Archives of Biochemistry and Biophysics 539 (2013) 110–116

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Biosynthesis of carotenoids in carrot: An underground story comes to light

Manuel Rodriguez-Concepcion [a,*], Claudia Stange [b,*]

[a] Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, 08193 Barcelona, Spain
[b] Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Casilla 653, Ñuñoa, Santiago, Chile

ARTICLE INFO

Article history:
Available online 19 July 2013

Keywords:
Biosynthesis
Carotenoid
Carrot
Development
Gene expression
Light
Regulation
Root

ABSTRACT

Carrot (*Daucus carota*) is a biannual plant that accumulates massive amounts of carotenoid pigments in the storage root. Although the root of carrot plants was white before domestication, intensive breeding generated the currently known carotenoid-rich varieties, including the widely popular orange carrots that accumulate very high levels of the pro-vitamin A carotenoids β-carotene and, to a lower extent, α-carotene. Recent studies have shown that the developmental program responsible for the accumulation of these health-promoting carotenes in underground roots can be completely altered when roots are exposed to light. Illuminated root sections do not enlarge as much as dark-grown roots, and they contain chloroplasts with high levels of lutein instead of the β-carotene-rich chromoplasts found in underground roots. Analysis of carotenoid gene expression in roots either exposed or not to light has contributed to better understand the contribution of developmental and environmental cues to the root carotenoid profile. In this review, we summarize the main conclusions of this work in the context of our current knowledge of how carotenoid biosynthesis and accumulation is regulated at transcriptional and post-transcriptional levels in carrot roots and other model systems for the study of plant carotenogenesis such as Arabidopsis de-etiolation and tomato fruit ripening.

Introduction

Daucus carota var sativus (2*n* = 18), the domesticated carrot, is a biennial plant of the botanical group Umbelliferae (or Apiaceae) that also includes parsley (*Petroselinum hortense*), celery (*Apium graveolens*), anise (*Pimpinella anisum*), caraway (*Carum carvi*), dill (*Anethum graveolens*), and thousands of other species. But unlike most plants, the storage root of many carrot cultivars displays a characteristic color due to the accumulation of high levels of carotenoids. Young carrot roots are pale but after the first month of growth they start accumulating carotenoids to reach highest levels in about 3 months, just before secondary growth is completed [1–3]. It is likely that wild carrot plants had uncolored roots of a bitter taste and a woody core but were initially cultivated because of their aromatic leaves and seeds. Carrot domestication probably took place around the 10th century [4] but despite intensive breeding procedures since the 19th century, the background structure coming from demographic and early cultivation history still persists in currently cultivated carrot germplasm [5]. At present, carrots (i.e. mature *D. carota* roots) are available in a range of colors, although orange varieties are most popular. Even though the high carotene content in carrots makes them one of the richest pro-vitamin A sources in the human diet, the mechanisms regulating their production remained poorly known until recently. In this review, we will summarize recent advances in our knowledge of how carotenoid biosynthesis is orchestrated in this unique system.

Genes and enzymes of the carotenoid pathway in carrot

Carotenoids are a group of isoprenoid molecules synthesized by all photosynthetic organisms (including plants) and some non-photosynthetic fungi and bacteria [6]. Their characteristic yellow, orange, and red colors are due to the presence of a number of conjugated double bonds in a polyene chain that functions as a chromophore. The hundreds of carotenoid structures known to date can be divided into two major groups (Fig. 1): carotenes (non-oxygenated molecules) and xanthophylls (oxygenated carotenoids). The core pathway for the biosynthesis of the major carotenoid species found in plants is well established [6–8]. They are produced in plastids from isoprenoid precursors supplied by the MEP pathway [8]. The first committed step in carotenoid biosynthesis (Fig. 1) is the production of 15-*cis*-phytoene catalyzed by the enzyme phytoene synthase[1] (PSY). This colorless carotenoid is then desaturated and isomerized to form the reddish all-*trans* lycopene by the

* Corresponding authors.
E-mail addresses: manuel.rodriguez@cragenomica.es (M. Rodriguez-Concepcion), cstange@uchile.cl (C. Stange).

[1] *Abbreviations used:* PSY, phytoene synthase; PDS, phytoene desaturase; ZISO, 15-cis-ζ–carotene isomerase; ZDS, ζ–carotene desaturase; LCYB, lycopene β cyclase; LCYE, lycopene ε cyclase; CHYB, carotenoid β hydroxylase.

0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.07.009

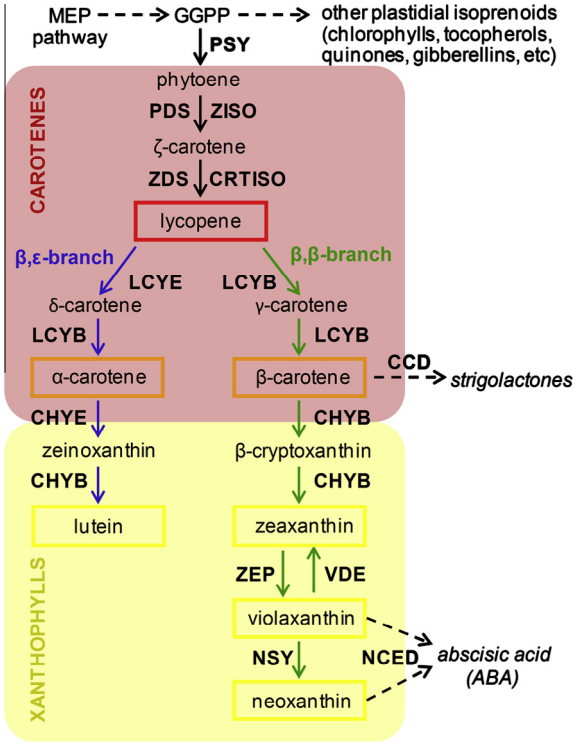

Fig. 1. Pathway for carotenoid biosynthesis in plants. MEP, methylerythritol 4-phosphate; GGPP, geranylgeranyl diphosphate. Dashed arrows represent multiple steps. The main carotenoids found in plant tissues are boxed. Enzymes are indicated in bold. PSY, phytoene synthase; PDS, phytoene desaturase; ZISO, 15-*cis*-ζ–carotene isomerase; ZDS, ζ–carotene desaturase; CRTISO, carotenoid (pro-lycopene) isomerase; LCYB, lycopene β cyclase; LCYE, lycopene ε cyclase; CHYB, carotenoid β hydroxylase; CHYE, carotenoid ε hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin deepoxidase; NSY, neoxanthin synthase; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase.

Table 1
Genes and proteins of the carotenoid pathway in cultivated carrot (*Daucus carota var. sativa*). Adapted from [12]. N.I., not identified; P.S., partial sequence.

Enzyme	Name	cDNA	Protein	Length (aa)
Phytoene synthase	PSY1	DQ192186	ABB52067	398
	PSY2	DQ192187	ABB52068	438
Phytoene desaturase	PDS	DQ222429	ABB52082	573
15-cis-ζ–carotene isomerase	ZISO	N.I.	N.I.	
ζ–Carotene desaturase	ZDS1	DQ222430	ABB52083	573
	ZDS2	DQ192189	ABB52070	575
Carotenoid isomerase	CRTISO	DQ192188	ABB52069	615
Lycopene β-cyclase	LCYB1	DQ192190	ABB52071	508
	LCYB2	DQ192191	ABB52072	492
Lycopene ε-cyclase	LCYE	DQ192192	ABB52073	530
Carotenoid β-hydroxylase (BCH)	CHYB1	DQ192193	ABB52074	309
	CHYB2	DQ192194	ABB52075	303
	CHYB3	DQ192195	P.S.	
Carotenoid ε-hydroxylase (CYP97)	CHYE	DQ192196	ABB52076	548
Zeaxanthin epoxidase	ZEP	DQ192197	ABB52077	668
Violaxanthin de-epoxidase	VDE	DQ192198	P.S.	
Neoxanthin synthase	NSY	N.I	N.I	
9-cis-epoxycarotenoid dioxigenase	NCED1	DQ192200	ABB52078	573
	NCED2	DQ192201	ABB52079	588
	NCED3	DQ192202	ABB52080	588
Carotenoid cleavage dioxigenase	CCD1	DQ192203	ABB52081	547
	CCD2	DQ192204	P.S.	
	CCD3	DQ192205	P.S.	

enzymes phytoene desaturase (PDS), 15-cis-ζ–carotene isomerase (ZISO), ζ–carotene desaturase (ZDS), and carotenoid (pro-lycopene) isomerase (CRTISO). Next, lycopene cyclases of the β (LCYB) or/and ε (LCYE) type produce two types of orange carotenes from lycopene: β-carotene (by the cyclization of the two ends of the lycopene molecule to form two β rings) or α-carotene (with one β ring in one end and one ε ring in the other). The two pathway branches that result from these cyclization reactions are named β,β-branch and β,ε-branch (Fig. 1). Hydroxylation of β-carotene by carotenoid β hydroxylase (CHYB) enzymes preferentially of the nonheme di-iron (BCH) type leads to the production of zeaxanthin, whereas hydroxylation of α-carotene carried out by β and ε hydroxylase (CHYB and CHYE) enzymes mainly of the cytochrome P450 (CYP97) type results in the production of the yellowish xanthophyll lutein. However, BCH-type CHYB enzymes show some activity toward the β and ε rings of α-carotene, and the CYP97 enzymes can also hydroxylate the β rings of β-carotene [9,10]. The β,β-branch produces additional xanthophylls such as violaxanthin, produced from zeaxanthin by the activity of zeaxanthin epoxidase (ZEP), and neoxanthin, produced from violaxanthin by a neoxanthin synthase (NSY) enzyme (Fig. 1). Violaxanthin deepoxidase (VDE) can transform violaxanthin back into zeaxanthin in the so called xanthophyll cycle. As shown in Fig. 1, some carotenoids can be cleaved by carotenoid cleavage dioxygenase (CCD) and 9-cis-epoxycarotenoid dioxygenase (NCED) enzymes to produce apocarotenoids such as the hormones strigolactones and abscisic acid (ABA) [11].

Table 1 summarizes the sequences showing homology to carotenoid biosynthesis genes in carrot [12]. No sequences encoding ZISO and NSY have been reported yet, whereas only partial sequences are available for VDE and some isoforms of CHYB (BCH type) and CCD enzymes (Table 1). The available information suggests that, similar to that found in the model plant *Arabidopsis thaliana* and other plants [7], only one carrot gene might encode the enzymes PDS, CRTISO, LCYE, ZEP, and VDE, whereas small gene families exist for carotenoid hydroxylases and dioxygenases (CHYB, CHYE, NCED, CCD). But unlike that observed in Arabidopsis, at least two isoforms are found in carrot for PSY, ZDS, and LCYB [7,12]. The presence of more than one PSY or LCYB enzymes is common in plants, but carrot might be one of the few plants with more than one gene encoding ZDS [6,7,13,14]. The presence of several enzyme isoforms in a particular organism often implies that different isozymes are involved in the production of carotenoids in specific plastid types. For example, the tomato PSY1 and LCYB2(-CYCB) isoforms are required for the production of carotenoids in the chromoplasts of ripe fruit, whereas PSY2 and LCYB1 are involved in the biosynthesis of carotenoids in chloroplasts of photosynthetic tissues [15–17]. The analysis of gene expression in carrot led to propose a preferential contribution of the PSY1, ZDS1, and LCYB2 isoforms to the production of carotenoids in chloroplasts of photosynthetic tissues, whereas the PSY2, ZDS2, and LCYB1 isoforms could be involved in the biosynthesis of carotenoids in chromoplasts of storage roots [1–3]. The model does not exclude that at least some isoforms could participate in carotenoid biosynthesis in different organs. This might be the case of LCYB1, which was recently shown to enhance carotenoid accumulation in both leaves and roots when overexpressed in carrot [18]. However, clear-cut evidence supporting this model in carrot is still missing. Most strikingly, the biochemical activity of the encoded sequences also awaits experimental demonstration in most cases. Thus, the carrot sequence annotated as CHYE (Table 1) shows homology to CYP97-type hydroxylases, which might also function as CHYB enzymes [9,10]. Also, it is possible that some of the identified isoforms of ZDS might not be active. A genome-wide search for sequences with homology to carotenoid biosynthetic enzymes, together with functional studies as the one recently demonstrating the enzymatic activity of the LCYB1 isoform [18], would be required to have a clearer picture of the structure of the carotenoid biosynthetic pathway in carrot. In this context, work in our labs is in progress to

functionally characterize the role of the two isoforms for PSY, LCYB, and ZDS that are found in carrot.

Carrot carotenoid profiles depend on genetic background, developmental program, and environmental signals

Although all plastid types synthesize carotenoids, their quantitative and qualitative profiles vary widely. In photosynthetic tissues, high levels of carotenoids accumulate in chloroplasts to contribute to light-harvesting and protect the photosynthetic apparatus against photooxidative damage caused by an excess of light energy [9,19–21]. Chloroplasts mainly accumulate lutein, β-carotene, and lower levels of β,β xanthophylls in most plant species. By contrast, completely different carotenoid profiles can be found in chromoplasts, which are plastids specialized in accumulating high levels of carotenoids in non-photosynthetic organs such as flowers, fruits, or seeds [6,11,13,22–24]. For example, the chromoplasts of marigold (*Tagetes erecta*) flowers mainly synthesize lutein, those of ripe tomato (*Solanum lycopersicum*) fruit accumulate high levels of lycopene, and maize (*Zea mays*) kernels are rich in zeaxanthin. The accumulation of carotenoids in reproductive organs contribute to their color and therefore functions to attract animals for pollination and seed dispersal [22,23]. Very low levels of carotenoids (typically lutein, β,β xanthophylls, and β-carotene) are present in other non-photosynthetic plastids such as the etioplasts of dark-grown seedlings, where they facilitate greening when soil-emerging seedlings perceive the light and de-etiolate [25,26], and the leucoplasts of roots, where they serve as precursors for the production of hormones such as strigolactones and ABA that can later be transported to aerial tissues to regulate development or trigger appropriate stress responses [27,28].

As indicated above, chromoplasts differentiate in the storage root of carrots during the late stages of growth underground. A remarkable diversity of carotenoid profiles are found in different *D. carota* varieties (Fig. 2) but also during root development and in response to light exposure (Fig. 3A). The level and type of the carotenoids produced and accumulated in storage roots result in white, yellow, orange or red carrots [29–34]. White carrots virtually lack carotenoids and only show traces of lutein and other carotenoids. Higher levels of lutein in yellow varieties are responsible for their color, whereas red varieties are rich in lycopene. The most popular orange varieties produce β-carotene and lower levels of α-carotene (Fig. 2). The color of purple carrots is caused by the accumulation of anthocyanins, although these varieties also accumulate carotenoids [32,33,35,36].

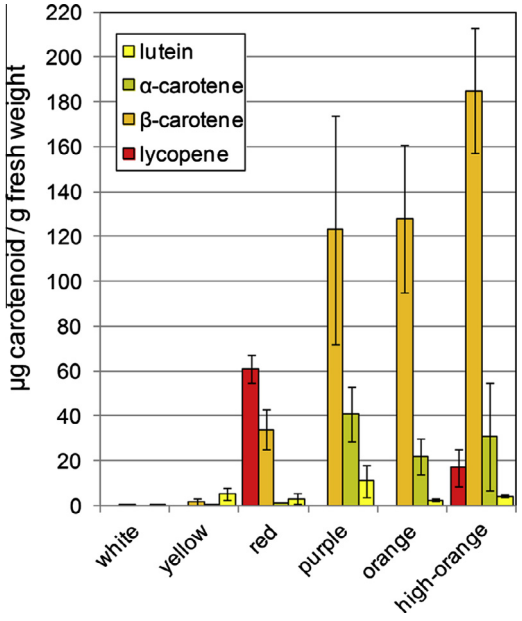

Fig. 2. Carotenoid profile of different carrot varieties. Data were acquired and adapted from [29]. Values correspond to the mean and standard deviation of three determinations of the main carotenoid species in fresh carrots.

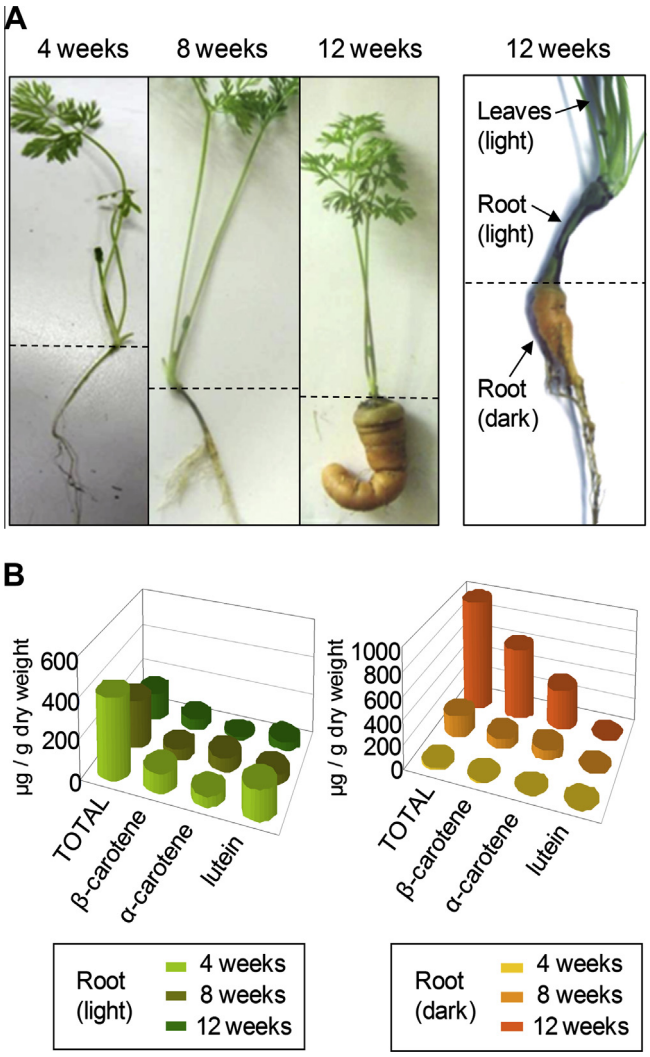

Fig. 3. Root development and carotenoid accumulation in Nantaise carrots. (A) Carrot plants grown for 4, 8 and 12 weeks. Dashed line separates light-exposed (upper) and underground (lower) sections of the plant. All panels are to the same scale except the one on the right, which shows a magnification of the root and shoot sections of a plant in which the upper segment of the root was left above ground. (B) Carotenoid content and composition during development of root segments either exposed to light (left) or grown in the dark (right). Data acquired and adapted from [2].

Carotenoid profiles also change dramatically during the development of roots of cultivars like the orange carrot Nantaise [2,3]. Under normal growth conditions (i.e. underground, in the dark), the root is thin and colorless at early stages of development but it thickens and starts accumulating carotenoids (β-carotene and α-carotene) after two months. At later stages, secondary root growth results in a dramatic enlargement (Fig. 3A) and a boosted production of carotenoids (Fig. 3B). In orange carrots, carotenoids mainly accumulate as large crystals inside chromoplasts [2,37,38]. Carrot chromoplasts, which likely derive from starch-containing leucoplasts (amyloplasts), are particularly abundant in the secondary

phloem of the root [31,37,39]. However, when the root is illuminated, chromoplast development is prevented and leucoplasts differentiate into chloroplasts [2,3]. Light-exposed carrot root sections display a carotenoid profile similar to that of leaves (i.e., with high lutein levels), whereas their total carotenoid levels do not increase but even decrease during root development (Fig. 3B). Besides promoting the differentiation of chloroplasts and preventing the differentiation of chromoplasts, light also impairs normal root growth, as illuminated root segments remain thin at late stages of development (Fig. 3A). When left again in the dark, dark-exposed segments are able to resume the chromoplast differentiation program and to enlarge, eventually reaching a morphological and metabolic phenotype similar to that of roots kept continuously underground [3,40].

Regulation of carotenoid biosynthesis in plants: learning from model systems

Despite the relevance of carotenoids for plant life and human health, our knowledge of how their synthesis and accumulation are regulated at the molecular level is still limited. The main mechanisms described to date can be grouped in three major categories: (1) control of expression of genes encoding enzymes involved in carotenoid biosynthesis and degradation, (2) regulation of enzyme activities, and (3) availability of storage structures. All three mechanisms are tightly coordinated throughout the plant life cycle by internal (developmental) signals but also in response to external (environmental) stimuli, with light having a major role [13,41,42]. The best studied examples of how gene expression, post-transcriptional mechanisms, and plastid differentiation act together to eventually determine carotenoid contents are Arabidopsis de-etiolation and tomato fruit ripening, two processes in which the production of carotenoids is boosted. In this section, we will summarize the information that these two model systems have contributed to our understanding of carotenoid regulation.

Arabidopsis de-etiolation (etioplasts to chloroplasts)

In Arabidopsis, the transcriptional control of genes encoding key biosynthetic enzymes can explain the carotenoid profiles of many organs and developmental stages [7]. Developmental control of gene expression results in a pre-established set of carotenoids in the etioplasts of seedlings germinated in the dark [25,43,44]. But when underground seedlings perceive the light, this environmental signal triggers a dramatic change in the composition of carotenoids aimed to protect the photosynthetic apparatus from excess light [25,45,46]. The quantitative (total levels) and qualitative (relative abundance) changes in carotenoid patterns during seedling de-etiolation are associated with concomitant changes in the expression of most Arabidopsis genes encoding carotenoid biosynthetic enzymes [43,47,48] but they also result from the differentiation of etioplasts into chloroplasts promoted by the new light-triggered developmental program. The development of thylakoid membranes and plastoglobules as well as the assembly of photosynthetic complexes in chloroplasts increases the capacity to accommodate the carotenoids required for photosynthesis and photoprotection [20,21]. In chloroplasts, light also modulates the metabolic flux through the carotenoid pathway by regulating the activity of biosynthetic enzymes [7]. Although de-etiolation triggers similar changes in carotenoid levels in other plant species [49–52], the use of Arabidopsis as a model has allowed to identify not only major regulators of the de-etiolation process but also direct regulators of the carotenoid pathway [44,46,53,54]. Among them, basic helix–loop–helix (bHLH) transcription factors of the Phytochrome-Interacting Factors (PIF) family were found to accumulate in the dark to repress chloroplast development and chlorophyll and carotenoid biosynthesis by directly binding to promoters like the one encoding PSY [46,55]. Light-mediated degradation of PIFs de-represses chloroplast development and *PSY* expression, therefore promoting the production and accumulation of carotenoids in coordination with chlorophylls to facilitate the transition to a photosynthetic metabolism.

Tomato fruit ripening (chloroplasts to chromoplasts)

Carotenoid biosynthesis during tomato fruit ripening is also determined by a developmental program that controls gene expression and plastid differentiation but influenced by environmental (light and temperature) conditions [56–58]. Mature green fruit contain chloroplasts similar to those of leaves. During ripening, chloroplasts differentiate into chromoplasts, chlorophylls are degraded, and a massive accumulation of carotenoids (particularly lycopene) changes the fruit color from green to red [59]. The transcriptional regulation of genes encoding carotenoid pathway enzymes appears to be instrumental for the changes in carotenoid levels and composition observed during ripening. While the expression of genes encoding fruit isoforms of PSY and lycopene-producing enzymes increases at the onset of carotenoid accumulation, the level of transcripts for lycopene cyclases decreases, resulting in an increased production of total carotenoids and a relative enrichment in lycopene at the late stages of ripening [50,60–63]. Analysis of mutants with altered fruit carotenoid profiles has led to the identification of regulators of the ripening process that also impact carotenoid gene expression [64,65]. For example, the identification of direct targets of the MADS-box transcription factor Ripening INhibitor (RIN) has revealed that it regulates carotenoid biosynthesis by directly binding to the promoter of *PSY1* (encoding the fruit-specific isoform of PSY in tomato) and other genes of the pathway, but it also controls the expression of genes involved in many other ripening-associated processes, including ethylene production and chlorophyll degradation [66,67]. Transcriptional regulation of the carotenoid pathway also appears to be the main mechanism responsible for the general carotenoid patterns of chromoplast-containing tissues of most other plant systems, including pepper or orange fruits [68–70] and marigold or chrysantemum flowers [71,72]. On the other hand, the level of transcripts encoding carotenoid-degrading CCD enzymes negatively correlates with carotenoid contents in chrysanthemum and orchid flowers [73,74], maize endosperm [75], and potato tubers [76], but not in Ipomea flowers [77], citrus fruit [78] or rice endosperm [79].

Besides the transcriptional regulation of gene expression, post-transcriptional control has been proposed to contribute to the final carotenoid profile of tomato fruits and other chromoplast-harboring systems [42,80]. Tomato *high pigment* (*hp*) mutants have revealed that deficient light signaling and ABA production increase the accumulation of carotenoids in ripe fruit because they result in higher number or volume of chromoplasts [56,81–85]. Besides illustrating how environmental cues such as light or water deficit can influence carotenoid contents, these results confirmed that carotenoid accumulation can be boosted in the absence of up-regulated carotenoid gene expression, just by triggering the synthesis of a plastid deposition sink to facilitate their storage. In fact, the development of large plastoglobules and carotenoid-sequestering structures in chromoplasts largely contributes to the deposition of massive amounts of carotenoids [11,86–88]. Although the specific factors controlling the differentiation of chromoplasts remain unknown, there are promising candidates such as the plastidial DnaJ-like protein encoded by the cauliflower (*Brassica oleracea var. botrytis*) *Orange* (*Or*) gene [89].

Mechanisms controlling carotenogenesis in carrot roots

The molecular mechanisms underlying the pigmentation of carrots were first analyzed with genetic approaches. Besides mapping several candidate genes and major QTLs for carrot color (i.e. carotenoid content), these studies have recently contributed to estimate heritabilities, gene numbers, and limiting steps for the production of carotenoids in storage roots [90–93]. The analysis of segregating populations resulting from the cross of white, orange, and dark orange carrot varieties provided evidence that inheritance of β-carotene and total carotenoids was continuous in the orange x dark orange cross but discrete in the white × orange cross, suggesting only two major loci as the minimum number separating white from orange carrots [91]. These studies led to propose that the primary difference between white and orange carrots resulted from a block in the production of phytoene early in the pathway [90]. Later work confirmed that the sole upregulation of PSY activity in white carrots was indeed sufficient to boost the production of β-carotene and other carotenoids and their accumulation as crystals in chromoplast-like plastids [38]. Consistent with the key relevance of the early steps of the pathway for carotenoid production in carrot roots, phylogenetic analyses have recently suggested that carrot domestication involved a preferential selection for mutations affecting upstream genes of the biosynthetic pathway, which could potentially have a greater effect on increasing metabolic flux to carotenoid biosynthesis [94].

The identification of genes putatively encoding carotenoid pathway enzymes in the first decade of this century opened the door to investigate the relevance of transcriptional regulation for the carotenoid profile of different carrot varieties [1,2,12]. In a pioneer study, the transcript levels of carrot genes encoding proteins with homology to PSY (isoforms PSY1 and PSY2), PDS, ZDS (isoforms ZDS1 and ZDS2 together), LCYE, LCYB1, and ZEP were quantified during the development of underground roots from white (Blanche), yellow (Yellowstone), orange (Bolero), and red (Nutrired) cultivars [1]. It was found that these genes were globally up-regulated during root development in all cultivars. This is consistent with the increased accumulation of total carotenoids observed during the same period in colored carrot varieties, even though the upregulation of transcript levels was only modest compared with the dramatic increase in carotenoid levels observed in Bolero and Nutrired cultivars. In addition, the levels of transcripts for LCYE and ZDS were highest in Yellowstone and Nutrired roots, respectively, in agreement with the preferential accumulation of lutein (Yellowstone) and lycopene (Nutrired) in these cultivars [1]. These results would support the conclusion that the regulation of carotenoid gene expression is instrumental for defining the general carotenoid profiles in colored carrot varieties. However, the lack of correlation between transcript and metabolite levels when carotenoid accumulation is boosted in the late stages of root development suggests that other mechanisms are also relevant to determine final carotenoid contents. The existence of additional control points regulating carotenogenesis in carrot roots is also evidenced by the absence of carotenoids in the white cultivar despite showing a profile of carotenoid gene expression similar to that of colored roots [1]. Although several mechanisms were proposed to explain this apparent paradox (including the existence of non-functional alleles, tissue-specific isoforms, impaired enzyme activity, or increased carotenoid degradation in white carrots), direct experimental evidence supporting any of them was not provided in the paper.

A second, more recent analysis of carotenoid gene expression during carrot root development, went a step forward to show the central influence of plastid identity for carotenoid accumulation in carrot roots [2]. Based on a previous observation that light had a dramatic influence on root development and carotenoid profiles in carrot [3,40], samples from root sections of the orange variety Nantaise that developed either underground or exposed to light (Fig. 3) were used to quantify the level of transcripts for a large set of carotenoid pathway enzymes (PSY1, PSY2, PDS, ZDS1, ZDS2, LCYE, LCYB1, LCYB2, CHYB2, ZEP, VDE, NCED1, and NCED3). This unique experimental system revealed a complex interaction of developmental and environmental (light) cues on defining plastid differentiation and expression of carotenoid genes [2]. The carotenoid profiles found in light-grown and dark-grown root tissues were completely different but they both partially correlated with the patterns of carotenoid gene expression. A decrease in total carotenoid levels was observed in illuminated root sections, whereas they increased in dark-grown segments during development. A similar pattern was observed for genes directly involved in the biosynthesis of carotenes, with the only exceptions of *ZDS1* (no significant changes), *LCYB2* (no significant changes), and *LCYE* (increased in both types of root samples). The strong increase in *LCYB1* and *LCYE* transcripts and decrease in *CHYB2* expression detected in late stages of underground root development also correlated with the preferential accumulation of α-carotene and β-carotene in mature roots [2]. But similar to that previously concluded with other carotenoid cultivars [1], transcriptional regulation could only explain part of the carotenoid profiles observed in carrot roots. A second key carotenoid-defining parameter regulated by both developmental and light cues was plastid identity. Differentiation of chloroplasts in light-exposed root sections also explained their carotenoid profile, similar to that of other chloroplast-containing organs such as leaves [2,3,40]. In dark-grown root segments, a dramatic increase in total carotenoid levels and a preferential accumulation of β-carotene and α-carotene correlated with the differentiation of chromoplasts during secondary growth [2,3,40]. Therefore, both carotenoid gene expression and chromoplast differentiation appear to be instrumental to define the carotenoid content of carrot storage roots.

The higher accumulation of carotenoids in chromoplasts of underground storage roots compared to that in chloroplasts of light-exposed tissues could be the result of an increased storage capacity, an enhanced metabolic flux by removal of carotenoid end-products, or the absence of photooxidative processes that degrade the accumulated carotenoids when exposed to light [24,31,87]. Plastid ultrastructure has also been shown to be relevant for the activity of key enzymes required for carotenoid biosynthesis [7]. For example, PSY activity in etioplasts is very low but it increases upon association to thylakoid membranes in the chloroplasts that differentiate during de-etiolation [95]. Because storage roots of white carrot varieties show levels of PSY-encoding transcripts similar to those in colored carrots [1] but phytoene synthesis is limiting only in white carrots [38,90], it is possible that PSY enzyme activity is reduced in leucoplasts but it increases when they differentiate into chromoplasts. On the other hand, increased PSY levels and activity in the root of white carrot cultivars but also in other plant systems was sufficient to promote the differentiation of chromoplasts [38,62,96,97], suggesting that both PSY activity and chromoplast differentiation might be mutually influenced.

These results together suggest that a complex feedback mechanism coordinates carotenoid gene expression, enzyme activities, and plastid differentiation to ensure an appropriate production of carotenoids. In agreement, a recent work showed that transgene-mediated alteration of LCYB1 levels caused concomitant changes in the expression of genes encoding PSY1, PSY2, and LCYB2 in the leaves (chloroplasts) and roots (chromoplasts) of carrot [18]. Feedback regulation of carotenoid gene expression and enzyme activities by phytoene, lycopene, or/and other carotenoid metabolites has been proposed to account for the observed carotenoid profiles

in many cases [98–108]. However, the molecular mechanisms and the specific metabolites involved in this regulation remain unclear.

Open questions and future perspectives

The described results indicate that carotenoid profiles in carrot roots are determined by the developmental and environmental control of carotenoid gene expression, enzyme activity, and plastid differentiation. However, the specific molecular factors involved in this control remain to be identified. Based on the key role of light for the regulation of carotenogenesis in carrot, a first step to identify regulatory factors could be the study of carrot homologues of light signaling proteins shown to control carotenoid accumulation in well-studied model systems such as Arabidopsis and tomato. In particular, transcription factors of the PIF family, which accumulate in the dark to repress carotenoid biosynthesis and chloroplast development [46,55], could be good candidates to test in carrot. Besides influencing carotenoid gene expression and plastid differentiation, light also impacts thickening and normal development of carrot storage roots [2,3,40], suggesting that the levels or activities of developmental regulators (i.e. hormones) might also be modulated by light signals, similar to that observed in other plant systems. Hormones like auxin, cytokinin, ABA, and ethylene are involved in regulating chromoplast differentiation and other physiological processes associated with tomato fruit ripening [24,64]. In particular, ABA has been shown to influence chromoplast number and volume in tomato fruit [81] but also to feedback-regulate the expression of genes encoding key carotenoid biosynthetic enzymes like PSY in the root of many plants, including Arabidopsis [47,109–111]. It has been proposed that an increased PSY activity provides carotenoid precursors for the enhanced production of ABA that takes place in roots under drought or saline stress, but it is unknown whether the presence of high carotenoid levels in carrot roots makes it unnecessary the ABA-mediated regulation of PSY gene expression observed in other plants. It would be interesting to test whether the presence of high levels of carotenoids available for ABA synthesis in colored carrots influences their fitness in response to abiotic stress under adverse environmental conditions compared to white carrot cultivars. The role of hormones in the coordination of root development and carotenoid accumulation represents an attractive challenge for future studies.

Carrots are consumed worldwide and are popular in a variety of foods because of their pleasant flavor, mainly due to the presence of volatile isoprenoids and sugars [33,112]. Although carotenoid pigments do not influence carrot flavor *per se* [29], they contribute to make the product appealing to consumers. But most importantly, carotenoids are powerful phytonutrients that contribute to human health [6,113,114]. Unlike plants, vertebrates are unable to produce carotenoids *de novo* but take them in their diet. Carotenoids contribute to prevent some types of cancer, cardiovascular diseases, and age-related macular degeneration, whereas those with β rings (specially β-carotene) are precursors of vitamin A. These properties make carotenoids of particular economic relevance not only as natural pigments in the industry but also as supplements in animal and human nutrition [6,114,115]. In particular, orange varieties represent a major source for pro-vitamin A in the Western diet. Future research focused on the identification of transcriptional factors and hormonal regulators of carotenogenesis in carrot should eventually provide additional tools to improve the visual appeal and nutritional content of this important vegetable.

Acknowledgements

Our current work is funded by grants from the Catalan AGAUR (2009SGR-26), Spanish DGI (BIO2011-23680 and PIM2010IPO-00660), and European Union FP7 (TiMet, contract 245143) to MRC, and Chilean Fondef (D10I1022) and Fondecyt (1130245) to CS. Collaboration between our labs was possible thanks to grants CSIC-2010CL0039 and CSIC/ESPAÑA 10/11-2, and it is currently ongoing in the frame of the IBERCAROT network funded by Programa Iberoamericano de Ciencia y Tecnologia para el Desarrollo (CYTED, code 112RT0445).

References

[1] J. Clotault, D. Peltier, R. Berruyer, M. Thomas, M. Briard, E. Geoffriau, J. Exp. Bot. 59 (2008) 3563–3573.
[2] P. Fuentes, L. Pizarro, J.C. Moreno, M. Handford, M. Rodriguez-Concepcion, C. Stange, Plant Mol. Biol. 79 (2012) 47–59.
[3] C. Stange, P. Fuentes, M. Handford, L. Pizarro, Biol. Res. 41 (2008) 289–301.
[4] V.E. Rubatzky, C.F. Quiros, P.W. Simon, Carrots and Related Vegetable Umbelliferae, CABI Publishing, New York, 1999. pp. 1–21.
[5] J. Clotault, E. Geoffriau, E. Lionneton, M. Briard, D. Peltier, Theor. Appl. Genet. 121 (2010) 659–672.
[6] P.D. Fraser, P.M. Bramley, Prog. Lipid Res. 43 (2004) 228–265.
[7] M.A. Ruiz-Sola, M. Rodriguez-Concepcion, Arabidopsis Book 10 (2012) e0158.
[8] M. Rodriguez-Concepcion, Arch. Biochem. Biophys. 504 (2010) 118–122.
[9] J. Kim, J.J. Smith, L. Tian, D. Dellapenna, Plant Cell Physiol. 50 (2009) 463–479.
[10] J.E. Kim, K.M. Cheng, N.E. Craft, B. Hamberger, C.J. Douglas, Phytochemistry 71 (2010) 168–178.
[11] M.H. Walter, D. Strack, Nat. Prod. Rep. 28 (2011) 663–692.
[12] B.J. Just, C.A. Santos, M.E. Fonseca, L.S. Boiteux, B.B. Oloizia, P.W. Simon, Theor. Appl. Genet. 114 (2007) 693–704.
[13] J. Hirschberg, Curr. Opin. Plant Biol. 4 (2001) 210–218.
[14] F.X. Cunningham, E. Gantt, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 557–583.
[15] P.D. Fraser, J.W. Kiano, M.R. Truesdale, W. Schuch, P.M. Bramley, Plant Mol. Biol. 40 (1999) 687–698.
[16] G. Giorio, A.L. Stigliani, C. D'Ambrosio, FEBS J. 275 (2008) 527–535.
[17] G. Ronen, L. Carmel-Goren, D. Zamir, J. Hirschberg, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 1102–1107.
[18] J.C. Moreno, L. Pizarro, P. Fuentes, M. Handford, V. Cifuentes, C. Stange, PLoS ONE 8 (2013) e58144.
[19] L. Dall'Osto, S. Cazzaniga, H. North, A. Marion-Poll, R. Bassi, Plant Cell 19 (2007) 1048–1064.
[20] B. Demmig-Adams, W.W. Adams, Science 298 (2002) 2149–2153.
[21] F.G. Xiao, L. Shen, H.F. Ji, Biochem. Biophys. Res. Commun. 414 (2011) 1–4.
[22] Y. Tanaka, N. Sasaki, A. Ohmiya, Plant J. 54 (2008) 733–749.
[23] C.A. Howitt, B.J. Pogson, Plant Cell Environ. 29 (2006) 435–445.
[24] I. Egea, C. Barsan, W. Bian, E. Purgatto, A. Latche, C. Chervin, M. Bouzayen, J.C. Pech, Plant Cell Physiol. 51 (2010) 1601–1611.
[25] H. Park, S.S. Kreunen, A.J. Cuttriss, D. DellaPenna, B.J. Pogson, Plant Cell 14 (2002) 321–332.
[26] A. Rodriguez-Villalon, E. Gas, M. Rodriguez-Concepcion, Plant J. 60 (2009) 424–435.
[27] J.M. Van Norman, L.E. Sieburth, Plant J. 49 (2007) 619–628.
[28] X. Xie, K. Yoneyama, K. Yoneyama, Annu. Rev. Phytopathol. 48 (2010) 93–117.
[29] R.L. Surles, N. Weng, P.W. Simon, S.A. Tanumihardjo, J. Agric. Food Chem. 52 (2004) 3417–3421.
[30] A. Mech-Nowak, A. Swiderski, M. Kruczek, I. Luczak, A. Kostecka-Gugala, Acta Biochim. Pol. 59 (2012) 139–141.
[31] M. Baranska, R. Baranski, H. Schulz, T. Nothnagel, Planta 224 (2006) 1028–1037.
[32] J. Grassmann, W.H. Schnitzler, R. Habegger, Int. J. Food Sci. Nutr. 58 (2007) 603–611.
[33] C. Alasalvar, J.M. Grigor, D. Zhang, P.C. Quantick, F. Shahidi, J. Agric. Food Chem. 49 (2001) 1410–1416.
[34] C. Nicolle, G. Simon, E. Rock, P. Amouroux, C. Remesy, J. Am. Soc. Hortic. Sci. 129 (2004) 523–529.
[35] E.C. Montilla, M.R. Arzaba, S. Hillebrand, P. Winterhalter, J. Agric. Food Chem. 59 (2011) 3385–3390.
[36] W.E. Glassgen, V. Wray, D. Strack, J.W. Metzger, H.U. Seitz, Phytochemistry 31 (1992) 1593–1601.
[37] J.E. Kim, K.H. Rensing, C.J. Douglas, K.M. Cheng, Planta 231 (2010) 549–558.
[38] D. Maass, J. Arango, F. Wust, P. Beyer, R. Welsch, PLoS ONE 4 (2009) e6373.
[39] T.C. Koch, I.L. Goldman, J. Agric. Food Chem. 53 (2005) 325–331.
[40] C. Stange, C. Flores, in: Advances in Photosynthesis-Fundamental Aspects, INTECH, Rijeka, Croatia, 2011, pp. 1–20.
[41] L. Pizarro, C. Stange, Cienc. Inv. Agr. 36 (2009) 143–162.
[42] C.I. Cazzonelli, B.J. Pogson, Trends Plant Sci. 15 (2010) 266–274.
[43] M. Ghassemian, J. Lutes, J.M. Tepperman, H.S. Chang, T. Zhu, X. Wang, P.H. Quail, B.M. Lange, Arch. Biochem. Biophys. 448 (2006) 45–59.
[44] A. Rodriguez-Villalon, E. Gas, M. Rodriguez-Concepcion, Plant Signal Behav. 4 (2009) 965–967.
[45] S. Woitsch, S. Römer, Plant Physiol. 132 (2003) 1508–1517.
[46] G. Toledo-Ortiz, E. Huq, M. Rodriguez-Concepcion, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 11626–11631.

[47] S. Meier, O. Tzfadia, R. Vallabhaneni, C. Gehring, E.T. Wurtzel, BMC Syst. Biol. 5 (2011) 77.
[48] M. Rodríguez-Concepción, Phytochem. Rev. 5 (2006) 1–15.
[49] S. Frosch, H. Mohr, Planta 148 (1980) 279–286.
[50] G. Giuliano, G.E. Bartley, P.A. Scolnik, Plant Cell 5 (1993) 379–387.
[51] M. Albrecht, G. Sandmann, Plant Physiol. 105 (1994) 529–534.
[52] P. Botella-Pavía, O. Besumbes, M.A. Phillips, L. Carretero-Paulet, A. Boronat, M. Rodríguez-Concepción, Plant J. 40 (2004) 188–199.
[53] J. von Lintig, R. Welsch, M. Bonk, G. Giuliano, A. Batschauer, H. Kleinig, Plant J. 12 (1997) 625–634.
[54] R. Welsch, J. Medina, G. Giuliano, P. Beyer, J. von Lintig, Planta 216 (2003) 523–534.
[55] P. Leivar, P.H. Quail, Trends Plant Sci. 16 (2010) 19–28.
[56] Y. Liu, S. Roof, Z. Ye, C. Barry, A. van Tuinen, J. Vrebalov, C. Bowler, J. Giovannoni, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 9897–9902.
[57] R. Alba, M.M. Cordonnier-Pratt, L.H. Pratt, Plant Physiol. 123 (2000) 363–370.
[58] I. Neta-Sharir, T. Isaacson, S. Lurie, D. Weiss, Plant Cell 17 (2005) 1829–1838.
[59] J. Giovannoni, Annu. Rev. Plant Physiol. Plant Mol. Biol. 52 (2001) 725–749.
[60] P.D. Fraser, M.R. Truesdale, C.R. Bird, W. Schuch, P.M. Bramley, Plant Physiol. 105 (1994) 405–413.
[61] I. Pecker, R. Gabbay, F.X. Cunningham Jr., J. Hirschberg, Plant Mol. Biol. 30 (1996) 807–819.
[62] G. Ronen, M. Cohen, D. Zamir, J. Hirschberg, Plant J. 17 (1999) 341–351.
[63] R. Alba, P. Payton, Z. Fei, R. McQuinn, P. Debbie, G.B. Martin, S.D. Tanksley, J.J. Giovannoni, Plant Cell 17 (2005) 2954–2965.
[64] H.J. Klee, J.J. Giovannoni, Annu. Rev. Genet. 45 (2011) 41–59.
[65] J.J. Giovannoni, Curr. Opin. Plant Biol. 10 (2007) 283–289.
[66] M. Fujisawa, T. Nakano, Y. Shima, Y. Ito, Plant Cell 25 (2013) 371–386.
[67] C. Martel, J. Vrebalov, P. Tafelmeyer, J.J. Giovannoni, Plant Physiol. 157 (2011) 1568–1579.
[68] M. Kato, Y. Ikoma, H. Matsumoto, M. Sugiura, H. Hyodo, M. Yano, Plant Physiol. 134 (2004) 824–837.
[69] M.J. Rodrigo, J.F. Marcos, L. Zacarias, J. Agric. Food Chem. 52 (2004) 6724–6731.
[70] P. Hugueney, F. Bouvier, A. Badillo, J. Quennemet, A. d'Harlingue, B. Camara, Plant Physiol. 111 (1996) 619–626.
[71] S. Kishimoto, A. Ohmiya, Physiol. Plant. 128 (2006) 436–447.
[72] C.P. Moehs, L. Tian, K.W. Osteryoung, D. Dellapenna, Plant Mol. Biol. 45 (2001) 281–293.
[73] A. Ohmiya, S. Kishimoto, R. Aida, S. Yoshioka, K. Sumitomo, Plant Physiol. 142 (2006) 1193–1201.
[74] C.Y. Chiou, H.A. Pan, Y.N. Chuang, K.W. Yeh, Planta 232 (2010) 937–948.
[75] R. Vallabhaneni, L.M. Bradbury, E.T. Wurtzel, Arch. Biochem. Biophys. 504 (2010) 104–111.
[76] R. Campbell, L.J. Ducreux, W.L. Morris, J.A. Morris, J.C. Suttle, G. Ramsay, G.J. Bryan, P.E. Hedley, M.A. Taylor, Plant Physiol. 154 (2010) 656–664.
[77] C. Yamamizo, S. Kishimoto, A. Ohmiya, J. Exp. Bot. 61 (2010) 709–719.
[78] M. Kato, H. Matsumoto, Y. Ikoma, H. Okuda, M. Yano, J. Exp. Bot. 57 (2006) 2153–2164.
[79] A. Ilg, Q. Yu, P. Schaub, P. Beyer, S. Al-Babili, Planta 232 (2010) 691–699.
[80] E.M. Enfissi, F. Barneche, I. Ahmed, C. Lichtle, C. Gerrish, R.P. McQuinn, J.J. Giovannoni, E. Lopez-Juez, C. Bowler, P.M. Bramley, P.D. Fraser, Plant Cell 22 (2010) 1190–1215.
[81] N. Galpaz, Q. Wang, N. Menda, D. Zamir, J. Hirschberg, Plant J. 53 (2008) 717–730.
[82] I. Kolotilin, H. Koltai, Y. Tadmor, C. Bar-Or, M. Reuveni, A. Meir, S. Nahon, H. Shlomo, L. Chen, I. Levin, Plant Physiol. 145 (2007) 389–401.
[83] H.V. Kilambi, R. Kumar, R. Sharma, Y. Sreelakshmi, Plant Physiol. 161 (2013) 2085–2101.
[84] G. Giuliano, G. Diretto, Trends Plant Sci. 12 (2007) 529–531.
[85] L. Li, J. Van Eck, Transgenic Res. 16 (2007) 581–585.
[86] J. Deruere, S. Romer, A. d'Harlingue, R.A. Backhaus, M. Kuntz, B. Camara, Plant Cell 6 (1994) 119–133.
[87] M. Vishnevetsky, M. Ovadis, A. Vainstein, Trends Plant Sci. 4 (1999) 232–235.
[88] A.J. Simkin, J. Gaffe, J.P. Alcaraz, J.P. Carde, P.M. Bramley, P.D. Fraser, M. Kuntz, Phytochemistry 68 (2007) 1545–1556.
[89] S. Lu, J. Van Eck, X. Zhou, A.B. Lopez, D.M. O'Halloran, K.M. Cosman, B.J. Conlin, D.J. Paolillo, D.F. Garvin, J. Vrebalov, L.V. Kochian, H. Kupper, E.D. Earle, J. Cao, L. Li, Plant Cell 18 (2006) 3594–3605.
[90] C.A.F. Santos, D. Senalik, P.W. Simon, Genet. Mol. Biol. 28 (2005) 287–293.
[91] C.A.F. Santos, P.W. Simon, Euphytica 151 (2006) 79–86.
[92] C.A. Santos, P.W. Simon, Mol. Genet. Genomics 268 (2002) 122–129.
[93] B.J. Just, C.A. Santos, B.S. Yandell, P.W. Simon, Theor. Appl. Genet. 119 (2009) 1155–1169.
[94] J. Clotault, D. Peltier, V. Soufflet-Freslon, M. Briard, E. Geoffriau, PLoS ONE 7 (2012) e38724.
[95] R. Welsch, P. Beyer, P. Hugueney, H. Kleinig, J. von Lintig, Planta 211 (2000) 846–854.
[96] C.K. Shewmaker, J.A. Sheehy, M. Daley, S. Colburn, D.Y. Ke, Plant J. 20 (1999) 401–412.
[97] P.M. Bramley, J. Exp. Bot. 53 (2002) 2107–2113.
[98] N. Misawa, K. Masamoto, T. Hori, T. Ohtani, P. Böger, G. Sandmann, Plant J. (1994) 6.
[99] V. Corona, B. Aracri, G. Kosturkova, G.E. Bartley, L. Pitto, L. Giorgetti, P.A. Scolnik, G. Giuliano, Plant J. 9 (1996) 505–512.
[100] S. Römer, P.D. Fraser, J.W. Kiano, C.A. Shipton, N. Misawa, W. Schuch, P.M. Bramley, Nat. Biotechnol. 18 (2000) 666–669.
[101] S. Romer, J. Lubeck, F. Kauder, S. Steiger, C. Adomat, G. Sandmann, Metab. Eng. 4 (2002) 263–272.
[102] A.J. Simkin, C. Zhu, M. Kuntz, G. Sandmann, J. Plant Physiol. 160 (2003) 439–443.
[103] G. Diretto, R. Tavazza, R. Welsch, D. Pizzichini, F. Mourgues, V. Papacchioli, P. Beyer, G. Giuliano, BMC Plant Biol. 6 (2006) 13.
[104] A.J. Cuttriss, A.C. Chubb, A. Alawady, B. Grimm, B.J. Pogson, Funct. Plant Biol. 34 (2007) 663–672.
[105] G. Qin, H. Gu, L. Ma, Y. Peng, X.W. Deng, Z. Chen, L.J. Qu, Cell Res. 17 (2007) 471–482.
[106] C.E. Harjes, T.R. Rocheford, L. Bai, T.P. Brutnell, C.B. Kandianis, S.G. Sowinski, A.E. Stapleton, R. Vallabhaneni, M. Williams, E.T. Wurtzel, J. Yan, E.S. Buckler, Science 319 (2008) 330–333.
[107] W. Apel, R. Bock, Plant Physiol. 151 (2009) 59–66.
[108] L. Bai, E.H. Kim, D. DellaPenna, T.P. Brutnell, Plant J. 59 (2009) 588–599.
[109] F. Li, R. Vallabhaneni, E.T. Wurtzel, Plant Physiol. 146 (2008) 1333–1345.
[110] R. Welsch, F. Wust, C. Bar, S. Al-Babili, P. Beyer, Plant Physiol. 147 (2008) 367–380.
[111] J. Arango, F. Wust, P. Beyer, R. Welsch, Planta 232 (2010) 1251–1262.
[112] P.W. Simon, R.C. Lindsay, C.E. Peterson, J. Agric. Food Chem. 28 (1980) 549–552.
[113] J. He, M.M. Giusti, Annu. Rev. Food Sci. Technol. 1 (2010) 163–187.
[114] U. Flores-Pérez, M. Rodríguez-Concepción, in: A Salter, H Wiseman, G Tucker (Eds.), Phytonutrients, Blackwell Publishing, Oxford (UK), 2012, pp. 89–109.
[115] K.J. Yeum, R.M. Russell, Annu. Rev. Nutr. 22 (2002) 483–504.