PLANT CAROTENOIDS: MOLECULAR GENETICS AND REGULATION

RASID, O A*; PARVEEZ, G K A*; HO, C L; RAVIGADEVI SAMBANTHAMURTHI* and NAPIS, S****

ABSTRACT

The potential health benefits of carotenoids as anti-cancer and antioxidant agents have recently been demonstrated. In particular, lycopene and β-carotene have lately been shown to be able to reduce the risk of chronic conditions of coronary heart disease, certain cancers and macular degeneration. The findings have led to rapid development in the field aimed at understanding the biosynthetic pathway and ultimately engineering the carotenoid content. This article reviews the recent progress made in the areas of molecular genetics and genetic engineering of plant carotenoids. The latest development in the regulatory mechanisms controlling the pathway is also highlighted. Finally, this review also highlights some recent progress made in oil palm carotenoid research, especially the molecular cloning of genes encoding key enzymes of the biosynthetic pathway and efforts to improve oil palm carotenoid content.

Keywords: carotenoids, biosynthesis, regulation, genetic engineering, oil palm.

Date received: 18 August 2008; **Sent for revision:** 9 January 2009; **Received in final form:** 22 April 2009; **Accepted:** 23 April 2009.

INTRODUCTION

Carotenoids are the most widespread group of pigments in nature. They are synthesized in a wide range of organisms including bacteria, fungi, algae and plants. In plants, about 600 different carotenoids have been identified. Carotenoids are 40-carbon isoprenoids that are derived from the central isoprenoid pathway. They contain polyene chains that may contain up to 15 conjugated double bonds. One or both ends of carotenoid molecules are cyclized. Carotenoids that contain one or more oxygen functions are known as xanthophylls. These properties are responsible for their colours and their essential roles in the photosynthetic reaction. They act as accessory light-harvesting pigments, which absorb light in the 450-570 nm region. They are also important for the assembly and stability of some of these light-harvesting complexes. Epoxidized carotenoids are essential to plants for protection from oxidative damage (Britton, 1995; Bartley and Scolnik, 1995; Armstrong and Hearst, 1996).

Carotenoids are also essential to human health. A number of carotenoids serve as pro-vitamin A which is important for development and the prevention of some diseases. Deficiency in vitamin A can cause severe health problems (West, 2002). Another interesting property of these compounds is their antioxidant activity, which has been suggested to potentially alleviate a number of chronic diseases. They also have anti-cancer properties. These properties have stimulated a huge interest in these compounds. Recent advances in the knowledge on the biosynthetic pathway have made the modification of the plant carotenoids feasible through genetic engineering. This review is to highlight the recent developments in the molecular genetics and genetic engineering of the plant carotenoid biosynthetic pathway. As oil palm is one of the richest sources of carotenoids, it is also our aim to provide readers with information on the recent developments in oil palm carotenoid work.

* Malaysian Palm Oil Board,
P. O. Box 10620,
50720 Kuala Lumpur,
Malaysia.
E-mail: omar@mpob.gov.my

** Department of Cell and Molecular Biology,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 Serdang, Selangor,
Malaysia.

BIOSYNTHETIC PATHWAY OF PLANT CAROTENOIDS

Carotenoids, as well as other isoprenoids, are built from the 5-carbon (5C) compound isopentenyl

pyrophosphate (IPP). In plants, there are two sources of IPP, plastid and cytosolic (*Figure 1*). In the cytosolic pathway, IPP is formed from three acetyl-CoA molecules via 3-hydroxy-3-methyl glutaryl-CoA, mevalonic acid (MVA), mevalonic acid 5-phosphate (MVAP) and mevalonic acid 5-diphosphate (MVAPP). Hence, this pathway is called the mevalonic pathway. IPP is converted into its isomer, dimethylallyl pyrophosphate (DMAPP), which acts as the substrate for the formation of sesquiterpenes (C15) and triterpenes (C30) such as sterols (Laule *et al.*, 2003; Hsieh and Goodman, 2005). The step is catalyzed by IPP isomerase (IDI). The condensation between IPP and the DMAPP molecule gives rise to the C10 compound, geranyl pyrophosphate (GPP). Further addition of two IPP units results in the formation of C20 geranylgeranyl pyrophosphate (GGPP) (Bramley, 2002; Cunningham, 2002; Frazer and Bramley, 2004).

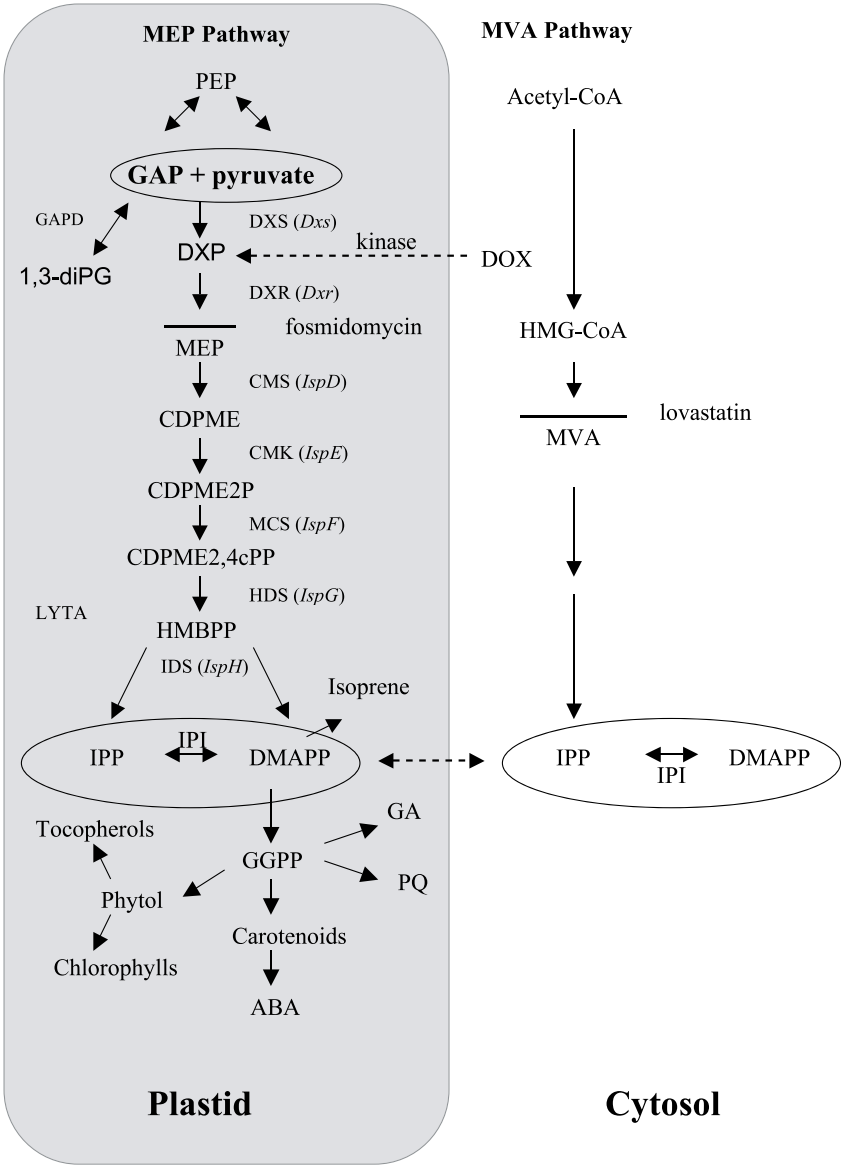

Figure 1. Schematic diagram depicting the formation of IPP via the MEP and MVA pathways in plants. The MEP pathway is localized in plastids, while the MVA pathway is localized in cytoplasm. Both MEP and MVA pathways occur in plants.

HMG-CoA = 3-hydroxy-3-methylglutaryl CoA
MVA = mevalonic acid
MVAP = mevalonic acid 5-phosphate
MVAPP = mevalonic acid 5-diphosphate
IPP = isopentenyl diphosphate
DMAPP = dimethylallyl diphosphate
FPP = farnesyl diphosphate

see overleaf

Mt = mitochondrion
UQ = ubiquinone
GA-3-P = glyceraldehyde 3-phosphate
DXP = 1-deoxy-D-xylulose-5-phosphate
MEP = 2-C-methyl-D-erythritol 4-phosphate
CDP-ME = 4-diphosphocytidyl-2-C-methyl-D-erythritol
CDP-ME2P = 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate
ME-2,4cPP = 2-C-methyl-D-erythritol 2,4-cyclodiphosphate
HMBPP = 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate
GGPP = geranylgeranyl diphosphate
GA = gibberellic acid
PQ = plastoquinone
ABA = abscisic acid.

Enzymes of the MVA pathway:
HMGS = HMG-CoA synthase
HMGR = HMG-CoA reductase
MVK = MVA kinase
PMK = MVAP kinase
MDD = MVAPP decarboxylase

Enzymes of the non-MVA pathway:
DXS = DXP synthase
DXR = DXP reductoisomerase
CMS = CDP-ME synthase
CMK = CDP-ME kinase
MCS = ME-2,4cPP synthase
HDS = HMBPP synthase
IDS = IPP/DMAPP synthase.

Notes: The names of their corresponding genes are indicated in parenthesis.
Adapted from Rodríguez-Concepción and Boronat (2002).

However, in plant plastids, the major source of IPP for carotenoid synthesis is formed via an alternative pathway referred to as the MEP (2-C-methyl-D-erythritol 4-phosphate) pathway. In contrast to the MVA pathway, the MEP pathway is localized in plastids. The pathway is important for the production of isoprenoids such as isoprene, carotenoids and plastoquinones. The MEP pathway was first elucidated in the early 1990s (Rohmer *et al.*, 1996; Lichtenthaler, 1999). The use of specific MVA and MEP pathway inhibitors and the *Arabidopsis* mutant in genes encoding 1-deoxy-D-xylulose 5-phosphate synthase (DXS), the first enzyme of the pathway, proved the role of the MEP pathway in plant isoprenoid biosynthesis. Application of a specific MEP pathway inhibitor, fosmidomycin, resulted in a reduction of lycopene content, whereas lovastatin, an MVA-specific inhibitor did not show any effect on lycopene accumulation (Elsenreich *et al.*, 2001; Rodríguez-Conceptión and Boronat, 2002). The *Arabidopsis CLA1* mutant, a knockout 1 of 3 *dxs* genes in *Arabidopsis*, exhibited a bleached phenotype. This phenotype could be rescued by application of deoxy-D-xylulose (DOX), a compound that can be phosphorylated to form deoxy-D-xylulose 5-phosphate (DXP). All of these evidence suggest that MEP pathway is the major source for plant isoprenoid biosynthesis in plastids (Lichtenthaler *et al.*, 1997; Bramley, 2002).

The pathway for the formation of IPP in plant plastids is schematically shown in *Figure 1*. The initial reaction of the pathway is the formation of 1-deoxyxylulose-5-phosphate (DXP) from pyruvate and glyceraldehyde-3-phosphate (GAP). This step is catalyzed by the enzyme DXP synthase (DXS). The formed DXP is converted to 2-C-methyl-D-erythritol 4-phosphate (MEP) by DXP reductoisomerase (DXR). Then MEP is further converted into isopentenyl pyrophosphate (IPP) and dimethylallyldiphosphate (DMAPP) via 4-diphosphocytidyl-2-C-methyl-D-erythritol (CDPME), 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate (CDPME2P), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (ME2,4cPP) and 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate (HMBPP). These steps are catalyzed by CDPME synthase (CMS), DPME kinase (CMK), ME2,4cPP synthase (MCS) and HMBPP synthase (HDS), respectively. The conversion of this final product into either IPP or DMAPP is catalyzed by IPP/DMAPP synthase (IDS) (Elsenreich *et al.*, 2001; Rodríguez-Concepción and Boronat, 2002).

The first committed step in the synthesis of carotenoids is the formation of phytoene (40-carbon compound) from two molecules of geranylgeranyl pyrophosphate (C20-GGPP). A general schematic diagram for the synthesis of phytoene and its derivatives in plants is given in *Figure 2*. The description and reviews of the pathway have been

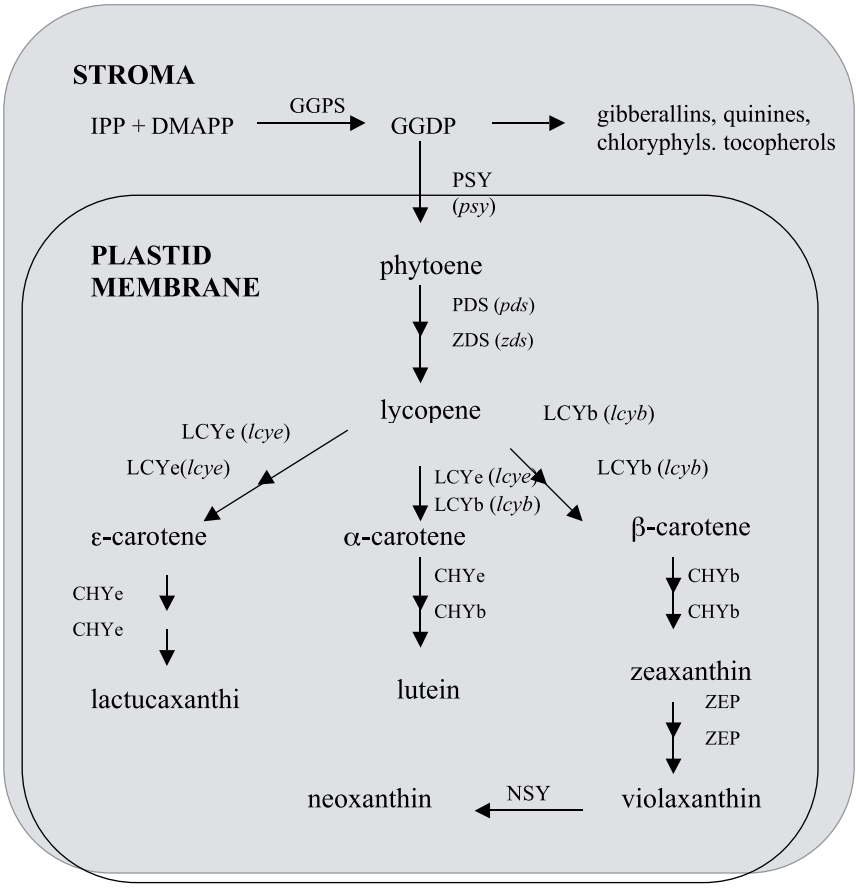

Figure 2. Schematic diagram depicting the carotenoid synthetic pathway in plants. The first committed step in plant carotenoid synthesis is the formation of phytoene from the condensation of two GGDP molecules. Phytoene is then converted into lycopene through a series of desaturation. In most plants, lycopene will be branched into α- and β-carotene. However in lettuce, most of the lycopene is converted into ε-carotene and ultimately lactucaxanthin.

PSY = phytoene synthase
PDS = phytoene desaturase
ZDS = zeta carotene desaturase
LCYb = lycopene β-cyclase
LCYe = lycopene ε-cyclase
CHYb = carotene β-hydroxylase
CHYe = carotene ε-hydroxylase
ZEP = zeaxanthin epoxidase
NSY = neoxanthin synthase.

Notes: The gene for each enzyme is given in the paranthesis next to the enzyme.
Adapted from Hirschberg *et al.,* (1997).

given in a number of publications (Cunningham and Gantt, 1998; Hirschberg, 2001; Frazer and Bramley, 2004). Phytoene is a colourless compound. The reaction for the formation of phytoene from GGPP is catalyzed by phytoene synthase (PSY). GGPP itself is derived from three molecules of isopentenyl pyrophosphate (IPP) and a DMAPP molecule. The sequential addition of three IPP molecules to a DMAPP molecule is catalyzed by GGPP synthase. In addition to its role as the immediate precursor to isoprenoid formation, GGPP also serves as the precursor to other compounds, including tocopherols, gibberellins, quinines and chlorophylls.

Phytoene is not a true pigment in that it is unable to absorb light at visible wavelengths. It undergoes four consecutive desaturation steps to form lycopene. Lycopene forms the main pigment in red tomato. In bacteria, these four desaturation steps are performed by a single enzyme, phytoene desaturase. In contrast, plants utilize two different desaturases to perform the reactions. The first two steps of the reaction are catalyzed by phytoene desaturase (PDS), while the latter two steps are catalyzed by (zeta) ζ–carotene desaturase (ZDS). As the number of double bonds increases, the carotenoids gain colour from pale yellow (ζ-carotene) to red (lycopene).

Cyclization of lycopene produces carotenoids with either two β-rings (β-carotene), or one β-ring and one ε-ring (α-carotene). Lycopene β-cyclase (LCYb) catalyzes the former reaction to produce β-carotene and its derivative xanthophylls (zeaxanthin, antheraxanthin, violaxanthin and neoxanthin). Alternatively, lycopene can be converted to α-carotene and its derivatives such as lutein by lycopene ε-cyclase (LCYe) and lycopene β-cyclase. LCYe first introduces an ε-ring to lycopene to produce δ-carotene. Then, LCYb adds the β-ring to the δ-carotene to produce β-ε-carotene (α-carotene). Thus, the formation of β-carotene requires only LCYb, whereas formation of α-carotene requires both LCYb and LCYe. The exception to this is found in lettuce. This plant accumulates a substantial amount of the carotenoid with two ε-rings, lactucaxanthin.

Zeaxanthin, an important carotenoid for photochemical quenching, is formed from hydroxylation of β-carotene. This reaction is catalyzed by β-carotene hydroxylase (CHYb). In addition to CHYb, formation of lutein also requires ε-carotene hydroxylase (CHYe) for the addition of the hydroxyl group to its ε-ring. Zeaxanthin will be further converted into violaxanthin through the introduction of an epoxy group into both of its β-rings. The conversion occurs via antheraxanthin as the intermediate and is catalyzed by zeaxanthin epoxidase (ZEP). The zeaxanthin formed from this pathway serves as the important precursor for abscisic acid biosynthesis.

REGULATION OF PLANT CAROTENOID BIOSYNTHESIS

Isolation of the first plant gene for carotenoids primarily relied on a bacterial heterologous probe. Exploitation of norflurazon, an inhibitor of phytoene desaturase (PDS), was successfully used to isolate a *Synechococus* mutant that was resistant to the inhibitor. The mutant was used to identify the *pds* gene based on its ability to confer resistance to the inhibitor (Chamovitz *et al.*, 1993). The gene was subsequently used as a probe to isolate the plant homologs (Scolnik and Bartley, 1993; Hirschberg, 2001).

The advent of molecular genetic techniques has further facilitated gene isolation, leading to functional complementation *in vivo*, characterization of recombinant enzymes and the creation of transgenic plants. These studies together have further improved our knowledge on carotenoid biosynthesis, its regulation and control, and the enzymes involved in the process. This recent progress has led to the isolation and characterization of genes or cDNAs encoding nearly all the enzymes for carotenoid biosynthesis (Cunningham and Gantt, 1998; Frazer and Bramley, 2004).

The amount and composition of carotenoids in green plastids are well conserved across plant species. They typically consist of lutein, β-carotene, violaxanthin, neoxanthin and zeaxanthin. These carotenoids account for most of the carotenoid pigments in the chloroplasts of many plants and algae. The synthesis and accumulation of these carotenoids are in concert with the developing chloroplast. The process thus must be highly regulated. However, the regulatory mechanism of the pathway is still poorly understood (Frazer and Bramley, 2004; Botella-Pavia and Rodríguez-Concepción, 2006). In contrast to leaves, the amount and composition of carotenoids in non-photosynthetic organs range broadly. The amount of carotenoids may vary from little or none, such as in any white flower, to quite substantial, such as in carrot. The pigments may include those that are common for photosynthesis such as the lutein of marigold flower petals or derivatives of chloroplast carotenoids such as capsanthin and capsorubin which are formed from violaxanthin in red pepper fruits (Cunningham, 2002). This clearly indicates that the chromoplast carotenoid pathway is highly regulated and the mechanisms of regulation must be distinct from those in the chloroplastic pathway (Bramley, 2002). Although the pathway is still not completely elucidated and understood, results from many recent studies have started to shed some light on the pathway.

IPP FORMATION

The regulation of carotenoid synthesis via the genes encoding the enzymes for the MEP pathway has been demonstrated in several studies. Expression modulation was demonstrated for the *dxs* gene in *Arabidopsis*. It was observed that the *CLA1* transcripts were especially abundant in developing seedlings, indicating the developmental modulation of the gene (Estevez *et al.*, 2000). Later, it was shown that in developing *Arabidopsis* seedlings, there was a correlation between *ipsH* expression and carotenoid accumulation (Botella-Pavia *et al.*, 2004). It was further demonstrated that transgenic *Arabidopsis* carrying an additional copy of the tomato *ispH* gene did not show any increase in the carotenoid level in etioplasts as compared to the wild type. However, light-grown transgenic plants showed an increase in their carotenoid content. Thus, in addition to supporting the role of light in regulating the MEP pathway, this finding also suggests the possible role of HDR in controlling the MEP pathway.

Besides regulation at the transcriptional level, post-transcriptional regulation has also been indicated for at least one of the enzymes in the MEP pathway. This was evident in a study using the *Arabidopsis clb6* mutant. The level of most genes in the MEP pathway was low in this mutant. Their

protein level was also low corresponding to their mRNA transcript level. However, the level of DXS protein did not correlate to its mRNA transcript. The DXS protein level was found to be 10 times higher in the mutant compared to the wild type during the early stage of plant development. This suggests that the post-transcriptional regulation of the protein is either by its translation or degradation (Guevara-Garcia *et al.*, 2005).

Although the knowledge in the regulation of the MEP pathway in plant carotenoid synthesis is limited, recent findings already suggest that the enzymes within the pathway are highly regulated and possibly have a regulatory role on the pathway. This was evident from the early studies on the enzymes involved in the pathway, especially the enzyme that catalyzes the first reaction, DXS. The expression of the *dxs* gene has been shown to be modulated during plant development. Pepper *dxs* was expressed at a higher level in the transition period of chloroplasts to chromoplasts (Bouvier *et al.*, 1998). Lois *et al.* (2000) also showed the organ-specific and developmental expression of tomato *dxs*. Its transcripts were abundant in young, developing and fully expanded leaves, inflorescences and stems. The transcripts also increased greatly during fruit ripening. In addition the limiting role of the enzyme in plant carotenoid biosynthesis was demonstrated by directly injecting DOX into green mature tomato fruits. The results indicate that DOX injection activated the accumulation of *dxs* and *psy* transcripts and eventually carotenoids in the tomato fruits. The possible regulatory role of *dxs* in plant carotenoid biosynthesis was further indicated in transgenic *Arabidopsis*. Overexpression of the *dxs* gene in this plant was accompanied by an increase in carotenoid accumulation (Estevez *et al.*, 2001).

The rate-limiting role of other enzymes in the MEP pathway is not clear. One of the possible candidates that may have a rate-limiting role within the MEP pathway is HDR. A strong up-regulation of *ispH* gene expression was shown to be correlated with carotenoid accumulation during tomato fruit development (Botella-Pavia *et al.*, 2004). This result may suggest some limiting role of the enzyme in the pathway. For DXR, the accumulation of carotenoids in ripening tomato fruits apparently did not require an increased level of *dxr* transcripts (Rodríguez-Concepción *et al.*, 2001). However, overexpression of *dxr* in peppermint was shown to result in an increase in carotenoid content (Mahmoud and Croteau, 2001). IPP isomerase (IPI) is very unlikely to have a regulatory role in plant carotenoids. However, *E. coli* engineered with IPP isomerase cDNAs from different plants, algae or yeast did indicate an enhanced accumulation in its carotenoid content (Kajiwara *et al.*, 1997; Sun *et al.*, 1998). This may indicate that this enzyme is rate-limiting to some extent in *E. coli*.

FORMATION OF LYCOPENE

As the enzyme that catalyzes the first committed step in the carotenoid biosynthetic pathway, phytoene sythase (PSY) has long been demonstrated to be highly regulated during the developmental stages of tomato fruits. It has also been postulated to be the rate-limiting enzyme and to substantially regulate the reaction flux. Early evidence to support this hypothesis came from the expression studies of the gene in ripening tomato fruits, *psy1.* A tomato fruit-specific PSY was shown to significantly increase at the breaker stage (Bartley *et al.*, 1992; Bartley and Scolnik, 1993). The increase in the *psy1* expression was followed by an increase in total carotenoid content in the fruits, mainly due to the accumulation of lycopene and β-carotene. In addition to *psy,* the levels of transcript for *pds* and *dxs* were also shown to increase during this transition stage (Frazer *et al.*, 1994; Lois *et al.*, 2000). These results clearly demonstrate that regulation of these three genes, and thus the accumulation of lycopene in tomato fruits occurred partly at the transcriptional level. Regulation by differential expression of the carotenoid genes was also illustrated in *Delta* and *Old-gold* tomato mutants. The *Delta* mutant accumulated δ-carotene as a result of an increased expression of *lcye* (Ronen *et al.*, 1999). In the *Old-gold* mutant, the up-regulation of one of the lycopene β-cyclase genes resulted in the accumulation of β-carotene (Ronen *et al.*, 2000). A similar transcriptional regulation of *psy* was also demonstrated during citrus fruit development (Ikoma *et al.*, 2001).

The regulation and rate-limiting role of PSY was also evident in transgenic tomato carrying an additional *psy* gene from the bacterium *Erwinia uredovoran* (Frazer *et al.*, 2002). The PSY activity in these transgenic plants was increased five-fold. This was accompanied by a two- to four-fold increase in the total carotenoid content. In addition, the flux control coefficients in these tomatoes were shown to reduce by half compared to untransformed fruits. In control plants, the flux control coefficient for PSY was about three times greater than other enzymes in the pathway. The value was equivalent to the regulatory sites of starch and sucrose synthesis. This finding strongly indicates the important role of PSY in regulating the flux. The introduction of an extra copy of *psy* from *E. uredovora* (*crtB*) into the transgenic plants resulted in less control of the enzyme on the pathway, allowing more carotenoids to accumulate.

In photosynthetic tissues, the gene has been shown to be modulated in response to different light intensities. For instance, in developing seedlings of mustard (*Sinapis alba* L.), the level of *psy* mRNA increases in light, but the levels of *pds* and *ggps* remain constant (von Lingtig *et al.*, 1997). It was

also shown that *psy* in the etioplasts is localized in the prolamella body and is inactive. The enzyme is activated upon illumination with light and relocalized into thylakoids (Welsch *et al.*, 2000).

FORMATION OF CYCLIC CAROTENOIDS AND XANTHOPHYLLS

The cyclization of lycopene into either carotenoids with one β-ring and one ε-ring, or with two β-rings, is a key branching point in plant carotenoid biosynthesis. This point has been suggested to play an important role in determining the composition of the carotenoid end-products. The regulation could be due to different substrate specificity or differential expression. In *Arabidopsis*, lycopene β-cyclase was found to efficiently add rings to both ends of the lycopene molecule. In contrast, lycopene ε-cyclase adds only a single ε-ring to this linear carotenoid. The presence of a ring at one end of the molecule somehow prevents the formation of a second ε-ring at the other end (Cunningham *et al.*, 1996). This inability restrains plants from accumulating carotenoids with two ε-rings. The exception to this is found in lettuce. This plant accumulates a substantial amount of a double ε-ring carotenoid (lactucaxanthin). The LCYe from this plant, which shares about 77% identity with *Arabidopsis* LCYe, was shown to efficiently add two ε-rings into lycopene (Cunningham and Gantt, 2001). The heterologous expression of its gene into *E. coli* resulted in 90% accumulation of bicyclic ε-carotene. By comparison, the expression of *Arabidopsis lcye* produced 98% accumulation of monocyclic ε-carotene (δ-carotene). Therefore, in addition to differential gene expression, the regulation of the carotenoid pathway could be partially due to the substrate specificity.

It has also been hypothesized that plant xanthophyll composition in photosynthetic tissues (zeaxanthin, antheraxanthin and violaxanthin) could be modulated through transcriptional regulation of the two cyclases (Hirschberg, 1999; Frazer and Bramley, 2004). It has been shown in *Arabidopsis* and tomato leaves that the ratio between the levels of *lcye* and *lcyb* mRNAs was increased five-fold in plants which were shifted from low to strong light. The difference in the mRNA ratio of these genes was accompanied by an increase in total xanthophyll content and a decrease in the ratio between lutein and xanthophylls. Conversely, the ratio was found to increase in plants under low light intensity (Hirschberg, 2001).

In ripening tomato fruits, the up-regulation of *dxs*, *psy* and *pds* was accompanied by down-regulation of both *lcyb* and *lcye* genes. The differential expression of these genes resulted in the accumulation of total carotenoids, particularly the formation of lycopene (Pecker *et al.*, 1996; Ronen *et al.*, 1999). Transcriptional regulation of the lycopene cyclase genes has also been demonstrated in transgenic plants with altered *lcyb* or *lcye* expression. Overexpression of *Arabidopsis lcyb* cDNA in tomato fruit has resulted in an increased expression of the gene and accumulation of β-carotene in the fruits (Rosati *et al.*, 2000).

MODIFICATION OF CAROTENOID CONTENTS IN PLANTS

The availability of genes and knowledge in the carotenoid pathway has opened up numerous possibilities for genetic modification aimed at improving the carotenoid content in plants, especially commercial crops. Carotenoid metabolic engineering has been accelerated within the past few years. Several significant achievements in the area have been reported recently (Ye *et al.*, 2000; Frazer *et al.*, 2002; Ducreux *et al.*, 2005; Paine *et al.*, 2005).

Due to the fact that PSY is the enzyme that catalyzes the first committed step in the plant carotenoid pathway, and is believed to be rate-limiting, it has been a preferred target for genetic manipulation especially in the earlier work. Fray *et al.* (1995) was the first to report the constitutive overexpression of the endogenous *psy* in transgenic tomato. The transgenic plants, especially the high expressors, were found to have a dwarf phenotype. It was suggested that this was due to the redirecting of the GGPP from the gibberellin pathway into carotenoid synthesis as indicated by an up to 30-fold reduction in gibberellin content. Furthermore, the growth of these plants was partially restored by exogenous gibberellin treatment. There was also a possibility of co-suppression as indicated by the reduced level in chlorophyll content in the transgenic plants.

In a later work, the transgene was retargeted into fruit tissues to overcome dwarfism, and a heterologous gene was used to overcome the co-suppression. Fruits of transgenic tomato plants carrying an extra copy of *E. uredovera psy* under the control of fruit-specific polygalacturonase were shown to have a five- to 10-fold increase in PSY activity. However, this increase was accompanied only by a two- to three-fold increase in the total carotenoid content, which was relatively low compared to the increase in the enzyme activity (Frazer *et al.*, 2002). It was speculated that this was due to a shift in the regulatory role to an enzyme located further downstream of the pathway.

A similar study was also carried out in *Brassica napus* (Shewmaker *et al.*, 1999). Interestingly, overexpression of the *E. uredovera psy* gene in this species resulted in a 50-fold increase in total carotenoid content, predominantly α-carotene,

β-carotene and phytoene. In another study, *E. uredovera crtB* was overexpressed in potato (Ducreux *et al.,* 2005). An increase in the total carotenoid content by about seven-fold, although relatively smaller compared to the increase in *B. napus,* was observed. These results indicate that modification of the *psy* gene in carotenoid-producing fruits may result in a shift in the regulatory control point within the pathway. However, seed-specific overexpression of an *Arabidopsis psy* substantially increased the total carotenoid content in the seeds of the transgenic plants (Lingren *et al.*, 2003). The β-carotene content was increased by up to 43-fold. The bigger increase in the carotenoid content in the latter case could be due to the use of a plant *psy*, as opposed to bacterial *psy* in the former studies. The bacterial *psy* may not exhibit effective protein-protein interaction compared to the plant *psy,* and thus limits the formation of the downstream carotenoids.

As DXS has been suggested to be another rate-limiting step for the whole isoprenoid synthesis, attempts have also been made to manipulate its genes in transgenic plants. Overexpression of the endogenous *dxs* gene in *Arabidopsis* resulted in an increase in the total carotenoid content by 112% to 131% relative to the wild-type (Estevez *et al*., 2001). The increase was shown to be due to an increase in the transcript and protein levels of *dxs*. In addition, increases in other MEP pathway end-products such as chlorophyll, (134%-142%), α-tocopherol (154%-142%) and ABA (259%-397%) were also observed. Enfissi *et al*. (2005) overexpressed bacterial *dxs* in transgenic tomato, using a fruit-specific promoter and a plastid-targeted sequence. The overexpression resulted in 1.6-fold increase in the total carotenoid content in the fruits of the transgenic plants. The results demonstrate the rate-limiting role of the IPI pool for the isoprenoid synthesis. The bacterial *dxs* gene has also been overexpressed in potato (Morris *et al*., 2006). Results show that the tuber carotenoid content was increased by seven-fold, largely attributed to an increase in phytoene level.

Attempts to genetically engineer plant carotenoid content using genes coding for other enzymes in the pathway have also been reported. Rosati *et al.* (2000) have overexpressed or down-regulated the *Arabidopsis* lycopene β-cyclase (*lcyb*) gene in tomato fruit. Transformed plants overexpressing *Arabidopsis lcyb* showed a significant increase in the β-carotene content. The total carotene content was also found to increase by two-fold in some of the transgenic lines. The introduction of an antisense copy of the gene resulted in a 50% reduction of *lcyb* expression. It was also observed that most of the resulting transformed plants had a slight increase in total carotenoid content. The increase was not accompanied by any major changes in the expression of the endogenous carotenoid genes. It was suggested that the increase was due to post-transcriptional regulation. An increase in β-carotene content in transgenic tomato fruits has also been observed by overexpressing *E. uredovora* phytoene desaturase (*crtI*) under the control of a constitutive promoter (Romer *et al.,* 2000). The overexpression of *crtI* resulted in an increase in the β-carotene content in the trangenic fruits by up to 45% of the total carotenoid content as compared to 14% in the control. However, the increase in β-carotene occurred at the expense of lycopene and total carotenoid contents. The total carotenoid content was shown to decrease by about 40% in a line producing the highest β-carotene content. It was suggested that this was due to feedback inhibition of the pathway.

One of the most successful stories of the genetic engineering work in plant carotenoids is the production of the famous Golden Rice (Ye *et al.,* 2000). The production of a detectable amount of carotenoids in the rice endosperm was achieved by overexpressing the phytoene synthase (*psy*) and carotene desaturase (*crtI*) genes from *E. uredovora*. The amount of carotenoids in the transgenic rice was rather low at about 1.6 $\mu g\ g^{-1}$. The low amount of carotenoid production in these transgenic plants has been a major hurdle in successfully extending the technology to the field, particularly for combating vitamin A deficiency (VAD). However, the success of the study has opened up the possibility of improving the pro-vitamin A content in the most important staple food of the South and Southeast Asians. Increasing the carotenoid content in rice to meet vitamin A requirements will reduce VAD incidence in the region significantly.

Efforts to improve Golden Rice by further increasing the amount of pro-vitamin A content has resulted in another recent breakthrough in the plant carotenoid genetic engineering programme (Paine *et al.,* 2005). It was hypothesized that the low amount of carotenoids in the first generation of Golden Rice was due to the rate-limiting PSY in the transgenic plants. In order to overcome this limitation, a group from Syngenta, USA, tested *psy* genes from different plant sources with the aim of finding a *psy* gene that can result in a higher content of carotenoids which can subsequently be used to increase the carotenoid content in rice. Results of the study suggest that *psy* from maize and rice gave higher amounts of carotenoids in the maize callus compared to *psy* from other tested sources. By overexpressing the maize *psy* in combination with *E. uredovora* carotenoid desaturase, the group successfully increased the amount of carotenoids in transgenic rice by 23-fold compared to the original Golden Rice. The second generation high carotene rice, designated Golden Rice 2, produced up to 37 $\mu g\ g^{-1}$ carotenoids with β-carotene contributing about 68% of the total carotenoid content.

The zeaxanthin level in our diet is very low. The only significant natural sources of zeaxanthin are some maize cultivars and special yellow/orange pepper varieties (Romer *et al.*, 2002). Thus, a number of attempts to increase the amount of zeaxanthin and other xanthophylls in food plants have been reported. A few of these studies have demonstrated some encouraging results. Dharmapuri *et al.* (2002) were able to increase the amount of zeaxanthin in transgenic tomato by overexpressing the lycopene β-cyclase and β-carotene hydroxylase. In potato, transgenic lines with higher zeaxanthin content (by up to 130-fold) were obtained by down-regulating the endogenous *zep* gene (Romer *et al.*, 2002). In addition, the down-regulation of the gene was accompanied by an unexpected increase in the total tuber carotenoid content.

These results clearly indicate that the plant carotenoid pathway is amenable to genetic engineering. This obviously provides opportunities for the improvement of the nutritional value of various food plants. It also opens up the possibility for the metabolic engineering of compounds further downstream in the carotenoid pathway, for which naturally accumulating mutants or genotypes are not currently available.

CAROTENOIDS IN OIL PALM

Oil palm is the most important economic crop in Malaysia. It has contributed prominently to the country's export revenues. Nevertheless, the industry faces a number of challenges, including land and labour shortages and competition from other vegetable oils. Therefore, proper strategies have to be put in place to ensure the sustainability of the industry in the future. One of the strategies is to produce high value-added products through genetic engineering (Cheah, 2000; Parveez *et al.*, 2000).

Crude palm oil is one of the richest natural resources of carotenoids. Palm oil from the current planting materials (DxP) contains 500-700 ppm carotenoids. This will provide great opportunities to further improve its superiority, value and quality by producing value-added products in its oil. The high content of carotenoids in oil palm clearly provides great advantages compared to other plants for genetic manipulation of the carotenoids. It can be specifically channeled to produce a large amount of a particular carotenoid. As lycopene is the most potent antioxidant, production of high lycopene transgenic oil palm has been one of the main targets of the oil palm genetic engineering programme at the Malaysian Palm Oil Board (MPOB) (Siti Nor Akmar *et al.*, 2001; Parveez *et al.*, 2003).

Reports on the biochemical studies as well as gene isolation for enzymes involved in carotenoid synthesis in oil palm are comparatively limited. Kaur and Sambanthamurthi (2000) carried out biochemical studies on carotenoids in oil palm fruits. They successfully developed an effective chromathography protocol for the separation of carotenoid components from the oil palm mesocarp. Their results also indicate that lycopene is only present in a negligible amount in the oil palm mesocarp tissues.

To date, the cDNAs coding for 1-deoxy-D-xylulose 5-phosphate synthase (Khemvong and Suvachittanont, 2005; GenBank Accession No. AY583783 and AY611205), 1-deoxy-D-xylulose 5-phosphate reductase (Khemvong and Suvachittanont, 2005; GenBank Accession No. AY583783 and AY611205), phytoene synthase (Wan Nur Syuhada, 2006; Rasid *et al.*, 2008), phytoene desaturase (Rasid *et al.*, 2006), zeaxanthin epoxidase (Rasid *et al.*, 2005), lycopene ε-cyclase (Rasid *et al.*, 2007) and lycopene β-cyclase (Rasid *et al.*, 2007) have been isolated and characterized. The level of these *cDNA* transcripts was found to be correlated to the accumulation of carotenoids in the developing oil palm fruits. For example, the expression of the two cyclases was shown to be correlated to the formation of α- and β-carotenes in oil palm. These results indicate that the total content and ratio of carotenoids in oil palm fruits are regulated, at least partly, at the transcriptional level.

Alpha-carotene and β-carotene are the two major components of oil palm carotenoids. These two carotenoids make up about 90% of the total carotenoid content in palm oil (Choo, 1995; Sambanthamurthi *et al.*, 2000b; Tay and Choo 2000; Sundram *et al.*, 2003). Other carotenoids found in palm oil are *cis-α*-carotene, phytoene and lycopene. The carotenoid content of oil palm fruits clearly indicates that lycopene is effectively converted into its derivatives, carotenes. The observation suggests that the two cyclases are very active in oil palm fruits. Therefore, an obvious strategy to increase the lycopene content is to block its conversion into carotenes (Sambanthamurti *et al.*, 2002; Rasid *et al.*, 2007). This can simply be carried out by introducing the antisense copy of the two cyclase genes in transgenic oil palms. The technique seems feasible, as the two genes have been shown to be at least partly regulated at the transcriptional level. Nevertheless, proper target is certainly required to ensure that there will be no detrimental effect on the growth of the transgenic oil palms produced.

In addition to modifications to the carotenoid components in the current planting materials, there is also a possibility to further increase the content of the carotenoids in oil palm. Studies on *Elaeis oleifera* (O), an oil palm species originating from South America, have shown that the species contains a higher content of carotenoids compared to *E. guineensis* (G). Some of these selected materials

were found to have total carotenoid content close to 4000 ppm (Choo *et al.*, 1997; Mohd Din *et al.*, 2000; 2004). These materials can be potentially introgressed into the current planting materials through breeding. However, this may be difficult to achieve as *E. oleifera* has very low oil content. Due to the imprecise nature of the technique, it may require several rounds of selection before the materials that retain both the high oil yield and carotenoid content can be obtained. Studies have shown that the progenies obtained from the OxG crosses were intermediate in their oil yield and carotenoid content (Choo *et al.*, 1997; Mohd Din *et al.*, 2000). Alternatively, the carotenoid content of current planting materials can potentially be increased by overexpressing the *psy* gene from *E. oleifera*. Although, the species has been shown to contain more carotenoids than *E. guineensis*, the percent components of their carotenoids are very similar. The higher carotenoid content could be due to a number of reasons. One possible explanation is a higher activity of *psy* in *E. oleifera* than in *E. guineensis*. Therefore, the use of *E. oleifera psy* may substantially increase the carotenoid accumulation in the transgenic oil palm. At present, a cDNA clone encoding the full coding sequence of *E. oleifera psy* has been obtained (unpublished data). The availability of the cDNA clone can facilitate the efforts to confirm the higher activity of *E. oleifera psy* in the near future.

CONCLUSION AND FUTURE PERSPECTIVES

Plants have been deemed to be potential renewable bioreactors. Oil palm, with an inherently high-yielding trait, is in an advantageous position for the application of genetic engineering. As oil palm is a perennial crop, concern for transgene instability is less for this crop. Once planted, the palms will remain in the field for 20-25 years.

The first generation transgenic technology emphasized improvements in agronomic traits such as herbicide and disease tolerance. Now, the focus has been widened to include the development of value-added products such as nutraceuticals and pharmaceuticals. An example of these products is carotenoids. The feasibility of manipulating plant carotenoid content has been demonstrated in a number of studies. In this respect, oil palm offers great advantages for exploitation. The high content of carotenoids in its fruits can be channeled towards the production of preferred carotenoid products.

Despite the progress, there is still an urgent need to gain further knowledge in a number of related areas. Better understanding on the regulatory mechanism of plant carotenoid biosynthesis will certainly be useful in developing strategies for genetic manipulation. It is not just the genes or enzymes that are directly involved in the pathway that need to be considered, but efforts should also include the regulatory sequences. The manipulation of regulatory sequences could eliminate the shift of the bottlenecks from one step to another, and could also result in an overall response in the pathway.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish this paper.

REFERENCES

ARMSTRONG, G A and HEARST, J E (1996). Genetics and molecular biology of carotenoid pigment biosynthesis. *FASEB Journal*, *10*: 228-237.

BARTLEY, G E and SCOLNIK, P A (1993). cDNA cloning, expression during development and genome mapping of PSY2, a second tomato gene encoding phytoene synthase. *The Journal of Biological Chemistry*, *268*: 25718-25721.

BARTLEY, G E and SCOLNIK, P A (1995). Plant carotenoids: pigments for photoprotection, visual attraction and human health. *The Plant Cell*, *7*: 1027-1038.

BARTLEY, G E; VIITANEN, P V; BACOT, K O and SCOLNIK, P A (1992). A tomato gene expressed during fruit ripening encodes an enzyme of the carotenoid biosynthesis pathway. *The Journal of Biological Chemistry*, *267*: 5036-5039.

BOTELLA-PAVIA, P; BESUMBES, O; PHILIPS, M A; CARRETERO-PALET, L; BORONAT, A and RODRIGUEZ-CONCEPTION, M (2004). Regulation of carotenoid bioysnthesis in plants; evidence for a key role of hydroxymethylbutenyl diphosphate reductase in controlling the supplyof plastidial isoprenoid precursors. *The Plant Journal*, *40*: 188-199.

BOTELLA-PAVIA, P and RODRIGUEZ-CONCEPTION, M (2006). Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum*, *126*: 369-381.

BOUVIER, F; BACKHAUS, R A and CAMARA, B (1998). Induction and control of chromoplast-specific carotenoid genes by oxidative stress. *The Journal of Biological Chemistry*, *273(46)*: 30652-30659.

BRAMLEY, P M (2002). Regulation of carotenoid formation during tomato fruit ropening and development. *J. Experimental Botany*, *53*: 2107-2113.

BRITTON, G (1995). Structure and properties of carotenoids in relation to function. *FASEB Journal*, *9*: 1551-1558.

CHAMOVITZ, D; SANDMAN, G and HIRSCHBERG, J (1993). Molecular and biochemical characterization of herbicide-resistant mutants of cyanobacteria reveals that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. *The Journal of Biological Chemistry*, *268*: 17384-17353.

CHEAH, S C (2000). Biotechnological strategies for improving plantation tree crop: the palm oil – a case study. *Proc. of the International Planters Conference*. p. 59-76.

CHOO, Y M (1995). Carotenoids from palm oil. *Palm Oil Developments No. 22*: 1-6.

CHOO, Y M; MA, A N and YAP, S C (1997). Carotenes, vitamin E and sterols in oils from *Elaeis giuneensis*, *Elaeis oleifera* and their hybrids. *Palm Oil Developments No. 27*: 1-9.

CUNNINGHAM, F X (2002). Regulation of carotenoid synthesis and accumulation in plants. *Pure and Applied Chemistry*, *74*: 1409-1417.

CUNNINGHAM, F X and GANTT, E (1998). Genes and enzymes of carotenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *49*: 557-583.

CUNNINGHAM, F X and GANTT, E (2001). One ring or two? Determination of ring number in carotenoids by lycopene ε-cyclase. *Proc. of the National Academy of Sciences USA*, *98*: 2905-2910.

CUNNINGHAM, F X; POGSON, B; SUN, Z; MCDONALD, K A; DELLAPENNA, D and GANTT, E (1996). Functional analysis of the β and ε lycopene cyclase enzymes of *Arabidopsis* reveals a mechanism for control of cyclic carotenoid formation. *The Plant Cell*, *8*: 1613-1626.

DHARMAPURI, S; ROSATI, C; PALLARA, P; AQUILQNI, R; BOUVIER, F; CAMARA, B and GIULIANO, G (2002). Metabolic engineering of xanthophyll content in tomato fruits. *FEBS Letters*, *519*: 30-34.

DUCREUX, L J M; MORRIS, A L.; HEDLEY, P E; SHEPERD, T; DAVIES, H V; MILLAM, S and TAYLOR, M A (2005). Metabolic engineering of high carotenoid potato tubers containing enhanced levels b-carotene and lutein. *J. Experimental Botany*, *56*: 81-89.

ELSENREICH, W; ROHDICH, F and BACHER, A (2001). Deoxyxylulose phosphate pathway to terpenoids. *Trends in Plant Science*, *6*: 78-84.

ENFISSI, E M; FRASER, P D; LOIS, L M; BORONAT, A; SCHUCH, W and BRAMLEY, P M (2005). Metabolic engineering of the mevalonate and non-mevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. *Plant Biotechnology Journal*, *3*: 17-27.

ESTEVEZ, J M; CANTERO, A; ROMERO, C; KAWAIDE, H; JIMENEZ, L F; KUZUYAMA, T; SETO, H; KAMIYA, Y and LEON, P (2000). Analysis of the expression of CLA1, a gene that encodes the 1-deoxyxylulose 5-phosphate synthase of the 2-C-methyl-D-erythritol-4-phosphate pathway in Arabidopsis. *Plant Physiology*, *124*: 95-103.

ESTEVEZ, J M; CANTERO, A; REINDL, A; REICHLER, S and LEON, P (2001). 1-Deoxyxylulose 5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. *The Journal of Biological Chemistry*, *276*: 22901-22909.

FRAY, R G; WALLACE, A; FRAZER, F D; VALERO, D; HADDEN, P; BRAMLEY, P M and GREISON, D (1995). Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes cause dwarfism by redirecting metabolites from the gibberellin pathway. *The Plant Journal*, *8*: 693-701.

FRAZER, F D and BRAMLEY, P M (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research*, *43*: 228-265.

FRAZER, P D; TRUESDALE, M R; BIRD, C R; SCHUCH, W and BRAMLEY, P M (1994). Carotenoid biosynthesis during tomato fruit development. *Plant Physiology*, *105*: 405-413.

FRAZER, F D; ROMER, S; SHIPTON, C A; MILLS, P B; KIANO, J W; MISAWA, N; DRAKE, R G; SCHUCH, W and BRAMLEY, P M (2002). Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruitspecific manner. *Proc. of the National Academy of Sciences USA*, *99*: 1092-1097.

GUEVARA-GARCIA, A; ROMAN, C S; ARROYO, A; CORTES, M E; GUTIERREZ-NAVA, M and LEON, P (2005). Characterization of the *Arabidopsis* clb6 mutant illustrates the importance of posttranscriptional regulation of the methyl-d-erythritol 4-phosphate pathway. *The Plant Cell*, *17*: 628-643.

HIRSCHBERG, J (1999). Production of high-value compounds: carotenoids and vitamin E. *Current Opinion in Biotechnology*, *10*: 186-191.

HIRSCHBERG, J (2001). Carotenoid biosynthesis in flowering plants. *Current Opinion in Plant Biology*, *4*: 210-218.

HSIEH, M H and GOODMAN, H M (2005). The *Arabidopsis* IspH homolog is involved in the plastid nonmevalonate pathway of isoprenoid biosynthesis. *Plant Physiology*, *138*: 641-653.

IKOMA, Y; KOMATSU, A; KITA, M; OGAWA, A; OMURA; MYANO, M and MORIGHUCI, T (2001). Expression of phytoene synthase gene and characteristic carotenoid accumulation during citrus fruit development. *Physiologia Plantarum*, *111*: 232-238.

KAJIWARA K; FRAZER, P D; KONDO, K and MISAWA, N (1997). Expression of an isopentenyl diphosphate isomerase gene enhances isoprenoid biosynthesis. *Biochemical Journal*, *324*: 421-426.

KAUR, J S and SAMBANTHAMURTHI, R (2000). Preliminary studies on carotene profiles in the developing oil palm mesocarp. First Malaysian MIT Biotechnology Partnership Programme (MMBPP) Symposium. Kuala Lumpur. 16-18 November 2000.

KHEMVONG, S and SUVACHITTANONT, W (2005). Molecular cloning and expression of a cDNA encoding 1-deoxy-D-xylulose 5-phosphate synthase from oil palm *Elaeis guineensis* Jacq. *Plant Science*, *169*: 571-578.

LAULE, O; FURHOLZ, A; CHANG, H; ZHU, T; WANG, X; HELFETZ, P B; GRUISSEM, W and LANGE, M B (2003). Crosstalk between cytosolic and plastidial patways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proc. of the National Academy of Sciences USA*, *100*: 6866-6871.

LICHTENTHALER, H K (1999). The 1-deoxy-D-xylulose 5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *50*: 47-65.

LICHTENTHALER, H K: ROHMER, M and SCHWENDER, J (1997). Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. *Physiologia Plantarum*, *101*: 643-652.

LINGREN, L O; STALBERG, K G and HOGLUND, A (2003). Seed-specific overexpression of an endogenos *Arabidopsis* phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and abscisic acid. *Plant Physiology*, *132*: 779-785.

LOIS, L M; RODRÍGUEZ-CONCEPCIÓN, M; GALLEGO, F; CAMPOS, M and BORONAT, A (2000). Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. *Plant Journal*, *22*: 503-513.

MAHMOUD, S S and CROTEAU, R B (2001). Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proc. of the National Academy of Sciences USA, 98*: 8915-8920.

MOHD DIN, A; RAJANAIDU, N and JALANI, B S (2000). Performance of *Elaeis oleifera* from panama, Costa Rica, Colombia and Honduras in Malaysia. *J. Oil Palm Research Vol. 12*: 71-80.

MOHD DIN, A; KUSHAIRI, A A; NOH, A; ISA, Z A; JUNAIDAH, J and RAJANAIDU, N (2004). Innovations in oil palm breeding and genetics. *Book of Abstracts of AGRICONGRESS 2004, Selangor, Malaysia.* p. 448-449.

MORRIS, W L; DUCREUX, L J M; HEDDEN, P; MILLAM, S and TAYLOR, M A (2006). Overexpression of a bacterial 1-deoxy-D-xylulose 5-phosphate synthasegene in potato tubers pertubs the isoprenoidmetabolic network: implications for the control of the tuber life cycle. *J. Experimental Botany*, *57 (12)*: 3007-3018.

PAINE, J A; SHIPTON, C A; CHAGAR, S; HOWELLS, R M; KENNEDY, M J; VERNON, G; WRIGHT, S Y; HINCHLIFF, E; ADAMS, J L; SILVERSTONE, A L and DRAKE, R (2005). Improving the nutritional value of golden rice through increased pro-vitamin A content. *Nature Biotechnology*, *23*: 482-487.

PARVEEZ, G K A; MASNITA, M M; ALIZAH, Z; NA'IMATULAPIDAH, A M; ABDUL MASANI, M Y; HALIZA, H F; RASID, O and CHEAH, S C (2000). Transgenic oil palm: Production and projection. *Biochemical Society Transaction*, *28*: 969-972.

PARVEEZ, G K A; OMAR, R; MASANI, A M Y; HALIZA, H F; NA'IMATULAPIDAH, A M; KUSHAIRI, A D; TARMIZI, A H and ZAMZURI, I (2003). Transgenic oil palm: where are we? *Advanced Research on Plant Lipids* (Murata, N, ed.). Kluwer Academic Publishers. p. 415-418.

PECKER, I; GABBAY, R; CUNNINGHAM, F X and HIRSCBERG, J (1996). Cloning and caharacterization of the cDNA for lycopene β-cyclase from tomato reveals decrease in its expression during fruit ripening. *Plant Molecular Biology*, *30*: 807-819.

RASID, O A; SINGH, R; SAMBANTHAMURTHI, S and CHEAH S C (2005). RTPCR Amplification and Cloning of Partial DNA Sequence Coding for Oil Palm (*E. guineensis*) zeaxanthin epoxidase. *Proc. of the Conference on Biotechnology of Plantation Comodities*. p. 553.

RASID, O A; NOR HANIN, A; MASURA, S S; HO, C L; SUHAIMI, N and SAMBANTHAMURTHI, R (2006). Regulation of oil palm (*E. guineensis*) phytoene desaturase in developing mesocarp tissues determined by real-time PCR. *Proc. of the Agriculture Congress 2006*. p. 41-47.

RASID, O A; NOR HANIN, A; MASURA, S S; ABDUL MASANI, M Y; SINGH, R; HO, C L; SUHAIMI, N; PARVEEZ, G K A and SAMBANTHAMURTHI, R (2007). Lycopene: genetic engineering of a potential nutraceutical. *Proc. of the PIPOC 2007 International Palm Oil Congress - Agriculture, Biotechnology and Sustainability Conference.* p. 248-273.

RASID, O A; WAN NUR SYUHADA, W S; NOR HANIN, A; MASURA, S S; HO, C L; SUHAIMI, N and SAMBANTHAMURTHI, R (2008). RT-PCR amplification and cloning of partial DNA sequence coding for oil palm (*E. oleifera*) phytoene synthase. *Asia Pacific Journal of Molecular Biology and Biotechnology*, *16(1)*: 17-24.

RODRÍGUEZ-CONCEPCIÓN, M and BORONAT, A (2002). Elucidation of the metylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. *Plant Physiology*, *130*: 1079-1089.

RODRÍGUEZ-CONCEPCIÓN, M; AHUMADA, I; DIEZ-JUEZ, E; SAURET-GÜETO, S; LOIS, L M; GALLEGO, F; CARRETERO-PAULET, L; CAMPOS, N and BORONAT, A (2001). 1-Deoxy-D-xylulose 5-phosphate reductoisomerase and plastid isoprenoid biosynthesis during tomato fruit ripening. *The Plant Journal*, *27*: 213-222.

ROMER, M; SEEMAN, M; HORBACH, S; BRINGER-MEYER, S and SAHM, H (1996). Glyceraldehyde 3-phosphate and pyruvate as presursor of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis. *J. American Chemistry Society*, *118*: 2564-2566.

ROMER, S; FRASER, P D; KIANO, J W; SHIPTON, C A; MISAWA, N; SCHUCH, W and BRAMLEY, P (2000). Elevation of the provitamin A content of transgenic tomato plants. *Nature Biotechnology*, *18*: 666-669.

ROMER, S; LUBECK, J; KAUDER, F; STEIGER, S; ADOMAT, C and SANDMANN, G (2002). Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. *Metabolic Engineering*, *4*: 263-272.

RONEN, G; COHEN, M; ZAMIR, D and HIRSCHBERG, J (1999). Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant *Delta*. *The Plant Journal*, *17*: 341-351.

RONEN, G; CARMEL-GOREN, L; ZAMIR, D and HIRSCHBERG, J (2000). An alternative pathway to β-carotene formation in plant chromoplasts discovered by map-based cloning of *Beta* and *old-gold* color mutations in tomato. *Proc. of the National Academy of Sciences USA*, *97*: 11102-11107.

ROSATI, C; AQUILANI, R; DHARMAPURI, S; PALLARA, P; MARUSIC, C; TAVAZZA, R; BOUVIER, F; CAMARA, B and GIULIANO, G (2000). Metabolic engineering of beta-carotene and lycopene content in tomato fruit. *The Plant Journal*, *24*: 413-419.

SAMBANTHAMURTHI, R; SITI NOR AKMAR, A and PARVEEZ, G K A (2002). Genetic manipulation of the oil palm: challenges and future prospects. *The Planter*, *78(919)*: 547-562.

SCOLNIK, P A and BARTLEY, G E (1993). Phytoene desaturase from *Arabidopsis*. *Plant Physiology*, *103*: 1475.

SHEWMAKER, C K; SHEEHY, J; DALEY, M; COLBURN, S and KE, D Y (1999). Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. *The Plant Journal*, *20*: 402-412.

SITI NOR AKMAR, A; SAMBANTHAMURTHI, R and PARVEEZ, G K A (2001). Genetic modification of oil palm for producing novel oils. *Proc. of the 2001 PIPOC International Palm Oil Congress*. p. 18-30.

SUN, Z R; CUNNINGHAM, F X and GANTT, E (1998). Differential expression of two isopentenyl pyrophosphate isomerases and enhanced carotenoid accumulation in a unicellular chlorophyte. *Proc. of the National Academy of Sciences USA*, *95*: 11482-11488.

SUNDRAM, K; SAMBANTHANMURTHI, R and TAN, Y A (2003). Palm fruit chemistry and nutrition. *Asia Pacific Journal of Clinical Nutrition*, *12*: 355-362.

TAY, Y P and CHOO, Y M (2000). Valuable polynutrients in commercial red palm olein. *Palm Oil Developments No. 32*: 20-25.

VON LINGTIG, J; WELSCH, R; BONK, M; GIULIANO, G; BATSCHAUER, A and KLEINIG, H (1997). Light-dependent regulation of carotenoid biosynthesis occurs at the level of phytoene synthase expression and is mediated by phytochrome in Sinapis alba and *Arabidopsis thaliana* seedlings. *The Plant Journal, 12*: 625-634.

WAN NUR SYUHADA, W S (2006). *Isolation of cDNA Clone Coding for Oil Palm Phytoene Synthase*. Undergraduate thesis. University of Malaya, Kuala Lumpur, Malaysia.

WELSCH, R; BEYER, P; HUGUENEY, P; KLEINIG, H and VON LINTIG, J (2000). Regulation and activation of phytoene synthase, a key enzyme in carotenoid biosynthesis, during photomorphogenesis. *Planta*, *211*: 846-854.

WEST, K P (2002). Extent of vitamin A deficiency among pre-school children and women of reproductive age. *J. Nutrition, 132*: 2857-2866.

YE, X; AL-BABILI, S; KLOTI, A; ZHANG, J; LUCCA, P; BEYER, P and POTRYKUS, I (2000). Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, *287*: 303-305.