

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

WAN NUR SYUHADA WAN SULAIMAN; OMAR ABD RASID and AHMAD PARVEEZ GHULAM KADIR



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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).

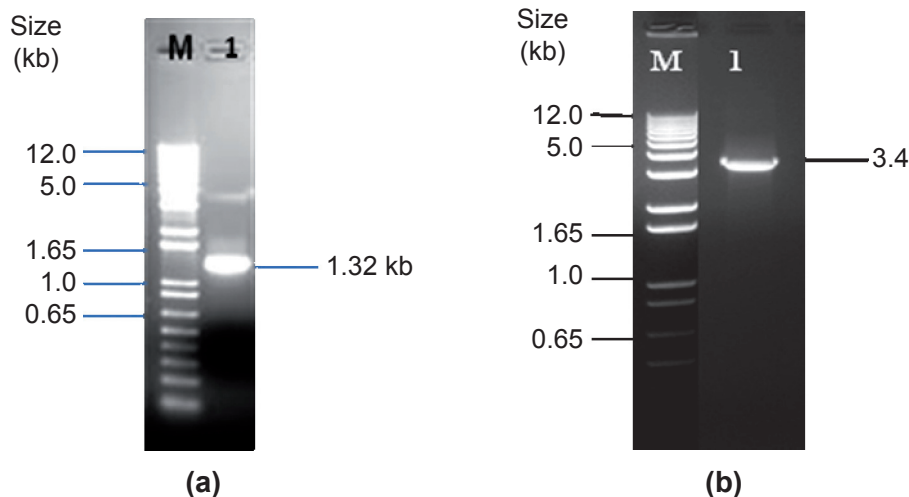


Figure 1. (a): Amplification of the full-length region for *Elaeis guineensis* cDNA using PSYA and PSYB. Arrow indicates a major band product of about 1.32 kb. (b): Amplification of *E. guineensis* genomic DNA using the phytoene synthase specific primers PSYA1 and PSYAB4. Lane M: 1 KB plus DNA.

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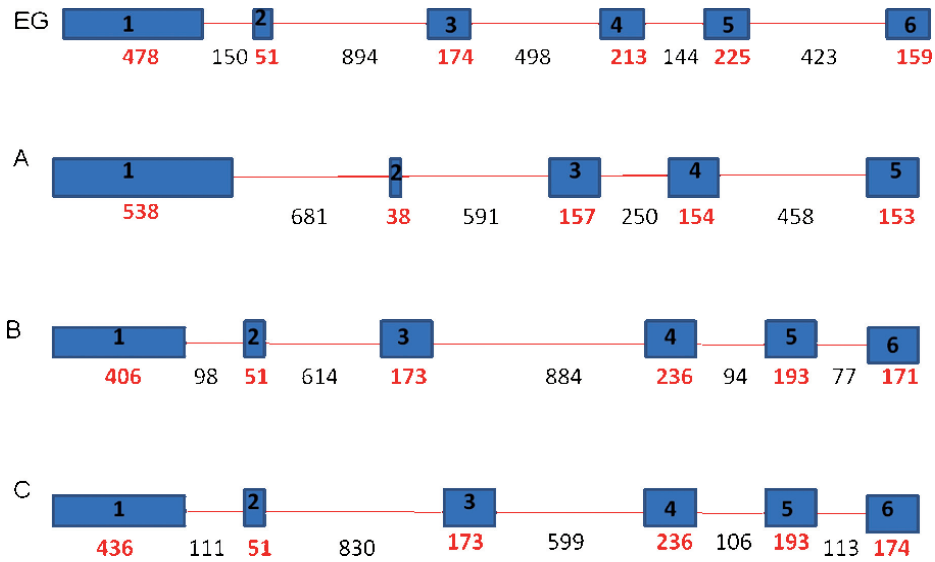


Figure 2. Diagram of exons and introns of other psy plants. Thick bars and thin bars indicate exons (red numbers) and introns, respectively, sizes of which are in bp. EG is *E. guineensis* psy; A is psy of *Dunaliella salina* (Accession number: AY601075); B is psy of *Zea mays* (Accession number: AY324431);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

	1	65
<i>Elaeis guineensis</i>	(1) -MAILMLRVVSAVHTSASLGFLEAIREGGRLSDTSRPFGRVOT--PSYNERLQ----RKRRRWSS	
<i>Carica papaya</i>	(1) MSVALFWVASTSELSNSFGFFDLSLDGRLDSSRLGSRDRS--LFSSRAK---KGRSQRLNP	
<i>Citrus unshiu</i>	(1) MSVTLWVSPNSQSLNCFVDSVRENRFPYSRFLYQHQTRTAVNSRKPQFNNSNRQRNS	
<i>Daucus carota</i>	(1) MSVAMSWIVTPSELVSNCFGYLETAREGTRVLDPSRLGSRDRN--MRCGGRLE---KGLLRWSS	
<i>N.pseudonarcissus</i>	(1) -MVAALRVVSAIEIPRLGFSEAN-----WRFS--PKYDNLG----RKKSLSV	
<i>Oncidium Gower Ramsey</i>	(1) -----MAASQLCFVGFLEGRGG-----DRLWN--AKNDCRFS---QRKKMRWF	
<i>Ricinus communis</i>	(1) MVALLVIANPTTEVSSSFGLLHSIR---VLDSKFGFIDRN--LMEKGREK---KDKKQKWS	
Consensus	(1) MSV LLWIV A EIS FGFLESIREG RL DSSR R S F R KK RWSS	66 130
<i>Elaeis guineensis</i>	(59) YSLYADSKYACVGFPEQEN-DNEPILSSFVANTAGEVAISSEKQVYDVVLKQAALVKKQLRSN-T	
<i>Carica papaya</i>	(61) GSICTDLRLGCSNFBGQ---SNMPLTSSMVSPAGEMAISSSEKVVNVVLRQAALVKKQLRSN-T	
<i>Citrus unshiu</i>	(66) YPLDTRDLRHFPCSSGID-----LPEITSCMVASTAGEVAMSSSEMVNVVLRQAALVKKQPSGVTR	
<i>Daucus carota</i>	(61) KSFNAEYSYSLGGSSELENGSIFPVHSSMVVSADGMAVSSSEKVVYDVVLKQAALVKKQRF-SDE	
<i>N.pseudonarcissus</i>	(45) YSLYITTSKYACVGFEAENN-GKFLIRSSLVANPAGEATIISSEKQVYDVVLKQAALVKKQPSVSRK	
<i>Oncidium Gower Ramsey</i>	(41) YCLFTNFNYACVSOEPEKD-LKEPTYSLSLVNVPGEVAISSEKQVYDVVLKQAALVEQQLRNR--	
<i>Ricinus communis</i>	(57) SSVNIDLNRHCTGSG----SKLPLISSMVASAGEMAISSSEKVVNVVLRQAALVKKQLR-MSG	
Consensus	(66) YSL TD RYACVG E FPI SSMVAS AGEMAISSSE KVDVVLKQAALVKKQLR S	131 195
<i>Elaeis guineensis</i>	(122) ALDVKPDMVLPGLTLLNEAYDRCGEVCAEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	
<i>Carica papaya</i>	(123) ELDVKPDIVLPGTLLSMTLAYDRCGEVCAEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	
<i>Citrus unshiu</i>	(125) DLDVNPDIAPGLTLLSEAYDRCGEVCAEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	
<i>Daucus carota</i>	(125) ELVKEPEMILPGLTLLSEAYDRCGEVCAEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	
<i>N.pseudonarcissus</i>	(109) STDVVKPDIVLPGTLYLLKDAYDRCGEVCAEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	
<i>Oncidium Gower Ramsey</i>	(103) -TVL---EERTGTTFLLNEAYDRCGVCEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	
<i>Ricinus communis</i>	(116) ALDVKPDMVLPGLTLLSMDAYDRCGEVCAEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	
Consensus	(131) ALDVKPDMVLPGLTLLSEAYDRCGEVCAEYAKTFYLGTLTLPERRRAIWAIVYWCRRTDELVD	196 260
<i>Elaeis guineensis</i>	(187) GPNASHITPTALDRWEARLEDFAGRPYDMFADALSHVTSKFPVDIQPFKDMIEGMRMLKKSRY	
<i>Carica papaya</i>	(188) GPNASHITPTALDRWEARLEDFQGRPFDMFADALSDTVAKFPVDIQPFKDMIEGMRMLKKSRY	
<i>Citrus unshiu</i>	(190) GPNASHITPTALDRWEARLEDFRGRPFDMFADALSDTVAKFPVDIQPFKDMIEGMRMLKKSRY	
<i>Daucus carota</i>	(190) GPNASHITPTALDRWEARLEDFKGRPFDMFADALSDTVAKFPVDIQPFKDMIEGMRMLKKSRY	
<i>N.pseudonarcissus</i>	(174) GPNASHITPTALDRWEARLEDFAGRPYDMFADALSDTVAKFPVDIQPFKDMIEGMRMLKKSRY	
<i>Oncidium Gower Ramsey</i>	(164) GPNASHITPTALDRWEARLEDFAGRPYDMFADALSDTVAKFPVDIQPFKDMIEGMRMLKKSRY	
<i>Ricinus communis</i>	(181) GPNASHITPTALDRWEARLEDFQGRPFDMFADALSDTVAKFPVDIQPFKDMIEGMRMLKKSRY	
Consensus	(196) GPNASHITPTALDRWEARLEDF GRPFDMFADALSDTVAKFPVDIQPFKDMIEGMRMLKKSRY	261 325
<i>Elaeis guineensis</i>	(252) KNFDELYLYCYVAGTVGLMSVPMGIAPESQATTESVYNAALALGIANQLTNILRDVGEDARRG	
<i>Carica papaya</i>	(253) KNFDELYLYCYVAGTVGLMSVPMGIAPESQATTESVYNAALALGIANQLTNILRDVGEDARRG	
<i>Citrus unshiu</i>	(255) KNFDELYLYCYVAGTVGLMSVPMGIAPDSQATTESVYNAALALGIANQLTNILRDVGEDARRG	
<i>Daucus carota</i>	(255) KNFDELYLYCYVAGTVGLMSVPMGIAPNSQATTESVYNAALALGIANQLTNILRDVGEDARRG	
<i>N.pseudonarcissus</i>	(239) KNFDELYLYCYVAGTVGLMSVPMGIAPESAEASVYNAALALGIANQLTNILRDVGEDARRG	
<i>Oncidium Gower Ramsey</i>	(229) KNFDELYLYCYVAGTVGLMSVPMGIDPESDATTESVYNAALS LGIANQLTNILRDVGEDTRRG	
<i>Ricinus communis</i>	(246) KNFDELYLYCYVAGTVGLMSVPMGIAPESQATTESVYNAALALGIANQLTNILRDVGEDARRG	
Consensus	(261) KNFDELYLYCYVAGTVGLMSVPMGIAPESQATTESVYNAALALGIANQLTNILRDVGEDARRG	326 390
<i>Elaeis guineensis</i>	(317) RYLPQDELAQAAGLSNEDVFNKQVTDGWSFMKNQIKRARMFFQEAEGVTELSAASRPVWASL	
<i>Carica papaya</i>	(318) RYLPQDELAQAAGLSDDDFAGTVTDKWRNFMKQIKRARMFFDEAEKGVTELSAASRPVWASL	
<i>Citrus unshiu</i>	(320) RYLPQDELAQAAGLSDDDFAGVETIKWRNFMKNQIKRARMFFDEAEKGVTELSAASRPVWASL	
<i>Daucus carota</i>	(320) RYLPQDELAQAAGLSDEDFAGKVTDKWRNFMKQIKRARMFFDEAQIGVRELSAASRPVWASL	
<i>N.pseudonarcissus</i>	(304) RYLPQDELAQAAGLSDEDFVTKQVTDKWRNFMKQIKRARTFFQEAEGVTELSAASRPVWASL	
<i>Oncidium Gower Ramsey</i>	(294) RYLPQDELAQAAGLSDEDFNGKVTDRWRNFMKNQIKRARTFFQEAEGVTELSAASRPVWASL	
<i>Ricinus communis</i>	(311) RYLPQDELAQAAGLSDDDFAGRVTDKWRNFMKNQIKRARMFFDEAEKGVTELSAASRPVWASL	
Consensus	(326) RYLPQDELAQAAGLSDEDFAGKVTDKWRNFMKNQIKRARMFFDEAEKGVTELS ASRPVWASL	391 446
<i>Elaeis guineensis</i>	(382) LLYRQILDEIEANDYNNFTKRAYVSKAKKIALP IAYGKSLIRPSSRNO-SL----	
<i>Carica papaya</i>	(383) LLYRKILDEIEANDYNNFTKRAYVSKAKKILALPISYARSLGCSRTSSANKIQI	
<i>Citrus unshiu</i>	(385) LLYRQILDEIEANDYNNFTKRAYVSKAKKIALP IAYAKSLRPSRIYTSKA----	
<i>Daucus carota</i>	(385) LLYRQILDEIEANDYNNFTKRAYVSKPKKILALP VAYAKAFAPTARTSSFTLKT--	
<i>N.pseudonarcissus</i>	(369) LLYRQILDEIEANDYNNFTKRAYVSKVKRLAALPLAYGKSLILPLSLRPPSLSKA-	
<i>Oncidium Gower Ramsey</i>	(359) LLYRQILDEIEANDYNNFTKRAYVSKAKKILMAYVAYGRSLIRPSSMKKP-SLVKP-	
<i>Ricinus communis</i>	(376) LLYRQILDEIEANDYNNFTTRAYVSKAKKILALP MAYSAYGPARLPSLTKA----	
Consensus	(391) LLYRQILDEIEANDYNNFTKRAYVSKAKKIALP IAYAKSLI PSR S SL	

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.

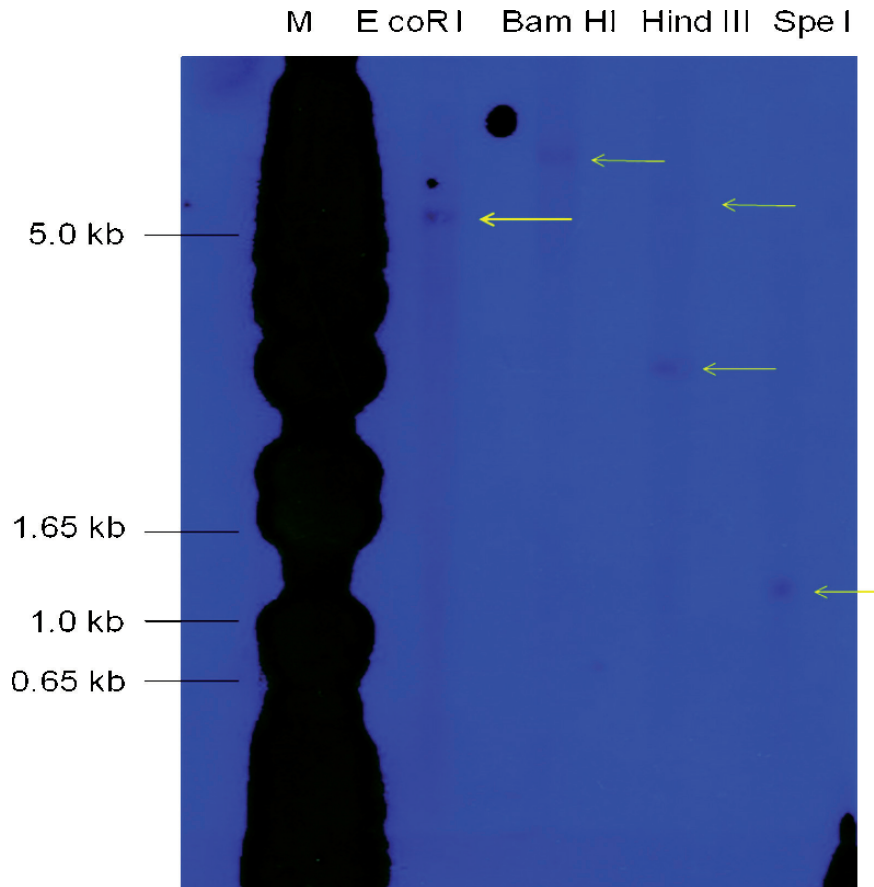


Figure 4. Southern blot analysis of *E. guineensis psy* suggests that oil palm *psy* is a single copy gene. Thirty micrograms of total DNA samples of *E. guineensis* were digested using four different restriction enzymes namely *EcoR I*, *Hind III*, *Bam H I*, and *Spe I*. M is 1kb plus DNA ladder; 1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

Director-General
MPOB
P. O. Box 10620
50720 Kuala Lumpur, Malaysia.
Tel: 03-8769 4400
Fax: 03-8925 9446
www.mpob.gov.my