

**ISOLATION AND SEQUENCE ANALYSIS OF GENE AND PROMOTER
STEAROYL ACYL CARRIER PROTEIN DESATURASE FROM OIL PALM
(*Elaeis guineensis* Jacq.)**

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Abstract

Stearoyl -acyl carrier protein desaturase (SAD) is an important enzyme of fatty acids biosynthesis in higher plants. SAD plays a key role in determining the ratio of saturated fatty acids to unsaturated fatty acids in plants. The expression level of SAD gene has been known to be highest in young fruit and seed. Engineering of fatty acids biosynthesis in *Elaeis guineensis* Jacq. requires appropriate fruit and seed specific promoters, so that the expression of transgenic gene will only occur in fruit and seed. In this research we isolated and cloned putative promoters of Stearoyl-acyl carrier protein desaturase (SAD) gene from *Elaeis guineensis* Jacq. The isolation of SAD gene and promoters was done by PCR-based genome walking method. PCR from genomic DNA of *E. guineensis* resulted in a fragment with 1000 bp in size (E-SAD). The genome was randomly cut by *EcoRV* and *StuI*, and then used as template in genome walking PCR. Four fragments in lengths of 800, 1000 and 1200 bp resulted from the *EcoRV* sample, while three fragments in lengths of 700, 1200 and 1500 bp were produced by the *StuI* sample. PCR results were cloned into pGEMT-Easy (Promega) in *E. coli* strain DH5 α prior to sequencing with SP6 and T7 primer. Sequence analysis using BLASTn indicated that the 1000 bp E-SAD had a high similarity with SAD genes from some other plants. The three fragments of putative E-SAD gene promoter, with lengths of 800, 700 and 1000 bp, have been cloned and sequenced. Sequence analysis using Geneious, Bioedit, BLASTn and promoter database showed that two fragments in lengths of 800 and 700 bp had no specificity for SAD gene promoters. Whereas a 907 bp fragment showed positive as 5' flanking region of SAD gene that probably contained a promoter region.

Keyword : promoter, SAD gene, PCR genome walking, *Elaeis guineensis* Jacq.

ISOLASI DAN ANALISIS SEKUEN GEN DAN PROMOTER *STEAROYL ACYL CARRIER PROTEIN DESATURASE* KELAPA SAWIT (*Elaeis guineensis* Jacq.)

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Abstrak

Gen *Stearoyl-acyl carrier protein desaturase* (SAD) adalah enzim yang penting dalam biosintesis asam lemak pada tanaman tingkat tinggi, berperan dalam menentukan rasio asam lemak jenuh terhadap asam lemak tidak jenuh. Gen SAD telah diketahui memberikan ekspresi yang kuat pada biji dan buah. Rekayasa biosintesis asam lemak pada kelapa sawit (*Elaeis guineensis* Jacq.) membutuhkan promoter spesifik biji dan buah, sehingga ekspresi gen transgenik hanya terjadi di biji dan buah, bukan di bagian tanaman lainnya. Pada penelitian ini telah dilakukan isolasi gen dan promoter *Stearoyl-acyl carrier protein desaturase* (SAD) dari kelapa sawit (*Elaeis guineensis* Jacq.). Isolasi promoter *gen* SAD dilakukan dengan menggunakan metode *genome walking*. Hasil isolasi gen SAD didapatkan fragmen DNA berukuran 1000 pb (E-SAD), sedangkan isolasi putatif promoter gen SAD didapatkan fragmen berukuran 800 bp, 1000 pb, dan 1200 pb dari sampel pemotongan enzim *EcoRV* dan fragmen berukuran 700 pb, 1200 pb dan 1500 pb dari sampel pemotongan enzim *StuI*. Gen dan promoter yang telah berhasil diisolasi kemudian diklon pada vector plasmid pGEM[®]-T Easy (Promega) dalam *Escherichia coli* galur DH5 α . Selanjutnya gen dan promoter disekuensing dengan menggunakan primer SP6 dan T7. Hasil penjajaran menggunakan BLASTn menunjukkan sekuen E-SAD mempunyai kemiripan dengan gen SAD dari beberapa tanaman. Promoter Putatif gen SAD berukuran 800 pb, 700 pb dan 1000 pb berhasil diklon dan disekuensing kemudian dianalisis dengan studi bioinformatik menggunakan program Geneious, Bioedit, BLASTn dan database promoter. Fragmen berukuran 800 pb dan 700 pb tidak teridentifikasi sebagai promoter gen SAD, sedangkan fragmen berukuran 907 pb positif sebagai 5' *flanking region* gen SAD yang kemungkinan memiliki daerah promoter gen SAD.

Kata kunci : promoter, gen SAD, PCR *genome walking*, kelapa sawit