LYCOPENE CYCLASE GENES FOR MODIFICATION OF CAROTENOID CONTENT

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553

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Modification of carotenoid content especially towards increasing lycopene content is one of the main targets of the oil palm genetic engineering programme at the Malaysian Palm Oil Board (MPOB) (Parveez et al., 2003). This is due to the potential nutraceutical properties of lycopene. It has been shown that this carotenoid has the greatest ability of quenching singlet oxygen compared to other carotenoids (Di Mascio *et al.*, 1989). Its protective effects against various cancer types have been demonstrated in a number of studies. These include tissue culture studies on different cell lines examining anti-cell proliferative activities, anti-tumorogenic studies using animal models, and a number of epidemiological studies (Gann *et al.*, 1999; Giovannucci *et al.*, 2002; Tang *et al.*, 2005).

The carotenoids contained in oil palm fruits are made up predominantly of α- and β-carotenes (Tay and Choo, 2000). This observation suggests that lycopene is effectively converted into its derivatives, and that the two lycopene cyclases are very active in oil palm fruits (*Figure 1*). Therefore, an obvious strategy to increase the lycopene content is to block its conversion into carotenes (Sambanthamurthi *et al.*, 2002; Rasid *et al.*, 2007). This may be achieved by silencing the two cyclase genes in transgenic oil palm, either by the anti-sense or RNAi strategy. Both procedures require the characterisation of the genes.

OIL PALM LYCOPENE CYCLASES

In plants, the conversion of lycopene into β-carotene is catalysed by lycopene β-cyclase (LCYb). In addition to lycopene β-cyclase, the conversion of lycopene into α-carotene requires another related cyclase enzyme, lycopene ε-cyclase. The cDNA clones coding for lycopene β-cyclase (*lcyb*) and lycopene ε-cyclase (*lcye*) from oil palm have been successfully amplified and cloned through this work. Consensus sequences of 1962 bp and 1759 bp were generated for oil palm *lcye* and *lcyb*, respectively. An open reading frame (ORF) of 1617 bp encoding 539 amino acid (AA) residues was identified for *lcye*. Similarly, ORF of 1509 bp encoding for 503 AA residues was identified for *lcyb*. Deduced AA sequences were shown to be highly identical to their respective counterparts in other plant species at about 80% identity (*Figures 2* and *3*). Although the enzymes were functionally equivalent, they were shown to share little

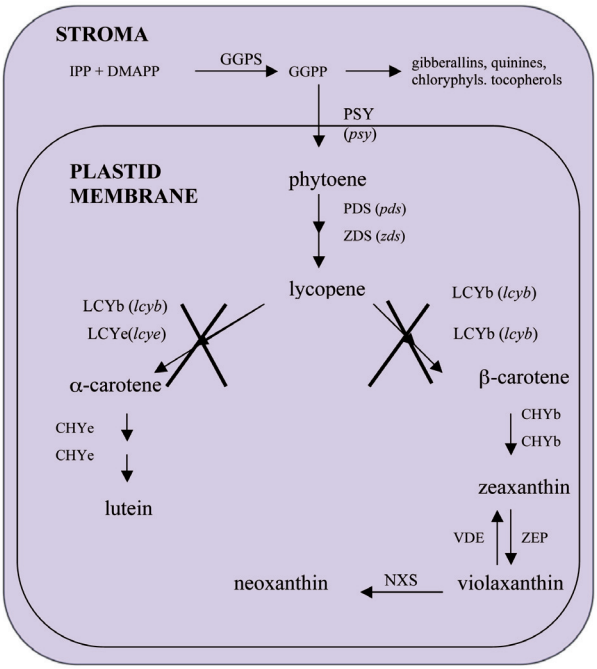

Figure 1. Schematic diagram depicting the carotenoid synthetic pathway in plants. The first committed in plant carotenoid synthesis is the formation of phytoene from the condensation of 2 GGPP molecules. Phytoene is then converted into lycopene through a series of desaturation. In most plants, lycopene will be branched into α- and β-carotene. Lycopene accumulation can be achieved by blocking this conversion.

Note: 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; VDE = violaxanthin de-epoxidase; NXS = neoxanthin synthase. The gene for each enzyme is given in parentheses and in italics next to the enzyme. Adapted from Cunningham (2002).

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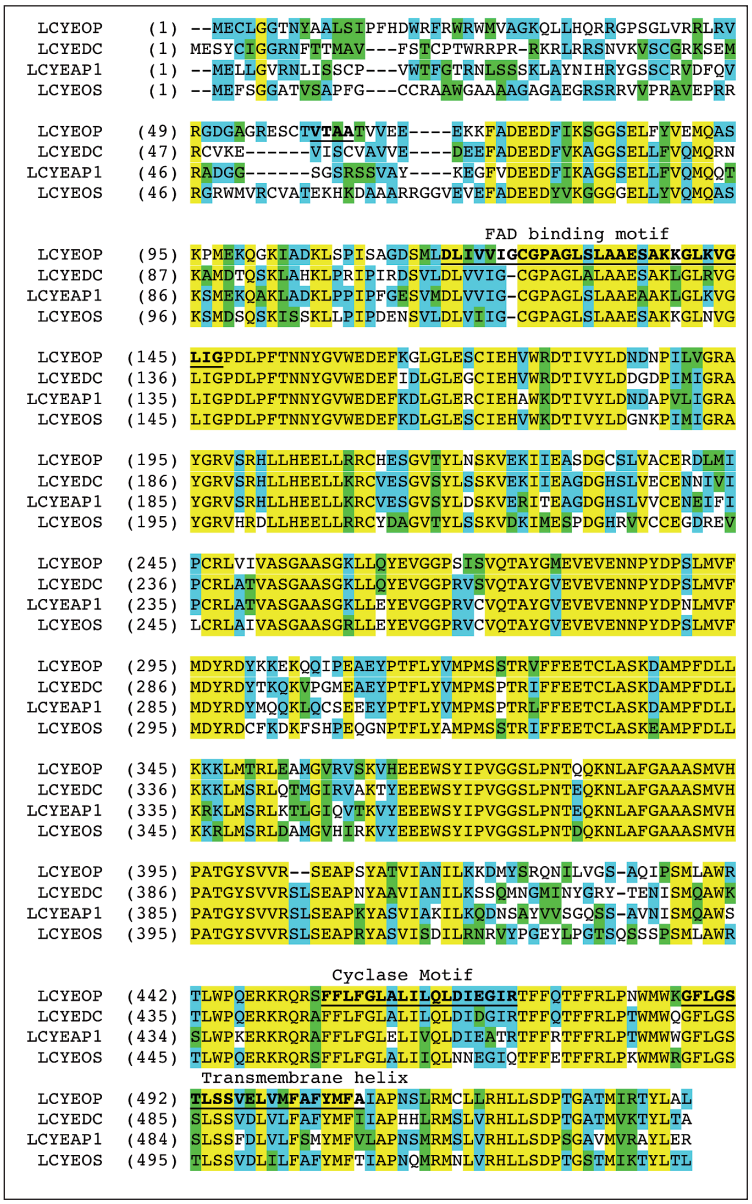

Figure 2. Comparison of amino acid sequences of oil palm lycopene ε-cyclase (LCYEOP) to representative lycopene ε-cyclase sequences from other plant species. The predicted transit peptide cleavage site (VTAA) is underlined and in bold. The FAD/NAD(P) binucleotide binding motif [residue 118 (D) to residue 147 (G)] for oil palm is shown to have an extra residue (G) at position 124AA. Transmembrane helixes and cyclase motif are underlined and indicated on top of the sequences. These two motifs are essential for structural functionality. Yellow indicates an exact match; blue denotes greater than 50% match; and green indicates weak similarity.
Note: LCYEDC = *Daucus carota*; LCYEAP1 = *A. palaestina*; LCYEOS = *Oryza sativa*.

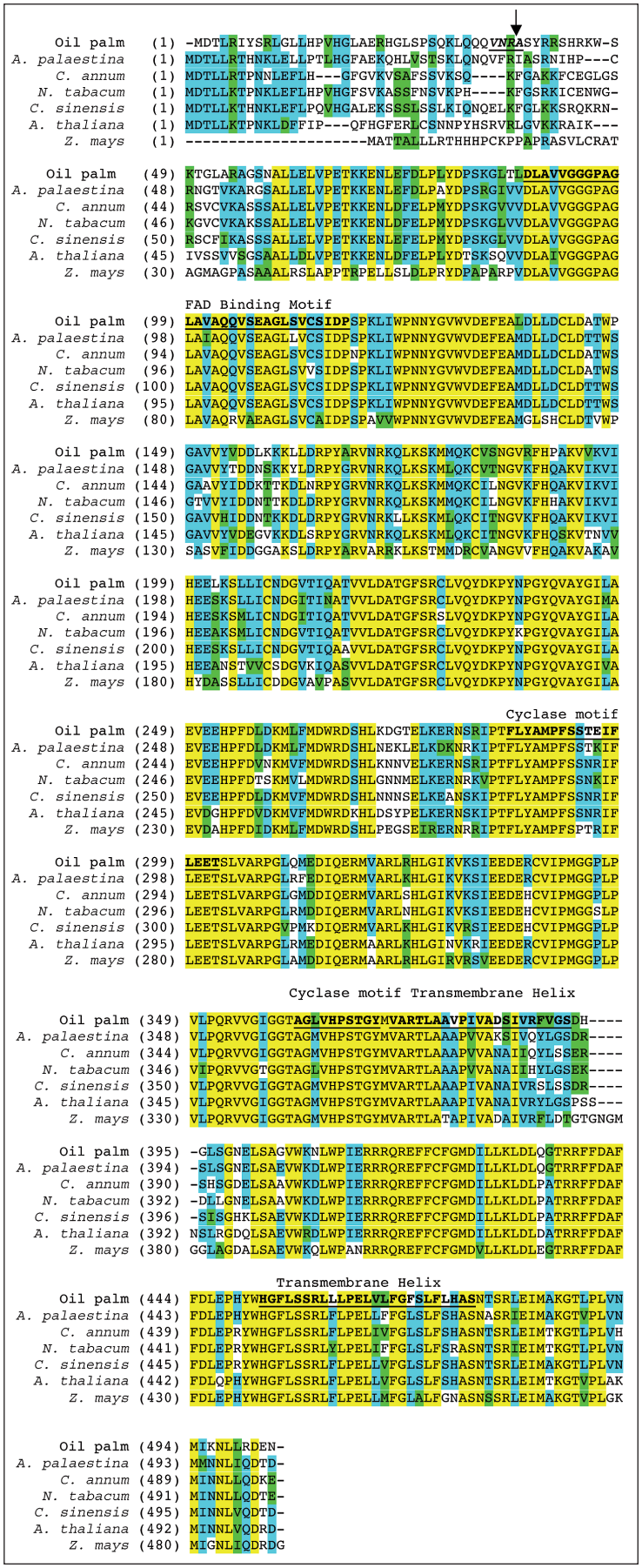

Figure 3. Comparison of deduced oil palm LCYb sequence to representative LCYb sequences from other plant species. The predicted transit peptide cleavage site is underlined and in italics. The arrow indicates the predicted transit peptide cleavage site. The FAD/NAD(P) binucleotide binding motif for oil palm is from residue 118 (D) to residue 147 (G), cyclase motifs and trans-membrane helixes are underlined and indicated below each of the motifs. Yellow indicates an exact match; blue denotes greater than 50% match; and green indicates weak similarity.

resemblance at about 30% identity. However, oil palm LCYb was shown to share a relatively high identity to plant neoxanthin and capxanthin-capsorubin synthases, suggesting a common ancestor for the cyclases and synthases. The full length coding sequences were later amplified, cloned and verified by sequencing (*Figure 4*).

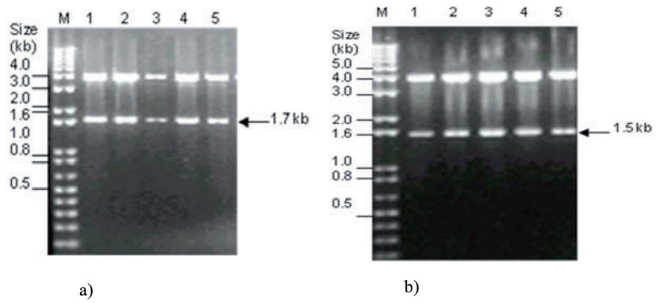

Figure 4. RTPCR amplification and cloning of the coding region of oil palm lycopene ε-cyclase and lycopene β-cyclase. Representative clones (1 to 5) containing the full length coding region of oil palm lycopene ε-cyclase (a) and lycopene β-cyclase (b) obtained through end-to-end RTPCR amplification. All of these clones were shown to carry the insert of about 1.7 kb for lycopene ε-cyclase (arrow) and 1.5 kb for lycopene β-cyclase.

Note: M = 1 kb plus DNA ladder.

EXPRESSION ANALYSIS

The regulation and expression of these cyclase genes were studied in developing mesocarp tissues using real-time PCR analysis (Livak and Schmittgen, 2001). Overall, the expression pattern of the target genes indicated some level of differential regulation of these genes with respect to the developmental stages as well as to tissue types (*Figure 5*). Within the mesocarp tissues, *lcyb* was expressed at a relatively high level in young mesocarp tissue [at five weeks after anthesis (WAA)]. The expression level then declined in the mesocarp tissues at 7, 9, 11, 13 and 15 WAA. The expression level increased again in the tissues at 17 and 19 WAA to almost the same level as in the mesocarp tissue at 5 WAA. Comparatively, the expression level of *lcye* was low in mesocarp tissues at all stages. Nevertheless, its expression level also seemed to be modulated during the developmental stages of the oil palm fruits. The level of *lcye* was shown to be relatively low in the late developmental stages compared to its expression in the young mesocarp tissues.

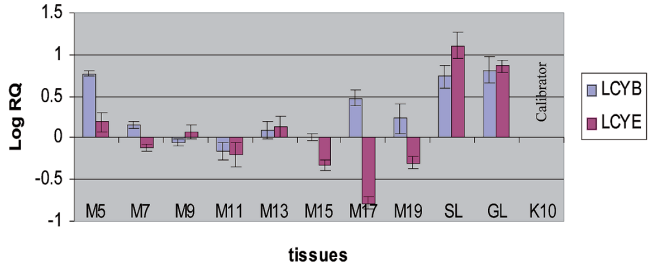

Figure 5. Expression of oil palm lcyb *and* lcye *genes in developing mesocarp tissues at different developmental stages (5-19 WAA), in spear (SL) and in green (GL) leaves relative to kernel (K10) obtained from the real-time PCR analysis. The expression level was expressed in fold (log10 RQ).*

The expression levels of the cyclase genes were well correlated to the accumulation of carotene in the fruit tissues. Tay and Gwendoline (2006) have shown that the major carotenoid component in the young mesocarp (at 1 WAA) is lutein. It remained high until 8 WAA. Thus, it may be expected that the genes required for the formation of this carotenoid will be expressed at relatively high levels at these earlier stages. In this work, it was shown that *lcye* which leads to the formation of lutein was expressed at a higher level in the mesocarp tissue at 5 WAA. Nevertheless, the expression of *lcyb* was also shown to be comparable to *lcye* (*i.e.* higher) at that stage. This may be explained by the requirement of abscisic acid (ABA) for the development and β-carotene derived xanthophylls associated with photosynthesis. The expression of these genes also seemed to be correlated to the accumulation of α- and β-carotenes in the oil palm fruits. These carotenoids have been shown to significantly increase at 12 WAA and to reach a maximum level at 18 WAA (Khemvong and Suvachittanont, 2005; Tay and Gwendoline, 2006). The raised levels were accompanied by the reincreased expression of lycopene ε-cyclases at 13 WAA and lycopene β-cyclase at 17 WAA.

WHO SHOULD BENEFIT

Molecular biologists or biotechnologists from the oil palm industry can benefit from using the cDNAs to manipulate the carotenoid content in oil palm fruits. Similarly, molecular biologists and biotechnologists from local universities, research institutions and research-based companies can benefit from use of the cDNAs in heterologous systems. This is in addition to the services previously offered by MPOB to make transformation vectors and RNAi constructs as well as to regenerate transgenic plants using both microprojectile bombardment and *Agrobacterium*-mediated transformation approaches (Parveez 2003; Masani and Parveez, 2005; Dayang *et al.*, 2008; Masani and Parveez, 2008).

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