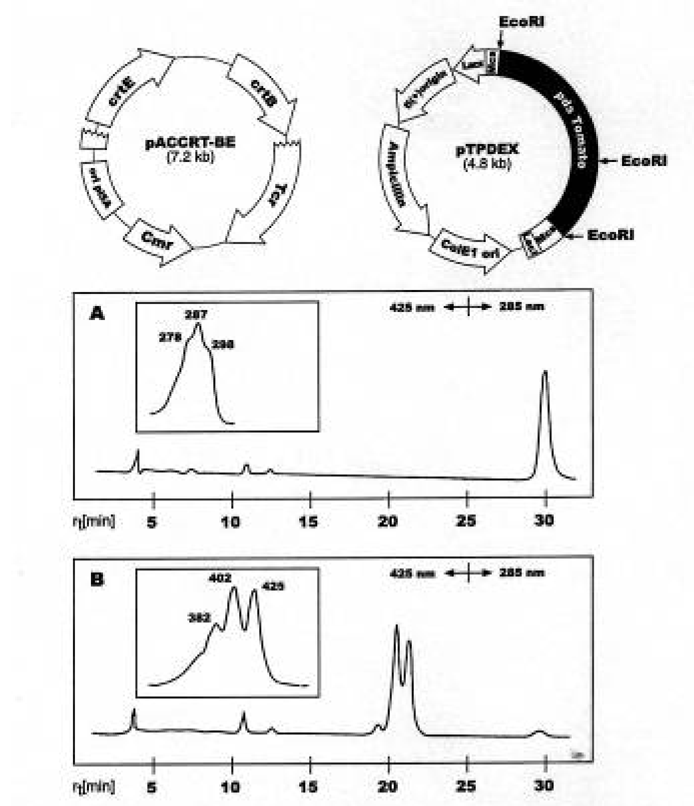
Klein et al. Annu. Rev. Plant Biol. 2009

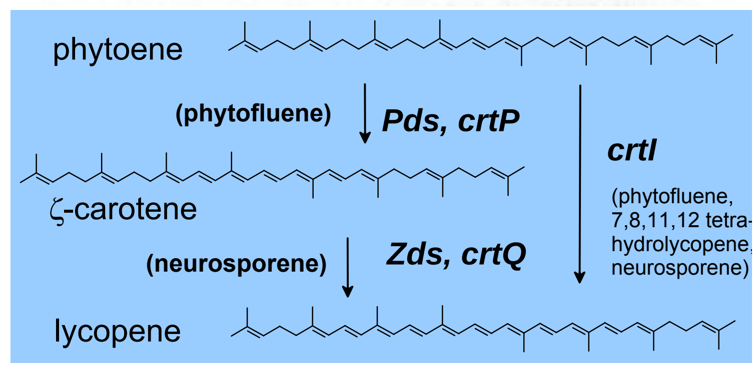
Cloning genes through inhibitor resistance

Isolating norflurazon-resistant mutations in *Synechococcus* 7942

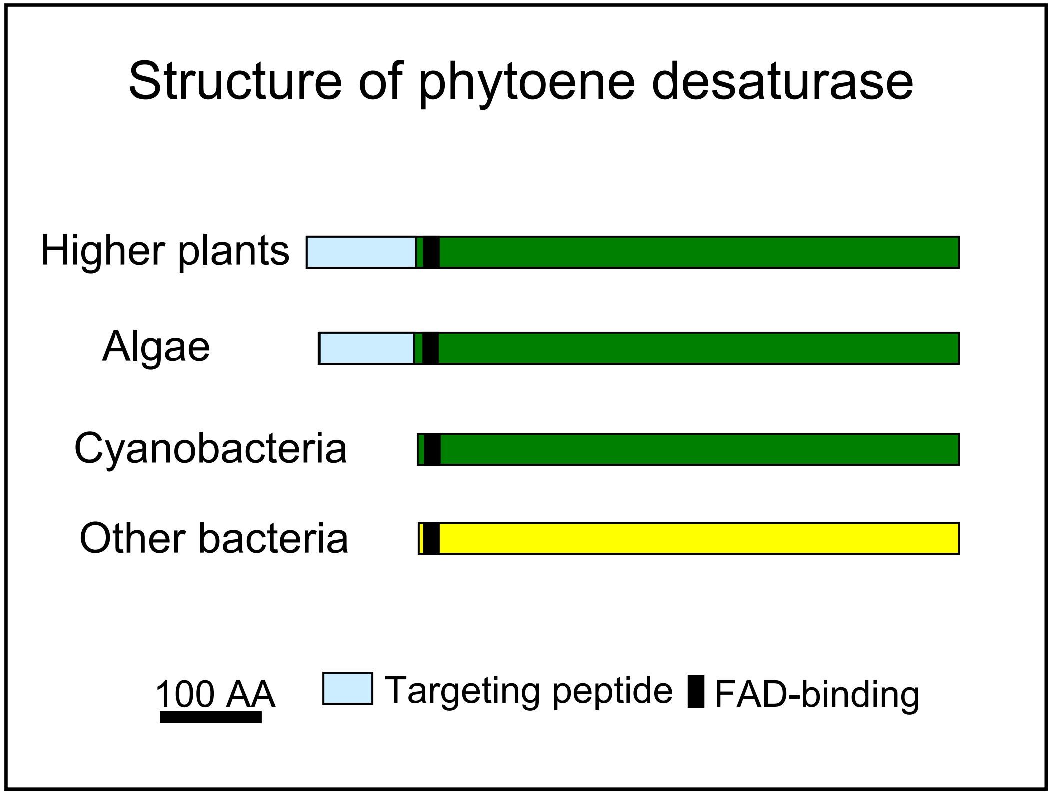


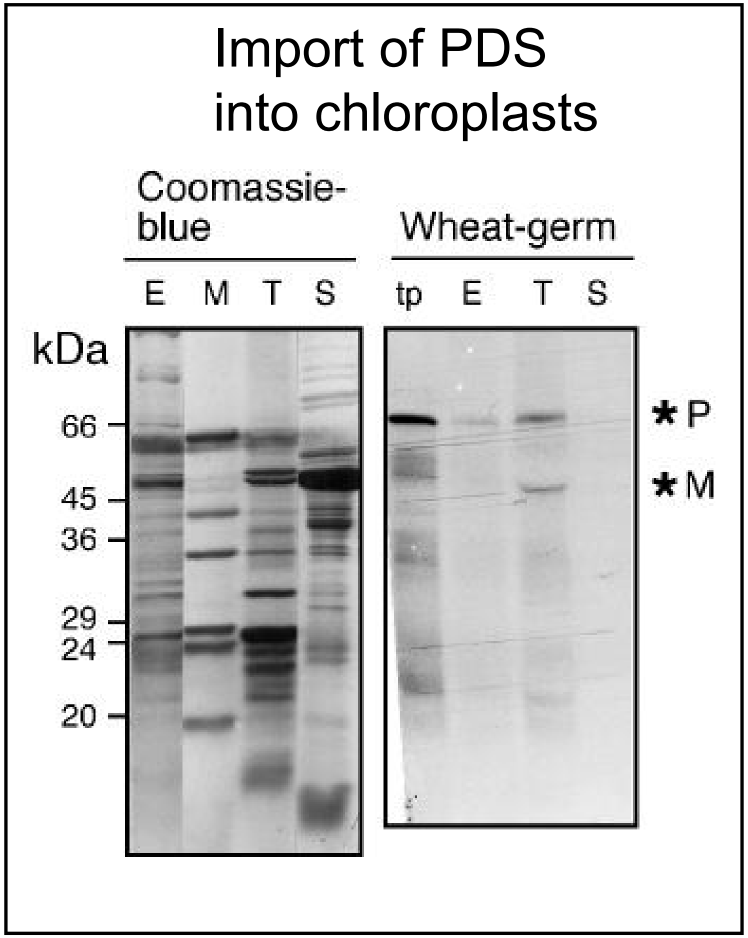
Chamovitz et al. 1990





Phytoene desaturase in plants





Pecker et al. 1992

Cloning genes by color complementation

Construction of a cDNA library from the carotenoid-producing tissue in the vector λ ZAP II

↓

Excision of plasmids in pBluescript vector

↓

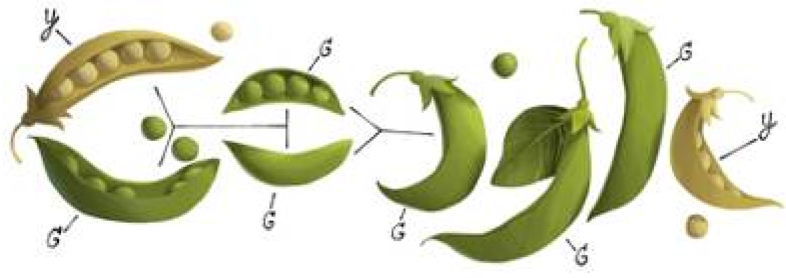
Transfection of plasmids into *E. coli* cells that produce a carotenoid precursor

↓

Screening for a colored colony

Lotan & Hirschberg 1995

The genetic approach

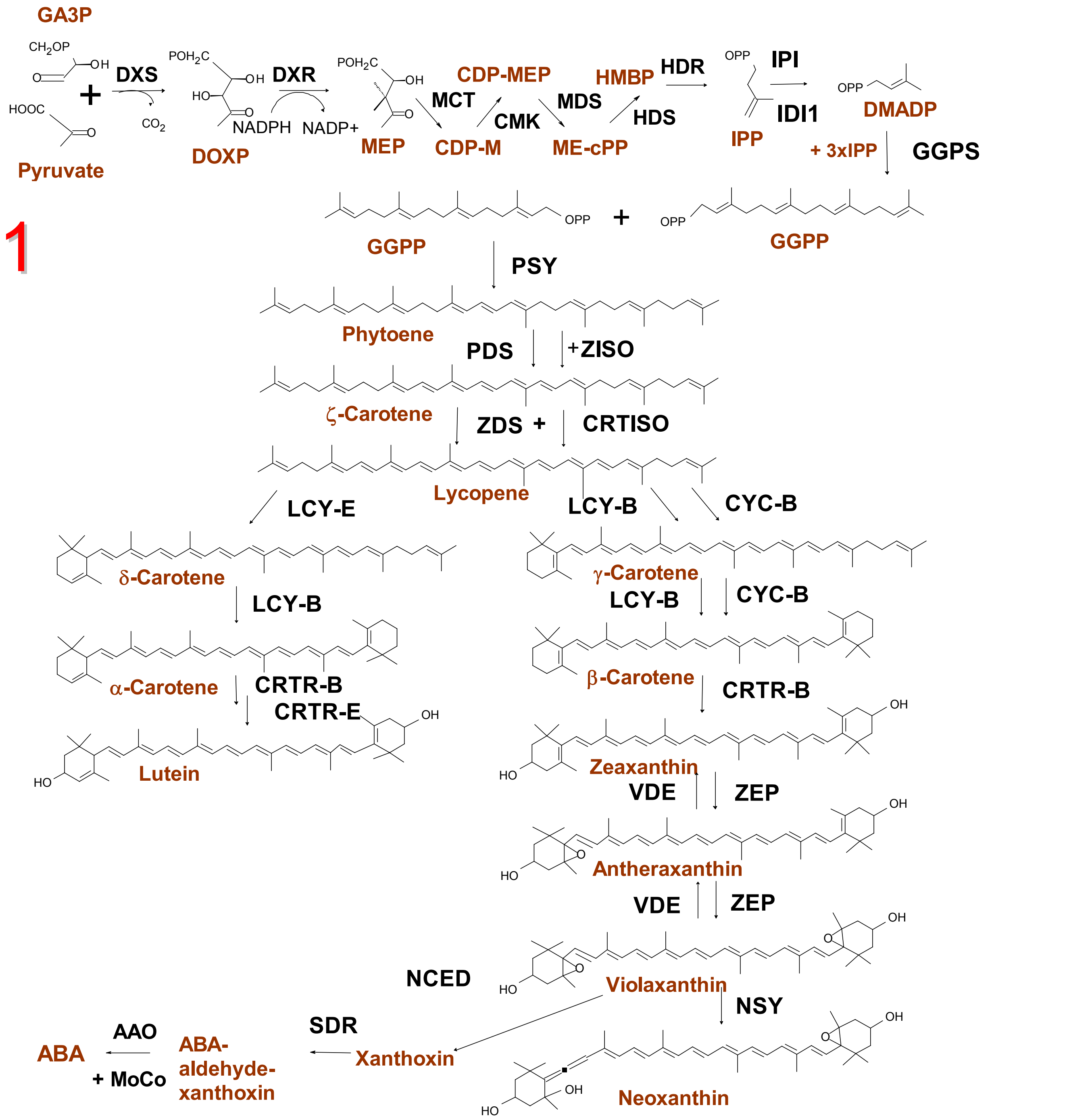


1. Map-based (“positional”) cloning of mutations
Arabidopsis, Tomato, Rice

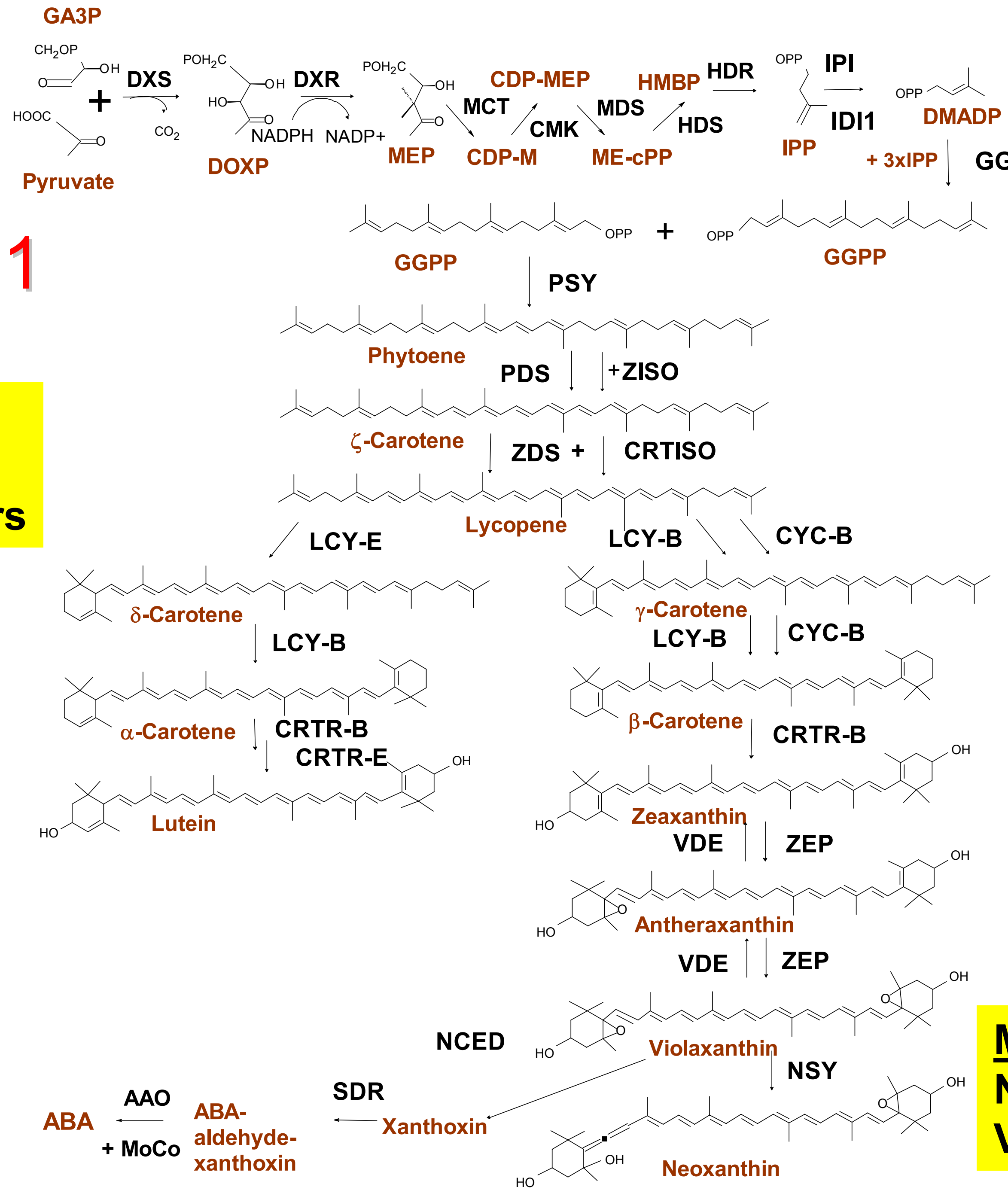
2. Genomics
Sequence-based candidate genes
Gene expression profiles (transcriptomics)

Classic Biochemistry: Capsanthin-capsorubin synthase (CCS)

The plant carotenoid pathway, 2011



The plant carotenoid pathway, 2011

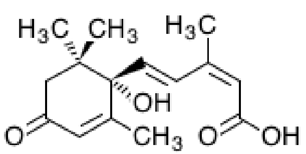


Missing:
Regulatory genes
Transcription factors

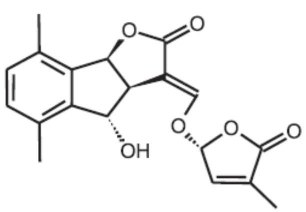
Missing:
NSY
VISO

The plant carotenoid pathway, 2011

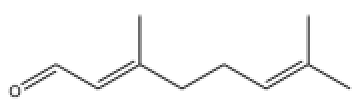
Abscisic acid (ABA)



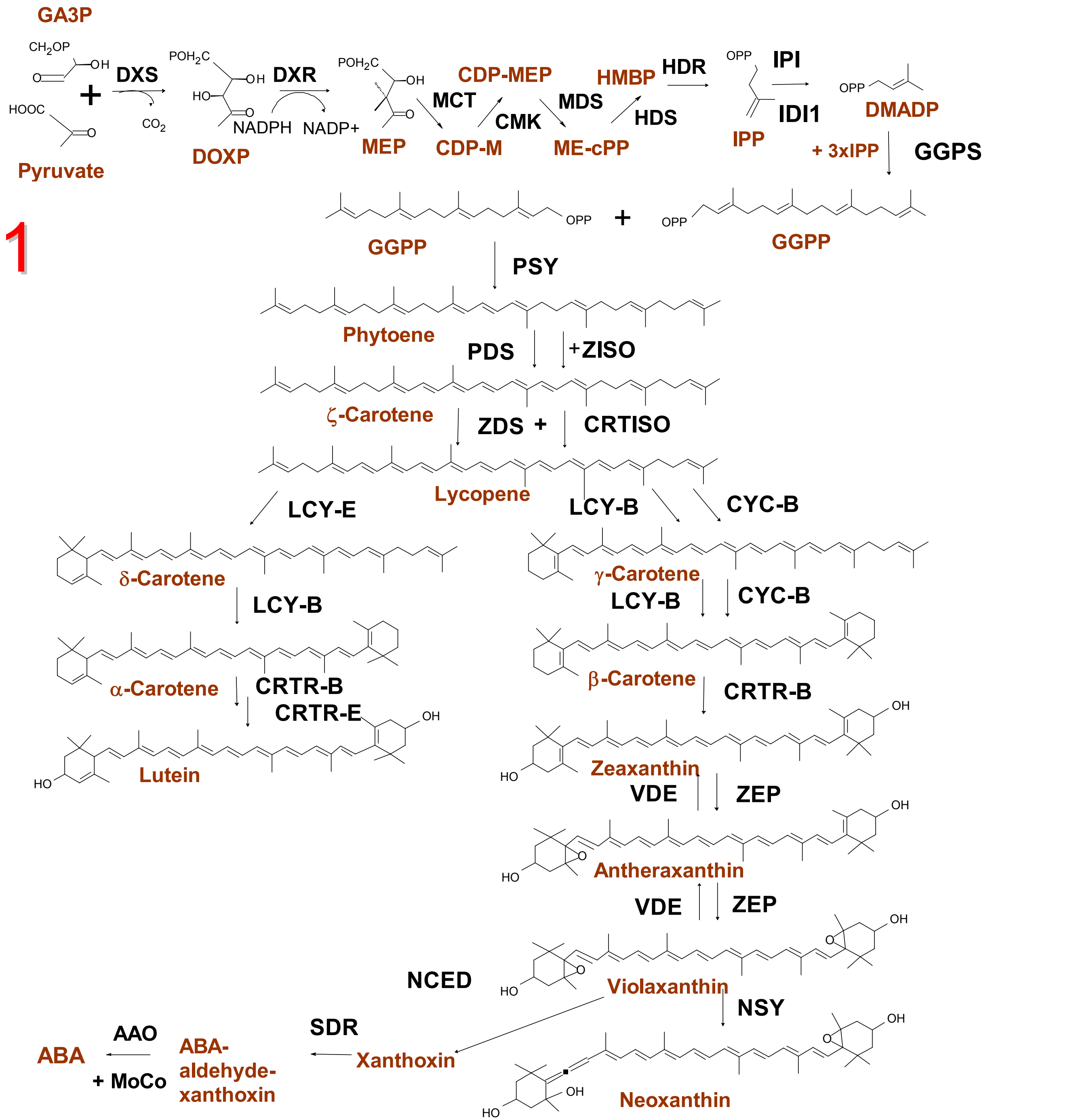
Strigolactones



Volatile compounds



Geranial



דע את האויב(ים)

מימין: ג'ורג' בוטלין, אלי ורדו, ג'ין דלפונה, גארי פולגסון,
גארי קנינגהם, לואיס שליפליוס

Cairns, 1999

The genetic approach: Map-based cloning

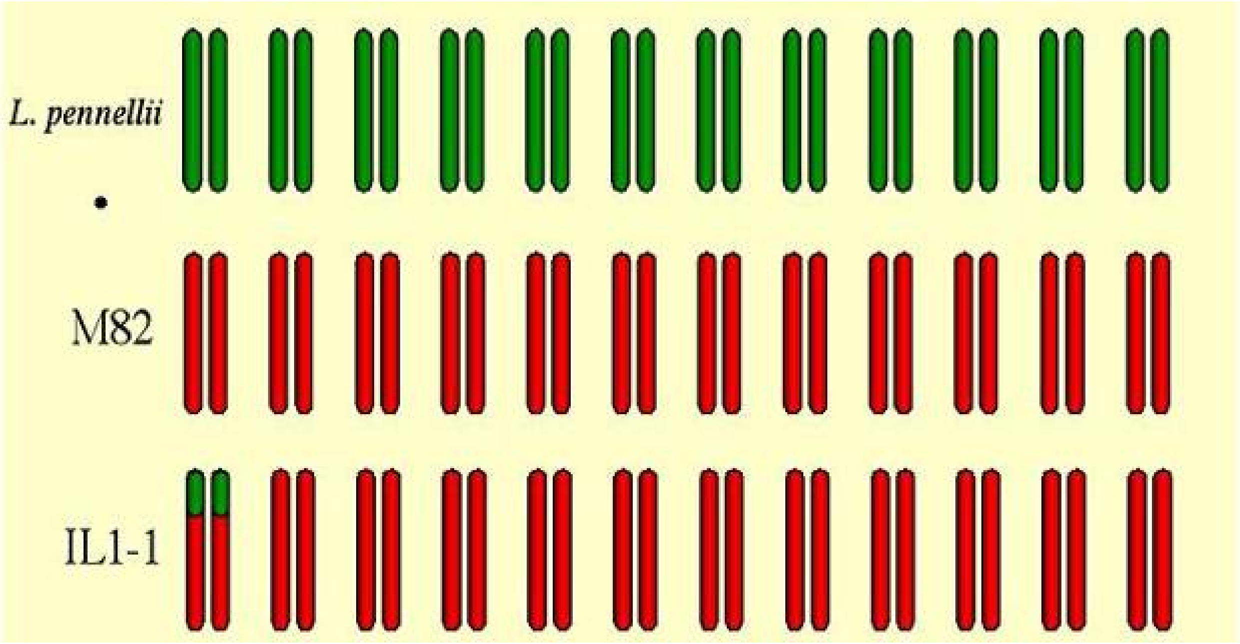
New mutations isolated in the line M82

Navot Galpaz, Naama Menda, Dani Zamir

The tomato introgression lines (IL)

S. pennellii ***S. lycopersicum***

Unique chromosomal segments from *S. pennellii* were introduced in to the genome of *S. lycopersicum*.

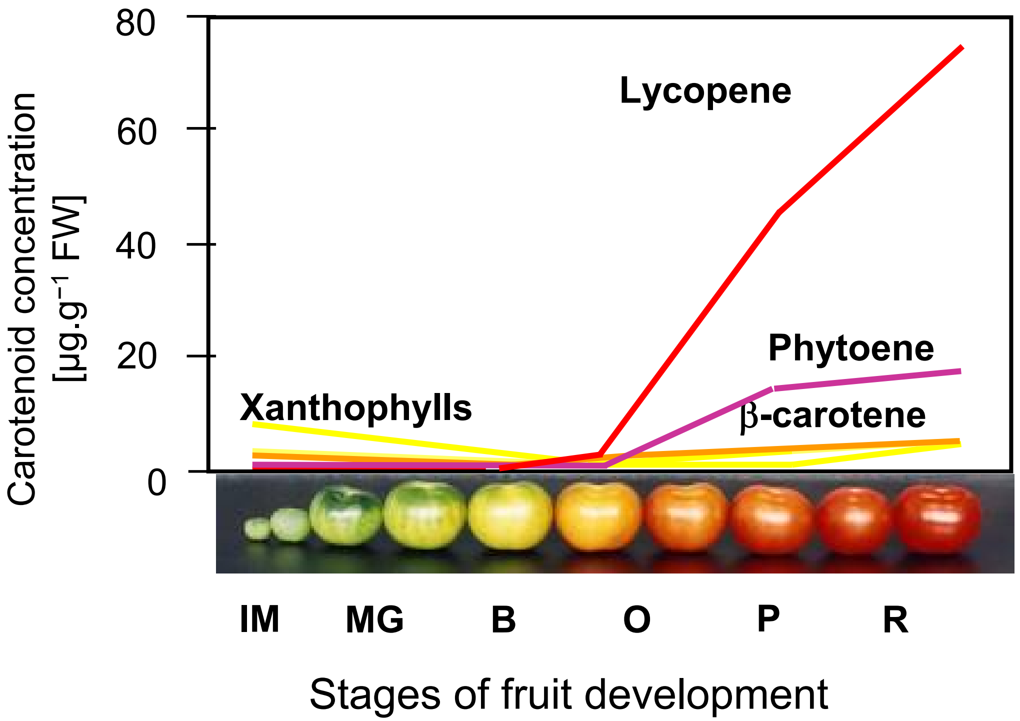


Eshed & Zamir, 1995

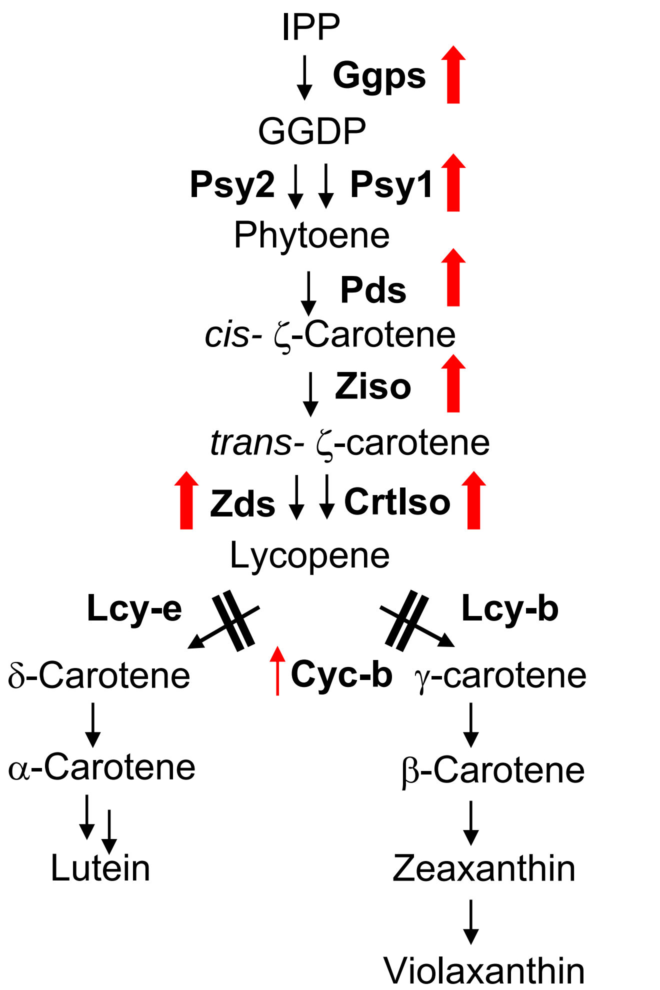
<u>The tomato genome:</u>
Size: 950 Mb
Map: ~750 cM

Carotenoid biosynthesis is genetically regulated

Carotenoid composition in fruits



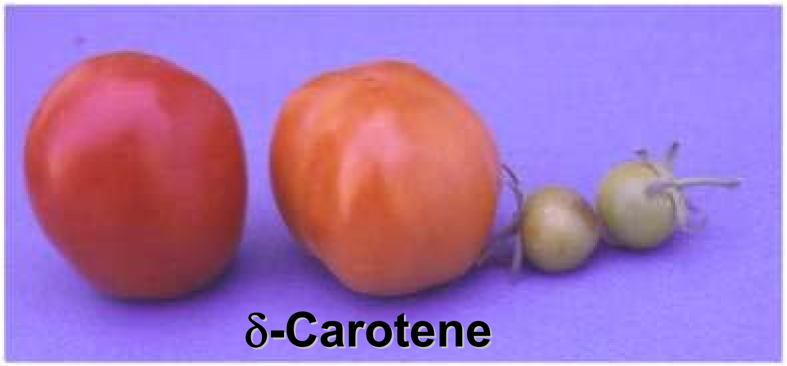
Gene expression during "Breaker"



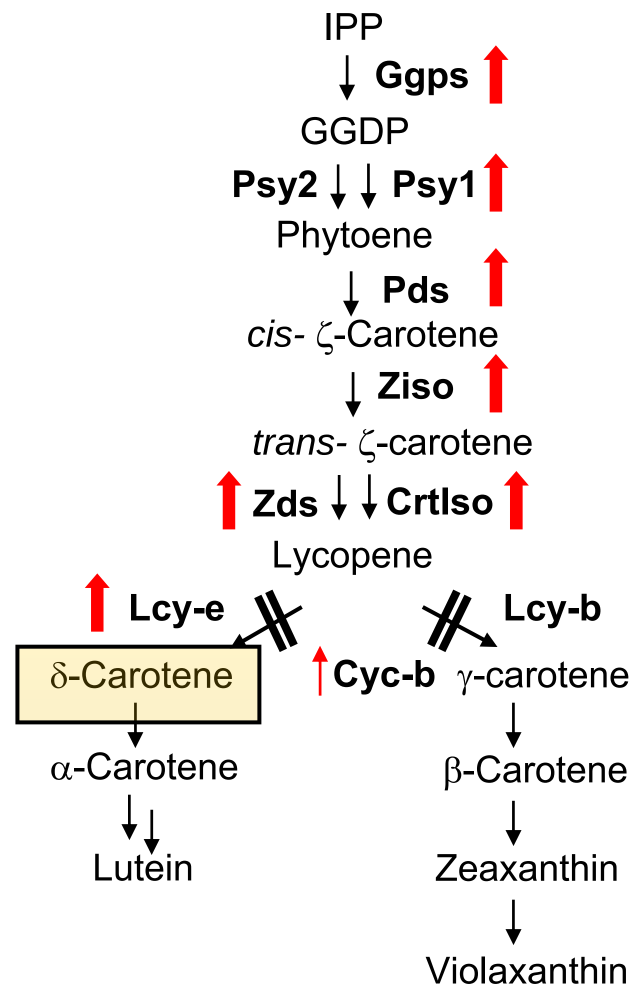
Pecker et al. 1996

Carotenoid biosynthesis is genetically regulated

WT *Delta* (*Del*) *S. pennellii*



Gene expression during "Breaker"

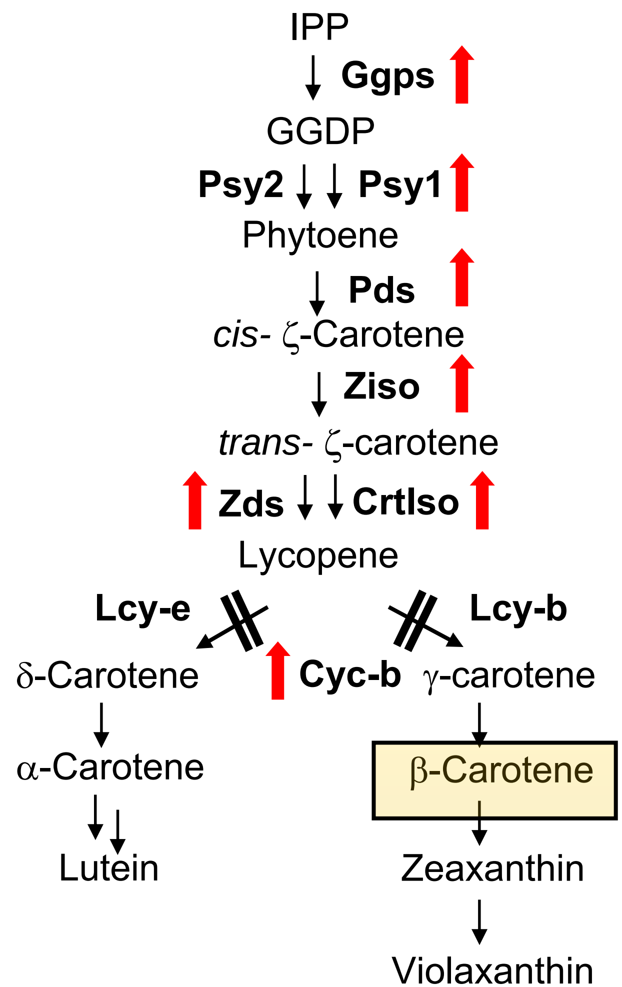


Ronen et al. 1999

Carotenoid biosynthesis is genetically regulated

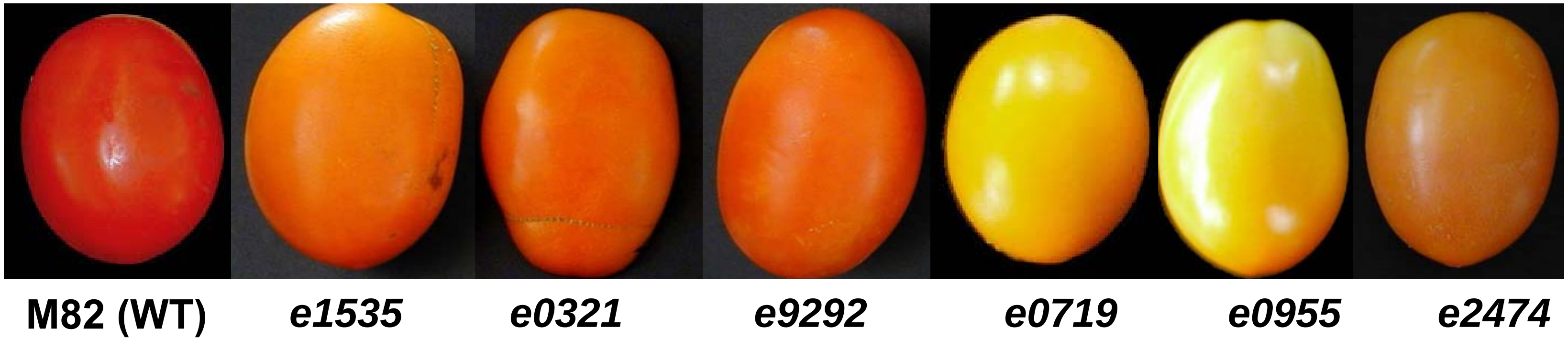
WT *Beta* (*B*)

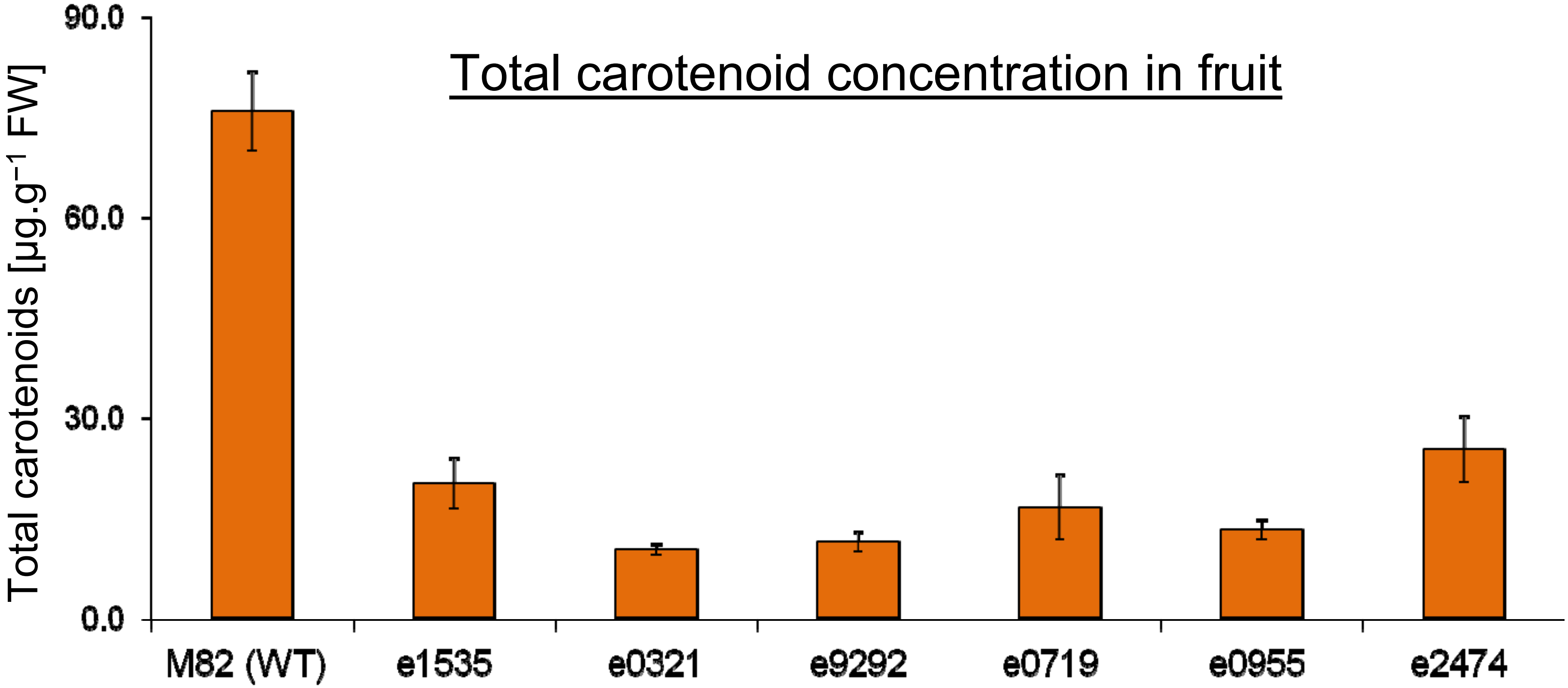
Gene expression during “Breaker”



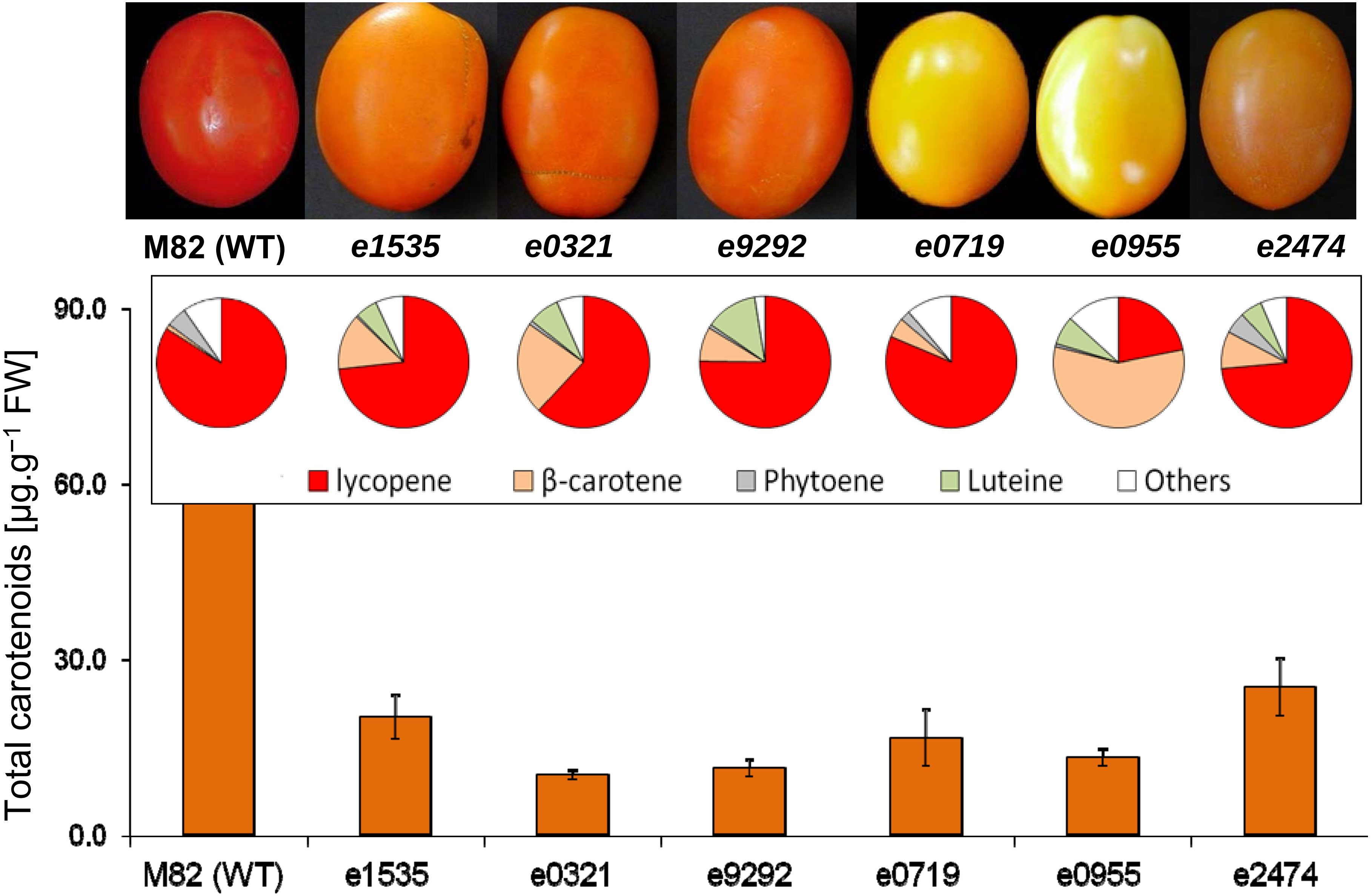
Ronen et al. 2000

Fruit-carotenoid-deficient (*Fcd*) mutants

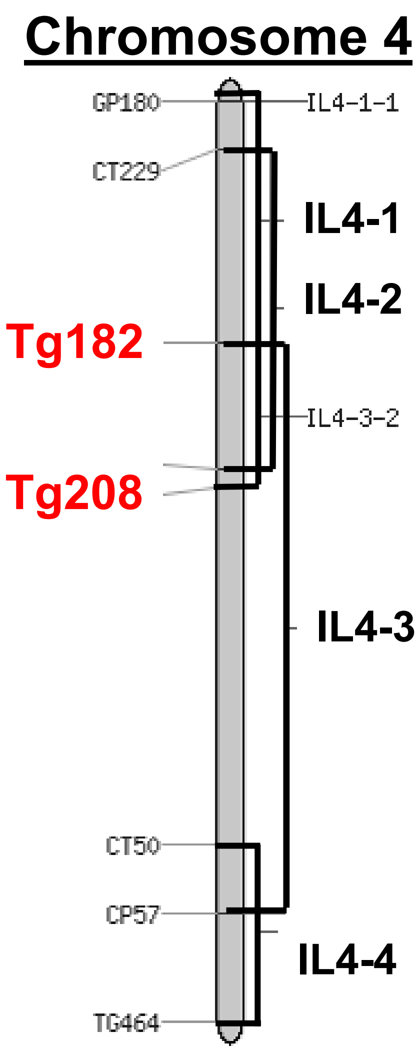




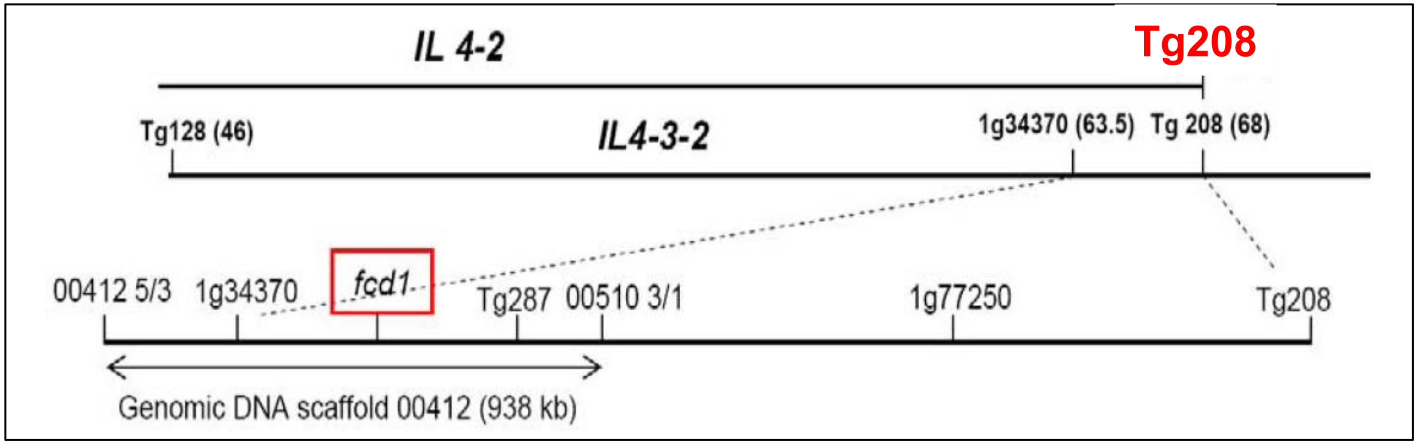
Fruit-carotenoid-deficient (*Fcd*) mutants



Map-based cloning of *Fcd1*



1. Crossing *Fcd1* (M82) X IL4-2 (*S. pennellii*).
2. F1 selfing.
3. 5000 F2 seedlings genotyped with markers Tg182 and Tg208.
4. 486 genetic recombinant plants identified, grown and phenotyped.
5. Genetic locus fine-mapped with new DNA markers
6. Candidate gene identified.



Fcd1 encodes IPP isomerase (IDI)

1 MSLTTASASLQFLRRFIASPITSHSSLRLPKSSLLPNNTL

41 PVSSLRCRFRCYSAASTTTMADAISDANMDAVQRRLMFED

81 ECILVDENDHVVGHDTKYNCHLMEKIEAENLLHRAFSVFI

*Fcd1-1

121 FNSKYELLLQQRSATKVTFPLVWTNTCCSHPLYRESELIE

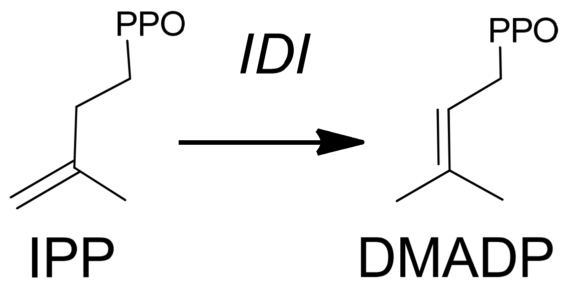
161 ENSLGVRNAAQRKLLDELGIPGEDVPVDQFIPLGRILYKA

ΔFcd1-2

201 PSDGKWGEHELDYLLFMVREVNMKPNPDEVAEVKYVNREQ

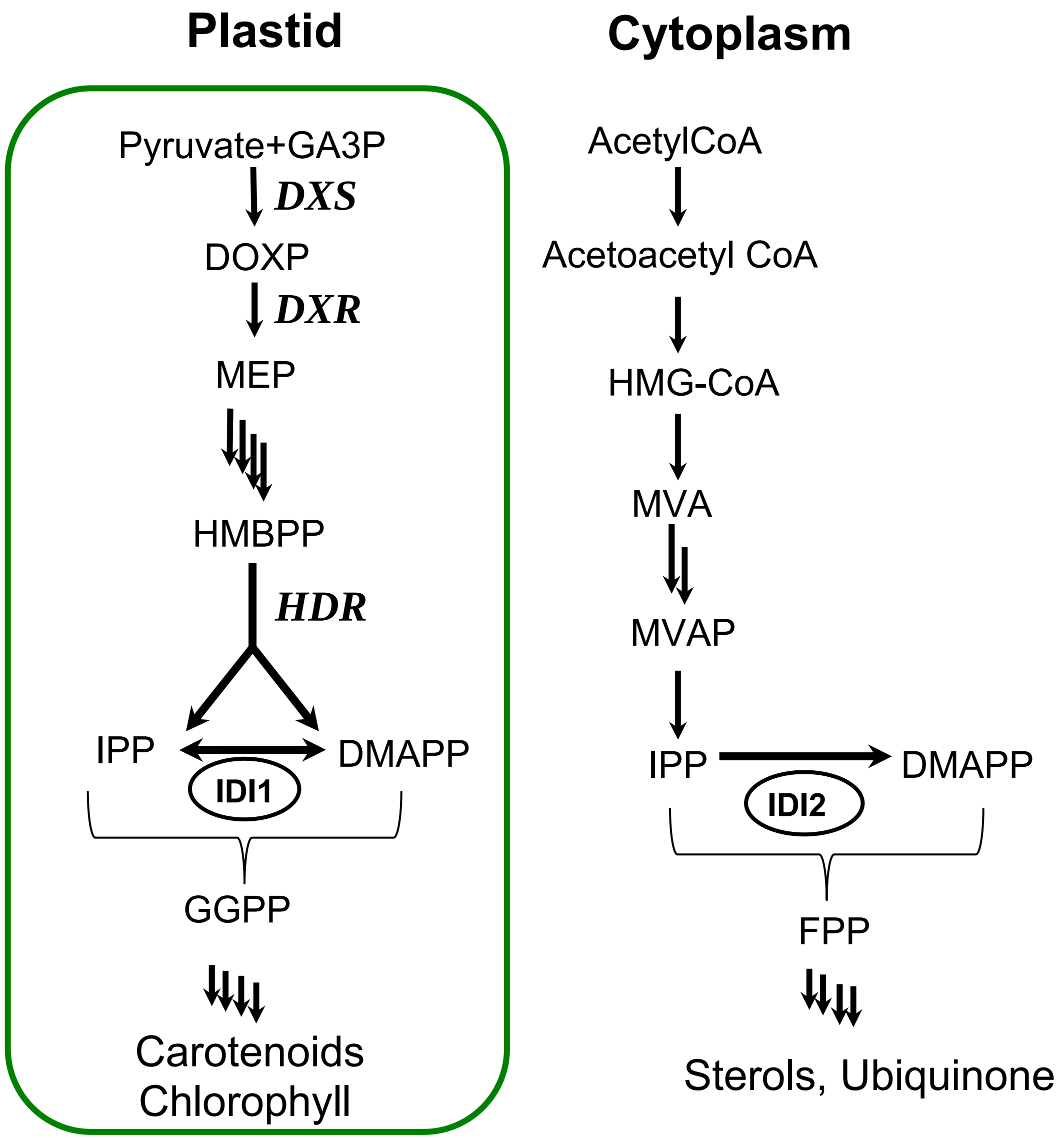
241 LKELLRKADAGEEGLKLSPWFRLVVDNFLFKWWDHLEKGT

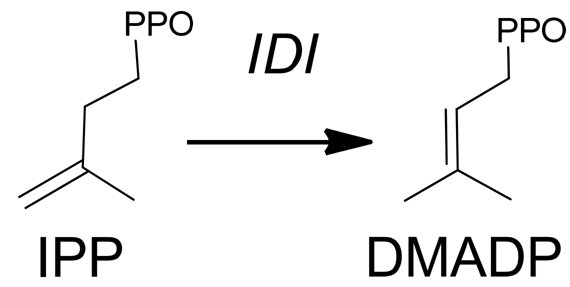
281 LKEVIDMKTIHKLT



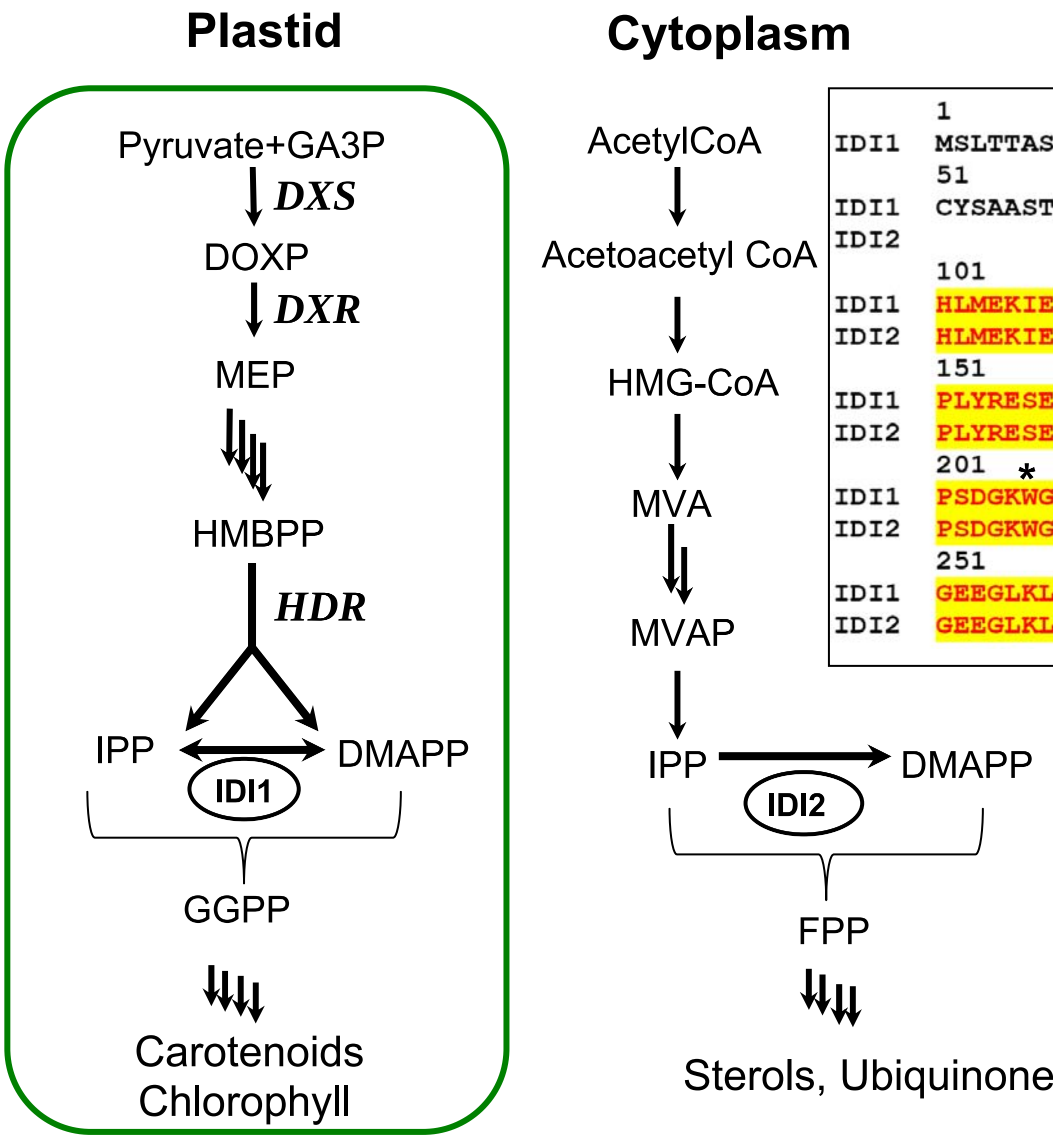
***Fcd1-1*: W143 stop** ***Fcd1-2*: W206 Δ**

Two IDI enzymes exist in plants



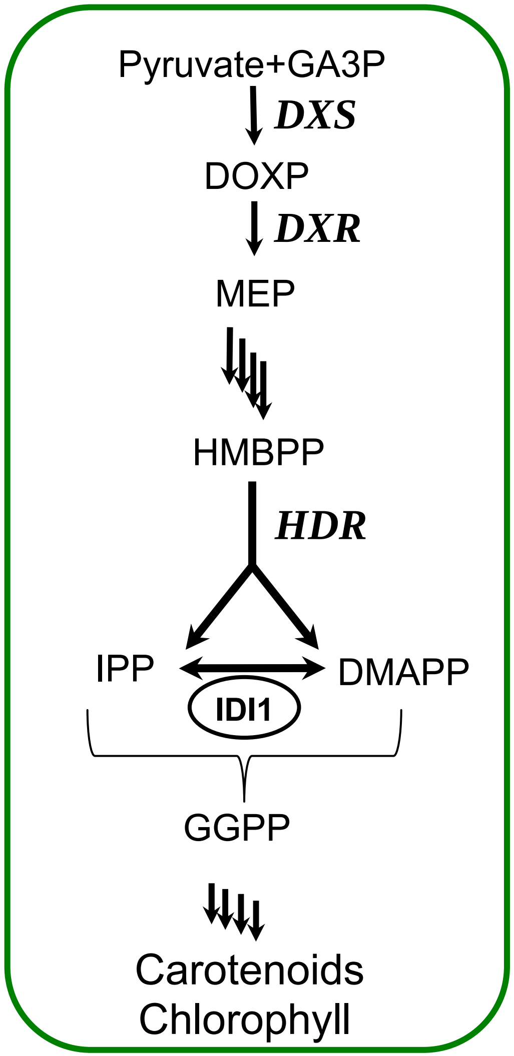


Two IDI enzymes exist in tomato



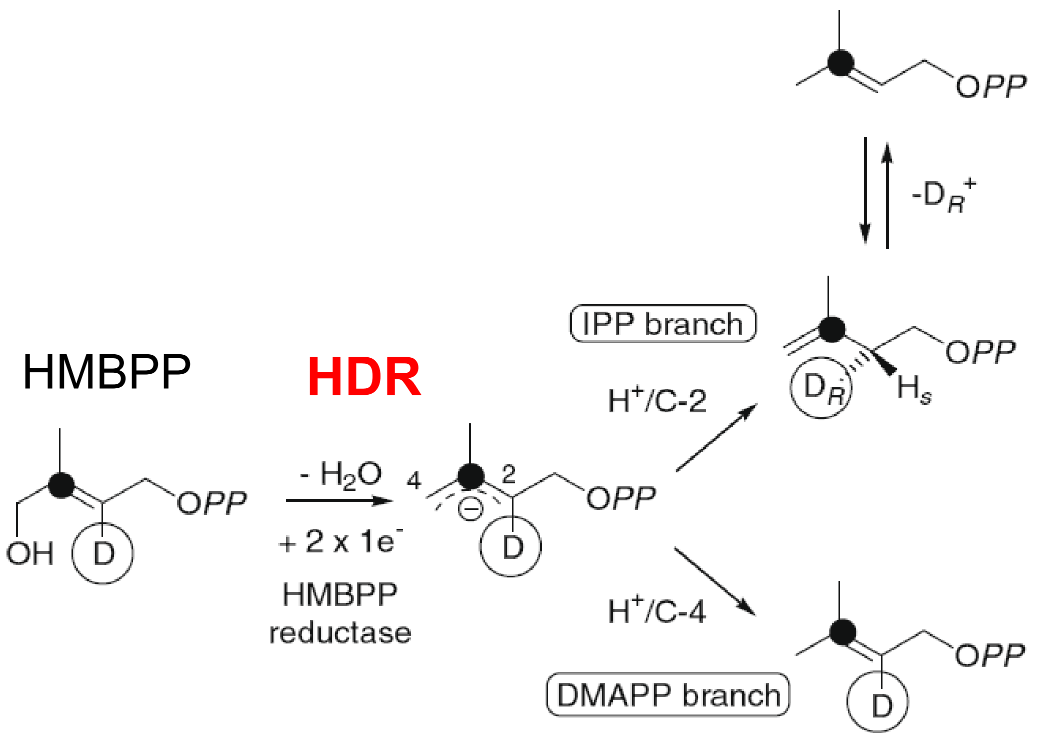
Do plastids need IDI?

Plastid



Tritsch et al, FEBS Lett. 2010

IPP (6)



DMADP (1)

Do plastids need IDI?

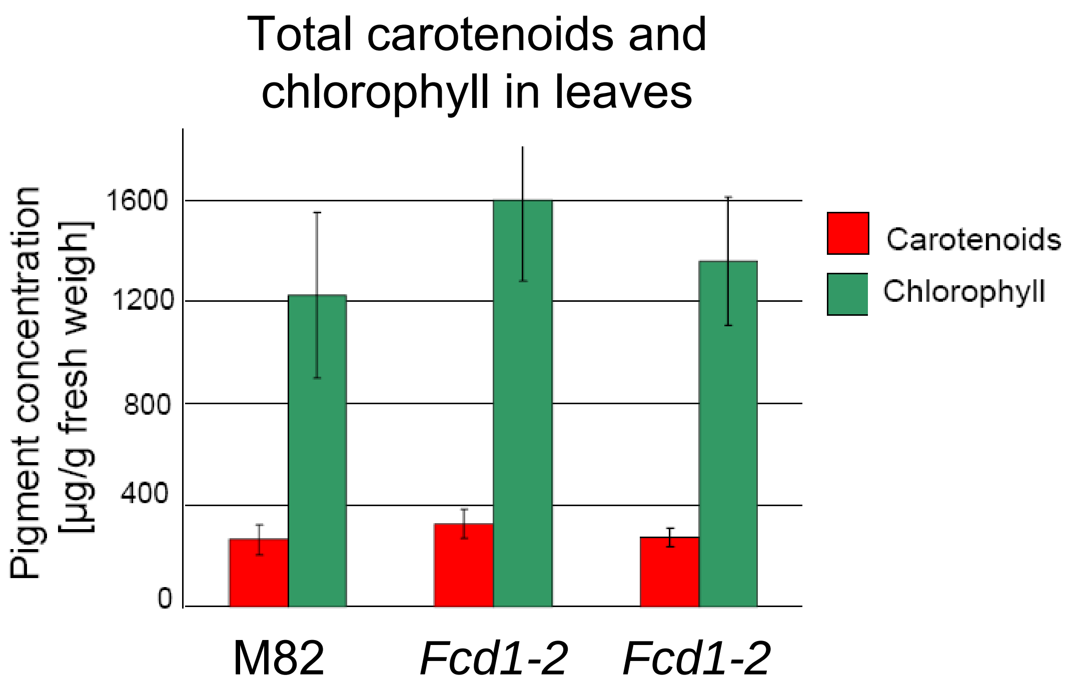
Mutants in Arabidopsis lacking IDI1 do not show a clear phenotype.

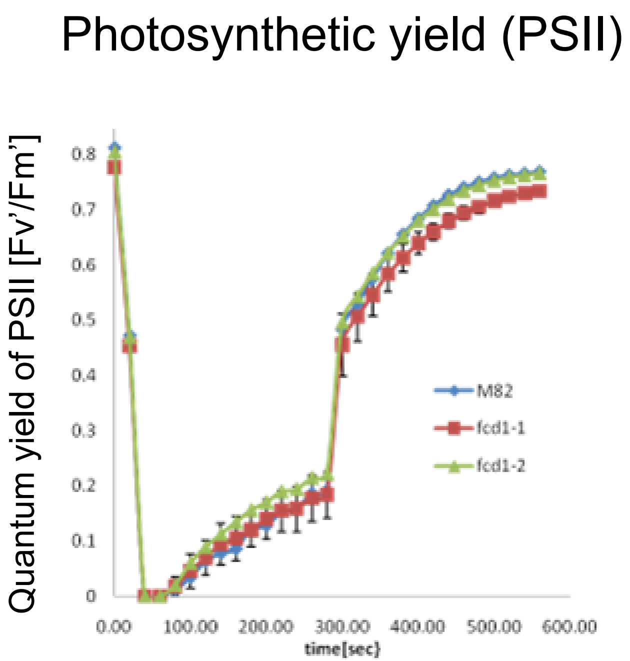
Okada et al. (2008) Plant Cell Physiol. 49:604; Phillips et al. (2008) Plant Cell, 20:677.

"Mutants specifically defective in IDI1 do not show changes in carotenoid levels, consistent with the dispensable role of this enzyme in plastids."

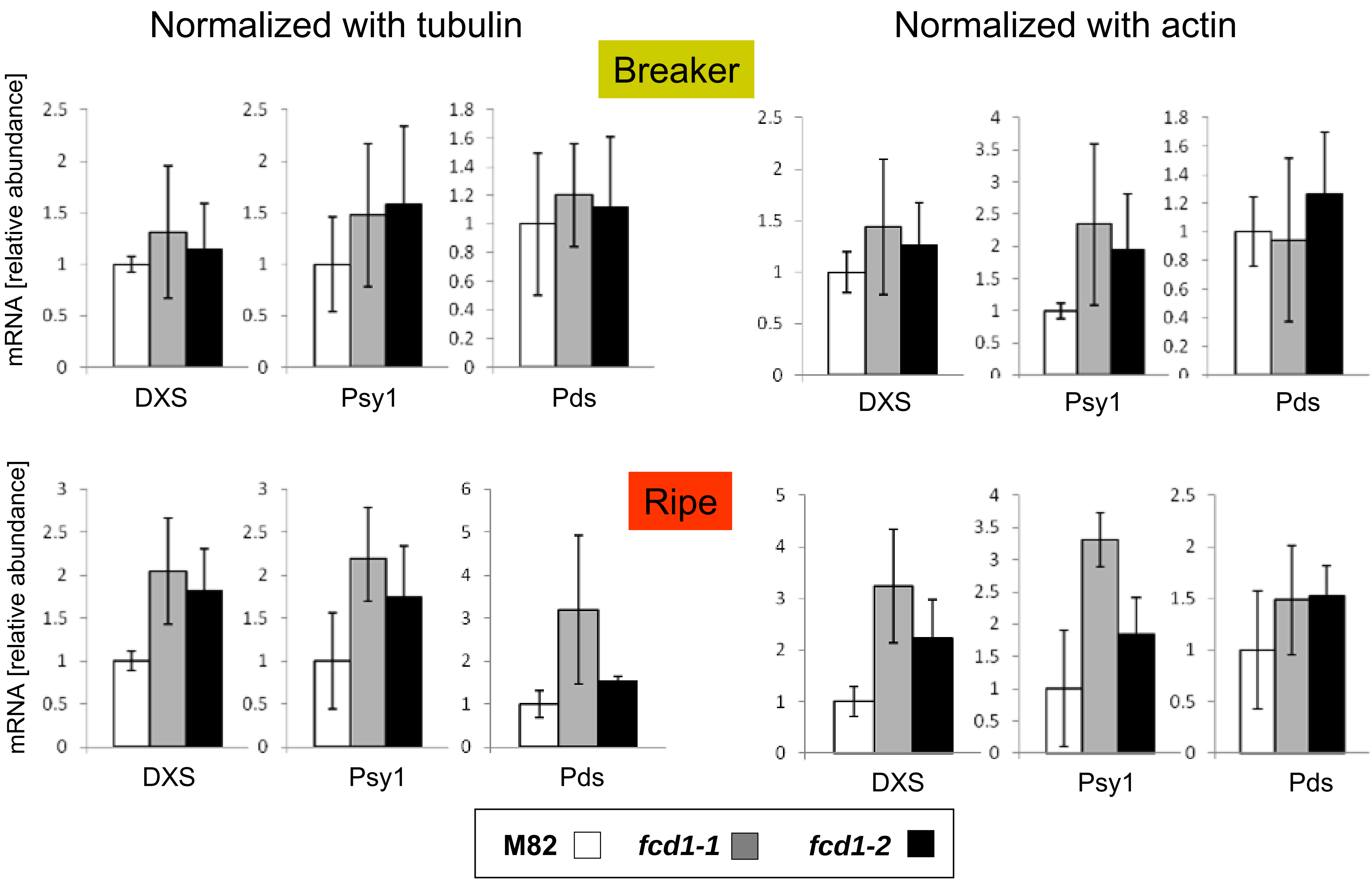
Rodriguez-Concepcion, M. (2010) Arch. Biochem. Biophys. 504:118-122.

No apparent phenotype in leaves of *Fcd1-1*

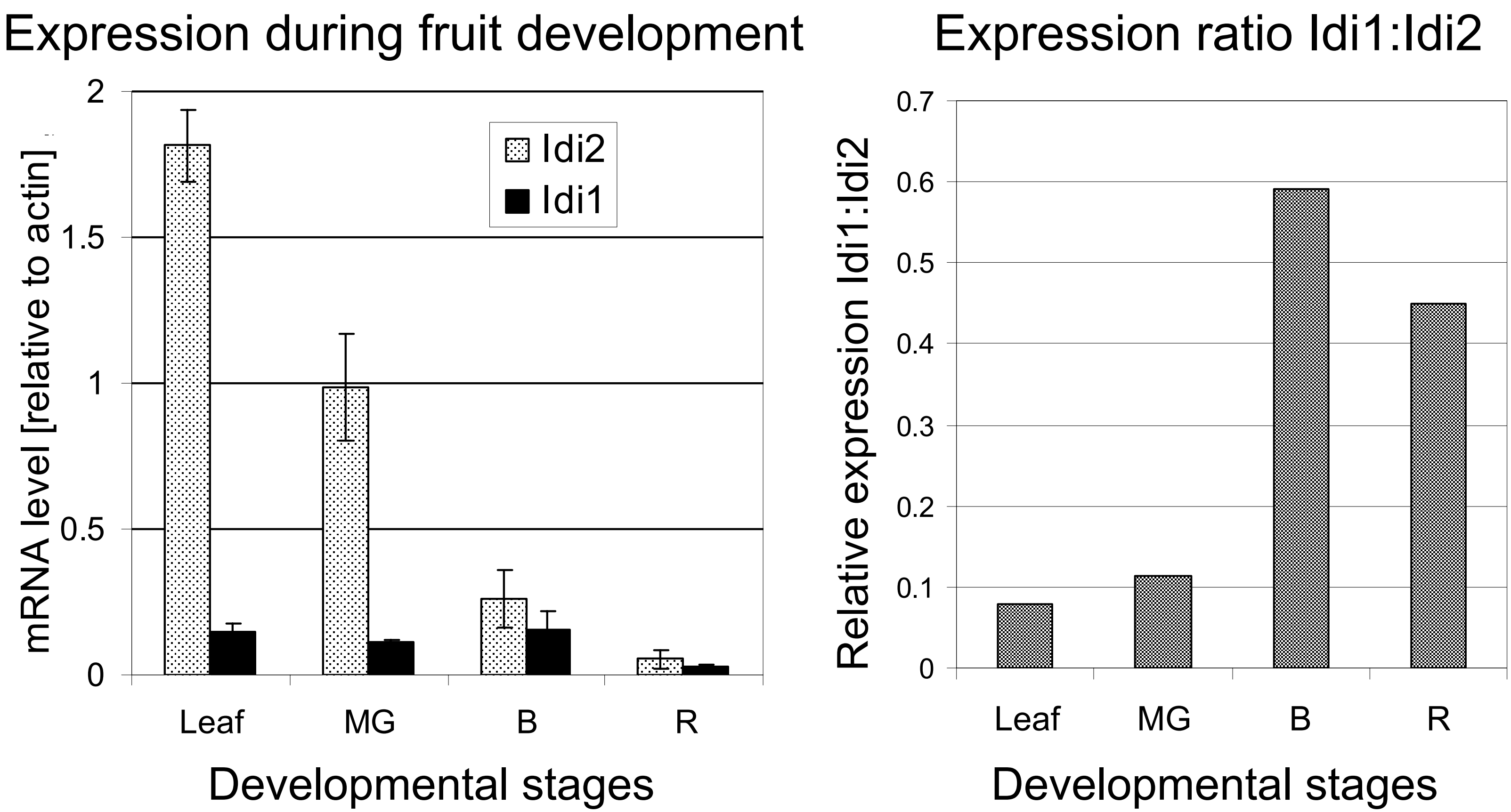




Expression of carotenoid genes in *Fcd1* fruit

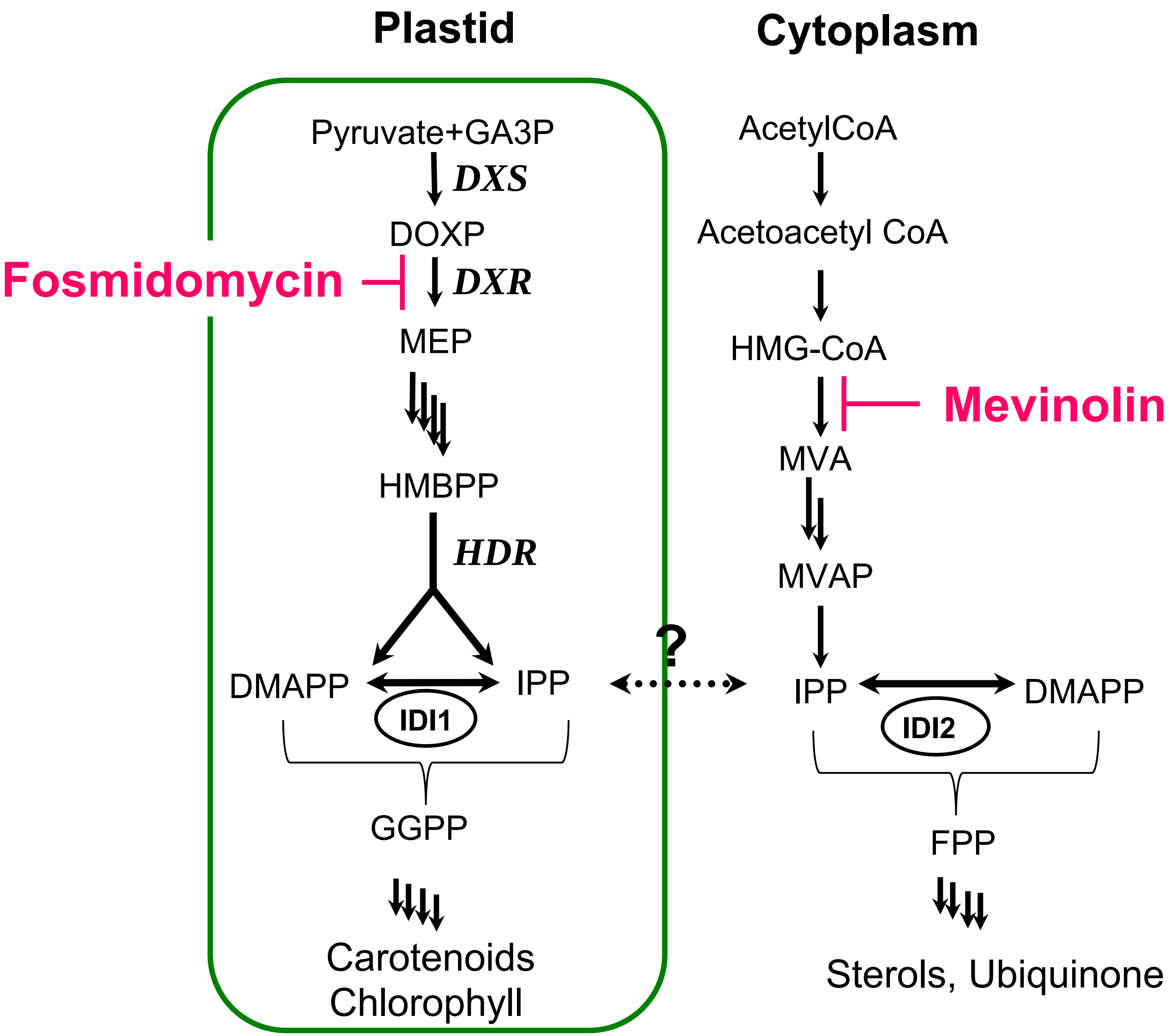


Differential expression of Idi1 and Idi2

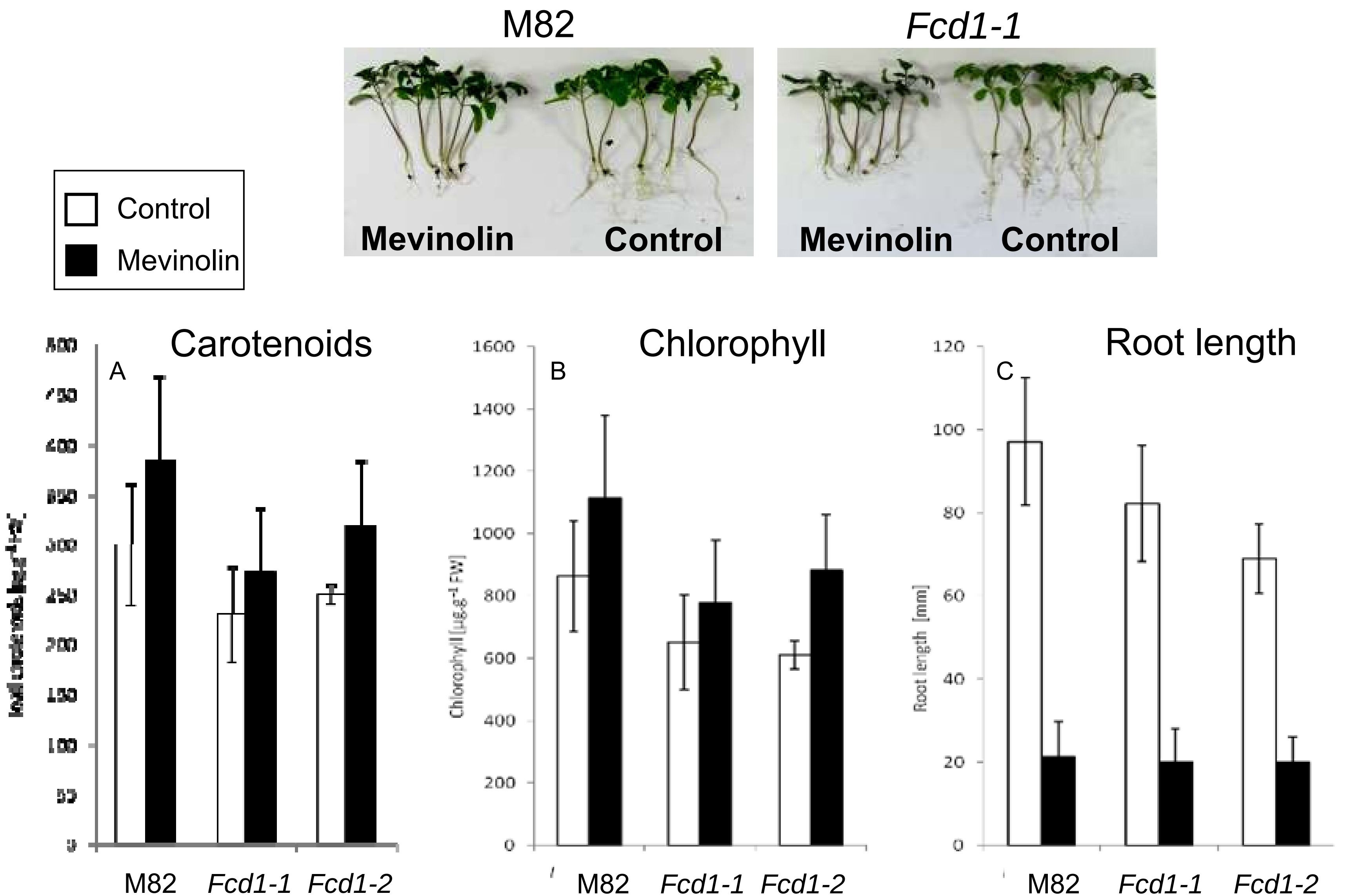


Stages in fruit development: MG- mature green; B- breaker; R- ripe

Specific inhibition of IPP biosynthesis

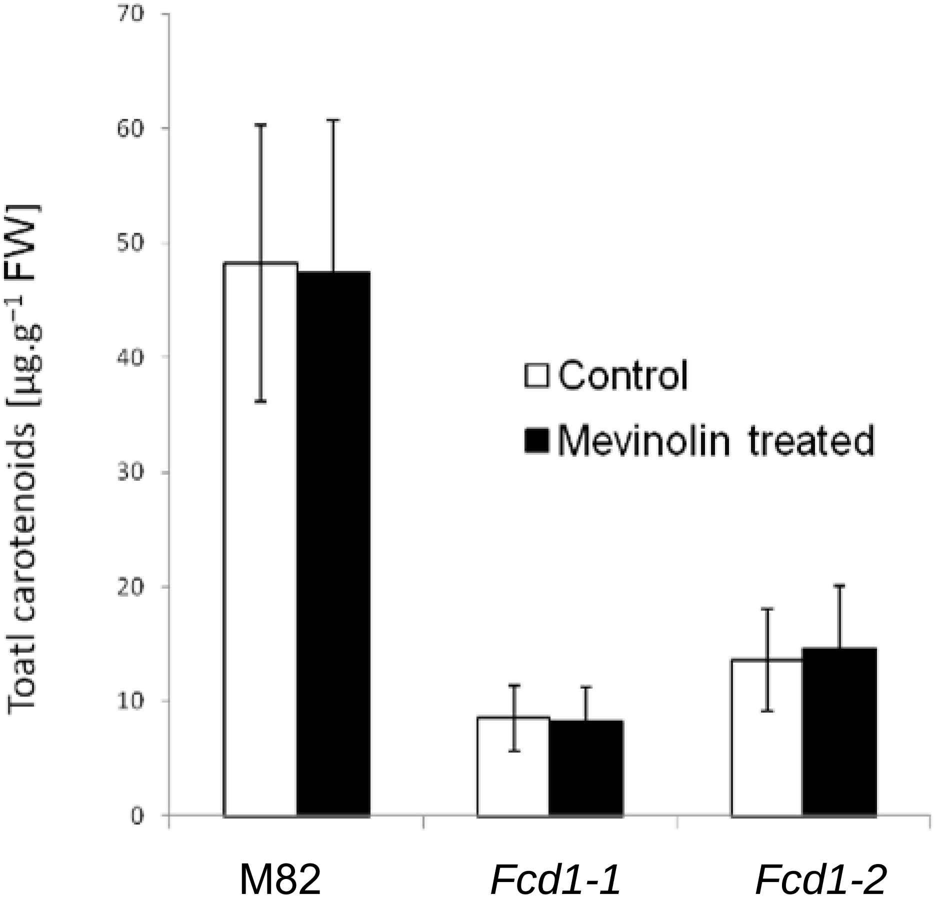


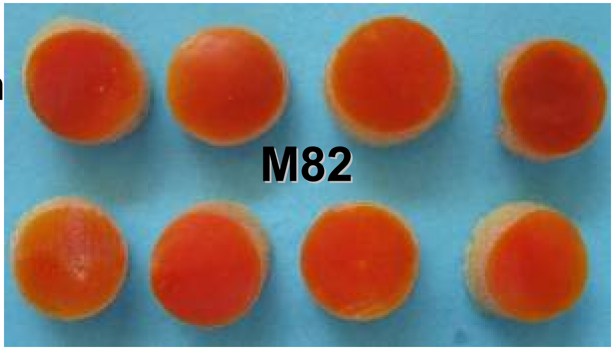
Inhibition of cytoplasmic IPP synthesis in seedlings

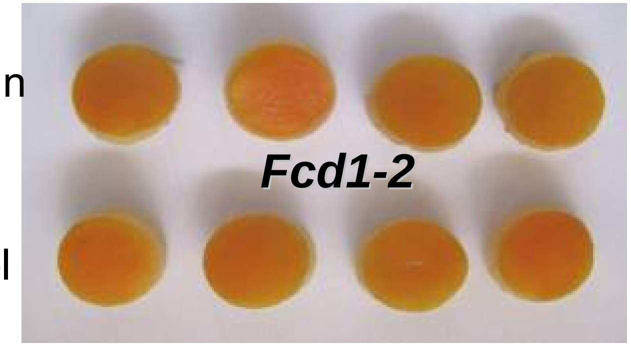


Inhibition of cytoplasmic IPP synthesis in fruit

Total carotenoids in fruit explants

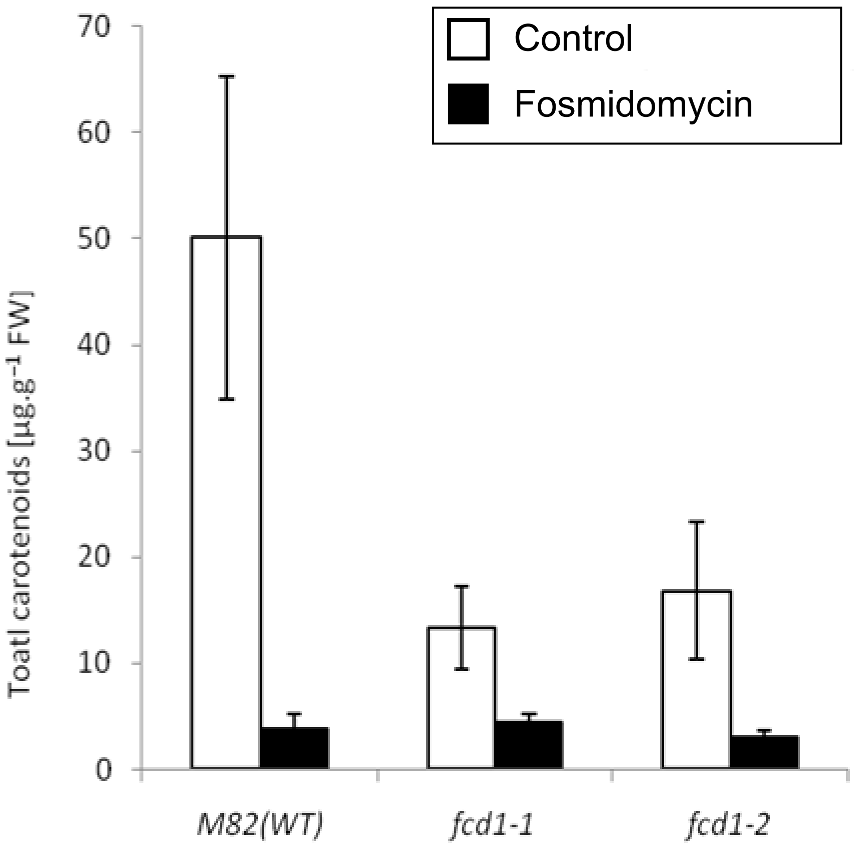






Inhibition of plastidial IPP synthesis in fruit

Total carotenoids in fruit explants



M82

Fosmidomycin

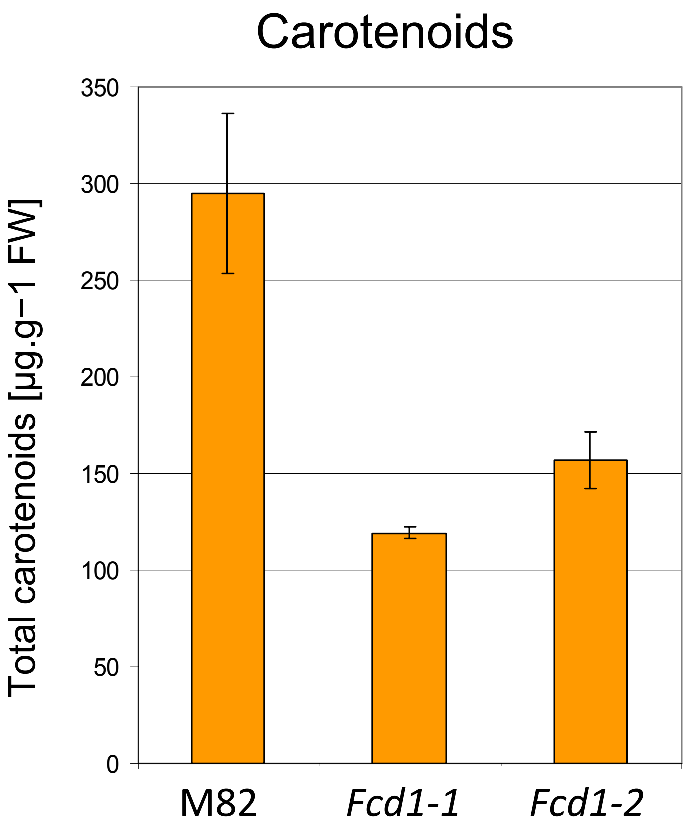
Control

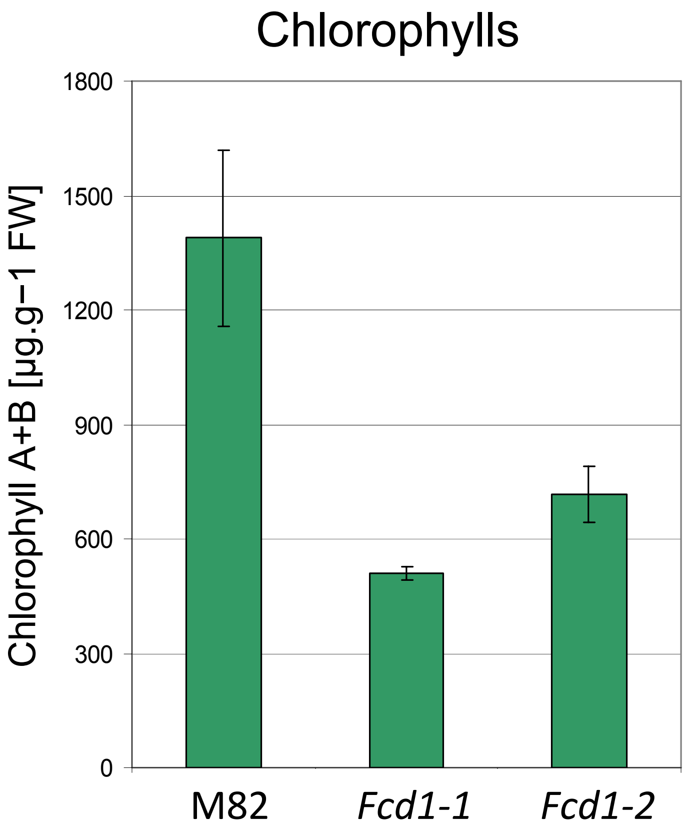
Fcd1

Fosmidomycin

Control

IDI1 is required in cotyledons





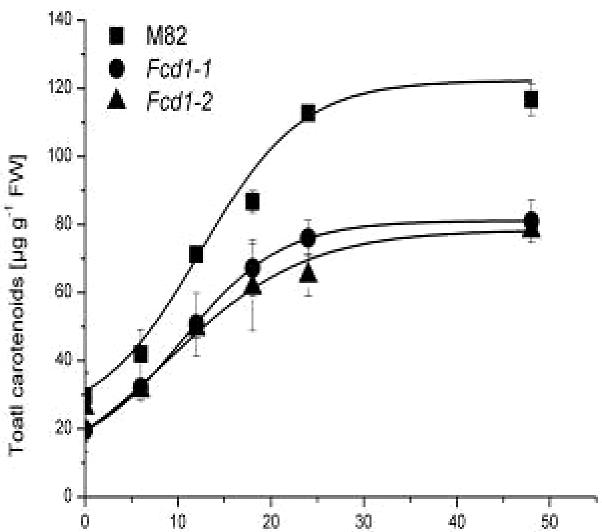
IDI1 is required during deetiolation

Etiolated seedling (14 days dark)

Light

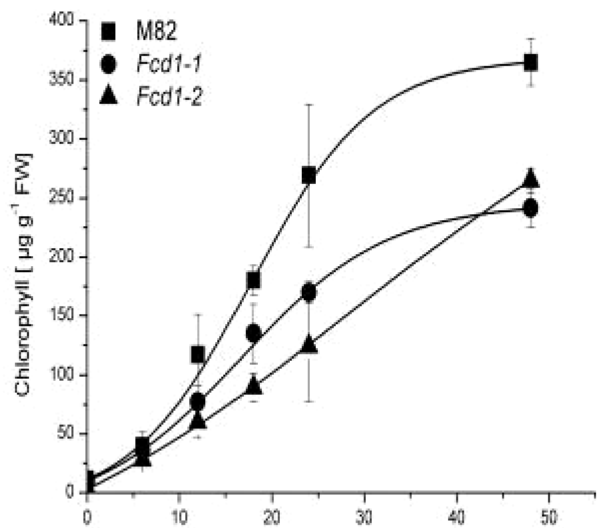
De-etiolation

Carotenoid accumulation



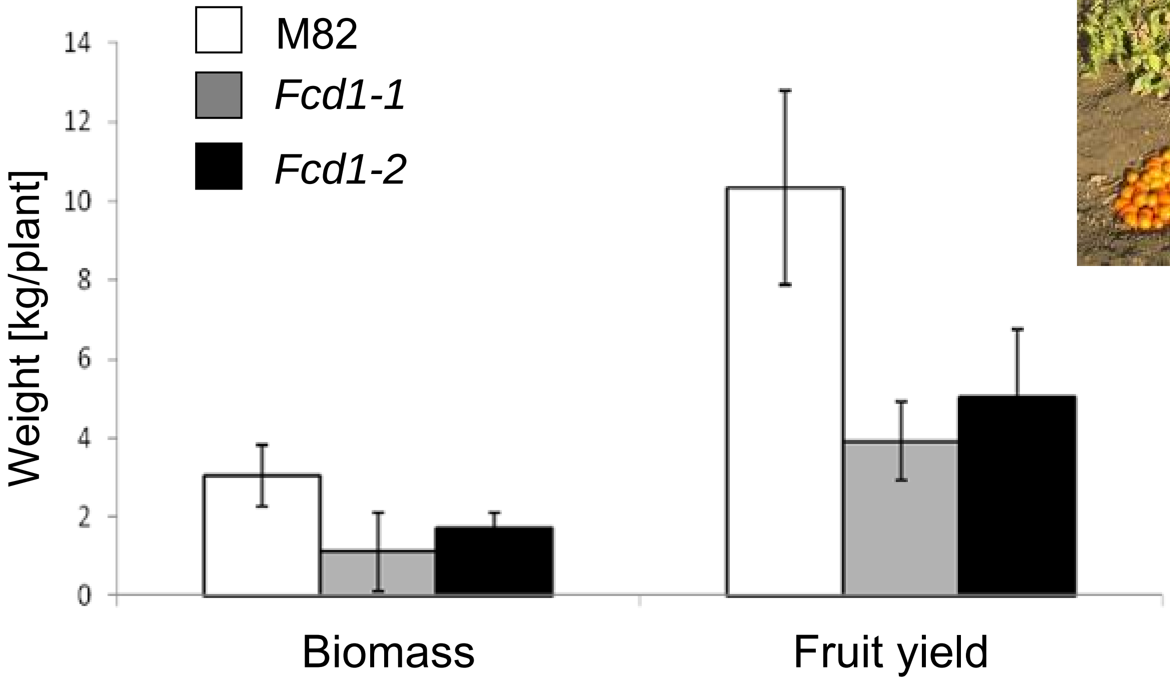
Hours after exposure to light

Chlorophyll accumulation

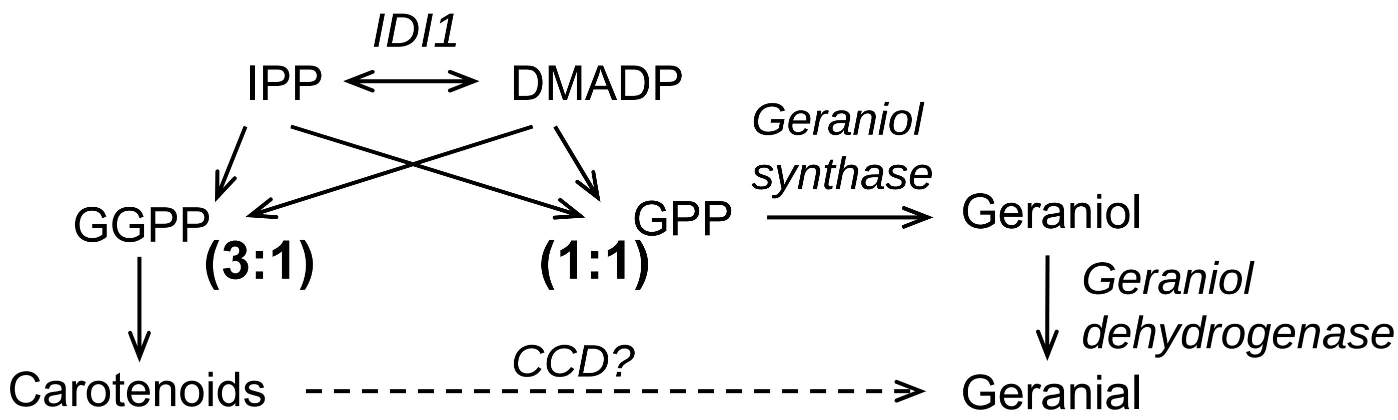


Hours after exposure to light

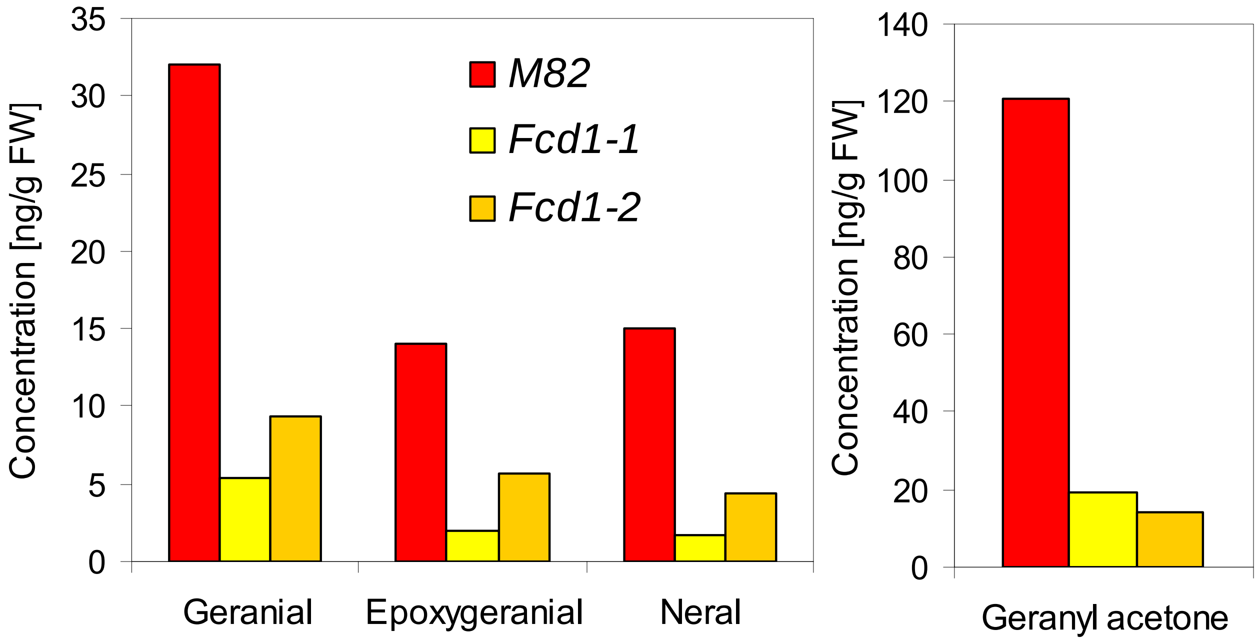
Reduced yield in *Fcd1* mutants



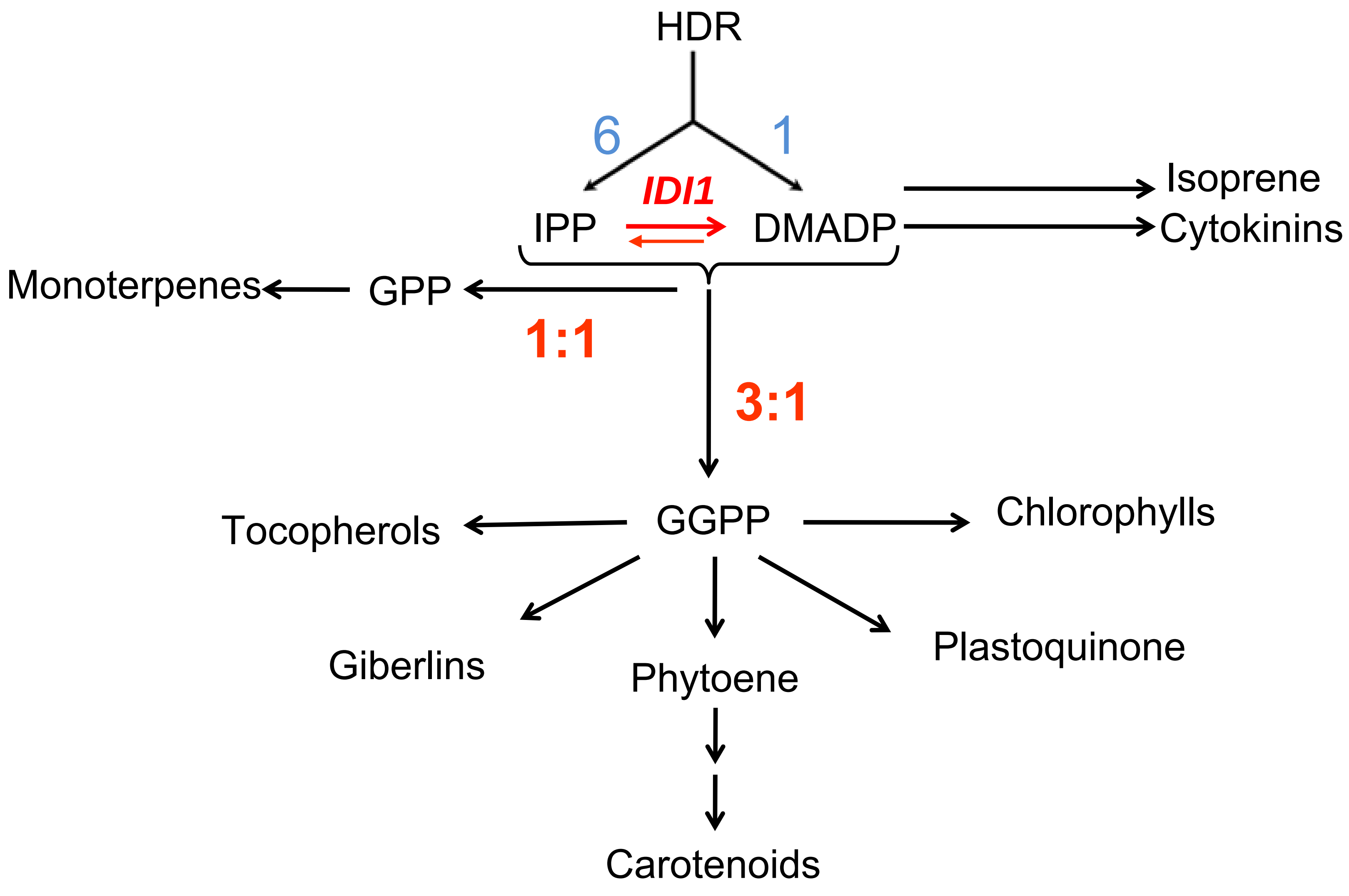
Alterations in fruit volatiles in *Fcd1*



Headspace solid phase micro-extraction GC-MS (SPME-GC-MS)



IDI1 regulates isoprenoid production by adjusting the IPP:DMADP ratio



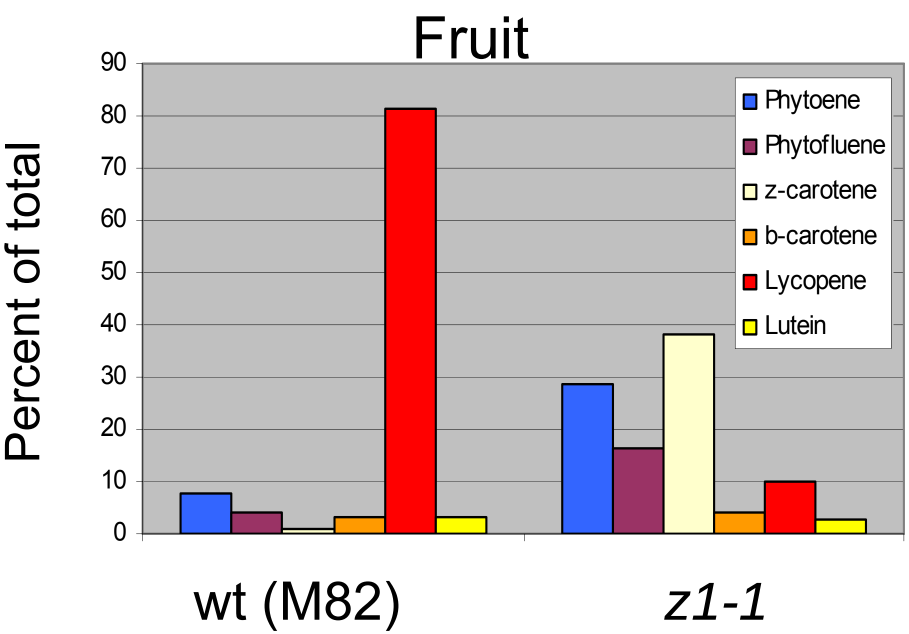
Conclusions from *Fcd1* characterization

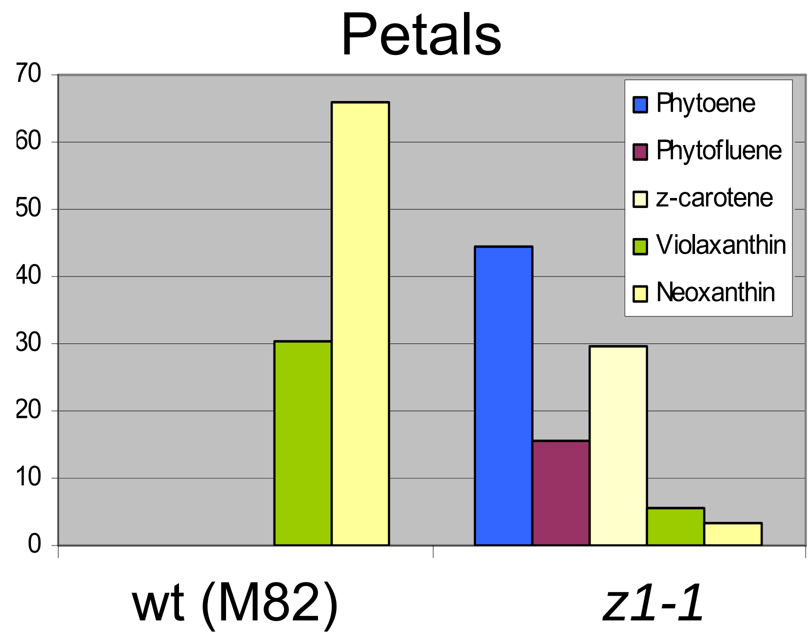
- There are two IPP isomerase enzymes in tomato- IDI1 in the plastids and IDI2 in the cytoplasm.
- IDI2 is mainly expressed in vegetative tissues and is probably dispensable during fruit ripening.
- F*cd1* impairs the plastidial IPP isomerase (IDI1).
- Cytoplasmic IPP does not contribute to carotenoid biosynthesis.
- **IDI1 is required to adjust the ratio of IPP:DMAPP in cases of high flux of isoprenoids toward GGPP, such as during carotenoid accumulation in chromoplasts and carotenoid and chlorophyll biosynthesis in developing leaves.**

The mutant *zeta* (*z*) accumulates ζ-carotene

wt (M82) *z-1*

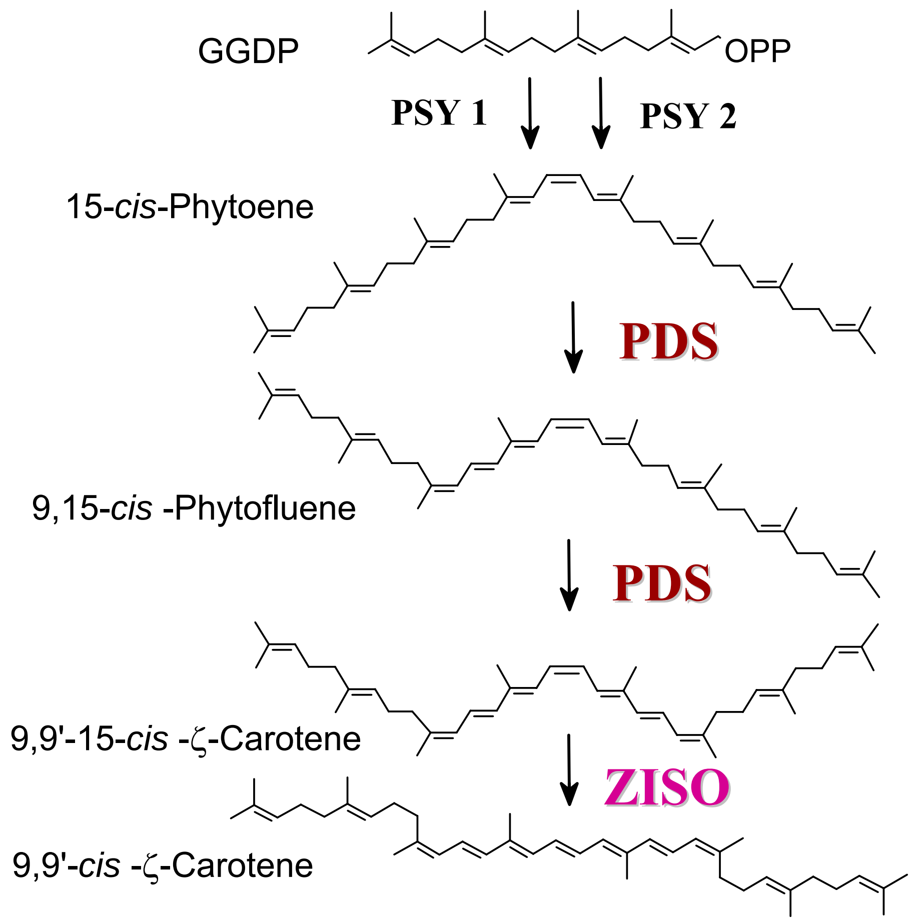
wt (M82) *z-1*



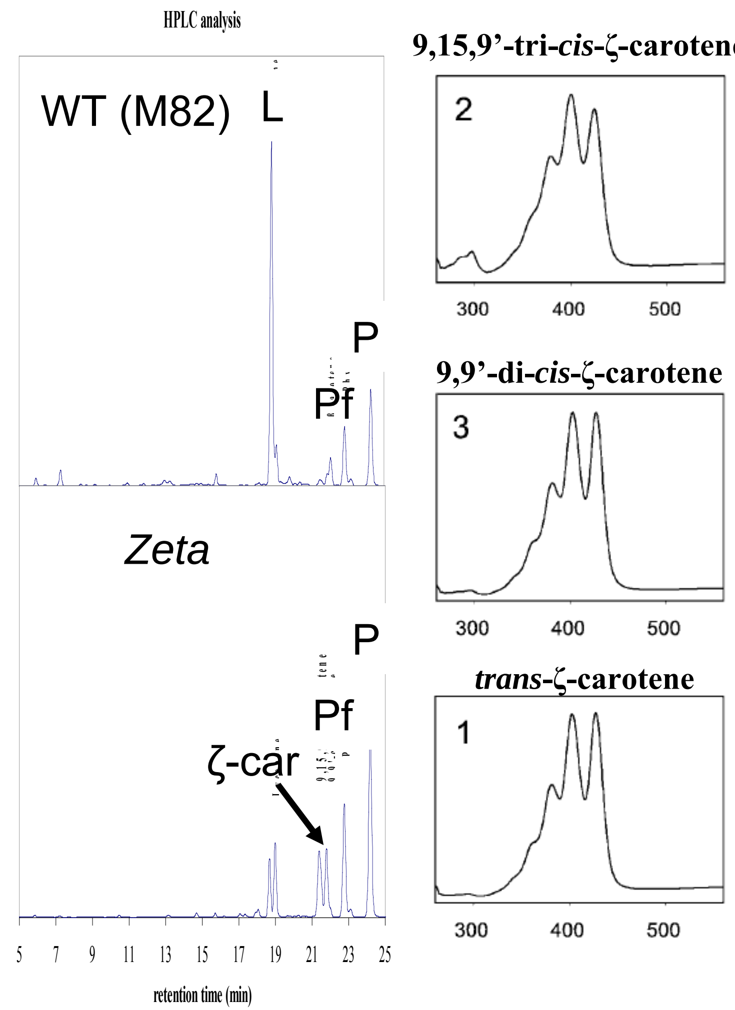


Shira Glasner, Chen Gafni-Amsalem

Zeta impairs the 15-*cis* ζ-carotene isomerization



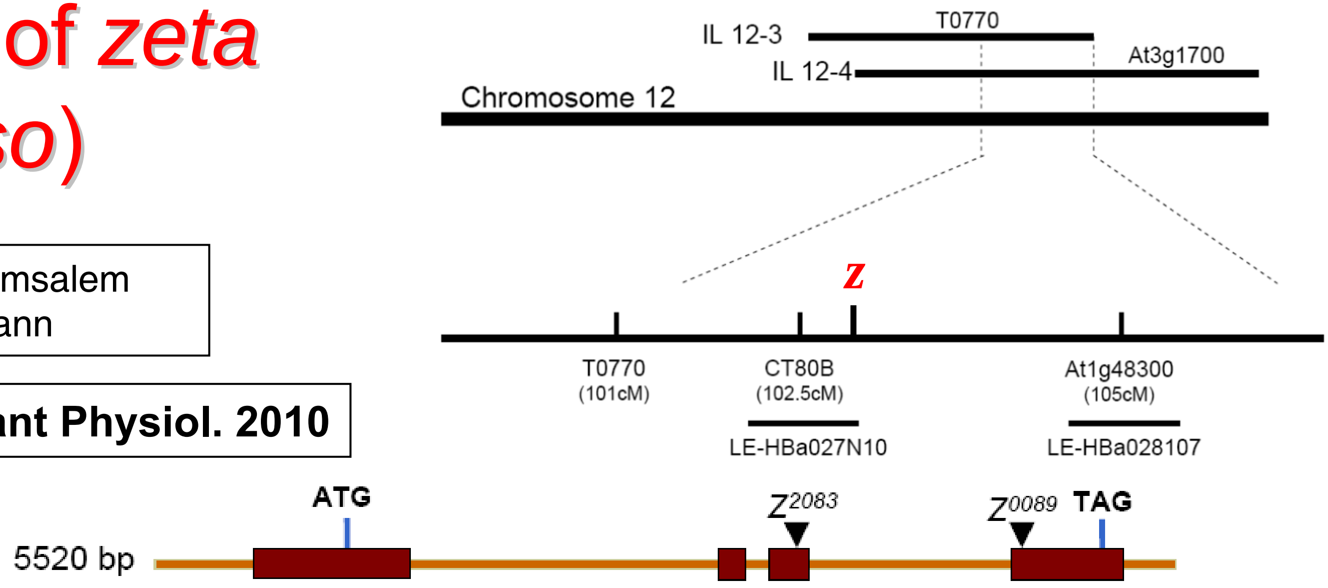
HPLC analysis of fruit carotenoids



Cloning of *zeta* (*Ziso*)

Chen Gafni-Amsalem
Varda Mann

Chen et al. Plant Physiol. 2010

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          1                                                                          75
Ziso M82  MATSIFLSHPFSHLLSKHHKIPSPKQTIAIAYHSTNKPTTKTPFLPLPTSFFPFPSNPRKEFWPISVGRTQTDEK
ZISO 89   MATSIFLSHPFSHLLSKHHKIPSPKQTIAIAYHSTNKPTTKTPFLPLPTSFFPFPSNPRKEFWPISVGRTQTDEK
Ziso2083  MATSIFLSHPFSHLLSKHHKIPSPKQTIAIAYHSTNKPTTKTPFLPLPTSFFPFPSNPRKEFWPISVGRTQTDEK

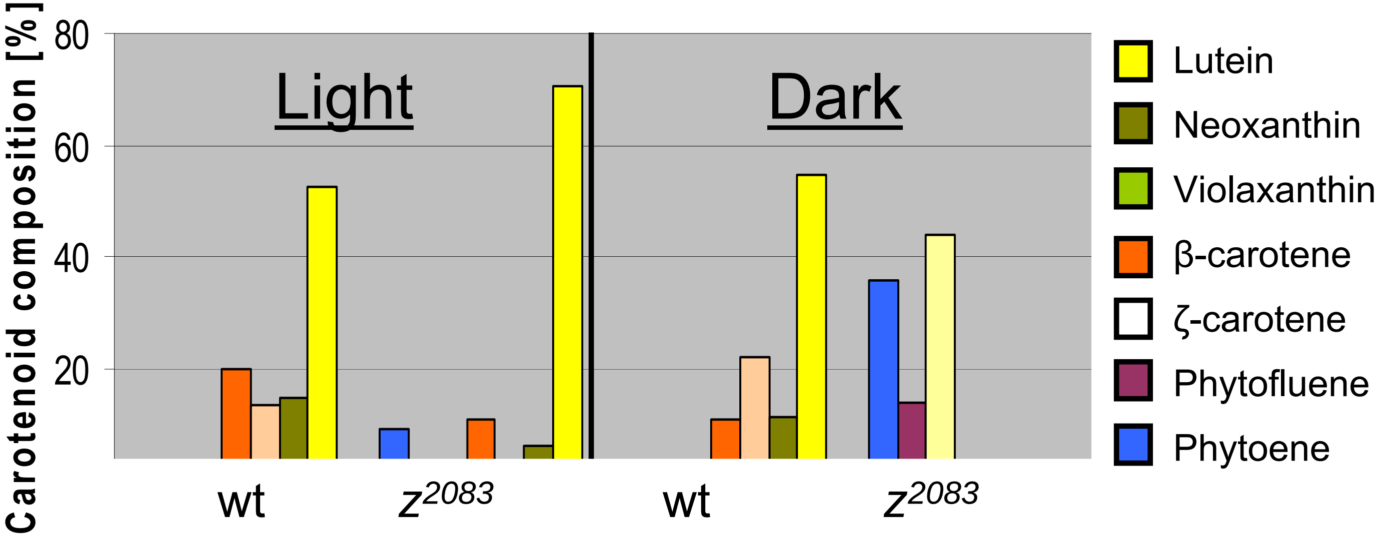
          76                                                                        150
Ziso M82  DEILVVGEDSAEFELSKQKISSWVYFAGVLGVVLYVLNVVWIDNSTGFGKSFIDSVSSISDSPEIVMLSLTLIFA
ZISO 89   DEILVVGEDSAEFELSKQKISSWVYFAGVLGVVLYVLNVVWIDNSTGFGKSFIDSVSSISDSPEIVMLSLTLIFA
Ziso2083  DEILVVGEDSAEFELSKQKISSWVYFAGVLGVVLYVLNVVWIDNSTGFGKSFIDSVSSISDSPEIVMLSLTLIFA

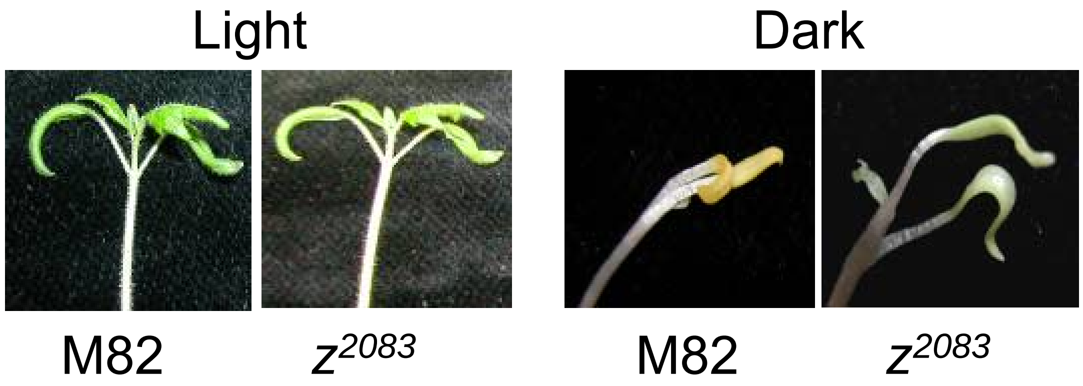
          151                                                                       225
Ziso M82  IVHSGLASLRDKGEELIGERAFRVLFAGVSLPLAVSTIVYFINHRYDGVQLWQLNSVAGIHELVWISNFVSFFFL
ZISO 89   IVHSGLASLRDKGEELIGERAFRVLFAGVSLPLAVSTIVYFINHRYDGVQLWQLNSVAGIHELVWISNFVSFFFL
Ziso2083  IVHSGLASLRDKGEELIGERAFRVLFAGVSLPLAVSTIVYFINHRYDGVQLWQLNSVAGIHELVWISNFVSFFFL

          226                                                                       300
Ziso M82  YPSTFNLLEVAAVDKPKMHLWETGIMRITRHPQLVGQVIWCLAHTLWIGNSVAVAASVGLIGHHLFGAWNGDRRL
ZISO 89   YPSTFNLLEVAAVDKPKMHLWETGIMRITRHPQLVGQVIWCLAHTLWIGNSVAVAASVGLIGHHLFGA*
Ziso2083  YPSTFNLLEVAAVDKPKMHL*

          301                                                                  369
Ziso M82  AIRYGEAFEVVKNRTSIIPFAAILDGRQKLPEDYYKEFIRLPYLSITTLTLGAYFLHPIMQAASYRLHW
```

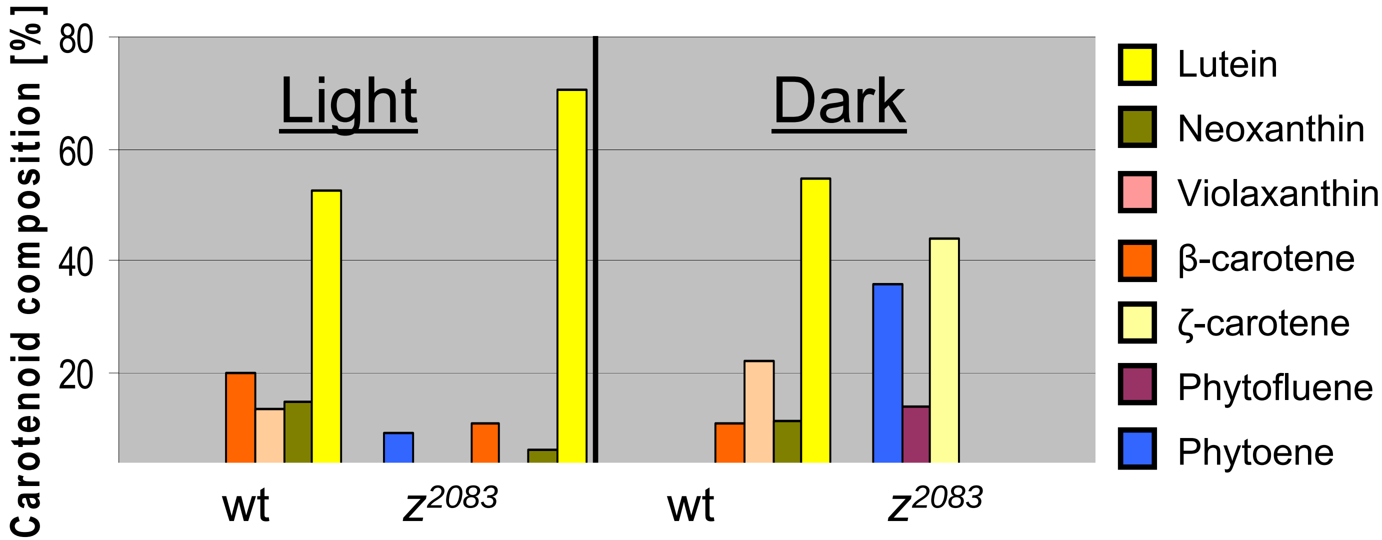
Light-driven isomerization of ζ-carotene in *zeta* leaves





Chen Gafni-Amsalem

Photosynthesis-generated redox compensates for the mutation *zeta*

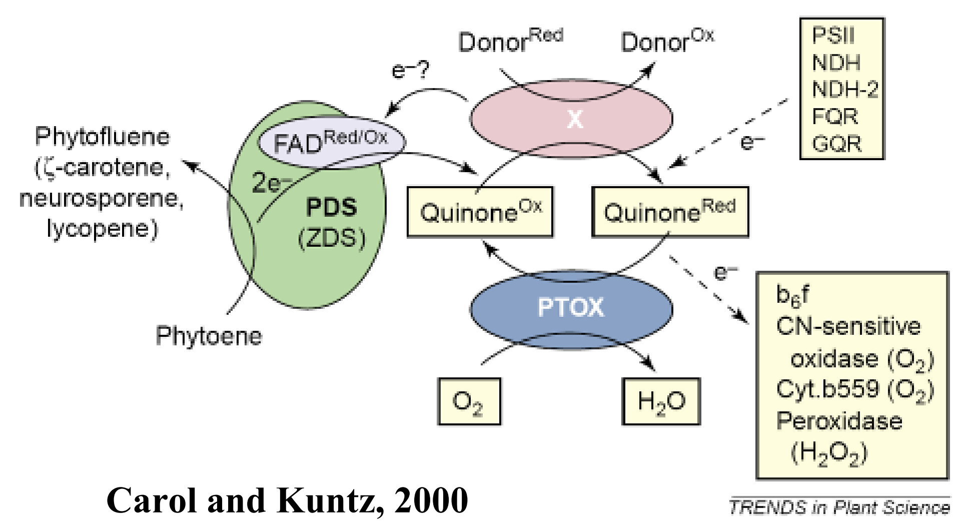


15,9,9'-*cis* ζ-Carotene

↓ **ZISO**

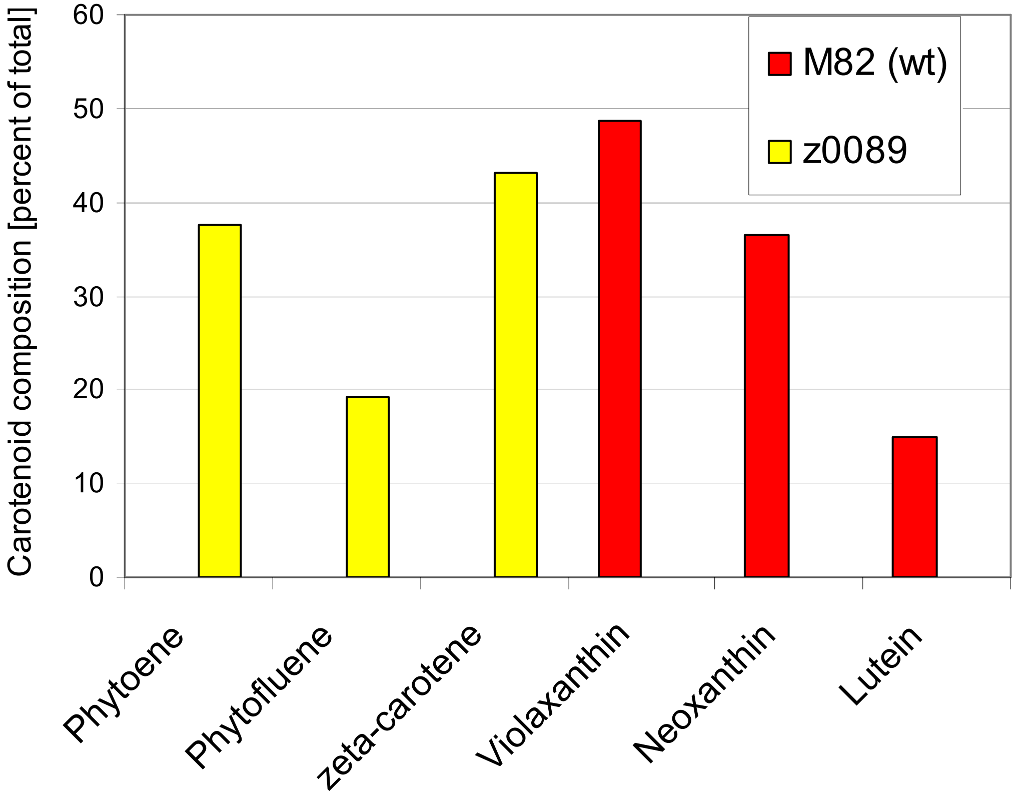
9,9'-*cis* ζ-Carotene

? ⟷



Carol and Kuntz, 2000

Carotenoid in roots of *zeta* plants

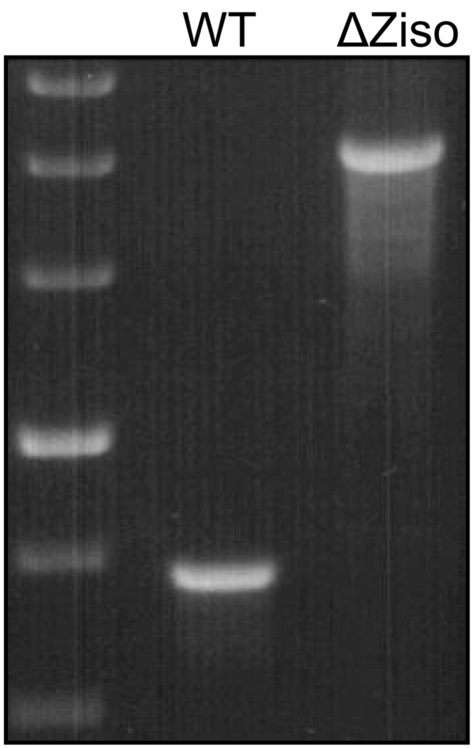


A Ziso-like gene exists in cyanobacteria

```
                      1                                                          60
 Ziso 7942     (1)    ------------------------------------------------------------
Ziso M82 f     (1)    MATSIFLSHPFSHLLSKHHKIPSPKQTIAIAYHSTNKPTTKTPFLPLPTSFFPFPSNPRK
                      61                                                        120
 Ziso 7942     (1)    ------------------------------------------------------------
Ziso M82 f    (61)    EFWPISVGRTQTDEKDEILVVGEDSAEFELSKQKISSWVYFAGVLGVVLYVLNVVWIDNS
                      121                                                       180
 Ziso 7942     (1)    ---------MPLSWWTPSHTIMLALLLLFAIAHSGLAALRPWGETKIGARGYRILFALVS
Ziso M82 f   (121)    TGFGKSFIDSVSSISDSPEIVMLSLTLIFAIVHSGLASLRDKGEELIGERAFRVLFAGVS
                      181                                                       240
 Ziso 7942    (52)    LPLAVVTISYFILHRYDGALLWQLQGIPWIAPLVWVLTAISFLLLYPATFNLLEIAAIAQ
Ziso M82 f   (181)    LPLAVSTIVYFINHRYDGVQLWQLNSVAGIHELVWISNFVSFFFLYPSTFNLLEVAAVDK
                      241                                                       300
 Ziso 7942   (112)    PQVRLYETGITRITRHPQTFGQIILWCLAHSLWLGTSFMMVASAGLIAHHLFSIWHGDRRL
Ziso M82 f   (241)    PKMHLWETGIMRITRHPQLVGQVIWCLAHTLWIGNSVAVAASVGLIGHHLFGAWNGDRRL
                      301                                                       360
 Ziso 7942   (172)    QKRYGEAFEALKSRTSIIPFLAIAQGKQTLVWKEFLRPAYLGVAIAIGLFWFAHRWIPQA
Ziso M82 f   (301)    AIRYGEAFEVVKNRTSIIPFAAILDGRQKLPEDYYKEFIRLPYLSITTLTLGAYFLHPIM
                      361
 Ziso 7942   (232)    TAALAEIGW
Ziso M82 f   (361)    QAASYRLHW
```

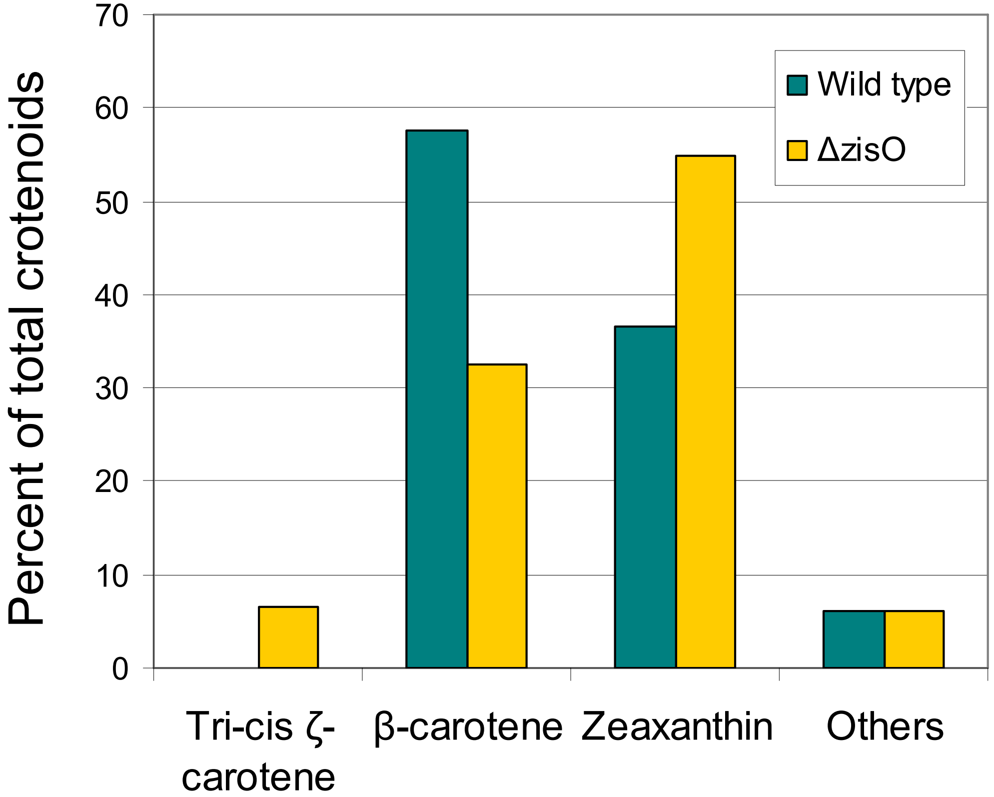
ZISO is a 15-*cis*-ζ-carotene in cyanobacteria

Knockout of *Ziso* in
S. elongatus PCC7942



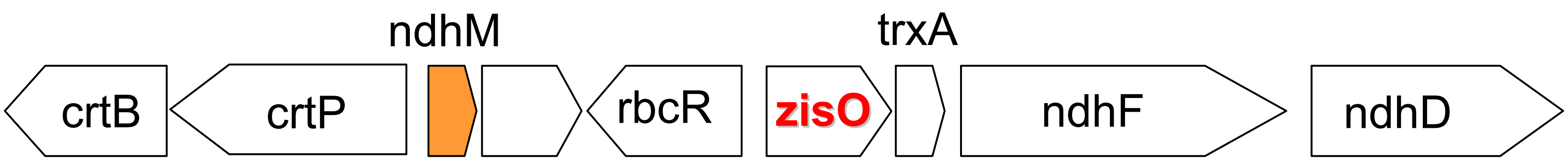
Varda Mann

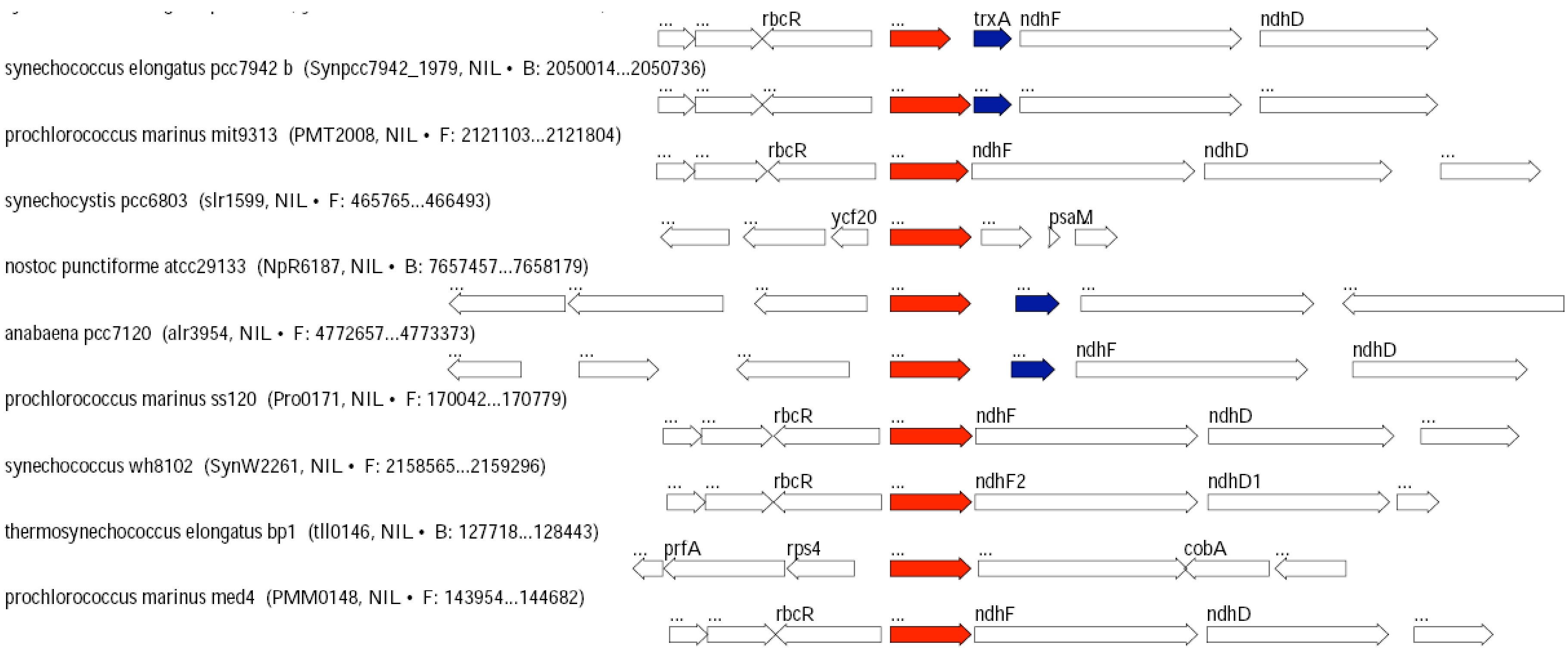
Carotenoid composition in
S. elongatus PCC7942



Ziso in cyanobacteria is part of a "redox operon"

Location of *zisO* in the genome of *S. elongatus* PCC7942



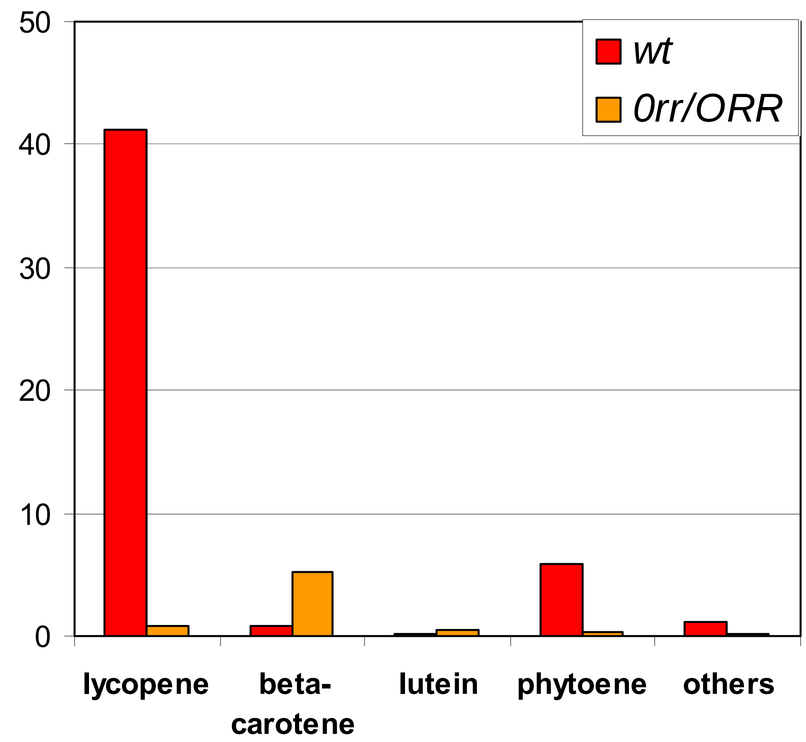


A tomato mutant deficient of NDH-M

Nashilevitz et al. The Plant Cell, 2010

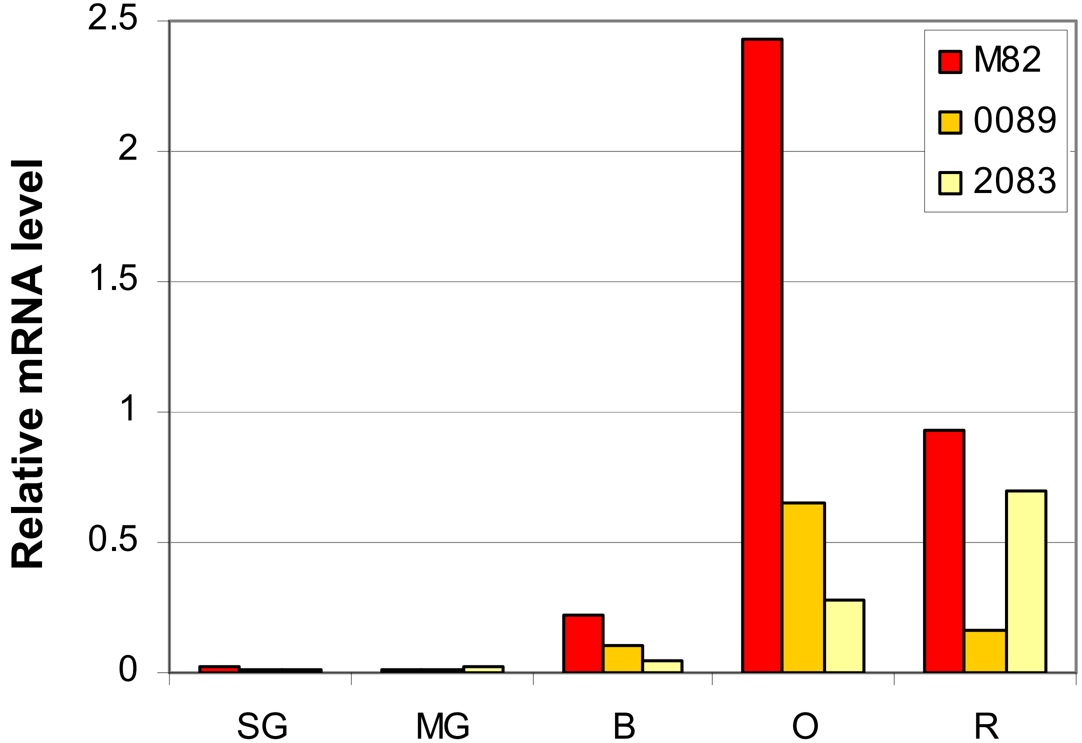
A mutation in subunit M of NAD(P)H oxidoreductase provided evidence that specialized (secondary) metabolism in tomato fruit, including carotenoid biosynthesis, depends on the complex activity that modulates the redox state of PQ.

Orr^Ds/ORR *ORR/ORR*



Expression of Ziso is upregulated during fruit ripening

Relative mRNA of *Ziso* was determined in fruits of M82 and two alleles of *zeta*.



Stages in fruit development. SG, small green; MG, mature green; B, Breaker; O, orange; R, ripe.

Conclusions on Ziso

- ZISO is a trans-membrane protein, possibly a transporter, that provides a redox-related functions essential for *cis-trans* isomerization of 15,15' ζ-carotene.
- Photosynthetic redox can compensate for ZISO deficiency.
- ZISO is indispensable in non-photosynthetic tissues (fruit, flower, root).
- **ZISO underscores the involvement of redox in plastid secondary metabolism.**

Open questions in carotenoid biosynthesis

1. Biochemistry

- Mechanism of reactions (structure-function)
- Do enzyme function in complexes?
- Where exactly in the plastids?

2. Regulation

- Gene expression (developmental; environmental)
- Metabolic regulation at the enzymatic level
- Interaction with other metabolic pathways

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