

Carotenoids in nature: insights from plants and beyond

Christopher I. Cazzonelli

Australian Research Council Centre of Excellence in Plant Energy Biology, College of Medicine, Biology and Environment, Research School of Biology, The Australian National University, Building 134, Linnaeus Way, Canberra, ACT 0200, Australia. Email: christopher.cazzonelli@anu.edu.au

This review originates from the Peter Goldacre Award 2010 of the Australian Society of Plant Scientists that was received by the author.

Abstract. Carotenoids are natural isoprenoid pigments that provide leaves, fruits, vegetables and flowers with distinctive yellow, orange and some reddish colours as well as several aromas in plants. Their bright colours serve as attractants for pollination and seed dispersal. Carotenoids comprise a large family of C₄₀ polyenes and are synthesised by all photosynthetic organisms, aphids, some bacteria and fungi alike. In animals carotenoid derivatives promote health, improve sexual behaviour and are essential for reproduction. As such, carotenoids are commercially important in agriculture, food, health and the cosmetic industries. In plants, carotenoids are essential components required for photosynthesis, photoprotection and the production of carotenoid-derived phytohormones, including ABA and strigolactone. The carotenoid biosynthetic pathway has been extensively studied in a range of organisms providing an almost complete pathway for carotenogenesis. A new wave in carotenoid biology has revealed implications for epigenetic and metabolic feedback control of carotenogenesis. Developmental and environmental signals can regulate carotenoid gene expression thereby affecting carotenoid accumulation. This review highlights mechanisms controlling (1) the first committed step in phytoene biosynthesis, (2) flux through the branch to synthesis of α - and β -carotenes and (3) metabolic feedback signalling within and between the carotenoid, MEP and ABA pathways.

Additional keywords: abscisic acid, apocarotenoids, carotenoid, chloroplast, chromatin, epigenetic, hormones, isoprenoid, metabolic feedback, plant, photoisomerisation, regulation, signal molecule, strigolactone.

Received 19 August 2011, accepted 30 August 2011, published online 18 October 2011

Functions for carotenoids in nature

Vitamins, antioxidants and spices

Animals are generally unable to synthesise carotenoids and require a dietary intake of plant products to meet daily health demands. There are exceptions such as the synthesis of carotenoids in human protist parasites (e.g. *Plasmodium* and *Toxoplasma*) and aphids, which can be explained by the existence of a remnant plastid and lateral gene transfer of carotenoid biosynthesis genes from a fungus, respectively (Tonhosolo *et al.* 2009; Moran and Jarvik 2010). In most animals, dietary carotenoids are cleaved to provide precursors for vitamin A biosynthesis (of which a deficiency leads to blindness) and are valuable for many physiological functions and thus promote human health (e.g. antioxidant activity, immunostimulants, yolk nourishment to embryos, photoprotection, visual tuning as well as limiting age-related macular degeneration of the eye) (Johnson 2002; Krinsky and Johnson 2005).

A deficiency in vitamin A is responsible for child (and maternal) mortality and it is estimated that a 23–34% reduction in preschool mortality can be expected from vitamin

A program reaching children in undernourished settings (The State of the World's Children, UNICEF, www.un.org/en/mdg/summit2010, accessed 19 August 2011). The development of high yielding β -carotene crops such as 'golden rice' could provide close to the recommended daily allowance of vitamin A for malnourished children and help combat vitamin A deficiency-induced mortality and morbidity (Fig. 1) (www.goldenrice.org, accessed 19 August 2011).

The nutraceutical industry synthetically manufactures five major carotenoids on an industrial scale (e.g. lycopene, β -carotene, canthaxanthin, zeaxanthin and astaxanthin) for use in a range of food products and cosmetics, such as vitamin supplements and health products and as feed additives for poultry, livestock, fish and crustaceans (reviewed by Del Campo *et al.* 2007; Jackson *et al.* 2008). One of the most commercially valuable pigments, astaxanthin, is primarily synthesised by marine microorganisms, such as the green alga *Haematococcus pluvialis* and accumulates in fish such as salmon, thus, colouring their flesh red. Astaxanthin has been implicated as a potential therapeutic agent treating cardiovascular disease and prostatic cancer (Fassett and Coombes 2011).

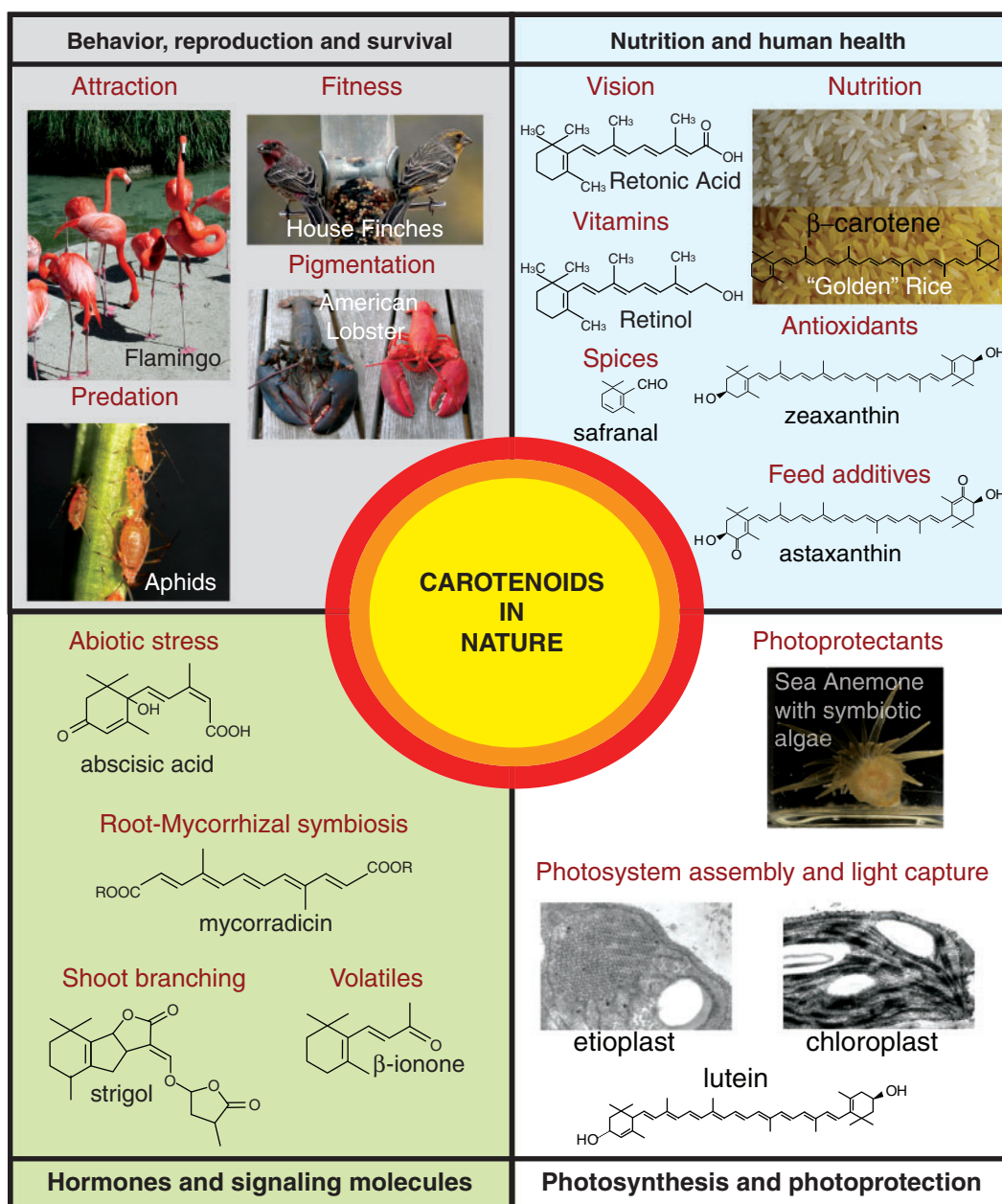


Fig. 1. The many essential functions for carotenoids in nature. Carotenoids play important roles in (1) promoting animal behaviour, reproduction and survival, (2) improving nutrition and human health, (3) assembly of photosystems, light capture and photoprotection and (4) providing substrates for the biosynthesis of plant hormones and signalling molecules.

Carotenoids accumulate in light exposed tissues, such as skin and as such have gained increased value in the cosmetic industries as suitable compounds for photoprotection due to their scavenging action on reactive oxygen species (ROS) and anti-inflammatory properties (Stahl and Sies 2007). Photo-oxidative damage affects cellular lipids, proteins and DNA and is involved in the patho-biochemistry of erythema formation, premature aging of the skin, development of photodermatoses and skin cancer. Evidence shows that β-Carotene, lutein and perhaps even lycopene, can prevent UV-induced erythema formation

and contribute to life-long protection against exposure to harmful affects of sunlight (Stahl and Sies 2007).

Apocarotenoids are also highly valued as additives in the food industry. Spices such as bixin (annatto), a red-collared, dicarboxylic monomethyl ester apocarotenoid is traditionally derived from the plant *Bixa orellana* (also known as achiote). Saffron comes from the thread-like reddish coloured female reproductive organs of the *Crocus sativa* flower (petals are coloured light purple), which is considered one of the worlds most expensive spices and widely used as a natural colourant. The

colour is due to the degradation of carotenoids (e.g. zeaxanthin to crocin and crocetin), whereas the flavour arises from the accumulation of carotenoid cleavage oxidation products from zeaxanthin (e.g. mainly safranal and the bitter glucoside, picrocrocin). Safranal (2,6,6-trimethylcyclohexa-1,3-dien-1-carboxaldehyde) is easily synthesised by de-glucosylation of picrocrocin and composes ~70% of total volatiles from crocin flowers (Fig. 1) (Leffingwell 2002; Bouvier *et al.* 2003a; Schwab *et al.* 2008). There is a growing interest in the impact of saffron carotenoids on human health due to their high antioxidant capacity (Verma and Bordia 1998). Other carotenoid-derived volatiles, such as α - and β -ionone are predominant norisoprenoid volatiles in the mature stigma tissue that are also important components of aroma and taste produced during flower and fruit development (Goff and Klee 2006).

Animal behaviour, reproduction and survival

Animals accumulate carotenoids where they are critical in determining sexual behaviour, reproduction and avoiding predation as well as parasitism. Fish and birds accumulate dietary carotenoids, which boost their immune system and advertise health, often leading to preferential selection by the sexual partner (McGraw *et al.* 2006b; Baron *et al.* 2008). Animals typically place different priorities on fitness-enhancing activities (e.g. gametic investment in females, sexual attraction in males) and carotenoid allocation appears to track such investment patterns in the two sexes (McGraw and Toomey 2010). For example, environmental and physiological factors influence colour expression in house finches (Fig. 1) and the type of dietary carotenoids is one determinant of their ability to become bright red rather than drab yellow (McGraw *et al.* 2006a). A particular primacy of higher levels of β -cryptoxanthin was assigned to the coloration in the male house finches that become red and more sexually attractive. It is possible that the red house finches adopt selective foraging strategies for the most β -cryptoxanthin-rich foods rather than grains and fruits, which typically contain the more common dietary yellow xanthophylls and carotenes (Fig. 2) (McGraw *et al.* 2006a). Finally, Hill (2000) demonstrated that birds fed diets rich in lutein and zeaxanthin turn only yellow, but grow red feathers when fed β -cryptoxanthin-rich foods (e.g. tangerine juice). Perhaps, it is the metabolic precursors of β -cryptoxanthin such as 3-hydroxyechinenone, which provide the red carotenoid pigmentation (Hill 2000).

Another intriguing example is the flamingo, whose feathers are coloured bright rosy pink (Fig. 1) from the carotenoid pigments (derivatives such as canthaxanthin, main pigment found in the secretions, as well as small quantities of β -cryptoxanthin are likely to contribute to pink colouration) in the algae and various invertebrates (e.g. crayfish) that make up the bulk of a flamingo's diet. Curiously, the crustaceans accumulate astaxanthin whose spectral properties become modified by the β -barrel protein, crustacyanin, resulting in blue pigmentation that shifts to red upon protein–pigment denaturation during cooking (Fig. 1; American lobster) (Krawczyk and Britton 2001). A dietary source of carotenoids by the flamingo was found in the oils of uropygial secretions, which deposit over the plumage and growing feathers more frequently during periods when in a group, contributing some

colour and possibly indicating a cosmetic function for mate choice. The cosmetic nature of accumulating carotenoids by painting canthaxanthin-rich secretions onto their feathers to create a visually more attractive breeding partner appeared to enhance the frequency of their courting behaviour during mating seasons (Amat *et al.* 2010).

Carotenoids also play important roles in animals to avoid predation and reduce parasitism. For example, aphids (e.g. *Acyrtosiphon pisum*) are the first known animal to have acquired the carotenoid biosynthetic machinery to produce carotenoids such as torulene and dehydro- γ , ψ -carotene, which provide a reddish colouration distinguished them from their green forms, which accumulate γ -carotene, β -carotene and α -carotene (Moran and Jarvik 2010). The colour polymorphism is maintained by frequency-dependent selection imposed by natural predators that preferentially prey upon the red morphs (Fig. 1) and higher rates of parasitism in the green forms (Losey *et al.* 1997).

Photosynthesis and photoprotection in plants

Carotenoids are essential for energy capture from the solar emission spectrum. Of the many naturally occurring carotenoids, less than 50 play a light-harvesting role in photosynthetic organisms (e.g. plants; protists such as the human parasites *Plasmodium* and *Toxoplasma* as well as the coral endosymbiotic *Dinoflagellates*; and the *Symbiodinium* marine algae which forms a relationship with the sea anemone, *Aiptasia insignis*) (Fig. 1) (Chidambara Murthy *et al.* 2005; Polívka and Frank 2010). Carotenoid colours range from pale yellow to a reddish brown depending upon the number of conjugated double bonds along the C₄₀ backbone, as well as other cyclic and oxygenic modifications. The distinctive yellow colour of light-harvesting carotenoids become more apparent in leaves during autumn when chlorophyll degrades revealing their strong colours. Carotenoids are required for the correct assembly of photosystems (Pogson *et al.* 2005; Li *et al.* 2009b). Here they absorb light across a broader range of the spectral region in which the sun irradiates maximally and transfer the energy to chlorophyll, initiating the photochemical events of photosynthesis (Polívka and Frank 2010). In plants, carotenoids are bound in discrete pigment–protein complexes referred to as the LHCII trimeric complex, which typically benefits from lutein, neoxanthin, as well as violaxanthin to transfer energy to chlorophyll (Pogson *et al.* 2005; Demmig-Adams and Adams 2006; Dall'Osto *et al.* 2010). A curious marine dinoflagellate, *Amphidinium carterae* alternatively evolved a peridinin–chlorophyll protein complex that contains more of the bound carotenoid (peridinin) when compared with chlorophyll (Hofmann *et al.* 1996; Polívka and Frank 2010) (Fig. 1).

Perhaps the roles that carotenoids play to protect the photosynthetic machinery from excessive light are among key biological functions of carotenoids relevant for life on earth. Plants are able to balance between absorbing sufficient light for photosynthetic processes while avoiding photo oxidative damage to membranes and proteins caused by excessive light. To meet this balance carotenoids (1) quench triplet chlorophyll, (2) scavenge ROS like singlet oxygen which damage membranes

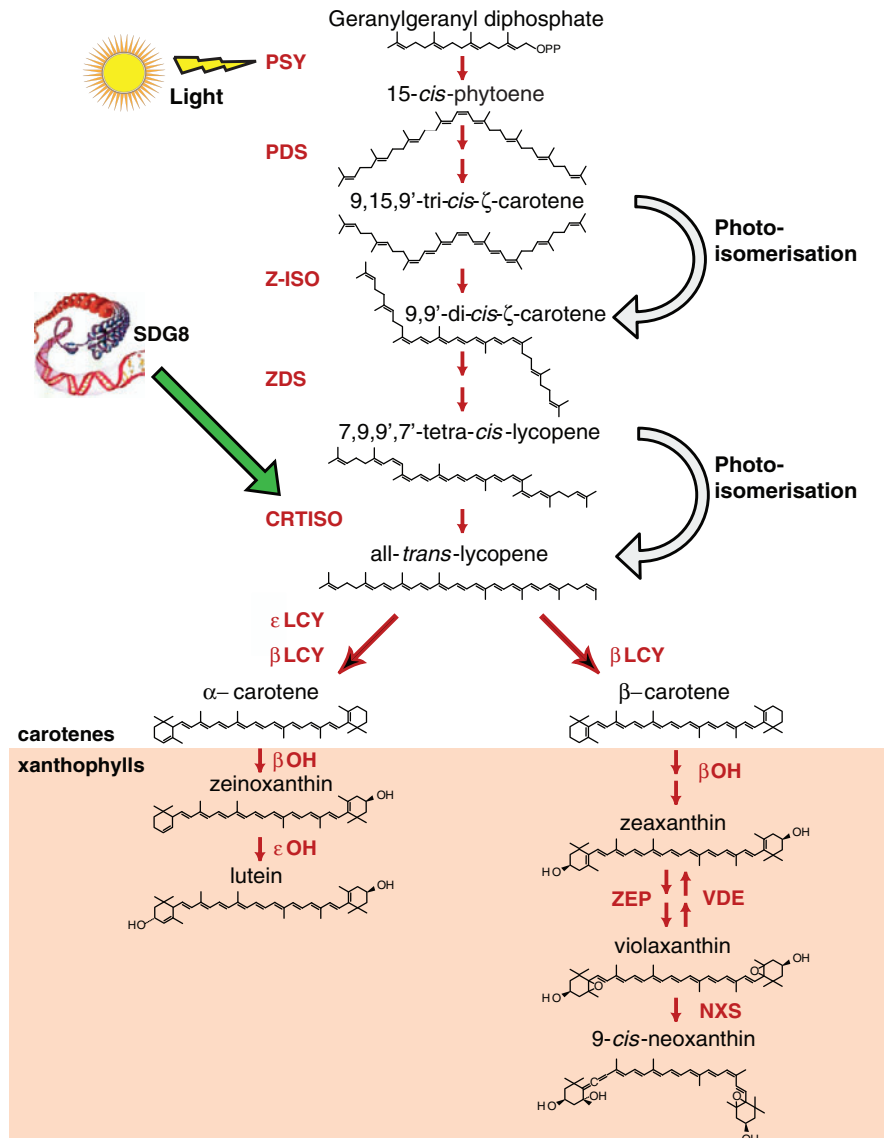


Fig. 2. Carotenoid biosynthesis and regulation in *Arabidopsis*. The light responsive enzyme, phytoene synthase (PSY) catalyses the first committed step in the synthesis phytoene from geranylgeranyl pyrophosphate and is a major bottleneck in the pathway. The branch in carotenoid synthesis is another key regulatory step. The isomerisation of tetra-*cis*-lycopene (pro-lycopene) to lycopene requires the carotenoid isomerase (CRTISO) and light-mediated photoisomerisation. Modification of chromatin surrounding the *CRTISO* locus by the histone lysine methyltransferase, SET DOMAIN GROUP8 (SDG8), is rate limiting for lutein production. Finally, lycopene undergoes modifications by εLCY (epsilon cyclase) and β-LCY (β-cyclase) to produce α- and β-carotene respectively. The carotenes serve as substrates for the production of xanthophylls including lutein, violaxanthin and neoxanthin. The xanthophyll cycle (interconversion between zeaxanthin, antheraxanthin and violaxanthin) is responsive to light and important in photoprotection. PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; Z-ISO ζ-carotene isomerase; βOH, β-hydroxylase; εOH, ε-hydroxylase; NXS, neoxanthin synthase; VDE, violaxanthin de-epoxidase; ZEP, zeaxanthin epoxidase; CCD: carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase.

and proteins, thereby behaving as antioxidants (along with ascorbate and tocopherols) and (3) dissipate excess energy via xanthophyll-mediated non-photochemical quenching (NPQ) (e.g. xanthophyll carotenoids include zeaxanthin, antheraxanthin and lutein). The physiological relevance of xanthophylls to

photosynthesis and plant biology in general is displayed by bleaching, delayed greening, viviparous and semi-lethal phenotypes observed in several carotenoid and NPQ-deficient mutants (Niyogi 1999; Pogson *et al.* 2005; Bailey and Grossman 2008; Alboresi *et al.* 2011).

Colour, volatiles, phytohormones and signalling molecules in plants

The bright colours of carotenoids serve as powerful visual attractants for birds and insects, which assist in the dispersal of pollen and seeds aiding plant reproduction. For example, a single locus, YELLOW UPPER5–7 (YUP) controls the presence or absence of yellow carotenoid pigments in the petals of pink-flowered *Mimulus lewisii* (monkey flower), which is pollinated by bumblebees and its red-flowered sister species *M. cardinalis*, which is pollinated by hummingbirds (Schemske and Bradshaw 1999). Researchers creating near-isogenic lines (NILs) in which the YUP allele from each species was substituted altering flower colour and revealed an adaptive shift in pollinator preference as a result of a single mutation altering carotenoid composition (Bradshaw and Schemske 2003). The next question from a plant regulatory perspective is what is the underlying molecular nature or mutation associated with the YUP allele? The regulation of carotenoid colours in flowers has been reviewed recently (Zhu *et al.* 2010). In addition to providing colour to flowers and fruits, C₄₀ carotenoid derivatives serve as substrates in the biosynthesis of plant volatile scents and aroma constituents (geranyl acetone and β -ionone) that attract insects and animals for pollination as well as seed dispersal (Simkin *et al.* 2004; Walter *et al.* 2010).

In plants, carotenoids serve as substrates for the production of phytohormones such as abscisic acid and strigolactone as well as other signalling molecules (e.g. blumenin and mycorradicin) (Figs 1, 3). ABA is a cleavage product of 9-*cis*-violaxanthin and/or 9'-*cis*-neoxanthin by 9-*cis*-epoxycarotenoid dioxygenase (NCED), which was first identified in the maize viviparous14 (vp14) mutant (Schwartz *et al.* 1997; Seo and Koshiba 2002; Tan *et al.* 2003; Chinnusamy *et al.* 2008). In some situations, carotenoid biosynthetic genes, such as β -carotene hydroxylase from rice, were shown to be rate-limiting for ABA biosynthesis and can alter the plants resistance to drought and oxidative stress by modulating the levels of xanthophylls and abscisic acid synthesis (Du *et al.* 2010). ABA mediates responses to environmental stresses, notably the control of stomatal aperture and transpiration during drought, also the induction of many stress-related gene products. Finally, ABA promotes developmental processes such as seed maturation and dormancy (Seo and Koshiba 2002; Nambara and Marion-Poll 2005; Chinnusamy *et al.* 2008).

The strigolactone class of carotenoid-derived terpenoids functions to (1) inhibit shoot branching, presumably by inhibiting bud outgrowth (Gomez-Roldan *et al.* 2008; Umehara *et al.* 2008), (2) influence mycorrhizal hyphal branching in order to stimulate a symbiotic relationship in the root rhizosphere (Akiyama *et al.* 2005) and (3) encourage germination of parasitic plant seeds such as striga (Matusova *et al.* 2005). Indeed, strigolactone application of GR24 can restore wild-type branching phenotype in pea *ccd8* mutants and also in the orthologous *ccd8 Arabidopsis*, in a dose-dependent manner (Gomez-Roldan *et al.* 2008). Beta-carotene has been proposed as the substrate for strigolactone biosynthesis (Matusova *et al.* 2005; Rani *et al.* 2008) and it has yet to be determined if changes in β -carotene levels perturb strigolactone production.

Other carotenoid-derived signalling molecules such as C₁₃ cyclohexenone derivatives (e.g. blumenol) and C₁₄

apocarotenoids (e.g. mycorradicin) play important roles in controlling beneficial arbuscule turnover during arbuscular mycorrhizal (AM) fungi symbiosis as well as triggering hyphal branching in the rhizosphere (Fig. 1) (Giuliano *et al.* 2003; Akiyama *et al.* 2005; Walter *et al.* 2010). Further, evidence exists for the potential for a novel mobile carotenoid-derived signalling metabolite required for normal root and shoot development. The *Arabidopsis* *BYPASS1* (*BPS1*) gene encodes an unknown protein and roots of *bps1* mutants produce a graft transmissible signal arresting shoot development (Van Norman *et al.* 2004). That fluridone (blocks phytoene production) can partially rescue both leaf and root defects displayed by *bps1* and CPTA (a lycopene cyclase inhibitor) treatment indicates that the signal is likely to require the biosynthesis of β -carotene and its derivatives (Fig. 2). The mobile signal is neither ABA- nor strigolactone related and the signal does not require the activity of any single carotenoid cleavage dioxygenase (Van Norman *et al.* 2004; Van Norman and Sieburth 2007). Unravelling the biosynthesis and regulation of carotenoid-derived signalling molecules will undoubtedly provide new insights into the essential roles that carotenoids play in nature.

Carotenoid biosynthesis and regulation

Carotenoid biosynthesis requires an available source of isoprenoid substrates derived from the plastid-localised 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Rodriguez-Concepcion 2010). Isoprenoids (or terpenoids) are naturally-occurring organic chemicals that serve as precursors to produce a diverse range of compounds such as tocopherols, chlorophylls, phyloquinone (PhQ), gibberellins, abscisic acid, monoterpenes and plastoquinone (PQ). Glyceraldehyde-3-phosphate and pyruvate (generated from the Calvin cycle or glycolysis) act as initial substrates leading to five-carbon isoprene isomers, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are then condensed to synthesise geranylgeranyl diphosphate (GGPP) (Fig. 3). The first steps in the MEP pathway are catalysed by 1-deoxyxylulose-5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR). 1-Hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (HDR) then catalyses the production of IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate). Abiotic and biotic factors are likely to influence the production of isoprenoid substrates for carotenogenesis. Most importantly, light and circadian oscillations appear to regulate the expression of most MEP and several carotenoid biosynthetic genes. The biosynthesis and regulation of the MEP pathway and isoprenoid substrates required for carotenogenesis has been covered elsewhere (Cordoba *et al.* 2009).

The condensation of two GGPP molecules catalysed by the phytoene synthase (PSY) enzyme produces 15-*cis*-phytoene, the first colourless carotenoid (Fig. 2). PSY is an important rate-limiting regulatory enzyme in the pathway, forming the first committed step and a bottleneck in carotenogenesis. In addition to high light, PSY can be transcriptionally responsive to ABA, salt, drought, temperature, photoperiod, development cues and post-transcriptional feedback regulation (Cazzonelli and Pogson 2010). Carotenoid pathway flux can be controlled by

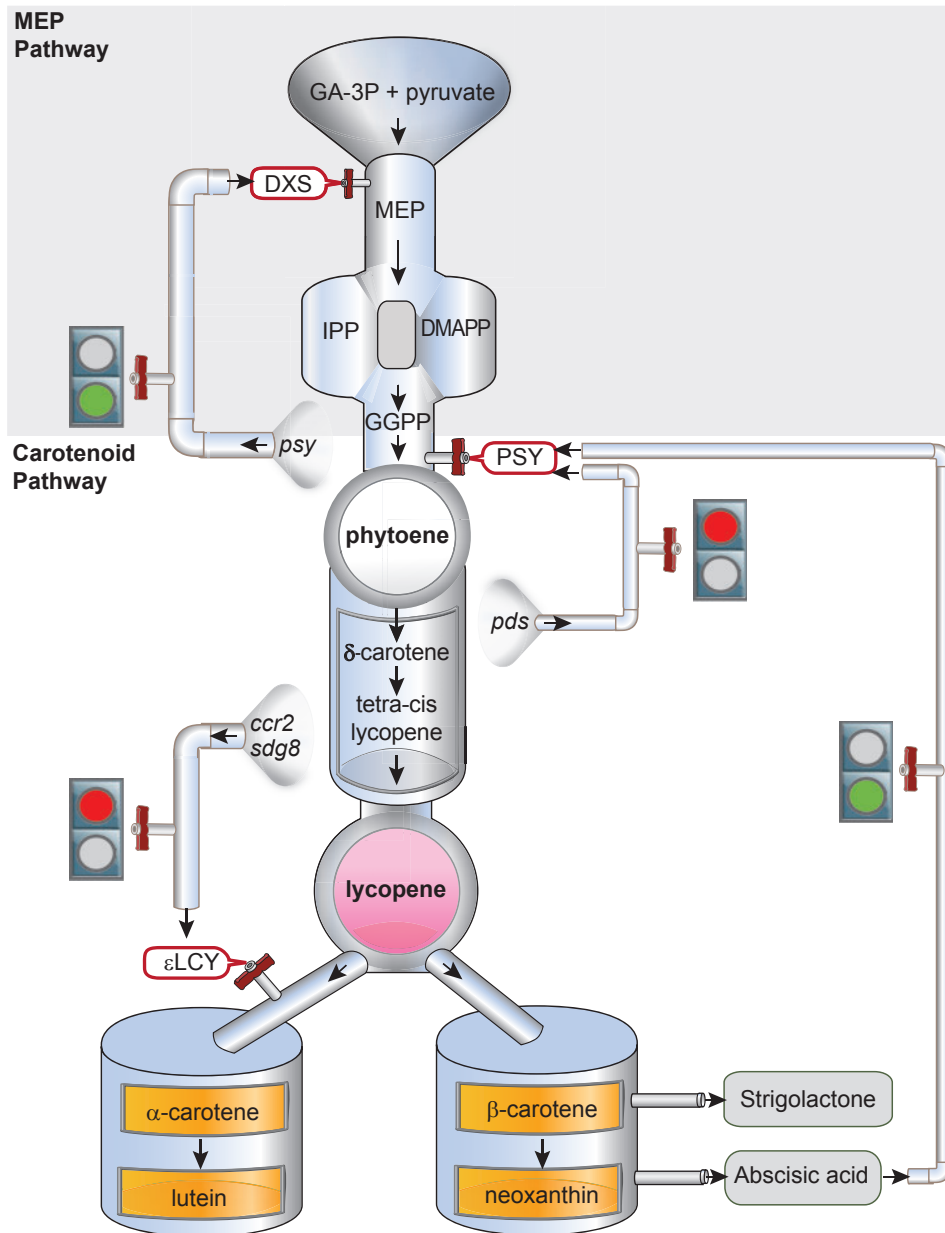


Fig. 3. Model for carotenoid metabolite feedback regulation in plants. Positive (green light) and negative (red light) metabolite feedback mechanisms exist between and within the carotenoid, MEP and ABA pathways. Post-transcriptional and transcriptional processes modulate the expression and/or activity of three key genes. (1) The overexpression of *PSY* leads to a positive upregulation of *DXS* activity in the MEP pathway. (2) The expression of the rate-limiting *PSY* gene is negatively downregulated by the loss-of-function in *pds* and positively upregulated by ABA. (3) A block in carotenoid flux through the branch to synthesis of α - and β -carotenes via the loss-of-function of CRTISO or SDG8, transcriptionally reduces *εLCY* gene expression. The 2-C-methyl-D-erythritol 4-P (MEP) pathway in chloroplasts provides C_{20} isoprenoid substrates for carotenoid biosynthesis. Only major enzymes and metabolites that play a key role in metabolite feedback regulation are displayed. GA-3P, D-glyceraldehyde-3-P; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate.

multiple *PSY*s, as is the case for tomato, rice and maize, whereas only a single allele exists in *Arabidopsis* (Welsch *et al.* 2000, 2008; Giorio *et al.* 2008; Li *et al.* 2008a). Multiple homologues of *PSY* are likely to be somewhat redundant; however, individual *PSY* alleles are expressed in a tissue-specific manner and only

some alleles show a unique response to abiotic stress-induced ABA signalling of *PSY* gene expression (Li *et al.* 2008b; Welsch *et al.* 2008; Qin *et al.* 2011). Alternative splicing as well as allelic variation are mechanisms by which to titrate *PSY* enzyme activity and are likely to underlie a major QTL determinant of flour colour

in bread and durum wheats (Howitt *et al.* 2009; Rodríguez-Suárez *et al.* 2011). The expression of *PSY* is highly co-regulated with photosynthesis-related genes and isoprenoid biosynthesis pathway genes insuring an optimum photosynthetic metabolism (Meier *et al.* 2011). Environmental, developmental and metabolic cues are major regulators of *PSY* gene expression and will be considered later in the review.

The production of all-*trans*-lycopene from 15-*cis*-phytoene involves four enzymes, phytoene desaturase (PDS), ζ -carotene isomerase (ζ -ISO), ζ -carotene desaturase (ZDS) and carotenoid isomerase (CRTISO) as well as a light-mediated photoisomerisation (Fig. 2) (Bartley *et al.* 1999; Park *et al.* 2002; Isaacson *et al.* 2004; Breitenbach and Sandmann 2005; Dong *et al.* 2007; Chen *et al.* 2010). *PDS* mRNA transcript levels are slightly upregulated during photomorphogenesis via the phytochrome-mediated pathway, which could imply that PDS plays a rate-limiting role in the generation of 9,15,9'-tri-*cis*- ζ -carotene (ζ -carotene) (Welsch *et al.* 2000; Qin *et al.* 2007). The plastid-targeted alternative oxidase (PTOX) protein was required for phytoene desaturase (PDS) activity and links desaturation to chloroplast electron transport (Yu *et al.* 2007). Regulatory roles for ZDS and ζ -ISO in the catalysis of ζ -carotene to tetra-*cis*-lycopene (pro-lycopene), the substrate for CRTISO are beginning to emerge. *ZISO* gene expression appears to be regulated by an extended dark period and the loss-of-function of *ZDS* affects carotenoid accumulation as well as plastid-to-nucleus communications (referred to as retrograde signalling; Pogson *et al.* 2008), most likely due to impaired plastid development (Dong *et al.* 2007; Chen *et al.* 2010).

CRTISO catalyses the *cis*-*trans* reactions to isomerase the four *cis*-bonds introduced by the desaturases and emerges as a major regulatory node in the pathway (Cazzonelli *et al.* 2009c). *criso* Mutants accumulate poly-*cis*-isomers (e.g. phytoene, phytofluene, ζ -carotene and neurosporene in addition to prolycopene) instead of the typical xanthophyll profile in non-photosynthetic tissues such as flowers (e.g. petals), the inner pericarp of green fruit and etiolated seedlings (Isaacson *et al.* 2002; Park *et al.* 2002). The function of these *cis*-carotenoids remains largely unknown and it is easy to speculate that they could play a role as novel signalling molecules. Most recently, CRTISO was shown to be essential for establishing an equilibrium between *cis* and *trans* carotenoid isomers (Yu *et al.* 2011). Perhaps CRTISO has other functions, such as to control the production of apocarotenoid precursors required for phytohormone biosynthesis (e.g. ABA or strigolactone) or to establish an equilibrium between *cis*- to *trans*-isomerisation and, hence, control the accumulation of *cis*-carotenoids. A recent insight revealed that the OsCRTISO may function to regulate the expression of the PSII core proteins (CP43 and CP47), thereby affecting the redox state of the PQ to stabilise oxygen-evolving extrinsic proteins and activity of photosynthetic oxygen evolution in rice (Wei *et al.* 2010).

Despite the block from *cis*- to an all-*trans*-configuration in *criso* mutants, carotenogenesis can proceed in chloroplasts via photoisomerisation, but there is a delay in the greening of etiolated seedlings and a substantial reduction in lutein in *Arabidopsis* as well as some degree of chlorosis in tomato and rice (Isaacson *et al.* 2002; Park *et al.* 2002; Fang *et al.* 2008; Wei *et al.* 2010; Chai *et al.* 2011). Perhaps, CRTISO plays important

roles in non-green tissues (e.g. fruit and root appear to be protected from photoisomerisation) and in germinating seeds as they grow through the soil towards the light (Isaacson *et al.* 2002; Park *et al.* 2002). The role of light in substituting for the absence of CRTISO activity in green tissues is under debate and now emerges as an exciting area of intense carotenoid research to unravel the elusive chemical mechanisms and regulatory nature associated with photoisomerisation (Fig. 2) (Cunningham and Schiff 1985; Sandmann 1991; Park *et al.* 2002; Isaacson *et al.* 2004; Yu *et al.* 2011).

Carotenoid biosynthesis bifurcates after lycopene to produce epsilon- and beta-carotenoids by enzymatic activity of two lycopene cyclases, ϵ LCY and β LCY (Fig. 2). Regulation by ϵ LCY is the first committed step coordinating carotenoid flux through the β - ϵ branch and modulates the ratio of the most abundant carotenoid, lutein to the β -carotenoids leading to changes in lutein content (Cuttriss *et al.* 2007; Harjes *et al.* 2008; Cazzonelli *et al.* 2009a; Howitt *et al.* 2009). A molecular synergism between ϵ LCY and β LCY activities is an overall major determinant of flux through the branch leading to production of lutein, β -carotene and other xanthophylls cycle (XC) carotenoids (Fig. 1) (Yu *et al.* 2008; Bai *et al.* 2009). Investigations have yielded mutants perturbing lutein biosynthesis including *lut1*, ϵ -hydroxylase (Tian *et al.* 2004); *lut2*, ϵ LCY (Cunningham *et al.* 1996; Pogson *et al.* 1996); *ccr2*, CRTISO (Park *et al.* 2002); and *lut5*, an additional β -hydroxylase (Kim and DellaPenna 2006) as well as the SDG8 chromatin regulatory mutant, *ccr1* (Cazzonelli *et al.* 2009a). Implications for the epigenetic regulation of carotenoid flux through the β - ϵ branch are discussed below.

The xanthophyll carotenoids are major carotenoid sink metabolites accumulating in plant leaf tissues produced by the oxidative functionalisation of either α -carotene (lutein) or β -carotenes (zeaxanthin, violaxanthin and neoxanthin) (Fig. 2). Modulation of xanthophyll composition can greatly affect photosystem assembly light harvesting, photoprotection and impact a plant's response to stressful conditions (Pogson *et al.* 1998; Dall'Osto *et al.* 2006). Zeaxanthin can be epoxidised to produce violaxanthin by zeaxanthin epoxidase (ZEP) (Marin *et al.* 1996). Under high-light stress, this reaction is reversed by violaxanthin de-epoxidase (VDE) (Pfundel *et al.* 1994). Violaxanthin is converted to neoxanthin by neoxanthin synthase (NXS) (Al-Babili *et al.* 2000). Neoxanthin is the final carotenoid of the β - β branch of the classical biosynthetic pathway (Fig. 2). Many other xanthophyll carotenoids have been identified in some plant tissues and these include the highly valued ketocarotenoids (e.g. astaxanthin, capsanthin, capsorubin and canthaxanthin) and their biosynthesis has been reviewed elsewhere (Giuliano *et al.* 2008; Jackson *et al.* 2008).

Turnover and degradation of carotenoids

Carotenoids are relatively stable compounds that accumulate in diverse types of tissues (photosynthetic and non-photosynthetic). Recently, it was demonstrated by $^{14}\text{CO}_2$ uptake experiments that carotenoid turnover appears to be much greater than expected (Beisel *et al.* 2010). Given the continued synthesis in mature leaves the active degradation of carotenoids by CCD (carotenoid cleavage dioxygenases) and NCED (9-*cis*-epoxycarotenoid dioxygenase) enzymatic turnover has emerged an exciting area

of discovery (Bouvier *et al.* 2005; Walter *et al.* 2010; Lewinsohn *et al.* 2005). Members of these gene families are involved in the biosynthesis of the phytohormone ABA (NCEDs), which controls abiotic stress signalling pathways and strigolactone (CCDs), which controls shoot growth and root-mycorrhizal symbiosis (Figs 1, 3).

The *NCED* gene family (*NCED2*, 3, 5, 6, 9) show different substrate specificity as well as tissue distribution and changes in *NCED* transcript levels regulate carotenoid composition and abundance (Schwartz *et al.* 1997, 2003; Tan *et al.* 1997, 2003). Further, *NCED3* gene expression appears to be controlled by chromatin modifying protein, ATX1 (homologue of the *Drosophila* Trithorax, TRX protein also referred to as SDG27), which is implicated in diverse dehydration stress-response mechanisms in *Arabidopsis* (Ding *et al.* 2011; Thorstensen *et al.* 2011). ATX1 was shown to affect the quantity of RNA polymerase II bound to the *NCED3* locus and is necessary for the increased levels of *NCED3* transcripts and nucleosomal histone-3-lysine-4 trimethylation (H3K4 me3) that occur during dehydration stress. In the *atx1* mutant the *NCED3* transcript levels decline and this in part reduces ABA production. The loss of function of *atx1* mutants shows a reduced germination rate, enlarged stomatal aperture, increased transpiration rates and a lower tolerance to dehydration stress (Ding *et al.* 2011).

The *CCD* gene family (*CCD1*, 4, 7 and 8) play essential roles in synthesis of colour, apocarotenoid flavour, aroma volatiles and phytohormones such as strigolactone. The active degradation of the xanthophylls by CCD activity can reduce lutein content in strawberries as well as cause changes in the pigmentation in chrysanthemums from white to yellow (Ohmiya *et al.* 2006; García-Limones *et al.* 2008). In maturing *Arabidopsis* seeds a loss of function of *CCD1* activity leads to higher carotenoid levels and may have a role in synthesis of apocarotenoid flavour and aroma volatiles (Auldrige *et al.* 2006). Similarly, in tomato (*Lycopersicon esculentum*) LeCCD1 activity contributes to the formation of the flavour volatiles β -ionone, pseudoionone and geranylacetone (Simkin *et al.* 2004). The *Crocus*, zeaxanthin 7,8(7',8')-cleavage dioxygenase (CsZCD) and 9,10(9',10')-cleavage dioxygenase (CsCCD) initiate the biogenesis of carotenoid derivatives such as crocetin glycosides, picrocrocin and safranal (saffron). Indeed, the expression of *CsZCD* mRNA was mostly restricted to the style branch tissues and transcript levels were inducible under dehydration stress (Bouvier *et al.* 2003b). Two of the genes that affect shoot branching encoding *CCD7* and *CCD8* can sequentially cleave β -carotene to form the C₁₈ compound 13-apo-carotenone (Schwartz *et al.* 2004). *CCD7* appears to be a biosynthetic cross point, controlling both strigolactone and AM-induced C₁₃ cyclohexenone and C₁₄ mycorradicin apocarotenoids (Vogel *et al.* 2010). Finally, there are examples of cross talk where inhibition of ABA biosynthesis reduces *CCD7* and *CCD8* transcript abundance as well as strigolactone levels (López-Ráez *et al.* 2010).

Accumulation, storage and insights from biofortification

The storage of carotenoids requires a lipophilic environment, usually within the membranes (envelope and in some cases the thylakoid membranes) of plastid organelles, which behave as a

sink for carotenoid accumulation. The biogenesis of organelles is a major determinant of the storage compartment size of plastids and can affect the accumulation of carotenoids (reviewed by Pogson and Albrecht 2011). The colourless pluripotent progenitor proplastid differentiates into specialised plastids that can store chlorophylls and carotenoids (Kirk and Tilney-Bassett 1978). Carotenoids are usually synthesised *de novo* in differentiated plastids of roots, flowers, fruits and seeds, accumulating mostly in chloroplasts (green photosynthetic plastids) and chromoplasts (coloured plastids), but also in amyloplasts (starch-storing plastids), leucoplasts (colourless plastids), etioplasts (dark-grown precursors of the chloroplast) and elaioplasts (lipid-storing plastids) (Cazzonelli and Pogson 2010). There are links between changes in carotenoid composition and plastid biogenesis, morphology and protein translocation, in particular, it is noteworthy that carotenoids (e.g. lutein) are necessary for the differentiation of an etioplast into a chloroplast (Fig. 1) (Park *et al.* 2002).

The regulation of carotenoid targeting, storage and sequestration within various plastid types is a process by which to modulate a sink for carotenoid accumulation (Lu *et al.* 2006; Cuttriss *et al.* 2007; Cazzonelli and Pogson 2010). For example, the *high-pigment 1* tomato mutant, *hp1*, displays an increased pigmentation because of increased chromoplast compartment size (Cookson *et al.* 2003). A naturally occurring mutation in the *Brassica oleracea* orange-curd (*or*) gene changes a normally white cauliflower curd into an orange *Or* mutant, which accumulates high levels of β -carotene (Lu and Li 2008). The *OR* gene encodes a DnaJ cysteine-rich domain-containing protein and plays a role in coordinating the differentiation of proplastids in the shoot and inflorescence meristems of the curd into chromoplasts (Li *et al.* 2001; Lu *et al.* 2006). Carotenoids accumulate in lipoprotein structures within the chromoplast (Bartley and Scolnik 1995; Vishnevetsky *et al.* 1999), which might allow for additional carotenoid biosynthesis. Therefore, chromoplasts serve as a metabolic sink to control carotenoid accumulation in plants and reveals the importance of plastid differentiation in controlling carotenoid accumulation in plants (Lu *et al.* 2006; Li and Van Eck 2007). The *Crocus* chromoplast localised enzymes, CsZCD and CsCCD control the accumulation of saffron metabolites as well as the differentiation of amyloplasts and chromoplasts (Bouvier *et al.* 2003b). The oxidative cleavage of zeaxanthin in chromoplasts is intriguingly followed by the sequestration of modified water-soluble derivatives into the central vacuole (Bouvier *et al.* 2003b).

The introduction of foreign exogenous carotenoid biosynthetic genes into crops is another means by which to modulate the sink for carotenoid storage. Carotenoid accumulation has been achieved in oil seeds of canola (*Brassica napus*) through the overexpression of PSY, which resulted in 43–50-fold increase in total seed carotenoid content (Shewmaker *et al.* 1999; Lindgren *et al.* 2003). 'Golden rice 2' is a high β -carotene accumulating line overexpressing PSY from maize as well as bacterial CrtI (from *Erwinia uredovora*) and accumulates 23-fold more carotenoids (37 $\mu\text{g g}^{-1}$) than was the case for the initial 'golden rice', which overexpressed PSY from daffodil (Beyer *et al.* 2002; Paine *et al.* 2005). Underground food crops, such as potato and carrot have also been engineered to have increased carotenoid content, in particular β -carotene and

ketocarotenoids (e.g. astaxanthin), which produce a deep yellow ('golden') potato tuber phenotype and a distinct pink to deep red colour compared with the typical orange colour in wild-type carrot roots (Taylor and Ramsay 2005; Diretto *et al.* 2007; Jayaraj *et al.* 2008). Finally, the overexpression of *PSY* in *Arabidopsis* increased carotenoid content by 10- to 100-fold in nonphotosynthetic calli and roots, predominantly β -carotene and its derivatives, which were deposited as crystals in storage plastids (Maass *et al.* 2009).

Photostimulation of carotenoid gene expression

Light regulates the key rate-limiting step in phytoene biosynthesis. *PSY* transcript abundance increases during photomorphogenesis via a phytochrome-mediated (red-light) pathway and this response was abolished in the *phyA* mutant (Welsch *et al.* 2000, 2008). Phytochrome-interacting factor 1 (PIF1) has been shown to bind to the *PSY* promoter and represses *PSY* mRNA expression. The authors show that light triggers the degradation of PIF1 after interacting with photoactivated phytochromes during de-etiolation and that a rapid derepression of *PSY* gene expression enhances carotenoid production during the optimal transition to photosynthetic metabolism (Toledo-Ortiz *et al.* 2010). The light-triggered degradation of PIFs causes a rapid accumulation of carotenoids, which is coordinated with chlorophyll biosynthesis to enable the rapid assembly of the photosynthetic machinery (Toledo-Ortiz *et al.* 2010). Very recently, the DELLA proteins were shown to function in part through the regulation of PIF activity, by a DELLA-PIF complex that coordinates the levels of POR, chlorophyll precursors and carotenoids in cotyledons of dark-grown seedlings (Cheminant *et al.* 2011). *PSY* is strongly light-induced in greening seedlings (Welsch *et al.* 2000) and a transcription factor, RAP2.2, (AP2/EREBP family) which binds to the *PSY* promoter was able to slightly perturb pigment alterations in *Arabidopsis* root calli (Welsch *et al.* 2007).

High light stress also alters the synthesis of β -xanthophylls, such as the synthesis of zeaxanthin from β -carotene (Depka *et al.* 1998). Plants with reduced zeaxanthin exhibit increased sensitivity to light stress (Havaux and Niyogi 1999). The amount of zeaxanthin can be modulated by regulating the expression of the β -hydroxylase (β -OH) gene, which is photo-inducible and violaxanthin de-epoxidase (*VDE*), whose transcript levels are slightly downregulated by light (Rossel *et al.* 2002). In addition, the luminal pH and ascorbate content appear to affect post-translation modulation of *VDE* activity and is critical for determining the levels of zeaxanthin upon exposure to excessive light (Rockholm and Yamamoto 1996). Further, the *βLCY* mutant (suppressor of *zeaxanthin-less1*, *szl1*) lacks zeaxanthin, yet accumulates higher levels of lutein and α -carotene, which partially restores quenching efficiency, revealing that lutein could substitute for zeaxanthin (Li *et al.* 2009a). The modulation of zeaxanthin levels by the violaxanthin de-epoxidase (*VDE*) is thought to be required for the thermal dissipation of excess light energy and the protection of photosynthetic membranes against lipid peroxidation, a process known as the xanthophyll cycle (Havaux and Niyogi 1999).

Epigenetic maintenance of carotenoid composition

Beyond the heavy demands from developmental and environmental cues to coordinate carotenoid gene expression (Cazzonelli and Pogson 2010), there are further implications for the epigenetic control over apocarotenoid formation (described above) and especially carotenoid biosynthesis (Cazzonelli *et al.* 2009c; Ding *et al.* 2011). A chromatin modifying protein was found to regulate *CRTISO* expression and carotenoid flux through the branch to α and β -carotenoids, thereby limiting lutein production. The SET DOMAIN GROUP (SDG) family of histone lysine methyltransferases are chromatin modifying proteins that methylate lysine residues (chromatin marks) on the tails of histone proteins in order to recruit other regulatory factors and make the DNA either more or less accessible to RNA polymerase II transcriptional complex (Cazzonelli *et al.* 2009b; Thorstensen *et al.* 2011). The methylation of lysine residues can be maintained through cell division (mitosis) establishing long-term memory required for adaptation and these marks are usually reset during gamete formation (meiosis) (Saze 2008).

SDG8 (homologue of the *Drosophila* absent, small or homeotic disks 2 often referred to as ASH2 or in plants as carotenoid and chloroplast regulation, *ccr1*, or early flowering in short days, *efs*) maintains a transcriptionally permissive chromatin state surrounding the *CRTISO* locus and thus is able to regulate carotenoid composition, particularly accumulation of the most abundant carotenoid, lutein (Fig. 2) (Cazzonelli *et al.* 2009a). In non-photosynthetic tissues, SDG8 is required for *CRTISO* activity and in the absence of SDG8 activity, etiolated tissues accumulate *cis*-isomers (Park *et al.* 2002). SDG8 maintains H3K4 trimethylation of regions surrounding the *CRTISO* promoter and SDG8 is necessary for regulating the *CRTISO* promoter in rapidly dividing tissues during epigenetic programming (e.g. mitosis in the shoot apex) and reprogramming (e.g. meiosis in pollen) events (Cazzonelli *et al.* 2010). The reporter gene expression patterns enabled by *SDG8* and *CRTISO* promoters overlap considerably, with the strongest expression being apparent in many tissues essential for defining plant architecture and development, including germinating seedlings, vasculature, meristems, shoot apices, floral anthers and pollen (Cazzonelli *et al.* 2010).

The *sdg8* mutant shows several pleiotropic phenotypes, such as early flowering, altered shoot and root architecture, altered pathogen resistance, male sterility, abnormal sporophyte and gametophyte development (Kim *et al.* 2005; Cazzonelli *et al.* 2009a; Grini *et al.* 2009; Berr *et al.* 2010). A few putative target genes responsible for these phenotypes have been described and target gene loci show a lower abundance of H3K4 or H3K36 methylation marks, as well as reduced mRNA levels in the *sdg8* mutant. However, only the early flowering and lutein deficient phenotypes have been sufficiently complemented by overexpression of the corresponding target genes, *FLOWERING LOCUS C* (*FLC*) and *CRTISO*, respectively (Kim *et al.* 2005; Cazzonelli *et al.* 2009a). The affect of the chromatin marks can spread and appear to only have a slight affect on the relative transcript abundance of genes neighbouring *CRTISO* and *FLC*, suggesting that indeed both *CRTISO* and *FLC* are likely to be direct targets of SDG8 activity (Kim *et al.* 2005; Cazzonelli *et al.* 2009a).

The chromatin regulatory proteins required to recruit and/or interact with SDG8 in order to post-translationally modify the *CRTISO* locus with permissive chromatin marks is yet to be discovered. The epigenetic regulation of *FLC* by vernalisation has been extensively studied and should pave the way to discover new insights into the epigenetic regulatory mechanisms controlling *CRTISO* expression and perhaps link carotenoids with the developmental regulation of floral initiation (Dennis and Peacock 2007). It was recently demonstrated that SDG8 interacts with FRIGIDA (FRI), a protein that delays flowering by activating *FLC* expression and in the absence of a prolonged cold winter period (a process known as vernalisation) FRI promotes a vegetative state of growth (Choi *et al.* 2011). SDG8 is essential for FRI function and SDG8 was recently demonstrated to have a novel dual substrate specificity for H3K4 and H3K36 specific histone lysine methylation activity (Ko *et al.* 2010). The effect of FRI on carotenoid accumulation, if any, remains to be determined.

There are bigger questions that remain to be answered. First, does SDG8 also affect H3K36 chromatin marks surrounding the *CRTISO* locus? Second, what is the molecular nature of the mechanisms used to target SDG8 to key target genes like *CRTISO* and *FLC*? Third, is carotenoid composition regulated in response to an epigenetic phenomena? Finally, what novel roles might SDG8 and *CRTISO* play in fine tuning plant development, architecture and the production of precursors for phytohormone biosynthesis? It is possible to speculate the role, if any, for photoisomerisation in overriding SDG8 regulation in green tissues, however, in non-photosynthetic tissues, perhaps SDG8 and *CRTISO* serve important functions in coordinating organelle differentiation, cellular specialisation and plastid to nucleus communications during development. The discovery that carotenoid biogenesis and apocarotenoid formation are regulated by chromatin modification opens the door to begin exploring what new roles might carotenoids and apocarotenoids (e.g. ABA) play in memory forming processes.

Carotenoid signals and metabolite feedback control

Several genes encoding enzymes in isoprenoid and carotenoid biosynthesis are the subject of negative and positive feedback regulatory processes and are likely to be mediated by a carotenoid or a molecule derived from a carotenoid (Römer *et al.* 2000; Beyer *et al.* 2002; Cuttriss *et al.* 2007; Qin *et al.* 2007; Bai *et al.* 2009). There is strong evidence to show carotenoid metabolite feedback regulation requires post-transcriptional and transcriptional processes to modulate target genes that (1) supply isoprenoid precursors for carotenogenesis, (2) control activity of the rate-limiting phytoene synthase step and (3) control carotenoid flux through the branch to synthesis of α - and β -carotenes. Essentially, positive and negative metabolite feedback mechanisms exist within the carotenoid pathway and among carotenoids, MEP and ABA (Fig. 3).

The overexpression of *PSY* transcript levels enhanced carotenoid levels in etiolated *Arabidopsis* seedlings via the concomitant post-transcriptional accumulation of *DXS* protein, which initiated feedback stimulating the supply of MEP substrates (Fig. 3) (Rodríguez-Villalón *et al.* 2009). Additional evidence that *PSY* controls metabolic flux was obtained through

the paclobutrazol treatment of *Arabidopsis* seedlings, which not only inhibits gibberellin synthesis but stimulates de-etiolation despite the absence of light. *PSY* activity and carotenoid levels were shown to increase in the dark following paclobutrazol treatment and this increase was supported by feedback regulation of *DXS* and *DXR* protein abundance (Rodríguez-Villalón *et al.* 2009). Surprisingly, overexpression of *DXS* mRNA in dark-grown seedlings does not affect carotenoid accumulation (Rodríguez-Villalón *et al.* 2009). An increase in *DXS* protein activity (but not gene expression levels) was also detected in tomato fruit containing increased *PSY* transcript levels and it was speculated that this could occur through the enhanced accumulation of active *DXS* enzymes in chromoplasts (Fraser *et al.* 2007; Rodríguez-Villalón *et al.* 2009). Finally, the establishment of plant root arbuscular mycorrhizal (AM) fungi in the rhizosphere activates the MEP pathway enhancing transcript levels of the first two enzymes of the MEP pathway (e.g. *DXS* and *DXR*), the two desaturases in the carotenoid pathway (e.g. *PDS* and *ZDS*) as well as the carotenoid-cleaving dioxygenases in AM roots (Strack and Fester 2006). The molecular nature of the metabolite feedback regulatory mechanisms controlling the accumulation of MEP precursors and *DXS* at the protein or gene expression levels, remain open for discovery.

PSY regulates the first committed step in carotenogenesis (e.g. phytoene production) and appears to be controlled by source and sink metabolites (Fig. 3). For example, in the *pds3* mutant, downstream (e.g. *ZDS* and β *LCY*) and upstream (*IP1*, *GGPS* and *PSY*) pathway genes were downregulated, but also those related to the biosynthesis of gibberellins as well as chlorophylls (Qin *et al.* 2007). The absence of downstream carotenoids in *pds3* mutants could provide the signal as evidence from the analysis of a tomato *PDS* promoter::GUS fusion demonstrated end product regulation in photosynthetic tissues (Corona *et al.* 1996). *OsPSY3* gene expression is upregulated in rice during increased abscisic acid (ABA) formation as well as upon salt treatment and drought, especially in roots indicating that the third *PSY* allele in rice plays a specialised role in abiotic stress-induced ABA formation (Welsch *et al.* 2008). A positive feedback regulation mediated by the application of ABA induces *OsPSY3* as well as *OsNCED* gene expression. An ABA-response element as well as a coupling element was identified in the promoter region for *OsPSY3*, but its function remains to be validated (Welsch *et al.* 2008). Therefore, the regulation of phytoene synthesis in plants appears to involve both negative and positive feedback regulatory mechanisms.

The control of carotenoid flux through the branch from all-*trans*-lycopene to synthesis of α - and β -carotenes is also under negative metabolite feedback regulation and mediated through the transcription control of ϵ *LCY* gene expression (Cuttriss *et al.* 2007). Investigations have indicated that both *CRTISO* (*ccr2*) and *SDG8* (*ccr1*) mutants have an inhibitory effect on ϵ *LCY* transcript levels (Fig. 3), revealing that feedback may partially explain the reduced lutein observed in *ccr2* and *ccr1* mutants (Cuttriss *et al.* 2007; Cazzonelli *et al.* 2009a). Further, a reduction in seed ϵ *LCY* mRNA levels show higher total carotenoid content, specifically increased levels of β -carotene, zeaxanthin, violaxanthin and unexpectedly, lutein (Yu *et al.* 2008). However, another report showed that tuber specific silencing of ϵ *LCY* resulted in an expected increase in β -carotene levels in potato (*Solanum tuberosum*) (Diretto *et al.* 2006). In *B. napus*, total carotenoids

increased in the β LCY (*lcyB-m2.1*) mutant by nearly 200 and 40% in mutant embryo and endosperm tissues of maize respectively (Bai *et al.* 2009). Lycopene was the primary carotenoid that accumulated in *lcyB-m2.1* embryo tissues, together with a small amount of δ -carotene (Bai *et al.* 2009) and perhaps these molecules are candidates for degradation and subsequent feedback regulation. Together, these lines of evidence support a hypothesis that lutein composition is largely rate-determined by ϵ LCY expression and that metabolite feedback might be another key regulator of the branch node in carotenogenesis (Fig. 3).

Future prospects

Carotenoids are everywhere and are essential in nature, clearly serving numerous functions during animal and plant development (Fig. 1). A deeper investigation into the control of carotenoid biosynthesis will undoubtedly provide new regulatory mechanisms by which to facilitate improvements to crop nutrition. Understanding how carotenoids are sequestered within plastid types is an area ripe for discovery and has the potential to further improve metabolic engineering attempts to enhance the composition of essential dietary micronutrients, such as the xanthophylls and carotenes. The discovery of new regulators of carotenoid biosynthesis that modulate the production of strigolactones and ABA will really highlight the important roles that carotenoids play in plants, especially in response to developmental and environmental signals. Two major nodes in the pathway play essential roles in regulating carotenoid composition and flux. One node affects the production of phytoene at the bottleneck in the pathway and the other modulates the accumulation of *cis*-carotenoids and lutein at the branch to α - and β -carotenoids. The cross-talk between and within the MEP, carotenoid and ABA biosynthetic pathways is an intriguing example of metabolite feedback control. This could involve a signalling protein bound to specific carotenoids or their *cis-trans*-isomers and perhaps even soluble or volatile carotenoid cleavage products could mediate signalling between the plastid and nucleus. For now the nature of the different forms of metabolic feedback regulation remain unknown. It will be necessary to establish an evolutionary importance for some of the carotenoid regulatory processes described in this review using non-model plant species. Finally, a deeper understanding of the molecular nature by which metabolite feedback and chromatin regulatory mechanisms control carotenoid abundance and composition is paramount to determine if indeed there is a memory forming process epigenetically regulating carotenogenesis in plants.

Acknowledgements

CC was supported by the Australian Research Council Centre of Excellence in Plant Energy Biology (CE0561495). A special thanks goes to Barry Pogson (Australian National University) in nominating me for the Peter Goldacre Award and for introducing me to the brighter world of carotenoids. Many thanks also to Mark Taylor (The James Hutton Institute) and Li Li (USDA-ARS) for reviewing this article and providing constructive comments. The author would finally like to give a strong appreciation to the following contributors for providing images in Fig. 1: aphids (Nancy Moran, Yale University), etioplast and chloroplast (Reprinted from *Trends in Plant Sciences*, 15(5), 266–274, 2010, with permission from Elsevier), finches (Kevin McGraw, Arizona State

University), golden rice (Courtesy Golden Rice Humanitarian Board: www.goldenrice.org, accessed 29 July 2011), sea anemone (Shunichi Takahashi, Australian National University) and flamingos as well as crayfish (Harry Frank, University of Connecticut).

References

- Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**, 824–827. doi:10.1038/nature03608
- Al-Babili S, Huguency P, Schledz M, Welsch R, Frohnmeyer H, Laule O, Beyer P (2000) Identification of a novel gene coding for neoxanthin synthase from *Solanum tuberosum*. *FEBS Letters* **485**, 168–172. doi:10.1016/S0014-5793(00)02193-1
- Alboresi A, Dall'Osto L, Aprile A, Carillo P, Roncaglia E, Cattivelli L, Bassi R (2011) Reactive oxygen species and transcript analysis upon excess light treatment in wild-type *Arabidopsis thaliana* vs a photosensitive mutant lacking zeaxanthin and lutein. *BMC Plant Biology* **11**, 62. doi:10.1186/1471-2229-11-62
- Amat J, Rendón M, Garrido-Fernández J, Garrido A, Rendón-Martos M, Pérez-Gálvez A (2010) Greater flamingos *Phoenicopterus roseus* use uropygial secretions as makeup. *Behavioural Ecology and Sociobiology* **65**, 665–673. doi:10.1007/s00265-010-1068-z
- Auldrige ME, Block A, Vogel JT, Dabney-Smith C, Mila I, Bouzayen M, Magallanes-Lundback M, DellaPenna D, McCarty DR, Klee HJ (2006) Characterisation of three members of the *Arabidopsis* carotenoid cleavage dioxygenase family demonstrates the divergent roles of this multifunctional enzyme family. *The Plant Journal* **45**, 982–993. doi:10.1111/j.1365-3113X.2006.02666.x
- Bai L, Kim EH, DellaPenna D, Brutnell TP (2009) Novel lycopene epsilon cyclase activities in maize revealed through perturbation of carotenoid biosynthesis. *The Plant Journal* **59**, 588–599. doi:10.1111/j.1365-3113X.2009.03899.x
- Bailey S, Grossman A (2008) Photoprotection in cyanobacteria: regulation of light harvesting. *Photochemistry and Photobiology* **84**, 1410–1420. doi:10.1111/j.1751-1097.2008.00453.x
- Baron M, Davies S, Alexander L, Snellgrove D, Sloman KA (2008) The effect of dietary pigments on the coloration and behaviour of flame-red dwarf gourami, *Colisa lalia*. *Animal Behaviour* **75**, 1041–1051. doi:10.1016/j.anbehav.2007.08.014
- Bartley GE, Scolnik PA (1995) Plant carotenoids: pigments for photoprotection, visual attraction and human health. *The Plant Cell* **7**, 1027–1038.
- Bartley GE, Scolnik PA, Beyer P (1999) Two *Arabidopsis thaliana* carotene desaturases, phytoene desaturase and zeta-carotene desaturase, expressed in *Escherichia coli*, catalyse a poly-*cis* pathway to yield pro-lycopene. *European Journal of Biochemistry* **259**, 396–403. doi:10.1046/j.1432-1327.1999.00051.x
- Beisel KG, Jahnke S, Hofmann D, Koppchen S, Schurr U, Matsubara S (2010) Continuous turnover of carotenes and chlorophyll a in mature leaves of *Arabidopsis* revealed by $^{14}\text{CO}_2$ pulse-chase labelling. *Plant Physiology* **152**, 2188–2199. doi:10.1104/pp.109.151647
- Berr A, McCallum EJ, Alioua A, Heintz D, Heitz T, Shen WH (2010) *Arabidopsis* histone methyltransferase SET DOMAIN GROUP8 mediates induction of the jasmonate/ethylene pathway genes in plant defense response to necrotrophic fungi. *Plant Physiology* **154**, 1403–1414. doi:10.1104/pp.110.161497
- Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Potrykus I (2002) Golden rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *Journal of Nutrition* **132**, 506S–510S.
- Bouvier F, Dogbo O, Camara B (2003a) Biosynthesis of the food and cosmetic plant pigment bixin (annatto). *Science* **300**, 2089–2091. doi:10.1126/science.1085162

- Bouvier F, Suire C, Mutterer J, Camara B (2003b) Oxidative remodelling of chromoplast carotenoids: identification of the carotenoid dioxygenase *CsCCD* and *CsZCD* genes involved in *Crocus* secondary metabolite biogenesis. *The Plant Cell* **15**, 47–62. doi:10.1105/tpc.006536
- Bouvier F, Isner JC, Dogbo O, Camara B (2005) Oxidative tailoring of carotenoids: a prospect towards novel functions in plants. *Trends in Plant Science* **10**, 187–194. doi:10.1016/j.tplants.2005.02.007
- Bradshaw HD, Schemske DW (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**, 176–178. doi:10.1038/nature02106
- Breitenbach J, Sandmann G (2005) Zeta-carotene *cis*-isomers as products and substrates in the plant poly-*cis* carotenoid biosynthetic pathway to lycopene. *Planta* **220**, 785–793. doi:10.1007/s00425-004-1395-2
- Cazzonelli CI, Pogson BJ (2010) Source to sink: regulation of carotenoid biosynthesis in plants. *Trends in Plant Science* **15**, 266–274. doi:10.1016/j.tplants.2010.02.003
- Cazzonelli CI, Cuttriss AJ, Cossetto SB, Pye W, Crisp P, Whelan J, Finnegan EJ, Turnbull C, Pogson BJ (2009a) Regulation of carotenoid composition and shoot branching in *Arabidopsis* by a chromatin modifying histone methyltransferase, SDG8. *The Plant Cell* **21**, 39–53. doi:10.1105/tpc.108.063131
- Cazzonelli CI, Millar T, Finnegan EJ, Pogson BJ (2009b) Promoting gene expression in plants by permissive histone lysine methylation. *Plant Signalling & Behaviour* **4**, 484–488. doi:10.4161/psb.4.6.8316
- Cazzonelli CI, Yin K, Pogson BJ (2009c) Potential implications for epigenetic regulation of carotenoid biosynthesis during root and shoot development. *Plant Signalling & Behaviour* **4**, 339–341. doi:10.4161/psb.4.4.8193
- Cazzonelli CI, Roberts AC, Carmody ME, Pogson BJ (2010) Transcriptional control of SET DOMAIN GROUP8 and CAROTENOID ISOMERASE during *Arabidopsis* development. *Molecular Plant* **3**, 174–191. doi:10.1093/mp/ssp092
- Chai C, Fang J, Liu Y, Tong H, Gong Y, Wang Y, Liu M, Wang Y, Qian Q, Cheng Z, Chu C (2011) *ZEBRA2*, encoding a carotenoid isomerase, is involved in photoprotection in rice. *Plant Molecular Biology* **75**, 211–221. doi:10.1007/s11103-010-9719-z
- Cheminant S, Wild M, Bouvier F, Pelletier S, Renou JP, Erhardt M, Hayes S, Terry MJ, Genschik P, Achard P (2011) DELLAs regulate chlorophyll and carotenoid biosynthesis to prevent photo-oxidative damage during seedling de-etiolation in *Arabidopsis*. *The Plant Cell* **23**, 1849–1860. doi:10.1105/tpc.111.085233
- Chen Y, Li F, Wurtzel ET (2010) Isolation and characterisation of the *Z-ISO* gene encoding a missing component of carotenoid biosynthesis in plants. *Plant Physiology* **153**, 66–79. doi:10.1104/pp.110.153916
- Chidambara Murthy KN, Vanitha A, Rajesha J, Mahadeva Swamy M, Sowmya PR, Ravishankar GA (2005) *In vivo* antioxidant activity of carotenoids from *Dunaliella salina* – a green microalga. *Life Sciences* **76**, 1381–1390. doi:10.1016/j.lfs.2004.10.015
- Chinnusamy V, Gong Z, Zhu JK (2008) Abscisic acid-mediated epigenetic processes in plant development and stress responses. *Journal of Integrative Plant Biology* **50**, 1187–1195. doi:10.1111/j.1744-7909.2008.00727.x
- Choi K, Kim J, Hwang HJ, Kim S, Park C, Kim SY, Lee I (2011) The FRIGIDA complex activates transcription of FLC, a strong flowering repressor in *Arabidopsis*, by recruiting chromatin modification factors. *The Plant Cell* **23**, 289–303. doi:10.1105/tpc.110.075911
- Cookson PJ, Kiano JW, Shipton CA, Fraser PD, Romer S, Schuch W, Bramley PM, Pyke KA (2003) Increases in cell elongation, plastid compartment size and phytoene synthase activity underlie the phenotype of the *high pigment-1* mutant of tomato. *Planta* **217**, 896–903. doi:10.1007/s00425-003-1065-9
- Cordoba E, Salmi M, Leon P (2009) Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. *Journal of Experimental Botany* **60**, 2933–2943. doi:10.1093/jxb/erp190
- Corona V, Aracri B, Kosturkova G, Bartley GE, Pitto L, Giorgetti L, Scolnik PA, Giuliano G (1996) Regulation of a carotenoid biosynthesis gene promoter during plant development. *The Plant Journal* **9**, 505–512. doi:10.1046/j.1365-3113X.1996.09040505.x
- Cunningham FX, Jr, Schiff JA (1985) Photoisomerisation of ζ -carotene stereoisomers in cells of *euglena gracilis* mutant W3BUL and in solution. *Photochemistry and Photobiology* **42**, 295–307. doi:10.1111/j.1751-1097.1985.tb08945.x
- Cunningham FX, Pogson B, Sun ZR, McDonald KA, DellaPenna D, Gantt E (1996) Functional analysis of the beta and epsilon lycopene cyclase enzymes of *Arabidopsis* reveals a mechanism for control of cyclic carotenoid formation. *The Plant Cell* **8**, 1613–1626. doi:10.2307/3870254
- Cuttriss AJ, Chubb A, Alawady A, Grimm B, Pogson B (2007) Regulation of lutein biosynthesis and prolamellar body formation in *Arabidopsis*. *Functional Plant Biology* **34**, 663–672. doi:10.1071/FP07034
- Dall'Osto L, Lico C, Alric J, Giuliano G, Havaux M, Bassi R (2006) Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection *in vivo* under strong light. *BMC Plant Biology* **6**, 32. doi:10.1186/1471-2229-6-32
- Dall'Osto L, Cazzaniga S, Havaux M, Bassi R (2010) Enhanced photoprotection by protein-bound vs free xanthophyll pools: a comparative analysis of chlorophyll *b* and xanthophyll biosynthesis mutants. *Molecular Plant* **3**, 576–593. doi:10.1093/mp/ssp117
- Del Campo JA, García-González M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Applied Microbiology and Biotechnology* **74**, 1163–1174. doi:10.1007/s00253-007-0844-9
- Demmig-Adams B, Adams WW (2006) Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytologist* **172**, 11–21. doi:10.1111/j.1469-8137.2006.01835.x
- Dennis ES, Peacock WJ (2007) Epigenetic regulation of flowering. *Current Opinion in Plant Biology* **10**, 520–527. doi:10.1016/j.pbi.2007.06.009
- Depka B, Jahns P, Trebst A (1998) Beta-carotene to zeaxanthin conversion in the rapid turnover of the D1 protein of PSII. *FEBS Letters* **424**, 267–270. doi:10.1016/S0014-5793(98)00188-4
- Ding Y, Avramova Z, Fromm M (2011) The *Arabidopsis* trithorax-like factor ATX1 functions in dehydration stress responses via ABA-dependent and ABA-independent pathways. *The Plant Journal* **66**, 735–744. doi:10.1111/j.1365-3113X.2011.04534.x
- Diretto G, Tavazza R, Welsch R, Pizzichini D, Mourgues F, Papacchioli V, Beyer P, Giuliano G (2006) Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. *BMC Plant Biology* **6**, 13. doi:10.1186/1471-2229-6-13
- Diretto G, Al-Babili S, Tavazza R, Papacchioli V, Beyer P, Giuliano G (2007) Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. *PLoS ONE* **2**, e350. doi:10.1371/journal.pone.0000350
- Dong H, Deng Y, Mu J, Lu Q, Wang Y, Xu Y, Chu C, Chong K, Lu C, Zuo J (2007) The *Arabidopsis* *Spontaneous Cell Death1* gene, encoding a ζ -carotene desaturase essential for carotenoid biosynthesis, is involved in chloroplast development, photoprotection and retrograde signalling. *Cell Research* **17**, 458–470. doi:10.1038/cr.2007.51
- Du H, Wang N, Cui F, Li X, Xiao J, Xiong L (2010) Characterisation of the beta-carotene hydroxylase gene *DSM2* conferring drought and oxidative stress resistance by increasing xanthophylls and abscisic acid synthesis in rice. *Plant Physiology* **154**, 1304–1318. doi:10.1104/pp.110.163741
- Fang J, Chai C, Qian Q, Li C, Tang J, Sun L, Huang Z, Guo X, Sun C, Liu M, Zhang Y, Lu Q, Wang Y, Lu C, Han B, Chen F, Cheng Z, Chu C (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. doi:10.1111/j.1365-3113X.2008.03411.x
- Fassett RG, Coombes JS (2011) Astaxanthin: a potential therapeutic agent in cardiovascular disease. *Marine Drugs* **9**, 447–465. doi:10.3390/md9030447

- Fraser PD, Enfissi EM, Halket JM, Truesdale MR, Yu D, Gerrish C, Bramley PM (2007) Manipulation of phytoene levels in tomato fruit: effects on isoprenoids, plastids and intermediary metabolism. *The Plant Cell* **19**, 3194–3211. doi:10.1105/tpc.106.049817
- García-Limones C, Schnäbele K, Blanco-Portales R, Luz Bellido M, Caballero JL, Schwab W, Muñoz-Blanco J (2008) Functional characterisation of FaCCD1: a carotenoid cleavage dioxygenase from strawberry involved in lutein degradation during fruit ripening. *Journal of Agricultural and Food Chemistry* **56**, 9277–9285. doi:10.1021/jf801096t
- Giorio G, Stigliani AL, D'Ambrosio C (2008) Phytoene synthase genes in tomato (*Solanum lycopersicum* L.) – new data on the structures, the deduced amino acid sequences and the expression patterns. *FEBS Journal* **275**, 527–535. doi:10.1111/j.1742-4658.2007.06219.x
- Giuliano G, Al-Babili S, von Lintig J (2003) Carotenoid oxygenases: cleave it or leave it. *Trends in Plant Science* **8**, 145–149. doi:10.1016/S1360-1385(03)00053-0
- Giuliano G, Tavazza R, Diretto G, Beyer P, Taylor MA (2008) Metabolic engineering of carotenoid biosynthesis in plants. *Trends in Biotechnology* **26**, 139–145. doi:10.1016/j.tibtech.2007.12.003
- Goff SA, Klee HJ (2006) Plant volatile compounds: sensory cues for health and nutritional value? *Science* **311**, 815–819. doi:10.1126/science.1112614
- Gomez-Roldan V, Feras S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. *Nature* **455**, 189–194. doi:10.1038/nature07271
- Grini PE, Thorstensen T, Alm V, Vizcay-Barrena G, Windju SS, Jorstad TS, Wilson ZA, Aalen RB (2009) The ASH1 HOMOLOG 2 (ASHH2) histone H3 methyltransferase is required for ovule and anther development in *Arabidopsis*. *PLoS ONE* **4**, e7817. doi:10.1371/journal.pone.0007817
- Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET, Yan J, Buckler ES (2008) Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science* **319**, 330–333. doi:10.1126/science.1150255
- Havaux M, Niyogi KK (1999) The violaxanthin cycle protects plants from photo-oxidative damage by more than one mechanism. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 8762–8767. doi:10.1073/pnas.96.15.8762
- Hill GE (2000) Energetic constraints on expression of carotenoid-based plumage coloration. *Journal of Avian Biology* **31**, 559–566. doi:10.1034/j.1600-048X.2000.310415.x
- Hofmann E, Wrench PM, Sharples FP, Hiller RG, Welte W, Diederichs K (1996) Structural basis of light harvesting by carotenoids: peridinin-chlorophyll-protein from *Amphidinium carterae*. *Science* **272**, 1788–1791. doi:10.1126/science.272.5269.1788
- Howitt CA, Cavanagh CR, Bowerman AF, Cazzonelli C, Rampling L, Mimica JL, Pogson BJ (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. doi:10.1007/s10142-009-0121-3
- Isaacson T, Ronen G, Zamir D, Hirschberg J (2002) Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of beta-carotene and xanthophylls in plants. *The Plant Cell* **14**, 333–342. doi:10.1105/tpc.010303
- Isaacson T, Ohad I, Beyer P, Hirschberg J (2004) Analysis *in vitro* of the enzyme CRTISO establishes a poly-*cis*-carotenoid biosynthesis pathway in plants. *Plant Physiology* **136**, 4246–4255. doi:10.1104/pp.104.052092
- Jackson H, Braun CL, Ernst H (2008) The chemistry of novel xanthophyll carotenoids. *American Journal of Cardiology* **101**, S50–S57. doi:10.1016/j.amjcard.2008.02.008
- Jayaraj J, Devlin R, Punja Z (2008) Metabolic engineering of novel ketocarotenoid production in carrot plants. *Transgenic Research* **17**, 489–501. doi:10.1007/s11248-007-9120-0
- Johnson EJ (2002) The role of carotenoids in human health. *Nutrition in Clinical Care* **5**, 56–65. doi:10.1046/j.1523-5408.2002.00004.x
- Kim J, DellaPenna D (2006) Defining the primary route for lutein synthesis in plants: the role of *Arabidopsis* carotenoid beta-ring hydroxylase CYP97A3. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 3474–3479. doi:10.1073/pnas.0511207103
- Kim SY, He Y, Jacob Y, Noh YS, Michaels S, Amasino R (2005) Establishment of the vernalisation-responsive, winter-annual habit in *Arabidopsis* requires a putative histone H3 methyl transferase. *The Plant Cell* **17**, 3301–3310. doi:10.1105/tpc.105.034645
- Kirk J, Tilney-Bassett R (1978) Proplastids, etioplasts, amyloplasts, chromoplasts and other plastids. In 'The plastids: their chemistry, structure, growth and inheritance'. (Eds ST Kirk, RA Tilney-Bassett) pp. 217–239. (Elsevier: Amsterdam)
- Ko JH, Mitina I, Tamada Y, Hyun Y, Choi Y, Amasino RM, Noh B, Noh YS (2010) Growth habit determination by the balance of histone methylation activities in *Arabidopsis*. *EMBO Journal* **29**, 3208–3215. doi:10.1038/emboj.2010.198
- Krawczyk S, Britton G (2001) A study of protein-carotenoid interactions in the astaxanthin-protein crustacyanin by absorption and Stark spectroscopy; evidence for the presence of three spectrally distinct species. *Biochimica et Biophysica Acta* **1544**, 301–310. doi:10.1016/S0167-4838(00)00242-9
- Krinsky NI, Johnson EJ (2005) Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine* **26**, 459–516. doi:10.1016/j.mam.2005.10.001
- Leffingwell JC (2002) Saffron. *Leffingwell Reports* **2**, 1–6.
- Lewinsohn E, Sitrit Y, Bar E, Azulay Y, Ibdah M, Meir A, Yosef E, Zamir D, Tadmor Y (2005) Not just colours – carotenoid degradation as a link between pigmentation and aroma in tomato and watermelon fruit. *Trends in Food Science & Technology* **16**, 407–415. doi:10.1016/j.tifs.2005.04.004
- Li L, Van Eck J (2007) Metabolic engineering of carotenoid accumulation by creating a metabolic sink. *Transgenic Research* **16**, 581–585. doi:10.1007/s11248-007-9111-1
- Li L, Paolillo DJ, Parthasarathy MV, Dimuzio EM, Garvin DF (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. doi:10.1046/j.1365-3113x.2001.01008.x
- Li F, Vallabhaneni R, Wurtzel ET (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. doi:10.1104/pp.107.111120
- Li F, Vallabhaneni R, Yu J, Rocheford T, Wurtzel ET (2008b) The maize phytoene synthase gene family: overlapping roles for carotenogenesis in endosperm, photomorphogenesis and thermal stress tolerance. *Plant Physiology* **147**, 1334–1346. doi:10.1104/pp.108.122119
- Li Z, Ahn TK, Avenso TJ, Ballottari M, Cruz JA, Kramer DM, Bassi R, Fleming GR, Keasling JD, Niyogi KK (2009a) Lutein accumulation in the absence of zeaxanthin restores non-photochemical quenching in the *Arabidopsis thaliana* *npq1* mutant. *The Plant Cell* **21**, 1798–1812. doi:10.1105/tpc.109.066571
- Li Z, Wakao S, Fischer BB, Niyogi KK (2009b) Sensing and responding to excess light. *Annual Review of Plant Biology* **60**, 239–260. doi:10.1146/annurev.arplant.58.032806.103844
- Lindgren LO, Stalberg KG, 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. doi:10.1104/pp.102.017053

- López-Ráez JA, Kohlen W, Charnikhova T, Mulder P, Undas AK, Sergeant MJ, Verstappen F, Bugg TD, Thompson AJ, Ruyter-Spira C, Bouwmeester H (2010) Does abscisic acid affect strigolactone biosynthesis? *New Phytologist* **187**, 343–354. doi:10.1111/j.1469-8137.2010.03291.x
- Losey J, Ives A, Harmon J, Ballantyne F, Brown C (1997) A polymorphism maintained by opposite patterns of parasitism and predation. *Nature* **388**, 269–272. doi:10.1038/40849
- Lu S, Li L (2008) Carotenoid metabolism: biosynthesis, regulation and beyond. *Journal of Integrative Plant Biology* **50**, 778–785. doi:10.1111/j.1744-7909.2008.00708.x
- Lu S, Van Eck J, Zhou X, Lopez AB, O'Halloran DM, Cosman KM, Conlin BJ, Paolillo DJ, Garvin DF, Vrebalov J, Kochian LV, Kupper H, Earle ED, Cao J, Li L (2006) The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of beta-carotene accumulation. *The Plant Cell* **18**, 3594–3605. doi:10.1105/tpc.106.046417
- Maass D, Arango J, Wust F, Beyer P, Welsch R (2009) Carotenoid crystal formation in *Arabidopsis* and carrot roots caused by increased phytoene synthase protein levels. *PLoS ONE* **4**, e6373. doi:10.1371/journal.pone.0006373
- Marin E, Nussaume L, Quesada A, Gonneau M, Sotta B, Huguency P, Frey A, Marion-Poll A (1996) Molecular identification of zeaxanthin epoxidase of *Nicotiana plumbaginifolia*, a gene involved in abscisic acid biosynthesis and corresponding to the ABA locus of *Arabidopsis thaliana*. *EMBO Journal* **15**, 2331–2342.
- Matusova R, Rani K, Verstappen FW, Franssen MC, Beale MH, Bouwmeester HJ (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobancha* spp. are derived from the carotenoid pathway. *Plant Physiology* **139**, 920–934. doi:10.1104/pp.105.061382
- McGraw KJ, Toomey MB (2010) Carotenoid accumulation in the tissues of zebra finches: predictors of integumentary pigmentation and implications for carotenoid allocation strategies. *Physiological and Biochemical Zoology* **83**, 97–109. doi:10.1086/648396
- McGraw K, Nolan P, Crino O (2006a) Carotenoid accumulation strategies for becoming a colourful house finch: analyses of plasma and liver pigments in wild moulting birds. *Functional Ecology* **20**, 678–688. doi:10.1111/j.1365-2435.2006.01121.x
- McGraw KJ, Crino OL, Medina-Jerez W, Nolan PM (2006b) Effect of dietary carotenoid supplementation on food intake and immune function in a songbird with no carotenoid coloration. *Ethology* **112**, 1209–1216. doi:10.1111/j.1439-0310.2006.01280.x
- Meier S, Tzfadia O, Vallabhaneni R, Gehring C, Wurtzel ET (2011) A transcriptional analysis of carotenoid, chlorophyll and plastidial isoprenoid biosynthesis genes during development and osmotic stress responses in *Arabidopsis thaliana*. *BMC Systems Biology* **5**, 77. doi:10.1186/1752-0509-5-77
- Moran NA, Jarvik T (2010) Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science* **328**, 624–627. doi:10.1126/science.1187113
- Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56**, 165–185. doi:10.1146/annurev.arplant.56.032604.144046
- Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 333–359. doi:10.1146/annurev.arplant.50.1.333
- Ohmiya A, Kishimoto S, Aida R, Yoshioka S, Sumitomo K (2006) Carotenoid cleavage dioxygenase (CmCCD4a) contributes to white colour formation in *chrysanthemum* petals. *Plant Physiology* **142**, 1193–1201. doi:10.1104/pp.106.087130
- Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Improving the nutritional value of golden rice through increased pro-vitamin A content. *Nature Biotechnology* **23**, 482–487. doi:10.1038/nbt1082
- Park H, Kreunen SS, Cuttriss AJ, DellaPenna D, Pogson BJ (2002) Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation and photomorphogenesis. *The Plant Cell* **14**, 321–332. doi:10.1105/tpc.010302
- Pfundel EE, Renganathan M, Gilmore AM, Yamamoto HY, Dilley RA (1994) Intrathylakoid pH in isolated pea chloroplasts as probed by violaxanthin de-epoxidation. *Plant Physiology* **106**, 1647–1658.
- Pogson BJ, Albrecht V (2011) Genetic dissection of chloroplast biogenesis and development: an overview. *Plant Physiology* **155**, 1545–1551. doi:10.1104/pp.110.170365
- Pogson B, McDonald KA, Truong M, Britton G, DellaPenna D (1996) *Arabidopsis* carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. *The Plant Cell* **8**, 1627–1639.
- Pogson BJ, Niyogi KK, Bjorkman O, DellaPenna D (1998) Altered xanthophyll compositions adversely affect chlorophyll accumulation and non-photochemical quenching in *Arabidopsis* mutants. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 13324–13329. doi:10.1073/pnas.95.22.13324
- Pogson BJ, Rissler HM, Frank HA (2005) The roles of carotenoids in photosystem II of higher plants. In 'Photosystem II: the light-driven water: plastoquinone oxidoreductase'. (Eds T Wydrzynski, K Satoh) pp. 515–537. (Springer-Verlag: Dordrecht, The Netherlands)
- Pogson BJ, Woo NS, Forster B, Small ID (2008) Plastid signalling to the nucleus and beyond. *Trends in Plant Science* **13**, 602–609. doi:10.1016/j.tplants.2008.08.008
- Polívka T, Frank HA (2010) Molecular factors controlling photosynthetic light harvesting by carotenoids. *Accounts of Chemical Research* **43**, 1125–1134. doi:10.1021/ar100030m
- Qin G, Gu H, Ma L, Peng Y, Deng XW, Chen Z, Qu LJ (2007) Disruption of phytoene desaturase gene results in albino and dwarf phenotypes in *Arabidopsis* by impairing chlorophyll, carotenoid and gibberellin biosynthesis. *Cell Research* **17**, 471–482. doi:10.1038/cr.2007.40
- Qin X, Coku A, Inoue K, Tian L (2011) Expression, subcellular localisation and cis-regulatory structure of duplicated phytoene synthase genes in melon (*Cucumis melo* L.). *Planta* **234**, 737–748. doi:10.1007/s00425-011-1442-8
- Rani K, Zwanenburg B, Sugimoto Y, Yoneyama K, Bouwmeester HJ (2008) Biosynthetic considerations could assist the structure elucidation of host plant produced rhizosphere signalling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants. *Plant Physiology and Biochemistry* **46**, 617–626. doi:10.1016/j.plaphy.2008.04.012
- Rockholm DC, Yamamoto HY (1996) Violaxanthin de-epoxidase. *Plant Physiology* **110**, 697–703. doi:10.1104/pp.110.2.697
- Rodríguez-Concepción M (2010) Supply of precursors for carotenoid biosynthesis in plants. *Archives of Biochemistry and Biophysics* **504**, 118–122.
- Rodríguez-Suárez C, Atienza SG, Pistón F (2011) Allelic variation, alternative splicing and expression analysis of *Psy1* gene in *Hordeum chilense* Roem. et Schult. *PLoS ONE* **6**, e19885. doi:10.1371/journal.pone.0019885
- Rodríguez-Villalón A, Gas E, Rodríguez-Concepción 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. doi:10.1111/j.1365-3113.2009.03966.x
- Römer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM (2000) Elevation of the pro-vitamin A content of transgenic tomato plants. *Nature Biotechnology* **18**, 666–669. doi:10.1038/76523
- Rossel JB, Wilson IW, Pogson BJ (2002) Global changes in gene expression in response to high light in *Arabidopsis*. *Plant Physiology* **130**, 1109–1120. doi:10.1104/pp.005595
- Sandmann G (1991) Light-dependent switch from formation of poly-*cis* carotenes to all-*trans* carotenoids in the *Scenedesmus* mutant *C-6D*. *Archives of Microbiology* **155**, 229–233. doi:10.1007/BF00252205

- Saze H (2008) Epigenetic memory transmission through mitosis and meiosis in plants. *Seminars in Cell & Developmental Biology* **19**, 527–536. doi:10.1016/j.semcdb.2008.07.017
- Schemske DW, Bradshaw HD, Jr (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences of the United States of America* **96**, 11910–11915. doi:10.1073/pnas.96.21.11910
- Schwab W, Davidovich-Rikanati R, Lewinsohn E (2008) Biosynthesis of plant-derived flavor compounds. *The Plant Journal* **54**, 712–732. doi:10.1111/j.1365-313X.2008.03446.x
- Schwartz SH, Tan BC, Gage DA, Zeevaart JA, McCarty DR (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. *Science* **276**, 1872–1874. doi:10.1126/science.276.5320.1872
- Schwartz SH, Tan BC, McCarty DR, Welch W, Zeevaart JAD (2003) Substrate specificity and kinetics for VP14, a carotenoid cleavage dioxygenase in the ABA biosynthetic pathway. *Biochimica et Biophysica Acta* **1619**, 9–14.
- Schwartz SH, Qin X, Loewen MC (2004) The biochemical characterisation of two carotenoid cleavage enzymes from *Arabidopsis* indicates that a carotenoid-derived compound inhibits lateral branching. *Journal of Biological Chemistry* **279**, 46940–46945. doi:10.1074/jbc.M409004200
- Seo M, Koshiba T (2002) Complex regulation of ABA biosynthesis in plants. *Trends in Plant Science* **7**, 41–48. doi:10.1016/S1360-1385(01)02187-2
- Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY (1999) Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. *The Plant Journal* **20**, 401–412. doi:10.1046/j.1365-313x.1999.00611.x
- Simkin AJ, Schwartz SH, Auldrige M, Taylor MG, Klee HJ (2004) The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles beta-ionone, pseudoionone and geranylacetone. *The Plant Journal* **40**, 882–892. doi:10.1111/j.1365-313X.2004.02263.x
- Stahl W, Sies H (2007) Carotenoids and flavonoids contribute to nutritional protection against skin damage from sunlight. *Molecular Biotechnology* **37**, 26–30. doi:10.1007/s12033-007-0051-z
- Strack D, Fester T (2006) Isoprenoid metabolism and plastid reorganisation in arbuscular mycorrhizal roots. *New Phytologist* **172**, 22–34. doi:10.1111/j.1469-8137.2006.01837.x
- Tan BC, Schwartz SH, Zeevaart JA, McCarty DR (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. doi:10.1073/pnas.94.22.12235
- Tan BC, Joseph LM, Deng WT, Liu L, Li QB, Cline K, McCarty DR (2003) Molecular characterisation of the *Arabidopsis* 9-*cis* epoxycarotenoid dioxygenase gene family. *The Plant Journal* **35**, 44–56. doi:10.1046/j.1365-313X.2003.01786.x
- Taylor M, Ramsay G (2005) Carotenoid biosynthesis in plant storage organs: recent advances and prospects for improving plant food quality. *Physiologia Plantarum* **124**, 143–151. doi:10.1111/j.1399-3054.2005.00509.x
- Thorstensen T, Grini PE, Aalen RB (2011) SET domain proteins in plant development. *Biochimica et Biophysica Acta* **1809**, 407–420.
- Tian L, Musetti V, Kim J, Magallanes-Lundback M, DellaPenna D (2004) The *Arabidopsis* LUT1 locus encodes a member of the cytochrome P450 family that is required for carotenoid epsilon-ring hydroxylation activity. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 402–407. doi:10.1073/pnas.2237237100
- Toledo-Ortiz G, Huq E, 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. doi:10.1073/pnas.0914428107
- Tonhosolo R, D'Alexandri FL, de Rosso VV, Gazarini ML, Matsumura MY, Peres VJ, Merino EF, Carlton JM, Wunderlich G, Mercadante AZ, Kimura EA, Katzin AM (2009) Carotenoid biosynthesis in intraerythrocytic stages of *Plasmodium falciparum*. *The Journal of Biological Chemistry* **284**, 9974–9985. doi:10.1074/jbc.M807464200
- Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyoizuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**, 195–200. doi:10.1038/nature07272
- Van Norman JM, Sieburth LE (2007) Dissecting the biosynthetic pathway for the *bypass1* root-derived signal. *The Plant Journal* **49**, 619–628. doi:10.1111/j.1365-313X.2006.02982.x
- Van Norman JM, Frederick RL, Sieburth LE (2004) *BYPASS1* negatively regulates a root-derived signal that controls plant architecture. *Current Biology* **14**, 1739–1746. doi:10.1016/j.cub.2004.09.045
- Verma SK, Bordia A (1998) Antioxidant property of saffron in man. *Indian Journal of Medical Sciences* **52**, 205–207.
- Vishnevetsky M, Ovadis M, Vainstein A (1999) Carotenoid sequestration in plants: the role of carotenoid-associated proteins. *Trends in Plant Science* **4**, 232–235. doi:10.1016/S1360-1385(99)01414-4
- Vogel JT, Walter MH, Gialvalisco P, Lytovchenko A, Kohlen W, Charnikhova T, Simkin AJ, Goulet C, Strack D, Bouwmeester HJ, Fernie AR, Klee HJ (2010) *SICCD7* controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. *The Plant Journal* **61**, 300–311. doi:10.1111/j.1365-313X.2009.04056.x
- Walter MH, Floss DS, Strack D (2010) Apocarotenoids: hormones, mycorrhizal metabolites and aroma volatiles. *Planta* **232**, 1–17. doi:10.1007/s00425-010-1156-3
- Wei J, Xu M, Zhang D, Mi H (2010) The role of carotenoid isomerase in maintenance of photosynthetic oxygen evolution in rice plant. *Acta Biochimica et Biophysica Sinica* **42**, 457–463. doi:10.1093/abbs/gmq044
- Welsch R, Beyer P, Huguency P, Kleinig H, von Lintig J (2000) Regulation and activation of phytoene synthase, a key enzyme in carotenoid biosynthesis, during photomorphogenesis. *Planta* **211**, 846–854. doi:10.1007/s004250000352
- Welsch R, Maass D, Voegel T, Dellapenna D, 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. doi:10.1104/pp.107.104828
- Welsch R, Wust F, Bar C, Al-Babili S, 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. doi:10.1104/pp.108.117028
- Yu F, Fu A, Aluru M, Park S, Xu Y, Liu H, Liu X, Foudree A, Nambogga M, Rodermeier S (2007) Variegation mutants and mechanisms of chloroplast biogenesis. *Plant, Cell & Environment* **30**, 350–365. doi:10.1111/j.1365-3040.2006.01630.x
- Yu B, Lydiat DJ, Young LW, Schafer UA, Hannoufa A (2008) Enhancing the carotenoid content of *Brassica napus* seeds by downregulating lycopene epsilon cyclase. *Transgenic Research* **17**, 573–585. doi:10.1007/s11248-007-9131-x
- Yu Q, Ghisla S, Hirschberg J, Mann V, Beyer P (2011) Plant carotene *cis-trans* isomerase CRTISO: a new member of the FAD(RED)-dependent flavoproteins catalysing non-redox reactions. *Journal of Biological Chemistry* **286**, 8666–8676. doi:10.1074/jbc.M110.208017
- Zhu C, Bai C, Sanahuja G, Yuan D, Farre G, Naqvi S, Shi L, Capell T, Christou P (2010) The regulation of carotenoid pigmentation in flowers. *Archives of Biochemistry and Biophysics* **504**, 132–141.