Agrotech Journal
Url: http://usnsj.com/index.php/ATJ
Email: editor.atj@usnsj.com

Variations on The Concentration and Purity of Oil Palm Flowers (*Elais Guineensis* Jac.) in Some Extraction Methods for Identification of the Flowering Gene

AUTHORS INFO

Winda Nawfetrias
Pusat Teknologi Produksi Pertanian
OR Pengkajian dan Penerapan Teknologi
Badan Riset Inovasi Nasional
winda.nawfetrias@bppt.go.id

Yuda Purwana Roswanjaya
Pusat Teknologi Produksi Pertanian
OR Pengkajian dan Penerapan Teknologi
Badan Riset Inovasi Nasional
yuda.purwana@bppt.go.id

ARTICLE INFO

e-ISSN: 2548-5148
p-ISSN: 2548-5121
Vol. 6 No. 2, December 2021
URL: https://doi.org/10.31327/atj.v6i2.1658

Abstract

Palm oil production is related to the number of fruit and is thought to be determined since the beginning of the flowering phase as the initial stage of fruit formation. Identification of oil palm flowing genes needs to be done as the first step of knowing the mechanism of flowering in oil palm molecular. Oil male and palm females contain high polysaccharides and polyphenolic compounds so they can inhibit the process of molecular identification. Molecular studies provide accurate and fast information about the potential of oil palm genetics as a commercial commercial commodity. The purity and concentration of DNA and the temperature of annealing are the main requirements for PCR-based molecular studies. The purpose of this study obtained the extraction method of oil palm flowers which produced high DNA concentration and purity and optimized the temperature of annealing to identify the flowering gene. DNA from male flower spike and female flowers is extracted using the A (SDS) method, method B (CTAB), method C (CTAB + PVP), and method D (commercial kit). Primary BMS annealing temperature for amplification of optimized flowering genes using PCR gradients. The extraction of male flowers and female flowers using the C (CTAB + PVP) method produces the best DNA concentration and purity compared to other methods. The best annealing temperature for male flower DNA amplification and female flowers using the BMS primer is 61.1 ° C. Amplification of male flowers and female flowers using Primary BMS produces 1200 PB products. The results of DNA extraction and PCR amplification using the BMS primers in female flower samples are better than male flowers

Keywords: CTAB, DNA Extraction, female flower, flowering gene, male flower, PVP

A. Introduction

Palm oil (Elaeis Guineensis Jacq.) Is one of the national superior commodities because it contributes greatly to the Indonesian economy. Based on Badan Pusat Statistik (2020) the area of oil palm plantations in Indonesia reached 14.33 million hectares with production reaching 42.9 million tons in 2018. The area of oil palm plantations is estimated to increase by 1.88% to 14.60 million hectares with Production increase of 12.92% to 48.42 million tons in 2019. This caused Indonesia to become the largest palm oil exporter in the world during 2020 and contributed 53.3% of the total value of world palm oil exports (workman, 2021).

Palm oil production is related to the number of fruit which is thought to be determined since the beginning of the flowering phase as the initial stage of fruit formation. Palm oil is referred to as a temporal dioecious for male and female flower production because the cycle of emergence of male and female flowers alternately on the same plant so as to produce alloogamus reproduction models. Male and female flower production alternately in one tree is influenced by various complex factors such as abiotic factors, metabolic factors and genetic factors (Adam, H., M. Collin, F. Richaud, T. Beule, D. Cros, A. Omore, L. Nodichao, B. Nouy, & J.W.Tregear, 2011). Genetic factors that influence flowering are groups of genes included in the large family of mads-box genes. Mads-Box genes encode the familial of the transcription factor that regulates developments in high-level plants including flower and fruit formation in angiosperme plants (Gramzow, L., L. Weilandt, & G. TheiBen, 2014). Until now the emergence of male and palm male and female palm females cannot be predicted accurately. Identification of oil palm flowing genes needs to be done as the first step of knowing the mechanism of flowering in oil palm molecular.

Molecular studies, especially DNA provide information quite accurately and quickly regarding the potential of oil palm genetics. DNA-based research on the potential of genes that influence a character related to organs that express the character. Research on the flow of flowering is carried out by extracting DNA from male and female flower organs to avoid the sequence of the targeted target gene. Some oil palm DNA extraction protocols generally use leaves as samples (Ihase, L.O., R. Horn, A.G. Anoliefo, C.R. Eke, A.S. Afolabi, & O. Asemota, 2016; Suzana, M., A.R. Rahimah, I. Maizura, & R. Singh, 2015), while the extraction method of oil palm flowers has not been widely published by researchers.

Palm oil plants have a network with high polysaccharide and phenolic content (Sasidharan, S., R. Nilawatyi, R. Xavier, L.Y. Latha, & R. Amala, 2010; Rosalina, T.R.T, S. Mohamed, G.F. Samaneh, M.M. Moordin, Y.M. Goh, & M.Y.A. Manap, 2011). Polysaccharides and proteins are the biggest contaminants during DNA extraction because the two compounds bind DNA and must be eliminated to obtain pure DNA (Samprook and Russell, 2001). The phenolic component in the sample will be quickly oxidized and binding DNA. Samples contaminated with polysaccharides and polyphenolics caused DNA degraded, thus disrupting the analysis of DNA sequencing. Optimization of the DNA extraction of oil palm flower organs needs to be done because every organ organ requires different DNA extraction protocols.

DNA Extraction Methods Are Always Related To Several Extraction Stages To Produce High Quality And Quantity Of DNA So That It Can Be Used For The Next Molecular Identification Process. The Cell Lysis Stage US The Initial Stage of DNA Extraction Generally Uses Chemical Compounds That Can Damage The Barrier Integrity of Cells Walls Such As Cetyl Trimethyl Ammonium Bromide (CTAB) and Sodium Dodecyl Sulfate (SDS) (Cheng, Y.J., W.W. Guo, H.L. Yi, X.M. Pang, & X. Deng, 2003), Especially For The Wall Lysis of Plant Cells With High Polysaccharide and Polyphenol Content. Polyvinyl Pyrrolidone (PVP) Is Often Used To Reduce Phenol Compounds That Can Degrade DNA. PVP Through Hydrogen Bonds, Makes Complex Bonds With Polyphenolic, And Can Effectively Eliminate Polyphenolics From Homogenate (Cheng et al., 2003). Every Plant Has A Secondary Compound Content In Different Plant Cells, Every Plant Requires An Optimum Extraction Procedure. Procedure Optimization can be made to the Composition of the Buffer License Solution or Physical Handling Technique for DNA Separation from other compounds, so that DNA degradation can avoided.

DNA with good concentration and purity is expected to be continued for the amplification process. One factor that affects the amplification process is the temperature of annealing. The appropriate annealing temperature affects the results of amplification. According to Asy'ari & Noer (2005) The temperature of annealing that is too high causes the release of primers that have attached to the DNA template so that the amplicon is not formed, while the annealing temperature is too low, it will cause a primary attachment that is not specific to the DNA template. This study aims to obtain the extraction method of oil palm flowers that produce high DNA concentration and purity and optimize the temperature of annealing to identify the flowering gene.

B. Methology

The study was conducted in biomolecular, PTPP, Laptiab, BPPT, Serpong laboratories. Plant sampling is carried out in Puspitek, Serpong. The material used in this study was the spikelet of male and individual flowers from the spike of the teneral female flower varieties taken from the Puspiptek trial garden, Serpong (Figure 1). Flower samples after being removed from the midrib are immediately stored at -20 oC for DNA extraction.

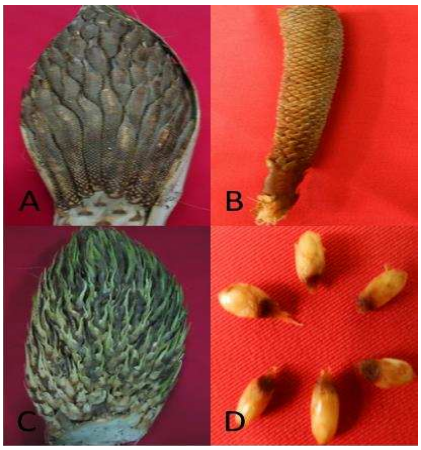

Figure 1. Male flower sign (a), male flower spike (b), female flower bunches (C), individual female flowers (D)

1. DNA Extraction

As many as 0.5 grams of spicles of male flowers and individual flowers from female flower spiketets are crushed using liquid nitrogen until smooth. The method used is as follows:

Method A (SDS). Sample powder is inserted into a centrifuable tube that contains 5 ml extraction buffer (10% SDS; EDTA 0.5 M pH 8; Tris HCl 1 m pH 8; NaCl 5 m; DDH2O; 1% MercaptoenaTOL) that has been incubated earlier at 65 OC.

2. Method B (CTAB)

Sample powder is inserted into a centrifued tube that contains 5 ml extraction buffer (CTAB 10%; EDTA 0.5 M pH 8; Tris HCl 1 m pH 8; NaCl 5 m; DDH2O; 1% MercaptTaHanol) which has been incubated before at 65 OC.

3. Method C

CTAB + PVP). Samples are crushed by adding 0.1 grams of PVP, then the powder is inserted into a centrifuge tube which contains 5 ml extraction buffer (CTAB 10%; EDTA 0.5 M pH 8; Tris HCl 1 m pH 8; NaCl 5 m; DDH2O; 1 % Mercaptoenatol) which had been incubated earlier at a temperature of 65 oC

4. The extraction procedure method A to the C method

The solution is homogenized with vortex then incubated at 65 oC for 30 minutes and turned back every 5 minutes. The solution was incubated at room temperature then added 1 Volume CI (Chloroform: isoamil alcohol = 24: 1) and centrifuged 14000 rpm for 20 seconds at 4 oC. This step is repeated three times. The supernatant was taken then added 1 volume of isopropanol that had been incubated at 4 oC. After being incubated at a temperature of -20 OC for 30 minutes, the solution was centrifuged at 14000 rpm for 10 minutes 4 oC. The supernatant was discarded then the pellet was dried, then added 500 µL, 1/10 volume nach3COO3 3 m pH 7 cold, 2 volume ethanol absolute that has been incubated at 4 oC, then stored at -20 OC overnight. Disentrized solution at 4 oC at a speed of 14000 rpm for 10 minutes. DNA pellets were washed using 400 µL 70% ethanol that had been incubated at 4 ^{o}C, then centrifuged at 4 ^{o}C at a speed of 14000 rpm for 5 minutes. The dried pellets are then added 100 µL DDH2O and 1/10 volume of RNASE, then incubated at 37 ^{o}C for 1 hour to actively in the RNase.

5. Method D

(Commercial Kit). The sample powder was crushed by adding 0.1 grams of PVP, subsequently extracted using a commercial DNA extraction kit in accordance with the procedure recommended by the manufacturer. Each extraction method is repeated three times.

6. Quality and quantitative DNA analysis

The quality of DNA was analyzed using an electrophoresis of gel agarose 0.8% with a tae buffer at a 100 volt voltage for 30 minutes. The electrophoresis results visualized using Gel Doc UV Transilluminator. Analysis of the quantity of DNA extraction was carried out using a 2000 thermo scientific production spectrophotometer. Data of purity and concentration are analyzed using R studio.

7. Amplification of flowering genes

The PCR reaction consists of DNA male flower genomes and female flowers as much as 150-180 ng as templates, 1 µl of Mix 2 mm, 1 µl 10 x Dream Taq Buffer, 0.1 Uream Taq DNA polymerase, 0.5 µl Primary Forward 0 , 2 µm, 0.5 µL primer reverse 0.2 µm, ddh2o nuclease-free to 10 µL. The condition of the PCR used is the initials denaturation of 95 oC 5 minutes, denaturation of 95 oC 30 minutes, the annealing temperature is 65.4 ± 06 oC 30 seconds, the 72 OC extension is 1 minute, this condition is repeated to 35 cycles, and the final extension 72 OC 5 minutes. The primary used for the amplification of the flowering gene is the Primary BMS F AgncacyaCcagnaggcn / r acncactyayggytcccacn for amplification of squamosa flowering genes

C. Result and Discussion

Palm oil flowers are very easy to oxidize, causing brown (browning) that causes degraded DNA, this causes the extraction of DNA to oil palm flowers is quite difficult. The solution to avoid browning on samples of male flowers and palm oil is a room, mortar and pestle must be cold when the sample fleeces. Addition of liquid nitrogen when the grade can prevent degraded oxidation and DNA occurrence. Fresh samples of male and female flowers are soaked in liquid nitrogen and crushed quickly to help break cell walls mechanically, this is done to keep DNA degraded. According to Xin and Chen (2012) the use of fresh tissue, liquid nitrogen and cold mortar on DNA extraction can reduce DNA degradation.

According to Chi, M.H., S.Y. Park, & Y.H. Lee (2009) The DNA extraction process consists of several stages, namely the preparation of the material to be used, the process of destruction of cells (lysis), removal of contaminant compounds, and DNA collection. The phase of the destruction of plant cells greatly affects the purity of DNA so that the appropriate technique and reagent material is needed so that the resulting DNA has a high purity. SDS and CTAB are detergent solutions that function to reduce the surface voltage of the liquid and dissolve lipids at the lysis stage, this causes cell membranes to degradate, so the cell organelets can come out. According to the Bintang (2010), CTAB is a cationic detejen that is destroying cells, unraveling proteins, and separating carbohydrates from nucleic acid. The interaction between SDS and cell membrane proteins affects cell lyzers in DNA extraction, so that cell membrane proteins do not hinder the next DNA process (Perumal, N.V., X. Zhang, M. Yuki, I. Fumio, dan F. Wang, 2016). The addition of SDS and Positively charged CTAB serves to separate the polysaccharide from DNA by binding negatively charged DNA.

Buffer extraction method A, B and C heat is heated at a temperature of 65 oC, because the ability of SDS and CTAB to melisis the cell will be active in conditions of 65 oC. According to Lade, B.D., A.S. Patil, & H.M. Paikrao (2014) Addition of extraction buffers incubated less than 65 oC causes imperfect denaturation in proteins that can produce contamination at the next extraction stage. The addition of heated extraction buffers in frozen samples by liquid nitrogen causes the network to change temperatures quickly so as to avoid damage to DNA. This is in accordance with the opinion of Purchooa (2004) that the addition of a heated CTAB buffer on frozen tissue by liquid nitrogen causes the network to experience a temperature difference from -80 oC to a temperature of 60 oC quickly resulting in high quality DNA.

Analysis of the quality of male and female DNA extraction using the A (SDS) method, method B (CTAB) and method C (CTAB + PVP) on agile gel 0.8% shows the quality of DNA is quite good even though there is still contaminant, while DNA extraction uses a kit Commercial shows degraded DNA ribbons (Figure 2). DNA extraction methods with SDS / CTAB extraction buffers produce good quality DNA from plants containing high concentrations of polysaccharides and polyphenols for Downstream analysis (Niu, C., H. Kebede, D.L. Auld, J.E. Woodward, G. Burrow, & R.J. Wright, 2008).

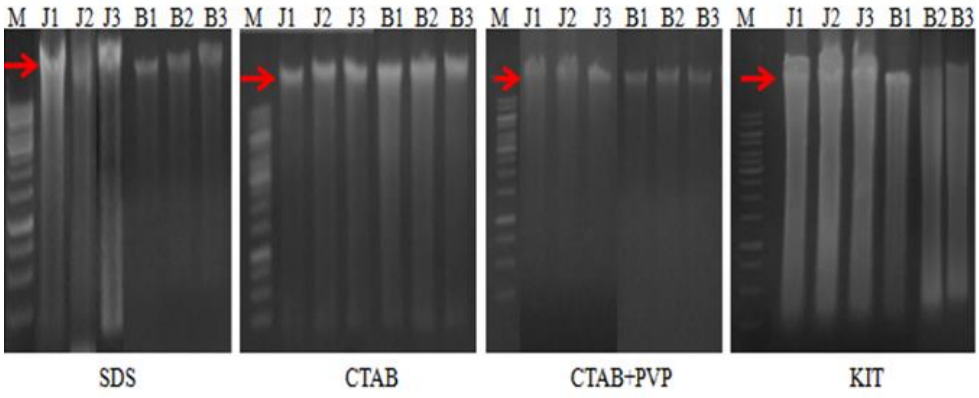

Figure 2. Analysis of the quality of male and female flowers DNA results using the A (SDS) method, method B (CTAB), C method (CTAB + PVP) and D method (kit)

Extraction using the D method (Kit method) produces degraded DNA shown by the existence of smears on the DNA band. The use of kits to extract DNA male and female flower organs is not recommended because this organ contains a fairly high phenolic compound. The reagents used on kits cannot reduce phenolic compounds produced by flower organs so that DNA is degraded. Commercial DNA extraction kit is an ideal procedure for DNA extraction because it does not use dangerous reagents, but in some commercial research kits produce low DNA results and varied quality (buldewo and jaufeerally-fakim 2002; Keb-Llanes, M., G. González, B. Chi-Manzanero, & D. Infante, 2002; Horne, E.C., S.P. Kumpatla, K.A. Patterson, M. Gupta, & S.A. Thompson, 2004).

Quantitative analysis shows that as much as 50% of male flower samples and extracted female flowers use the A (SDS) method, method B (CTAB), and the C (CTAB + PVP) method produces a concentration of DNA more than 500 ng / µL and 50% more DNA purity Of the 1.5 (Figure 3). Aboul-Maaty and Oraby (2019) recommended the ratio of DNA purity greater than 1.5 and better close to 1.8, while according to Healey, A., A. Furtado, A. Cooper, & R.A. Henry (2014) for NGS-based Molecular activities (next generation sequencing) needed DNA with a comparison of A260 / A280 between 1.8 to 2.0. The DNA extraction using the D method (Kit Method) is not quantified due to degradation.

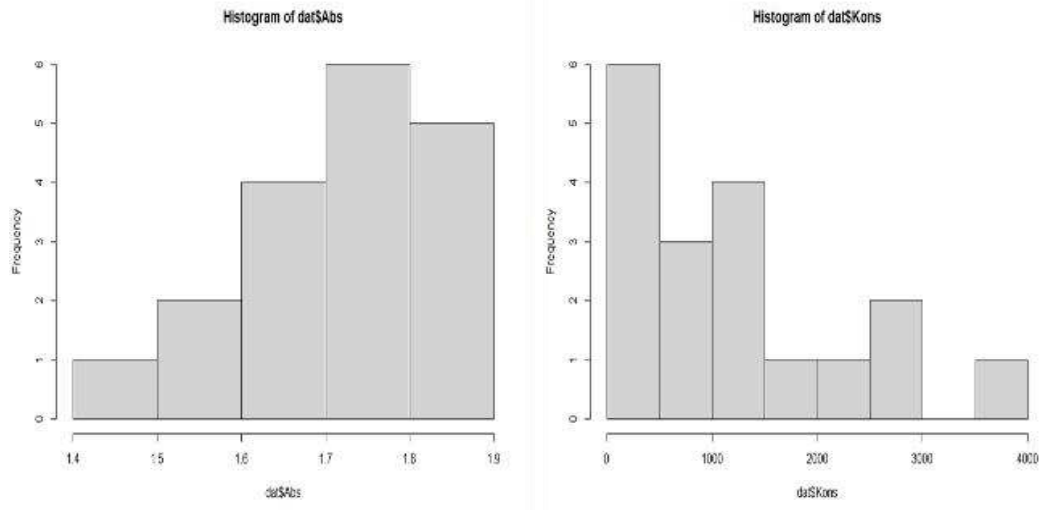

Figure 3. Histogram concentration and purity DNA extraction results using the A (SDS) method, B (CTAB) method, and method C (CTAB + PVP)

The male flower DNA extraction using the B (CTAB) method produces the lowest concentration and purity of DNA compared to samples and other methods (Figure 4). The extraction of female flower DNA uses the B (CTAB) method and the C (CTAB + PVP) method shows good purity, while the male flower extraction using the C (CTAB + PVP) method produces better DNA purity than male interest extracted using other methods. Method C (CTAB + PVP) produces the concentration of DNA from male flowers and the highest female interest compared to the A (SDS) method and B (CTAB) method. This shows the method C (CTAB + PVP) is able to produce the concentration and purity of male flower DNA and oil palm female flowers higher than other methods. This result is in accordance with the results of Murtiyaningsih's research (2017) that

the extraction of DNA pineapple leaves using CTAB and PVP buffers show good purity between 1.8 - 1.9.

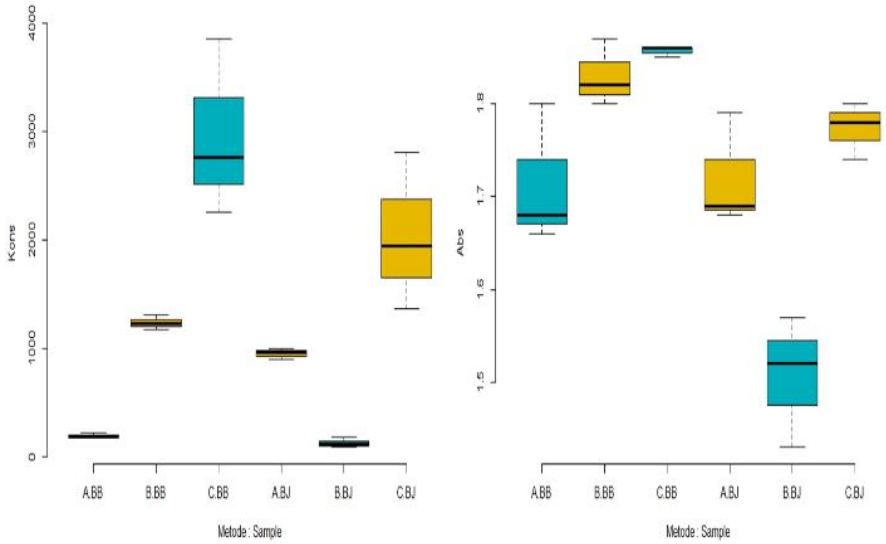

Figure 4. Boxplot concentration and purity DNA male and female flowers extraction using method A (SDS), method B (CTAB), and method C (CTAB + PVP)

The purity of female flowers DNA differs significantly with the purity of male flowers DNA, while DNA purity is no different between method A (SDS), method B (CTAB) and C method (CTAB + PVP) (Figure 5). The concentration of male flower DNA is no different from the concentration of female flower DNA, but the sample extracted using the C (CTAB + PVP) method produces a significant different concentration with other methods. Addition of PVP when the grade is thought to be able to inhibit the oxidase polyphenol enzyme. The polyphenol enzyme can degrade DNA and cause the oxidation of phenol compounds. Oxidation is characterized by the formation of brown color on the plant tissue to be isolated. This is in accordance with the opinion of Utami, A., R. Meryalita, N.A. Prihatin, L. Ambasari, P.A. Kurniatin, & W. Nurcholis (2012) Insulation with CTAB as an extraction buffer requires additional PVP to eliminate phenol contamination.

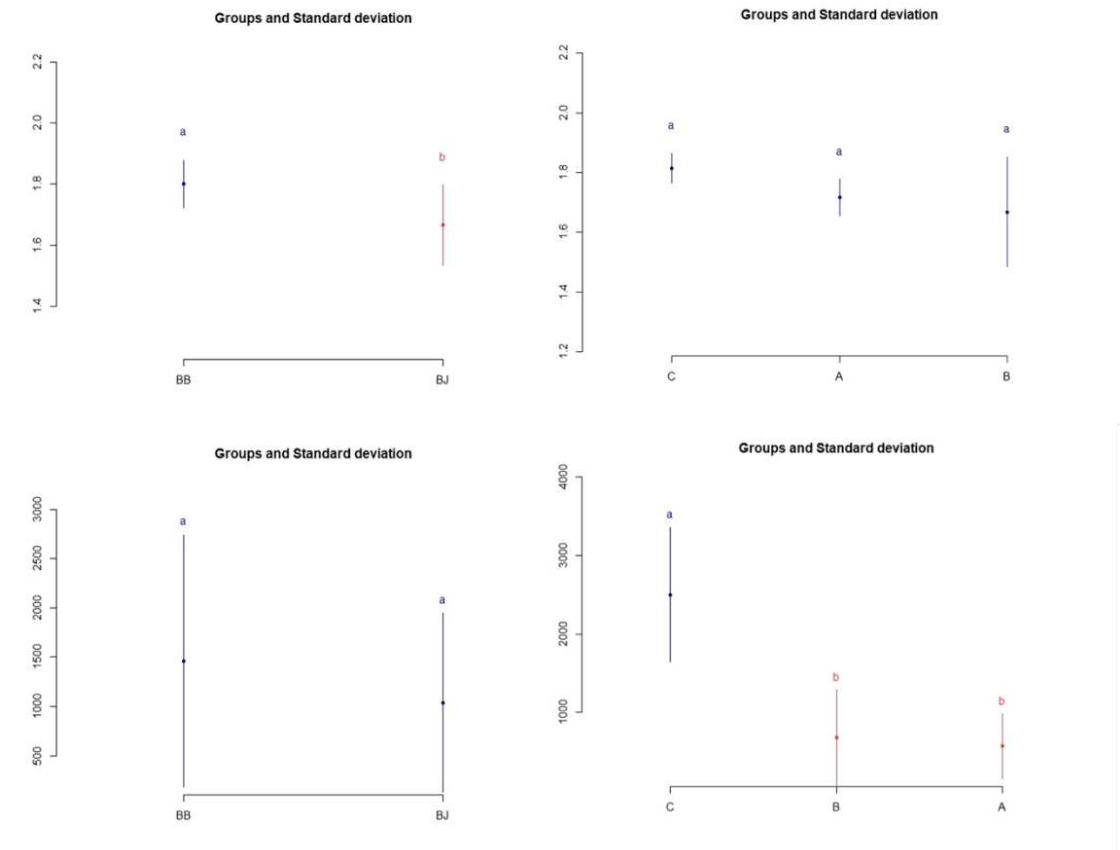

Figure 5. Advanced Test Duncan Purity and Concentration of Male Flower DNA and Female Interest Results Using the A (SDS) method, method B (CTAB), and method C (CTAB + PVP)

Extraction of male flowers and female flowers use method B (CTAB) and C methods (CTAB + PVP) show the same pattern, while the A (SDS) method shows a different direction in the parameters of purity and DNA concentration (Figure 6). The extracted female flowers using the B (CTAB) and C (CTAB + PVP) method shows higher purity and concentration than male flowers, while extracted female flowers using the A (SDS) method shows lower purity and concentrations than male flowers. This shows a CTAB buffer that is applied to a single and modified with the addition of PVP capable of producing the concentration and purity of oil palm female DNA better than the SDS buffer application. The extracted male flowers using SDS buffers produce purity and concentration of DNA better than Buffer CTAB and CTAB + PVP.

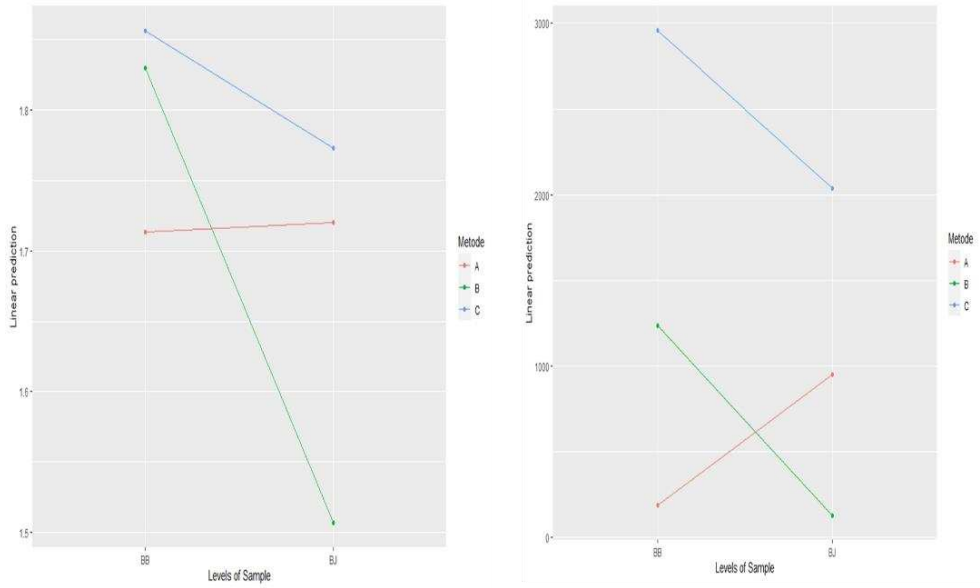

Figure 6. Pattern of interaction methods and types of samples on the extraction of male flowers and female flowers

DNA male flowers and female flowers extraction using the C (CTAB + PVP) method are widelyified using the BMS primer for the amplification of squamosa flowering gene. The PCR reaction uses DNA male flower genomes and female flowers as templates, the PCR reaction of each sample is repeated three times to get consistent results. The results of the amplification of male flowers and female flowers at various temperatures of primary BMS annealing using PCR gradients showing female flower DNA produces better PCR product quality than male flower DNA (Figure 7). PCR products female flower samples look more clear than male flowers.

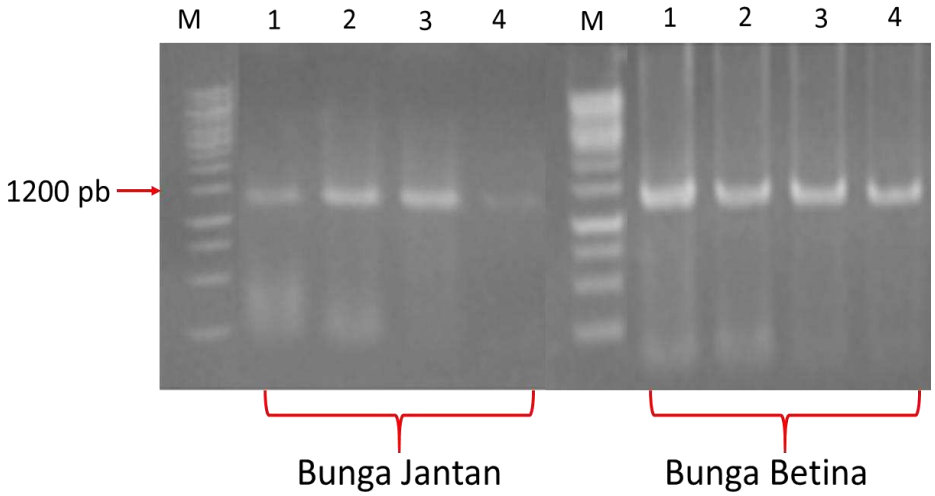

Figure 7. PCR Gradient Primary BMS at 59.4 oC (1); 60.2 oC (2); 61.1 OC (3); 62.1 OC (4)

The results of the Gradient PCR show amplification of male flower DNA using the BMS primary produces PCR products at 59.4 oC annealing temperatures; 60.2 oC: 61.1 OC and not produced PCR products at annealing temperature 62.1 oC, whereas in DNA female flower PCR products are

produced at all annealing temperatures. These results show a type of sample and annealing temperature allegedly influencing the quality of PCR products. The best annealing temperature for male and female DNA amplification using the BMS primer is 61.1 oC. Amplification of male flowers and female flowers using Primary BMS produces 1200 PB products.

In general, the results of this study show extraction of DNA and PCR reactions to identify the flowering gene from female flower samples producing purity and concentration and and PCR products are better than male flowers, because they are suspected of male flowers containing more contaminants that can reduce DNA purity and concentration, thus disrupting the PCR reaction. One source of contaminants in male flowers is the presence of pollen composed of polysaccharide compounds. The high polysaccharide male flower causes male flower DNA extraction and the PCR reaction is more difficult than female flowers.

D. Conclusion

The extraction of male and female flowers DNA uses the CTAB + PVP method produces the best DNA concentration and purity compared to the SDS method and the CTAB method. The best annealing temperature for male and female DNA amplification using the BMS primer is 61.1 oC. Amplification of male flowers and female flowers using Primary BMS produces 1200 PB products. DNA extraction and PCR amplification using primary BMS on female flower samples is better than male flowers.

E. Acknowledgement

The author expressed gratitude to the Agricultural Production Technology Center - Deputy Technology of Agroindustry and Biotechnology - The Assessment Agency and Application of Technology that facilitate this research.

F. References

Adam, H., M. Collin, F. Richaud, T. Beule, D. Cros, A. Omore, L. Nodichao, B. Nouy, & J.W.Tregear. (2011). Enviromental regulation of sex determination in oil palm : current knowledge and insights from other spesies. Ann. Bot. 108:1529-1537. DOI:10.1093/aob/mcr151.

Aboul-Maaty, N.A. & H.A. Oraby. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bulletin of the National Research Centre. 43(25):1-10. https://bnrc.springeropen.com/articles/10.1186/s42269-019-0066-1.

Asy'ari, M. & A.S. Noer. (2005). Optimasi konsentrasi MgCl2 dan suhu annealing pada proses amplifikasi multifragmens mtDNA dengan metode PCR. JKSA VIII (1):24-28.

Badan Pusat Statistik. (2020). Statistik Kelapa Sawit Indonesia Indonesian Oil Palm Statistics 2019. Subdirektorat Statistik Tanaman Perkebunan. BPS. Indonesia. ISSN:1978-9947.

Bintang, M. (2010). Biokimia Teknik Penelitian. Jakarta:Penerbit Erlangga.

Buldewo, S., and Y.F. Jaufeerally-Fakim. (2002). Isolation of clean and PCR amplifiable DNA from Anthurium andreanum. Plant Mol. Biol. Rep. 20: 71a-71g.

Cheng, Y.J., W.W. Guo, H.L. Yi, X.M. Pang, & X. Deng. (2003). An efficient protocol for genomic DNA extraction from citrus species. Plant Molecular Biology Reporter 21: 177a-177g.

Chi, M.H., S.Y. Park, & Y.H. Lee. (2009). A quick and safe method for fungal DNA extraction. Plant Pathol. J. 25(1):108–111.

Gramzow, L., L. Weilandt, & G. TheiBen. (2014). MADS goes genomic in conifers:towards determining the ancestral set of MADS-box genes in seed plants. Annals of Botany. 114:1407-1429. DOI:10.1093/aob/mcu066.

Healey, A., A. Furtado, A. Cooper, & R.A. Henry. (2014). Protocol: a simple method for extracting next–generation sequencing quality genomic DNA from recalcitrant plant species. Plant Methods 10:21.

Horne, E.C., S.P. Kumpatla, K.A. Patterson, M. Gupta, & S.A. Thompson. (2004). Improved high-throughput sunflower and cotton genomic DNA extraction and PCR fidelity. Plant Mol. Biol. Rep. 22:83a-83i.

Ihase, L.O., R. Horn, A.G. Anoliefo, C.R. Eke, A.S. Afolabi, & O. Asemota. (2016). Development of a method for DNA extraction from oil palm leaves and the effects of pH and ionic and strength on nucleic acid quantification. Journal of Biological Methods. 3(2):1-6. DOI:10.14440/jbm.2016.80.

Keb-Llanes, M., G. González, B. Chi-Manzanero, & D. Infante. (2002). A rapid and simple method for small-scale DNA extraction in Agavaceae and other tropical plants. Plant Mol. Biol. Rep. 20:299a-299e.

Lade, B.D., A.S. Patil, & H.M. Paikrao. (2014). Efficient genomic DNA extraction protocol from medicinal rich Passiflora foetida containing high level of polysaccharide and polyphenol. SpringerPlus 3:457.

Murtiyaninsih, H. (2017). Isolasi DNA genom dan identifikasi kekerabatan genetik nanas menggunakan RAPD (Random Amplified Polimorfic DNA). Agritop. 15(1):83-93. ISSN 1693-2877 EISSN 2502-0455.

Niu, C., H. Kebede, D.L. Auld, J.E. Woodward, G. Burrow, & R.J. Wright. (2008). A safe inexpensive method to islate high quality plan and fungal DNA in an open laboratory environment. Afr. J. Biotechnol. 7(16):2818-2822.

Perumal, N.V., X. Zhang, M. Yuki, I. Fumio, dan F. Wang. (2016). A modified SDS-based DNA extraction method for high quality environmental DNA from seafloor environments. Front. Microbiol. 7:1–13.

Purchooa, D. (2004). A simple, rapid and efficient method for the extraction of genomic DNA from lychee (*Litchi chinensis* Sonn.). Afr. J. Biotechnol. 3:253-255.

Rosalina, T.R.T, S. Mohamed, G.F. Samaneh, M.M. Moordin, Y.M. Goh, & M.Y.A. Manap. (2011). Polyphenol rich oil palm leaves extract reduce hyperglycaemia and lipid oxidation in STZ-rats. Int. Food Res. K. 18:179-188.

Sambrook, J., & D.W. Russell. (2001). Molecular Cloning: A Laboratory Manual. 3rd edn. New York: Spring Harbor Laboratory Press. New York.

Sasidharan, S., R. Nilawatyi, R. Xavier, L.Y. Latha, & R. Amala. (2010). Wound healing potential of Elaeis guineensis Jacq. Leave in an infected albino rat model. Molecules. 15:3186-3199.

Suzana, M., A.R. Rahimah, I. Maizura, & R. Singh. (2015). A simple and rapid protocol for isolation of genomic DNA from oil palm leaf tissue. Journal of Oil Palm Research. 27(3):282-287.

Utami, A., R. Meryalita, N.A. Prihatin, L. Ambasari, P.A. Kurniatin, & W. Nurcholis. (2012). Variasi metode isolasi DNA daun temulawak (Curcuma xanthorrhiza Roxb.). Prosiding seminar nasional kimia unesa. 25 Februari 2012. ISBN : 978-979-028-550-7.

Workman, D., (2021). Palm Oil Exports by Country. Available at: https://www.worldstopexports.com/palm-oil-exports-by-country.

Xin, Z., & J. Chen. (2012). A high troughput DNA extraction method with high yield and quality. Plant Methods. 8:26.