Matoa

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Molecular identification of mother trees of four matoa cultivars (*Pometia pinnata* Forst & Forst) from Pekanbaru City, Indonesia using RAPD markers

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Abstract. *Zulfahmi, Pertiwi SA, Rosmaina, Elfianis R, Gulnar Z, Zhaxybay T, Bekzat M, Zhaparkulova G. 2023. Molecular identification of mother trees of four matoa cultivars (*Pometia pinnata *Forst & Forst) from Pekanbaru City, Indonesia using RAPD markers. Biodiversitas 24: 1524-1530.* Pekanbaru city has four matoa (*Pometia pinnata* Forst & Forst) cultivars, i.e. red matoa, yellow matoa, green matoa, and black matoa, but the knowledge of the genetic variation among matoa cultivars is not available. The objective of this study was to assess the genetic variation among matoa cultivars using Random amplified polymorphism DNA (RAPD) markers and to identify the specific marker to distinguish among matoa cultivars. Eighteen primers were initially screened and four matoa cultivars were analyzed with twelve primers using the RAPD marker. The results of this study found that the twelve selected RAPD primers generated 39 fragments, with fragment sizes ranging from 200 to 1500 bp. The percentage of fragment polymorphic was 80.41%, indicating the high genetic variation of the matoa cultivar. The high genetic variation of matoa in this study is caused by cross-pollinated among matoa cultivars in the field. Eleven primers can differentiate four matoa cultivars with specific bands. The obtaining unique bands can be utilized by breeders and farmers as a basis to select the parents for the genetic improvement of Matoa, cultivars or clones protection, detection of seedling purity in a nursery, and to verify the originality of the seedlings that will be cultivated.

Keywords: Cultivar identification, genetic improvement, *Pometia pinnata*, RAPD marker

INTRODUCTION

Matoa (*Pometia pinnata* Forst & Forst) is a member of the Sapindaceae family. Matoa is widely distributed in Indonesia, including Papua, Maluku, Sulawesi, Kalimantan, Jawa, and Sumatra up to an altitude of 1.400 meters above sea level. Matoa trees can reach 20-40 meters in height and have a trunk diameter of up to 1.8 meters. Matoa fruit contains many nutrients such as carbohydrates, protein, fat, and other nutrients. Matoa is an important fruit tree economically, not only fruits but also leaves and timber of Matoa have high economic value. The fruit peel of Matoa has a component of bioactive compounds such as alkaloids, saponins, tannins, triterpenoids, flavonoids, and phenolic compounds which can be used as antioxidant, antibacterial, antidiabetic and anti-obesity (Irawan et al. 2017; Hanafi et al. 2020; Pridyantari et al. 2020; Suzuki et al. 2021), meanwhile, the hardwood of Matoa can be used for construction, doors, flooring, furniture, and window panels because it has high physical and mechanical properties (Belleville et al. 2020).

Nowadays, Matoa has becomes one of the popular fruit tree species in the Riau province, Indonesia because Matoa have fruits delicious with a distinctive taste from the flavors combination of rambutan, longan, and durian fruits. Pekanbaru city is one of the regions in Riau Province - Indonesia that has four matoa cultivars, namely red matoa, green matoa, yellow matoa, and black matoa based on rind morphological identification. Meanwhile, Kadir & Raodah (2014) reported that three matoa cultivars in Papua island, namely, red rind matoa (Emme Bhanggahe), green rind matoa (Emme Anokhong), and yellow rind matoa (Emme Khabhelaw). Three cultivars (red matoa, yellow matoa, and black matoa) in Pekanbaru have been registered in the Ministry of Agriculture of the Republic of Indonesia, with the registration numbers: 1623/PVL/2020, 1622/PVL/2020, 1650/PVL/2021, respectively. However, knowledge of the genetic variation among matoa cultivars is not available, so it is urgent to be done. In addition, identification of Matoa cultivars that refer to certain morphological characteristics, such as fruit rind color, is difficult because the fruit is not available all the time, is influenced by environmental factors, and plant age, so it will appear confusion and doubt in determining Matoa cultivars. Rosmaina et al. (2016) stated that morphological traits have the weakness to differentiate genetically similar individuals because traits do not cover the entire genome and require extensive observation in mature plants.

The information on the genetic variation among Matoa cultivars is important for plant improvement programs.

This information is required by breeders to plan hybridization and development of superior varieties more quickly and commercially in the future. In addition, the availability of genetic variation is attractive to plant breeders because allele and genotype reserves can be utilized to deal with climate change and create desired new varieties in the future (Capblancq et al. 2020). Genetic characterization of germplasm is important for the conservation and utilization of plant genetic resources (Renganathan et al. 2017). Until now, information on the genetic variation among Matoa cultivars using molecular techniques is not available. Therefore, this study is urged to be implemented as a basic consideration to formulate the improvement strategies of Matoa in the future.

The identification and understanding of the relationship among plant cultivars using the DNA molecular approach are more effective than morphological markers because DNA analysis allows direct access to the hereditary material, not influenced by the environment, independent from the season, and no limit of plant stage (Nadeem et al. 2018; Mirzaei 2021). RAPD markers have been known as one of the most commonly used techniques for cultivar identification, for example, apple (Antic et al. 2016), Cherry (Atnafu 2016), durian (Rosmaina et al. 2016), cashew (Bhadra et al. 2019), citrus (El-Khayat 2019), hybrid purity identification (Rajesh et al. 2014; Mylonas et al. 2014; Pujar et al. 2017), estimating the genetic diversity of plants and the population genetic structure of *Panax ginseng* (Wang et al. 2016), *Dalbergia sissoo* (Dobhal et al. 2019), *Eurycoma apiculata* (Zulfahmi et al. 2021) due to their advantages compared to other DNA molecular techniques such as its simplicity, fast, cost-effective, short primers of arbitrary sequences, require small amounts of target DNA as well as no need of prior sequence information on the target species and radioactivity free procedure (Williams et al. 1990; Nadeem et al. 2018; Amiteye 2021). On the other hand, RAPD has some weaknesses, such as low reproducibility and dominant properties (heterozygotes and homozygotes can not be differentiated) (Kumari & Thakur 2014). However, this weakness can be overcome by optimizing the reaction conditions. The purpose of this study was to assess the genetic variation among Matoa cultivars using RAPD markers and to identify the specific marker to distinguish among matoa cultivars.

MATERIAL AND METHODS

Sample collection

The young leaves of four matoa cultivars, namely Red Matoa, Yellow Matoa, Green Matoa, and Black Matoa were taken from the Matoa farmer's garden in Palas village (latitude: 0°35'3" N and longitude: 101°22' E), Rumbai subdistrict, Pekanbaru City, Riau, Indonesia as shown in Figure 1. The leaves samples were stored in a cool box and then carried to the laboratory and stored in the freezer (-20°C) until DNA isolation was performed. Laboratory work was conducted from September 2020 to January 2021 at the Genetic and Breeding Laboratory, Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau, and the Biotechnology laboratory of Riau University, Indonesia.

Genomic DNA isolation

Total genomic DNA was isolated from young leaves of four matoa cultivars (each cultivar was represented by one selected mother trees) using CTAB (cetyl trimethyl ammonium bromide) method (Doyle & Doyle 1990) with slight modification, namely adding 0.2% PVP (polyvinyl pyrolidone) and 1.0% mercaptoethanol into extraction buffer.

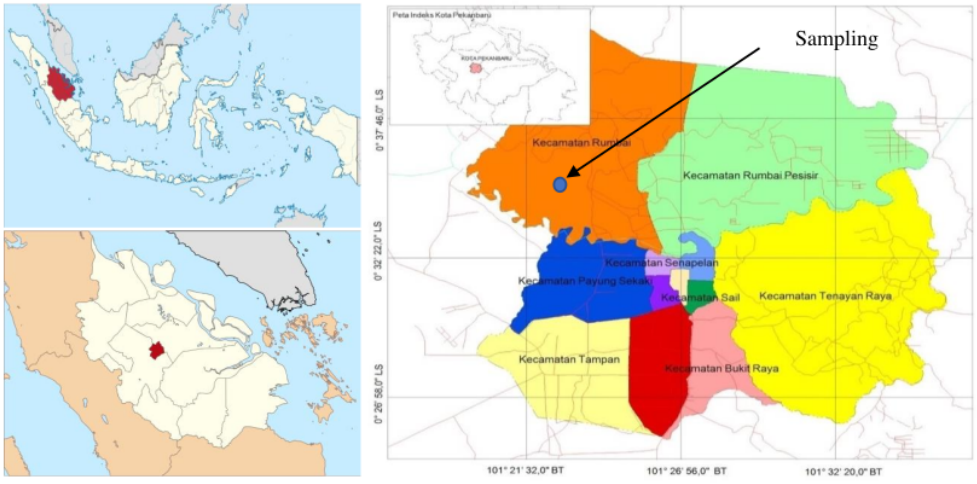

Figure 1. Map of sampling location of four matoa cultivars

Table 1. The list of primers and sequences for initial screening in this study

No	Primers	Primer Sequences
1.	OPD-08	5'-GTGTGCCCCA-'3
2.	D-08	5'-GTGTGCCCCA-'3
3.	OPO-13	5'-GTCAGAGTCC-'3
4.	X-01	5'-CTCACCGTCC-'3
5.	C-09	5'-CTCACCGTCC-'3
6.	OPY-19	5'-TGAGGGTCCC-'3
7.	OPO-11	5'-GACAGGAGGT-'3
8.	OPD-13	5'-GGGGTGACGA-'3
9.	D-12	5'-CACCGTATCC-'3
10.	OPJ-20	5'-AAGCGGCCTC-'3
11.	OPO-16	5'-TCGGCGGTTC-'3
12.	P-08	5'-ACATCGCCCA-'3
13.	K-02	5'-GTCTCCGCAA-'3
14.	K-14	5'-CCCGCTACAC-'3
15.	Z-13	5'-GACTAAGCCC-'3
16.	OPY-15	5'-AGTCGCCCTT-'3
17.	D-06	5'-ACCTGAACGG-'3
18.	A-16	5'-AGCCAGCGAA-'3

The quality of extracted DNA was determined by electrophoresis on agarose gel with an agarose concentration of 0.8% (w/v) and ethidium bromide (1.0%). Electrophoresis was carried out using 1xTAE (Tris Acetate EDTA) solution for 45 minutes at a voltage of 120 volts. The DNA banding patterns were observed under ultraviolet (UV) light equipment and the gel documentation was performed using GelDoc (Biorad). The extracted DNA was stored in a freezer at -20 °C until PCR amplification was performed.

PCR amplification and electrophoresis

Eighteen random primers (Operon Technologies) were initially screened to determine the matching of each primer for the study (Table 1). The total volume of polymerase chain reaction (PCR) was 10 μl, which consisted of Hot Star Taq Master Mix Qiagen (5 μl), primer (1 μl), DNA template (1.3 μl), and water-free nuclease (2.7 μl). The DNA amplification was performed in a PCR machine (Labcycler 48 gradient thermocycler, Sensoquest). The PCR condition was programmed, followed by Zulfahmi et al. (2021), as follows: pre-denaturation at 95°C for 5 minutes, followed by 39 cycles consisting of denaturation at 94°C for 1 minute, annealing at 37°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 8 minutes. The amplification products were electrophoresed in 1.2% agarose gel (w/v) and ethidium bromide (1.0%) in 1 x Tris Acetate EDTA (TAE) buffer at 120 volts for 45 minutes. The banding patterns of the gel were observed under UV light and gel documentation was carried out using Gel Doc™ XR and Image Lab™ Software (BIO-RAD). The length of DNA banding was determined according to the 100 bp DNA Ladder (Vivantis).

Data analysis

The DNA banding pattern obtained from PCR products of each sample was scored manually, with a value of 1 for the presence and 0 for the absence of the band. The scoring results are compiled as binary data to be analyzed by software. The percentage of the polymorphic fragments was calculated by dividing the number of the polymorphic fragment at the cultivar by the total of fragments amplified.

RESULTS AND DISCUSSION

Genetic variation

Out of eighteen primers screened, twelve primers were selected, as shown in Table 2, due to clear bands and polymorphic. The remaining six primers exhibited poor/no amplification of DNA fragments and generated a smear. It may be caused by an unmatching annealing temperature during PCR. Our result was in line with those previous studies by Amiteye (2021) that explained the failure of primer to be amplified the DNA template due to the unmatched annealing temperature of the primer. Primer concentration also affects the success of amplification, primer concentrations that are too low or too high cause amplification not to occur accurately (Hasanah et al. 2022). The amplification results of 12 RAPD primers produced 39 DNA fragments. The DNA fragment of each primer ranged from 1-6 fragments and fragment size ranged from 200 - 1500 bp (Table 2), depending on the cultivar tested and primer used. The differences in the size of the resulting DNA band are caused by differences in the length of DNA sites in plants that can be extended by primers. The farther the distance between the primer site and the others in the DNA template will generate a new band with a size of longer (Uslan & Pharmawati 2020). A higher number of fragments amplified was generated by OPD-08 and OPJ-20 primers with six fragments, and a lower number of fragments was observed in OPO-13, D-12, OPO-16, and P-08 primers. The polymorphic fragment was 29 fragments and average of 2.4 fragments were detected per primer.

Polymorphism is characterized by the presence and absence of bands in the sample and the difference in the size of the band produced by each sample. The percentage of polymorphic fragments showed a high degree of genetic variation among the genotypes tested. The higher the percentage of polymorphic fragments, the higher the

genetic variation. The percentage of polymorphic fragments in this study ranged from 25-100%, with an average was 80.41%. The highest percentage of polymorphism fragments was 100%, observed in the primer of OPD-08, D-08, OPO-13, OPO-11, OPD-13, D-12, OPO-16, and P-O8. The lowest percentage of polymorphism fragments was found in the C-09 primer (25%) followed by the OPY-19 primer (33.33%). The level of polymorphism was classified as high if the percentage of polymorphism loci produced was greater than 50% (Ho & Ngo 2017). The genetic variation of matoa in this study was a high category based on the category mentioned above. The high genetic variation of matoa in the present study is caused by cross-pollinated among matoa cultivars in the field. In general, cross-pollinated species exhibited a high level of genetic variation compared to self-pollinated species. Our result was in line with other cross-pollinated species, such as *Fritillaria tubiformis* ssp. *moggridgei* Rix (Mucciarelli et al. 2014), *Dimocarpus longan* (Ho & Ngo 2017), and Sago palm (Riyanto et al. 2018).

The percentage of fragment polymorphic in this study was higher than previously reported by Sheet et al. (2018) in mulberry cultivars (59.62%), Kumar et al. (2019) in papaya (58.36%), and El-Khayat & Aseel (2020) in four Egyptian Mandarin cultivars (53.81%). The level of polymorphism obtained in the present study is lower than in those previous studies reported by Sundari et al. (2017) in durian (86.50%), Hannum et al. (2020) in durian (100%), Fadillah et al. (2022) in Rukam (93.42%). However, the degree of polymorphism obtained in the present study is almost similar to that obtained in an earlier study using the RAPD marker on the citrus cultivar (80.63%) (Shahzadi et al. 2016), *Prunus salisina* L (81.60%) (Li et al. 2022). A diverse degree of polymorphism between this study and previous studies can be explained by differences in the number, type, and origin of the cultivars tested, marker selection criteria, and the number and type of primers used.

Identification of matoa cultivar

Out of 12 primers selected, 11 primers can differentiate the four cultivars of Matoa in this study as shown in Figure 2 and Table 3. The primer X-01 can be used to distinguish the black matoa cultivar and the red matoa cultivar. The black matoa cultivar can be identified specifically with a band size of 450 bp meanwhile the red matoa cultivar can be identified specifically with a band size of 550 bp (Figure 2A). Yellow Matoa cultivar can also be identified by C-09 primer with the presence of a band size of 550 bp. The OPY-19 primer can distinguish the red Matoa cultivar from others with produced a band size of 400 bp (Figure 2A). The OPD-08 primer can differentiate four matoa cultivars, the black matoa cultivar has no amplified fragment with this primer, the green matoa cultivar produced a specific band size of 1000 bp, the yellow matoa produced bands with sizes of 1500 bp and 400 bp, whereas the red matoa cultivar produced band with sizes of 750 bp and 550 bp. The primer of D-08 can distinguish three matoa cultivars the red matoa cultivar can be identified specifically with a band size of 750 bp, the yellow matoa cultivar with a band size of 1500 bp, and the black matoa cultivar with a band size of 600 bp (Figure 2B).

Red Matoa can be identified using the D-12 primer that generated a band size of 700 bp and the rest cultivars with no band amplified. The Primer of OPO-11 can distinguish the Red Matoa cultivar and the Green Matoa cultivar. The Red Matoa generated a specific band size of 800 bp, whereas the green matoa cultivar showed no band amplified (Figure 2C). The Red Matoa cultivar was not generated bands when amplified with OPD-13 primer, and this can act as a marker for the Red Matoa cultivar. The Yellow Matoa cultivar and the Black Matoa cultivar can be differentiated by OPD-13 primer with the presence of band sizes of 600 bp and 200 bp, respectively (Figure 2C).

The green matoa cultivar can be identified with P-08 primer that no band produced when amplified, whereas the rest cultivars generate fragment size of 200 bp. Red Matoa can be distinguished from other matoa cultivars using OPO-16, which generates fragment size 1000 bp, whereas the rest of the cultivars are not produced band when amplified.

Table 2. The results of PCR amplification of 12 primers selected

Primer name	Sequences	Fragment size (bp)	Number of fragment	Number of fragment polymorphic	Percentage of fragment polymorphic (%)
OPD-08	5'-GTGTGCCCCA-'3	400 - 1500	6	6	100%
D-08	5'-GTGTGCCCCA-'3	500 - 1500	5	5	100%
OPO-13	5'-GTCAGAGTCC-'3	900	1	1	100%
X-01	5'-CTCACCGTCC-'3	450 - 1000	5	2	40%
C-09	5'-CTCACCGTCC-'3	550 - 1000	4	1	25%
OPY-19	5'-TGAGGGTCCC-'3	400 - 900	3	1	33.33%
OPO-11	5'-GACAGGAGGT-'3	200 - 1300	3	3	100%
OPD-13	5'-GGGGTGACGA-'3	200 - 1200	3	3	100%
D-12	5'-CACCGTATCC-'3	700	1	1	100%
OPJ-20	5'-AAGCGGCCTC-'3	400 - 1500	6	4	66.66%
OPO-16	5'-TCGGCGGTTC-'3	1000	1	1	100%
P-08	5'-ACATCGCCCA-'3	200	1	1	100%
Total		200-1500	39	29	964.99
average			3.3	2.4	80.41%

Table 3. Unique band to distinguish the matoa cultivars and criteria used

Cultivars	Unique band	Criteria
Red Matoa	X-01 (550 bp)	Present
Black Matoa	X-01 (450 bp)	Present
Yellow Matoa	C-09 (550 bp)	present
Red Matoa	OPY-19 (400 bp)	Present
Black Matoa	OPD-08	Not amplified
Green Matoa	OPD-08 (1000)	Present
Yellow Matoa	OPD-08 (900, 400)	Present
Red Matoa	OPD-08 (750, 550)	Present
Yellow Matoa	D-08 (1500 bp)	Present
Red Matoa	D-08 (750 bp)	Present
Red Matoa	OPD-13	Not amplified
Yellow Matoa	OPD-13 (600 bp)	Present
Black Matoa	OPD-13 (200 bp)	Present
Red Matoa	OPO-11 (800 bp)	Present
Green Matoa	OPO-11	Not amplified
Green Matoa	P-08 (200 bp)	absent
Red Matoa	OPO-16 (1000 bp)	Present
Red Matoa	D-12 (700 bp)	Present
Black Matoa	OPJ-20 (600)	Absent

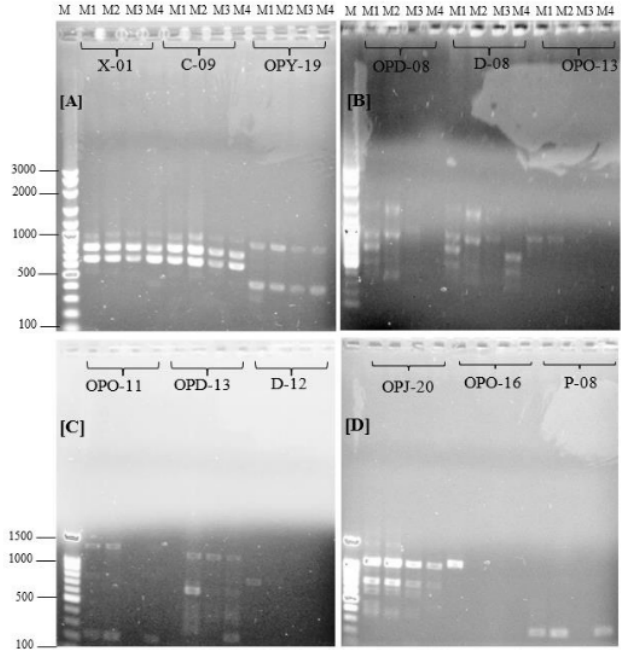

Figure 2. Product amplification of twelve primers, L: DNA Marker, M1: red matoa, M2: yellow matoa, M3: green matoa, M4: black matoa

The black matoa cultivar can be identified by no band size of 600, 500, and 400 bp at primer OPJ-20, meanwhile the red matoa cultivar can be identified with the appearing band size of 500 bp (Figure 2D). The present study revealed that RAPD was a valuable tool for cultivar identification. Our results are in agreement with previous research on coconut (Rajesh et al. 2014), peach (Han et al. 2014), durian (Rosmaina et al. 2016) and cashew (Bhadra et al. 2019). This is the first report on utilizing of DNA marker to identify Matoa (*Pometia pinnata*). Information on the unique bands can be utilized by breeders and farmers. The breeder can use this information as a basis to select the parents for the genetic improvement of Matoa, cultivars or clones protection, and detection of seedling purity in a nursery. Meanwhile, for farmers, this information could be used to verify the originality of the

seedlings that will be cultivated. Matoa (*Pometia pinnata*) is a long-lived plant, so the seedlings that are cultivated do not be mistaken because it will be disadvantageous economically and at times.

In conclusion, The level of polymorphism of the matoa cultivar in this study was high (80.41%). Eleven primers can distinguish the four matoa cultivars in this study.

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