Plant Mol Biol (2006) 62:453–469
DOI 10.1007/s11103-006-9033-y

Sucrose deficiency delays lycopene accumulation in tomato fruit pericarp discs

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Received: 24 February 2006 / Accepted: 13 June 2006 / Published online: 17 August 2006

Abstract Tomato (*Solanum lycopersicum*) fruit ripening is characterized by a massive accumulation of carotenoids (mainly lycopene) as chloroplasts change to chromoplasts. To address the question of the role of sugars in controlling carotenoid accumulation, fruit pericarp discs (mature green fruits) were cultured in vitro in the presence of various sucrose concentrations. A significant difference in soluble sugar content was achieved depending on external sucrose availability. Sucrose limitation delayed and reduced lycopene and phytoene accumulation, with no significant effect on other carotenoids. Chlorophyll degradation and starch catabolism were not affected by variations of sucrose availability. The reduction of lycopene synthesis observed in sucrose-limited conditions was mediated through metabolic changes illustrated by reduced hexose accumulation levels. In addition, variations of sucrose availability modulated *PSY1* gene expression. Taken together our results suggest that the modulation of carotenoid accumulation by sucrose availability occurs at the metabolic level and involves the differential regulation of genes involved in carotenoid biosynthesis.

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Keywords Tomato fruit · Isoprenoids · Carotenoid · Lycopene · Sucrose · Pericarp discs

Abbreviations

ACS	1-aminocyclopropane 1-carboxylic acid synthase
B	breaker
cnr	colourless non-ripening
dpa	days post-anthesis
del	delta
DMAPP	dimethyl-allyl-pyrophosphate
DXS	1-deoxy-D-xylulose-5-phosphate synthase
GABA	γ-amino butyric acid
GA3P	glyceraldehyde-3-Phosphate
GGPP	geranylgeranyl pyrophosphate
GGPS	geranylgeranyl pyrophosphate synthase
Glc	glucose
HDR	1-hydroxy-2-methyl-(E)-butenyl-4-phosphate reductase
hp	high pigment
IPP	isopentenyl-pyrophosphate
LCY-B	lycopene β-cyclase
LCY-E	lycopene ϵ-cyclase
MEP	2-C-methyl-D-erythritol
MG	mature green
nor	non-ripening
Nr	never ripe
PDS	phytoene desaturase
PSY	phytoene synthase
RR	red ripe
rin	ripening inhibitor
T	turning
ZDS	ζ-carotene desaturase

Introduction

Carotenoids are a widespread class of isoprenoid-derived molecules produced by photosynthetic organisms, fungi and some bacteria. In plants, they are synthesized in chloroplasts and play essential functions as accessory pigments in light harvesting and dissipating excess excitation energy of chlorophylls (Niyogi 1999). Carotenoids also accumulate to high levels in fruit and flower chromoplasts, contributing to the characteristic red, orange or yellow colours of these organs. These colours are involved in animal and insect attraction for pollination and seed dispersal. In human nutrition, they may protect against certain cancers and may prevent eye degenerative disease (Jonhson 2002).

A general scheme of the carotenoid biosynthetic pathway is shown in Fig. 1. Carotenoid biosynthesis requires the synthesis of isopentenyl-pyrophosphate (IPP), the common precursor of all isoprenoids, following the 2-C-methyl-D-erythritol (MEP) pathway (Lichtenthaler 1999). IPP is sequentially condensed with its isomer dimethyl-allyl-pyrophosphate (DMAPP) leading to the formation of geranylgeranyl pyrophosphate (GGPP).

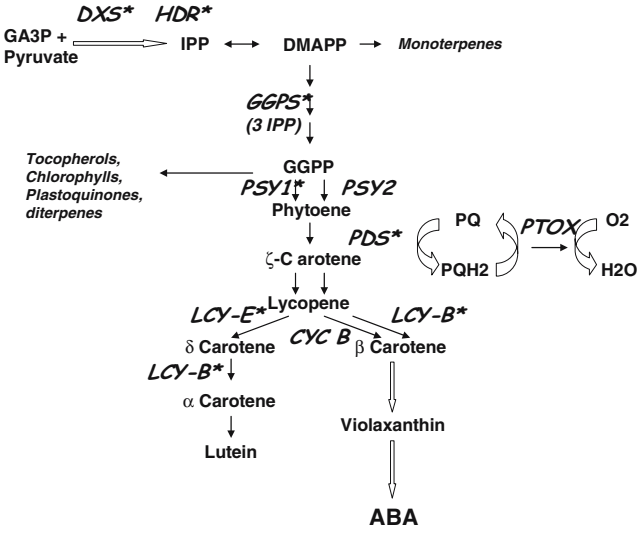

Fig. 1 Overview of the carotenoid metabolic pathway localised into the tomato plastid. Solid arrows represent single step reactions and open arrows indicate multiple enzymatic steps. Expression of the genes marked with * have been analyzed in this study. The following enzyme and intermediate compounds are represented: DXS: 1-deoxy-D-xylulose-5-Phosphate synthase, HDR: 1-hydroxy-2-methyl-2-(E)-butenyl-4-phosphate reductase, GGPS: geranylgeranyl pyrophosphate synthase, PSY: phytoene synthase, PDS: phytoene desaturase, PTOX: plastid terminal oxidase, LCY-E: lycopene-epsilon cyclase, LCY-B: lycopene beta cyclase, CYC B: lycopene beta cyclase, GA3P: glyceraldehyde-3-phosphate, IPP: isopentenyl pyrophosphate, DMAPP: dimethylallyl pyrophosphate, GGPP: geranylgeranyl pyrophosphate, ABA: abscissic acid

The first committed step of carotenoid synthesis is the head-to-head condensation of two GGPP molecules, catalysed by phytoene synthase (PSY). The product of this reaction, phytoene, is an acyclic colourless compound. Phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) then catalyse four successive desaturations of phytoene leading to the synthesis of the red acyclic compound lycopene. Finally, lycopene is metabolized by lycopene cyclases producing α or β carotene that are precursors of xanthophylls and abscissic acid (for a review see Cunningham and Gantt 1998).

During tomato fruit ripening, the total carotenoid content increases more than 15-fold, mainly due to a massive accumulation of lycopene and to a lower extent β-carotene (Fraser et al. 1994). So far, most of the enzymatic steps of the carotenoid pathway have been elucidated in tomato using combined molecular, biochemical and genetic approaches (Hirschberg 2001; Bramley 2002). Genes encoding the first and the last enzyme of the MEP pathway, the deoxyxylulose-5-P-synthase (DXS) and the hydroxybutenyl diphosphate reductase (HDR), and a geranylgeranyl pyrophosphate synthase (GGPS), are up-regulated during fruit ripening (Lois et al. 2000; Botella-Pavia et al. 2004; Bartley and Ishida 2003) which is consistent with the higher requirement for isoprenoid precursors at this developmental stage. *PSY1*, one of the two tomato genes encoding phytoene synthase is also strongly induced during fruit ripening (Ray et al. 1992). Additionaly, flux measurements have shown that phytoene synthase is a major control point of the carotenoid biosynthesis in tomato fruits (Fraser et al. 2002). *PDS* gene expression also increases (Pecker et al. 1992; Corona et al. 1996) as well as the expression of the genes *CRT ISO* and *PTOX* encoding respectively a carotene isomerase and a plastid alternative oxidase necessary for phytoene desaturation (Isaacson et al. 2002; Josse et al. 2000). In contrast to the up-regulation of genes involved in lycopene synthesis those encoding lycopene β-cyclase (*LCY-B*) and ϵ-cyclase (*LCY-E*) are down regulated (Fig. 1, Pecker et al. 1996; Ronen et al. 1999). The differential regulation of the genes involved in carotenoid biosynthesis has been taken as evidence that the accumulation of lycopene in tomato fruit is primarily controlled at the level of gene expression (Ronen et al. 1999). Additional evidence that transcriptional regulation is the main mechanism that controls lycopene accumulation during ripening comes from the identification of novel isoform of lycopene β-cyclase encoded by the *B* gene. The *B* gene is transiently expressed at the onset of ripening and is involved in β carotene accumulation during tomato fruit maturation (Ronen et al. 2000).