Genetic manipulation of carotenoid pathway in higher plants

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Food security in developing countries has both the quantity and quality components. The major identified malnutritions are iron > iodine > vitamin A. Each year, more than one million vitamin-A deficiency (VAD)-associated childhood deaths occur. According to the World Health Organization, as many as 230 million children are at the risk of clinical or subclinical VAD, which can largely be prevented. VAD makes children vulnerable to infections and worsens the course of many infections. VAD is also the single most important cause of blindness among children in developing countries; about 0.5 million per year. β-carotene, which naturally occurs as coloured pigment in plants, is a dietary precursor of vitamin A. VAD-associated risks can be reduced to a greater extent by regular intake of β-carotene-rich food. Besides, some carotenoids have also been found to function as antioxidants that reduce risk of certain cancers and help regulate the immune system. Recent researches, therefore, have been focused to elevate β-carotene content, especially in crop plants. Elucidation of the carotenoid biosynthetic pathway in plants, identification of enzymes and cloning of genes involved in the regulation of carotenoid biosynthesis led to production of transgenic plants with increased carotenoid content. This article briefly reviews the carotenoid biosynthesis pathway, cloning of carotenogenic genes and genetic manipulation of carotenoid biosynthesis in plants, with special emphasis on β-carotene.

CAROTENOIDS are a large class of isoprenoid-derived pigments that are synthesized *de novo* by all photosynthetic and many non-photosynthetic organisms. In plants, the carotenoid pigments are essential components of photosynthetic membranes and assist in harvesting light energy, function as photoprotectants and antioxidants, serve as precursors for biosynthesis of plant-growth regulator abscisic acid and protect the photosynthetic apparatus by quenching the harmful reactive oxygen species that are produced by overexcitation of chlorophyll[1–3]. A role of carotenoids in the prevention of lipid peroxidation has also been suggested[4]. Carotenoids are also exploited as colouring agents, furnishing distinctive yellow, orange and red colours to flowers and fruits to attract pollinating insects and animals involved in seed dispersal. The colours provided by the pigments are of important agronomic value in many horticultural crops. Carotenoids are mainly C_{40} isoprenoids, which consist of eight isoprene units. The polyene chain in carotenoids contains up to 15 conjugated double bonds, a feature that is responsible for their characteristic absorption spectra and specific photochemical properties[5,6].

Besides their indispensable and prominent role in plants, carotenoids also play an important role in human health and nutrition. Vertebrates do not synthesize carotenoids and depend on dietary carotenoid sources for making their retenoids, such as retinal (the main visual pigment), retinol (vitamin A) and retenoic acid (a substance controlling morphogenesis). The main precursor of retenoids is β-carotene, also called provitamin A, which contains two unsubstituted beta-ionone rings at the two ends of the molecule. β-carotene deficiency in human diet causes symptoms ranging from night-blindness to those of xerophthalmia and keratomalacia, leading to total blindness. Furthermore, vitamin-A deficiency exacerbates afflictions like diarrhoea, respiratory diseases and childhood diseases such as measles[7,8]. Human diet supplemented with carotenoids, lycopene and β-carotene has been shown to be beneficial in reducing chronic conditions related to coronary heart diseases (CHD), certain cancers and macular degeneration[9]. It is estimated that 124 million children worldwide are deficient in vitamin A, and UNICEF advocates that improved vitamin-A nutrition could prevent 1–2 million deaths annually among children aged one to four years[10].

Recent researches have focused upon manipulation of carotenoid content and composition in crop plants to improve their nutritional value for human consumption. Although the sequence of biochemical reactions that constitute the pathway of carotenoid biosynthesis in plants was well-established by mid-1960s, genes and cDNAs encoding nearly all enzymes of the pathway have been identified, sequenced and their products characterized only in the last few years. Thus, the enormous progress in cloning of carotenogenic genes has opened up the possibility of genetic manipulation of carotenoid biosynthetic pathway in plants.

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Carotenoid biosynthetic pathway

Carotenoids of higher plants are C_{40} tetraterpenes biosynthesized from the central isoprenoid pathway[11] (Figure 1). They are formed from a C_5 building block isopentenyl pyrophosphate (IPP) that is the common precursor of all isoprenoid compounds. IPP, in turn, is formed from three molecules of acetyl-CoA via 3-hydroxy-3-methyl glutaryl-CoA (HMG-CoA) and mevalonic acid (MVA). Before chain elongation begins, IPP isomerises to its allylic isomer, dimethylallyl pyrophosphate (DMAPP). DMAPP is the initial, activated substrate for formation of long-chain polyisoprenoid compounds. DMAPP condenses with a molecule of IPP to give the C_{10} compound, geranyl pyrophosphate. Further addition of two IPP units produces the C_{20} geranyl geranyl pyrophosphate (GGPP), a common precursor of other essential biosynthetic pathways. Two molecules of GGPP react tail-to-tail to form phytoene, the first C_{40} hydrocarbon in the biosynthetic sequence. Phytoene undergoes a series of four desaturation reactions that result in the formation of the first phytofluene and then, in turn, zeta-carotene (**z**carotene), neurosporene and lycopene. These desaturation reactions introduce a series of carbon–carbon double bonds that constitute the chromophore in carotenoid pigments, and they transform the colourless phytoene into pink-coloured lycopene (Figure 2). The linear, symmetrical lycopene then cyclizes to yield carotenes with two types of rings – **b** and **e** rings. The mechanism involved in the formation of **b** and **e** rings has been dealt with in detail by Britton *et al.*[6]. Cyclization of lycopene into carotene is a branch point in carotenoid biosynthesis. **b**carotene, with two **b** rings (Figure 2), is an essential end-product that serves as a precursor for several other carotenoids in plants like xanthophylls. **a**carotene, with one **b** ring and one **e** ring, is the immediate precursor of lutein, the predominant carotenoid pigment in the photosynthetic membranes of green plants.

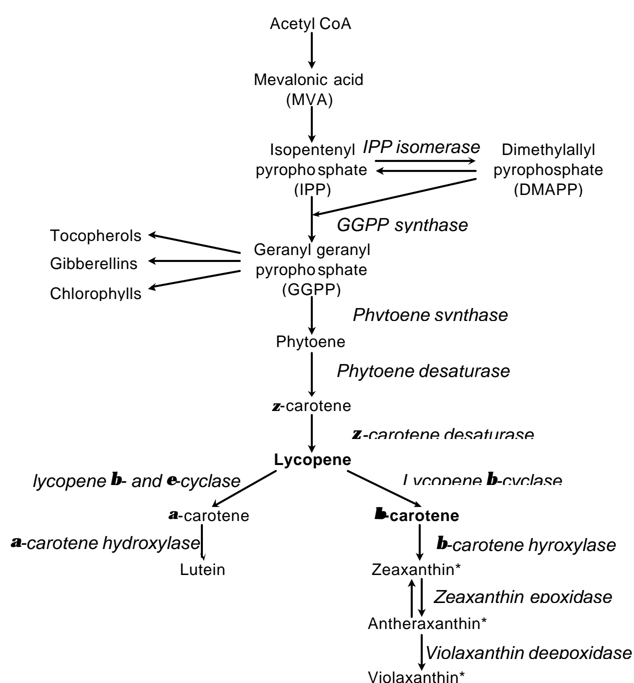

Figure 1. Carotenoid biosynthetic pathway. Isopentenyl pyrophosphate is the common precursor of all isoprenoid compounds. Formation of phytoene from two molecules of geranyl geranyl pyrophosphate is the first committed step in carotenoid biosynthesis. Compounds marked * are xanthophylls.

In bacteria and plastids of plants, formation of IPP proceeds via an alternative pathway, referred to as 1-deoxyxylulose-5-phosphate (DOXP) pathway. Not all of the reaction steps are elucidated to date. The starting substrates of the DOXP pathway are glyceraldehyde-3-phosphate (GA-3-P) and pyruvate. Initially, a C_2-unit from pyruvate is condensed to GA-3-P to form DOXP. DOXP is further converted to 2-C-methyl-D-erythritol-4-P, which is the branching point for independent routes to IPP and DMAPP[12].

The rapid progress in the study of carotenoid biosynthetic pathway in plants can perhaps be attributed most of all, to the pioneering work on carotenogenesis in bacterial systems and in well-chosen plant experimental systems (pepper and daffodil chromoplasts). Earlier work of Marrs[13] and Armstrong *et al.*[14] genetically defined the pathway and determined the sequences of genes encoding the enzymes of carotenoid biosynthesis in the photosynthetic bacterium *Rhodobacter capsulatus*. Thereafter, Misawa *et al.*[15] reported the sequences and functions of the products of the carotenogenic genes in the bacterium *Erwinia uredovora*. The pathway in *Erwinia herbicola* was also genetically dissected. These bacterial carotenogenic genes have proven to be indispensable tools in elucidation of the pathway in plants. Studies on carotenoids biosynthesis in cyanobacteria have also been useful models for carotenogenesis in plants. Gene-cloning approaches like heterologous hybridization, differential cDNA screening, transposon tagging, screening of expression cDNA libraries with antibodies, visual screening of cDNA and genomic libraries by colour complementation (the different colours displayed by colonies of *Escherichia coli* that accumulate carotenoids) have been used for cloning genes involved in carotenoid biosynthesis in

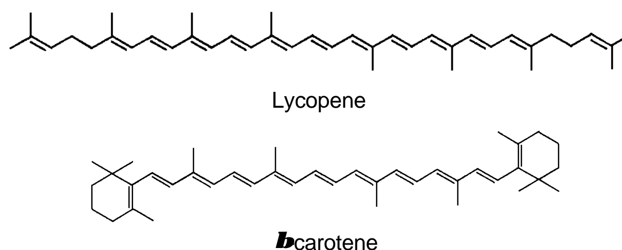

Figure 2. Chemical structures of lycopene and **b**carotene.

plants. The genes and major enzymes involved in carotenoid pathway are discussed in the following section.

Genes and enzymes of carotenoid biosynthesis

IPP isomerase

IPP isomerase catalyses the formation of DMAPP from IPP, a reversible isomerization reaction. cDNAs for IPP isomerase have been identified in *Clarkia brewerii*[16], *Arabidopsis* and lettuce[17]. Two distinct cDNAs for this enzyme, *Ipp1* and *Ipp2* (GenBank accession numbers U47324 and U49259), have been identified in *Arabidopsis*. While *Ipp2* polypeptide sequence has an N-terminal extension that has been suggested to target this enzyme to the chloroplast, *Ipp1* lacks the N-terminal extension, suggesting a cytosolic localization. As yet, no more than two different cDNAs or genes have been identified for this enzyme in any plant. Although IPP isomerase would seem to be an unlikely candidate for the controlling or regulating step in carotenoid biosynthesis, it has been found that the activity of this enzyme in *E. coli* is a limiting step for carotenoid production. *E. coli* engineered with different plant, algal or yeast IPP isomerase cDNAs showed enhanced accumulation of carotenoid pigments[18,19]. A possibility that IPP isomerase activity might also limit biosynthesis of carotenoids and other isoprenoids in plants, remains to be established.

GGPP synthase

GGPP synthase, a multifunctional enzyme, catalyses the formation of GGPP by sequential and linear addition of three molecules of IPP to one molecule of DMAPP. Antibodies against GGPP synthase purified from *Capsicum annuum* (pepper) chromoplasts have been used to clone the corresponding cDNA from an expression library made from ripening-fruit mRNA[20]. In *Arabidopsis*, five different cDNA or genomic clones with substantial sequential similarity to the pepper GGPP synthase have been identified, of which, two cDNAs *Ggps5* and *Ggps1* showed GGPP synthase activity by functional complementation in *E. coli*[21–24]. *Ggps1* contains an N-terminal extension of 76 amino acids that has been suggested to target this enzyme to the chloroplast. However, the specific roles and subcellular locations of various *Arabidopsis* GGPP synthases have not been yet ascertained.

Phytoene synthase

Phytoene synthase (PSY) converts two molecules of GGPP into phytoene and it is the first dedicated enzyme of the carotenoid biosynthesis pathway. In ripening fruits of tomato, transcription of PSY was found to be up-regulated[25]. A cDNA clone TOM5 showing significant homology to bacterial phytoene synthase gene (*crtB*) has been identified in tomato[14]. Transgenic tomato plants expressing a 5′ segment of TOM5 in antisense orientation showed significant accumulation of GGPP in fruits, and decreased levels of carotenoids in flowers and fruits, indicating that TOM5 clone encodes phytoene synthase[26]. While fruits of antisense TOM5 plants showed 97% reduction in carotenoid levels, leaf carotenoids remained unaltered, suggesting that a second gene may be active in somatic tissues. An mRNA encoding active phytoene synthase (Psy2), with lower homology to TOM5, has been identified in tomato leaves and its corresponding genomic sequence cloned[27]. Phytoene synthase genes have also been cloned from maize, pepper, *Arabidopsis* and *Narcissus*[21,28–30].

Phytoene desaturase and ζ-carotene desaturase

The four sequential desaturations from phytoene to lycopene are catalysed by two related enzymes in plants: phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS). The enzymes create additional double bonds in the C_{40} backbone of phytoene, thus converting this colourless carotenoid into a coloured compound. In bacteria and fungi, a single gene product (CRT1) catalyses these reactions. A soybean cDNA encoding PDS was cloned using a heterologous probe derived from *Synechococcus* PDS gene[31]. The tomato PDS cDNA was cloned by a similar approach and its identity was confirmed by expression in *E. coli*, which resulted in the formation of ζ-carotene[32]. Pepper PDS cDNA, isolated using antibodies raised against the purified protein, showed high homology to soybean and tomato PDS genes and its overexpression in *E. coli* resulted in the synthesis of the active PDS protein[33]. cDNAs encoding ZDS have also been identified in *Arabidopsis* and pepper. Both these PDS and ZDS clones isolated so far in plants have N-terminal transit peptides sequences for plastid targetting.

Lycopene β-cyclase

Lycopene β-cyclase catalyses the formation of the bicyclic β-carotene from lycopene in plants and cyanobacteria. This enzyme introduces two β-rings at the ends of the linear lycopene molecule. cDNA encoding lycopene β-cyclase has been cloned from *C. annuum* and its corresponding gene was found to be constitutively expressed during fruit development. A role for this enzyme in the specific rechannelling of linear carotenoids into β-cyclic carotenoids in ripening fruits of *C. annuum* is suggested[34]. The cDNA which encodes lycopene β-cyclase (crtL) was cloned from tomato and tobacco and functionally expressed in *E. coli*[35]. Unlike enzymes involved in early steps of carotenoid pathway, mRNA levels of crtL

were found to decrease in ripening fruits of tomato at 'breaker stage'. This transcriptional down-regulation of lycopene β-cyclase leads to accumulation of lycopene in tomato during ripening.

Some of the plant-originated genes involved in plant carotenoid biosynthesis are given in Table 1. Other enzymes involved in the conversion of β-carotene and α-carotene to xanthophyll pigments, such as hydroxylases, β-C-4-oxygenase, epoxidase, de-epoxidase and epoxy-carotenoid cleavage enzymes have been studied and their cDNAs or genomic sequences have been identified[17].

Genetic manipulation of carotenoid biosynthesis

The elucidation of carotenoid biosynthesis pathway in plants and cloning of genes involved in the control and regulation of the pathway provide possibilities for genetic manipulation of carotenoid content and composition in plants. In the past few years, several molecular approaches have been used for carotenoid production in heterologous microorganisms. Non-carotenogenic yeast, *Candida utilis* has been genetically engineered using four carotenogenic genes to synthesize lycopene, β-carotene and astaxanthin[36]. Metabolic engineering of early mevalonate pathway to increase supply of terpenoid precursors has also led to increased carotenoid formation in heterologous microorganisms[37].

In plants, different molecular approaches have been used to increase or modify the carotenoid content. However, a successful manipulation of the pathway for higher carotenoid levels faces three basic problems[38]. These are as follows:

- The synthesis of β-carotene is induced by GGPP, a precursor of other essential metabolic pathways that lead to synthesis of vitamin E, gibberellic acid and chlorophylls. Redirection of GGPP towards carotenoid biosynthesis may lead to decrease in the synthesis of other compounds.
- Interference with the well-balanced regulatory mechanism of the pathway might occur.
- As carotenoids are highly lipophilic, product storage in the plants should be ensured.

Therefore, high carotenoid production should focus on increased precursor supply, maintaining the balance of interacting metabolic pathways and targetting of tissues that are capable of incorporating lipophilic molecules.

Transgenic plants with elevated β-carotene

Phytoene synthase (PSY) is a branching enzyme that directs substrates irreversibly to carotenoids. Hence, it has been the target in several genetic manipulation studies.

Table 1. Cloned genes for carotenoid biosynthesis enzymes in plants

Enzyme	Plant source	Gene	Clone type	GenBank accession number
IPP isomerase	*Arabidopsis thaliana*	*Ipp1*	cDNA	U47324
		Ipp2	cDNA	U49259
	Clarkia brewerii	*Ipp1*	cDNA	X82627
GGPP synthase	*A. thaliana*	*GGPS2*	cDNA	U44876
		GGPS3	cDNA	U44877
	Catharanthus roseus	*GGPS*	Genomic	X92893
	Capsicum annum	*GGPS*	cDNA	P80042
	Nicotiana tabacum	*GGPS1*	cDNA	AB041632
		GGPS2	cDNA	AB041633
Phytoene synthase	*A. thaliana*	*PSY*	cDNA	AY056287
	C. annum	*PSY*	cDNA	X68017
	Helianthus annus	*PSY*	cDNA	AJ308385
		PSY	cDNA	AJ304825
	Lycopersicon esculentum	*PSY1*	Genomic	X60441
		PSY1	cDNA	M84744
	Narcissus pseudonarcissus	*PSY*	cDNA	X78814
Phytoene desaturase	*C. annum*	*PDS*	cDNA	X68058
	Glycine max	*PDS*	cDNA	M64704
	Hordeum vulgare	*PDS*	cDNA	AY062039
	L. esculentum	*PDS*	Genomic	U64919
	Zea mays	*PDS*	cDNA	U37285
Zeta-carotene desaturase	*L. esculentum*	*ZDS*	cDNA	AF195507
	Z. mays	*ZDS*	cDNA	AF047490
Lycopene beta-cyclase	*A. thaliana*	*LCY*	Genomic	AF117256
		LCY	cDNA	AY091396
	L. esculentum	*LCY*	cDNA	AF254793
		LCY	cDNA	X86452
	N. pseudonarcissus	*LCY*	cDNA	X98796
	Z. mays	*PS-1*	Genomic	AY206862

Expression of antisense RNA to the PSY gene (*Psy1*) of tomato was found to reduce the accumulation of carotenoids in fruits by 97%, without noticeable effect on carotenoids in leaf tissue[39]. However, the levels of gibberellins and other isoprenoids were perturbed in these plants[40]. The constitutive overexpression of PSY led to substantial increase in carotenoid accumulation even in plant tissues that do not normally produce carotenoid pigments, as in tobacco[41] and rice[42]. Overexpression of PSY in tomato resulted in carotenoid-rich seed coats, cotyledons and hypocotyls[40]. However, plants were reduced in stature because of changes in gibberellic acid due to competition for prenyl pyrophosphates by both pathways. This work illustrates how problems arise when interfering with a well-balanced metabolism[43].

However, the genetic manipulation of rape seeds (*Brassica napus*) using a bacterial PSY gene (*crtB*) to increase carotenoid content to high levels was a success. *crtB* was overexpressed in a seed-specific manner and the protein product targetted to the plastid. The resultant embryos from the transgenic plants were visibly orange, and mature seeds contained up to a 50-fold increase in carotenoids, α and β-carotenes being the predominant ones. In seeds, concentrations of carotenoids of more than 1 mg per g fresh weight accumulated, yielding an oil with 2 mg per g carotenoids. When other metabolites of the isoprenoid pathway were examined in seeds, levels of tocopherols and chlorophylls decreased significantly compared to non-transgenic controls. Although in lower light conditions the transgenic seedlings appeared pink to orange, in the field where light intensity is higher, 10–12-day-old transgenic seedlings look similar to normal plants. Additionally, the fatty acyl composition was altered, with the transgenic seed having relatively higher percentage of the 18:1 (oleic acid) component and a decreased percentage of the 18:2 (linoleic acid) and 18:3 (linolenic acid) components. The alteration in fatty-acid composition could not be accounted for and needs further experimentation[44].

Manipulation of desaturation activity in plants also produced transgenic plants with increased β-carotene contents. The phytoene desaturase gene of *E. uredovora*, the 5′ region of which was fused to the sequence for the transit peptide of pea Rubisco small unit, was expressed in tobacco plants under the control of CaMV 35S promoter. The chimeric gene product was targetted into chloroplast, and radioactive labelling study using the leaves demonstrated enhanced activity for the synthesis of β-carotene[45]. *E. uredovora* PDS gene (*crt1*) was introduced into tomato under the control of CaMV 35S promoter by *Agrobacterium*-mediated transformation. β-carotene in *crt1*-transformed tomato fruit increased threefold, up to 45% of the total carotenoid content, which was unpredicted. Since the end-product of CRT1 activity is lycopene, Misawa *et al.*[45] predicted an increase in lycopene. Determination of gene expression by RT–PCR showed that endogenous PDS, ZDS and lycopene β-cyclase were concurrently upregulated, converting the lycopene to β-carotene[46]. In a similar study, transgenic tomato lines expressing *E. uredovora* PDS gene (*crt1*) under the control of CaMV 35S promoter showed significant increase in β-carotene content (threefold), but a reduction in total carotenoids. The contents of biosynthetically related isoprenoids, including tocopherols, vitamin K, ubiquinones and plastoquinones were unaltered by manipulation of the carotenoid pathway[47].

DNA constructs aimed at upregulating (OE construct) the expression of lycopene β-cyclase gene in a fruit-specific fashion, were introduced via *Agrobacterium*-mediated transformation in tomato. Fruits of three transformants showed a significant increase in β-carotene content and displayed different colour phenotypes, from orange to red, depending upon the lycopene/β-carotene ratio[48]. Both lycopene β-cyclase and β-carotene hydroxylase genes were overexpressed under the control of fruit-specific phytoene desaturase promoter in tomato. Transgene and protein expression studies by semi-quantitative RT–PCR, Western blotting and enzyme assays showed that fruits of the transformants had increased levels of β-carotene, β-cryptoxanthin and zeaxanthin. The carotenoid levels of leaves remained unaltered[49].

A different approach, using antisense gene constructs, can also be utilized for increasing β-carotene in plants. β-hydroxylase enzyme catalyses the hydroxylation of the β rings of β-carotene to form xanthophylls. Antisense construct for β-hydroxylase gene can be introduced into plants to block the conversion of β-carotene to xanthophylls, thereby increasing accumulation of β-carotene. Expression of the antisense β-hydroxylase in *Arabidopsis* resulted in a 22% increase in β-carotene[50].

A major breakthrough in the genetic manipulation of carotenoid biosynthesis in crop plants to increase their nutritional value, has been made in rice. Although half of the world's population consumes rice daily and depends on it as staple food, rice is a poor source of many essential micronutrients and vitamins. Rice endosperm contains neither β-carotene nor C_{40} carotenoid precursors. To improve the nutritional content of rice, especially in terms of provitamin-A content, genetic engineering was chosen as a means to introduce the ability to make β-carotene into rice endosperm tissue. Immature rice endosperm synthesizes GGPP, the C_{20} isoprenoid precursor necessary for C_{40} carotenoid biosynthesis. Burkhardt *et al.*[42] transformed japonica rice cv. Tapei 309 by microprojectile bombardment with a cDNA coding for PSY from daffodil (*Narcissus pseudonarcissus*) under the control of endosperm-specific promoter. Transgenic rice plants expressed an active daffodil enzyme and accumulated lycopene in rice endosperm. This was the first time that the possibility of engineering a critical step in provitamin-A biosynthesis in a non-photosynthetic plant tissue was demonstrated.

Ye *et al.*[51] used *Agrobacterium*-mediated transformation to introduce plant PSY (*N. pseudonarcissus*) under the control of CaMV 35S promoter and a bacterial PDS gene (*E. uredovora*) under the control of endosperm-specific glutelin (*Gt1*) promoter constructed in a single plasmid into rice plants. The PSY cDNA contained a 5′-sequence coding for a functional transit peptide and the PDS gene contained the transit peptide (tp) sequence of pea Rubisco small subunit. Although this plasmid should have directed the formation of lycopene in the endosperm, analysis of seeds from transformants by photometry and high performance liquid chromatography showed no detectable amounts of lycopene. Instead, β-carotene, and to some extent, lutein and zeaxanthin were formed, suggesting that the lycopene α- and β-cyclases and the hydroxylases are either constitutively expressed in normal rice endosperm or induced upon lycopene formation. Co-transformation with a lycopene β-cyclase-containing plasmid increased the β-carotene content of the rice endosperm to a maximum level of 1.6 μg per g dry weight. It is not yet clear whether lines producing β-carotene or lines possessing additionally zeaxanthin and lutein would be nutritious, because the latter have been implicated in the maintenance of healthy macula within the retina.

In addition to the improvement of nutritional content and composition of crop plants, genetic manipulation of carotenoid biosynthesis has also been used to increase stress tolerance, herbicide resistance, photoprotection and synthesize novel carotenoids in crop plants. Exposure of plants to high-light conditions and UV radiation leads to photooxidative stress, changes in plant morphology, peroxidation of membrane lipids and DNA dimerization[52]. Xanthophyll cycle, which involves the synthesis of β-carotene-derived xanthophylls zeaxanthin, antheraxanthin, violaxanthin and their interconversion, has a key photoprotective role in plants[53] and is therefore a promising target for genetic engineering to enhance stress tolerance. Overexpression of the *chyB* gene that encodes β-carotene hydroxylase (an enzyme in the zeaxanthin biosynthetic pathway) in *Arabidopsis* causes an increase in size of the xanthophyll cycle pool[54]. The plants were more tolerant to conditions of high light and high temperature, as shown by reduced necrosis, reduced production of the stress indicator anthocyanin and reduced lipid peroxidation. Transformation of tobacco with a bacterial β-carotene hydroxylase gene under the control of a constitutive promoter showed a rapid and increased production of zeaxanthin[55]. UV-exposed, transformed plants maintained a higher biomass and a greater amount of photosynthetic pigments than the control.

Bleaching herbicides like norflurazon, fluridone, flurtamone and fluorochloridone are known to inhibit plant PDS. However, bacterial phytoene desaturases (*crtI*) are not inhibited. Herbicide-resistant crop plants have been developed using the bacterial PDS gene. Expression of *E. uredovora* PDS gene (*crtI*) in tobacco produced transgenic plants showing multiple resistance to these bleaching herbicides[56]. While levels of β-carotene and its xanthophyll derivatives were increased, the level of lutein was reduced in modified plants, but the total amount of carotenoids was unaffected.

Novel carotenoid pigments can be synthesized in plants using genes from different organisms. Astaxanthin, a red pigment is of considerable economic value. Astaxanthin is synthesized via canthaxanthin. The enzyme β-carotene ketolase catalyses the conversion of β-carotene to canthaxanthin via echinenone. The carotenoid biosynthetic pathway in tobacco was modified to produce astaxanthin[57]. cDNA of the gene *crt*O from the alga *Haematococcus pluvialis*, encoding β-carotene ketolase, was transformed to tobacco under the regulation of the tomato PDS promoter. Chromoplasts in the nectary tissue of transgenic plants accumulated astaxanthin and other keto carotenoids, changing the colour of the nectary from yellow to red. This may serve as the platform technology for future genetic manipulations of pigmentation of fruits and flowers of horticultural and floricultural importance.

Conclusion and prospects

Crops that would serve as better sources of essential micronutrients and vitamins will help in improving human health and nutritional deficiency-related diseases, especially in developing countries where poverty limits food availability. Over the years, classical breeding approaches have been primarily targetted to increase crop productivity, disease resistance, stress tolerance etc., while the nutrient content and composition of crops have often been overlooked. Studies on the biochemical and molecular mechanisms of biosynthetic pathways of nutrients and vitamins in plants and cloning of genes involved in these pathways have led to production of transgenic plants with improved nutritional value. Grains of 'Golden Rice' that produce provitamin A, exemplify the best that agricultural biotechnology can offer to the nutritional security in the world.

Genetic manipulations of carotenoid biosynthesis, especially for increased provitamin-A content, face many challenges. Shunting more of the common precursor GGPP into carotenoid production might result in a decrease in other compounds whose synthesis is dependant upon GGPP. This can be avoided by targetting tissues in which primary metabolism is rather low, such as fruits and seeds, with organ-specific promoters. Another major obstacle in precisely modifying carotenoid metabolism by genetic engineering is our limited knowledge of how expression of endogenous carotenogenic genes is regulated in higher plants. Although genetic engineering for higher β-carotene content in food plants has been targetted towards addressing vitamin-A deficiency, the concentrations of β-carotene reached in some transgenic plants

were low. However, tremendous increase in β-carotene concentration in modified rape seeds yielding a β-carotene-enriched oil is an encouraging milestone in this direction.

Production of various carotenoids, other than β-carotene, in transgenic plants could provide additional health benefits by reducing the risk of cancer, cardiovascular disease and age-related macular degeneration. Moreover, excess dietary β-carotene, in contrast to excess dietary vitamin A, has no harmful effects, making transgenic plants with increased β-carotene a safe and effective means of vitamin-A delivery. Possibilities of engineering the pathways for many of the 13 essential vitamins into plants have also been explored. The model plant *Arabidopsis* has already been successfully engineered to synthesize vitamin E. Current efforts are also centred on improving mineral content of crop plants. Iron deficiency is one of the leading nutritional disorders in the world today. Development of golden rice that has high levels of iron in grain storage protein ferritin, is a step forward.

Today there are some 800 million food insecure people mainly in the developing countries. About 30,000 people, half of them children, die everyday due to hunger and malnutrition. Iron deficiency anaemia (IDA) among 3.7 billion people (mainly women) and annual blindness among 0.5 million children due to vitamin-A deficiency are stark realities that mankind faces today. Under such a scenario, novel techniques like molecular-genetic modification may play a prominent role in future development of plant varieties that can produce more food and nutrients per unit area and time.

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