Carotenoids

ABBY J. CUTTRISS,[*,†] CHRISTOPHER I. CAZZONELLI,[‡] ELEANORE T. WURTZEL[†] AND BARRY J. POGSON[‡,1]

*Molecular Biosciences and Bioengineering, University of Hawai'i at Mānoa, Honolulu, HI, USA
[†]Department of Biological Sciences, Lehman College, The City University of New York, Bronx, New York, USA
[‡]ARC Centre of Excellence in Plant Energy Biology, Research School of Biology, Australian National University, Canberra, ACT 0200, Australia

[1]Corresponding author: E-mail: barry.pogson@anu.edu.au

Advances in Botanical Research, Vol. 58 0065-2296/11 $35.00

DOI: 10.1016/B978-0-12-386479-6.00005-6

ABSTRACT

Carotenoid pigments provide fruits and flowers with distinctive red, orange and yellow colours as well as a number of aromas, which make them commercially important in agriculture, food, health and the cosmetic industries. Carotenoids comprise a large family of C_{40} polyenes that are critical for the survival of plants and animals alike. β-carotene and its derivatives contain unmodified β-ionone groups, which serve as precursors for vitamin A and are therefore essential dietary components for mammals. Significant progress has been made towards producing staple food crops with elevated provitamin A carotenoids, an important first step in alleviating worldwide vitamin A deficiency. Recent insights into the regulatory processes that control carotenoid composition and content may further advance biofortification projects.

ABBREVIATIONS

βLCY	lycopene β-cyclase
εLCY	lycopene ε-cyclase
βOH	β-hydroxylase
εOH	ε-hydroxylase
ABA	abscisic acid
CCD	carotenoid cleavage dioxygenases
CRTISO	carotenoid isomerase
CsZCD	crocus zeaxanthin 7,8(7′,8′)-cleavage dioxygenase
DMAPP	dimethylallyl diphosphate
DXP	deoxy-D-xylulose 5-phosphate
DXS	deoxy-D-xylulose 5-phosphate synthase
GGPP	geranylgeranyl diphosphate
IPP	isopentenyl diphosphate
MEP	methylerythritol 4-phosphate
MVA	mevalonic acid
NCED	9-cis-epoxycarotenoid dioxygenase
NPQ	non-photochemical quenching
NXS	neoxanthin synthase

PDS	phytoene desaturase
PSY	phytoene synthase
VDE	violaxanthin de-epoxidase
ZDS	ζ-carotene desaturase
ZEP	zeaxanthin epoxidase
Z-ISO	15-cis-ζ-carotene isomerase

I. BIOLOGICAL FUNCTION

A. DIETARY CAROTENOIDS

Carotenoids are a vital component of mammalian diets, providing precursors for vitamin A biosynthesis. Antioxidants and their dietary uptake can pigment the tissues of animals such as fish, crustaceans and birds. Vitamin A (all-*trans*-retinol) is generated from unmodified β-ring containing provitamin A carotenoids in the diet (von Lintig, 2010), of which β-carotene (two nonhydroxylated β-ionone rings), is the most efficient, because it can generate up to two retinol molecules. α-carotene and β-cryptoxanthin also contain provitamin A potential, but only have a single nonhydroxylated β-ring (Davis *et al*., 2008).

Vitamin A deficiency is responsible for a number of disorders that range from impaired iron mobilization, growth retardation and blindness to a depressed immune response, as well as increased susceptibility to infectious disease (Sommer and Davidson, 2002). Between 140 and 250 million children are at risk of vitamin A deficiency (Underwood, 2004); 250,000–500,000 become blind every year and half will die within 12 months after losing their sight (http://www.who.int/nut/vad.htm). Simply improving the vitamin A status of children, by increasing the uptake of provitamin A (e.g. β- and α-carotene), can reduce overall child mortality by 25% (http://www.unicef.org/immunization/facts_vitamina.html).

Low serum levels of vitamin A (less than 0.7 μmol L^{-1}) can be used as a population-based indicator of health risks (Underwood, 2004). Recommended daily allowances for vitamin A range from 300–600 μg for children to 900–1300 μg for adults of retinol activity equivalents (retinol and provitamin A carotenoids; Fig. 1). There is no recommended daily allowance for provitamin A carotenoids, as the conversion efficiency remains imprecise; however, between 3 and 6 mg of β-carotene daily is sufficient to maintain healthy serum carotenoid levels, as would five or more servings of fruits and vegetables per day (Panel on Micronutrients, 2001).

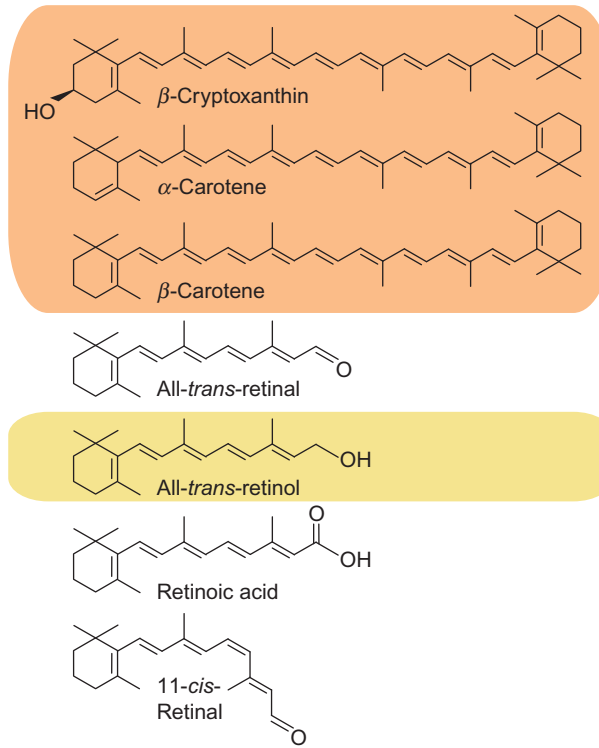

Fig. 1. Vitamin A and carotenoid precursor structures. Common dietary provitamin A carotenoids with unmodified β-ionone rings (highlighted in orange/dark grey) are processed to form C_{20} retinoids, including all-*trans*-retinol (vitamin A, highlighted in yellow/light grey), all-*trans*-retinal, retinoic acid and 11-*cis*-retinal, a photoreceptor chromophore.

B. CAROTENOIDS IN PHOTOSYNTHETIC ORGANISMS

Carotenoids play a variety of crucial roles in photosynthetic organisms. Carotenoids are involved in photosystem assembly where they contribute to harvesting light in a broader range of wavelengths in the blue region of the visible light spectrum and subsequently transfer the energy to chlorophyll (Fig. 2). The distinctive yellow colours of light-harvesting carotenoids become visible during autumn when chlorophyll degrades. The colour of carotenoids, typically ranging from pale yellow to red is defined by the number of conjugated double bonds along the C_{40} backbone as well as other structural and oxygenic modifications that impart different spectral properties. Carotenoids also provide protection from excessive light via

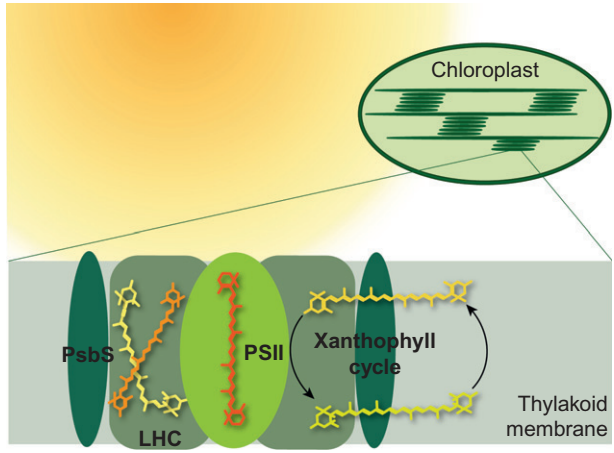

Fig. 2. Photoprotective carotenoids in chloroplast membranes and proteins. Carotenoids accumulate in chloroplast thylakoid membranes, as indicated by this simplified schematic. Xanthophylls, such as lutein, zeaxanthin, violaxanthin and neoxanthin, accumulate in light-harvesting complex proteins (LHC) where they have a structural role and contribute to light harvesting. β-carotene molecules in the photosystem II reaction centre (PSII) could quench singlet oxygen or possibly have a role in electron transfer. In high light, zeaxanthin is formed from violaxanthin via the xanthophyll cycle. Zeaxanthin, lutein, PsbS and specific antenna proteins all contribute to non-photochemical quenching of chlorophyll fluorescence; note, the exact locations of each are not depicted in this cartoon.

energy dissipation and free radical detoxification, which limits damage to membranes and proteins (DellaPenna and Pogson, 2006).

Plants need to maintain a balance between absorbing sufficient light for photosynthetic processes and avoiding oxidative damage caused by high light. Complementary photoprotective mechanisms are employed to minimize photodamage induced by exposure to high light and these include (1) the harmless dissipation of excess energy via non-photochemical quenching (NPQ) that is mediated by certain xanthophylls (zeaxanthin, antheraxanthin and lutein), (2) quenching of triplet chlorophylls by carotenoids, (3) accumulation of antioxidants (ascorbate, tocopherols and carotenoids) and (4) activation of antioxidant enzymes such as ascorbate peroxidase that de-toxify free radicals, as well as repair damaged proteins (Bailey and Grossman, 2008; Niyogi, 1999).

The physiological relevance of xanthophylls is exemplified by the bleaching, delayed greening, viviparous and semi-lethal phenotypes observed in several carotenoid- and NPQ-deficient mutants (Neill *et al.*, 1986; Niyogi *et al.*, 1997; Pogson *et al.*, 1998; Robertson *et al.*, 1966; Treharne *et al.*, 1966;

Wurtzel, 2004). Alterations in the carotenoid pool size make the xanthophyll cycle affect plant fitness. Increasing the xanthophyll cycle pool by overexpressing the bacterial *βOH* gene (*chyB*) enhances stress tolerance in Arabidopsis (Johnson *et al.*, 2008). Zeaxanthin prevents oxidative damage of the thylakoid membranes and plants with reduced zeaxanthin exhibit increased sensitivity to light stress (Havaux and Niyogi, 1999; Verhoeven *et al.*, 2001). Conversely, a lycopene β-cyclase (βLCY) mutant that lacks zeaxanthin but accumulates additional lutein and α-carotene (suppressor of zeaxanthin-less1, *szl1*) exhibits a partially restored quenching efficiency, suggesting that lutein may substitute for zeaxanthin (Li *et al.*, 2009).

II. DISTRIBUTION

Carotenoids are synthesized by all photosynthetic organisms, some bacteria and fungi. Other organisms, such as humans, must acquire carotenoids through dietary intake. For instance, the commercially significant pigment astaxanthin is primarily synthesized by microorganisms, such as the green alga *Haematococcus pluvialis* and is accumulated by fish such as salmon, thus colouring their flesh red. In the case of lobster and other crustaceans, astaxanthin's spectral properties are modified by the protein, crustacyanin, which results in blue pigmentation that shifts to red upon cooking, which causes protein-pigment denaturation (Britton *et al.*, 1997). Flamingos can also make use of carotenoids cosmetically and when the birds applied canthaxanthin-rich secretions onto their feathers, their courting behaviour became more frequent during mating seasons due to a visually more attractive breeding partner (Amat *et al.*, 2010). Humans have been using carotenoids and their derivatives, such as bixin, as food additives, as well as for cosmetic purposes (Bouvier *et al.*, 2003a).

Curious exceptions to the lack of synthesis of carotenoids by animals include the synthesis of carotenoids in the human protist parasites, *Plasmodium* and *Toxoplasma* (Tonhosolo *et al.*, 2009), which is explained by the existence of a remnant plastid, known as an apicoplast. An aphid genome was found to encode enzymes for carotenoid biosynthesis, which was the result of lateral gene transfer from a fungus, thus making aphids the only known animal to date capable of synthesizing their own carotenoids (Moran and Jarvik, 2010).

Carotenoid accumulation relies on the presence of structures capable of storing and retaining carotenoids. During the transformation of a chloroplast into a chromoplast, carotenoids become localized in plastoglobuli before incorporation into the chromoplast (Tevini and Steinmuller, 1985). Carotenoids within plastoglobuli exhibit much higher light stability than carotenoids within

chloroplast membranes, suggesting that pigments are better protected from light destruction in these structures (Merzlyak and Solovchenko, 2002). Cyanobacterial mutants with inactivated plastoglobulin-like genes are especially sensitive to altered light regimes, and the plastoglobulin-like peptides accumulate to a greater extent in wild-type cultures that are exposed to high light (Cunningham *et al.*, 2010). Chromoplasts also accumulate carotenoids in lipoprotein structures (Bartley and Scolnik, 1995; Vishnevetsky *et al.*, 1999) that are sequestered as crystals. For example, in a novel cauliflower mutant with orange curd, *Or*, β-carotene accumulates in the plastids of the pith and curd as sheets, ribbons and crystals (Li *et al.*, 2001; Lu *et al.*, 2006).

There are other plastid organelles capable of storing carotenoids. These include the 'colourless' amyloplasts, which store starch granules (Kirk and Tiliney-Bassett, 1978). Lutein is the predominant carotenoid present in many seed amyloplasts such as wheat (Hentschel *et al.*, 2002; Howitt *et al.*, 2009), whereas maize exhibits great diversity in terms of pigment composition (Harjes *et al.*, 2008). Leucoplasts are characteristic of mature root cells and accumulate trace levels of neoxanthin and violaxanthin, which amount to only 0.03–0.07% of the levels in light-grown leaves (Parry and Horgan, 1992). Elaioplasts are specialized lipid-storing plastids and provide an ideal hydrophobic sink for accumulation of carotenoids. The dark-grown etioplast is distinguished by the prolamellar body, a uniformly curved lattice of tubular membranes, which contains several of the biochemical building blocks required for the chloroplast (Gunning and Jagoe, 1967) including the xanthophylls, lutein and violaxanthin (Joyard *et al.*, 1998). The Arabidopsis *crtiso* (*ccr2*) mutant accumulates tetra-*cis*-lycopene and lacks a prolamellar body. Thus, a mutation in carotenoid biosynthesis apparently disrupts membrane curvature and stabilization of the prolamellar body (Park *et al.*, 2002). The absence of this structure in *CRTISO* mutants suggests that different carotenoids either directly or indirectly impede formation of the membrane lattices, which results in a delay in plastid development and greening upon exposure to light. These data demonstrate an important role for carotenoids in plastid differentiation (Park *et al.*, 2002).

III. CAROTENOID BIOSYNTHESIS

A. ISOPRENOID PRECURSORS

Isoprenoids (or terpenoids) are a large and diverse class of naturally occurring organic chemicals derived from five-carbon isoprene units. Carotenoids are derived from two isoprene isomers, isopentenyl diphosphate (IPP) and

dimethylallyl diphosphate (DMAPP). The same precursors are used to make a diverse range of compounds that include tocopherols, chlorophylls, phylloquinone, gibberellins, abscisic acid (ABA), monoterpenes and plastoquinone. The biosynthesis of isoprenoid precursors has been covered in detail elsewhere (Rodriguez-Concepcion, 2010).

Two distinct pathways exist for IPP production: the mevalonic acid (MVA) pathway and the mevalonate-independent, methylerythritol 4-phosphate (MEP) pathway (Lange *et al.*, 2000). The plastid-localized MEP pathway combines glyceraldehyde-3-phosphate and pyruvate to form deoxy-D-xylulose 5-phosphate (DXP), a reaction catalysed by DXP synthase (DXS). A number of steps are then required to form geranylgeranyl diphosphate (GGPP), the precursor to carotenoid biosynthesis. The Arabidopsis *Cla1* mutant, in which the *DXS* gene of the MEP pathway is disrupted, is photobleached because of the absence of protective carotenoids (Araki *et al.*, 2000; Estevez *et al.*, 2000). Conversely, overexpression of *PSY* (phytoene synthase) resulted in increased carotenoid accumulation and a concomitant accumulation of the DXS enzyme (Rodriguez-Villalon *et al.*, 2009).

B. CAROTENE SYNTHESIS

1. *Phytoene synthase*

The first committed step is the condensation of two molecules of GGPP to produce phytoene (Fig. 3). This reaction is catalysed by PSY in higher plants and bacteria (CrtB; Armstrong, 1994). *PSY* is a single-copy gene in Arabidopsis but present in multiple copies in other plants such as rice, maize and cassava, all of which have three copies that are expressed in different tissues and show differential responses to environmental stimuli (Arango *et al.*, 2010; Li *et al.*, 2008a,b; Welsch *et al.*, 2008). PSY is a rate-limiting step and a dosage effect of the maize Y1 allele was noted as early as 1940 (Randolph and Hand, 1940). Overexpression of an exogenous daffodil PSY in rice endosperm leads to phytoene accumulation, the first instance of carotenoid engineering in rice (Burkhardt *et al.*, 1997).

2. *Desaturases (PDS and ZDS)*

Phytoene is produced as a 15-*cis* isomer, which is subsequently converted to all-*trans* isomer derivatives (Beyer *et al.*, 1989; Chen *et al.*, 2010). Two desaturases, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), catalyse a series of dehydrogenation reactions by introducing four double bonds to form lycopene. Desaturation is linked to a plastidic respiratory

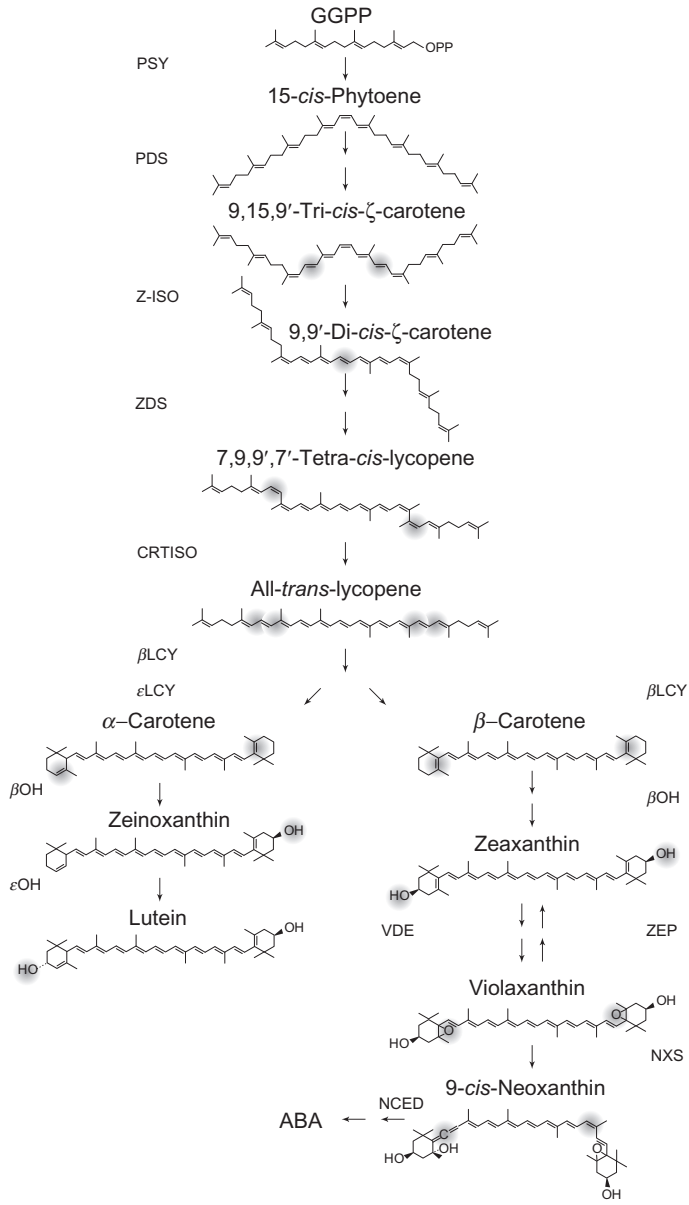

Fig. 3. Carotenoid biosynthetic pathway in higher plants. The pathway shows the primary reactions found in nearly all plant species. Grey shaded areas on carotenoid structures indicate site of activity for each biosynthetic enzyme. ABA, abscisic acid; βLCY, lycopene β-cyclase; βOH, β-hydroxylase; CRTISO, carotenoid isomerase; εLCY, lycopene ε-cyclase; εOH, ε-hydroxylase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; NXS, neoxanthin synthase; PDS, phytoene desaturase; PSY, phytoene synthase; VDE, violaxanthin de-epoxidase; ZDS, ζ-carotene desaturase; ZEP, zeaxanthin epoxidase; Z-ISO, 15-cis-ζ-carotene isomerase.

redox chain (Nievelstein *et al.*, 1995) and evidence for a quinone requirement was demonstrated in daffodil and Arabidopsis (Beyer, 1989; Norris *et al.*, 1995).

3. Isomerases (Z-ISO and CRTISO)

Recent biochemical evidence confirms that the desaturation reactions in plants proceed via various *cis* intermediates, including 9,15,9′-tri-*cis*-ζ-carotene, 9,9′-di-*cis*-ζ-carotene and 7,9,9′-tri-*cis*-neurosporene (Chen *et al.*, 2010; Isaacson *et al.*, 2004). Thus, all-*trans*-lycopene, the preferred substrate for the cyclases, is produced by the desaturases in concert with two isomerases. The first isomerase was identified in Arabidopsis and tomato (Isaacson *et al.*, 2002; Park *et al.*, 2002). Lesions in *CRTISO* result in accumulation of *cis*-carotene isomers in dark-grown plants (Park *et al.*, 2002). Characterization of the maize recessive *y9* mutant demonstrated that, like *crtiso* mutants, the phenotype could be rescued by light exposure, to form 9,9′-di-*cis*-zeta-carotene, the substrate for ZDS (Li *et al.*, 2007). The *Z-ISO* gene was identified in both maize and Arabidopsis and found to be similar to *NnrU* (for nitrite and nitric oxide reductase U), which is required for bacterial denitrification, a pathway that produces nitrogen oxides as alternate electron acceptors for anaerobic growth. An *Escherichia coli* assay proved that Z-ISO was capable of 15-*cis* bond isomerization in 9,15,9′-tri-*cis*-ζ-carotene (Chen *et al.*, 2010).

In the Arabidopsis *CRTISO* (*ccr2*) and *Z-ISO* mutants, *cis* intermediates are photoisomerized in the light, which raises questions about the necessity of carotenoid isomerases in plants and why there are four genes required for the synthesis of lycopene in plants but only one in bacteria. In chromoplasts, CRTISO activity is required for all-*trans*-lycopene accumulation, regardless of the light regime, because the *tangerine* mutant accumulates tetra-*cis*-lycopene in the light (Isaacson *et al.*, 2002). Carotenoids are deposited in a crystalline form in tomato chromoplasts and these may be more resistant to photoisomerization. Further, although the biosynthetic pathway proceeds in chloroplasts, a delayed greening and substantial reduction in lutein occurs in mutants defective in CRTISO in Arabidopsis and some chlorosis occurs in rice and tomato leaves (Fang *et al.*, 2008; Isaacson *et al.*, 2002; Park *et al.*, 2002). Thus, carotenoid synthesis in dark-grown tissues absolutely requires isomerase activity. Such tissues include the endosperm, a target for provitamin A carotenoid biofortification.

4. Cyclases

After lycopene, the carotenoid biosynthetic pathway divides into two branches, distinguished by different cyclic end groups, namely beta or epsilon. Two β-rings form the β,β branch (β-carotene and its derivatives) with

one β- and one ε- forming the β,ε branch (α-carotene and its derivatives). βLCY introduces a β-ionone ring to either end of all-*trans*-lycopene to produce β-carotene, whereas both the β-cyclase and ε-cyclase enzymes are required to form α-carotene (Cunningham and Gantt, 2001). Curiously, mutated maize endosperm tissue lacking βLCY activity was also found to accumulate lactucaxanthin (ε,ε-ring) and other unusual carotenes, including δ-carotene, and ε-carotene. The ratio of *βLCY:εLCY* transcripts correlated with the accumulation of different cyclization products in embryo and endosperm tissues (Bai *et al.*, 2009). *εLCY* expression is important in controlling pathway flux to carotenes with higher provitamin A value and the breeding alleles that have been developed for breeding high-provitamin A maize (Harjes *et al.*, 2008).

Other cyclase activities include the capsanthin–capsorubin synthase (CCS) (Lefebvre *et al.*, 1998) in capsicum that cyclizes lycopene to produce the κ-cyclic carotenoids, capsanthin and capsorubin. CCS was found to contain a noncovalently bound flavin adenine dinucleotide (FAD), though it was only required for activity in the presence of NADPH, which functions as the FAD reductant. The CCS flavoproteins catalyse reactions with no net redox change as the reaction did not transfer hydrogen from the dinucleotide cofactors to β-carotene or capsanthin. Thus, FAD in its reduced form could be implicated in the stabilization of the carbocation intermediate (Mialoundama *et al.*, 2010).

C. XANTHOPHYLL SYNTHESIS

Xanthophylls are oxygenated derivatives of carotenes and play important roles in photoprotection and light-harvesting antennae formation (Niyogi, 1999).

1. Hydroxylases

Nearly all xanthophylls in higher plants have hydroxyl moieties on the 3-carbon in the β- or α-carotene rings to form zeaxanthin and lutein, respectively. There are two distinct hydroxylation reactions of the ε- and β-rings, confirmed by the identification of the ε-hydroxylase (εOH) locus, *lut1* (Pogson *et al.*, 1996), and the β-hydroxylase (βOH) genes in higher plants (Sun *et al.*, 1996). βOH enzymes are ferredoxin dependent and contain an iron-coordinating histidine cluster that is required for activity (Bouvier *et al.*, 1998). In contrast, εOH is a plastid-targeted cytochrome P450-type monooxygenase with a distinctly different enzymatic mechanism from the βOHs (Tian *et al.*, 2004).

βOH activity is an important provitamin A biofortification target, as hydroxylation or any other modification of β-ionone rings depletes vitamin A potential. Thus, reduced hydroxylase activity will result in fewer β-rings modifications, thereby maintaining β-carotene pool and maximum vitamin A potential. Of the six loci encoding this enzyme, one locus, *HYD3*, was found to be critical for maize endosperm β-carotene levels and alleles were identified in a population of 51 maize lines (Vallabhaneni *et al.*, 2009) and further association and linkage population studies in maize found that this gene was indeed responsible for a QTL associated with β-carotene accumulation (Yan *et al.*, 2010), and in combination with ϵLCY alleles (Harjes *et al.*, 2008), it is now possible to use molecular markers to select for high-provitamin A carotenoid maize seeds.

2. Zeaxanthin epoxidase and violaxanthin de-epoxidase

An epoxide group is introduced into both rings of zeaxanthin by zeaxanthin epoxidase (ZEP) to form violaxanthin. Under high light stress, the reverse reaction is rapidly undertaken by the violaxanthin de-epoxidase (VDE; Yamamoto, 1979). Light is critical in modulating the interconversion of zeaxanthin and violaxanthin. Under normal light conditions, when the incident light can be safely utilized for photosynthetic electron transport, ZEP converts zeaxanthin to violaxanthin by introducing 5,6-epoxy groups to the 3-hydroxy-β-rings. However, when incident light is in excess, VDE converts a substantial pool of violaxanthin to zeaxanthin (Pfundel *et al.*, 1994).

VDE is soluble and inactive at neutral pH, but following acidification (below pH 6.5) it attaches to the thylakoid membrane and its violaxanthin substrate (Hager and Holocher, 1994). The thylakoid membrane lipid monogalactosyldiacylglycerol is needed for optimal VDE activity when assayed *in vitro* and it requires ascorbate as a reductant (Schaller *et al.*, 2010). Structural analyses revealed that at neutral pH, VDE is monomeric and its active site occluded within a lipocalin barrel, but acidification causes the barrel to open and the enzyme dimerizes. The carotenoid substrate could fit in a channel linking the two active sites of the dimer enabling de-epoxidation of both violaxanthin β-rings, thus forming zeaxanthin (Arnoux *et al.*, 2009). Site-directed mutagenesis of amino acid residues lying in close contact with the two substrates supported the proposed substrate-binding sites and identified two residues, Asp-177 and Tyr-198, that are required for catalytic activity (Saga *et al.*, 2010).

ZEP mutants, *aba1,* are deficient in ABA and display a partially de-etiolated phenotype, including reduced hypocotyl growth, cotyledon expansion and the development of true leaves during late skotomorphogenic growth. However, other ABA-deficient mutants lack this phenotype and

ABA application did not rescue the skotomorphogenesis, though it could be phenocopied by the addition of fluridone, a carotenoid inhibitor that blocks PDS activity. Thus, ZEP appears to have a role in skotomorphogenic growth (Barrero *et al.*, 2008).

3. *Neoxanthin synthase*

Conversion of violaxanthin to neoxanthin is performed by the enzyme neoxanthin synthase (NXS), which was unequivocally identified in a novel ABA-deficient Arabidopsis mutant, *aba4*. The predicted gene product is a novel chloroplast membrane protein, and constitutive expression of *ABA4* in Arabidopsis led to increased accumulation of *trans*-neoxanthin. Significantly reduced levels of ABA were synthesized in dehydrated *aba4* mutants, demonstrating that ABA biosynthesis in response to stress must occur mainly via neoxanthin isomer precursors (North *et al.*, 2007). Detached *aba4.1* leaves were more sensitive to oxidative stress than the wild type and *aba4.1 npq1* double mutants, lacking both zeaxanthin and neoxanthin, underwent stronger PSII photoinhibition (Dall'Osto *et al.*, 2007).

D. CLEAVAGE PRODUCTS

Characterization of the carotenoid-cleavage gene family has yielded some interesting results in recent years. The enzyme products are varyingly referred to as carotenoid-cleavage dioxygenases (CCD) or 9-*cis*-epoxycarotenoid dioxygenases (NCED), reflecting the first characterized member of this gene family (Schwartz *et al.*, 1997; Tan, 1997). The nine members of the gene family in Arabidopsis show different substrate specificity and tissue distribution (Schwartz *et al.*, 2001, 2003; Tan *et al.*, 2003). The CCD gene family is responsible for the formation of vitamin A, phytohormones (e.g. ABA and strigolactones), coloured spices (e.g. saffron and bixin) and novel signalling molecules as well as plant volatiles used in the perfume industry (Fig. 4).

1. *Vitamin A*

Vitamin A is a C_{20} cleavage product of carotenoids, which, in addition to its retinoid derivatives, is essential for animal survival and vitamin A biosynthesis has recently been reviewed in detail (von Lintig, 2010). Cleavage of β-carotene was postulated as an important step in the formation on vitamin A, but it was not until 2000 that a β-carotene 15,15′-dioxygenase was cloned from *Drosophila melanogaster* (von Lintig and Vogt, 2000) and chicken (Wyss *et al.*, 2000). The deduced amino acid sequence showed homology to the maize carotenoid dioxygenase, VP14, involved in the synthesis of ABA.

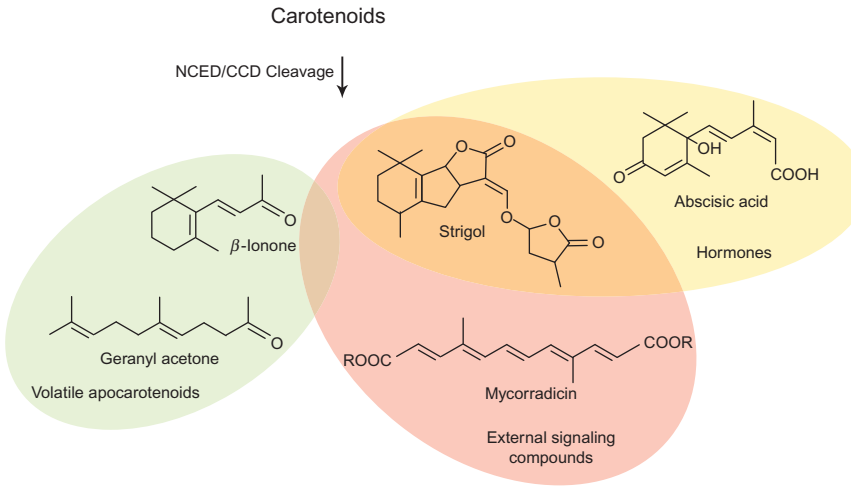

Fig. 4. Carotenoid cleavage products have diverse roles. Carotenoids are cleaved by 9-*cis*-epoxycarotenoid dioxygenase (NCED) or carotenoid cleavage dioxygenase (CCD) enzymes and further modified to form apocarotenoids with diverse functions. Geranyl acetone and β-ionone are volatile apocarotenoids that are commonly used in fragrance manufacture. Mycorradicin is involved in recruiting beneficial fungi. Strigolactones such as strigol enhance the germination of harmful parasitic plant seeds and modulate shoot branching as well as stimulate beneficial mycorrhizal fungi symbiosis. Abscisic acid mediates plant stress responses, playing an important role in controlling stomatal aperture and transpiration as well as promoting seed development and dormancy.

Any carotenoid containing an unmodified β-ionone ring has provitamin A activity; thus, β-carotene is one of the most active because a single β-carotene molecule is cleaved to form two all-*trans*-retinal molecules, which are reduced to form all-*trans*-retinol (vitamin A). All retinoids are derived from this compound and maintain the characteristic β-ionone ring. Different end groups or β-ionone ring modifications characterize the various retinoids. For example, retinoic acid (or 11-*cis*-retinal), which is required for reproduction, embryonic development, cell differentiation, immunity and other biological processes, binds to opsin to provide a chromophore for the visual pigments that mediate phototransduction (von Lintig, 2010).

2. *Phytohormones*

The plant hormone ABA is primarily involved in plant stress responses, seed development and dormancy (Seo and Koshiba, 2002). ABA is a cleavage product of 9-*cis*-violaxanthin and/or 9′-*cis*-neoxanthin, an idea that was first proposed by Taylor and Smith (1967). Cleavage of 9′-*cis*-neoxanthin by

NCED produces xanthoxin and was first identified in the maize *viviparous14* (*vp14*) mutant (Schwartz *et al*., 1997; Tan, 1997). Xanthoxin is followed in the pathway by a number of further modified products that are required to produce ABA (Seo and Koshiba, 2002). For the ABA signal to be transmitted, it must first bind a receptor molecule. The putative identification of such receptors has been the topic of recent controversy, though the recent crystal structure of a PYR/PYL (pyrabactin resistance/pyrabactin resistance-like) or RCAR (regulatory component of ABA receptor) protein appears to resolve this question (Park *et al*., 2009). ABA-bound PYR/PYL/RCAR protein inhibits a phosphatase 2C that is known to participate in ABA signalling (Ma *et al*., 2009).

Strigolactones are carotenoid-derived terpenoid lactones that inhibit shoot branching and can be exuded from plant roots to recruit beneficial mycorrhizal fungi. This apocarotenoid signal has been hijacked by parasitic plant seeds to encourage germination (Dun *et al*., 2009; Matusova *et al*., 2005). Such a signal was initially proposed after novel *CCD* mutants were found to exhibit increased shoot branching in Arabidopsis *max4* and pea *rms1* mutants (Sorefan *et al*., 2003). MAX3 (CCD7) (Booker *et al*., 2004) and MAX4 (CCD8) can sequentially cleave β-carotene to form the C_{18} compound 13-apo-carotenone (Schwartz *et al*., 2004). The recent discovery that both rice and pea branching mutants were deficient in strigolactones resolved years of speculation about the nature of the branching signal. It has been shown that strigolactone application restores the wild-type branching phenotype in pea *CCD8* mutants, confirming that strigolactones are necessary and sufficient to inhibit shoot branching in plants. Further, the *CCD8* mutants exhibited additional typical strigolactone-deficient phenotypes including alterations to mycorrhizal symbiosis and parasitic weed interaction (Gomez-Roldan *et al*., 2008). Concurrent studies confirmed that synthetic strigolactone application inhibits tillering in rice *D10* (*CCD8*) and *D17* (*CCD7*) mutants as well as rescuing the equivalent Arabidopsis mutants. An elegant indirect assay confirmed that these mutants were deficient in strigolactone synthesis, as root exudates did not stimulate germination of parasitic *Striga* seeds to the same extent as wild-type exudates (Umehara *et al*., 2008). The *CCD7* knockdown in tomato exhibited increased branching, but a metabolic screen did not identify any significant changes in root carotenoid substrate. However, C_{13} cyclohexenone and C_{14} mycorradicin apocarotenoids were reduced in response to mycorrhizal colonization, indicating that CCD7 is required for arbuscular mycorrhiza-induced apocarotenoid synthesis (Vogel *et al*., 2010).

Other components of the strigolactone biosynthetic pathway have been identified, including *MAX1*, which encodes a cytochrome p450 that modifies

an apocarotenoid product of the CCD7 and CCD8 cleavage reactions to produce another mobile intermediate (Booker *et al.*, 2005). *MAX2/RMS4/D3* encode F-box proteins and the mutants are not rescued by exogenous strigolactones and are thus predicted to have a role in signalling via ubiquitin-mediated protein degradation (Beveridge *et al.*, 1996; Stirnberg *et al.*, 2002). Additional steps have been identified in rice, including another high-tillering rice mutant, *d27*, which does not exude strigolactones. *D27* is chloroplast localized, though its enzymatic activity has not been described. Crosses with *d10* (*CCD8*) are not additive and the *d27* mutant can be rescued by strigolactone application, thus is thought to be required for the biosynthesis of strigolactones (Lin *et al.*, 2009). The *D14* gene encodes a α/β-fold hydrolase, and the *d14* mutant is strigolactone insensitive, but exhibits increased tillering and does not show an additive phenotype when crossed with *d10* (Arite *et al.*, 2009). Characterization of this curious mutant could provide insights into strigolactone signalling or have a role in producing a bioactive strigolactone-derived hormone.

Strigolactone and ABA composition were analysed in plants treated with inhibitors of specific carotenoid-cleavage enzymes. Strigolactone content was reduced in plants treated with the CCD inhibitor, D2, but root ABA levels were maintained. Conversely, plants with genetically or chemically inhibited ABA biosynthesis also had reduced strigolactones and a concomitant reduction in *CCD7* and *CCD8* transcript abundance, implying a potential cross-talk role for ABA in the regulation of strigolactone biosynthesis (Lopez-Raez *et al.*, 2010). Finally, strigolactone biosynthesis and the concomitant branching phenotype are responsive to phosphate deficiency in Arabidopsis (Kohlen *et al.*, 2010). The role of strigolactones in controlling plant morphology and response to the environment has become an exciting area of active research.

3. *Bixin, saffron and plant volatiles*

Carotenoid cleavage metabolites are vital for plants and animals. They are also highly prized in the food and cosmetic industries. Bixin (annatto) is a red-coloured, di-carboxylic monomethyl ester apocarotenoid, traditionally derived from the plant *Bixa orellana.* Bouvier and colleagues identified a lycopene cleavage dioxygenase, bixin aldehyde dehydrogenase and norbixin carboxyl methyltransferase that are required to produce bixin from lycopene. Co-transforming the appropriate constructs into *E. coli,* engineered to produce lycopene, resulted in bixin production at a level of 5 mg g^{-1} dry weight (Bouvier *et al.*, 2003a).

Saffron, another commercially important coloured compound, can attribute the majority of its characteristic colour, flavour and aroma to the

accumulation of carotenoid derivatives. A crocus (*Crocus sativus*) zeaxanthin 7,8(7′,8′)-cleavage dioxygenase (CsZCD) was cloned and found to be targeted to the chromoplast and initiated the production of the cleavage products. Another enzyme, 9,10(9′,10′)-cleavage dioxygenase was also cloned and found to be a less specific cleavage enzyme (Bouvier *et al*., 2003b).

Beta-ionone is the predominant norisoprenoid volatile in the mature stigma tissue. Four *CCD* genes were isolated from crocus that were capable of cleaving β-carotene at the 9,10(9′,10′) positions to yield β-ionone, though with different expression patterns indicative of sub-functionalization (Rubio *et al*., 2008). Differential expression was also observed for β*LCY* genes, *CstLcyB1* and *CstLcyB2a*. The CstLcyB2a is chromoplast specific and conspicuously absent in crocus species with low apocarotenoid content, suggesting that it encodes an important step in determining the accumulation of β-carotene substrate that is required to produce the distinctive saffron apocarotenoids (Ahrazem *et al*., 2010).

4. Novel-signalling molecules

A putative novel signal has been observed in Arabidopsis *bps1* mutants, which are developmentally defective but the shoot can be rescued if the roots are removed or carotenoid biosynthesis is chemically blocked with norflurazon. It is hypothesized that an unknown substance moves constitutively from the root to the shoot to arrest growth, and this is supported by experiments demonstrating that mutant roots are sufficient to arrest wild-type shoot development (Van Norman *et al*., 2004). *BYPASS1* encodes a novel protein of unknown function that is widespread in plant genomes (Sieburth and Lee, 2010), though the tobacco homologue contains a transmembrane domain and GFP fusion proteins were endoplasmic reticulum associated (Kang *et al*., 2008). It is likely that more novel carotenoid-derived signalling molecules remain to be identified.

IV. REGULATION OF CAROTENOID BIOSYNTHESIS

A. TRANSCRIPTIONAL REGULATION

Carotenoid composition is responsive to environmental stimuli, oxidative stress, redox poise and metabolite feedback regulation. In general, increases in carotenoid accumulation, be it during fruit ripening, flower development or production of stress-induced carotenoids in algae, coincide with increased transcript abundance of some key (but not all) steps in the pathway.

Phytoene biosynthesis is a rate-limiting step in carotenogenesis and transcript abundance can dramatically alter carotenoid pool size, thus making PSY a logical target in the study of carotenoid regulation. Changes in transcript abundance are particularly evident during morphogenic changes from etioplast to chloroplast or chloroplast to chromoplast. PSY transcript abundance is upregulated during photomorphogenesis via a phytochrome-mediated (red-light) pathway, a response that is abolished in the *phyA* mutant (Welsch *et al.*, 2000, 2008). Phytochrome-mediated light signals regulate carotenoid biosynthesis in plants by way of phytochrome-interacting factor 1 (PIF1), which directly binds to the *PSY* promoter, thus repressing *PSY* expression. Light-triggered degradation of PIFs by photoactivated phytochromes during deetiolation permits *PSY* expression, which enables rapid production of carotenoids (Toledo-Ortiz *et al.*, 2010).

Further evidence that *PSY* controls metabolic flux was obtained by paclobutrazol treatment, which inhibits gibberellin synthesis and enables deetiolation despite the absence of light. PSY activity and carotenoid levels increased in the dark following treatment with paclobutrazol, and this increase was supported by feedback regulation of DXS protein abundance. Overexpression of *DXS* alone in etiolated tissue did not increase carotenoid accumulation; however, *PSY* overexpression resulted in increased carotenoid accumulation and a concomitant post-transcriptional accumulation of DXS (Rodriguez-Villalon *et al.*, 2009).

PSY is present as a single copy in Arabidopsis, but additional homologues have been identified in tomato, poplar and cereal crops such as rice, wheat and maize (Chaudhary *et al.*, 2010; Howitt *et al.*, 2009; Li *et al.*, 2008a,b; Welsch *et al.*, 2008). *PSY* homologues respond differently to abiotic stimuli and have unique tissue specificities though their function remains redundant. For example, salt and drought induce *PSY3* transcript abundance in maize roots, which correlated with increased carotenoid flux and ABA in maize roots (Li *et al.*, 2008a). Rapid disappearance of *PSY2* and *PSY3* mRNA after rewatering suggests mRNA instability or strict control of transcription (Li *et al.*, 2008a). Similar responses were observed in rice *PSY* homologues (Welsch *et al.*, 2008). Cassava also has three sub-functionalized *PSY* genes; however, it was not *PSY3*, but a *PSY1* paralogue that responded to abiotic stress (Arango *et al.*, 2010). Perhaps the most dramatic enhancement of carotenoid accumulation has been achieved in the oil seeds of canola (*Brassica napus*) and Arabidopsis, where overexpression of *PSY* in seeds resulted in a 43- to 50-fold increase in total carotenoid content (Lindgren *et al.*, 2003; Shewmaker *et al.*, 1999). *PSY* overexpression in Arabidopsis seedlings did not alter carotenoid content. However, non-photosynthetic calli and roots overexpressing *PSY* accumulated 10- to 100-fold more carotenoids than

corresponding wild-type tissues, predominantly β-carotene and its derivatives, which were deposited as crystals. Similarly, overexpression of the bacterial *PSY*, *crtB*, in white carrot roots also initiated carotenoid crystal formation (Maass *et al*., 2009).

The complexity of carotenoid regulation is further demonstrated by the analysis of the *PSY* promoter where a *cis*-acting motif (ATCTA) was identified to be involved in mediating the transcriptional regulation of photosynthetic genes, including *PSY* (Welsch *et al*., 2003). Manipulation of *RAP2.2*, APETALA2 transcription factors that bind to the *PSY* promoter, resulted in only minor carotenoid alterations in root calli (Welsch *et al*., 2007).

The relative activities of the εLCY and βLCY at the branch point of the pathway have a major regulatory role in modulating the ratio of lutein to that of the β-branch carotenoids (Cuttriss *et al*., 2007). CRTISO is a major regulatory node at the branch point of the biosynthetic pathway (Cazzonelli *et al*., 2009; Isaacson *et al*., 2004). A chromatin-modifying histone methyltransferase enzyme (SET DOMAIN GROUP 8, SDG8) has been shown to be necessary for maintaining *CRTISO* gene expression (Cazzonelli *et al*., 2009). The *CRTISO* and *SDG8* promoters show overlapping patterns of expression specifically in the shoot apical meristem and pollen, which are active sites of cell division and epigenetic programming (Cazzonelli and Pogson, 2010). The absence of SDG8 reduces *CRTISO* transcript abundance, thereby altering carotenoid flux through the pathway, which might potentially impair strigolactone biosynthesis. This was the first report implicating epigenetic regulatory mechanisms in the control of carotenoid composition (Cazzonelli *et al*., 2009).

Allelic variation is another important source of carotenoid regulation. For example, alternative splicing of the *PSY-A1* allele altered the relative abundance of functional *PSY* transcript and appeared to be a major QTL determinant of flour colour in bread wheat (Howitt *et al*., 2009). This was reiterated by a detailed analysis of natural genetic variation in maize. Association analysis, linkage mapping, expression analysis and mutagenesis confirmed that variation at the εLCY locus altered flux partitioning. Four polymorphisms controlled 58% of the variation between α- and β-branch accumulation, thus enabling the selection of alleles that confer high-provitamin A status for improved maize varieties (Harjes *et al*., 2008). Natural variation in βOH activity also has a significant impact on carotenoid composition (Vallabhaneni *et al*., 2009; Yan *et al*., 2010). Multiple control points both within the carotenoid pathway and MEP precursor pathway were identified in maize, and the timing of gene expression was found to be critical in determining carotenoid composition (Vallabhaneni and Wurtzel, 2009).

B. METABOLITE FEEDBACK

Feedback regulation by ABA increases *PSY3* gene expression in rice and plays a critical role in the formation of a positive feedback loop that mediates abiotic stress-induced ABA formation (Welsch *et al.*, 2008). The *βLCY* gene from the eubacterium *Erwinia herbicola* and daffodil (*Narcissus pseudonarcissus*) flowers were introduced into the tomato plastid genome resulting in increased accumulation of xanthophyll cycle pigments in leaves and β-carotene in fruits. Surprisingly, transplastomic tomatoes showed > 50% increase in total carotenoid accumulation (Apel and Bock, 2009), which may be due to a carotenoid product or intermediate feedback.

Lutein levels are altered when the higher plant desaturases and isomerases are bypassed, and thus *cis*-carotene intermediates are not produced (Misawa *et al.*, 1994). Similarly, the absence of CRTISO or specific carotene isomers results in less lutein (Isaacson *et al.*, 2002; Park *et al.*, 2002). The mechanism of this flux partitioning is unclear, though flux through the two branches can be determined by *εLCY* mRNA levels (Cuttriss *et al.*, 2007; Harjes *et al.*, 2008; Pogson *et al.*, 1996; Pogson and Rissler, 2000) and recent experiments indicate that both CRTISO (*ccr2*) and SDG8 (*ccr1*) mutants have aberrant *εLCY* transcript levels. It is thus possible that feedback may account for at least part of the reduction in lutein (Cazzonelli *et al.*, 2009; Cuttriss *et al.*, 2007).

C. CATABOLISM

Accumulation of carotenoids in photosynthetic tissue requires a balance between their rate of synthesis and catabolism. Recent $^{14}CO_2$ uptake data demonstrates that synthesis, and by inference, turnover, is much more rapid than expected (Beisel *et al.*, 2010). The incorporation of ^{14}C into different carotenoids was not uniform and varied between mutants and under high light (Beisel *et al.*, 2010), implying active degradation both enzymatically and by oxidative damage.

Studies in Arabidopsis, strawberry (*Fragaria ananassa*) and chrysanthemum (*Chrysanthemum morifolium*) petals have all demonstrated that the pool of carotenoids is determined in part by CCD catalysed degradation (Auldridge *et al.*, 2006; Garcia-Limones *et al.*, 2008; Ohmiya *et al.*, 2006). In Arabidopsis seeds, loss of CCD function leads to significantly higher carotenoid levels (Auldridge *et al.*, 2006).

CCD1 expression levels in strawberry correlate with ripening and a decrease in lutein content, which suggests that lutein could constitute the main natural substrate of FaCCD1 activity (Garcia-Limones *et al.*, 2008). High

expression of CCD1 associated with certain maize alleles was correlated with low carotenoid levels in maize endosperm (Vallabhaneni *et al*., 2010). Petal colour in chrysanthemums is also regulated by CCD activity; white petals contain elevated transcript levels of *CmCCD4a*, which catabolizes the yellow carotenoid pigments (Ohmiya *et al*., 2006). Curiously, when *CCD1* was overexpressed in high carotenoid golden rice lines (GR2), there appeared to be little impact on carotenoid levels in the endosperm. In fact, a similar carotenoid content was observed in both GR2 and antisense lines. Surprisingly, *in vitro* analyses suggested that apocarotenoids were the primary substrates of OsCCD1 (Ilg *et al*., 2010).

D. STORAGE CAPACITY

Carotenoid biosynthesis appears to take place largely at the chloroplast envelope and, in some cases, the thylakoid membrane (Joyard *et al*., 2009). Storage capacity is a major determinant of carotenoid pool size; the *high pigment2* (*hp2*) tomato mutant (DEETIOLATED1, a negative regulator of light signalling) has a larger plastid and thus increased pigmentation (Kolotilin *et al*., 2007). Similarly, the *hp3* tomato mutant (ZE) revealed an ABA deficiency, an enlarged plastid compartment and 30% more carotenoids in mature fruit (Galpaz *et al*., 2008). Plastid differentiation is an important mechanism in determining storage capacity, as demonstrated by the cauliflower (*Brassica oleracea*) Orange (*Or*) gene that creates a metabolic sink to accumulate β-carotene in the chromoplast (Li *et al*., 2001; Li and Van Eck, 2007; Lu *et al*., 2006). During the chloroplast to chromoplast transformation process, carotenoids become localized in plastoglobuli (Steinmuller and Tevini, 1985). Carotenoids within plastoglobuli exhibit much higher light stability than carotenoids within chloroplast membranes (Merzlyak and Solovchenko, 2002).

V. NUTRITION

A. RICE

Golden rice (*Oryza sativa*) was developed to alleviate vitamin A deficiency as this important staple crop does not typically accumulate any carotenoids in edible endosperm tissue. Daffodil *PSY* and bacterial desaturases (*crtI, Erwinia uredovora*) were targeted to endosperm tissue, where they produced up to 1.6 μg g^{-1} carotenoids, predominantly β-carotene due to endogenous cyclase activity (Ye *et al*., 2000). A second generation line 'Golden Rice 2' overcame

a metabolic bottleneck by incorporating a more active *PSY* gene from maize, which substantially improved carotenoid biosynthesis, with some lines accumulating up to 37 μg g^{-1} (Paine *et al.*, 2005). More recent work has focused on transgene stability and the transformation of high-yielding cultivars (Datta *et al.*, 2006, 2007). A dietary study of Golden Rice confirmed that deuterium-labelled [^{2}H]$\hat{a}$-carotene produced by these plants could be converted to retinol and is thus an effective biofortification strategy (Tang *et al.*, 2009).

B. MAIZE

Zea mays is an essential staple cereal crop that naturally accumulates provitamin A carotenoids in the endosperm of the seed. There are vast diverse collections from which to source favourable alleles for plant breeding programmes. Such collections have been extensively utilized to identify important regulatory points in determining provitamin A potential. A significant QTL analysis determined that *PSY1* was responsible for 6.6–27.2% of phenotypic variation in carotenoid content (Chander *et al.*, 2008). Genetic variation in ε*LCY* was responsible for 58% of the variation in flux between the two branches of the pathway and is critical for driving provitamin A levels (Harjes *et al.*, 2008). Two recent studies identified different βOH alleles of one locus that were important in determining the extent of β-ionone ring hydroxylation, and thus loss of provitamin A activity (Vallabhaneni *et al.*, 2009; Yan *et al.*, 2010). The most favourable alleles were found in temperate varieties and will be bred into tropical maize germplasm to help alleviate vitamin A deficiency in third world countries (Yan *et al.*, 2010). Recent studies also identified additional control points that offer future possibilities for further enhancing carotenoid levels in maize (Vallabhaneni *et al.*, 2010; Vallabhaneni and Wurtzel, 2009). Transgenic approaches to maize biofortification have also played a significant role in modifying β-carotene content (Aluru *et al.*, 2008; Zhu *et al.*, 2008) and laid the foundation for targeting alternative approaches. Analyses of tropical varieties (Menkir *et al.*, 2008) and sweet corn (Fanning *et al.*, 2010) have identified further diversity for carotenoid enhancement projects.

C. WHEAT

Triticum spp. endosperm colour is an important agronomic trait and thus has been the focus of several QTL studies. Lutein is the predominant carotenoid in wheat endosperm tissue and is frequently heavily esterified (Atienza *et al.*, 2007; Howitt *et al.*, 2009). A targeted molecular marker was developed for

the *PSY1* gene on wheat chromosome 7A, and found to co-segregate with yellow pigmentation in a collection of Chinese wheat cultivars (He *et al.*, 2008). Further, the total carotenoid pool size was found to be modulated by ε*LCY* alleles and/or *PSY-A1* spice variants (Howitt *et al.*, 2009). Transgenic wheat expressing endosperm-specific PSY1 from maize and bacterial CRTI (desaturases) produced a 10.8-fold increase (up to 4.96 μg g^{-1} dry weight) in total seed carotenoid content (Cong *et al.*, 2009). Thus, both targeted breeding and transgenic approaches are likely to improve wheat lutein content, which is correlated with protection against age-related macular degeneration (AMD) of the eye—the leading cause of blindness in the developed world. Whether such strategies can increase provitamin A levels in wheat has not been reported thus far.

D. CASSAVA

Manihot esculenta is an important staple crop, especially in arid regions such as sub-Saharan Africa, though it is nutrient poor and typically accumulates very little provitamin A. Analysis of diversity collections identified landraces that accumulate lycopene (5 mg kg^{-1}) or β-carotene (4 mg kg^{-1}) (Nassar *et al.*, 2007) and such variation was harnessed to identify natural *PSY* alleles that altered metabolic flux (Welsch *et al.*, 2010). Cassava has three *PSY* genes, one of which (*PSY1*) responded strongly to abiotic stress (Arango *et al.*, 2010). A single nucleotide polymorphism in *PSY2* was found to co-segregate with yellow-rooted cultivars in a breeding population that accumulated between 6.0 and 11.5 μg g^{-1} carotenoids in fresh tissue. This genetic variant was used to successfully produce transgenic cassava with increased carotenoid accumulation in the roots (Welsch *et al.*, 2010). Bioavailability of β-carotene in cassava was analysed and found to be as efficacious as β-carotene supplementation; thus, biofortification of cassava is a valid approach to alleviating vitamin A deficiencies (Howe *et al.*, 2009)

E. SORGHUM

Sorghum bicolor is a major staple crop grown in semiarid regions due to its drought tolerance, which makes it a good candidate for biofortification. Yellow endosperm varieties contain provitamin A carotenoids and diverse collections of sorghum landraces have been analysed to quantify pigment diversity, including a collection of 164 landraces from Niger and Nigeria (Fernandez *et al.*, 2009). Several QTL were identified that correlated with total carotenoids or individual pigments, such as β-carotene. A strong QTL that accounted for between 11% and 15% of phenotypic variation was

associated with *PSY3*, thus pinpointing a focal point for breeding high-provitamin A sorghums (Fernandez *et al.*, 2008).

F. BANANA AND PLANTAIN

Banana and plantain (*Musa* spp.) are tropical crops and some of the most highly consumed fruits in the world. They have a high genetic diversity, as exemplified by the Embrapa international germplasm collection of more than 400 accessions, including wild diploids, triploids and tetraploids; however, they are not readily bred. Analysis of pigment composition identified 42 high pigment lines that accumulate between 1.06 and 19.24 $\mu g\ g^{-1}$ of total carotenoids. Genetic variability was estimated using Diversity Arrays Technology molecular markers to establish a biofortification programme (Amorim *et al.*, 2009). A similar study identified broad pigment diversity but limited accumulation of mineral micronutrients in a 171 genotype collection (Davey *et al.*, 2009).

G. SWEET POTATO

Proof of the biofortification principle was established in Kenya where consumption of the orange-fleshed sweet potato (*Ipomoea batatas*) increased the vitamin A status of women and children (Hagenimana *et al.*, 1999). A similar study in South Africa demonstrated a reduction in vitamin A deficiency of children (van Jaarsveld *et al.*, 2005). However, analysis of carotenoid degradation in stored sweet potato, which is typically dried and stored for months, indicated losses of around 70% of the total carotenoid pool after 4 months' storage in Uganda. This demonstrates the necessity for establishing diversity in carotenoid-rich agricultural products and underlines the difficulty in maintaining provitamin A intake outside of the growing season (Bechoff *et al.*, 2010).

H. POTATO

Another staple food crop with limited micronutrient content is potato (*Solanum tuberosum*). Potato has been successfully fortified to produce provitamin A carotenoids. Overexpression of three bacterial genes for β-carotene synthesis (*CrtB, CrtI* and *CrtY*, encoding PSY, PDS and βLCY, respectively) from Erwinia were targeted to the tuber. The transgenic lines accumulated up to 47 $\mu g\ g^{-1}$ β-carotene (Diretto *et al.*, 2007). Detailed transcript analyses of lines carrying various combinations of transgenes found that β-cyclase had the greatest impact on regulating the amount of carotenoid accumulation (Diretto *et al.*, 2010).

VI. CONCLUSIONS

The essential roles that carotenoids play in human health, as well as plant photosynthesis, photoprotection and reproduction, make them obvious candidates for enhancement and manipulation. To this end, molecular genetics, in concert with classical biochemistry, has facilitated an advanced understanding of the biosynthetic pathway. Breakthroughs in understanding the regulation of carotenoid accumulation are paving the way for improving the provitamin A content of staple food crops that would otherwise be of low nutritional value. This is of utmost importance for developing countries, where food storage is a problem and effective agriculture practices are still being developed. Further characterisation of regulatory processes that determine carotenoid accumulation, composition and storage capacity, as well as developing new transgenic technologies and breeding varieties, will all continue to strengthen biofortification projects in diverse crop species.

ACKNOWLEDGEMENTS

The authors acknowledge the support of Professor David Christopher, the New Zealand Foundation for Research Science and Technology to A. J. C.; funding from the United States National Institutes of Health (GM081160) and New York State to E. T. W. and A. J. C; and the Australian Research Council Centre of Excellence in Plant Energy Biology (CE0561495) to C. I. C. and B. J. P.

REFERENCES

Ahrazem, O., Rubio-Moraga, A., Lopez, R. C. and Gomez-Gomez, L. (2010). The expression of a chromoplast-specific lycopene beta cyclase gene is involved in the high production of saffron's apocarotenoid precursors. *Journal of Experimental Botany* **61**, 105–119.

Aluru, M., Xu, Y., Guo, R., Wang, Z. G., Li, S. S., White, W., Wang, K. and Rodermel, S. (2008). Generation of transgenic maize with enhanced provitamin A content. *Journal of Experimental Botany* **59**, 3551–3562.

Amat, J. A., Rendón, M. A., Garrido-Fernández, J., Garrido, A., Rendón-Martos, M. and Pérez-Gálvez, A. (2010). Greater flamingos Phoenicopterus roseus use uropygial secretions as make-up. *Behaviour Ecology and Sociobiology* **50**, 55–60.

Amorim, E. P., Vilarinhos, A. D., Cohen, K. O., Amorim, V. B. O., dos Santos-Serejo, J. A., Oliveira e Silva, S., Pestana, K. N., dos Santos, V. J., Paes, N. S., Monte, D. C. and dos Reis, R. V. (2009). Genetic diversity of carotenoid-rich bananas evaluated by Diversity Arrays Technology (DArT). *Genetics and Molecular Biology* **32**, 96–103.

Apel, W. and Bock, R. (2009). Enhancement of carotenoid biosynthesis in transplastomic tomatoes by induced lycopene-to-provitamin A conversion. *Plant Physiology* **151**, 59–66.

Araki, N., Kusumi, K., Masamoto, K., Niwa, Y. and Iba, K. (2000). Temperature-sensitive Arabidopsis mutant defective in 1-deoxy-D-xylulose-5-phosphate synthase within the plastid non-mevalonate pathway of isoprenoid biosynthesis. *Physiologia Plantarum* **108**, 19–24.

Arango, J., Wust, F., Beyer, P. and Welsch, R. (2010). Characterization of phytoene synthases from cassava and their involvement in abiotic stress-mediated responses. *Planta* **232**, 1251–1256.

Arite, T., Umehara, M., Ishikawa, S., Hanada, A., Maekawa, M., Yamaguchi, S. and Kyozuka, J. (2009). d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant & Cell Physiology* **50**, 1416–1424.

Armstrong, G. A. (1994). Eubacteria show their true colors: Genetics of carotenoid pigment biosynthesis from microbes to plants. *Journal of Bacteriology* **176**, 4795–4802.

Arnoux, P., Morosinotto, T., Saga, G., Bassi, R. and Pignol, D. (2009). A structural basis for the pH-dependent xanthophyll cycle in Arabidopsis thaliana. *The Plant Cell* **21**, 2036–2044.

Atienza, S. G., Ballesteros, J., Martin, A. and Hornero-Méndez, D. (2007). Genetic variability of carotenoid concentration and degree of esterification among tritordeum (× Tritordeum Ascherson et Graebner) and durum wheat accessions. *Journal of Agricultural and Food Chemistry* **55**, 4244–4251.

Auldridge, M. E., McCarty, D. R. and Klee, H. J. (2006). Plant carotenoid cleavage oxygenases and their apocarotenoid products. *Current Opinion in Plant Biology* **9**, 315–321.

Bai, L., Kim, E. H., DellaPenna, D. and Brutnell, T. P. (2009). Novel lycopene epsilon cyclase activities in maize revealed through perturbation of carotenoid biosynthesis. *The Plant Journal* **59**, 588–599.

Bailey, S. and Grossman, A. (2008). Photoprotection in cyanobacteria: Regulation of light harvesting. *Photochemistry and Photobiology* **84**, 1410–1420.

Barrero, J. M., Rodriguez, P. L., Quesada, V., Alabadi, D., Blazquez, M. A., Boutin, J. P., Marion-Poll, A., Ponce, M. R. and Micol, J. L. (2008). The ABA1 gene and carotenoid biosynthesis are required for late skotomorphogenic growth in Arabidopsis thaliana. *Plant, Cell & Environment* **31**, 227–234.

Bartley, G. and Scolnik, P. (1995). Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. *The Plant Cell* **7**, 1027–1038.

Bechoff, A., Westby, A., Owori, C., Menya, G., Dhuique-Mayer, C., Dufour, D. and Tomlins, K. (2010). Effect of drying and storage on the degradation of total carotenoids in orange-fleshed sweetpotato cultivars. *Journal of the Science of Food and Agriculture* **90**, 622–629.

Beisel, K. G., Jahnke, S., Hofmann, D., Köppchen, S., Schurr, U. and Matsubara, S. (2010). Continuous turnover of carotenes and chlorophyll a in mature leaves of Arabidopsis thaliana revealed by 14CO2 pulse-chase labeling. *Plant Physiology* **152**, 2188–2199.

Beveridge, C. A., Ross, J. J. and Murfet, I. C. (1996). Branching in pea—Action of genes rms3 and rms4. *Plant Physiology* **110**, 859–865.

Beyer, P. (1989). Carotene biosynthesis in daffodil chromoplasts: On the membrane-integral desaturation and cyclization reactions. *In* Physiology, Biochemistry, and Genetics of Nongreen Plastids, (C. D. Boyer, J. C. Shannon and R. C. Hardison, eds.), pp. 157–170. The American Society of Plant Physiologists, Rockville, MD.

Beyer, P., Mayer, M. and Kleinig, H. (1989). Molecular oxygen and the state of geometric isomerism of intermediates are essential in the carotene desaturation and cyclization reactions in daffodil chromoplasts. *European Journal of Biochemistry* **184**, 141–150.

Booker, J., Auldridge, M., Wills, S., McCarty, D., Klee, H. and Leyser, C. (2004). MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. *Current Biology* **14**, 1232–1238.

Booker, J., Sieberer, T., Wright, W., Williamson, L., Willett, B., Stirnberg, P., Turnbull, C., Srinivasan, M., Goddard, P. and Leyser, O. (2005). MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. *Developmental Cell* **8**, 443–449.

Bouvier, F., Backhaus, R. A. and Camara, B. (1998). Induction and control of chromoplast-specific carotenoid genes by oxidative stress. *The Journal of Biological Chemistry* **273**, 30651–30659.

Bouvier, F., Dogbo, O. and Camara, B. (2003a). Biosynthesis of the food and cosmetic plant pigment bixin (annatto). *Science* **300**, 2089–2091.

Bouvier, F., Suire, C., Mutterer, J. and Camara, B. (2003b). Oxidative remodeling of chromoplast carotenoids: Identification of the carotenoid dioxygenase CsCCD and CsZCD genes involved in Crocus secondary metabolite biogenesis. *The Plant Cell* **15**, 47–62.

Britton, G., Weesie, R. J., Askin, D., Warburton, J. D., GallardoGuerrero, L., Jansen, F. J., de Groot, H. J. M., Lugtenburg, J., Cornard, J. P. and Merlin, J. C. (1997). Carotenoid blues: Structural studies on carotenoproteins. *Pure and Applied Chemistry* **69**, 2075–2084.

Burkhardt, P. K., Beyer, P., Wünn, J., Klöti, A., Armstrong, G. A., Schledz, M., von Lintig, J. and Potrykus, I. (1997). Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. *The Plant Journal* **11**, 1071–1078.

Cazzonelli, C. I. and Pogson, B. J. (2010). Source to sink: Regulation of carotenoid biosynthesis in plants. *Trends in Plant Science* **15**, 266–274.

Cazzonelli, C. I., Cuttriss, A. J., Cossetto, S. B., Pye, W., Crisp, P., Whelan, J., Finnegan, E. J., Turnbull, C. and Pogson, B. J. (2009). Regulation of carotenoid composition and shoot branching in Arabidopsis by a chromatin modifying histone methyltransferase, SDG8. *The Plant Cell* **21**, 39–53.

Chander, S., Guo, Y. Q., Yang, X. H., Zhang, J., Lu, X. Q., Yan, J. B., Song, T. M., Rocheford, T. R. and Li, J. S. (2008). Using molecular markers to identify two major loci controlling carotenoid contents in maize grain. *Theoretical and Applied Genetics* **116**, 223–233.

Chaudhary, N., Nijhawan, A., Khurana, J. P. and Khurana, P. (2010). Carotenoid biosynthesis genes in rice: Structural analysis, genome-wide expression profiling and phylogenetic analysis. *Molecular Genetics and Genomics* **283**, 13–33.

Chen, Y., Li, F. Q. and Wurtzel, E. T. (2010). Isolation and characterization of the Z-ISO gene encoding a missing component of carotenoid biosynthesis in plants. *Plant Physiology* **153**, 66–79.

Cong, L., Wang, C., Chen, L., Liu, H. J., Yang, G. X. and He, G. Y. (2009). Expression of phytoene synthase1 and carotene desaturase crtl genes result in an increase in the total carotenoids content in transgenic elite wheat (Triticum aestivum L.). *Journal of Agricultural and Food Chemistry* **57**, 8652–8660.

Cunningham, F. X. and Gantt, E. (2001). One ring or two? Determination of ring number in carotenoids by lycopene epsilon-cyclases. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 2905.

Cunningham, F. X., Tice, A. B., Pham, C. and Gantt, E. (2010). Inactivation of genes encoding plastoglobulin-like proteins in Synechocystis sp PCC 6803 leads to a light-sensitive phenotype. *Journal of Bacteriology* **192**, 1700–1709.

Cuttriss, A. J., Chubb, A. C., Alawady, A., Grimm, B. and Pogson, B. J. (2007). Regulation of lutein biosynthesis and prolamellar body formation in Arabidopsis. *Functional Plant Biology* **34**, 663–672.

Dall'Osto, L., Cazzaniga, S., North, H., Marion-Poll, A. and Bassi, R. (2007). The Arabidopsis aba4-1 mutant reveals a specific function for neoxanthin in protection against photooxidative stress. *The Plant Cell* **19**, 1048–1064.

Datta, K., Rai, M., Parkhi, V., Oliva, N., Tan, J. and Datta, S. K. (2006). Improved 'golden' indica rice and post-transgeneration enhancement of metabolic target products of carotenoids (beta-carotene) in transgenic elite cultivars (IR64 and BR29). *Current Science* **91**, 935–939.

Datta, S. K., Datta, K., Parkhi, V., Rai, M., Baisakh, N., Sahoo, G., Rehana, S., Bandyopadhyay, A., Alamgir, M., Ali, M. S., Abrigo, E. Oliva, N. *et al.* (2007). Golden rice: Introgression, breeding, and field evaluation. *Euphytica* **154**, 271–278.

Davey, M. W., Van den Bergh, I., Markham, R., Swennen, R. and Keulemans, J. (2009). Genetic variability in Musa fruit provitamin A carotenoids, lutein and mineral micronutrient contents. *Food Chemistry* **115**, 806–813.

Davis, C. R., Howe, J. A., Rocheford, T. R. and Tanumihardjo, S. A. (2008). The xanthophyll composition of biofortified maize (Zea mays Sp.) does not influence the bioefficacy of provitamin A carotenoids in Mongolian gerbils (Meriones unguiculatus). *Journal of Agricultural and Food Chemistry* **56**, 6745–6750.

DellaPenna, D. and Pogson, B. J. (2006). Vitamin synthesis in plants: Tocopherols and carotenoids. *Annual Review of Plant Biology* **57**, 711–738.

Diretto, G., Al-Babili, S., Tavazza, R., Papacchioli, V., Beyer, P. and Giuliano, G. (2007). Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. *PloS One* **2**(4), e350.

Diretto, G., Al-Babili, S., Tavazza, R., Scossa, F., Papacchioli, V., Migliore, M., Beyer, P. and Giuliano, G. (2010). Transcriptional-metabolic networks in beta-carotene-enriched potato tubers: The long and winding road to the golden phenotype. *Plant Physiology* **154**, 899–912.

Dun, E. A., Brewer, P. B. and Beveridge, C. A. (2009). Strigolactones: Discovery of the elusive shoot branching hormone. *Trends in Plant Science* **14**, 364–372.

Estevez, J. M., Cantero, A., Romero, C., Kawaide, H., Jimenez, L. F., Kuzuyama, T., Seto, H., Kamiya, Y. and Leon, P. (2000). Analysis of the expression of *CLA1*, a gene that encodes the 1- deoxyxylulose 5-phosphate synthase of the 2-C-methyl-D-erythritol-4- phosphate pathway in Arabidopsis. *Plant Physiology* **124**, 95–104.

Fang, J., Chai, C. L., Qian, Q., Li, C. L., Tang, J. Y., Sun, L., Huang, Z. J., Guo, X. L., Sun, C. H., Liu, M., Zhang, Y. Lu, Q. T. *et al.* (2008). Mutations of genes in synthesis of the carotenoid precursors of ABA lead to pre-harvest sprouting and photo-oxidation in rice. *The Plant Journal* **54**, 177–189.

Fanning, K. J., Martin, I., Wong, L., Keating, V., Pun, S. and O'Hare, T. (2010). Screening sweetcorn for enhanced zeaxanthin concentration. *Journal of the Science of Food and Agriculture* **90**, 91–96.

Fernandez, M. G. S., Hamblin, M. T., Li, L., Rooney, W. L., Tuinstra, M. P. and Kresovich, S. (2008). Quantitative trait loci analysis of endosperm color and carotenoid content in sorghum grain. *Crop Science* **48**, 1732–1743.

Fernandez, M. G. S., Kapran, I., Souley, S., Abdou, M., Maiga, I. H., Acharya, C. B., Hamblin, M. T. and Kresovich, S. (2009). Collection and characterization of yellow endosperm sorghums from West Africa for biofortification. *Genetic Resources and Crop Evolution* **56**, 991–1000.

Galpaz, N., Wang, Q., Menda, N., Zamir, D. and Hirschberg, J. (2008). Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. *The Plant Journal* **53**, 717–730.

Garcia-Limones, C., Schnabele, K., Blanco-Portales, R., Bellido, M. L., Caballero, J. L., Schwab, W. and Munoz-Blanco, J. (2008). Functional characterization of FaCCD1: A carotenoid cleavage dioxygenase from strawberry involved in lutein degradation during fruit ripening. *Journal of Agricultural and Food Chemistry* **56**, 9277–9285.

Gomez-Roldan, V., Fermas, S., Brewer, P. B., Puech-Pages, V., Dun, E. A., Pillot, J. P., Letisse, F., Matusova, R., Danoun, S., Portais, J. C., Bouwmeester, H. Becard, G. *et al.* (2008). Strigolactone inhibition of shoot branching. *Nature* **455**, 189–194.

Gunning, B. and Jagoe, M. (1967). The prolamellar body. *In* Biochemistry of Chloroplasts, (T. Goodwin, ed.), pp. 655–676. Academic Press, London.

Hagenimana, V., Anyango-Oyunga, M., Low, J., Njdroge, S. M., Gichuki, S. T. and Kabira, J. (1999). The effects of women farmers' adoption of orange-fleshed sweet potatoes: Raising vitamin A intake in Kenya. *In* Research Report Series, pp. 1–28. International Center for Research on Women, Washington, DC.

Hager, A. and Holocher, K. (1994). Localization of the xanthophyll-cycle enzyme violaxanthin de-epoxidase within the thylakoid lumen and abolition of its mobility by a (light-dependent) pH decrease. *Planta* **192**, 581–589.

Harjes, C. E., Rocheford, T. R., Bai, L., Brutnell, T. P., Kandianis, C. B., Sowinski, S. G., Stapleton, A. E., Vallabhaneni, R., Williams, M., Wurtzel, E. T., Yan, J. B. and Buckler, E. S. (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science* **319**, 330–333.

Havaux, M. and Niyogi, K. K. (1999). The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 8762–8767.

He, X. Y., Zhang, Y. L., He, Z. H., Wu, Y. P., Xiao, Y. G., Ma, C. X. and Xia, X. C. (2008). Characterization of phytoene synthase 1 gene (Psy1) located on common wheat chromosome 7A and development of a functional marker. *Theoretical and Applied Genetics* **116**, 213–221.

Hentschel, V., Kranl, K., Hollmann, J., Lindhauer, M. G., Bohm, V. and Bitsch, R. (2002). Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *Journal of Agricultural and Food Chemistry* **50**, 6663–6668.

Howe, J. A., Maziya-Dixon, B. and Tanumihardjo, S. A. (2009). Cassava with enhanced beta-carotene maintains adequate vitamin A status in Mongolian gerbils (Meriones unguiculatus) despite substantial cis-isomer content. *The British Journal of Nutrition* **102**, 342–349.

Howitt, C. A., Cavanagh, C. R., Bowerman, A. F., Cazzonelli, C., Rampling, L., Mimica, J. L. and Pogson, B. J. (2009). Alternative splicing, activation of cryptic exons and amino acid substitutions in carotenoid biosynthetic genes

are associated with lutein accumulation in wheat endosperm. *Functional & Integrative Genomics* **9**, 363–376.

Ilg, A., Yu, Q. J., Schaub, P., Beyer, P. and Al-Babili, S. (2010). Overexpression of the rice carotenoid cleavage dioxygenase 1 gene in Golden Rice endosperm suggests apocarotenoids as substrates in planta. *Planta* **232**, 691–699.

Isaacson, T., Ronen, G., Zamir, D. and Hirschberg, J. (2002). Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of b-carotene and xanthophylls in plants. *The Plant Cell* **14**, 333–342.

Isaacson, T., Ohad, I., Beyer, P. and Hirschberg, J. (2004). Analysis in vitro of the enzyme CRTISO establishes a poly-cis-carotenoid biosynthesis pathway in plants. *Plant Physiology* **136**, 4246–4255.

Johnson, M. P., Davidson, P. A., Ruban, A. V. and Horton, P. (2008). The xanthophyll pool size controls the kinetics of nonphotochemical chlorophyll quenching in Arabidopsis thaliana. *FEBS Letters* **582**, 262–266.

Joyard, J., Teyssier, E., Miege, C., Berny-Seigneurin, D., Marechal, E., Block, M. A., Dorne, A. J., Rolland, N., Ajlani, G. and Douce, R. (1998). The biochemical machinery of plastid envelope membranes. *Plant Physiology* **118**, 715–723.

Joyard, J., Ferro, M., Masselon, C., Seigneurin-Berny, D., Salvi, D., Garin, J. and Rolland, N. (2009). Chloroplast proteomics and the compartmentation of plastidial isoprenoid biosynthetic pathways. *Molecular Plant* **2**, 1154–1180.

Kang, Y. W., Kim, R. N., Cho, H. S., Kim, W. T., Choi, D. and Pai, H. S. (2008). Silencing of a BYPASS1 homolog results in root-independent pleiotrophic developmental defects in Nicotiana benthamiana. *Plant Molecular Biology* **68**, 423–437.

Kirk, J. T. O. and Tiliney-Bassett, R. A. E. (1978). Proplastids, etioplasts, amyloplasts, chromoplasts and other plastids. *In* The Plastids: Their Chemistry, Structure, Growth and Inheritance, (J. T. O. Kirk and R. A. E. Tinley-Bassett, eds.), pp. 217–239. Elsevier/North Holland Biomedical Press, Amsterdam.

Kohlen, W., Charnikhova, T., Liu, Q., Bours, R., Domagalska, M. A., Beguerie, S., Verstappen, F., Leyser, O., Bouwmeester, H. J. and Ruyter-Spira, C. (2010). Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in non-AM host Arabidopsis thaliana. *Plant Physiology* **55**, 974–987.

Kolotilin, I., Koltai, H., Tadmor, Y., Bar-Or, C., Reuveni, M., Meir, A., Nahon, S., Shlomo, H., Chen, L. and Levin, I. (2007). Transcriptional profiling of high pigment-2dg tomato mutant links early fruit plastid biogenesis with its overproduction of phytonutrients. *Plant Physiology* **145**, 389–401.

Lange, B. M., Rujan, T., Martin, W. and Croteau, R. (2000). Isoprenoid biosynthesis: The evolution of two ancient and distinct pathways across genomes. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 13172–13177.

Lefebvre, V., Kuntz, M., Camara, B. and Palloix, A. (1998). The capsanthin-capsorubin synthase gene: A candidate gene for the y locus controlling the red fruit colour in pepper. *Plant Molecular Biology* **36**, 785–789.

Li, L. and Van Eck, J. (2007). Metabolic engineering of carotenoid accumulation by creating a metabolic sink. *Transgenic Research* **16**, 581–585.

Li, L., Paolillo, D. J., Parthasarathy, M. V., Dimuzio, E. M. and Garvin, D. F. (2001). A novel gene mutation that confers abnormal patterns of beta-carotene accumulation in cauliflower (*Brassica oleracea* var. botrytis). *The Plant Journal* **26**, 59–67.

Li, F. Q., Murillo, C. and Wurtzel, E. T. (2007). Maize Y9 encodes a product essential for 15-cis-zeta-carotene isomerization. *Plant Physiology* **144**, 1181–1189.

Li, F. Q., Vallabhaneni, R. and Wurtzel, E. T. (2008a). PSY3, a new member of the phytoene synthase gene family conserved in the poaceae and regulator of abiotic stress-induced root carotenogenesis. *Plant Physiology* **146**, 1333–1345.

Li, F. Q., Vallabhaneni, R., Yu, J., Rocheford, T. and Wurtzel, E. T. (2008b). The maize phytoene synthase gene family: Overlapping roles for carotenogenesis in endosperm, photomorphogenesis, and thermal stress tolerance. *Plant Physiology* **147**, 1334–1346.

Li, Z. R., Ahn, T. K., Avenson, T. J., Ballottari, M., Cruz, J. A., Kramer, D. M., Bassi, R., Fleming, G. R., Keasling, J. D. and Niyogi, K. K. (2009). Lutein accumulation in the absence of zeaxanthin restores nonphotochemical quenching in the Arabidopsis thaliana npq1 mutant. *The Plant Cell* **21**, 1798–1812.

Lin, H., Wang, R. X., Qian, Q., Yan, M. X., Meng, X. B., Fu, Z. M., Yan, C. Y., Jiang, B., Su, Z., Li, J. Y. and Wang, Y. H. (2009). DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. *The Plant Cell* **21**, 1512–1525.

Lindgren, L., Stahlberg, K. G. and Hoglund, A.-S. (2003). Seed-specific overexpression of an endogenous Arabidopsis phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and abscisic acid. *Plant Physiology* **132**, 779–785.

Lopez-Raez, J. A., Kohlen, W., Charnikhova, T., Mulder, P., Undas, A. K., Sergeant, M. J., Verstappen, F., Bugg, T. D. H., Thompson, A. J., Ruyter-Spira, C. and Bouwmeester, H. (2010). Does abscisic acid affect strigolactone biosynthesis? *The New Phytologist* **187**, 343–354.

Lu, S., Van Eck, J., Zhou, X., Lopez, A., O'Halloran, D. M., Cosman, K. M., Conlin, B. J., Paolillo, D. J., Garvin, D. F., Vrebalov, J., Kochian, L. V. Küpper, H. *et al*. (2006). The cauliflower Or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of betacarotene accumulation. *The Plant Cell* **18**, 3594–3605.

Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A. and Grill, E. (2009). Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**, 1064–1068.

Maass, D., Arango, J., Wuest, F., Beyer, P. and Welsch, R. (2009). Carotenoid crystal formation in Arabidopsis and carrot roots caused by increased phytoene synthase protein levels. *PloS One* **4,** Article No. e6373.

Matusova, R., Rani, K., Verstappen, F. W. A., Franssen, M. C. R., Beale, M. H. and Bouwmeester, H. J. (2005). The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant Physiology* **139**, 920–934.

Menkir, A., Liu, W. P., White, W. S., Mazlya-Dixon, B. and Rocheford, T. (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. *Food Chemistry* **109**, 521–529.

Merzlyak, M. N. and Solovchenko, A. E. (2002). Photostability of pigments in ripening apple fruit: A possible photoprotective role of carotenoids during plant senescence. *Plant Science* **163**, 881–888.

Mialoundama, A. S., Heintz, D., Jadid, N., Nkeng, P., Rahier, A., Deli, J., Camara, B. and Bouvier, F. (2010). Characterization of plant carotenoid cyclases as members of the flavoprotein family functioning with no net redox change. *Plant Physiology* **153**, 970–979.

Misawa, N., Masamoto, K., Hori, T., Ohtani, T., Boger, P. and Sandmann, G. (1994). Expression of an Erwinia phytoene desaturase gene not only confers multiple resistance to herbicides interfering with carotenoid biosynthesis but

also alters xanthophyll metabolism in transgenic plants. *The Plant Journal* **6**, 481.

Moran, N. A. and Jarvik, T. (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science* **328**, 624–627.

Nassar, N., Vizzotto, C. S., Schwartz, C. A. and Pires, O. R. (2007). Cassava diversity in Brazil: The case of carotenoid-rich landraces. *Genetics and Molecular Research* **6**, 116–121.

Neill, S. J., Horgan, R. and Parry, A. D. (1986). The carotenoid and abscisic acid content of viviparous kernels and seedlings of *Zea mays* L. *Planta* **169**, 87–96.

Nievelstein, V., Vandekerchove, J., Tadros, M., Lintig, J., Nitschke, W. and Beyer, P. (1995). Carotene desaturation is linked to a respiratory redox pathway in Narcissus pseudonarcissus chromoplast membranes. Involvement of a 23-kDa oxygene-evolving-complex-like protein. *European Journal of Biochemistry* **233**, 864–872.

Niyogi, K. K. (1999). Photoprotection revisited: Genetic and molecular approaches. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 333–359.

Niyogi, K. K., Bjorkman, O. and Grossman, A. R. (1997). The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 14162–14167.

Norris, S. R., Barrette, T. R. and DellaPenna, D. (1995). Genetic dissection of carotenoid synthesis in *Arabidopsis* defines plastoquinone as an essential component of phytoene desaturation. *The Plant Cell* **7**, 2139–2149.

North, H. M., De Almeida, A., Boutin, J. P., Frey, A., To, A., Botran, L., Sotta, B. and Marion-Poll, A. (2007). The Arabidopsis ABA-deficient mutant aba4 demonstrates that the major route for stress-induced ABA accumulation is via neoxanthin isomers. *The Plant Journal* **50**, 810–824.

Ohmiya, A., Kishimoto, S., Aida, R., Yoshioka, S. and Sumitomo, K. (2006). Carotenoid cleavage dioxygenase (CmCCD4a) contributes to white color formation in Chrysanthemum petals. *Plant Physiology* **142**, 1193–1201.

Paine, J. A., Shipton, C. A., Chaggar, S., Howells, R. M., Kennedy, M. J., Vernon, G., Wright, S. Y., Hinchliffe, E., Adams, J. L., Silverstone, A. L. and Drake, R. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology* **23**, 482–487.

Panel on Micronutrients (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Food and Nutrition Board. Institute of Medicine, Washington, DC, pp. 82–69.

Park, H., Kreunen, S. S., Cuttriss, A. J., DellaPenna, D. and Pogson, B. J. (2002). Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation, and photomorphogenesis. *The Plant Cell* **14**, 321–332.

Park, S., Fung, P., Nishimura, N., Jensen, D., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T., Alfred, S. Bonetta, D. *et al.* (2009). Abscisic acid inhibits type 2 C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**, 1068–1071.

Parry, A. D. and Horgan, R. (1992). Abscisic acid biosynthesis in roots I. The identification of potential abscisic acid precursors, and other carotenoids. *Planta* **187**, 185–191.

Pfundel, E. E., Renganathan, M., Gilmore, A. M., Yamamoto, H. Y. and Dilley, R. A. (1994). Intrathylakoid Ph in isolated pea-chloroplasts as probed by violaxanthin deepoxidation. *Plant Physiology* **106**, 1647–1658.

Pogson, B. J. and Rissler, H. M. (2000). Genetic manipulation of carotenoid biosynthesis and photoprotection. *Philosophical Transactions of the Royal Society London B: Biological Sciences* **355**, 1395–1403.

Pogson, B., McDonald, K., Truong, M., Britton, G. and DellaPenna, D. (1996). *Arabidopsis* carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. *The Plant Cell* **8**, 1627–1639.

Pogson, B. J., Niyogi, K. K., Bjorkman, O. and DellaPenna, D. (1998). Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in Arabidopsis mutants. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 13324–13329.

Randolph, L. F. and Hand, D. B. (1940). Relation between carotenoid content and number of genes per cell in diploid and tetraploid corn. *Journal of Agricultural Research* **60**, 51–64.

Robertson, D., Bachmann, M. and Anderson, I. (1966). Role of carotenoids in protecting chlorophyll from photodestruction-II. Studies on the effect of four modifiers of the albino *cl1* mutant of maize. *Photochemistry and Photobiology* **5**, 797–805.

Rodriguez-Concepcion, M. (2010). Supply of precursors for carotenoid biosynthesis in plants. *Archives of Biochemistry and Biophysics* **504**, 118–122.

Rodriguez-Villalon, A., Gas, E. and Rodriguez-Concepcion, M. (2009). Phytoene synthase activity controls the biosynthesis of carotenoids and the supply of their metabolic precursors in dark-grown Arabidopsis seedlings. *The Plant Journal* **60**, 424–435.

Rubio, A., Rambla, J. L., Santaella, M., Gomez, M. D., Orzaez, D., Granell, A. and Gomez-Gomez, L. (2008). Cytosolic and plastoglobule-targeted carotenoid dioxygenases from Crocus sativus are both involved in beta-ionone release. *The Journal of Biological Chemistry* **283**, 24816–24825.

Saga, G., Giorgetti, A., Fufezan, C., Giacometti, G. M., Bassi, R. and Morosinotto, T. (2010). Mutation analysis of violaxanthin de-epoxidase identifies substrate-binding sites and residues involved in catalysis. *The Journal of Biological Chemistry* **285**, 23763–23778.

Schaller, S., Latowski, D., Jemiola-Rzeminska, M., Wilhelm, C., Strzalka, K. and Goss, R. (2010). The main thylakoid membrane lipid monogalactosyldiacylglycerol (MGDG) promotes the de-epoxidation of violaxanthin associated with the light-harvesting complex of photosystem II (LHCII). *Biochimica et Biophysica Acta-Bioenergetics* **1797**, 414–424.

Schwartz, S. H., Tan, B. C., Cage, D. A., Zeevaart, J. A. D. and McCarty, D. R. (1997). Specific oxidative cleavage of carotenoids by VP14 of maize. *Science* **276**, 1872–1874.

Schwartz, S. H., Qin, X. Q. and Zeevaart, J. A. D. (2001). Characterization of a novel carotenoid cleavage dioxygenase from plants. *The Journal of Biological Chemistry* **276**, 25208–25211.

Schwartz, S. H., Tan, B. C., McCarty, D. R., Welch, W. and Zeevaart, J. A. (2003). Substrate specificity and kinetics for VP14, a carotenoid cleavage dioxygenase in the ABA biosynthetic pathway. *Biochimica et Biophysica Acta* **1619**, 9–14.

Schwartz, S. H., Qin, X. and Loewen, M. C. (2004). The biochemical characterization of two carotenoid cleavage enzymes from Arabidopsis indicates that a carotenoid-derived compound Inhibits lateral branching. *The Journal of Biological Chemistry* **279**, 46940–46945.

Seo, M. and Koshiba, T. (2002). Complex regulation of ABA biosynthesis in plants. *Trends in Plant Science* **7**, 41–48.

Shewmaker, C. K., Sheehy, J. A., Daley, M., Colburn, S. and Ke, D. Y. (1999). Seed-specific overexpression of phytoene synthase: Increase in carotenoids and other metabolic effects. *The Plant Journal* **20**, 401–412.

Sieburth, L. E. and Lee, D. K. (2010). BYPASS1: How a tiny mutant tells a big story about root-to-shoot signaling. *Journal of Integrative Plant Biology* **52**, 77–85.

Sommer, A. and Davidson, F. R. (2002). Assessment and control of vitamin A deficiency: The annecy accords. *The Journal of Nutrition* **132**, S2845.

Sorefan, K., Booker, J., Haurogne, K., Goussot, M. and Bainbridge, K. (2003). MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. *Genes & Development* **17**, 1469.

Steinmuller, D. and Tevini, M. (1985). Composition and function of plastoglobuli. 1. Isolation and purification from chloroplasts and chromoplasts. *Planta* **163**, 201–207.

Stirnberg, P., van de Sande, K. and Leyser, H. M. O. (2002). MAX1 and MAX2 control shoot lateral branching in Arabidopsis. *Development* **129**, 1131–1141.

Sun, Z., Gantt, E. and Cunningham, F. X., Jr. (1996). Cloning and functional analysis of the beta-carotene hydroxylase of *Arabidopsis thaliana*. *The Journal of Biological Chemistry* **271**, 24349–24352.

Tan, B. C. (1997). Genetic control of abscisic acid biosynthesis in maize. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12235–12240.

Tan, B. C., Joseph, L. M., Deng, W. T., Liu, L., Li, Q. B., Cline, K. and McCarty, D. R. (2003). Molecular characterization of the Arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. *The Plant Journal* **35**, 44–56.

Tang, G. W., Qin, J., Dolnikowski, G. G., Russell, R. M. and Grusak, M. A. (2009). Golden Rice is an effective source of vitamin A. *The American Journal of Clinical Nutrition* **89**, 1776–1783.

Taylor, H. F. and Smith, T. A. (1967). Production of plant growth inhibitors from xanthophylls: A possible source of dormin. *Nature* **215**, 1513–1514.

Tevini, M. and Steinmuller, D. (1985). Composition and function of plastoglobuli. II. Lipid composition of leaves and plastoglobuli during senescence. *Planta* **163**, 91.

Tian, L., Musetti, V., Kim, J., Magallanes-Lundback, M. and DellaPenna, D. (2004). The Arabidopsis *LUT1* locus encodes a member of the cytochrome P450 family that is required for carotenoid e-ring hydroxylation activity. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 402–407.

Toledo-Ortiz, G., Huq, E. and Rodriguez-Concepcion, M. (2010). Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 11626–11631.

Tonhosolo, R., D'Alexandri, F. L., de Rosso, V. V., Gazarini, M. L., Matsumura, M. Y., Peres, V. J., Merino, E. F., Carlton, J. M., Wunderlich, G., Mercadante, A. Z., Kimura, E. A. and Katzin, A. M. (2009). Carotenoid biosynthesis in intraerythrocytic stages of Plasmodium falciparum. *The Journal of Biological Chemistry* **284**, 9974–9985.

Treharne, K. J., Mercer, E. I. and Goodwin, T. W. (1966). Carotenoid biosynthesis in some maize mutants. *Phytochemistry* **5**, 581–587.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyozuka, J. and

Yamaguchi, S. (2008). Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**, 195–200.

Underwood, B. A. (2004). Vitamin A deficiency disorders: International efforts to control a preventable ''pox''. *The Journal of Nutrition* **134**, 231S–236S.

Vallabhaneni, R. and Wurtzel, E. T. (2009). Timing and biosynthetic potential for carotenoid accumulation in genetically diverse germplasm of maize. *Plant Physiology* **150**, 562–572.

Vallabhaneni, R., Gallagher, C. E., Licciardello, N., Cuttriss, A. J., Quinlan, R. F. and Wurtzel, E. T. (2009). Metabolite sorting of a germplasm collection reveals the Hydroxylase3 locus as a new target for maize provitamin A biofortification. *Plant Physiology* **151**, 1635–1645.

Vallabhaneni, R., Bradbury, L. and Wurtzel, E. T. (2010). The carotenoid dioxygenase gene family in maize, sorghum, and rice. *Archives in Biochemistry and Biophysics* **504**, 104–111, (Carotenoids Highlight edition).

van Jaarsveld, P., Faber, M., Tanumihardjo, S., Nestel, P., Lombard, C. and Benade, A. (2005). Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose–response test. *The American Journal of Clinical Nutrition* **81**, 1080–1087.

Van Norman, J. M., Frederick, R. L. and Sieburth, L. E. (2004). BYPASS1 negatively regulates a root-derived signal that controls plant architecture. *Current Biology* **14**, 1739–1746.

Verhoeven, A. S., Bugos, R. C. and Yamamoto, H. Y. (2001). Transgenic tobacco with suppressed zeaxanthin formation is susceptible to stress-induced photoinhibition. *Photosynthesis Research* **67**, 27–39.

Vishnevetsky, M., Ovadis, M., Zuker, A. and Vainstein, A. (1999). Molecular mechanisms underlying carotenogenesis in the chromoplast: Multilevel regulation of carotenoid-associated genes. *The Plant Journal* **20**, 423–431.

Vogel, J. T., Walter, M. H., Giavalisco, P., Lytovchenko, A., Kohlen, W., Charnikhova, T., Simkin, A. J., Goulet, C., Strack, D., Bouwmeester, H. J., Fernie, A. R. and Klee, H. J. (2010). SlCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. *The Plant Journal* **61**, 300–311.

von Lintig, J. (2010). Colors with functions: Elucidating the biochemical and molecular basis of carotenoid metabolism. *Annual Review of Nutrition* **30**, 35–56.

von Lintig, J. and Vogt, K. (2000). Filling the gap in vitamin a research: Molecular identification of an enzyme cleaving beta-carotene to retinal. *The Journal of Biological Chemistry* **275**, 11915–11920.

Welsch, R., Beyer, P., Hugueney, P., Kleinig, H. and von Lintig, J. (2000). Regulation and activation of phytoene synthase, a key enzyme in carotenoid biosynthesis, during photomorphogenesis. *Planta* **211**, 846–854.

Welsch, R., Medina, J., Giuliano, G., Beyer, P. and Von Lintig, J. (2003). Structural and functional characterization of the phytoene synthase promoter from Arabidopsis thaliana. *Planta* **216**, 523–534.

Welsch, R., Maass, D., Voegel, T., DellaPenna, D. and Beyer, P. (2007). Transcription factor RAP2.2 and its interacting partner SINAT2: Stable elements in the carotenogenesis of Arabidopsis leaves. *Plant Physiology* **145**, 1073–1085.

Welsch, R., Wust, F., Bar, C., Al-Babili, S. and Beyer, P. (2008). A third phytoene synthase is devoted to abiotic stress-induced abscisic acid formation in rice and defines functional diversification of phytoene synthase genes. *Plant Physiology* **147**, 367–380.

Welsch, R., Arango, J., Bar, C., Salazar, B., Al-Babili, S., Beltran, J., Chavarriaga, P., Ceballos, H., Tohme, J. and Beyer, P. (2010). Provitamin A accumulation in cassava (Manihot esculenta) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. *The Plant Cell* **22**, 3348–3356.

Wurtzel, E. T. (2004). Genomics, genetics, and biochemistry of maize carotenoid biosynthesis. *In* Recent Advances in Phytochemistry, (J. Romeo, ed.), **38**, pp. 85–110. Elsevier Ltd., Oxford, UK.

Wyss, A., Wirtz, G., Woggon, W., Brugger, R., Wyss, M., Friedlein, A., Bachmann, H. and Hunziker, W. (2000). Cloning and expression of beta, beta-carotene 15,15′-dioxygenase. *Biochemical and Biophysical Research Communications* **271**, 334–336.

Yamamoto, H. Y. (1979). Biochemistry of the xanthophyll cycle in higher plants. *Pure and Applied Chemistry* **51**, 639–648.

Yan, J. B., Kandianis, C. B., Harjes, C. E., Bai, L., Kim, E. H., Yang, X. H., Skinner, D. J., Fu, Z. Y., Mitchell, S., Li, Q., Fernandez, M. G. S. Zaharieva, M. *et al*. (2010). Rare genetic variation at Zea mays crtRB1 increases beta-carotene in maize grain. *Nature Genetics* **42**, 322–327.

Ye, X., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P. and Potrykus, I. (2000). Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **287**, 303–305.

Zhu, C. F., Naqvi, S., Breitenbach, J., Sandmann, G., Christou, P. and Capell, T. (2008). Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 18232–18237.