Varietal Identification of Liberica Coffee in Kepulauan Meranti Riau using RAPD Marker: A Preliminary Study

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Abstract. Identity of plant variety is essential for crop production system. Two liberica coffee varieties, Lim 1 and Lim 2, have been cultivated in Kepulauan Meranti where peat lands area predominantly found. In addition to their adaptability to peat lands, the two varieties also were reported resistant against *Hemileia vastatrix*, one of main fungal pathogens in coffee plants. Molecular-based techniques, such as Random Amplified Polymorphic DNA (RAPD) marker, are considered as reliable approaches compared to morphological-based method for identifying plant varieties. This study aimed to analyze RAPD profiles to identify Lim coffee varieties. We sampled Lim 1, Lim 2, and local coffee genotype from Kabupaten Bengkalis and extracted genomic DNA from young leaves and carried out PCR (Polymerase Chain Reaction) using three primers (OPA-04, OPE-03, OPE-18). Results of electrophoresis showed that all three primers produced polymorphic bands. RAPD profiles generated by OPE-03 and OPE-18 could dishtinguish the three different coffee varieties or genotype. In addition, two bands (1,500 and 2,500 bp) amplified by PCR with OPA-04 were specifically observed in Bengkalis coffee, while 650 bp band revealed by PCR with OPE-03 was only present in Lim 1. Based on our result, we suggest that RAPD marker is suitable for identifying liberica coffee varieties and can be used for further investigation to develop liberica varietal specific markers.

Keywords: Liberica · Lim 1 · Lim 2 · Meranti · RAPD

1 Introduction

Coffee is one of important agricultural commodities in numerous countries including Indonesia. Commercial coffee production has been dominated by robusta coffee (*Coffea canephora*) and arabica (*C. arabica*), while liberica coffee (*C. liberica*) is recognized to possess less economic value particularly due to lower rendement. However, liberica coffee has a unique characteristic flavor and low caffeine content. Furthermore, it has

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been reported to be more adaptive grown on peat land area and resistant against leaf rust disease caused by *Hemileia vastatrix*, one of notorious pathogenic fungi in coffee plants.

It has been considered that using resistant varieties is the most effectual aspect strategy in crop protection against plant diseases [1]. Two liberica resistant varieties, Lim 1 and Lim 2, has been officially released by Indonesia Agricultural Department and cultivated for several decades in Kabupaten Meranti, Riau Province where peat land predominantly found [2, 3]. The two varieties has been featured by their resistance trait against leaf rust disease caused by *H. vastatrix* [4, 5].

One of essential elements in crop production including coffee is genetic purity or the trueness of crop cultivar or variety. Conventionally, determination of crop variety is commonly based on phenotypic traits which generally need more time and prone to be influenced by environmental factors [6]. In addition, as perennial trees, it is not easy to distinguish different coffee varieties under morphological basis particularly in earlier growth stage. Therefore, morphology approach should be accompanied by molecular marker such as Random Amplified Polymorphic DNA (RAPD).

RAPD technique has some advantages, for example it does not involve cloning, sequencing, or hybridization process. Additionally, only small DNA quantity is sufficient f RAPD template which can be sampled at any stage of plant growth [7]. RAPD has been applied in a various analysis such as assessment of genetic relationship of sweet potatoes and and their hybrid [8], detection of pepper varieties *Capsicum annuum* [9], identifying coffea arabica seed [6], assessment of hybrid identity of hippeastrum plants [10] and seven varieties of olive plants [11]. RAPD could distinguish blueberry cultivars [12] and detect five closely related peanut cultivars [13]. Thus, RAPD may lead to alleviate time consumed to identify plant varieties.

Several approaches can be applied to determine plant cultivar identity such as morpological markers and molecular-based techniques [14]. DNA-based methods such as RAPD markers are demonstrated as more reliable and environmentally stable. Specific DNA band profiles produced by Polymerase Chain Reaction (PCR) can be generated for each plant variety and useful for varietal identification [14].

For this reason, we used RAPD in our present study for analyzing specific RAPD bands related to liberica coffee. Thus, our study aimed to analyze RAPD band profiles to detect different coffee varieties or genotype namely Lim 1, Lim 2 and coffee genotype from Bengkalis area.

2 Material and Method

2.1 Plant Materials and Genomic DNA Extraction

A total of three coffee varieties were used for this study, consisted of two liberica varieties (Lim 1 and Lim 2) originating from Kabupaten Kepulauan Meranti and one local coffee genotype sampled from Kabupaten Bengkalis, Riau Province. Every variety was analysed three times using each RAPD primer ($n = 3$). Total genomic DNA was extracted from young, fresh and healthy coffee leaves. Leaf samples were grounded using mortar and pestle with liquid nitrogen. An amount of 0.5 mg of leaf fine powder was used for isolating total DNA and the steps of DNA extraction referred to Geneaid Genomic

DNA Mini Kit (Plant) protocol (Geneaid Biotech Ltd., Taipei, Taiwan). Result of DNA isolation was checked by 0.8% agarose gel electrophoresis.

2.2 Amplification of Genomic DNA using RAPD

All DNA samples were amplified using three RAPD primers: OPA-04 (5'-AATCGGGCTG-3'), OPE-03 (5'-CCAGACGCAC-3') and OPE-18 (5'-GGACTGCAGA-3'). PCR mixed for random amplification reaction consisted of 1x DreamTaq buffer, 0.2 mM dNTP mix, 2 units of DreamTaq Polymerase (Thermo Fisher Scientific, Vinius Lithuania), 1 μM primers, ddH_2O and 2 μl gDNA in a total reaction volume of 20 μl. The PCR reaction program used was initial denaturation for 3 min at 95 °C followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 35 °C, extension for 1 min at 72 °C and final extension for 10 min at 72 °C [15]. PCR products were separated by electrophoresis in 1% agarose gel with the additional of 1x FluoroVue ™ Nucleic Acid Gel Stain (10,000X) (SMOBIO Technology Inc.). The electrophoresis performed for 40 min in 50 V and the RAPD band profile was visualized under UV transilluminator and photographed using a UV filtered camera (Olympus SP-500 UZ).

2.3 Data Analysis

The amplified DNA band pattern was identified manually and scored for the presence (1) and absence (0) band. The binary data was then converted into a genetic similarity according to Nei's coefficient. This genetic similarity data was then used to construct a dendrogram. Data analysis was carried out by NTSys 2.02 software.

3 Results and Discussion

The visualization of PCR product on electrophoresis showed that all three arbitrary 10-mer primers used in this study generated polymorphic band patterns. A total of 17 amplicons produced by three primers. Out of 17 amplicons, 12 bands were polymorphic (70% polymorphism). Number of amplified bands per primer was ranged from three to eight bands with the size varying from around 400 to 2,500 bp (Table 1). In addition, the average of band amplified each primer was 5.7 band per primer with the average of 4 polymorphic bands per primer. This result indicates that RAPD may be sufficiently able to detect genomic DNA variation amongst the samples. Report on the analysis of RAPD band patterns of 19 oil palm moderately resistant against ganoderma disease using three primers (OPA-13, OPD13, OPH-13) produced 100% of polymorphic bands with PCR product sized about 424 to 2572 bp [16].

DNA amplification using OPA-04 primer generated two band patterns (Fig. 1). It means that OPA-04 primer could not distinguish three coffee varieties since Lim 1 and Lim 2 shared an identic pattern. Using OPA-04, we obtained 8 bands with 6 of them are polymorphic. With the same primer, there was only one polymorphic band of 10 RAPD bands on C. arabica [6]. Based on the band size, there were two different bands (sized around 2500 and 1500 bp) presented only by Bengkalis coffee (Fig. 1). These

Table 1. Number of polymorphic bands and band size amplified by three RAPD primers in this study

Primers	Number of bands	Polymorphic bands	Band size (bp)
OPA-04	8	6	650–2500
OPE-03	3	6	400–650
OPE-18	6	3	500–2000
Total	17	12 (70%)	400–2500

bands might be considered as candidate of varietal specific bands to coffe genotype from Bengkalis.

Electrophoretic bands produced by PCR with OPE-03 primer showed that the primer could identify three different coffee genotypes (Fig. 2). This figure depicts that Lim 1 can be recognized by a single band sized around 650 bp that might be potential as a varietal specific marker for Lim 1. While two other bands (400 and 500 bp) generated by Lim 1 were not specific to Lim 1 as they were also amplified by Lim 2 and Bengkalis genotype, respectively.

Another primer we used in this study, OPE-18, also generated band patterns which could discriminate three different coffee genotypes (Fig. 3). In addition, PCR with OPE-18 did not reveal any band specific to particular coffee variety or genotype in this study.

Although it still requires further analysis, results of our current preliminiary study indicated that RAPD marker is likely potential to be applied for identifying varieties or cultivars in liberica coffee plants. Several earlier studies have reported the success RAPD marker to detect different plant varieties in various plant species. For example, RAPD marker revealed potential particular RAPD bands for detecting different olive varieties [11]. In addition, 34 apricot cultivars could be recognized by using five RAPD primers [17]. Furthermore, RAPD markers were also able to identify "Superjohn" potato variety using nine primers [18]. Amplification using only two RAPD primers was also able to clearly distinguish four different rice cultivars [19]. A total of 12 commercial *C.*

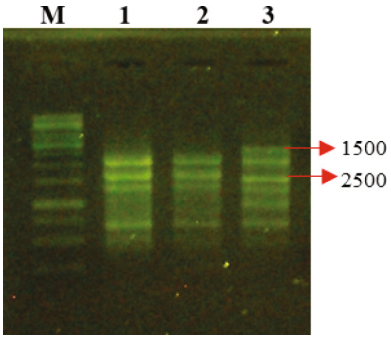

Fig. 1. RAPD profiles of genomic DNA from different three *C. liberica* using primer **OPA-04**. Lane M: 1kb DNA Ladder, 1: Lim 1, 2: Lim 2, 3: Bengkalis

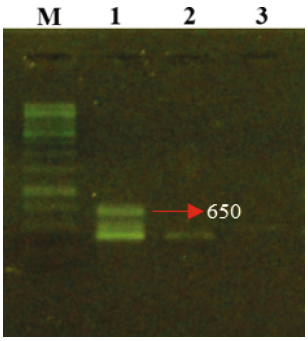

Fig. 2. RAPD profiles of genomic DNA from different three *C. liberica* using primer OPE-03. Lane M: 1kb DNA Ladder, 1: Lim 1, 2: Lim 2, 3: Bengkalis

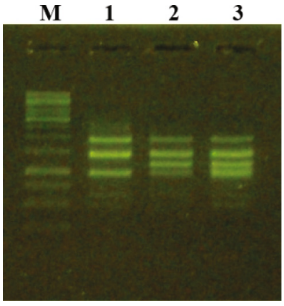

Fig. 3. RAPD profiles of genomic DNA from different three *C. liberica* using primer OPE-18. Lane M: 1kb DNA Ladder, 1: Lim 1, 2: Lim 2, 3: Bengkalis

arabica coffee varieties has been succesfully detected using four RAPD primers [6]. By using template DNA extracted from coffee seeds, RAPD technique has been reported efficiently assessed genetic relationship coffee genotypes [20]. These reports reveal that RAPD is a suitably potential technique for identifying plant varieties or genotypes.

Identification of plant variety or cultivar is one of the essential factors in agricultural system [21, 22]. Varietal identification is crucial to ensure the identity of particular genotypes or plant varieties. As we mentioned above, RAPD marker will be useful and helpful to distinguish liberica coffee. It also will be beneficial for coffee nursery industry due to allowing identification at the earlier growth stage of seedlings. Furthermore, it is necessary to guarantee the particular quality level of plant product as expected by farmers and consumers. Variations of soybean mutants induced with EMS could be detected by RAPD marker showing that the marker is still a promising alternative approach amongst other various molecular markers [23]. RAPD marker also successfully revealed particular band linked to *Fusarium* wilt resistance in castor plant [24]. Based on the present results, we suggest that RAPD technique could be applied for varietal assessment in liberica coffee, particularly for Lim 1 and Lim 2.

Based on our RAPD data, we determined genetic similarity among our samples (Table 2) and constructed UPGMA dendrogram derived from similarity matrix (Fig. 4).

Table 2. Similarity index among Lim 1, Lim2, and Bengkalis genotype based on RAPD bands using three primers

Var	Lim 1	Lim 2	B1
Lim 1	1.00		
Lim 2	0.71	1.00	
B1	0.47	0.41	1.00

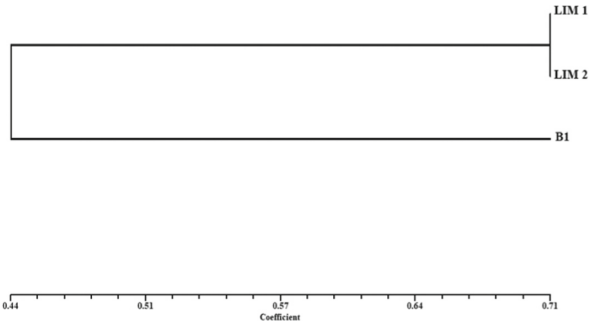

Fig. 4. Dendrogram of the genetic relationships among Lim 1, Lim2, and Bengkalis genotype based on RAPD bands using three primers. Lim 1, Lim 2, and B1 indicate Lim 1 variety, Lim 2 variety and Bengkalis variety, respectively.

As we expected, Lim 1 and Lim 2 showed higher similarity (71%) compared to genetic relationship either between Lim 1 and Bengkalis (47%) or between Lim 2 and Bengkalis (41%). In addition, Lim and Lim 2 are in the same group in the dendrogram while Bengkalis genotype is separated from Lim varieties. This result supports that liberica coffee Lim 1 and Lim 2 are from same region, Kepulauan Meranti. On the other hand, Bengkalis genotype is grown in another different island, Pulau Bengkalis. Therefore, Lim 1 and Lim 2 most likely have similar genetical background.

Our results indicated that RAPD method is promising to be developed for identifying liberica coffee varieties particularly Lim 1 and Lim 2 varieties.

4 Conclusions

This study aimed to analyze RAPD profiles to identify Lim coffee varieties. We sampled Lim 1, Lim 2, and local coffee genotype from Kabupaten Bengkalis and extracted genomic DNA from young leaves and carried out PCR (Polymerase Chain Reaction) using three primers (OPA-04, OPE-03, OPE-18). Results of electrophoresis showed that all three primers produced polymorphic bands. RAPD profiles generated by OPE-03 and OPE-18 could dishtinguish the three different coffee varieties or genotype. In addition, two bands (1,500 and 2,500 bp) amplified by PCR with OPA-04 were specifically observed in Bengkalis coffee, while 650 bp band revealed by PCR with OPE-03 was only present in Lim 1. Based on our result, we suggest that RAPD marker is suitable for identifying liberica coffee varieties and can be used for further investigation to develop liberica varietal specific markers.

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References

1. P. Talhinhas , D. Batista, I Dinis, The coffee leaf rust pathogen Hemileia vastatrix: one and a half centuries around the tropics, Mol. Plant Pathol. 18 (2017) 1039–1051. doi: https://doi.org/10.1111/mpp.12512
2. R. Harni, E. Taufiq, B. Martono, Ketahanan pohon induk kopi liberika terhadap penyakit karat daun (Hemileia vastatrix B. Et Br.) di Kepulauan Meranti J. Tanam. Ind. dan Penyegar 2(1) (2015) 35. DOI:https://doi.org/10.21082/jtidp.v2n1.2015.p35-42
3. B. Martono, B. Sudjarmoko, L. Udarno, The potential of liberoid coffee cultivation on the peatlands (a case study: The peatlands in the Meranti island, Riau), IOP Conf. Ser: Earth Environ. Sci., 2020. DOI: https://doi.org/10.1088/1755-1315/418/1/012022.
4. Balitbangtan, Kopi Liberoid Meranti-1 Lim 1, (2016) http://www.litbang.pertanian.go.id/varietas.php/1136/
5. Balitbangtan, Liberoid Meranti 2: Varietas unggul kopi spesifik lahan gambut bercitarasa tinggi, InfoTek Perkeb.10 (2018) http://www.litbang.pertanian.go.id/info-teknologi/3513/
6. M.L. Crochemore, L.M Nunes, G.A. Andrade, H.B.C Molinari, M.E. Vasconcellos, Varietal identification of coffee seeds by RAPD technique Brazilian Arch. Biol. Technol. 47(1) (2004) 7–11. https://doi.org/10.1590/S1516-89132004000100002
7. S. Avivi, E.N. Suliswanto, D.P. Restanto, A Syamsunihar, Morphological diversity and molecular RAPD markers of sugarcane mutant, AGRIVITA 1(2) (2019) 21–29. DOI: https://doi.org/10.17503/agrivita.v41i2.1304
8. N.N. Wahibah, Daya silang ubi jalar berdaging umbi jingga dengan Ipomoea trifida diploid dan hubungan genetiknya, Natur 5(1) (2002) 1–8
9. H. Ilbi, RAPD markers assisted varietal identification and genetic purity test in pepper, Capsicum annuum, Sci. Hortic. (Amsterdam) 97 (2003) 211–218. https://doi.org/10.1016/S0304-4238(02)00158-9
10. D. Chakrabarty, V.N. Gupta, S.K. Datta, Varietal identification and assessment of genetic relationships in Hippeastrum using RAPD markers, Plant Biotechnol. Rep., 1 (2007) 211–217. https://doi.org/10.1007/s11816-007-0034-3
11. M.Z. Iqbal, R. Shahzad, S. Jamil, Identification of seven olive varieties using RAPD molecular markers, J Agric Res 57 (2019) 7–14
12. K.H. Cho, S.J. Park, S.J. Kim, S.H. Kim, H.C. Lee, M.Y. Kim, J.A. Chun, Blueberry cultivar identification using random amplified polymorphic DNA and sequence-characterized amplified region markers, HortScience 52(11) (2017) 1483–1489. https://doi.org/10.21273/HORTSCI12105-17
13. G.M. Samaha, M.A. Ahmed, A.R. El-Hameid, Assessment of growth and productivity of five peanut cultivars and genetic diversity using RAPD markers, Bull. Natl. Res. Cent. 43(168) (2019) 1–11. DOI https://doi.org/10.1186/s42269-019-0201-z
14. H. Tanwar, Morphological and molecular methods of varietal identification, Agrobios 17(2) (2018) 1–4.

15. M. Silvestrini, M.P. Maluf, M.B. Silvarolla, O. Guerreiro-Filho, H.P. Medina-Filho, M.M.T. Vanini, A.S. Oliveira, C. de Gaspari-Pezzopore, L.C. Fazuoli, Genetic diversity of a coffea germplasm collection assessed by RAPD markers, Genet. Resour. Crop Evol. 55 (2008) 901–910. DOI: https://doi.org/10.1007/s10722-007-9295-5
16. F.P. Girsang, L.A.P. Putri, Rosmayati, Analisis keragaman genetik varietas mtg kelapa sawit (Elaeis guineensis jacq.) berdasarkan tiga marka RAPD, J. Agroekoteknologi 7(2) (2019) 330–338
17. L. Shangguan, Y. Wang, X. Li, Y. Wang, C. Song, J. Fang, Identification of selected apricot cultivars using RAPD and EST-SSR markers, Caryologia 65 (2) (2012) 130–139. DOI: https://doi.org/10.1080/00087114.2012.711668
18. D.S. Runtunuwu, J.E.X. Rogi, J.H. Palendeng, Identifikasi varietas kentang 'Superjohn' berdasarkan penanda RAPD (Random Amplified Polymorphic DNA), Eugenia 17(1) (2011) 1–8, DOI: https://doi.org/10.35791/eug.17.1.2011.100
19. P. Mani, T.M.M.J. Bastin, R. Arun, A. Bakrudeen, A. Ahmed, RAPD-analysis of geneyic variation of four important rice varieties using two OPR primers ARPN, J. Agric. Biol. Sci. 5(4) (2010) 12–15
20. T.A.Teixeira-Cabral, N.S. Sakiyama, L. Zambolim, A.A. Pereira, E.G. Barros, C.C. Sakiyama, Reproducibility of the RAPD marker and its efficiency in coffee tree genotype grouping analysis, Crop. Breed. Appl. Biotechnol. 2 (2002) 121–129
21. N.K Korir, J. Han, L. Shangguan, C. Wang, Plant variety and cultivar identification : advances and prospects, Crit. Rev. Biotechnol. 1 (2012) 1–5
22. H. Rai, O.H. Peerzada, O.S. Dahiya, S.S. Jakhar, Varietal identification based on chemical methods in different varieties of indian mustard (Brassica juncea (L.) Czern. & Coss.), Int. J.Curr. Microbiol. Appl. Sci. 8(5) (2019) 2391–2396. DOI: https://doi.org/10.20546/ijcmas.2019.805.283
23. D. Wahyudi, L. Hapsari, Sundari, RAPD analysis for genetic variability detection of mutant soybean (Glycine max (L.) Merr), J. Trop. Biodivers. Biotechnol. 5(1) (2020) 68–77. https://doi.org/10.22146/jtbb.53653
24. M. Singh, I. Chaudhuri, S.K. Mandal, R.K. Chaudhuri, Development of RAPD markers linked to Fusarium wilt resistance gene in castor bean (Ricinus communis L), Genet. Eng. Biotechnol. J. (2011) 1–9