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Genetic diversity and its association with *Phytophthora palmivora* resistance in durian (*Durio zibethinus*) using RAPD markers

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Abstract. *Numba S. 2023. Genetic diversity and its association with* Phytophthora palmivora *resistance in durian (*Durio zibethinus*) using RAPD markers. Biodiversitas 24: 4542-4548. Durian* (*Durio zibethinus* L.) is a popular tropical fruit that contributes significantly to regional economic value. Root and stem rot disease caused by *Phytophthora palmivora* has become a serious threat to the growth and development of durian. Indonesia has various local durian varieties that have the potential of resistant to *P. palmivora*. This study aims to analyze genetic diversity and its association with the resistance of durian to root and stem rot disease caused by *P. palmivora* using RAPD markers. Ten varieties of durian were compared with those of 2 wild species, namely *D. kutejensis* (Hassk.) Becc. and *Boschia excelsa* Korth.. The DNA profile analysis was performed by using 10 RAPD primers. Furthermore, cluster analysis was done using the NTSYS (Numerical Taxonomic and Multivariate Analysis System) ver. 2.0. Observation of the DNA profile (DNA banding pattern) showed that all the primers succeeded in producing amplified DNA fragments. Of the total 78 bands produced, 10 bands were considered monomorphic, while 68 bands (87.20%) were considered polymorphic. The high level of polymorphism causes a low level of similarity between varieties so that not all resistant varieties are grouped in the same cluster. This study found that the 1kb band produced by the OPA-2 primer was a potential gen associated with resistance to *P. palmivora*. It is suggested to carry out further tests on crosses of resistant and susceptible varieties.

Keywords: *Durio zibethinus*, genetic diversity, NTSYS, *Phytophtora palmivora*, RAPD markers

INTRODUCTION

durian (*Durio zibethinus* L.) is a popular tropical fruit that contributes significantly to regional economic value (Somsri 2015; Aziz et al. 2017). Durian has a unique taste and texture as well as its high nutrient content (Aziz and Jalil 2019). According to Devalaraja et al. (2011), durian is a good source of protein (1.47%), fat (5.33%), fiber (3.1%), and carbohydrates (27%). Durian is also known as the "king of tropical fruit" due to its superlative flesh, high nutritional contents, and appearance which resembles the thorny thrones of Asian kings (Ali et al. 2020). In Indonesia, most plants are not commercially cultivated, and the differences between the cultivars have not been widely studied (Tan 2022).

durian is a plant native to Southeast Asia (Zhou et al. 2021). Indonesia is listed among the major durian-producing countries along with Malaysia, Thailand, Cambodia, and Vietnam. Durian production in Indonesia has increased since 2016 and peaked in 2021, with production reaching 1.35 million tons. That number increased by 19.40% compared to the previous year which amounted to 1.13 million tons (Central Bureau of Statistics 2022). Furthermore, the Indonesian government has opened 5,000 ha of durian plantations throughout Indonesia, because of the increasing domestic demand for durian in 2017 (Sulistiyo et al. 2022). These plantation areas led to a remarkable increase in durian production in 2018. Therefore, one of the priorities of research in the field of horticulture is to collect and identify the parent tree fruit native to Indonesia's superior worth in terms of both production and resistance to pests and diseases.

The genetic diversity of durian has attracted much interest from researchers from Southeast Asia (Vanijajiva 2011; Mursyidin and Daryono 2016; Prakoso and Retnoningsih 2021; Mursyidin et al. 2022) as well as from Cina (Wang et al. 2019; Lin et al. 2022). The majority of these studies seek to uncover the potential superiority of durian characters because germplasm collection is a vital component of the success of plant breeding programs and the development of superior cultivars (Cowling et al. 2016; Wambugu et al. 2018).

Root and stem rot disease caused by *Phytophthora palmivora* has become a serious threat to the development of durian plants in Indonesia. This disease causes the death of plants, both juvenile and mature plants (Santoso et al. 2015). This disease has damaged up to 30% of durian farms (Drent and Guest 2004). Attacks of this disease have been reported in Australia (O'Gara et al. 2004), Phillipines (Abad and Cruz 2013), and Thailand (Ritmontree and Kongtragoul 2020). However, few studies were associated with the resistance of durian varieties to that disease (Camellia et al. 2019). Some wild durian species were reported to be resistant to the disease (Shamsudin et al. 2000). Previous research assessed the resistance of durian to the missing lesion diameter parameter (Vawdrey et al. 2005). Considering that the resistance of plant variety is influenced by both environmental and genetic factors, it is

necessary to conduct a study at the genetic level. One method that can be applied is the Random Amplified Polymorphic DNA (RAPD) marker. The marker has been used in several previous studies to determine genes associated with resistance to *Phytophthora fragariae* (Rpf1) in cultivated strawberries (Haymes et al. 1997), resisting Quantitative Trait Loci tolerance to root rot in soybean (Han et al. 2008), and developing molecular markers related to resistance of *Phytophthora capsici* to pepper (*Capsicum annuum* L.) (Truong et al. 2013).

It is expected to obtain information about the DNA profile of disease-resistant varieties by analyzing several durian cultivars. This information can be used in the assembly program for durian varieties resistant to *P. palmivora*. This study aimed to analyze genetic diversity of durian and its association with resistance to root and stem rot disease caused by *P. palmivora* using RAPD markers. Furthermore, from the genetic information that can be planned further studies to detect genes that encode resistance to cancer and cancer stem to root, and can eventually be used for the assembly of durian varieties that are resistant to *P. palmivora.*

MATERIALS AND METHODS

Plant materials

This study was conducted in the Laboratory of Molecular Biology, Center for Plant Breeding, Department of Agronomy, Institut Pertanian Bogor, Bogor, Indonesia. Durian leaves were collected from the nursery and germplasm in Cipaku, Bogor, West Java (varieties), and from Kalimantan (wild species). Identification of the level of resistance to *P. palmivora* was performed by using a data description of each variety released by the Ministry of Agriculture (Table 1).

Procedures

DNA isolation

Total plant genomic DNA extraction was performed according to the method described by Dellaporta et al. (1983). Fresh leaf samples (2.5 g) were crushed with liquid nitrogen in a porcelain dish until a fine powder, then put in a tube (50 mL). A total of 15 mL of lysis buffer (Tris-HCl pH 8.0, 2.0 MNaCl and 50 mM EDTA, 90 mM PVP, 30 mM SDS, and β-Mercaptho-ethanol 15 mM) was added to the tube containing the powder, then incubated at 65°C for 20 minutes with shaking slowly. A total of 5 mL of 5 M sodium acetate solution was added and mixed thoroughly by shaking gently, then the suspension was incubated at -20°C. The suspension was centrifuged with 15,000 xg for 25 min at 8°C. The supernatant was removed by using a micropipette whose tip had been cut to have a wide diameter. A solution of chloroform/isoamyl alcohol (24/1; v/v) was added to the DNA suspension. The suspension was homogenized carefully to avoid destruction of DNA, then centrifuged with 15,000 xg for 25 minutes at 8°C. The supernatant was then transferred to a new tube (extraction was repeated 2 times). The DNA in the suspension was added with ether and inverted the tube slowly so that mixed evenly, then centrifuged at 15,000 xg for 15 min at room temperature. A total of 2-3 volumes of absolute ethanol was added to the DNA suspension, and then the tube was inverted gently and incubated at -20°C for 2 hours. The threads of DNA were taken and transferred to a new Eppendorf tube. DNA precipitate was rinsed with the addition of 70% ethanol, then centrifuged with 10,000 xg for 5-10 minutes at 4°C, and then the supernatant was discarded. The DNA precipitate was dried quickly at room temperature by placing the test tube using a vacuum pump. The DNA precipitate is suspended in 5-10 mL of TE or ddH_2O.

Table 1. Plant materials used in the study and the identification results of each variety resistance against *P. palmivora*

Species	Varieties/ local name	Source	Status	Identification
D. zibethinus L.	Sukun	Semarang, Central Java, Indonesia	Indonesia variety, released in 1984	Sensitive
D. zibethinus L.	Sunan	Boyolali, Central Java, Indonesia	Indonesia variety, released in 1984	Sensitive
D. zibethinus L.	Monthong	Bangkok, Thailand	Thailand variety, introduced in 1987	Resistant
D. zibethinus L.	Kani	Bangkok, Thailand	Thailand variety, introduced in 1987	Sensitive
D. zibethinus L.	Sihijau	Banjar, South Kalimantan, Indonesia	Indonesia variety, released in 1990	Resistant
D. zibethinus L.	Perwira	Majalengka, West Java, Indonesia	Indonesia variety, released in 1993	Resistant
D. zibethinus L.	Siwirig	Majalengka, West Java, Indonesia	Indonesia variety, released in 1993	Resistant
D. zibethinus L.	Bakul	Muara, South Sumatra, Indonesia	Indonesia variety, released in 1994	Resistant
D. zibethinus L.	Hepe	Bogor, West Java, Indonesia	Indonesia variety, released in 1994	Sensitive
D. zibethinus L,	Matahari	Bogor, West Java, Indonesia	Indonesia variety, released in 1994	Sensitive
D. kutejensis (Hassk.) Becc.	Lai Kute	East Kalimantan, Indonesia	Wild	Resistant
Boschia excelsa Korth.	Lai Bengang	Mabah, West Kalimantan, Indonesia	Wild	Resistant

RNase (DNase free) was added to a final concentration of 10-100 µg/mL, then incubated at 37°C for 1 hour. Proteinase K was added to a concentration of 100 µg/mL to remove all the protein after RNase treatment, then the suspension was incubated at 37°C for 1 hour. The suspension was extracted 1-2 times with chloroform/isoamyl alcohol. DNA contained in the tube precipitated by the addition of 0.1 volume 3 M sodium acetate and 2-3 volumes of cold absolute ethanol, then allowed at -20°C for 2 hours. The DNA solution was centrifuged with 10,000 xg for 10 min at 4°C, then precipitated DNA was dried at room temperature by turning on a paper tube with a suction or vacuum pump. The DNA was suspended in TE or ddH_2O and stored at -20°C. The DNA samples are stored in the storage buffer precipitated with cold ethanol 4x volumes, then centrifuged with 10,000 xg for 3 minutes. DNA precipitate was dried and then redissolved in ddH_2O to achieve a 50 ng/µL final concentration. This DNA was used as a DNA template for DNA amplification. Pure DNA has been obtained in the extraction of DNA, then diluted to a concentration of 25 ng/mL.

DNA amplification and electrophoresis

The primers used in this study were 10 RAPD primers (Operon Tech). The DNA profile analysis was done using the RAPD method by performing amplification of the total genomic DNA of plants. This stage was carried out following the method reported by Williams et al. (1990). The reaction mixture for Polymerase Chain Reaction (PCR) was prepared with a total volume of 25 µL of each containing 16.8 µL ddH_2O, 2 µL (50 µg/ml) DNA template, 2.5 µL dNTP Solution Mix (Bio-Rad), 1 µL primer (15 ng) of OPA-1 – OPA-10 (Table 3), 0.2 µL (0.5 U) Taq DNA Polymerase (Bio-Rad), and 2.5 µL (50 ng) PCR buffer solution. Mineral oil was added to cover the surface of each tube perfectly. Amplification was done by placing the reaction mixture on the block and regulating the reaction cycle by step cycle program (DNA Thermal Cycler Bio-Rad CFX 96) up to 45 cycles, each cycle consisting of 3 stages, which begins with predenaturation at 94°C to 96°C for 3 minutes. Stage 1 was denatured by heating to 94°C for 60 seconds, stage 2 for primary adhesion at temperatures of 36°C for 30 seconds and stage 3 for the extension phase at 72° C for 2 minutes. After 45 cycles added to the incubation at 72°C for 5 minutes to ensure that the amplified DNA renaturation has undergone, is used for thermal program delay the incubation at 72°C for 5 min, followed by cooling.

A total of 10 µL aliquots of the amplified result of each primer combination was added with 3 µL of loading buffer containing bromophenol blue and sucrose (New England Biolabs). The suspension was migrated by electrophoresis on agarose gel (1%) at 50 volts for 2 hours. In the TAE electrophoresis buffer solution, 4 µg/µL ethidium bromide was added. The results of the gel electrophoresis were then observed using a UV-Trans illuminator (Major Science UV), then the band pattern (profile) of the results of the DNA amplification was observed and photographed using a Polaroid camera (FM UVT-A100-Vison).

Data analysis

The RAPD bands were scored for their presence (1) or absence (0). The RAPD data generated by reproducible primers were used to compile a matrix for clustering analysis using NTSYSpc (Numerical Taxonomic and Multivariate analysis System) Version 2.0. Genetic similarity among durian varieties and wild species was calculated using SIMQUAL (Similarity for Qualitative Data). The similarity coefficients were used to construct a dendrogram using UPGMA (Unweighted Pair-group Method with Arithmetical Averages) through SAHN (Sequential, Hierarchical, Agglomerative, and Nested Clustering). While the analysis of the relationship between genetic diversity and the level of resistance to *P. palmivora* was carried out by observing the specific banding patterns of the various test varieties and showing the level of resistance of each durian variety.

RESULTS AND DISCUSSION

Quantity and quality of DNA

The quality of the DNA obtained from the extraction was very good, because the values of the A260/A280 ratio ranged from 1.74 to 1.88. The overall DNA yield recorded ranged between 43.2 µg/mL (Sunan) and 108.0 µg/mL (Montong). Data of observations about the quality and quantity of DNA from each of the durian varieties and wild species by using a spectrophotometer are presented in Table 2.

According to Sambrook et al. (1989), suitable DNA purity for PCR ranged from 1.8 to 2.0. High-quality DNA available in DNA extraction was a basic rule requirement in molecular studies, particularly in DNA fingerprinting. Cetyl Trimethyl Ammonium Bromide (CTAB) was a method commonly used in many extractions of plant DNA polysaccharides and polyphenolic compounds. Furthermore, observational data on the quality of DNA from each wild variety by carrying out PCR amplification is presented in Figure 1. The quality of DNA obtained was considered appropriate because all the DNA samples can be amplified by PCR using random primers. Several factors affect the resulting pattern of intact bands and well, such as concentration and purity of DNA sample rate, size/length of the primer, primer composition (sequence of nucleotides), the concentration of magnesium ions, and the enzyme Taq-polymerase, and the optimal conditions for the PCR reaction for each type of plant.

Genetic polymorphisms

Observation of the DNA profile (DNA banding pattern) showed that all the primers used succeeded in producing amplified DNA fragments with the number of bands for each primer ranging from 7 to 9 bands. Of the total 78 bands produced, 10 bands were considered monomorphic, while 68 bands 87.20% were considered polymorphic (Table 3). The data showed that none of the DNA profiles produced a 100% monomorphic band. This means that there were huge variations in the genomes of plants, both intra- and inter-specific among varieties and wild species on durian. The examples of RAPD banding patterns of

durian studied generated by primers OPA-3, OPA-4, and OPA-5 was presented in Figure 1. The results of amplification using OPA 3, 4 and 5 showed that there were no specific DNA bands found for varieties that were resistant to disease resistance to *P. palmivora* (Figure 1.A-C).

There were differences in nucleotide sequence size or genetic variation in plants based on DNA banding pattern polymorphism generated by PCR. This was caused by a single random primer sticking to the DNA samples in 2 different places on the opposite DNA thread. One of the main requirements for DNA amplification with a single primer random is if the primer has a sequence of a nucleotide base that is complementary to the second genomic DNA strands in opposite positions (Idrees and Irshad 2014). DNA amplification with PCR occurs if the complement was alkaline primers with the DNA base sequence of the template DNA not exceeding 5,000 bp.

Table 3. Number of polymorphic and monomorphic bands generated by RAPD primers

Primer	Sequence (5' – 3')	Number of bands		Total bands
		Mono-morphic	Poly-morphic	
OPA-1	5'-CAGGCCCTTC-3'	0	9	9
OPA-2	5'-TGCCGAGCTG-3'	2	5	7
OPA-3	5'-AGTCAGCCAC-3'	1	6	7
OPA-4	5'-AATCGGGCTG-3'	2	6	8
OPA-5	5'-AGGGGTCTTG-3'	1	6	7
OPA-6	5'-GGTCCCTGAC-3'	0	6	6
OPA-7	5'-GAAACGGGTG-3'	0	9	9
OPA-8	5'-GTGACGTAGG-3'	2	6	8
OPA-9	5'-GGGTAACGCC-3'	1	8	9
OPA-10	5'-GTGATCGCAG-3'	1	7	8
	Total	10	68	78

Table 2. Quantity and quality of DNA obtained based on absorbance at a wavelength of 260 nm and 280 nm

Species	Absorbance		A_{260}/A_{280}	[DNA] (µg/mL)
	260 nm	280 nm		
Durio zibethinus var. Sukun	0.118	0.064	1.85	70.8
D. zibethinus var. Sunan	0.072	0.040	1.80	43.2
D. zibethinus var. Montong	0.180	0.101	1.78	108.0
D. zibethinus var. Kani	0.114	0.065	1.75	68.4
D. zibethinus var. Sihijau	0.080	0.045	1.77	48.0
D. zibethinus var. Perwira	0.126	0.072	1.75	75.6
D. zibethinus var. Siwirig	0.119	0.067	1.77	71.4
D. zibethinus var. Bakul	0.133	0.073	1.83	79.8
D. zibethinus var. Hepe	0.096	0.051	1.88	57.6
D. zibethinus var. Matahari	0.170	0.095	1.79	102.0
Durio kutejensis	0.132	0.071	1.86	79.2
Boschia exelsus	0.146	0.084	1.74	87.6

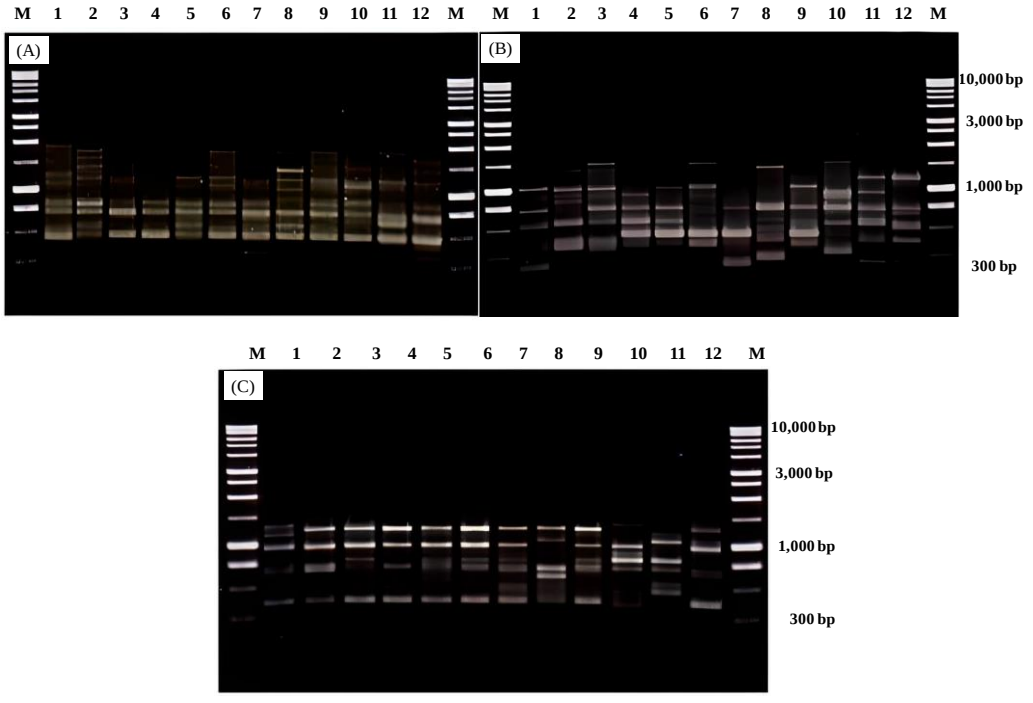

Figure 1. Banding patterns of durian generated by RAPD primers: (A) OPA-3, (B) OPA-4, (C) OPA-5, (M) 1 kb ladder, (1) Sihijau, (2) Sukun, (3) Montong, (4) Kani, (5) Sunan, (6) Perwira, (7) Siriwig, (8) Bakul, (9) Hepe, (10) Matahari, (11) Lai Kute, and (12) Lai Bengang

Previous studies have shown that local durian genetic polymorphism is generally quite high (> 69%). The number of polymorphism bands in this study is larger than in some previous studies. Ruwaida et al. (2009) reported five varieties of durian in Central Java, namely Sukun, Sunan, Montong, Petruk and Kani showed an average polymorphism of 81.9%. Mursyidin and Daryono (2016) was also reported 11 local durian cultivars from South Kalimantan's Province has indicated polymorphism degree of 82.17%. Research in Riau Province used seven durian cultivars, namely Empu Kunyit, Keong Mas, Ome Kampar, Montong, Tembaga, Bakul and Sijantung showed a polymorphism rate of 69.39% (Rosmaina et al. 2016). Furthermore, research on Tidore Island used Sixteen local durians namely Nona, Baba, Boso, Tusa, Batu/ gulinga, Mafu, Malal, Elephant, Butter, Blek, Malal Jaya, Jackfruit, Kusi, Kohori, Takate, Sambiki showed a polymorphism level of 75.22% (Sundari et al. 2021). In the study of several durian varieties in the Nonthaburi Province of Thailand, it was shown that the polymorphism level was quite low at 37.77% (Vanijajiva 2011). However, when compared to a study in Malaysia the level of polymorphism in this study was lower. samples from four durian orchards in Universiti Putra Malaysia 27 durian varieties showed a polymorphism level of more than 90% (Siew et al. 2018).

Cluster analysis

Out of 10 varieties of cultivated durian and 2 species of wild durian, three groups were formed at a similarity level of 57.5%. The first group consists of the varieties of Sukun, Sunan, Siwirig, Matahari, Hepe, Kani, Perwira, and Montong. This group is sensitive to *P. palmivora*, except for Perwira and Siwirig varieties. The second group consisted of varieties Bakul, Sigreen, and *D. kutejensis*. The second group was classified as resistant to *P. palmivora*. Outside these two groups, there is a species that does not belong to the two groups above, namely the species *D. exelcus*, which is resistant to *P. palmivora*. These results indicate that resistant characters have a low influence in determining the level of genetic similarity. This can be seen from the similarities between the Siwirig and Matahari varieties. these two species have a level of genetic similarity > 70%, but the character of resistance is different. Meanwhile, Bakul, Sigreen, and *D. kutejensis* are a group of species that are resistant but have a similarity of less than 60% (Figure 2). A similarity value of more than 0.70 indicates high similarity, while it is classified as low below 0.60 (Trimanto 2012). The high level of polymorphism causes a low level of similarity between varieties, so that not all resistant varieties are grouped in the same cluster. There are resistant varieties grouped with susceptible varieties. Clustering based on the level of similarity generated using a limited number of primers in the RAPD method often does not determine the level of closeness of relationship for a special character such as the level of disease resistance. Previous research in Central Java showed that Sukun, Sunan, Montong, and Petruk varieties were in a separate group from Kani variety. In contrast, this study showed a close relationship between Sukun and Sunan varieties, while that in Central Java showed a close relationship between Sunan and Montong varieties which reached 84% (Ruwaida et al. 2009). Other research in Riau Province showed that Bakul and Montong were in different clusters (Rosmaina et al. 2016). That result is in line with this study.

DNA band pattern with resistance to *Phytophthora palmivora*

Differences in DNA versus patterns were influenced by differences in the results of genomic DNA amplification. The polymorphic band is an illustration of DNA bands that appear at certain sizes in a sample. Polymorphism is caused by the absence of amplification at a locus which is triggered by differences in the sequence of nucleotide bases at the primary attachment point. The existence of a polymorphic DNA banding pattern is caused by differences in the base arrangement of each DNA sample. The choice of primers in the RAPD analysis affects the polymorphism of the resulting bands, because each primer has its attachment site, as a result, the polymorphic DNA bands produced by each primer are different, both in terms of the number of base pairs and the number of DNA bands (Poerba and Martanti 2008). Previous studies have focused on studies on *P. palmivora* attack and its diversity in plants, which have been carried out on several species of durian host plants (Masanto et al. 2019; Alsultan et al. 2021). The results of the report indicate that *P. palmivora* has attacked cocoa and durian crops in Malaysia. Previous research in Malaysia tested these 3 SCAR primers as markers of resistance to *Phytophthora* in durian and found that Primer 886-2 was effective in differentiating susceptible and resistant genes (Camellia et al. 2019).

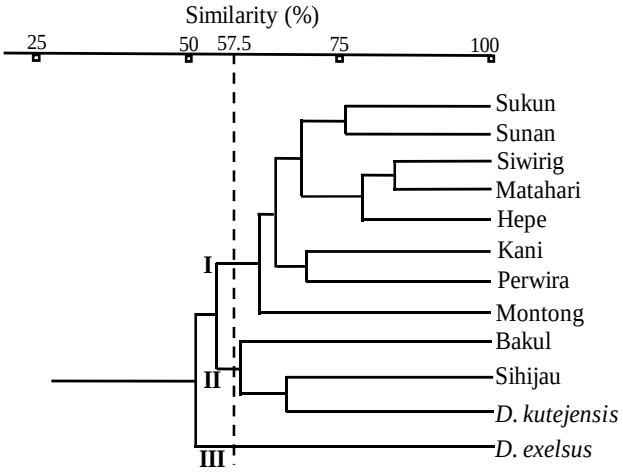

Figure 2. A dendrogram among 10 cultivated varieties of durian and 2 wild species of *Durio* generated by the UPGMA cluster analysis

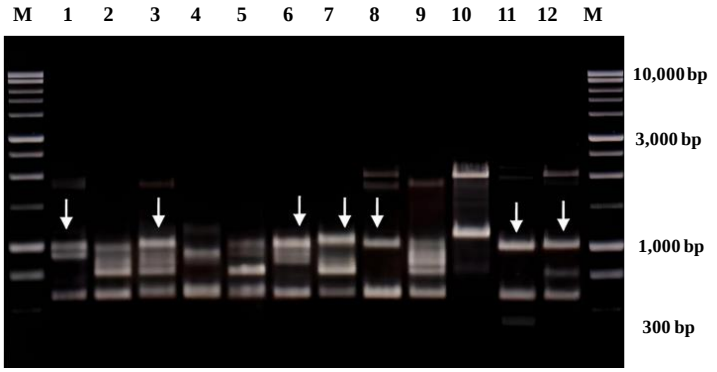

Figure 3. Specific bands 1 kb size of the PCR amplification by using Primer OPA-2 (arrow) found in resistant varieties: (M) 1kb ladder, (1) Sihijau, (2) Sukun, (3) Montong, (4) Kani, (5) Sunan, (6) Perwira, (7) Siriwig, (8) Bakul, (9) Hepe, (10) Matahari, (11) Lai Kute, and (12) Lai Bengang

One of the efforts to increase tolerance to *Phytophthora* root rot and stem rot can be done by overexpression of the plant antimicrobial peptide CaAMP1 gene. Antimicrobial peptides play an important role in the defense systems of plants and animals. Over-expression of CaAMP1 (*C. annuum* antimicrobial protein 1), an antimicrobial protein gene isolated from *C. annuum* leaves infected with *Xanthomonas campestris* pv. *vesicatoria* , confers broad-spectrum resistance to hemibiotrophic bacterial and necrotrophic fungal pathogens in *Arabidopsis* (Niu et al. 2020). Application of plant AMP has been shown to increase tolerance to many fungal diseases in several species, including chili (Tantong et al. 2016), pepper (Majid et al. 2017), rice (Weerawanich et al. 2018), soybean (Lin et al. 2013; Niu et al. 2020), and citrus (Kishi et al. 2018). This study found that the OPA-2 primer has produced specific bands of around 1 kb which may be associated with resistance to root/stem cancer (*P. palmivora*) (Figure 3). Further studies can test the effectiveness of crosses between sensitive and resistant varieties. Furthermore, these bands can be characterized as those immediately behind (associated) with resistance genes or gene bands directly responsible for resistance to *P. palmivora*. If their offspring produced band resistance properties, this band can be used as a marker/molecular marker to identify plants that are resistant to ***P. palmivora***. Furthermore, these bands can be characterized as directly behind (associated) with resistance genes or gene bands directly responsible for resistance to ***P. palmivora.***

Based on DNA profile analysis, OPA-2 has produced specific bands of around 1 kb (1000 bp) which may be associated with resistance to root/stem cancer (***P. palmivora***) (Figure 3). A special band with a size of about 1 kb appeared on the resistant varieties Montong, Sigreen, Officer, Siwirig, Bakul, *D. kutejensis*, and *D. exelsus*. Whereas, those were absent in the sensitive varieties (Sukun, Sunan, Kani, Hepe, and Matahari). The figure showed that the 1 kb band produced by primer OPA-2 is expected to determine the resistant character to root and stem disease caused by ***P. palmivora***. The size of the fragments obtained ranged from 300 to 1000 bp (Figure 3).

In conclusion, observation of the DNA profile (DNA banding pattern) showed that all the primers succeeded in producing amplified DNA fragments with the number of bands for each primer ranging from 7 to 9 bands. Of the total 78 bands produced, 68 bands 87.20% were considered polymorphic. The high level of polymorphism causes a low level of similarity between varieties, so that not all resistant varieties are grouped in the same cluster. This study found that the 1-kb band produced by the OPA-2 primer was a potential gen associated with resistance to ***P. palmivora***. It is suggested to carry out further tests on crosses of resistant and susceptible varieties.

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REFERENCES

Abad RG, Cruz KJT. 2013. Incidence of *Phytophthora* fruit rot on four durian cultivars in Davao City, Philippines. Acta Hortic 1006: 35-39. DOI: 10.17660/ActaHortic.2013.1006.2

Ali MM, Hashim N, Abd Aziz S, Lasekan O. 2020. Exploring the chemical composition, emerging applications, potential uses, and health benefits of durian: A review. Food Control 113: 107189. DOI: 10.1016/j.foodcont.2020.107189.

Alsultan W, Vadamalai G, Saud HM, Khairulmazmi A, Wong MY, Jaaffarand AKM, Al-Sadi AM, Rashed O, Nasehi, A. 2021. Phylogenetic analysis and genetic diversity of *Phytophthora palmivora* causing black pod disease of cocoa in Malaysia. Plant Heal Prog 22 (3): 260-271. DOI: 10.1094/PHP-02-21-0030-FI.

Aziz NAA, Jalil AMM. 2019. Bioactive compounds, nutritional value, and potential health benefits of indigenous durian (*Durio zibethinus* Murr.): A review. Foods 8 (3): 96. DOI: 10.3390/foods8030096.

Aziz SA, Clements GR, McConkey KR, Sritongchuay T, Pathil S, Abu Yazid MNH, Bumrungsri S. 2017. Pollination by the locally endangered island flying fox (*Pteropus hypomelanus*) enhances fruit production of the economically important durian (*Durio zibethinus*). Ecol Evol 7(21): 8670-8684. DOI: 10.1002/ece3.3213.

Camellia NAN, Salma I, Mohd Norfaizal G. 2019. Development of SCAR markers for rapid identification of resistance to *Phytophthora* in durian using inter simple sequence repeat markers. Asian J Adv Basic Sci 7 (1): 30-34. DOI: 10.33980/ajabs.2019.v07i01.005.

Central Bureau of Statistics. 2022. Statistical Year Book of Indonesia. BPS-Statistic Indonesia, Jakarta. http://www.bps.go.id/. [Indonesian]

Cowling WA, Li L, Siddique KHM, Henryon M, Berg P, Banks RG, Kinghorn BP. 2016. Evolving gene banks: Improving diverse populations of crop and exotic germplasm with optimal contribution selection. J Exp Bot 68: 1927-1939. DOI: 10.1093/jxb/erw406.

Dellaporta SL, Wood J, Hicks JB. 1983. A plant DNA minipreparation: Version II. Plant Mol Biol Rep 1: 19-21. DOI: 10.1007/BF02712670.

Devalaraja S, Jain S, Yadav H. 2011. Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. Food Res Intl 44 (7): 1856-1865. DOI: 10.1016/j.foodres.2011.04.008.

Drent A, Guest DI. 2004. Diversity and Management of *Phytophthora* in Southeast Asia. ACIAR Monograph, Canberra.

Han Y, Teng W, Yu K, Poysa V, Anderson T, Qiu L, Lightfoot DA, Li W. 2008. Mapping QTL tolerance to Phytophthora root rot in soybean using microsatellite and RAPD/SCAR derived markers. Euphytica 162: 231-239. DOI: 10.1007/s10681-007-9558-4.

Haymes KM, Henken B, Davis TM, van de Weg WE. 1997. Identification of RAPD markers linked to a *Phytophthora fragariae* resistance gene (Rpf1) in the cultivated strawberry. Theor Appl Genet 94: 1097-1101. DOI: 10.1007/s001220050521.

Idrees M, Irshad M. 2014. Molecular markers in plants for analysis of genetic diversity: A review. Eur Acad Res 2 (1): 1513-1540.

Kishi RNI, Stach-Machado D, Singulani JL, Dos Santos CT, Fusco-Almeida AM, Cilli EM, Freitas-Astúa J, Picchi SC, Machado MA. 2018. Evaluation of cytotoxicity features of antimicrobial peptides with potential to control bacterial diseases of citrus. PLoS ONE 13 (9): 0203451. DOI: 10.1371/journal.pone.0203451.

Lin F, Zhao M, Ping J, Johnson A, Zhang B, Abney TS, Hughes TJ, Ma J. 2013. Molecular mapping of two genes conferring resistance to Phytophthora sojae in a soybean landrace PI 567139B. Theor Appl Genet 126 (8): 2177-2185. DOI: 10.1007/s00122-013-2127-4.

Lin X, Liu X, Chen M, Gao H, Zhu Z, Ding Z, Zhou Z. 2022. Assessment of genetic diversity and discovery of molecular markers in durian (*Durio zibethinus* L.) in China. Diversity 14 (9): 769. DOI: 10.3390/d14090769.

Majid MU, Awan MF, Fatima K, Tahir MS, Ali Q, Rashid B, Husnain T. 2017. Genetic resources of chili pepper (*Capsicum annuum* L.) against *Phytophthora capsici* and their induction through various biotic and abiotic factors. Cytol Genet 51 (4): 296-304. DOI: 10.3103/S009545271704003X.

Masanto, Hieno A, Wibowo A, Subandiyah S, Shimizu M, Suga H, Kageyama, K. 2019. Genetic diversity of Phytophthora palmivora isolates from Indonesia and Japan using rep-PCR and microsatellite markers. J Gen Plant Pathol 85: 367-381. DOI: 10.1007/s10327-019-00853-x.

Mursyidin DH, Daryono BS. 2016. Genetic diversity of local durian (*Durio zibethinus* Murr.) cultivars of South Kalimantan's Province based on RAPD markers. AIP Conf Proc 1755 (1): 040008. DOI: 10.1063/1.4958483.

Mursyidin DH, Makruf MI, Noor A. 2022. Molecular diversity of exotic durian (*Durio* spp.) germplasm: A case study of Kalimantan, Indonesia. J Genet Eng Biotechnol 20 (1): 1-13. DOI: 10.1186/s43141-022-00321-8.

Niu L, Zhong X, Zhang Y, Yang J, Xing G, Li H, Yang X. 2020. Enhanced tolerance to *Phytophthora* root and stem rot by over-expression of the plant antimicrobial peptide CaAMP1 gene in soybean. BMC Genet 21 (1): 1-10. DOI: 10.1186/s12863-020-00872-0.

O'Gara E, Sangchote S, Fitzgerald L, Wood D, Seng WAC, Guest DI. 2004. Infection biology of *Phytophthora palmivora* Butl. in *Durio zibethinus* L. (durian) and responses induced by phosphonate. In: Drenth A (eds.). Diversity and Management of *Phytophthora* in Southeast Asia. Australian Centre for International Agricultural Research, Canberra.

Poerba YS, Martanti D. 2008. Genetic variability of *Amorphophallus muelleri* Blume in Java based on random amplified polymorphic DNA. Biodiversitas 9 (4): 245-249. DOI: 10.13057/biodiv/d090401.

Prakoso CNY, Retnoningsih A. 2021. Molecular based genetic diversity of Brongkol's superior durian germplasm of Semarang, Indonesia. Biodiversitas 22 (12): 5311-5316. DOI: 10.13057/biodiv/d221211.

Ritmontree S, Kongtragoul P. 2020. Antifungal effect of zinc oxide nanoparticles against disease in durian caused by *Phytophthora palmivora*. Acta Hortic 1312: 423-430. DOI: 10.17660/ActaHortic.2021.1312.61.

Rosmaina, Warino J, Suhaida, Zulfahmi. 2016. Genetic variability and relationship among durian cultivars (*Durio zibethinus* Murr) in the Kampar, Indonesia assessed by RAPD markers. Pak J Biotechnol 13 (2): 87-94.

Ruwaida IP, Supriyadi S, Parjanto P. 2009. Variability analysis of Sukun durian plant (*Durio zibethinus*) based on RAPD marker. Nusantara Biosci 1 (2): 84-91. DOI: 10.13057/nusbiosci/n010206.

Sambrook J, Fritsch ER, Maniatis T. 1989. Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor Laboratory Press, New York.

Santoso PJ, Aryantha, INP, Pancoro A, Suhandono S. 2015. Identification of *Pythium* and *Phytophthora* associated with durian (*Durio* sp.) in Indonesia: Their molecular and morphological characteristics and distribution. Asian J Plant Pathol 9 (2): 59-71. DOI: 10.3923/ajppaj.2015.59.71.

Shamsudin M, Redzuan A, Abidin Z, Zaharah T. 2000. The use of forest durian, *Durio lowianus* as a waiting tree. In: Mohamed ZA, Othman MS, Sapii AT, Mahmood Z, Idris S (eds.). Proc of the 2000 durian Seminar: Towards Stabilizing Production Quality and Markets, 1-3 Ogos 2000. Ipoh, Perak. [Malaysian]

Siew GY, Ng WL, Salleh MF, Tan SW, Ky H, Alitheen NBM, Mohammed NB, Tan SG, Yeap SK. 2018. Assessment of the genetic variation of Malaysian durian varieties using inter-simple sequence repeat markers and chloroplast DNA sequences. Pertanika J Trop Agric Sci 41 (1): 321-332.

Somsri S. 2015. Production, diversity and utilization of durian in Thailand. International Symposium on durian and Other Humid Tropical Fruits 1186: 1-14. DOI: 10.17660/ActaHortic.2017.1186.1.

Sulistiyo CD, Cheng KC, Su'andi HJ, Yuliana M, Hsieh CW, Ismadji S, Angkawijaya AE, Go AW, Hsu HY, Tran-Nguyen PL, Santoso SP. 2022. Removal of hexavalent chromium using durian in the form of rind, cellulose, and activated carbon: Comparison on adsorption performance and economic evaluation. J Clean Prod 380 (1): 382135010. DOI: 10.1016/j.jclepro.2022.135010.

Sundari, Mas'ud A, Wahyudi D, Arumingtyas EL, Hakim L, Azrianingsih R. 2021. Genetic diversity of local durian from Tidore Island based on morphological and moleculer data for tropical fruit conservation in North Maluku. IOP Conf Ser Earth Env Sci 739: 012073. DOI: 10.1088/1755-1315/739/1/012073.

Tan SS. 2022. Diversity of local Indonesian durian (*Durio zibenthimus* Murr.) with the cases of durian orange and buntat ali. Respati Sci J 13 (1): 21-33. DOI: 10.52643/jir.v13i1.2230.

Tantong S, Pringsulaka O, Weerawanich K, Meeprasert A, Rungrotmongkol T, Sarnthima R, Roytrakul S, Sirikantaramas S. 2016. Two novel antimicrobial defensins from rice identified by gene coexpression network analyses. Peptides 84: 7-16. DOI: 10.1016/j.peptides.2016.07.005.

Trimanto. 2012. Characterization and similarity distance of yam (*Dioscorea alata* L.) based on tuber morphology. Buletin Kebun Raya 15 (1): 47-59. [Indonesian]

Truong HTH, Kim JH, Cho MC, Chae SY, Lee HE. 2013. Identification and development of molecular markers linked to Phytophthora root rot resistance in pepper (*Capsicum annuum* L.). Eur J Plant Pathol 135: 289-297. DOI: 10.1007/s10658-012-0085-3.

Vanijajiva O. 2011. Genetic variability among durian (*Durio zibethinus* Murr.) cultivars in the Nonthaburi province, Thailand detected by RAPD analysis. J Agric Sci Technol 7 (4): 1107-1116.

Vawdrey LL, Martin TM, De Faveri J. 2005. A detached leaf bioassay to screen durian cultivars for susceptibility to *Phytophthora palmivora*. Australas Plant Pathol 34: 127-128. DOI: 10.1071/AP05005.

Wambugu PW, Ndjiondjop MN, Henry RJ. 2018. Role of genomics in promoting the utilization of plant genetic resources in genebanks. Brief Funct Genom 17: 198-206. DOI: 10.1093/bfgp/ely014.

Wang J, Yuan J, Yu J, Meng F, Sun P, Li Y, Xi Z. 2019. Recursive paleohexaploidization shaped the durian genome. Plant Physiol 179 (1): 209-219. DOI: 10.1104/pp.18.00921.

Weerawanich K, Webster G, Ma J, Phoolcharoen W, Sirikantaramas S. 2018. Gene expression analysis, subcellular localization, and in planta antimicrobial activity of the rice (*Oryza sativa* L.) defensin 7 and 8. Plant Physiol Biochem 124: 160-166. DOI: 10.1016/j.plaphy.2018.01.011.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18 (22): 6531-6535. DOI: 10.1093/nar/18.22.6531.

Zhou X, Wu H, Pan J, Chen H, Jin B, Yan Z, Rogers KM. 2021. Geographical traceability of South-east Asian durian: A chemometric study using stable isotopes and elemental compositions. J Food Compos Anal 101: 103940. DOI: 10.1016/j.foodchem.2019.126093.