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Identification of MADS-box Gene in Oil Palm (*Elaeis guineensis* Jacq.)

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Abstract

The bunch size represented by the fruit number is the main parameter of oil palm (*Elaeis guineensis* Jacq.) yield. The fruit number, which is determined during the initial phase of development, is related to various factors, including the genetic properties of the trees. Trees that have more pistillate flowers have more fruit. The diversity of MADS-box genes assumed can be used as a marker for trees that have a higher number of pistillate flowers. Therefore, the aims of this research were to isolate and identify the MADS-box genes from flowers of tenera oil palm using PCR techniques. The *SQUAMOSA* (*SQUA*) gene and the *GLOBOSA* (*GLO*) gene are members of the *MADS-box* genes family that are responsible for sepal, petal and stamen organ development. The genomic DNA of the staminate flowers of trees that have more staminate flowers (P1) and the genomic DNA of the pistillate flowers of trees that have more pistillate flowers (P2) were isolated using the CTAB+ PVP method. The CTAB+PVP method was more efficient for isolating pistillate flower genomic DNA than staminate flower genomic DNA. The genomic DNA of P1 and P2 was amplified with two primers: BMS and BMG. The BMS primers gave a PCR product size of 1250 bp for the genomic DNA of P1 and P2. Meanwhile, the BMG primers gave a PCR product size of 1250 bp and 1300 bp for P1 and P2, respectively. The PCR products were sequenced and analyzed for homology using the GenBank database. BLAST analysis showed the PCR products have high homology with the *SQUA1* gene and the *GLO2* gene. Alignment analysis showed that the DNA fragments amplified with the BMS primers of the P1 and P2 sequences have variations in the exons and introns, and the variations were observed only in the introns of the DNA fragments amplified with the BMG primers.

Abstrak

Identifikasi Gen MADS-box pada Kelapa Sawit (*Elaeis guineensis* Jacq.). Ukuran tandan yang dipresentasikan dengan jumlah buah merupakan parameter utama pada produksi kelapa sawit (*Elaeis guineensis* Jacq.). Jumlah buah, yang dapat diduga selama fase awal perkembangan tanaman, berkaitan dengan berbagai faktor, salah satunya adalah properti genetik pohon. Pohon yang mempunyai bunga betina lebih banyak mempunyai buah lebih banyak. Keragaman gen MADS-box diduga dapat digunakan sebagai marka untuk pohon yang mempunyai banyak bunga betina. Tujuan dari penelitian ini adalah mengisolasi dan mengidentifikasi gen MADS-box dari bunga kelapa sawit Tenera menggunakan teknik PCR. Gen SQUAMOSA (SQUA) dan gen GLOBOSA (GLO) termasuk dalam famili gen MADS-box yang berperan pada perkembangan organ sepal, petal dan stamen. DNA genom bunga jantan dari pohon yang mempunyai bunga jantan lebih banyak (P1) dan DNA genom bunga betina dari pohon yang mempunyai bunga betina lebih banyak (P2) diisolasi menggunakan metode CTAB+PVP. DNA genom P1 dan P2 diamplifikasi menggunakan dua primer: BMS dan BMG. Primer BMS menghasilkan produk PCR berukuran 1250 bp untuk DNA genomP1 dan P2. Primer BMG menghasilkan produk PCR berukuran 1250 bp dan 1300 bp untuk P1 dan P2. Produk PCR disekuensing dan dianalisis homologinya menggunakan database GenBank. Analisis BLAST menunjukkan bahwa produk PCR mempunyai homologi yang tinggi dengan gen SQUA1 dan gen GLO2. Analisis alignment menunjukkan fragmen DNA yang teramplifikasi primer BMS dari sekuen P1 dan P2 mempunyai keragaman pada ekson dan intron, keragaman hanya terdeteksi pada intron fragmen DNA yang teramplifikasi primer BMG.

Keywords: GLO2, elaeis guineensis, PCR, pistillate, staminate flower, SQUA1

Introduction

Oil palm (*Elaeis guineensis* Jacq.) produces the second most commonly used vegetable oil and contributes to almost 25% of the global oil production. The bunch size represented by the fruit number, which is determined during the initial phase of development, is related to various factors, including the trees' genetic properties.

Trees that have a higher number of pistillate flowers have higher fruit numbers.

Oil palm is a diœcious temporal plant in terms of pistillate and staminate flowers production. The pistillate and staminate flowers cycle alternately on the same plant and follow the allogamous reproduction model. This cycle is not predictable due to the influence of several complex process (abiotic factors, metabolic status, hormone, genetic factors, biochemical change and physiology) [1].

The *MADS-box* gene family affects flower development. MADS-box is an abbreviation of the initial letters of the first members of this family that were found: *MCM*, *AGAMOUS*, *DEFICIENS* and *SRF (MADS-box)* [2], [3]. The *MADS-box* gene encodes transcription factors that are responsible not only for controlling plant growth and development, including the formation of the flowering meristem, the male and female flower development organ [4], but also the mantled phenotype of oil palm [5]. The diversity of *MADS-box* genes can be used to determine flowering traits, including determining the trees with a larger number of pistillate flowers.

The MADS-box genes that determine male and female traits are the *SQUAMOSA (SQUA)* and *GLOBOSA (GLO)* genes. Based on the ABCDE flowering model for oil palm, *SQUA* genes are A class genes, and *GLO* genes are B class genes. A class genes are responsible for sepal formation, the combination of A, B and E class genes is responsible for petal formation, B, C and E class genes are responsible for stamen formation, class C and E genes are responsible for carpel formation, and C, D and E class genes responsible for determining the ovule identity [4].

The aims of this research were to isolate the MADS-box genes of the oil palm flowering organ and to identify the sequence diversity of the MADS-box genes. The outcomes of the sequence studies will contribute to improving oil palm through breeding programs that use marker-assisted selection, the development of diagnostic assays that use gene-targeted markers and the discovery of candidate genes related to important agronomic traits of oil palm [7]. The molecular mechanism and flower development as well as the genes that affect flower development can be used as selection markers for production traits. Therefore, knowledge about the molecular aspects of flower development can be used to create tools for predicting oil palm production.

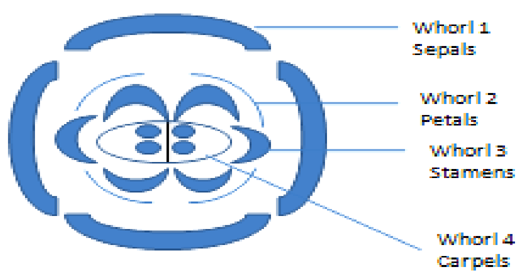

Figure 1. The Parts of a Flower [6]

Methods

Materials. The plant materials included staminate flowers from a tree that had more staminate flowers than pistillate flowers (P1) and pistillate flowers from a tree that had more pistillate flowers than staminate flowers (P2). Two staminate flowers P1 and two pistillate flowers P2 were collected from one oil palm plantations in Central Kalimantan and Kebun Percobaan Puspitek Serpong, respectively. The flowers were produced within tenera, derived from dura and pisifera.

DNA isolation. DNA was isolated by using the Cetyl Trimethyl Ammonium Bromide (CTAB) method [8] that was modified with Poly Vinyl Pyrrolidone (PVP). Staminate and pistillate flowers were first ground in liquid nitrogen with a mortar, and then 0.1 grams of PVP were added. Then 5 mL of CTAB was added to the sample and incubated at 65 °C for 30 min. Next, 1 volume of chloroform:isoamyl alcohol = 24:1; CI) was added, and the sample centrifuged at 14,000 rpm at 4 °C for 20 sec. This step was repeated three times. The collected supernatant was added to 1 volume of cold isopropanol, then incubated at –20 °C for 30 min. The sample was then centrifuged at 14,000 rpm at 4 °C for 10 min. This dried pellet was added with 500 µL TE pH 8, 1/10 volume of cold $NaCH_3COO_3$ 3 M pH 7 and 2 volumes of cold ethanol absolute and incubated overnight at –20 °C. The next following day, the dried pellet was washed with 400 µL of 70% cold ethanol and centrifuged at 14,000 rpm at 4 °C for 5 min. One hundred milliliters of double-distilled water (ddH_2O) and 1/10 volume RNAse were added to the dried pellet, and then the sample was incubated at 37 °C for 1 h.

PCR amplification. The PCR reaction consisted of 179 ng of genomic DNA template, 1 µL of dNTP mix 2 mM (Fermentas), 1 µL of 10X DreamTaq buffer (Fermentas), 0.1 U of DreamTaq DNA polymerase (Fermentas), 0.5 µL of primer 0.2 µM (forward and reverse), and ddH_2O of up to 10 µL total volume. The PCR process for the BMG primer (F RCACTAAYAGCRCA/R TCACTTAR TTCPCA) [9] consisted of early denaturation at 95 °C for 5 min followed by 30 cycles. Each cycle consisted of denaturation at 95 °C for 30 sec, annealing at 65.4 ± 6 °C for 30 sec and primer extension at 72 °C for 30 sec. Post-extension was performed at 72 °C for 5 min. The PCR process for the BMG primer (F VGCGITGAYCG/R ATTCTGTLGTGQ) [9] consisted of early denaturation at 95 °C for 5 min followed by 30 cycles. Each cycle consisted of denaturation at 95 °C for

30 sec, annealing at 61.7 ± 6 °C for 30 sec and primer extension at 72 °C for 30 sec. The final extension was performed at 72 °C for 5 min.

The PCR product was separated using 1.8% agarose gel via the electrophoresis technique in the TAE 1X buffer system. Voltage of 100 V was applied to the gel system. Then the gel was run for 30 min and visualized using ultraviolet (UV) light. The concentration and purity of extracted genomic DNAs was measured by NanoDrop Spectrophotometer 2000 (Thermo Scientific).

Data analysis. The PCR product was sequenced with an Applied Biosystem Hitachi Genetic analyzer 3130. BLASTn (www.ncbi.nlm.nih.gov/BLAST) showed the sequence was similar in the GenBank database. ApE (A Plasmid Editor) v2.0.47 was used for the alignment analysis. Softberry (http://linux1.softberry.com) was used for the exons analysis.

Results and Discussion

DNA isolation. The results showed that good genomic DNA was successfully isolated from the staminate and pistillate flowers using the CTAB+PVP method [10] (Figure 2). CTAB is an efficient extraction buffer for extracting genomic DNA, especially from plants and fungi that contain high phenolic compounds and polysaccharides [11]. The addition of PVP with CTAB may bind to the polyphenolic compounds by forming a complex with hydrogen bonds and may help in the removal of impurities [12].

The concentration and purity of extracted genomic DNAs was measured by NanoDrop Spectrophotometer 2000 (Thermo Scientific). The results are shown in Table 1. The results show that genomic DNA isolation with the CTAB+PVP method is more effective for pistillate flowers

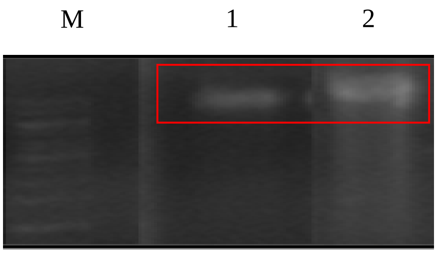

Figure 2. The Electrophoresis of Isolated Genomic DNA from Staminate (1) and Pistillate (2) Flowers M: 1 kb DNA Ladder

Table 1. The Quantification of DNA

Sample	Concentration (ng/µL)	A260/280
Staminate flower	179.1	1.57
Pistillate flower	1230.7	1.87

than for staminate flowers. We assumed that staminate flowers have more contaminants for genomic DNA isolation such as polysaccharides from pollen. Obtaining DNA is the essential first step for many genetic studies. Many plant species present various problems when attempting to isolate DNA, with polysaccharides one of the most frequently encountered problems [11]. The high viscosity of polysaccharides causes pipetting difficulties, and they hinderdownstream applications by interfering with the activity of enzymes, such as restriction endonucleases, ligases and polymerases [14]. Highly purified genomic DNA is the main requirement for the PCR process; therefore, the absorbance ratio of λ260/λ280 can be used as a parameter for detecting the purity of DNA from protein contaminant. A λ260/λ280 ratio of less than 1.8 indicates the absence of protein, phenol or other contaminants that absorbed approaches 280 nm [13]. Based on the NanoDrop measurement, genomic DNA was successfully used as the template for the PCR process although the λ260/λ280 ratio did not match the NanoDrop spectrophotometer recommendation.

PCR process for primers BMS and BMG. The results showed that the primer BMS successfully produced a single band with the product size of 1250 bp for the P1 and P2 DNA fragments (Figure 3). The PCR amplification results for the primer BMG showed that it successfully produced a single PCR product sized 1300 bp for P2 and 1250 bp for P1 (Figure 4). These results show that the DNA fragments have different sizes. The P2 DNA

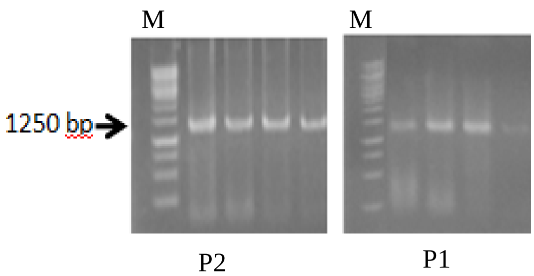

Figure 3. The Gel Electrophoresis Image PCR Amplification of Primer BMS on P2 and P1 DNA Fragments. M: 1 kb DNA Ladder

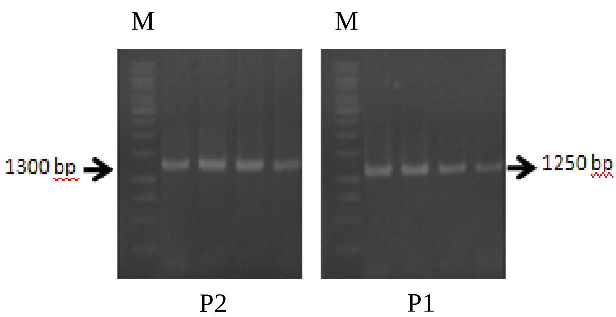

Figure 4. The Gel Electrophoresis Image PCR Amplification of Primer BMG on P2 and P1 DNA Fragments. M: 1 kb DNA Ladder

fragment produced a thicker band than the P1 DNA fragment at the same annealing temperature for primers BMS and BMG. The difference might be because P1 contained staminate flowers that had more contaminant in the genomic DNA for the samples.

Sequence analysis of the primer BMS amplicon. The PCR product from the primer BMS amplification was then subjected to the sequencer in order to reveal the order of the nucleotide bases. The sequencing coverage of the P1 and P2 DNA fragments was 1170 bp and 1158 bp, respectively. To annotate the sequences, several analyses were conducted. First, the BLASTn analysis determined the similarity of the sequences with sequences from GenBank. The Softberry analysis determined the exons' positions. The ApE software determined the differences in the sequences.

The BLASTn analysis of the P1 and P2 DNA fragments amplified by the primer BMS indicated one very high homology region (≥200 bp) that is indicated by a red line and one high homology region (80–200 bp) that is indicated with a pink line in Figure 5. The region indicated by the white line is assumed to be unregistered region sequences in GenBank.

The BLASTn analysis showed that the sequences have 99% point of similarity with the oil palm *SQUA1* gene (AF411840.1). The *SQUA1* gene is a member of the *SQUAMOSA* subfamily, which has an important role in determining the flowering meristem identity. The bit score score between the sequences and the *SQUA1* gene was more than 50. The bit score identifies the accuracy alignment point of the sequence and the nucleotide sequence database. A higher score indicates higher homology. The E-value showed a significant statistic point from the nucleotide sequence alignment and the GenBank nucleotide sequence. A low E-value point indicates higher homology for two sequences [15]. The BLASTn analysis showed that the E-value of both sequences was 4e-135, which means the sequences and the *SQUA1* gene from GenBank have high sequence homology (Tables 2 and 3).

The Softberry alignment analysis of the sequences and the *SQUA1* gene sequence from GenBank showed that the primer BMS successfully amplified two exons; CSDi and CDSl. CDSi (intermediate) means that the first exon is located at the center of the sequence, and CDSl (last) means that the second exon is located at the end of the sequence (Figure 6).

The alignment analysis of the sequences with ApE software showed the same results as the Softberry analysis. Two exons were amplified by the primer BMS in this analysis. The first exon is located at the beginning of the region (*), and the second exon is located at the end of the region (#). The sequence between the two exons is predicted to be the intron sequence. The first exon showed

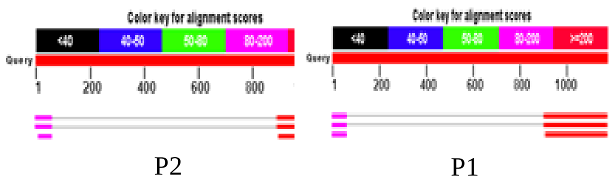

Figure 5. The Results of the BLASTn Analysis of the Primer BMS Amplicon of the P2 and P1 DNA Fragments

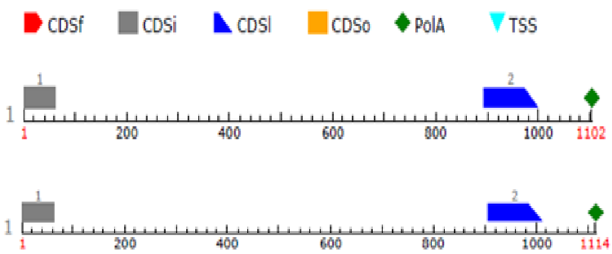

Figure 6. Softberry Alignment Analysis of the P2 (Top) and P1 (Bottom) DNA Fragments

Table 2. The BLASTn Result for the Similarity of the Amplicon Primer BMS for the P2 DNA Fragment and the GenBank Database

Accession	Description	Total score	E value	Max ident
AF411840.1	*Elaeis guineensis* MADS box transcription factor (SQUA1) mRNA, complete cds	604	4e-135	99%

Table 3. The BLASTn Result for the Similarity of the Amplicon Primer BMS between the P1 DNA Fragment and the GenBank Database

Accession	Description	Total score	E value	Max ident
AF411840.1	*Elaeis guineensis* MADS box transcription factor (SQUA1) mRNA, complete cds	609	4e-135	99%

one nucleotide difference between the P1 and P2 DNA fragments, while the second exon showed no nucleotide differences between the two sequences. The intron sequence showed nucleotide differences between the P1 and P2 DNA fragments (red region, Figure 7). The difference of one base for one exon does not cause a significant difference, although it causes a difference in amino acid formation. The similarity of the exon sequences for P1 and P2 showed that the DNA fragment amplified in this study was associated with the *SQUA1* gene but does not necessarily identify oil palm trees with high pistillate flowers.

These results are appropriate with the result that the *SQUA1* transcript abundance is constant during the development of pistillate and staminate flowers [4]. None of the identified oil palm MADS-box genes has an expression pattern specific to either male or female inflorescence [9]. This result showed exon sequences that produce expression cannot be used to distinguish female and male traits. Nevertheless, the differences between expected intron sequences can be used as a marker to identify trees that have more pistillate flowers than staminate flowers.

Sequence analysis of primer BMG amplicon. The sequencing coverage of the PCR products from the P2 and P1 samples was 1042 bp. The BLASTn analysis of the P2 and P1 DNA fragments amplified with primer BMG indicated a very high homology region (≥200 bp), indicated by the red line, two high homology regions (80–200 bp), indicated by the pink line, and a medium homology region, indicated by the green line; see Figure 8. The regions between the exons are indicated by the white line and identified in GenBank as unregistered sequences.

The results of the BLASTn analysis showed that the sequences of the P2 and P1 DNA fragments have 96% and 98% point of similarity with the oil palm *GLO2* gene (AF411848.1), respectively. In addition, the bit score and the E-value also showed that both sequences share high homology with the *GLO2* gene (Tables 4 and 5). The *GLO2* gene is a member of the *GLOBOSA* subfamily and is a B class gene based on the ABCDE flowering models. This gene is responsible for determining stamen and petal identities [4]

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        *******************************************************************
P2     1>ACAAGCTCAGCATCCCCAACCTCCTTCCTGATCGGAGACTCTCTCCCCACCCTGAATATTGGGTACGTTCTCATGCCACAACTCTTATTTTTAACCTTGT>100
P1     1>ACAAGCTCATCATCCCCAACCTCCTTCCTGATCGGAGACTCTCTCCCCACCCTGAATATTGGGTACGTTCTCATGCCACAACTCTTATTTTTAACCTTGT>100

     101>ATTTTATCCTGATAATATAGGCTCCATATGTCAACCTAGGCATATACCAGTGAATAAGTAATCAGTCATGAATGCACAGAGTGCTATTATGTACAACCAC>200
     101>ATTTTATCCTGATAATATAGGCTCCATATGTCAACCTAGGCATATACCAGTGAATAAGTAATCAGTCATGAATGCACAGAGTGCTATTATGTACAACCAC>200

     201>TAAGACTTTTATTAATACAAACTAAGGTTTTAAAGCTCAGAGGATAGAGATGTCTCGATTTCTGTATCAAATGAGATGTCCCACTATTCCTTCCTATCTC>300
     201>TAAGACTTTTATTAATACAAACTAAGGTTTTAAAGCTCAGAGGATAGAGATGTCTCGATTTCTGTATCAAATGAGATGTCCCACTATTCCTTCCTATCTC>300

     301>AATAGTAATTTTGATCATGCATCCCGATAGATATCCTCCGTCTCACTCTCCTTTCCTTCCTCTCTTTTCTTTTTT------------------------->375
     301>AATAGTAATTTTGATCATGCATCCCGATAGATATCCTCCGTCTCACTCTCCTTTCCTTCCTCTCTTTTCTTTTTTTTTTTTTTTTCCTCCTTTCTATTT>400

     376>-------------------------------------------TTTTTTTTTTTCCCCCTTCCAAATTTCCTTTTTCCAAAACCCATCCCTTCATTTTT>431
     401>TTTCTTTTTTCAAAACCCATTCCTCCTTTTTTTTTTCTCCCCCCTTTTTTTTTTTCCCCC----------CCTTTTTCC----CCC--CCCTT------->477

     432>TTTTCTTCCCCCT-TTTTTTTTTCCCTCCCTTTTCGCCCCCCTTTCCCCGTAACTTTTTTTTTGTCATTTTAAAACTTTTTCCCCCCCCCCCGG-AAA>529
     478>-----TTCCCCGTATTTTTTTTTT---T--------G--------GTCC---T---TTT---------A---AAAAATTTTTTCCCCCCCCCCCCGGGAAA>536

     530>AAAT--CCCCCTTTTT-AAAACCAAACGGGGG--TCCAAAAAAATCCCGCTTTCTTCTCTTTTCTACTCCTGACAATAATTCACACTATTTTCAAATCTAG>624
     537>AAATTACCAAATTTTTAAAAACCAAAAGGGGGGCTAAAAAAAACCCCGCTTTCTTCTCTTTTCTACTCCTGACAATAATTCACACTATTTTCAAATCTAG>636

     625>ATCTGGTTTCAATTAGATCTCATGTAATCAACATTTACACCATCTAGCTCTGTGAAAGTTTTCAGCAAATCTTCTAGCATTTAAGCTTGTCCATTAAATT>724
     637>ATCTGGTTTCAATTAGATCTCATGTAATCAACATTTACACCATCTAGCTCTGTGAAAGTTTTCAGCAAATCTTCTAGCATTTAAGCTTGTCCATTAAATT>736

     725>TTCTGCAAATTCTGCAAGTTGTGTTCATGACTGATTTTCAGTTCTCTAGGTTTGTGCTCAATTTTGCACATTGATCAGGTGCCTGAAGCATCTGCTAGGG>824
     737>TTCTGCAAATTCTGCAAGTTGTGTTCATGACTGATTTTCAGTTCTCTAGGTTTGTGCTCAATTTTGCACATTGATCAGGTGCCTGAAGCATCTGCTAGGG>836

                                                                              ##################################
     825>CTGCAGCATACGTGCATAAAGAAGAAACTAGGTGCGACTCTAAGCAAGTTTTTCACTTTGATGCAGGACATACCAATGTAGCGGAAATGAACATGGGGAG>924
     837>CTGCAGCATACGTGCATAAAGAAGAAACTAGGTGCGACTCTAAGCAAGTTTTTCACTTTGATGCAGGACATACCAATGTAGCGGAAATGAACATGGGGAG>936

        ####################################################################################################
     925>GAAGCAGCACAACCCCAGGTTCGTATAGGAAACAGCCTGTTACCACCTTGGATGCTTAGCCACTTGAACGGGTAGAAGCTACCGTTCCGCAGTCAACTTG>1024
     937>GAAGCAGCACAACCCCAGGTTCGTATAGGAAACAGCCTGTTACCACCTTGGATGCTTAGCCACTTGAACGGGTAGAAGCTACCGTTCCGCAGTCAACTTG>1036

        ####################################################################################################
    1025>AATGGTTTAAACATTTCACGAACTCTTTAGATTATACTGTATTTGGATGTTTGGAACACTCTCCAGCTACAATTTAAAATACATGCCCAGATGTCTCAGG>1124
    1037>AATGGTTTAAACATTTCACGAACTCTTTAGATTATACTGTATTTGGATGTTTGGAACACTCTCCAGCTACAATTTAAAATACATGCCCAGATGTCTCAGG>1136

        ##################################
    1125>GGAGTATTGAGATTAGGGGTTTCTCGTACGTTCC>1158
    1137>GGAGTATTGAGATTAGGGGTTTCTCGTACGTTCC>1170
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Figure 7. The Alignment Analysis of P2 (top) and P1 (bottom) DNA Fragment Sequence were Amplified by Primer BMS

The Softberry alignment analysis of both sequences and the *GLO2* gene sequence in GenBank showed that the primer BMG successfully amplified four CDSi exons. CDSi refers to all of the exons located at the center of the sequence (Figure 9).

The alignment analysis of both sequences by ApE showed the same result as the Softberry analysis. Four exons were amplified by the primer BMG. This analysis showed that the first exon is located at the beginning of the DNA fragment sequence (*), the second (#) and third (^) exons are located at the center of the DNA fragment sequence and the fourth exon is located at the end of the DNA fragment sequence (-). The sequence between the four exons is predicted to be an intron sequence. The intron sequence showed the differences in the nucleotides between the P1 and P2 DNA fragments (red in Figure 10). The similarity of the exon sequences of P1 and P2 showed that the DNA fragments amplified by the primer BMG are associated with the *GLO2* gene but not required to identify trees with high pistillate flowers. The expression of *GLO2* seems to be mainly localized in sepals, petals and staminodes or the stamens of pistillate and staminate flowers, respectively. *GLO2* genes in oil palm are expressed not only in male and female inflorescence but also in the roots [4]. The differences in the DNA fragment introns associated with the *GLO2* gene can be used as a marker to identify trees with more pistillate flowers than staminate flowers. Marker-assisted selection (MAS) could greatly assist plant breeders in attaining breeding program goals. The exploitation of the advantages of MAS relative to conventional breeding could have a great impact on crop improvement [16].

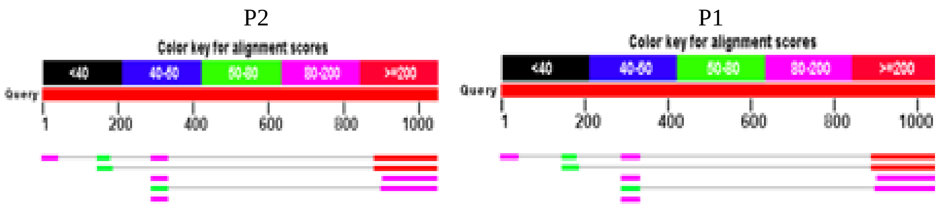

Figure 8. The Results of BLASTn Analysis of the Primer BMG Amplicon of the P2 and P1 DNA Fragments

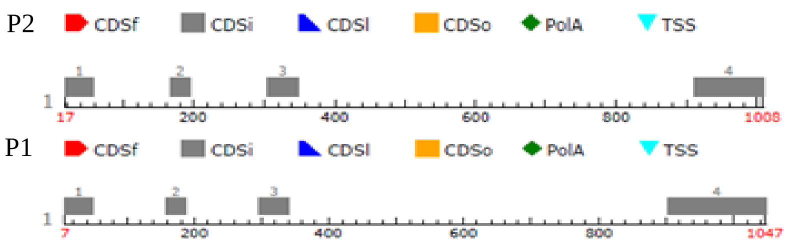

Figure 9. Softberry Alignment Analysis of the P2 (Top) and P1 (Bottom) DNA Fragments Amplified by Primer BMG

Table 4. The BLASTn Result for the Similarity of the Amplicon Primer BMG between the P2 DNA Fragment and the GenBank Database

Accession	Description	Total score	E value	Max ident
AF411848.1	*Elaeis guineensis* MADS box transcription factor (GLO2) mRNA, complete cds	503	2e-69	96%

Table 5. The BLASTn Result for the Similarity of the Amplicon Primer BMG between the P1 DNA Fragment and the GenBank Database

Accession	Description	Total score	E value	Max ident
AF411848.1	*Elaeis guineensis* MADS box transcription factor (GLO2) mRNA, complete cds	501	6e-69	98%

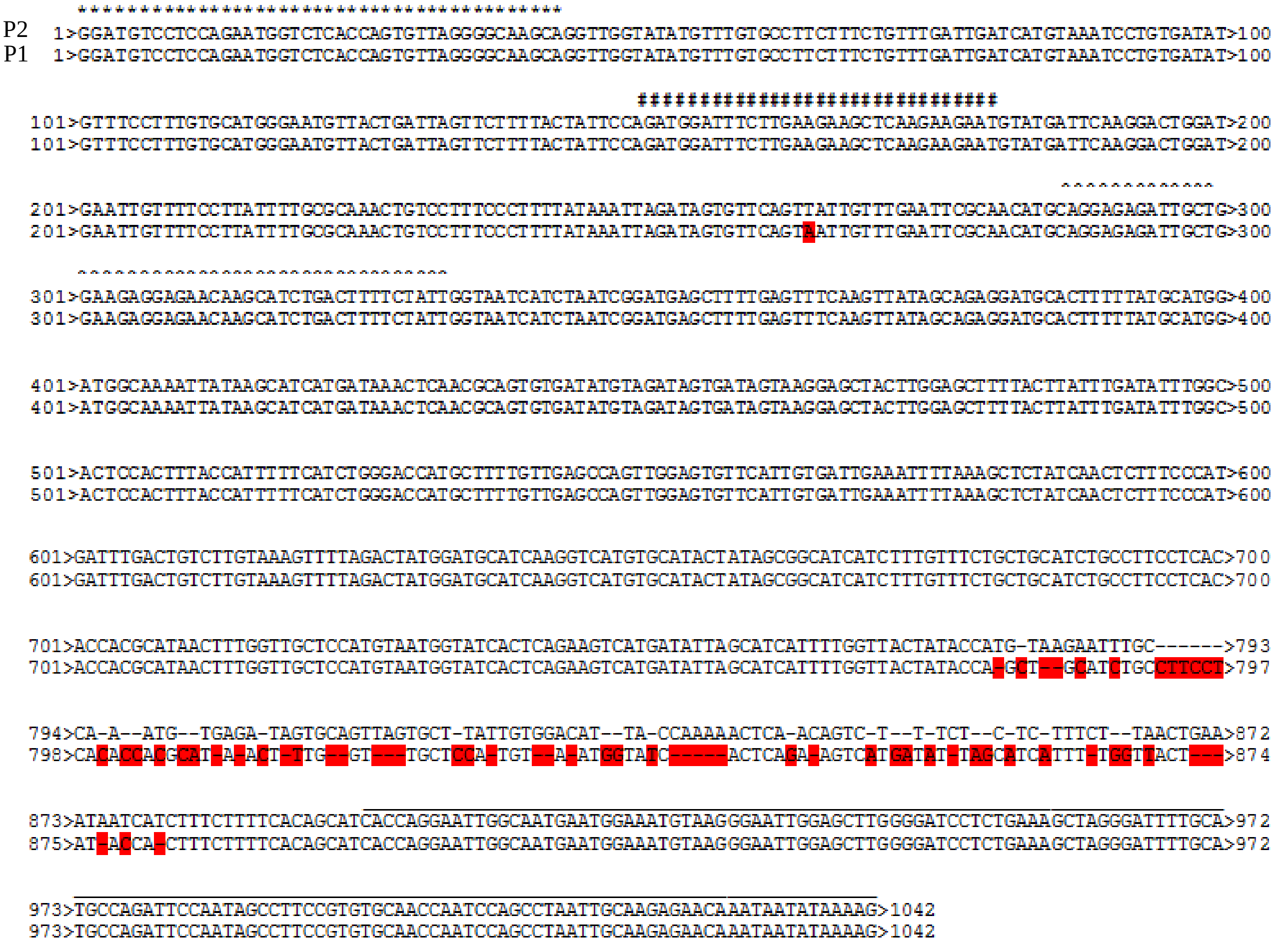

Figure 10. The Alignment Analysis of P2 (top) and P1 (bottom) DNA Fragment Sequence was Amplified with the Primer BMG

Conclusion

The CTAB+PVP method successfully isolated the genomic DNA of pistillate and staminate flowers of the oil palm tree. The genomic DNA isolation for pistillate flowers was more efficient than that for staminate flowers. BLASTn analysis showed that the P2 and P1 DNA fragments amplified with primer BMS have a 99% point of similarity with the oil palm *SQUA1* gene and the P2 and P1 DNA fragments amplified by primer BMG have 96% and 98% point of similarity, respectively, with the oil palm *GLO2* gene. Although the primer BMS produced a single band with the product size of 1250 bp on the genomic DNA of the P2 and P1 flowers, differences between the exon and intron nucleotides appear in both sequences. Primer BMG produced a single band with the product size of 1250 bp for the P1 genomic DNA sample and 1300 bp for the P2 genomic DNA sample. Differences between intron sequences detected for both sequences.

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