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Validated SNAP markers based on the CYP P450 87 A3 gene in coconut (*Cocos nucifera*) are associated with yearly stem height increase

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Abstract. *Hatta ANNL, Sukma D, Maskromo I, Sudarsono S. 2023. Validated SNAP markers based on the CYP P450 87 A3 gene in coconut (*Cocos nucifera*) are associated with yearly stem height increase. Biodiversitas 24: 2503-2512.* Tall and dwarf coconuts are coconut types used worldwide as the parents in hybrid coconut production, and they differ in their yearly stem height. The yearly stem height of coconuts is estimated as 11 leaf scars length. The CYP P450 87 A3 gene is essential in the yearly stem-height increase (YSI) character. This study aims to validate single nucleotide-amplified polymorphism (SNAP) markers based on sequence variabilities of the coconut CYP P450 87 A3 gene and analyze their association with the coconut YSI character. The CYP P450 87 A3 partial genomic fragments were PCR amplified, sequenced, and used to develop SNAP markers. The markers were subsequently validated using 155 samples of 31 coconut varieties. The analysis showed that at least 17 single nucleotide polymorphisms (SNPs) exist in the exon-1 and 2 of the coconut CYP P450 87 A3 gene, and SNAP markers were successfully developed. Single-locus analysis showed that the validated CYP2_SNP1 SNAP marker locus was associated with coconut YSI characters. The developed SNAP marker based on the partial CYP P450 87 A3 gene can potentially support future marker-assisted selection (MAS) in coconut breeding, especially for developing coconut with low YSI character.

Keywords: Dwarf coconut, leaf scars length, marker-assisted selection (MAS), single-locus analysis, tall coconut

INTRODUCTION

Coconut (*Cocos nucifera* L.) is divided into two types, namely tall and dwarf coconut (Pan et al. 2018). The two types of coconut classification are based on their differences in several characteristics, such as the presence of a ball, the yearly stem height increase (YSI), the nut size, and yield (Nair 2021). The YSI character is a vital parameter to differentiate the two coconut types (Boonkaew et al. 2018). Breeding of Indonesian coconut is carried out by crossing tall and dwarf coconuts to develop hybrid varieties (Mahayu et al. 2021). The hybrid coconut varieties combine the best characteristics of the tall and the dwarf parents, such as excellent quality endosperm for processing and high nut yield (Sudha et al. 2022). Moreover, the YSI character in hybrid coconuts is intermediate between the parents.

The YSI character is estimated by measuring the height of 11 leaf scars, and it is used to predict the increase in yearly stem height of the coconut palms over specific years (Martial et al. 2019). The productive period of the coconut palms is associated with the YSI character since the higher the coconut stem height, the more challenging to harvest the nuts. Therefore, coconut varieties having a low YSI character are more desirable. Tall coconuts show 100-115 cm of the YSI character, while dwarf coconuts show only 40-55 cm (Geethanjali et al. 2018).

The CYP gene is one of the many genes regulating plant stem height characteristics (Wang et al. 2017), and the CYP P450 87 A3 gene is an auxin response gene influencing auxin levels (Wei and Chen 2018). Auxin is a phytohormone essential in cell elongation (Vosolsobě et al. 2020). Moreover, auxin affects plant stem height (Emenecker and Strader 2020), and CYP P450 87 A3 is an auxin-induced gene affecting stem height increase (Sidhu et al. 2020). It would be interesting to evaluate nucleotide sequence variability of the CYP P450 87 A3 among dwarf and tall coconuts and their association with the differences in their YSI characteristics. Gene-based single nucleotide-amplified polymorphism (SNAP) molecular markers have been used in plant breeding since they are simultaneously co-dominant and bi-allelic (Pesik et al. 2017; Sukma et al. 2021). Moreover, the SNAP markers can efficiently distinguish homozygous from heterozygous genotypes (Devi et al. 2017). The SNAP markers were developed based on single nucleotide polymorphism (SNP) loci, arising as base differences at a specific locus due to the diversity of DNA sequences in the plant genome (Pesik et al. 2017; Larekeng et al. 2018; Rahmawati et al. 2022). The presence of nucleotide sequence variation in genes associated with plant height increase has been reported in

oil palm (Babu et al. 2019), soybean (Assefa et al. 2019), and wheat (Zhang et al. 2021).

Nucleotide sequence variabilities of various coconut genes have been reported (Dumhai et al. 2019; Muñoz-Pérez et al. 2022), but nucleotide sequence variabilities of genes associated with YSI character in coconut have never been previously reported. Such nucleotide sequence variability data should aid molecular marker development for predicting the YSI character in coconut. Therefore, isolation, sequencing, and characterization of genes associated with a YSI characteristic in coconut, such as the CYP P450 87 A3, are necessary. This study aims to validate single nucleotide-amplified polymorphism (SNAP) markers based on sequence variabilities of the coconut CYP P450 87 A3 gene and analyze their association with the coconut YSI characteristics.

MATERIALS AND METHODS

Plant materials

Young leaf of coconut accessions from the Indonesian Palm Research Institute (IPRI), Manado, Indonesia, was used for DNA isolation, including 31 varieties (9 tall varieties, 10 dwarf varieties, and 12 hybrid varieties). The Tall coconut varieties evaluated include Tenga Tall (TET), Bali Tall (BIT), Palu Tall (PUT), Mapanget Tall (MTT), Mamuaya Tall (MMT), Sawarna Tall (SWT), Banyuwangi Tall (BWT), Jepara Tall (JPT), and Takome Tall (TAT). The Dwarf coconut varieties used include Nias Yellow Dwarf (NYD), Bali Yellow Dwarf (BYD), Malaysia Yellow Dwarf (MYD), Malaysia Red Dwarf (MRD), Raja Dwarf (RAD), Salak Dwarf (SKD), Sweet Green Dwarf (SGD), Tebing Tinggi Dwarf (TTD), Jombang Green Dwarf (JGD), and Nias Green Dwarf (NGD). Meanwhile, the evaluated hybrid coconuts varieties include: KHINA-1, KHINA-2, KHINA-3, KHINA-5, HENGNIU-1, HENGNIU-2, IMA-1, IMA-2, IMA-3, IMA-4, IMA-5, and IMA-6 hybrids. Five accessions were sampled from each coconut variety. All coconut samples were used for marker validation and association with the targeted YSI phenotype. Most research activities were conducted at the Plant Molecular Biology (PMB) Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University. The field data collection and genotyping of coconut accessions were conducted from August 2021 to August 2022.

Procedures

Isolation of partial CYP P450 87 A3 gene

The CYP P450 87 A3 genomic sequences were searched in the annotated genome of coconut cv. Hainan Tall (ASM812446v1), publicly available in the NCBI genome database (https://www.ncbi.nlm.nih.gov/assembly/GCA_008124465.1). The CYP specific primers were designed based on the coconut CYP P450 87 A3 genomic sequences using Geneious software (https://www.geneious.com/prime/), and they were used to amplify the Exon 1 and Exon 2 of the CYP P450 87 A3 gene. Young leaf of coconut was used as samples, and a modified CTAB method (Larekeng et al. 2015) were used for DNA isolation. Subsequently, DNA quality and quantity were examined using 1% agarose gel electrophoresis in 1x sodium borate (SB) buffer. The agarose gel was stained with GelRed™ (Biotium, Fremont, California) for DNA fragment visualization and photographed for data recording.

The PCR amplification of partial CYP P450 87 A3 gene was conducted using the designed CYP specific primers and the total DNA of four tall and four dwarf coconut varieties (tall coconuts: MTT, PUT, BIT, and TET; dwarf coconuts: BYD, NYD, MYD, and MRD). These coconut varieties were selected because they were the parental lines used to generate the evaluated hybrid coconuts in this study. All PCR amplifications were carried out in a Takara PCR Thermal Cycler. The PCR mixes follow the standard procedures described in MyTaq Red Mix Bioline protocols (Meridian Bioscience, Cincinnati, USA). The total of 25 µL per PCR reaction comprised MyTaq Mix (12.5 µL), each forward and reverse primer of 4 µM (0.5 µL), DNA template (1.5 µL), and ddH2O (up to 25 µL). The PCR amplification conditions comprised initial denaturation at 95°C for 1 minute, 35 cycles of denaturation at 95°C for 15 seconds, primer annealing at the appropriate temperature for each primer pair (CYP1 primer pair of 51ºC, and CYP2 of 56.4ºC) for 15 seconds, primer extension at 72°C for 10 seconds, and one cycle of final primer extension at 72°C for 5 minutes. The PCR-amplified products were evaluated using 1% agarose gel electrophoresis in 1x SB buffer at a constant voltage (100 V) for 30 minutes. Staining and recording of agarose gel electrophoresis were as previously described.

Identification of SNP sites in the CYP P450 87 A3 gene

The single band of the PCR-amplified putative CYP P450 gene was sent for direct PCR product DNA sequencing to 1st BASE, Singapore (https://order.base-asia.com/). Meanwhile, since coconut cv. TET is heterozygous, so the TET partial CYP P450 DNA fragments were sequenced by cloning at 1st BASE, Singapore, and three independent sample colonies were selected for DNA sequencing, and the sequences were used as references for the direct PCR product sequencing results. All partial CYP P450 putative sequence raw data were processed using the Geneious Prime version 2021.2.2 (https://www.geneious.com/prime/). After removing low-quality sequences, the putative CYP P450 DNA sequences were subjected to the basic local alignment search tool (BLAST) analysis to confirm their identity as fragments of the CYP P450 gene.

The CYP P450 accessions from coconut cv. Catigan Green Dwarf (CaGD), Chowgat Green Dwarf (ChGD), Hainan Tall (HNT), and oil palm assembled genomes were also retrieved from NCBI Genome Database using BLAST for genome analysis. Multiple sequence analysis (MSA) for all CYP P450 genomic sequences downloaded from NCBI and the determined CYP P450 sequences from eight Indonesian coconut cultivars was carried out using Geneious Prime version 2021.2.2 (https://www.geneious.com/prime/). The MSA output was exported to Mega-X

version 10.0.5 (https://megasoftware.net/) for the phylogenetic tree construction. Subsequently, results of the CYP P450 MSA were also used to identify the presence of single nucleotide polymorphism (SNP) sites among the introns (intronic SNPs) and the exons (exonic SNPs). Hence, synonymous and non-synonymous SNPs in the exon regions were also evaluated.

SNAP primer design based on the CYP P450 87 A3 and marker validation

Primers used to generate single nucleotide amplified polymorphism (SNAP) markers were designed based on the identified SNPs in the CYP P450 sequences using WebSNAPER (https://pga.mgh.harvard.edu/cgi-bin/snap3/websnaper3.cgi). Three primers were selected for each of the identified SNP, namely reference (Ref) and alternate (Alt) forward primers and a reverse (Rev) primer. Genotyping for each SNAP marker locus of each coconut sample was carried out using two PCR reactions. The first PCR used a pair of Ref and Rev primers, and the second used a pair of Alt and Rev primers. As previously described, the generated PCR products were subjected to 1% agarose gel electrophoresis. Each coconut sample's genotypes were determined based on the profiles of the Ref-Rev and Alt-Rev PCR amplicons.

Data analysis

Recorded agarose gel photographs of the profiles of the PCR amplicons for each coconut accession were used to score the presence or absence of either PCR product using combinations of the Ref-Rev (Ref allele) or Alt-Rev (Alt allele) primers, and the score data were tabulated as binary data using an MS Excel spreadsheet software. For subsequent genetic analysis, the binary data were converted into genotypes for each SNAP marker locus and the evaluated individual. Subsequently, in this study, the predicted genotypes based on the evaluated SNAP marker loci of 155 coconut samples belonging to 31 coconut cultivars are used to analyze the marker and the YSI characteristic association. The association analysis was done using the STAR 2.0.1 application (http://bbi.irri.org/products).

RESULTS AND DISCUSSION

Isolation of partial CYP P450 87 A3 gene

The CYP P450 87 A3 gene structure in the assembled Hainan Tall coconut genome comprised 14,846 bp, with nine exons and eight introns. The diagrammatic structure of the CYP P450 87 A3 gene is presented in Figure 1A. CYP P450 87 A3 gene fragments were isolated using CYP P450 specific primers, and the primers were targeted to amplify Exon 1 (CYP-E1 forward and reverse primers) and Exon 2 (CYP-E2 forward and reverse primers) of the CYP P450 87 A3 (Table 1). The eight coconut samples used for CYP P450 87 A3 DNA fragment isolation comprised four dwarf coconuts (BYD, MRD, MYD, and MRD) and four tall coconuts (BIT, MTT, PUT, and TAT).

This study successfully amplified putative exons 1 and 2 of the CYP P450 87 A3 gene from eight Indonesian coconut genomes (Figure 1B). The putative CYP P450 87 A3 fragment containing exon 1 was approximately 700 bp, while that containing exon 1 was 600 bp (Figure 1B). The generated PCR amplicon sizes were as expected based on the CYP P450 87 A3 gene structure of Hainan Tall coconut (Figure 1A). The PCR amplicons were subsequently sequenced to determine their nucleotide compositions. Amplifying the target DNA using the designed specific primers is one of the essential steps in molecular biology research (Kim and Park 2019). DNA sequencing is carried out if the correct PCR amplicon sizes are obtained (Sun et al. 2020).

The two coconut types (dwarf and tall) were selected to represent low and high YSI characteristics. Meanwhile, the study focused on the CYP P450 87 A3 gene since the gene has been associated with plant height characteristics (Tu et al. 2020). The CYP P450 87 A3 is an auxin-induced gene essential in plant growth, development, and stress responses (Sekhar et al. 2022). Transgenic *Arabidopsis* expressing the ginseng CYP P450 showed phenotypically reduced plant height and was herbicide tolerant. The plant height reduction is associated with increased expression of various genes converting active gibberellic acid (GA) into inactive forms (Khanom et al. 2019). The association of palm height increment and SNPs in oil palm has also been evaluated (Ong et al. 2018).

DNA sequencing and analysis of the putative CYP P450 87 A3 fragments

The direct PCR DNA sequencing results of the E1 and E2 amplicons from eight coconut accessions confirmed they were partial CYP P450 87 A3 fragments. Representative samples of the putative coconut cv. Bali Tall (BIT) exon 1 containing fragment (CYP-E1, 667 bp) and exon 2 (CYP-E2, 620 bp) sequencing results of CYP P450 87 A3 were presented in Figures 2A and 2B. The nucleotide sequences of the exon 1 fragment (CYP-E1) of coconut CYP P450 87 A3 comprised 184 bp of 5' non-coding region (NCR), 331 bp of exon 1, and 152 bp of partial intron 1 (Figure 2A). Meanwhile, the nucleotide sequences of the exon 2 fragment (CYP-E2) of coconut CYP P450 87 A3 comprised 256 bp of partial intron 1, 96 bp of exon 2, and 268 bp of partial intron 2 (Figure 2B). CYP-E1 and CYP-E2 fragments from other coconut varieties show the same or similar sequences as the coconut cv. BIT.

The phylogenetic tree construction results based on the partial CYP P450 87 A3 nucleotide sequences show that the partial CYP P450 87 A3 of coconuts are more closely related than oil palm (*Elaeis guineensis* Jacq.) (Figure 3). The evaluated sequences of CYP P450 87 A3 of MTT, ChGD, and HNT are closely related (Cluster 1). Similarly, the CYP P450 87 A3 sequences of BYD-1 and -2 (Cluster 2), MRD-1, MRD 4, NYD-1, and NYD-2 (Cluster 3), and BIT, MYD, TAT-1 - 3 (Cluster 4) are closely related. The CYP P450 87 A3 of CaGD, PUT, and all coconut accessions belonging to Cluster 2-4 have identical progenitor sequences, which are more distantly related to those coconuts belonging to Cluster 1 (Figure 3).

Table 1. Primer pairs for amplification of the partial CYP P450 87 A3 gene

Primer	Sequence (5'-3')	Amplification target	Estimated product size (bp)*
CYP-E1_F	GAGTAACCCATCGACCATCC	Exon 1	700
CYP-E1_R	AGATTTGCTAAAAGGTCAGATATTT		
CYP-E2_F	CAGTCTAAATCTTTCTGCTATACTT	Exon 2	600
CYP-E2_R	TTTTCTCGTAGCATTTAATCAAGAT		

Note: *The estimated sizes are based on the agarose electrophoresis results

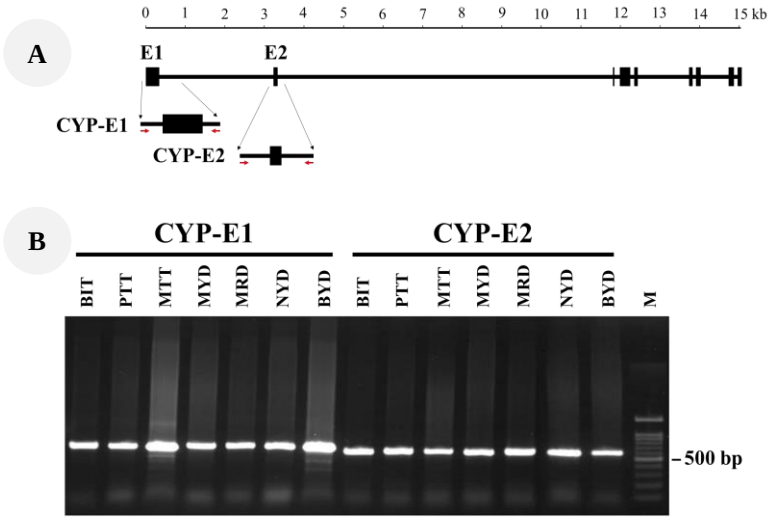

Figure 1. The CYP P450-87A3 gene structure, primer positions, and PCR amplification products. A. The structure of the CYP P450 87 A3 gene in the coconut cv. Hainan Tall genomes, primer positions, and the expected PCR amplification products. B. Visualisation of the PCR amplification products generated by the Indonesian and Malaysian coconut variety genomic DNA and the CYP-E1 and CYP-E2 specific primers

A CYP-E1 primers:

```
   1 gagtaaccca tcgaccatcc atcgtcgtcg tcagtcggcg aacatccatc ggtgtctggt 60
     ggtctgttgg ctgggttggt gagtcaggcg gcagattgat agcccctaac aagaagaaaa
 121 gaaacaaagg aaaaaataag aagaaaggaa agaaataaga agaaaaagaa caaagaaagg 180
     aaggATGGGA GAAGGAAATG AAAGGAAAGA AAGAAAGAAG GAAGAAGGAA AAGAAAGAAA
 241 AAGAAACAGA GAAGAAAGAA AGGGCGTGGA AGAAAACGAA GGAAAGGAAA GAAAGAAGGA 300
     AAAAGAGAAG GAAAGAAGAG GAAAAAGAAA GAAAGGAATG AAGGAAATAA GGAATAGGGG
 361 AACCAAAGAA GAAGAGAAAA AGAAAGGAAG GCAAAAGAGA AAGCAAGAAA GGAAAGAAAG 420
     AAGTAAAGAA AGAAAAAGAA AAACAAACAA TGAAAGAAAT AAAGAAAAGA AGGGGAGAAT
 481 GATGGAGGGT ATCTACCGGG ATCATGATCA AGAAGgtagc ttggatgggg tgggagaatg 540
     gggaatctgg ttccatggag aaacctggat gctgccatcc tataggatta aaaactctac
 601 catgaacaat aaggctactc aatagacgtt gatatttgag tcaagatcta tcttcctgtc 660
     aatctta
```

B CYP-E2 primers:

```
   1 cagtctaaat ctttctgcta tactttcaaa ccatttctgt ccaattatgt tatgcgagca 60
     atgaataatc agtagttcaa tatggaatac atattggttt gatgaactca aatttgtgta
 121 ttgctcacga acccattcaa atggcatacg gagcaagatt aaggtctcta accaaatatg 180
     ctcaaatgct ttaacattat gggatattag ggtcattgaa aatgttccca attgatactc
 241 aaaattctat gtgtagTTCT AGAACTGATT GACTTTGTGA GAAAACTGAG TAAGCAAGTT 300
     ATTGACAATT ATATGGCATT ATCAGTTATC AGCATTGTTC ATCCAAGCAC AGgtacattc
 361 tacacttgtc tggaattatt catatgtcta aaattttgat aaacatataa ttatagcacc 420
     tgattttaca tcggacaggt tttatgaaag acaggaggta agctgttttt cataaaactt
 481 aattcagatt ttgaggtcca tcagttcttt ctttgtccga atcaaaacac ttttaacaag 540
     agcaaccaag cctgtttttc cataaaactc attaatttga gattttgtgt gaccccatct
 601 tgattaaatg ctacgagaaa                                           620
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Figure 2. Representative nucleotide sequences of the putative CYP P450-87A3 amplicon of the PCR products using the Bali Tall (BIT) total genomic and the CYP-specific primers. The determined sequences for the amplicon generated using: A. CYP-E1 primers are 667 bp, B. CYP-E2 primers were 620 bp. Small letters indicate (A) the partial 5' non-coding region and intron-1 and (B) partial intron-1 and intron-2 sequences. The capital letters indicate (A) exon-1 and (B) exon-2 sequences

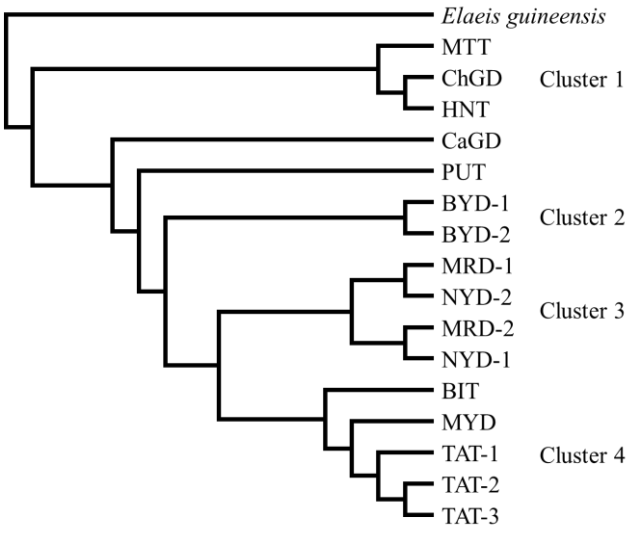

Figure 3. Phylogenetic tree based on the CYP-E1 and CYP-E2 amplicons containing Exon 1 and Exon 2 of the putative CYP P450 87 A3 gene among coconut varieties and oil palm (*E. guineensis*). The evaluated dwarf coconut varieties include BYD: Bali Yellow Dwarf, MRD: Malaysian Red Dwarf, Malaysian Yellow Dwarf, and NYD: Nias Yellow Dwarf. The evaluated tall coconut varieties include: BIT: Bali Tall, MTT: Mapanget Tall, PUT: Palu Tall, and TAT: Tenga Tall. The CYP P450 87 A3 fragments of the CaGD (Catigan Green Dwarf, Philippines), the ChGD (Chowgat Green Dwarf, India), the HNT (Hainan Tall, Taiwan), and the *E. guineensis* were obtained from the NCBI Genome databases

The nucleotide sequence diversity of a particular gene among evaluated samples could describe the kinship of tested samples (Zamudio et al. 2020). Phylogenetic tree construction aimed to see the grouping of the evaluated individuals based on their nucleotide or amino acid sequences (Chen et al. 2018). The phylogenetic tree construction based on exonic-SNPs was previously reported based on the nucleotide sequence variation of CnAMADH2, WRKY, and other genes in coconut (Larekeng et al. 2015; Pesik et al. 2017; Dumhai et al. 2019; Rahmawati et al. 2022). Genetic diversity analysis in coconut has also been carried out using SNP-based markers by Pesik et al. (2017), Santos et al. (2020), and Rahmawati et al. (2022). The dendrogram based on SNP site diversities can distinguish Japonica and Indica rice (Kurniasih et al. 2019).

Identification of SNP sites in partial CYP P450 87 A3 gene of coconuts

The presence of SNP in the intron and exon sequences was evaluated after multiple sequence alignment (MSA) analysis of the partial CYP P450 87 A3 gene from eight coconut varieties. Moreover, the partial CYP P450 87 A3 sequences originating from Hainan Tall (HNT, Taiwan), Chowgat Green Dwarf (ChGD, India), and Catigan Green Dwarf (CaGD, Philippine) were downloaded from NCBI DNA Database and used to reference sequences. Results of the MSA showed single nucleotide polymorphisms (SNPs) exist in the exon and intron parts of the CYP-E1 and CYP-E2 amplicons sequences (Table 2). Examples of exonic SNPs and intronic SNPs (IN-SNPs) are presented in Figure 4. In the exonic-SNP, synonymous (SN-SNP) and non-synonymous (NS-SNP) are identified (Figure 4 and Table 2). The identified SNP may be used to develop molecular markers, such as the PCR-based CAPS and SNAP markers (Pesik et al. 2017; Ong et al. 2018). The SNP-based markers are essential and highly potent markers widely distributed in genes and genomes (Ong et al. 2018). Generally, there is one SNP for every 330 bp of the plant genome (Zhao et al. 2017). Hence, the abundance of SNPs in the genome can be used to develop SNP-based markers in coconut plants and the SNAP marker is one of them (Pesik et al. 2017; Terryana et al. 2020). Similarly, SNPs in the partial CYP P450 87 A3 gene of coconuts may potentially be used to develop SNAP markers, as it has been proven for various coconut genes by Pesik et al. (2017). Since one of the CYP P450 87 A3 functions is associated with the plant height, the SNAP markers based on CYP P450 87 A3 could be used as gene-based SNAP markers associated with YSI characteristics in coconuts.

Most of the open-pollinated tall coconuts have heterogeneous genomes, and most of the loci genotypes are heterozygous. Therefore, sequence variations may occur even in the genomes of one tall coconut sample. To generate reference sequences for the evaluated coconuts, the CYP-E1 and CYP-E2 PCR amplicons used CYP-specific primers, and TET genomic sequences as the DNA template was cloned into plasmid vectors recombinant plasmids were sequenced. As expected, sequence diversities among the determined sequences of the cloned CYP-E1 and CYP-E2 PCR amplicons show the heterozygous nature of the CYP P450 87 A3 gene in the coconut cv. TET. The presence of sequence diversity in the cloned amplicon showed gene heterozygosity (Bogema et al. 2021).

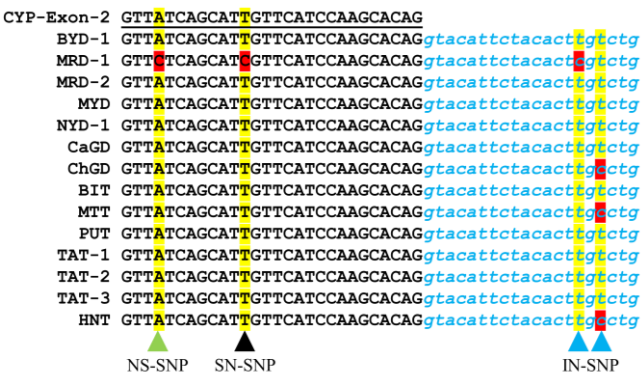

Figure 4. Examples of exonic- and intronic-single nucleotide polymorphisms (SNPs) are found in the amplicon having partial exon-2 (underlined capital letters) and intron-2 (small letters) of the CYP 450 87A3. The NS-SNP (green arrow) is a non-synonymous SNP, and SN-SNP (the black arrow) is a synonymous SNP. Both NS- and SN-SNPs are exonic-SNPs. IN-SNP (blue arrows): intronic-SNPs

Table 2. The single nucleotide polymorphisms (SNPs) in the CYP-E1 and CYP-E2 amplicon sequences of coconut CYP P450 87 A3 gene identified after multiple sequence alignment (MSA) analysis

SNP	SNP positions	SNP locations	SNP alleles	SNP types	Amino acid residue changes
CYP1_SNP1	59	Exon	G/C	Non-Synonymous	Arg - Thr
CYP1_SNP2	163	Exon	A/C	Non-Synonymous	Ile - Leu
CYP2_SNP1	115	Intron	T/C	-	-
CYP2_SNP3	328	Exon	A/C	Non-Synonymous	Ile - Leu
CYP2_SNP5	367	Exon	T/C	Non-Synonymous	Cys - Arg
CYP2_SNP7	413	Intron	A/C	-	-
CYP2_SNP8	445	Intron	T/C	-	-
CYP2_SNP10	552	Intron	C/G	-	-
CYP2_SNP11	554	Intron	G/A	-	-

The non-synonymous SNPs cause amino acid changes in the translation product, while non-synonymous did not change the amino acid composition in the translation product (Table 2). As previously suggested, non-synonymous SNP changes the amino acids, while synonymous SNP results in the same amino acid (Sujipuli et al. 2021). Nonetheless, the association of many non-synonymous SNPs has been studied for their association with specific traits (Babu et al. 2019).

CYP-specific SNAP primers and marker validation

The SNAP primers were designed based on selected SNP sites of the partial CYP P450 87 A3 gene, and the designed primers were validated for their ability to generate reference allele (Ref - using the Ref and Rev primer pair) and alternate allele (Alt - using the Alt and Rev primer pair). Alleles of the SNAP markers were generated using combinations of at least three primers, i.e., Ref and Alt forward primers and Rev reverse primer. PCR amplification using a combination of Ref and Rev primers generated a Ref allele, while those using a combination of Alt and Rev primers generated the Alt allele (Pesik et al. 2017; Raynalta et al. 2018). Any primer pairs unable to generate PCR amplicon during the validation steps were not used for association analysis. A list of SNAP primers selected after primer validation is listed in Table 3 and comprises three exonic-SNP loci and three intronic-SNPs of the CYP P450 87 A3 gene.

The SNAP marker amplification variations are associated with the presence of reference (Ref) and alternate (Alt) alleles in the evaluated DNA sequences (Jang and Lee 2021). The amplification results using coconut DNA and SNAP primers showed variations among individuals within the same accessions or between accessions, and the examples of amplified SNAP markers generated using the CYP2_SNP11 primers for four coconuts (SGD, TTD, JGD, and NGD) are presented in Figure 5A. Using CYP2_SNP11 primer pairs, one sample of coconut cv. SGD and two samples of JGD showed a positive amplicon for the Ref - Rev and negative for Alt - Rev primer pairs. Meanwhile, five samples of coconut cv. TTD, one JGD, and one sample of NGD yielded positive amplicons for Ref - Rev and Alt - Rev primer pairs. On the other hand, two coconut cv. JGD samples yielded a negative amplicon for the Ref - Rev and positive for Alt - Rev primer pairs (Figure 5A).

Table 3. Lists of the designed and validated single nucleotide amplified polymorphism (SNAP) primers based on the nucleotide variation in the partial CYP P450 87 A3 gene of coconuts

Primer names	Primer sequences (5' - 3')	SNP position	Expected product size (bp)
CYP1_SNP2_Ref	AAAGAAAGAAAGGAATGAAGTAAA	Exon, 163	332
CYP1_SNP2_Alt	GAAAGAAAGGAATGAAGCAAC		
CYP1_SNP2_Rev	GAAGATAGATCTTGACTCAAATATCA		
CYP2_SNP1_Ref	TGGTTTGATGAACTCAAGTTT	Intron, 115	238
CYP2_SNP1_Alt	TGGTTTGATGAACTCAACTTC		
CYP2_SNP1_Rev	CTGATAACTGATAATGCCATATAA		
CYP2_SNP3_Ref	ACAATTATATGGCATTATCACTTA	Exon, 328	275
CYP2_SNP3_Alt	CAATTATATGGCATTATCACTTC		
CYP2_SNP3_Rev	CAAATTAATGAGTTTTATGGAAA		
CYP2_SNP5_Ref	AAGCACAGGTACATTCTACTCTT	Exon, 367	274
CYP2_SNP5_Alt	AGCACAGGTACATTCTACTCTC		
CYP2_SNP5_Rev	TCTCGTAGCATTTAATCAAGAT		
CYP2_SNP8_Ref	AGCTTACCTCCTGTCTTTCA	Intron, 445	263
CYP2_SNP8_Alt	AGCTTACCTCCTGTCTATCG		
CYP2_SNP8_Rev	GGATATTAGGGTCATTGAAAA		
CYP2_SNP11_Ref	AAATTAATGAGTTTTATGGAATAAC	Intron, 554	405
CYP2_SNP11_Alt	AATTAATGAGTTTTATGGAAGAAT		
CYP2_SNP11_Rev	CAAATATGCTCAAATGCTTTA		

The examples of gel photographs, scoring of the SNAP markers, and the genotyping interpretation of the evaluated coconut samples based on the CYP2_SNP11 SNAP marker locus are presented in Figure 5B. The coconut cv. SGD scores 1/0 for Ref/Alt alleles, indicating the SGD sample is genotypically a homozygous individual having a 1/1 genotype for the CYP2_SNP11 SNAP marker locus (Figure 5B). The coconut cv. TTD scores 1/1 for Ref/Alt alleles, indicating the TTD sample is genotypically a heterozygous individual having a 1/2 genotype for the CYP2_SNP11 SNAP marker locus (Figure 5B). Meanwhile, the coconut cv. JGD scores 0/1 for Ref/Alt alleles, indicating the SGD sample is genotypically a homozygous individual having a 2/2 genotype for the CYP2_SNP11 SNAP marker locus (Figure 5B). Such conversion between scoring for the presence of Ref and Alt alleles and the genotypes has been previously proposed for the SNAP markers (Pesik et al. 2017; Terryana et al. 2020).

SNAP marker association with the YSI characteristic

The analysis of variance (ANOVA) of the yearly stem height increase (YSI) characteristics of the evaluated coconut populations is all statistically significant (Table 4). Such data indicated the presence of phenotypic variation for the YSI characteristics among the evaluated coconut populations. Moreover, the descriptive YSI characteristics among dwarf, tall, and hybrid coconuts further support the presence of genetic variation for the character (Table 5). For dwarf coconut, the mean YSI characteristic was 49.08 cm, the minimum was 25.00 cm, the maximum was 63.00 cm, and CV was 19.99 %. The mean YSI characteristic for the tall coconut was 99.73 cm, the minimum was 75.00 cm, the maximum was 125.00 cm, and CV was 12.60 %. Meanwhile, the mean YSI characteristic for the hybrid coconut was 90.83 cm, the minimum was 72.00 cm, the maximum was 117.00 cm, and CV was 11.34 % (Table 5).

Association analysis among SNAP markers developed based on the existing SNPs of partial CYP P450 87 A3 gene with the YSI characteristic was carried out using the single-locus analysis (Campa et al. 2020). Results of the association analysis among six SNAP loci on 155 coconut palms belonging to the 31 varieties of dwarf, tall, and hybrid coconuts are presented in Figure 6.

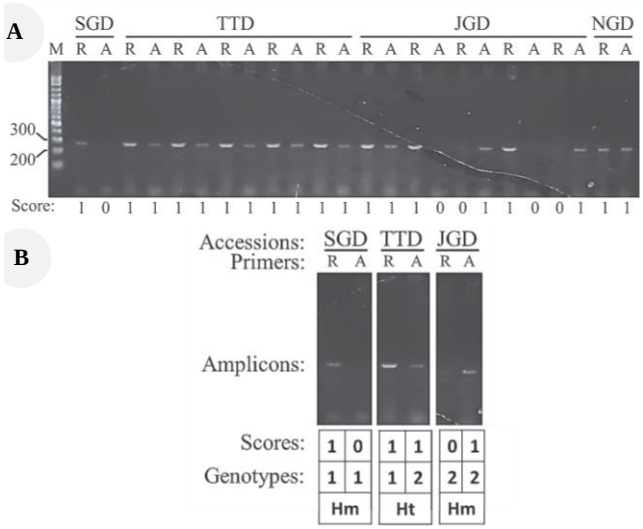

Figure 5. Examples of amplified single nucleotide amplified polymorphism (SNAP) markers generated using the CYP2_SNP11 primers, marker's scoring, and genotyping. A. Amplified SNAP markers and their scorings for four coconuts (coconut cv. SGD, TTD, JGD, and NGD). B. The examples of scoring of the SNAP markers, and the genotyping interpretation of the evaluated coconut samples based on the CYP2_SNP11 SNAP marker locus

Table 4. Analysis of variance (ANOVA) results for yearly stem-height increase characteristics among the evaluated dwarf, tall, and hybrid coconut populations

Source of variations	Df	Mean Square	Pr<F
Coconut types (tall, dwarf, and hybrids)	2	33979.621	**
Coconut varieties	24	3210.429	**
Samples numbers per coconut variety	4	55.072	**
Residual	94		

Note: Df: degrees of freedom; Pr<F: the probability of lower than F table, **statistically highly significant

Table 5. Descriptive yearly-stem-height increase characteristics among the evaluated dwarf, tall, and hybrid coconut populations

Populations	N	Yearly stem height increase			CV (%)
		Mean (cm)*	Minimum (cm)	Maximum (cm)	
Overall	125	77.34	25.00	125.00	33.39
Dwarf coconuts	50	49.08 c	25.00	63.00	19.99
Tall coconuts	45	99.73 a	75.00	125.00	12.60
Hybrid coconuts	30	90.83 b	72.00	117.00	11.34

Note: N: total number of samples, CV: coefficient of variation. *The numbers in a column, followed by the same letter, indicated they are not significantly different based on DMRT at α 0.05

The single locus association analysis results for the CYP2_SNP1 locus of the CYP P450 gene 87A3 were associated with the coconut YSI character. The CC homozygous genotype for the CYP2_SNP1 (Figure 6A) SNAP locus has the lowest YSI characteristics than the TC and TT genotypes. Four Dwarf coconut samples show the CC homozygous genotype for the CYP2_SNP1 marker locus, i.e., RAD-1 (YSI = 27.00 cm), RAD-5 (YSI = 26.00 cm), SGD-2 (YSI = 40.00 cm), and SGD4 (YSI = 50.00 cm). The RAD-2, RAD-3, and RAD-4 samples show the TC heterozygous genotype for the CYP2_SNP1 SNAP marker locus, with the average YSI characteristics of 25.33 cm (25.00-26.00 cm). Meanwhile, the SGD-1, SGD-3, and SGD-5 samples show the TC heterozygous genotype for the CYP2_SNP1 SNAP marker locus, with an average YSI of 48 cm (40.00-52.00 cm). The NYD and BYD show the TC heterozygous genotype for the CYP2_SNP1 SNAP marker locus, with the average YSI characteristic of 50.90 cm (42.00-57.00 cm).

The analysis results also show no genotype, and the YSI characteristic association exists for the CYP1_SNP2 SNAP marker locus among the evaluated coconuts (Figure 6B). On the other hand, the analysis results show that the TC heterozygous genotypes for the CYP2_SNP3 (Figure 6C), AC heterozygous for the CYP2_SNP5 (Figure 6D) and CYP2_SNP8 (Figure 6E) and CT heterozygous for the CYP2_SNP11 (Figure 6F) SNAP marker loci show lower YSI characteristics than their homozygous counterpart, indicating the presence of heterotic effects for these loci.

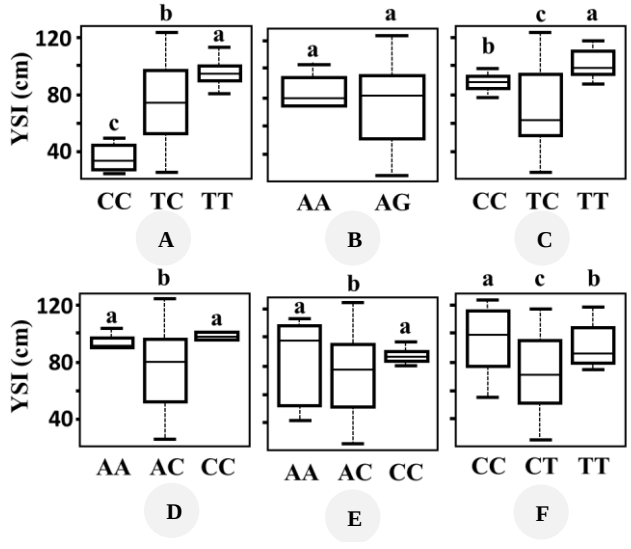

Figure 6. Genotypic constitution and yearly stem height increment (YSI) in 155 coconut samples were represented using the single-locus analysis of SNAP marker based on CYP P450 87 A3 sequence variations. The genotype association is based on the SNAP: A. CYP2_SNP1; B. CYP1_SNP2; C. CYP2_SNP3; D. CYP2_SNP5; E. CYP2_SNP8, and F. CYP2_SNP11 loci with yearly stem height increase (YSI) in coconut

Association among markers developed based on SNP loci has been investigated in oil palm (Ong et al. 2018; Babu et al. 2019; Rizal et al. 2020). Using genotyping by sequencing (GBS), the association between SNPs and oil palm height increment has previously been studied (Babu et al. 2019). Results of the association studies indicated that the proteins comprised families of auxin response factors (ARFs)-like and abscisic acid insensitive 3 (ABI3)-like genes were suggested as associated with the stem height increment in oil palm (Babu et al. 2019). However, such GBS studies need quite large funding support. Therefore, realizing similar aims for coconut studies may not be easy.

Another work in oil palms was started to evaluate the usefulness of the random and candidate gene SNP marker for studying marker and height increment association (Ong et al. 2018). Although it successfully found the association between SNP markers and height increment characteristics in oil palm, they suggested that their study should be expanded to include more candidate gene SNP markers (Ong et al. 2018). Association among SNAP markers developed based on the SMT1 nucleotide sequence variations have also been reported in oil palm (Rizal et al. 2020).

SNAP marker development based on the SNPs of various coconut genes and its uses have also been done in coconuts (Larekeng et al. 2015; Pesik et al. 2017; Rahmawati et al. 2022). However, association studies between SNAP markers and YSI characters in coconuts have not yet been reported. This report was the first attempt to associate SNAP markers based on SNP of the CYP P450 87 A3 gene in coconuts and successfully identify five SNAP marker loci associated with YSI characteristics in coconuts. The developed markers may be used for future marker-assisted selection (MAS) for low YSI characteristics in coconuts.

In conclusion, CYP P450 87 A3 is an auxin-induced gene involved in plant growth processes, and it exists in the genomes of various coconuts. PCR amplification using the designed CYP-specific primers pairs and genomic DNA of eight dwarf and tall coconuts resulted in the CYP-E1 (667 bp) and CYP-E2 (620 bp) amplicons. Upon DNA sequencing and analysis, the CYP-E1 was a DNA fragment having 5'-non-coding regions - exon-1 - partial intron-1, while the CYP-E2 fragment containing partial intron-1 - exon-2 - partial intron-2 of the target CYP P450 87 A3 gene. The analysis showed that at least 17 SNPs exist in the exon-1 and 2 of the coconut CYP P450 87 A3 gene, and the identified SNP sites consisted of synonymous and non-synonymous (exonic-SNPs) and intronic-SNPs. Six SNPs were selected, used to design SNAP primers, and validated to generate SNAP markers. Single-locus analysis showed that the validated CYP2_SNP1 SNAP marker locus was associated with coconut YSI characters. The developed SNAP marker based on the partial CYP P450 87 A3 gene in the present study has the potential to support future marker-assisted selection (MAS) in coconut breeding, especially for developing coconuts with low YSI character.

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REFERENCES

Assefa T, Otyama PI, Brown AV, Kalberer SR, Kulkarni RS, Cannon SB. 2019. Genome-wide associations and epistatic interactions for internode number, plant height, seed weight, and seed yield in soybean. BMC Genomics 20: 527. DOI: 10.1186/s12864-019-5907-7.

Babu BK, Mathur RK, Ravichandran G, Venu MVB. 2019. Genome-wide association study (GWAS) for stem height increment in oil palm (*Elaeis guineensis*) germplasm using SNP markers. Tree Genet Genomes 15: 40. DOI: 10.1007/s11295-019-1349-2.

Bogema DR, Yam J, Micallef ML, Gholipourkanani H, Go J, Jenkins C, Dang C. 2021. Draft genomes of *Perkinsus olseni* and *Perkinsus chesapeaki* reveal polyploidy and regional differences in heterozygosity. Genomics 113 (1): 677-688. DOI: 10.1016/j.ygeno.2020.09.064.

Boonkaew T, Mongkolsiriwatana C, Vongvanrungruang A, Srikulnath K, Peyachoknagul S. 2018. Characterisation of *GA20ox* genes in tall and dwarf types coconut (*Cocos nucifera* L.). Genes Genom 40: 735-745. DOI: 10.1007/s13258-018-0682-4.

Campa A, Fernandez CG, Ferreira JJ. 2020. Genome-wide association study (GWAS) for resistance to *Sclerotinia sclerotiorum* in common bean. Genes 11 (12): 1496. DOI: 10.3390/genes11121496.

Chen M, Chen J, Luo N, Qu R, Guo Z, Lu S. 2018. Cholesterol accumulation by suppression of SMT1 leads to dwarfism and improved drought tolerance in herbaceous plants. Plant Cell Environ 41: 1417-1426. DOI: 10.1111/pce.13168.

Devi EL, Devi CH, Kumar S, Sharma SK, Beemrote A, Chongtham SK, Singh CH, Tania C, Singh TB, Ningombam A, Akoijam R, Singh IM, Singh YR, Monteshori S, Omita Y, Prakash N, Ngachan SV. 2017. Marker-assisted selection (MAS) towards generating stress-tolerant crop plants. Plant Gene 11: 205-218. DOI: 10.1016/j.plgene.2017.05.014.

Dumhai R, Wanchana S, Saensuk C, Choowongkomon K, Mahatheeranon S, Kraithong T, Toojinda T, Vanavichit A, Arikit S. 2019. Discovery of a novel CnAMADH2 allele associated with higher levels of 2-acetyl-1-pyrroline (2AP) in yellow dwarf coconut (*Cocos nucifera* L.). Sci Hortic 243: 490-497. DOI: 10.1016/j.scienta.2018.09.005.

Emenecker RJ, Strader LC. 2020. Auxin-abscisic acid interactions in plant growth and development. Biomolecules 10 (2): 281. DOI: 10.3390/biom10020281.

Geethanjali S, Rukmani AJ, Rajakumar D, Kadirvel P, Viswanathan PL. 2018. Genetic diversity, population structure and association analysis in coconut (*Cocos nucifera* L.) germplasm using SSR markers. Plant Genet Resour 16 (2): 156-168. DOI: 10.1017/S1479262117000119.

Jang YE, Lee S. 2021. Gene-based allele-specific marker for resistance to *Phytophthora sojae* in soybean (*Glycine max* L.). Plant Breed Biotechnol 9: 164-169. DOI: 10.9787/PBB.2021.9.2.164.

Khanom S, Jang J, Lee OR. 2019. Overexpression of ginseng cytochrome P450 CYP736A12 alters plant growth and confers phenylurea herbicide tolerance in *Arabidopsis*. J Ginseng Res 43: 645-653. DOI: 10.1016/j.jgr.2019.04.005.

Kim S, Park TH. 2019. PCR-based markers developed by comparison of complete chloroplast genome sequences discriminate *Solanum chacoense* from other *Solanum* species. J Plant Biotechnol 46: 79-87. DOI: 10.5010/JPB.2019.46.2.079.

Kurniasih NS, Susandarini R, Susanto FA, Nuringtyas TR, Jenkins G, Purwestri YA. 2019. Characterisation of Indonesian pigmented rice (*Oryza sativa*) based on morphology and Single Nucleotide Polymorphisms. Biodiversitas 20 (4): 1208-1214. DOI: 10.13057/biodiv/d200437.

Larekeng SH, Maskromo I, Purwito A, Mattjik NA, Sudarsono. 2015. Pollen dispersal and pollination patterns study in Pati Kopyor coconut using molecular markers. Coconut Res Dev J 31 (1): 46-60. DOI: 10.37833/cord.v31i1.70.

Larekeng SH, Purwito A, Mattjik NA, Sudarsono. 2018. Microsatellite and SNAP markers used for evaluating pollen dispersal on Pati tall coconuts and Xenia effect on the production of 'Kopyor' fruits. IOP Conf Ser Earth Environ Sci 157: 012042. DOI: 10.1088/1755-1315/157/1/012042.

Mahayu WM, Taryono, Kumaunang J, Maskromo I. 2021. Identification of superior dwarf coconut (*Cocos nucifera* L.) parental cultivars for hybrid breeding. SABRAO J Breed Genet 53 (2): 278-289.

Martial YSD, Nafan D, Noël D, Zadjehi KEB, Jesus FI, Noel KKJ, Louis KKJ, Roland B, Syvère SR, Arsène ZBI. 2019. Genetic relationships among regenerated tall coconut (*Cocos nucifera* L.) accessions from multivariate analyses using reliable short list of morphological descriptors in Côte d'Ivoire. J Plant Breed Crop Sci 11 (5): 137-143. DOI: 10.5897/JPBCS2018.0788.

Muñoz-Pérez JM, Cañas GP, López, L, Arias T. 2022. Genome-wide diversity analysis to infer population structure and linkage disequilibrium among Colombian coconut germplasm. Sci Rep 12: 2958. DOI: 10.1038/s41598-022-07013-w.

Nair KP. 2021. The Coconut Palm (*Cocos nucifera* L.). In: Tree Crops. Springer, Cham. DOI: 10.1007/978-3-030-62140-7_3.

Ong PW, Maizura I, Marhalil M, Rajanaidu N, Abdullah NAP, Rafii MY, Ooi LCL, Low ETL, Singh R. 2018. Association of SNP markers with height increment in MPOB-Angolan natural oil palm populations. J Oil Palm Res 30 (1): 61-70. DOI: 10.21894/jopr.2017.0003.

Pan K, Wang W, Wang H, Fan H, Wu Y, Tang L. 2018. Genetic diversity and differentiation of the Hainan Tall coconut (*Cocos nucifera* L.) as revealed by inter-simple sequence repeat markers. Genet Resour Crop Evol 65: 1035-1048. DOI: 10.1007/s10722-017-0593-2.

Pesik A, Efendi D, Novarianto H, Dinarti D, Sudarsono. 2017. Development of SNAP markers based on nucleotide variability of WRKY genes in coconut and their validation using multiplex PCR. Biodiversitas 18 (2): 465-475. DOI: 10.13057/biodiv/d180204.

Rahmawati A, Dinarti D, Maskromo I, Volkaert HA, Sudarsono. 2022. Coconut diversity based on chloroplast Single Nucleotide Amplified Polymorphism (SNAP) and Insertion-Deletion (indel) markers. Biodiversitas 23 (8): 4073-4081. DOI: 10.13057/biodiv/d230827.

Raynalta E, Elina J, Sudarsono, Sukma D. 2018. Clonal fidelity of micro-propagated *Phalaenopsis* plantlets based on assessment using eighteen Ph-Pto SNAP marker loci. Agrivita J Agri Sci 40 (3): 390-402. DOI: 10.17503/agrivita.v40i3.1493.

Rizal S, Sukma D, Siberakuno R, Liwang T, Sudarsono. 2020. Isolation and characterization of Sterol Methyltransferase 1 (SMT1) gene from oil palm. Jurnal Agronomi Indonesia 48 (3): 348-354. DOI: 10.24831/jai.v48i3.32205. [Indonesian]

Santos PHAD, Venâncio TM, Santos PHD, Ramos HCC, Arêdes FAS, Azevedo AON, Boechat MSB, Filho GAS, Ramos SRR, Mirisola LA, Aragão WM, Pereira MG. 2020. Genotyping-by-sequencing technology reveals directions for coconut (*Cocos nucifera* L.) breeding strategies for water production. Euphytica 216: 45. DOI: 10.1007/s10681-020-02582-1.

Sekhar S, Das S, Panda D, Mohanty S, Mishra B, Kumar A, Navadagi DB, Sah RP, Pradhan SK, Samantaray S, Baig MJ, Behera L, Mohapatra T. 2022. Identification of microRNAs that provide a low light stress tolerance-mediated signaling pathway during vegetative growth in rice. Plants 11: 2558. DOI: 10.3390/plants11192558.

Sidhu JS, Singh D, Gill HS, Brar NK, Qiu Y, Halder J, Tameemi RA, Turnipseed B, Sehgal SK. 2020. Genome-wide association study uncovers novel genomic regions associated with coleoptile length in hard winter wheat. Front Genet 10: 1345. DOI: 10.3389/fgene.2019.01345.

Sudha R, Samsudeen K, Rajesh M, Niral V. 2022. Molecular marker assisted confirmation of hybrids in coconut (*Cocos nucifera* L.). Indian J Genet Plant Breed 82 (3): 369-372. DOI: 10.31742/ISGPB.82.3.15.

Sujipuli K, Kaeomani S, Kunpratum N. 2021. PCR-based SNP markers for sex identification in date palm (*Phoenix dactylifera* L.) cv. KL1. Trends Sci 18 (20): 26. DOI: 10.48048/tis.2021.26.

Sukma D, Elina J, Raynalta E, Aisyah S, Aziz SA, Sudarsono, Chan MT. 2021. Analysis of the genetic diversity of phalaenopsis orchids with single nucleotide polymorphisms and SNAP markers derived from the

Pto gene. SABRAO J Breed Genet 53 (4): 620-632. DOI: 10.54910/sabrao2021.53.4.6.

Sun C, Dong Z, Zhao L, Ren Y, Zhang N, Chen F. 2020. The Wheat 660K SNP array demonstrates great potential for marker-assisted selection in polyploid wheat. Plant Biotech J 18: 1354-1360. DOI: 10.1111/pbi.13361.

Terryana RT, Rijzaani H, Priyatno TP, Manzila I, Lestari P. 2020. Construction of DNA fingerprint for chili pepper varieties using SNAP markers. IOP Conf Ser Earth Environ Sci 482: 012038. DOI: 10.1088/1755-1315/482/1/012038.

Tu L, Su P, Zhang Z, Gao L, Wang J, Hu T, Zhou J, Zhang Y, Zhao Y, Liu Y, Song Y, Tong Y, Lu Y, Yang J, Xu C, Jia M, Peters RJ, Huang L, Gao W. 2020. Genome of *Tripterygium wilfordii* and identification of cytochrome P450 involved in triptolide biosynthesis. Nature Comm 11 (1): 1-12 DOI: 10.1038/s41467-020-19253-3. 10.1038/s41467-020-14776-1.

Vosolsobě S, Skokan R, Petrášek J. 2020. The evolutionary origins of auxin transport: What we know and what we need to know. J Exp Bot 71 (11): 3287-3295. DOI: 10.1093/jxb/eraa169.

Wang H, Li W, Qin Y, Pan Y, Wang X, Weng Y, Chen P, Li Y. 2017. The Cytochrome P450 Gene CsCYP85A1 Is a putative candidate for Super Compact-1 (Scp-1) plant architecture mutation in cucumber (*Cucumis sativus* L.). Front Plant Sci 8: 266. DOI: 10.3389/fpls.2017.00266.

Wei K, Chen H. 2018. Global identification, structural analysis and expression characterisation of cytochrome P450 monooxygenase superfamily in rice. BMC Genom 19: 35. DOI: 10.1186/s12864-017-4425-8.

Zamudio F, Yanez M, Guerra F, Fuentes D, Gonzales A. 2020. Comparative analysis of SNP data and hybrid taxa information by using a classificatory linear mixed model to study the genetic variation and heritability of initial height growth in selected poplar hybrids. Tree Gen Genomes 16 (69): 1-25. DOI: 10.1007/s11295-020-01435-1.

Zhang Y, Liu H, Yan G. 2021. Characterisation of near-isogenic lines confirmed QTL and revealed candidate genes for plant height and yield-related traits in common wheat. Mol Breed 41: 4. DOI: 10.1007/s11032-020-01196-8.

Zhao P, Zhang J, Qian C, Zhou Q, Zhao X, Chen G, Ma XF. 2017. SNP discovery and genetic variation of candidate genes relevant to heat tolerance and agronomic traits in natural populations of sand rice (*Agriophyllum squarrosum*). Front Plant Sci 8: 536. DOI: 10.3389/fpls.2017.00536.