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ANALYSIS OF GENETIC DIVERSITY OF PALM OIL PLANT (Elaeis guineensis Jacq.) PALM OIL RESEARCH CENTER (PPKS) BASED ON PRIMARY SSR (Simple Sequence Repeats)

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Abstract

The background of this research was to obtain information of genetic diversity within and among populations of E. guineensis using SSR markers. This research was conduted in Biology Molecular Laboratory, Indonesian Oil Palm Research Institute, Marihat, Pematangsiantar, North Sumatra from March 2016 to January 2017. A total of 100 palm oil samples used in this research, including 20 samples from the populations of E. guineensis TR01S, including 20 samples from the populations of E. guineensis BJ022S, including 20 samples from the populations of E. guineensis BJ42S, including 20 samples from the populations of E. guineensis BO52S, and including 20 samples from the populations of E. guineensis MA19S. Calculation and Desciptive analysis in this research using software GeneMarker® version 2.40, software DARwin version 6.0.13 and Microsoft Excel 2007. Research shows that the number of banding patterns detected 104 The banding patterns WITH range 8-9 per primary banding pattern, and the size of the banding patterns ranging from 101 bp -328 bp. mEgCIR2569 primary is the most widely primer to amplify DNA and the average of the percentage of polymorphism in the primer being used by 71%. Analysis dendogram 100 samples All 3 hearts big group of Jatropha genetic WITH BETWEEN Yang ranged from 0.03 to 1.00 Based on the findings of the analysis of genetic relationships WITH Matrix Method using differences Simple Matching Value factorial analysis (PCoA) declared WITH axis I and axis II ON 12 primary SSR of 20.3%. mEgCIR2569 primary is the most widely primer to amplify DNA and the average of the percentage of polymorphism in the primer being used by 71%. Analysis dendogram 100 samples All 3 hearts big group of Jatropha genetic WITH BETWEEN Yang ranged from 0.03 to 1.00 Based on the findings of the analysis of genetic relationships WITH Matrix Method using differences Simple Matching Value factorial analysis (PCoA) declared WITH axis I and axis II ON 12 primary SSR of 20.3%. mEgCIR2569 primary is the most widely primer to amplify DNA and the average of the percentage of polymorphism in the primer being used by 71%. Analysis dendogram 100 samples All 3 hearts big group of Jatropha genetic WITH BETWEEN Yang ranged from 0.03 to 1.00 Based on the findings of the analysis of genetic relationships WITH Matrix Method using differences Simple Matching Value factorial analysis (PCoA) declared WITH axis I and axis II ON 12 primary SSR of 20.3%.

Keywords: Genetic diversity, PPKS oil palm, SSR primers.

1. Introduction

Indonesia is the largest palm oil producer in the world with a total production of 24 million tons of crude palm oil in 2011, which is equivalent to 52% of the total world production. The total area for oil palm plantations in Indonesia reaches 9.1 million hectares with an export value of 19.6 million tons of crude oil (Indonesian Sustainable Palm Oil Commission, 2012). Taking into account the potential of land resources, human resources and the potential of oil palm as well as the geographical location of Indonesia, it can be understood that oil palm is one of the mainstay commodities for agro-industry in Indonesia. The use of palm oil in Indonesia is generally as a raw material for palm oil and most of it is exported as crude palm oil (Pangaribuan, 2003).

Sustainability of production and supply of world palm oil products needs to be maintained with more intensive breeding through genetic diversity studies to ensure that high-yielding planting material is available for cultivation. An understanding of genetic diversity and its relationship to oil palm germplasm material is very important in selecting superior planting material. Availability of genetic diversity in germplasm. really helps improve the efficiency of breeding activities that are able to produce the expected selection outcomes (Azrai, 2005).

Oil palm plant breeding is generally constrained by natural problems such as the length of the breeding cycle and the lack of genetic information (Asmono, 1998). Genetic diversity based on agromorphological information to evaluate genotypic diversity is currently felt to be inadequate. The integration of molecular markers into oil palm breeding programs can be used to solve these natural problems (Asmono et al, 1999) as well as to increase efficiency in analysis of kinship, gene mapping, and MAS in food crops such as plantation crops such as oil palm. (Billote et al. 2005), and forestry and horticultural crops.

Microsatellites or also known as SSR (Simple Sequence Repeat), are DNA strands consisting of several base repeats of 1 to 8 pairs (Bredemeijer et al., 1998; Narvel et al., 2000). SSR markers are currently still the most popular markers used in genetic studies and breeding because of their various advantages, including their location that is spread throughout the plant genome, multi-allelic, and easily amplified by PCR technique (Powel et al. 1996).

Microsatellite markers have been widely used in plants for fingerprinting, gene mapping, linkage, genetic analysis (Singh et al. 2007; Sayekti et al. 2015), genotype identification, population genetic studies in plants (Singh et al., 2007) , comparing genetics of individuals based on the results of the level of genetic similarity (Singh et al, 2007). Then grouped based on the level of existing genetic similarity using clustering analysis (Zulhermana, 2009)

Based on the description above, the authors are interested in knowing the genetic information of the oil palm population of E. guineensis, namely the TR01S and BJ022S populations representing the progeny population, the BJ42S population representing the

wild population introduced from Cameroon, the BO52S population representing the male population and the MA19S population representing the female population in the PPKS experimental garden. Things that can be done to find out the genetic information include analyzing kinship and genetic diversity using the Principal Coordinat Analysis (PCoA) approach and Neighbor Joining Phylogenetic Hierarchy analysis based on the Dissimilarity Simple Matching Matrix.

2. Materials and Methods

This research was conducted from March 2016 to January 2017 at the Molecular Biology Laboratory of the Marihat Oil Palm Research Center (PPKS), Pematangsiantar.

The plant material used was white-yellow-green spearhead leaves consisting of 100 individual oil palm plants from 5 oil palm populations, namely TR01S, BJ42S, BO52S, MA19S and BJ22S with a genetic background of E. gueneensis. The five populations of E. guineensis oil palm were BJ22S obtained from PPKS experimental garden in Bah Jambi PT Perkebunan Nusantara IV, BJ42S obtained from PPKS experimental garden in Bah Jambi PT Perkebunan Nusantara IV, TR01S obtained from PPKS experimental garden in Tanah Raja PT Perkebunan Nusantara III, BO52S obtained from the PPKS experimental garden in Bah Jambi. The tools used for molecular biology analysis in this study were Tissuelyser (Qiagen), PCR BIO-RAD (CFX96 real-time system), analytical balance (AdvanturerTM – Ohaus) electrophoresis, hot plate (Barnstead CIMAREC-Thermolyne), magnetic stirrer, erlenmeyer , measuring cup, water bath MGW LAUDA-RM 6, Centrifuge Thermo Scientific (Sorval Legend Micro 17R), vortex Thermolyne (Speed control Type 37600 Mixer),UV translumination (BIORAD), Tungstean Carbide beads (Qiagen).

3. Results and Discussion

Results

Bunch Quality Character

Physical characteristics of the population FFB e. guineensis based on the mean of the observed variables showed that the TR01S progeny population had superior bunch quality characteristics compared to the BJ022S progeny population. Figure 1 shows the character values of M/F, O/DM, O/B, O/FM, and OER in the TR01S population of 81.73; 79.60; 54.41; 28.59; and 24.39 while in the population of BJ022S each were 75.78; 63.01; 28.52; and 24.38. The average difference in the bunch character ratio in the population of TR01S and BJ022S is 1.21; 5.95; 16.59; 0.07, and 0.01. In general, the mean ratio of the bunch quality characteristics of the male parent population BO52S, the female parent population MA19S and the wild population BJ42S observed that the BO52S population outperformed the MA19S and BJ42S populations. Figure 1 shows the difference in the characters of M/F, O/DM, O/B, O/FM, and OER between the BO52S and MA19S populations of 3.91; 3.37; 8.36; 2.83; and 2.37 while the difference between the population of BO52S and BJ42S is 27.25; 6.77; 10.08; 11.46, and 9.75.

Visualization of Amplification Results with Agaros Gel Electrophoresis

Visualization of the amplification results using agaros electrophoresis was performed on several DNA amplified PCR-SSR results. The DNA visualized was the amplified DNA which was chosen randomly so that it represented 100 amplified DNA in each primer. The number of amplified DNA visualized was 3-4 DNA in each primer (Appendix 7). This visualization aims for the success of the DNA amplification primer so that the amplified DNA can be further analyzed with a cappilary sequencer by 1stBASE Malaysia.

DNA Fragment Analysis using a processed Cappilary Sequencer with GeneMarker software

There were 104 banding patterns produced by the 12 SSR primers used in this study. The average band produced ranged from 8 to 9 band patterns per primer. The highest number of banding patterns were found in the mEgCIR2569 primer with 15 band patterns and the lowest number of band patterns with 4 in the mEgCIR3705 primer. The size of the resulting band varies between 101 bp-328 bp. From the analysis of the fragments, it is known that 1068 DNA was amplified by the 12 primers used and 142 DNA was not amplified by the 12 primers. The most unamplified DNA was found in the mEgCIR3775 primer as many as 46 DNA and the most amplified DNA was 99 by the mEgCIR2569 primer.

The resulting polymorphic bands are 758 bands and the total monomorphic bands are 310 bands. The percentage of polymorphism bands of DNA amplified by the 12 primers used varied between 7%-100% with an average polymorphism of 71%. The highest percentage of polymorphisms was found in the mEgCIR3775 primer, which was 100% and the lowest polymorphism percentage was found in the mEgCIR3705 primer, which was 7%.

Table 1 DNA Fragment Analysis Data using Cappilary Sequencer processed with Gene Marker software

No	Primary	Ribbon Size	Total Ribbon Pattern	Total DNA amplified	Total DNA Not amplified	Amount Polymorphic	Number of Ribbons Monomorphic	Percentage polymorphic tape
1	mEgCIR2569	237-260	15	99	1	91	8	92%
2	mEgCIR3705	101-108	4	90	10	6	84	7%
3	mEgCIR0782	170-200	10	92	8	34	58	37%
4	mEgCIR3691	197-212	8	94	6	71	23	76%
5	mEgCIR3433	258-270	8	86	14	72	14	84%
6	mEgCIR3400	156-178	9	91	9	55	36	60%
7	mEgCIR3555	138-158	10	90	10	86	4	96%
8	mEgCIR2224	118-125	7	94	6	92	2	98%
9	mEgCIR3775	197-208	9	64	46	64	0	100%
10	mEgCIR0783	307-328	11	87	13	55	32	63%
11	mEgCIR2347	161-175	6	90	10	75	15	83%
12	mEgCIR3213	103-117	7	91	9	57	34	63%
Total			104	1068	142	758	310	858%
Average			8.7	89	11.83	63.17	25.83	71%

Note: The numbers followed by the same letter notation in the same column are not significantly different according to Duncan's test at the 5% level

Binary data from the amplification scoring of 12 primers processed with DARwin software resulted in a Neighbor-Joining Tree (NJTree) phylogenetic hierarchy showing the kinship of 100 individual oil palm plants from 5 populations in the PPKS experimental garden. according to their respective populations. For the population of E. guineensis TR01S characterized by analysis code (20160602001-20160602020), population of E. guineensis BJ022S characterized by analysis code (20160421007-20160426), population of E. guineensis BJ42S characterized by analysis code (20160413009-201604130029), population of E. guineensis BO52S was indicated by the analysis code (20160414009-20160414028), and the population of E. guineensis MA19S was indicated by the analysis code (2016052001-20160520018, 20160603001- 20160603002).

The results of grouping based on SSR primers showed that the genetic relationships of the populations of E. guineensis BJ022S, E. guineensis TR01S, E. guineensis BJ42S, E. guineensis BO52S, E. guineensis MA19S were observed to be spread at a genetic distance between 0.003 - 1.00. Based on the genetic distance observed using the Matrix Dissimilarity Simple Matching method, it is known that the closest and furthest genetic distances in each population of E. guineensis used in this study. The population of E. guineensis BJ022S has a genetic distance within the population ranging from 0.09–0.62, the closest genetic distance

indicated in the analysis code 20160421025 with 20160421019 of 0.09 and the farthest genetic distance was shown in the analysis code 20160421012 with 20160421011. The population of E. guineensis TR01S has a genetic distance within the population ranging from 0.07-1.00, the closest genetic distance is shown in the analysis code 20160602012 with 20160602006 of 0.07 while the farthest genetic distance is shown in the analysis code 20160602017 with 20160602001 of 1.00. The population of E. guineensis BJ42S has a genetic distance within the population ranging from 0.29 to 0.95, the closest genetic distance is shown in the 20160413019 analysis code and 20160413014 of 0.29 and the farthest genetic distance is shown in the 20160413018 analysis code with 20160413009 of 0.95. The population of E.

Discussion

The physical characteristics of fresh fruit bunches (FFB) can be seen from Figure 1 that overall the TR01S progeny showed better oil quality conditions than the populations of BJ022S, BO52S, BJ42S and MA19S. This is indicated by the oil character per dry mesocarp in TR01S progeny of 79.60. This indicates that projeni TR01S as a commercial variety has superior bunch quality compared to other populations used in this study. This is in accordance with the statement of Suprianto et al (2002) which states that the character of good bunch quality can be seen from the components of the percentage of fruit per bunch, mesocarp per fruit, nucleus per fruit, oil per dry mesocarp, oil per fresh mesocarp, oil per bunch and yield.

From the results of the analysis of the fragments processed with Gene Marker software, from 100 samples analyzed there were 1068 DNA that was successfully amplified by the 12 primers used but not all of them were amplified by the 12 SSR primers used in this

study, because the total amount of DNA that was not amplified was as much as 142 DNA. The mEgCIR2569 primer was able to amplify the most DNA with 99 amplified DNA while the least amplified DNA was 46 DNA in the mEgCIR3775 primer. Primer mEgCIR2569 and other primers that successfully amplify sample DNA show that the nucleotide base sequences between the template DNA and the primers used match, so that the nucleotide bases in the DNA stick to the target sequence. The unamplified DNA in the 12 primers was caused by the nucleotide bases not attached to the target sequence and it was suspected that the primers did not match the template DNA. This is in accordance with Ariani (2015) which states that nucleotide bases will only stick to the target sequence. Williams et al. (1990) Fragments that do not appear due to the absence of amplification, may occur because the primers used are not compatible with the template DNA. Some experimental evidence suggests that a single base pair difference is sufficient to cause a mismatch of the primer prints which then prevents amplification. (1990) Fragments that do not appear due to the absence of amplification, may occur because the primers used are not compatible with the template DNA. Some experimental evidence suggests that a single base pair difference is sufficient to cause a mismatch of the primer prints which then prevents amplification. (1990) Fragments that do not appear due to the absence of amplification, may occur because the primers used are not compatible with the template DNA. Some experimental evidence suggests that a single base pair difference is sufficient to cause a mismatch of the primer prints which then prevents amplification.

Based on the value of genetic diversity using Matrix Dissimilarity Simple Matching, in general, 5 populations of E. guineensis obtained the value of genetic dissimilarity for 100 samples from 5 populations of E. guineensis used in this study ranging from 0.03–1.00. The sample with the highest genetic distance indicates that the two samples have different genetic material, while the sample with the lowest genetic distance indicates that the two samples have a relationship in their genetic material.

4. Conclusion

Of the 12 SSR primers tested, a total of 104 band patterns were detected with a range of 8-9 band patterns per primer, and the band pattern size ranged from 101 bp–328 bp with an average percentage of polymorphism in the 12 SSR primers obtained 71%. The five populations of E. guineensis were divided into 3 large groups with genetic distances ranging from 0.03-1.00. 12 primary SSR by 20.3%

5. Reference

Pangaribuan, Y. 2003. Analisis Kadar β-Karoten Kelapa Sawit Tipe Dura Deli dan Dura Dumpy Berdasarkan Tingkat Kematangan Buah.J. Penelitian Kelapa sawit, 11 (1): 15-22.

Azrai, M. 2005. Pemanfaatan Markah Molekuler dalam Proses Seleksi Pemuliaan Tanaman. J. Agrobiogen. Vol. 1 (1):26-37.

Diwan, N. and P. B. Cregan. 1997. Automated Sizing of Fluorescent Labeled Simple Sequence Repeat (SSR) Markers to Assay Genetic Variation in Soybean. Theor. Appl. Genet. 95:723-733.

Powell, W., Machrax, G. C. & Provan, J. 1996. Polymorphism Reveald by Simple Sequence Repeats, Trends in Plant Sience. 1:215-222.

Singh R J. Nagappan S G. Tan J M. Panandam and Cheah S C. 2007. Development of Simple Sequence Repeat (SSR) markers for oil palm and their application in genetic mapping and finger printing of tissue culture clones. Asia Pacific Journal of Molecular Biology and Biotechnology. 15(3): 121- 131.

Zulhermana. 2009. Keragaman Genetik Intra dan Interpopulasi Kelapa Sawit (Elaeis guineensis Jacq) Pisifera Asal Nigeria Berdasarkan Analisis Marka Simple Sequence Repeats (SSR). Institut Pertanian Bogor, Bogor.

Kohlerschmidt, D.J., K.A. Musser, and N.B. Dumas. 'Identification of Aerobic Gram-Negative Bacteria'. Dalam Goldman, E. and L.H. Green (ed.). 2009. Practical Handbook of Microbiology, Second Edition. CRC Press, Boca Raton.