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Bioinformatics analysis in flowering gene for differentiation male and female flowersin african palm oil (*Elaeis guineensis*)

Abstract

Flowering stage in palm oil is regulated by MADS Box which is contained with the variety of genes in which one of them is squamosa. The squamosa gene is responsible for sepal and petal formation on the flower organs both on male and female sex. The existence of physical differences in male and female flower organs shows that the differences at the molecular level that is specifically indicated in base composition between males and females sex. Thus, it takes bioinformatics methods to see the difference between the base composition of male and female sex. The purpose of this study is to obtain squamosa gene based on the designed primer used, get on base composition of male and female sex, knowing the differences between males and females concerned on the bases composition, and primer characterization test used as molecular markers. Based on the previous study that managed to characterize MADS-box gene of African palm oil, a primer was designed and named GmG (Globosa-male-Gaps). In this study, 20 samples consisted of 10 males and 10 females, 2 samples taken based on the purity of the DNA through nanodrop test. Those two samples were further tested using electrophoresis to see where the squamosa gene and sequencing tools to determine the composition of the bases of the two samples. Visualization results of squamosa genes show that the gene is located between 1000 and 1500 base pairs. The sequencing results obtained were further analyzed using bioinformatics analysis such as UGENE V1.15.1, Geneious V8.05, and Perl Primer Free Version, shows that there are differences in base composition between the two samples in the intron.

Keywords: bioinformatics, *Elaeis guineensis*, MADS box, squamosa, palm flowers

Volume 2 Issue 3 - 2018

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Received: February 20, 2018 | **Published:** June 04, 2018

Introduction

Indonesia is one of the countries that consume palm oil that can be modified into multiple products such as frying oil, soap, chocolate butter, and biodiesel.[1] Recent years, the productivity of palm oil has increased significantly through the consumption of the modified products. In addition, there were several methods used in stages to increase the productivity such as superior seeds, fertilization and molecular. In the scope of molecular methods[2] in previous research used to design a primer that amplified the flowering gene in order to increase the productivity. Only the female flowers can be processed to modified products because fruit can only be produced by mating. However, there were difficulties to distinguish which are male and female's flowers on the palm tree before flowering stage occurred.

The previous study was explained by Adam et al.,[3] revealed that selection and preparation of primer was based on the flowering gene sequence resulted in four types of flowering genes that can be described as a whole as MADS box such as agamous, deficient, minichromosomes maintenance, and serum response factor. Squamosa, one of the genes within agamous, is responsible for sepal and petal formation on flower structure. The process to form sepal and petal are initiated by the transcription and translation of squamosa. The proteins formed will interact with the proteins from keratin-like domain to form a multimeric complexes protein. These complexes proteins can act to inhibit or to promote sepal and petal formation on the flower structure.

Nevertheless, these genes do not determine the sex of palm oil flower. The designed primer is created based on squamosa, which is in one of the genes that are within agamous. Squamosa plays role in regulating sepal and petal formation. The purpose of this study is to obtain squamosa gene based on the designed primer used, get on base composition of male and female sex, knowing the differences between males and females concerned on the bases composition, and primer characterization test used as molecular markers.

Materials and methods

Collection of male and female flowers as samples

Twenty samples, consist of 10 male and 10 female flowers were obtained from Agency for Assessment and Application of Technology (BPPT) palm oil field located in Serpong Area, Banten Province of Indonesia.

Whole genome isolation

This procedure was used to isolate the whole genome of palm oil using plant DNA isolation protocol.[4] As much 0.5gr of samples were soaked with liquid nitrogen and crushed in a mortar. During grinding, 0.1gr of PVP was added into the mortar. The powder formed was inserted into a centrifuge tube containing 5ml of extraction buffer and 50µl of 1% Mercaptoethanol. The mixture was shaken and heated for 30 minutes at 65°C. During heating, the mixture was shaken slowly for every 5 minutes interval and cooled to room temperature.

A 5ml of C:I was added to the mixture. The mixture were shaken vigorously and centrifuged at 13500rpm for 20 minutes at 4°C. A 4ml of supernatant formed was moved into a new centrifuge tube then 4 ml of C:I was added to the supernatant. The mixture formed was shaken and centrifuged at 13500rpm for 20 minutes at 4°C. As much 3ml of supernatant formed was inserted into a new centrifuge tube. 3ml of C:I was added to the supernatant. The mixture formed was shaken and centrifuged at 13500rpm for 20 minutes at 4°C. One ml of supernatant formed was moved into a new centrifuge tube while 1 ml of isopropanol was added to the centrifuge tube. The mixture formed was shaken slow and stored in a refrigerator for 30 minutes. After that re-centrifuged the mixture to 13500rpm for 10 minutes at 4°C. The supernatant formed was discarded and the pellets formed was dried, 500µl of TE buffer and 50µl of NaOCH3CO (3M, pH 5.2, cold condition) was added into the pellets. 1.1ml of ethanol absolute was added to the mixture and stored overnight at -20°C. The mixture formed was re-centrifuged to 16000rpm for 10 minutes at 4°C. The supernatant formed was discarded and the pellets formed containing the DNA was inserted with 400µl of 70% cold ethanol. The supernatant formed was discarded and the pellets formed was dried and inserted with 100µl of ddH2O and 10µl of RNAse. Last, the mixture formed was incubated for 1 hour at 37°C.

Nanodrop assay

As much 1µl of isolated DNA from each sample was quantified using Nanodrop assay to see a ratio of concentrated DNA between A_{260}/A_{280} and A_{260}/A_{230}.[5]

Genome visualization

This method was used to visualize the isolated genome. A 4µl of each sample was inserted into the agarose well. Also, 1µl of 1kb DNA ladder from FERMENTAS also inserted into the different well to act as a marker. The agarose well was made from 1gr of agarose and 100ml of water. The mixture was heated and poured into the electrophoresis case. The samples were inserted when the agarose gel had already hardened and cooled off in the electrophoresis case. After the electrophoresis had finished, the gel was soaked with Ethidium Bromide for 20 minutes to visualize the isolated genome.[6] Amplification and sequencing of squamosa gene. First, 1µl of isolated DNA from the samples was dissolved in PCR mastermix containing 4.9µl of ddH2O, 0.1µl of Dream Taq polymerase, 1µl of Taq buffer 10X, 0.5µl of EGSQUA forward primer, and 0.5µl of EGSQUA reverse primer. The PCR was conducted under the conditions shown in Table 1. The amplicon formed were sequenced at Agency for Assessment and Application of Technology (BPPT), Jakarta.

Table 1 The condition of the PCR to amplify squamosa gene sequence

	Temperature (°C)	Time
Pre-denaturation	95	5 minutes
Denaturation	95	30 seconds
Annealing	65.5±10	30 seconds
Extension	72	1 minute
Final-extension	72	5 minutes
Cycle	30	

Bioinformatics analysis

The sequenced amplicons were analyzed using bioinformatics software such as UGENE V1.15.1, Geneious V8.05, and Perl Primer Free Version.

Results and discussion

From 20 samples, 12 of them were visualized. Based on the results, the length of the isolated genome is longer than 50kbp that is higher than the marker used. All 20 samples were analyzed to quantify the purity of the isolated genomes. A good purity DNA is more than 1.8 for A_{260}/A_{280} and 1.2 for A_{260}/A_{230}. Based on this results, two samples were picked to be further analyzed which are CJ5 and CB3 (which has higher purity than others). The CB3 stand for a female sample, on the other hand, CJ5 stand for male sample. Based on the results, the amplicon formed using a designed primer is located between 1000bp and 1500bp revealed that on proper size of squamosa gene.[3]

Sequencing of squamosa gene amplicons

CB3 and CJ5 amplicons were sequenced to identify their bases composition. CJ5 amplicon could be sequenced up to 605bp by forward primer and 593bp by reverse primer. On the other hand, the CB3 amplicon could be sequenced up to 591bp by the forward primer and 601 by the reverse primer. Based on the result of genome isolation, the length of all 20 samples is higher than 50Kbp. This study proves that the length of whole genome of palm oil is 1.8Gbp.[7] The isolated genomes were quantified by Nanodrop assay. Based on the results, 2 samples, 1 male, and 1 female were taken, CB3 and CJ5. Those samples were taken because their DNA purity is good enough. From this results, 2 samples were picked, they were CB3 as female palm oil and CJ5 as male palm oil.CB3 and CJ5 were amplified using a couple of designed primer. The sequence of forward primer is GmG primer (F-RCACTAAYAGCRCA / R-TCACTTARTTCPCA). These primers were designed to amplified squamosa. The previous study shows that the length of squamosa is around 1400bp.[3] Thus, to know the differences between the male and female amplicon, sequencing was conducted. Based on the sequencing results, the sequencing tools could not fully sequence the whole amplicons. CJ5 amplicon could be sequenced up to 605bp by forward primer and 593bp by reverse primer. On the other hand, the CB3 amplicon could be sequenced up to 591bp by forward primer and 601 by reverse primer. Therefore, there is still an unsequenced region on both amplicons (Figure 1).

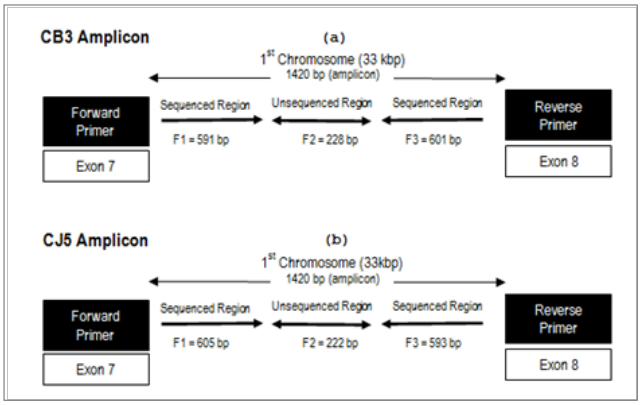


Figure 1 CB3. (A) and CJ5 (B) amplicons sequencing results. F1, Sequenced region using forward primer; F2, Unsequenced region; F3, Sequenced region using reverse primer.

First of all, *Elaeis guineensis* has 16 chromosomes.[8] Using a sequencing software, PerlPrimer Free Version, *squamosa* is located on the first chromosome based on the sequencing results. Also, from using PerlPrimer, eight exons of *squamosa* are found on the first chromosome. The length of the first chromosome is 33627bp (Table 2). The first chromosome contains exons and introns. Using another

sequencing software, UGENE V.1.15.1, forward primer is attached on the 7th exon, on the other hand, the reverse primer is attached on the 8th exon (Table 2). Based on the UGENE analysis results, the exact length of the sequenced amplicon is 1420bp whereas this region is located between the forward and the reverse primer (Figure 2).

Table 2 Eight exons of squamosa on the first chromosome based on the sequencing results

Exon	Gen	mRNA	Id	Mismatches	Gap	Splice site (d a)
1	67 - 366	32-331	100%	0	0	1 0
2	19624-19702	332-410	100%	0	0	1 1
3	30439-30503	411-475	100%	0	0	1 1
4	30582-30681	476-575	100%	0	0	1 1
5	31093-31134	576-617	97.60%	0	0	1 1
6	31922-31963	618-659	100%	0	0	1 1
7	32063-32193	660-790	100%	0	0	1 1
8	33192-33627	791-1226	100%	0	0	0 1

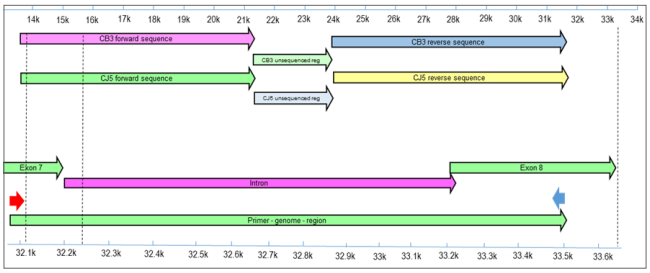
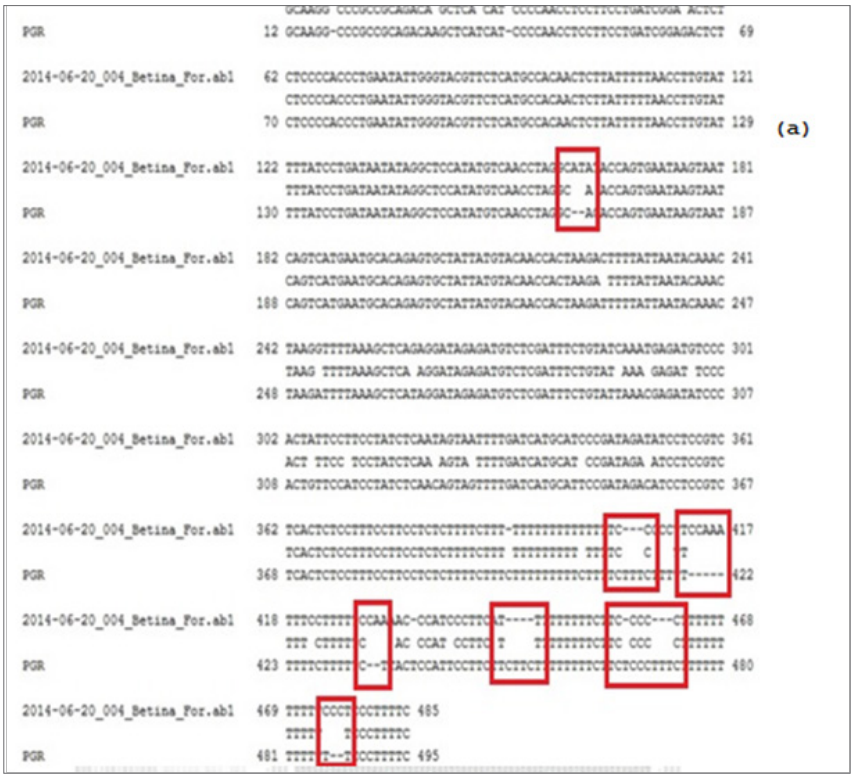


Figure 2 Gene mapping of the sequenced amplicon by UGENE V.1.15.1.

Based on the gene mapping, the sequenced region on both amplicons contains exons and introns. The forward sequenced amplicon on CB3 contains 89 exons and 503 introns, 89 exons and 517 introns on CJ5. The reverse sequenced amplicon on CB3 contains 312 introns and 288 exons, 304 introns and 288 exons on CJ5. Hereafter, the sequenced amplicons were compared with *squamosa* in order to know the differences on their bases sequence (Figure 3).

The results of the alignment show that there are differences between CB3 and CJ5 sequenced amplicon concerning their introns. Both on CB3 and CJ5 have different gaps position and composition. Further analysis is required to simplify their differences. These results also show that the designed primers have the potential to differentiate between male and female sex of palm oil. However, further analysis is needed to ensure their base differences. Full sequenced amplicons both on male and female sex are required to know precisely their base differences. Nevertheless, the sequencing condition need to be optimized to fully sequence the whole amplicons because their amplicons have many repeat bases, mainly on their intron part.



Citation: Faizal I, Febryan Y. Bioinformatics analysis in flowering gene for differentiation male and female flowersin african palm oil (Elaeis guineensis). *Biodiversity Int J.* 2018;2(3):265–270. DOI: 10.15406/bij.2018.02.00071

Figure 3 Four results of alignment between the amplicons and squamosal (PGR) using Geneious V8.05. (A) CB3 forward sequenced amplicon compared with squamosa. (B) CB3 reverse sequenced amplicon compared with squamosa. (C) CJ5 forward sequenced amplicon compared with squamosa. (D) CJ5 reverse sequenced amplicon compared with squamosa. Red squares (☐) stands for the differences between the amplicons and squamosa concerning their missing bases.

Conclusion

All twenty samples containing 10 male and 10 female palms oil show that the genome isolation is successful. 2 out of 20 samples were picked, one as the representative of male and the other as the representative of female palm oil based on the nanodrop assay. The length of the amplicons formed by PCR using designed primers show no differences between male and female amplicon. Thus, further analysis using sequencing tool, PerlPrimer, UGENE, and Geneious show that there are differences between the amplicons and squamosa concerning their gaps. However, further analysis, such as full sequenced amplicons is still needed to ensure their differences, especially on their introns part. The most important finding from this study is the finding of the differences between male and female amplicons, which shows the designed primers as a potential genetic marker in the future.[9–12]

Acknowledgments

I gratefully acknowledge the support of this work by the Agency for the Assessment and Application of Tehcnology (BPPT), Indonesia.

Conflict of interest

Author declares there is no conflict of interest in publishing the article.

References

1. Lutz G, Chavarria M, Arias ML, et al. Microbial degradation of palm (*Elaeis guineensis*) biodiesel. *Rev Biol Trop*. 2006;54(1):59–63.
2. Kok SY, Namasivayam P, Ee GC, et al. Biochemical characterisation during seed development of oil palm (*Elaeis guineensis*). *J Plant Res*. 2013;126(4):539–547.
3. Adam H, Jouannic S, Morcillo F, et al. MADS box genes in oil palm (*Elaeis guineensis*): patterns in the evolution of the SQUAMOSA, DEFICIENS, GLOBOSA, AGAMOUS, and SEPALLATA subfamilies. *K Mol Evol*. 2006;62(1):16–31.
4. Sarwat M, Madan SN, Malathi L, et al. A standardized protocol for genomic DNA isolation from Terminalia arjuna for genetic diversity analysis. *Electronic Journal of Biotechnology*. 2006;9(1):85–91.
5. Desjardins P, Conklin D. NanoDrop microvolume quantitation of Nucleic Acids. *J Vis Exp*. 2010;22(45):2565.
6. Yilmaz M, Ozic C, Gok I. *Principles of Nucleic Acid Separation by Agarose Gel Electrophoresis, Gel Electrophoresis*. In: Magdeldin S, editors. *Principles and Basics*. China: Shanghai. In Tech; 2012.
7. Singh R, Abdullah MO, Low ET, et al. Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds. *Nature*. 2013;500(7462):335–339.
8. Xiao Y, Zhou L, Xia W, et al. Exploiting transcriptome data for the development and characterization of gene-based SSR markers related to cold tolerance in oil palm (*Elaeis guineensis*). *BMC Pant Biol*. 2014;14:384.
9. Allegruci M, Hu FZ, Shen K, et al. Phenotypic characterization of *Streptococcus pneumonia* biofilm development. *J Bacteriol*. 2006;188(7):2325–2335.
10. Chan PL, Ma LS, Low ETL, et al. Normalized embryoid cDNA library of oil palm (*Elaeis guineensis*). *Electronic Journal of Biotechnology*. 2010;13(1):10–11.

11. Mukherjee S, Mitra A. Health Effects of Palm Oil. *J Hum Ecol*. 2009;26(3):197–203.

12. Robert EF. *RNA Methodologies: A Laboratory Guide for Isolation and Characterization*. 4th ed. Chicago: Illinois. Elsevier; 2010.

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