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Isolation and Analysis of DNA Fragment of Genes Related to *Kopyor* Trait in Coconut Plant

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Abstract

Kopyor coconut is a natural mutant that has abnormal endosperm development. For the first time several genes that were suspected to be related to *kopyor* trait were identified based on the chemical compounds of the endosperm that different from that of normal coconut. Sucrose synthase (*SUS*), Stearoyl acyl carrier protein desaturase (*SACPD*), and Absicid acid insensitive (*ABI*) genes were isolated and analyzed. Four DNA fragments with length of 746, 738, 780, and 687 bp (CnSus1A, CnSus1B, CnSus2A, and CnSus2B) were obtained from *SUS* gene. Sequence analysis at DNA and amino acid level showed that CnSus1A, CnSus1B, CnSus2A, and CnSus2B were classified into monocot SUS group with nongrass SUS type. Isolation of *SACPD* gene resulted in one DNA fragment with DNA length of 716 bp. CnSacpd shared a high homology with *SACPD* gene of oil palm and soybean. Isolation of *ABI* gene resulted in two DNA fragments, CnAbi3A and CnAbi3B, with DNA length of 760 and 728 bp, respectively. CnAbi3A and CnAbi3B showed a high homology with *ABI3* gene of several plants. All DNA fragment obtained from *SUS, SACPD, ABI* genes were used as templates to design spesific markers for each corresponding gene. There were 7 specific primer sets designed, i.e., CnSUS1A, CnSUS1B, CnSUS2A, CnSUS2B, CnSACPD, CnABI3A, and CnABI3B.

Key words: *Cocos nucifera*, DNA analysis, *SUS* gene, *SACPD* gene, *ABI3* gene

Introduction

In Indonesia heterozygote *kopyor* coconut is a rare species, naturally only can be found in Java Island. *Kopyor* coconut is a result of natural mutation that is expressed in the endosperm, in which the endosperm develops into a soft and jelly-like endosperm, all or some parts becoming detached from the shell and mix with the liquid endosperm. Although the nut has a normal embryo, it will not germinate due to lack of energy for the growth of embryo. In the normal coconut, the endosperm contains galactomannan as source of energy that is required for germination process.

Galactomannan is degraded into galactose and mannose that has a function as carbon source and energy in the beginning of germination process (DeMason *et al*., 1985; Dirk *et al*., 1999). It is one of the cell wall polysaccharide compounds. In the endosperm of normal coconut, 61% of total polysaccharide was galactomannan (Balasubrahmaniam, 1976). In the case of *kopyor* coconut, one of the degraded enzymes for galactomannan, á-D galactosidase, is not active (Mujer *et al*., 1984), so it

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will disturb the normally degradation of galactomannan.

Endosperm of *kopyor* coconut has some chemical compounds such as carbohydrate, sucrose, and lipid that differ from the normal one. Total carbohydrate in the normal coconut endosperm was 29.1%, and total lipid was 62.4 %. In contrast, *kopyor* coconut endosperm contained 62.3% carbohydrate and 30.71% lipid (Santoso *et al.*, 1996). Sucrose content in the endosperm of *kopyor* coconut about 2 fold of normal coconut, while in the coconut water it could increase to 8 fold compared to liquid endosperm of normal coconut. For fatty acid composition, oleic acid (C18:1) and linoleic acid (C18:2) contents were higher in *kopyor* coconut endosperm. However, oleic acid, linoleic acid, and stearic acid (C18:0) were lower in the coconut water of *kopyor* coconut.

High sucrose and lower lipid contents can make the endosperm of *kopyor* coconut as a healthy refreshing drink. One of the important enzymes that involves in the synthesis of sucrose is sucrose synthase (SUS). Meanwhile, stearoyl-acyl carrier protein desaturase (SACPD) is important enzyme in the synthesis of fatty acid. The role of SUS is catalyses a reversible reaction of UDP-glucose+fructose-P"!sucrose+UDP (Sturn and Tang 1999). The stearoyl-acyl carrier protein desaturase (SACPD) enzyme converts stearic to oleic acid through the insertion of double bond at C9 position (Kachroo *et al.*, 2008).

Mobilization of galactomanan in the process of embryo germination is under system of metabolic control that differs among plants. In the legume and non-legume plants, abscisic acid (ABA) has been shown to act as a modulator of the biochemical and physiological interactions between the embryo and the endosperm (Buckeridge *et al.*, 2000; Potomati and Buckeridge, 2002).

Molecular analysis and characterization of sucrose synthase, stearoyl-acyl carrier protein desaturase, and abscisic acid genes have been studied in many plants (Potomati and Buckeridge, 2002; Komatsu *et al.*, 2002; Chiu *et al*., 2005; Pramanik *et al*., 2005; Byfield *et al*., 2006; Geromel *et al*., 2006; Sivasudha and Kumar, 2007). However, no molecular analyses of those genes in the *kopyor* coconut have been carried out. In the present study, several genes such as *SUS* (*Sucrose synthase*), *SACPD* (*Stearoyl-acyl carrier protein desaturase*), and *ABI* (*Abscisic acid insensitive*) were isolated and analyzed from tall heterozygote *kopyor* coconut.

Materials and Methods

Plant Material and DNA Extraction

Young leave of tall heterozygote *kopyor* coconut that just emerging was used as material source of genomic DNA. Leaf samples were taken from tall heterozygote *kopyor* coconut population in Margoyoso and Tayu, Pati, Central Java, Indonesia. In each population, ten coconut trees were randomly taken.

Approximately 50-100 mg fresh leave from every sample was cut into small pieces and placed together with small gear in the eppendoft tube. It was grinded to a fine powder in the crushed machine (Retsch MM301) for 3 min at 300 frequency. The process of DNA extraction that consisted of lysis, DNA binding, washing, and elution was done according to Plant Genomic DNA Mini Kit protocol (Geneaid, Geneaid Biotech Ltd. http://www.geneaid.com).

Primer and PCR Amplification

Kopyor coconut *SACPD*s and *ABI*s were amplified with primer sets that have been developed by CAB (Central for Agricultural Biotechnology, Kasetsart University, Thailand) for oil palm. Gene-specific primers for *SACPD* were 5'TAT CTA TAC CTT TCT GGT AGA GT3'-forward and 5'ACA CCA TAT TAA CGG AGT CTA CT3'-reverse. Primers to amplify *ABI* gene were taken

from degenerate primers ABI3 for oil palm. The oligonucleotide primers for *ABI* were 5'AAG GTG TTG AAG CAR AGY GAY GT3'-forward and 5'ATC ACT ATG AAR TCT CCY TCY TG3'-reverse. For *SUS*, degenerate primers were designed to a region of variability in exon 6 to 9 and 11 to 12 using GeneDoc (http://www.psc.edu/biomed/genedoc), BLAST (http://www.ncbi.nlm.nih.gov/) and ClustalW (http://www.ebi.ac.uk/embl) programs. Primer sequences of *SUS* were designed by comparing 72 *SUS* genomic sequences of dicot and monocot plants available in several DNA GenBank database: http://www.ncbi.nlm.nih.gov/;http://www.comphio.dfci.harvard.edu/tgi/; and http://www.ebi.ac.uk/embl. The designing of *SUS* gene primers resulted in two sets of degenerate primers namely SUS1 and SUS2. Degenerate primers of SUS1 consisted of 5'GGT TAT CCT GAT ACY GGN GGN CA3'-forward and 5'ACA AGG TTT CCA TCA CTR TAR TTN CC3'-reverse. For degenerate primers of SUS2 consisted of 5'GAT CCC AAG TTC AAC ATT GTN TCN CC3'-forward and 5'CCA AAT GCT TCA TAR AAN GCN GG3'-reverse.

Amplification of each 15 µl PCR reaction contained 1 µl genomic DNA template, 0.75 µl each primer, 2.7 µl dNTPmix, 1.5 µl 10x buffer, 0.15 µl $MgCl_2$ (Fermentas), and 0.075 µl Taq DNA polymerase (RBC). The PCR cycling was initiated by a 4 min at 94°C denaturation followed by 40 cycles of 45 sec at 94°C, 45 sec at 55°C, 1.30-2.0 min at 72°C and a final extension at 72°C for 5 min. PCR product was confirmed by electrophoresis on 1% agarose gel and stained in ethidium bromide.

Cloning and Nucleotide Sequencing

The amount of 1.5 µl of purified PCR fragments were ligated into 0.5 µl pGem-T vector using 0.5 µl T4 DNA ligase enzyme and 2.5 µl buffer at 4-5°C overnight. DNA plasmid of 2.5 µl was cloned into 40 µl competen cell of *Escherichia coli* strain DH 10B by electricity shock. *E. coli* was shaken on the shaker machine at 220 rpm and 37°C for two hours. *E. coli* was spread out on a solid LB media contained amphysilin, IPTG, and X-gal and then incubated in the incubator at 37°C overnight. The recombinant plasmid was checked by electrophoresis in 1% agarose gel.

E. coli contained a recombinant plasmid of 5 µl was cultured in 5 ml liquid LB media and shaken on the shaker machine at 220 rpm for 16 h. The recombinant plasmid was extracted using High-Speed Plasmid Mini Kit (Geneaid). Each recombinant plasmid was sequenced at the First Base Laboratories Selangor Darul Ehsan, Malasyia.

Nucleotide Sequence Analysis

The nucleotide sequences of each amplified fragment were analyzed with Chromas Lite version 2.01 (http://www.technelysium.com.au). Sequences were aligned and manually edited to determine introns and exons position using GeneDoc software (http://www.psc.edu/biomed/genedoc). Translation to amino acid sequences were done with the Genebee tools for data mining: tblastx (http://www.genebee.msu.su/blastnew/blastform.php?program=tblastx). Multialignments of amino acid were obtained by ClustalW program (http://www.genebee.msu.su/clustal/advanced.html) to construct a phylogenetic tree based on the deduced amino acid and in the phylip-phylogram format.

Results and Discussion

Nucleotide Analysis of SUS, SACPD and ABI DNA Fragment

Nucleotide sequence analysis showed that the PCR product amplified by SUS1 and SUS 2 primers contained two different DNA sequences and sizes. The SUS1 primers

amplified two PCR fragments of 746 bp (CnSus1A) and 738 bp (CnSus1B) while primers for *SUS2* gene amplified two PCR fragments of 780 bp (CnSus2A) and 687 bp (CnSus2B) (Table 1).

Table 1. DNA fragment analysis of *SUS*, *SACPD*, and *ABI3* genes in *kopyor* heterozygote tall coconut

Genes	DNA fragments	Fragment length (bp)	Exon position	Intron position
Sucrose synthase	CnSus1A	373+3 intron (78+161+134)=746	E-6=1-24 E-7=103-198 E-8=360-532 E-9=667-746	I-6=25-102 I-7=199-359 I-8=533-666
	CnSus1B	373 + 3 intron (84+161+120) = 738	E-6=1-24 E-7=109-204 E-8=366-539 E-9=660-738	I-6=25-108 I-7=205-365 I-8=540-659
	CnSus2A	482+2 intron (160+138)= 780	E-11=1-140 E-12A=301- 619 E-12B=758-780	I-11A=141- 300 I-11B=620-757
	CnSus2B	482+2 intron (80+125)=687	E-11=1-140 E-12A=22-539 E-12B=665-687	I-11A=141-220 I-11B=540-664
Stearoyl Acyl Carrier Protein Desaturase	CnSacpd	229 + 1intron (487)= 716	E-2=1-75 E-3=563-716	I-2=76-562
Absicic Acid Insensitive	CnAbi3A	251+3 intron (138+259+112)= 760	E-1=1-54 E-2=193-293 E-3=553-599 E-4=712-760	I-1=55-192 I-2=294-552 I-3=600-711
	CnAbi3B	251+3 intron (129+138+210)=728	E-1=1-54 E-2=184-284 E-3=423-469 E-4=680-728	I-1=55-183 I-2=285-422 I-3=470-679

Nucleotide fragments of CnSus1A or CnSus1B that were amplified by primers SUS1 at from exon 6 to exon 9 contained three introns with different sizes (Figure 1A).

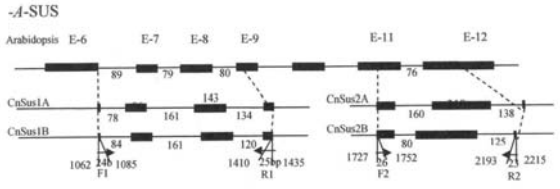

Figure 1A. Position of SUS1 (SUS1-F1 & SUS1-R1) and SUS2 (SUS2-F2 & SUS2-R2) primer sets in the partial structure of *SUS* gene of *Arabidopsis thaliana* (NM122090) and exon & intron position of CnSus1A, CnSus1B, CnSus2A and CnSus2B DNA fragments relatively to *Arabidopsis SUS* gene.

The open reading frame (translated sequence) of CnSu1A or CnSus1B started at the end of exon 6, exon 7, exon 8, and part of exon 9. That open reading frame consisted of nucleotides of 373 bp long (Figure 1A). The SUS2 primers which amplified a part of *SUS* gene from exon 11 to exon 12 contained two introns. Those primers were expected amplify one intron such as shown by the DNA fragment of Arabidopsis if it was amplified at the same exon position. The position of intron 11 of CnSus2A or CnSus2B was the same position as intron 11 of Arabidospis, while the position of the next intron was in the middle of exon 12 and split them into two parts (Figure 1A).

The SACPD primers amplified only one PCR fragment of 716 bp (CnSacpd) (Table 1). Meanwhile the ABI3 primers amplified two PCR fragments of 760 bp (CnAbi3A) and 728 bp (CnAbi3B). The amplified fragment of CnSacpd consisted of one intron (487 bp) and total translated sequence of 229 bp from exon 2 to exon 3 (Figure 1B).

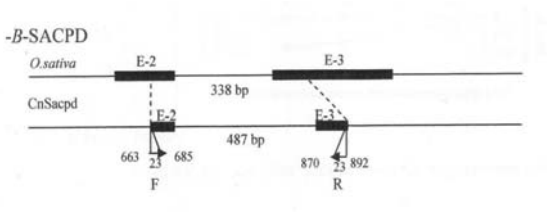

Figure 1B. Primer set of SACPD (SACPD-F & SACPD-R) in the partial structure of *SACPD* gene of *Oryza sativa* (NM0010517500) and exon & intron position of CnSacpd DNA fragments relatively to oryza *SACPD* gene.

Fragment of CnAbi3A or CnAbi3B that was amplified by ABI3 primers from exon 1 to exon 4 contained 3 introns. The total translated sequence of CnAbi3A or CnAbi3B was 251 bp (Figure 1C).

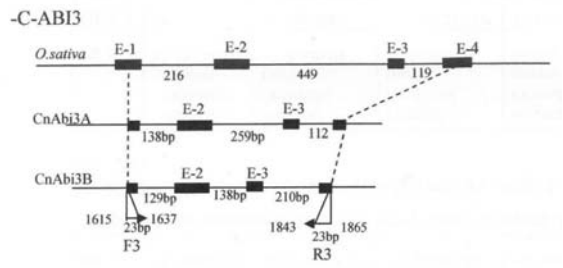

Figure 1C. Primer set of ABI3 (ABI3-F3 & ABI3-R3) in the partial structure of *ABI* gene of *O.sativa* (AP003436) and exon & intron position of CnAbi3A & CnAbi3B DNA fragments relatively to *Oryza ABI* gene.

Analysis of amino acid and phylogeny

Total exons of *SUS*, *SACPD*, and *ABI* DNA fragments were translated using tblastx program to know the composition of sequence and length of amino acid. The jointed exons of CnSus1 and CnSus2 were 808 bp long and encoded for a protein of 270 amino acids (Figure 2). This protein showed high similarity (90.5 and 88.5% identity) to the Sucrose synthase1 from *Oncidium* cv. Goldiana (AF530567) and *Tulipa gesneriana* (X96938) (Balk & de Boer, 1999). It also showed high similarity (85-87.5% identity) to other monocot plants such as *Bambusa oldhamii* (AF412037), *Oryza sativa* (Z15028), *Saccharum officinarum* (AF263384), *Zea mays* (L22296), and *Triticum aestivum* (AJ000153).

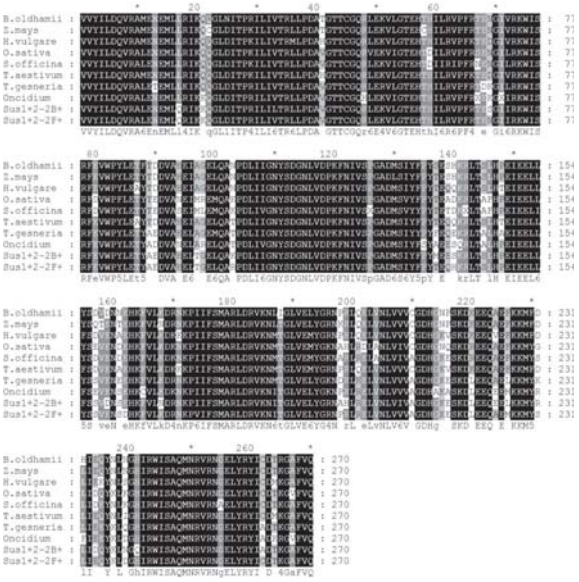

Figure 2. Aligment of the deduced amino acid sequences derived from *kopyor* heterozygote tall coconut Sucrose synthase (CnSus1A+B and CnSus2A+B) and other monocot plants *SUS* such as *Oncidium* AF530567, *Tulipa gesneriana* X96938, *Triticum aestivum* AJ000153, *Sacharrum officinarum* AF263384, *Oryza sativa* Z15028, *Hordeum vulgare* X69931, *Zea mays* L22296, dan *Bambusa oldhamii* AF412037.

The CnSacpd DNA fragment consisted of 229 bp long of coding region and encoded a protein of 76 amino acids (Figure 3A).

This amino acid sequence was 98% similar to *SACPD* amino acid of *Elaeis guineensis* var. tenera (AF143501 and EGU68756). It was also showed high homology (90-97% identity) with *SACPD* of high lipid seed plants such as *Glycine max* (L34346), *Arachis hypogea* (AF172728), Sesame (D42086), *Z. mays* (AY104235), *Helianthus annus* (U70374), and *Ricinus communis* (M59857).

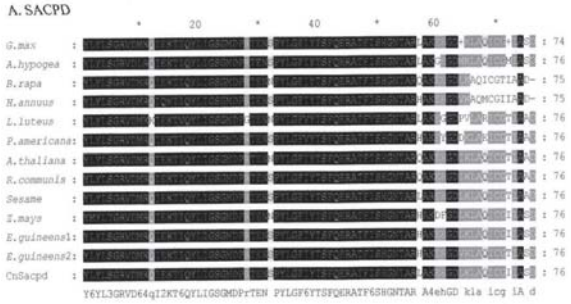

Figure 3A. Alignment of amino acid CnSacpd, *Elaeis guineensis* var. tenera AF143501 dan U68756, *Glycine max* L34346, Sesame D42086, *Arachis hypogaea* AF172728, *Ricinus communis* M59857, *Helianthus annuus* U70374, *Arabidopsis thaliana* AF395441, *Persea americana* AF116861, *Z. mays* AY104235, and *Lupinus luteus* AF139377.

Total coding region of CnAbi3A or CnAbi3B DNA fragment was 251 bp long in which encoded 82 amino acids (Figure 3B). The deduced amino acid sequences exhibited 92.3% identity between of the two CnAbi3. Sequence analysis of CnAbi3B amino acid showed high homology (96.5 % identity) with amino acid of *ABI3* (ABA insensitive 3) gene of *Pisum sativum* (Figure 3B).

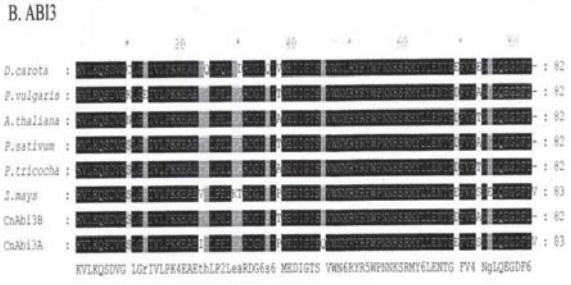

Figure 3B. Alignment of amino acid CnAbi3A and CnAbi3B, *Z. mays* M60214, *Populus trichocarpa* AJ003166, *Pisum sativum* AB080195, *A. thaliana* X68141, *Phaseolus vulgaris* AB085836, and *Daucus carota* AB005558.

A phylogenetic dendrogram of plant *SUS* including *kopyor* heterozygote tall coconut *SUS* was made based on the deduced amino acid sequences by multi-alignment analysis using ClustalW program in the Genebee tools (Figure 4). The dendrogram showed that plant SUS genes could be divided into three groups: group 1 was monocot plant *SUS* consisted of *B. oldhamii*, *O. sativa*, *S.officinarum*, *Z. mays*, *T. Aestivum*, and *H. vulgare*. Group 2 was dicot plant *SUS1* such as *Citrus lanatus*, *Vicus faba*, *Pisum sativum*, *Medicago truncale*, *Gossypium hirsutum*, *Citrus unshiu*, *Lycopersicon esculentum*, and *Daucus carota*. The third group was *kopyor* heterozygote tall coconut (CnSus1A+CnSus2A and CnSus1B+CnSus2B) and *Oncidium*.

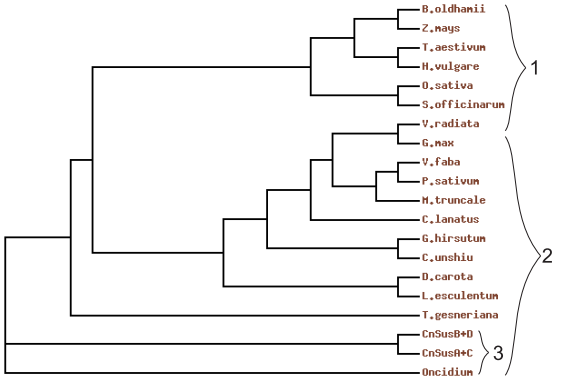

Figure 4. Alignment of the deduced amino acid of *kopyor* heterozygote tall coconut *SACPD* and *ABI* DNA with monocot & dicot plant *SACPD* and *ABI*. A. Alignment of amino acid CnSacpd, *Elaeis guineensis* var. tenera AF143501 dan U68756, *Glycine max* L34346, Sesame D42086, *Arachis hypogaea* AF172728, *Ricinus communis* M59857, *Helianthus annuus* U70374, *Arabidopsis thaliana* AF395441, *Persea americana* AF116861, *Z. mays* AY104235, and *Lupinus luteus* AF139377. B. Alignment of amino acid CnAbi3A & CnAbi3B, *Z. mays* M60214, *Populus trichocarpa* AJ003166, *Pisum sativum* AB080195, *A. thaliana* X68141, *Phaseolus vulgaris* AB085836, and *Daucus carota* AB005558.

Comparison of deduced amino acid sequences of plant *SACPD* showed that their phylogenetic dendrogram could be clustered into two groups. *Kopyor* heterozygote tall coconut *SACPD* (CnSacpd) was in the same group with *E.guineensis*, *G.max*, *H.annus*, *Z.mays* and *A.hypogea* (Figure 5A). A Phylogenetic dendrogram of plant *ABI* was clustered into two groups which were CnAbi3A and CnAbi3B separated in the different group. Absicic acid insensitive of CnAbi3A gene was in the same group with *Z.mays*, *A.thaliana*, *P.trichocarpa*, and *D.carota* while CnAbiC clustered with *P.sativum* and *P.vulgaris* (Figure 5B).

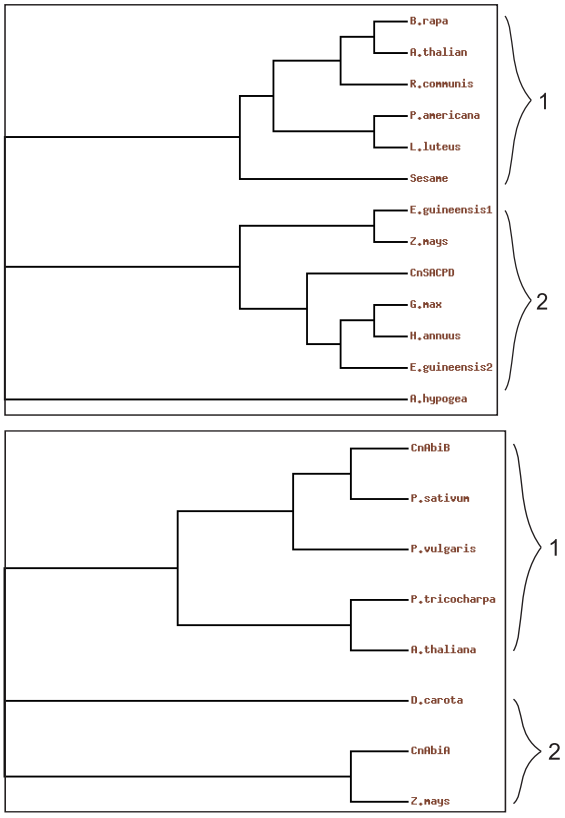

Figure 5. Dendrogram of *SACPD* and *ABI* amino acid sequences with other monocot and dicot plants that was available in data base. A. Dendrogram of CnSacpd with *Brassica rapa* X60978, *Arabidopsis thaliana* AF395441, *Ricinus communis* M59857, *Persea americana* AF116861, *Lupinus luteus* AF139377, Sesame D42086, *Elaeis guineensis* AF143501 dan U68756, *Z. mays* AY104235, *G. max* L34346, *Helianthus annuus* U70374, and *Arachis hypogaea* AF172728. B. Dendrogram of CnAbi3A and CnAbi3B with *P. sativum* AB080195, *Phaseolus vulgaris* AB085836, *Populus trichocarpa* AJ003166, *A.thaliana* X68141, *D. carota* AB005558, and *Z. mays* M60214

Kopyor coconut fruits can not be used as seed due to its endosperm will be decay after the nuts harvest from the tree. Consequently, the embryo can not utilize this endosperm as source of energy in the germination process.

Coconut endosperm mainly consisted of galactomannan. Galactomannan was accumulated in the cell wall of endosperm as a cell wall storage polysaccharide (Buckeridge *et al.*, 2000). The endosperm of palmae family has very thick cell wall that function as carbohydrate storage included the galactomannan, while lipid and protein are stored in the endosperm cell (DeMason *et al*., 1983). During the germination process of embryos, the energy and nutrients are supported by liquid coconut (sucrose) and endosperm (carbohydrate/ galactomannan and lipid) (Balachandran and Arumughan, 1995).

The endosperm of *kopyor* coconut contained sucrose that was higher compared to normal coconut (Santoso *et al*., 1996). In contrast, the lipid content was lower than the normal one. One of the fatty acids, oleic acid, also was found very high. Base on the those composition of chemical compounds, sucrose synthase (*SUS*), stearoyl acyl carrier protein desaturase (*SACPD*), and absicid acid insensitive (*ABI*) are genes that predicted associated to *kopyor* trait in the coconut plant. In the coffee fruit, the activity of sucrose synthase increased during the end of endosperm development and parallel to the accumulation of sucrose (Geromel *et al*. 2006). In the several studies, the synthesis of sucroce was done concomitant with degradation of galactomannan into mannose and galactose. Meanwhile, stearoyl acyl carrier protein desaturase (*SACPD*) is a key enzyme that converts stearic to oleic acid (Kachroo *et al*., 2008). Absicic acid insensitive (*ABI*) gene has an important role in the mobilization of galactomannan (Potomati and Buckeridge, 2002).

In this study, four DNA fragments of CnSus could be isolated and analyzed. Two fragments, CnSus1A and CnSus1B, were amplified by degenerate SUS1 primers from exon 6 to exon 9 and two fragments, CnSus2A dan CnSus2B, amplified by degenerate SUS2 primers from exon 11 to exon 12. Sequence alignment of the two CnSus1 showed 88.8% similarity to one another at the DNA level and 91.6% at the amino acid level. The sequence alignment of CnSus2A and CnSus2B showed more nucleotide variation in the DNA level and only had 78.5% sequence similarity, however, the similarity of the two fragments were high in the amino acid level (90.5%). The differences between CnSus1A and CnSus1B or CnSus2A and CnSus2B were not only in their nucleotide sequences but also in the length of their fragments.

The different fragment length of the sequences was because of the short-long variation in the intron. Exon/intron structures of two sucrose synthase (CnSus1A and CnSus1B) of *kopyor* heterozygote tall cconut showed that the length of exon 7 and 8 were 96 and 174 bp long, respectively. The two exons had the similar length to those exons of citrus sucrose synthase (Citrus SUS1 dan Citrus SUSA) (Komatsu *et al.,* 2002). At the 12th exon on the sucrose synthase of CnSus2A and CnSus2B was split at the reverse primer position and divided them into two exons with one intron insertion. This intron insertion is typical of the monocot *SUS* group which is not be found at the 12th exon of dicot *SUS* group (Komatsu *et al.,* 2002). At the numerous of plant species, the coding region of SUS gene consisted of 806-812 amino acids (Komatsu *et al.,* 2002; Chiu *et al.,* 2006; Geromel *et al.,* 2006). These coding region was separated on the 13 exons for dicot plants while for monocot plants was separated on the 15 exons. Two extra exons in the monocot plants were resulting from intron insertion at the exon 6 and 12 (Komatsu *et al.* 2002).

Plant sucrose synthase is classified into four groups: a monocot group, a dicot SUS1 group, a dicot SUSA group, and New group (Fu and Park, 1995; Sturm *et al.*, 1999; Komatsu *et al.*, 2002). Sucrose synthase gene of monocot plants can be further divided into three groups: grass SUS1, grass SUS2, and nongrass SUS (Chiu *et al.*, 2006). Phylogenetic analysis of amino acid sequence (Figure 4) revealed that sucrose synthase of CnSus1A+CnSus2A and CnSus1B+CnSus2B were classified into nongrass SUS. It seems that sucrose synthase of *kopyor* heterozygote tall coconut was closer to the orchid spesies than the grass plant.

In this study, we obtained one fragment of *SACPD* (CnSacpd) with the length of 716 bp. Amino acid sequence analysis showed that CnSacpd had 965 and 97% sequence similarity with *E. guineensis*1 (U68756) and *E. guineensis*2 (AF143501) *SACPD*s. Amino acid variations between CnSacpd and other plants including oil palm were found mostly in exon 3 (Figure 3A). Isolation of soybean *SACPD* genes revealed that SACPD proteins consisted of 386-411 amino acids which were distributed in 3 exons (Byfield *et al*. 2006; Kachroo *et al*. 2008) and the amino acid variations were also found in exon 3, not in exon 1 and 2 of the soybean *SACPD*s (Byfield *et al.*, 2006).

There were two fragments of ABI genes that obtained, CnAbi3A (760 bp) and CnAbi3B (728 bp). Nucleotides of CnAbi3A and CnAbi3B shared 66.5% identity, however, at the amino acids level the similarity were very high (92.3% identity). Perhaps the variations occurred in the third codon so there were not changes the translations of their amino acids. Interestingly they were separated in the different groups, CnAbi3A was in the same group with *Z. mays*, while CnAbi3B was in one group with *P. sativum* and *P. vulgaris*. Both CnAbi3A and CnAbi3B showed 90% nucleotides similarity with *ABI3* genes from other plant species such as *A. thaliana*, *P. sativum*, *P. tricocharpa*, and *Z. mays*. In the Arabidopsis has been identified 3 loci of *ABI*, i.e. *ABI1*, *ABI2*, dan *ABI3*. The *ABI1* and *ABI2* have important role in the growing phase of plants but *ABI3* is in the development of plant seeds (Koornneef *et al.*, 1984). These findings were consistent with *ABI3* gene in the *P. tricocharpa* species which its expression found only in the developing seeds. It consists of 736 amino acids and contains 5 introns (Rohde *et al*., 1998).

Based on the several DNA fragments of *SUS*, *SACPD*, and *ABI* genes that have been isolated, we made them as a DNA template to create a specific marker molecular for its corresponding genes as presented at the Table 2.

Table 2. Specific primers of Sucrose synthase (*SUS*), Stearoyl Acyl Carrier Protein Desaturase (*SACPD*), and Absicic Acid Insensitive (*ABI*) genes that was designed based on DNA fragment sequences from *kopyor* heterozygote tall coconut

Genes	Primer name	Sequence of primer set 5′ .....3′
Sucrose synthase	-CnSUS1A	F-GGTTATCCTGATACYGGNGGNCA
		R-TTCCGGATGATCCCATTCTCCGT
	-CnSUS1B	F- GGTTATCCTGATACYGGNGGNCA
		R-TTTCGGATAATACCAATCTCTGT
	-CnSUS2A	F-TCTATTTACTTCCCTTACATGG
		R-TAGAAGGCAGGCTTCAACCAAC
	-CnSUS2B	F-TCCATCTACTTCCCATACACCG
		R-TAGAAGGCCGGCTAAGCAAAGG
Stearoyl Acyl Carrier Protein Desaturase	-CnSACPD	F-CTTTCTGGTAGAGTGGACATGAA
		R-GTCCTAGGATCCAAAGACAGC
Abscisic Acid Insensitive	-CnAbi3B	F-GGGAGAATTGTTCTGCCAAAGG
		R-CATTGGCTTTCACAAAGTCCC

These specific primers will be used for analysis diversity of *SUS*, *SACPD*, and *ABI* genes in the *kopyor* homozygote coconut and the normal one in our future works and one of these markers are expected can be used to differentiate between kopyor and normal coconut.

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