554

PHYTOENE SYNTHASE GENE FROM OIL PALM FOR THE MODIFICATION OF CAROTENOID CONTENT

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Carotenoids are a group of isoprenoid pigments which are widely distributed in nature. They are synthesized by all photosynthetic organisms and some non-photosynthetic bacteria and fungi. Carotenoids protect the photosynthetic apparatus from photo-oxidation, and represent structural components of light-harvesting antenna and reaction-centre complexes. Dietary carotenoids fulfil essential requirements for human and animal nutrition. β-carotene is the most potent dietary precursor of vitamin A, the deficiency of which leads to xerophthalmia, blindness and premature death (Mayne, 1996). Other carotenoids have been shown to alleviate age-related diseases when taken in sufficient quantities in the diet, probably because of their powerful properties as lipophilic antioxidants (Mordi, 1993).

The modification of carotenoid content especially towards increasing lycopene content is one of the main targets of the oil palm genetic engineering program at the Malaysian Palm Oil Board (MPOB) (Parveez *et al*., 2003; Parveez and Na'imatulpidah, 2008). The carotenoid biosynthetic pathway takes place within the plastid, and the first committed step is the head-to-head condensation of two geranylgeranyl diphosphate (GGDP) molecules to produce phytoene (a colourless carotenoid). This step is catalyzed by the enzyme phytoene synthase (*psy*) (Cunningham and Gantt, 1998).

PHYTOENE SYNTHASE *(PSY)* GENE FROM OIL PALM

The full length *psy* cDNA and genomic clones were isolated from oil palm mesocarp (*Figure 1*). The genomic distribution is shown in *Figure 2*. Sequence analysis revealed that the *psy* clone is a 1296 bp nucleotide, encoding a protein of 432 amino acids. The oil palm *psy* cDNA sequence shows very good homology to other *psy* cDNAs in the Genbank database (*Figure 3*). Southern blot analysis suggests that *psy* is a single copy gene in oil palm (*Figure 4*).

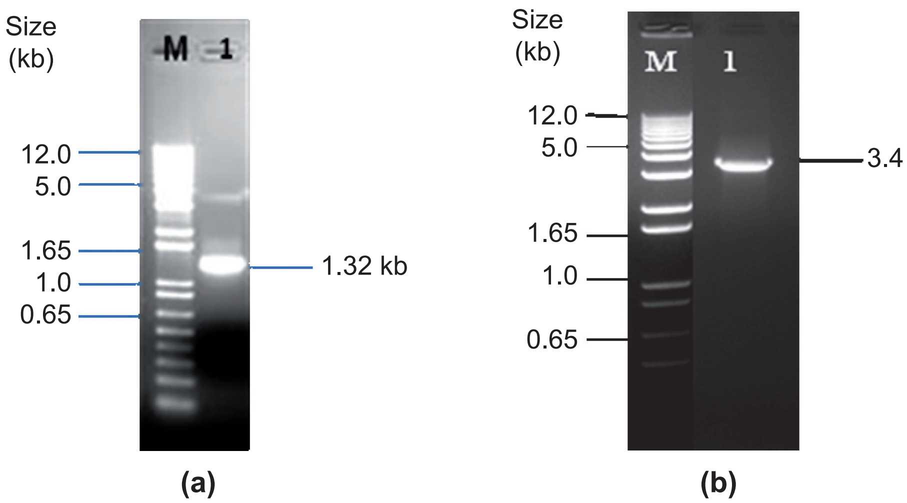

Figure 1. (a): Amplification of the full-length region for Elaeis guineensis *cDNA using PSYA and PSYB. Arrow indicates a major band product of about 1.32 kb. (b): Amplification of* E. guineensis *genomic DNA using the phytoene synthase specific primers PSYA1 and PSYAB4. Lane M: 1 KB plus DNA.*

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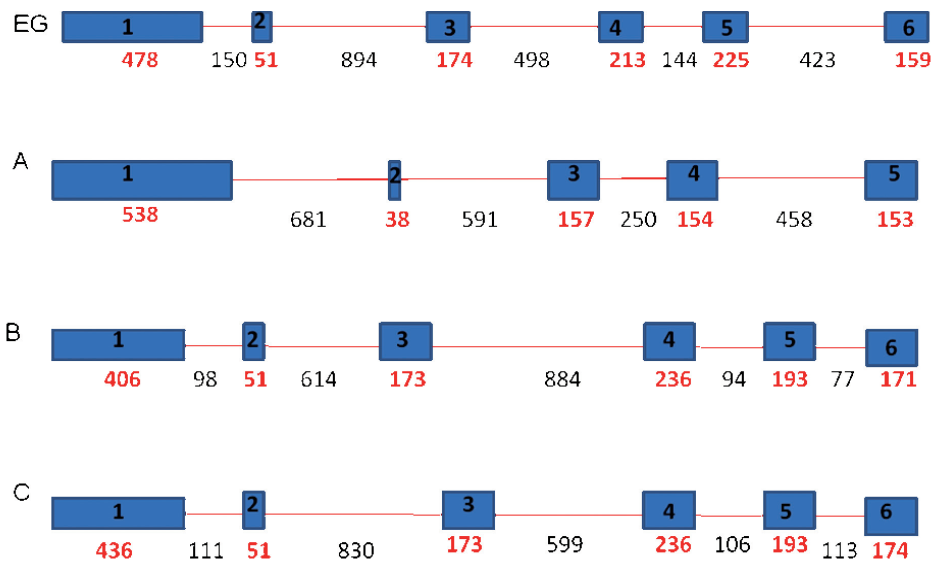

Figure 2. Diagram of exons and introns of other psy *plants. Thick bars and thin bars indicate exons (red numbers) and introns, respectively, sizes of which are in bp. EG is* E. guineensis psy*; A is* psy *of* Dunaliella salina *(Accession number: AY601075); B is* psy *of Zea mays (Accession number: AY324431); C is* psy *of rice (Accession number: AP005750).*

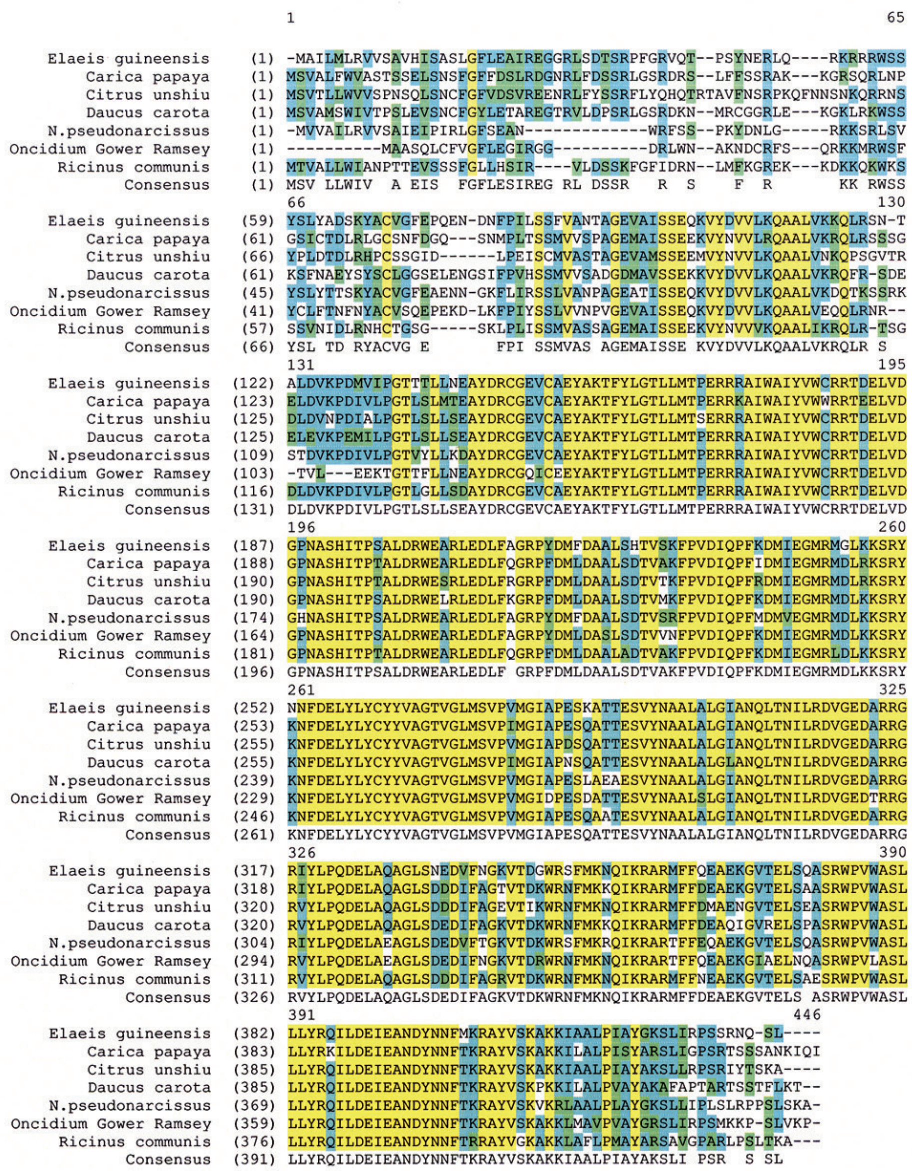

Figure 3. Alignment of the amino acid sequence of phytoene synthase from E. guineensis *with other plant phytoene synthases. Conserved nucleotides are indicated in yellow.*

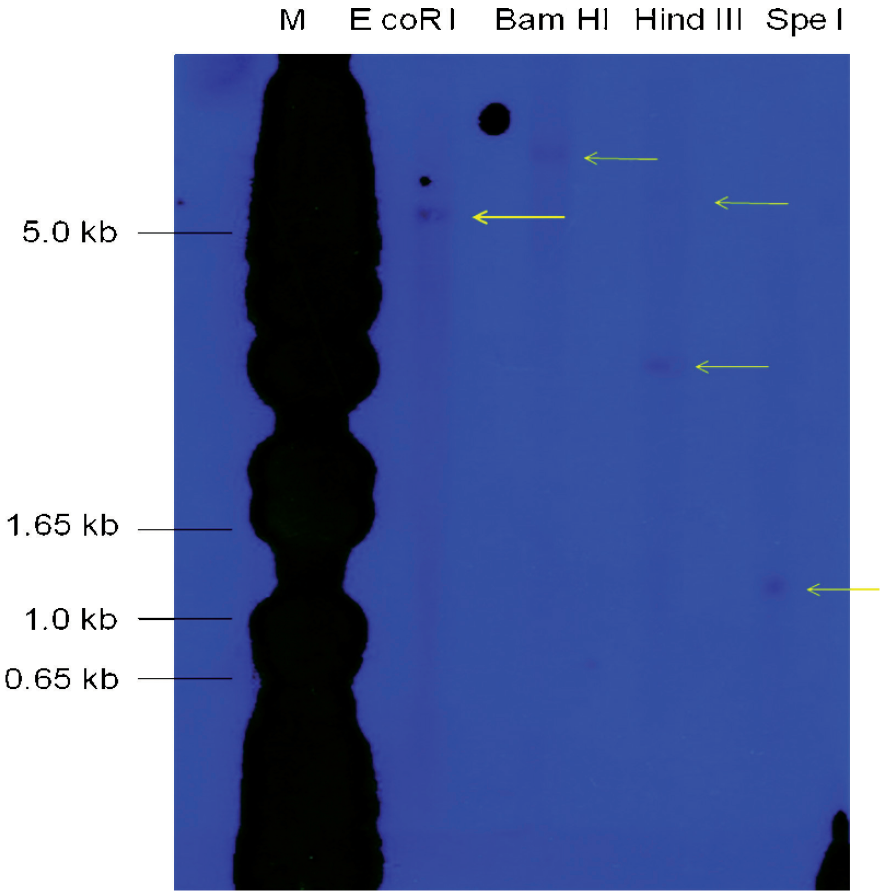

Figure 4. Southern blot analysis of E. guineensis psy *suggests that oil palm psy is a single copy gene. Thirty micrograms of total DNA samples of* E. guineensis *were digested using four different restriction enzymes namely EcoR I, Hind III, Bam H I, and Spe I. M is 1kb plus DNA ladder; 1 is DNA* E. guineensis *digested with EcoR I; 2 is DNA* E. guineensis *digested with Bam HI; 3 is DNA* E. guineensis *digested with Hind III; 4 is DNA* E. guineensis *digested with Spe I.*

BENEFITS OF THE PHYTOENE SYNTHASE GENE

Genes encoding phytoene synthase have been identified from numerous higher plants. Many studies have shown that *psy* activity is rate-limiting in several carotenogenic tissues such as the tomato fruit (Fraser *et al.*, 2002), canola seeds (Shewmaker *et al.*, 1999) and marigold petals (Moehs *et al.*, 2001). It is also well-known that in many species *psy* affects the flux of the carotenoid pathway. The regulation is at the transcriptional level (Li *et al.*, 2008). Besides, genes encoding *psy* have been the first target genes for improving carotenoid content and components in transgenic plants (Zhu *et al.*, 2004). The phytoene synthase cDNAs can be used to facilitate lycopene accumulation in transgenic oil palm by up-regulation of phytoene synthase and down-regulation of lycopene β-cyclase. *Psy* is a key enzyme of the pathway, and as such any modulation in its activity is likely to cause a modification of the content of other carotenoids in oil palm such as zeaxanthin. Besides using this gene in oil palm, the gene can also be used in heterologous systems and in other carotenoid-producing crops such as rice, tomato, and pepper.

WHO WOULD BENEFIT

Molecular biologists or biotechnologists from the oil palm industry can benefit from using the cDNAs to manipulate carotenoid content. Similarly, molecular biologists and biotechnologists from local universities, research institutions and research-based companies can benefit from the cDNAs in heterologous systems.

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