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SNo.: **BBRA/5243**
M/S. Recieved On: **02 January 2023**

Dear Dr. **Susilo Susilo**
Department of Biology Education, Universitas Muhammadiyah Prof. DR. HAMKA, East Jakarta, DKI Jakarta, Indonesia.

(A) Your manuscript **Deciphering the Analysis of the Genetic Diversity of Mangosteen (Garcinia mangostana L.) from Various Regions of Origin in Java, Indonesia**
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Best Wishes,

Editorial Assistant
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Thank you
Regards,

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Pada hari Kamis, 5 Januari 2023 pukul 14:04:26 GMT+7, info@biotech-asia.org <info@biotech-asia.org> menulis:

Dear Dr Susilo Susilo,

Terima kasih telah mengirimkan naskah Anda yang berjudul " **Menguraikan Analisis Keanekaragaman Genetik Manggis (Garcinia mangostana L.) dari Berbagai Daerah Asal di Jawa, Indonesia** ." untuk Biosciences Biotechnology Research Asia. Makalah Anda diberi **Naskah No-BBRA/5243** (Harap sebutkan nomor ini dalam semua korespondensi di masa mendatang)

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Dikirim: 02 Januari 2023 12:19
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Date : 2 January 2023 (mm/dd/yyyy) Signature Susilo.

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Deciphering the Analysis of the Genetic Diversity of Mangosteen (Garcinia mangostana L.) from Various Regions of Origin in Java, Indonesia

Abstract

The genetic improvement of a plant to produce high-yielding varieties depends on genetic variability. It is widely known that the mangosteen plant (*Garcinia mangostana*L.) does not have genetic variability because it has an apomictic reproductive mechanism. Analysis of genetic diversity can be carried out by analysis of similarities and clusters between accessions of plants or with their close relatives of the Garcinia genus. This research was carried out to analyze the genetic diversity of50 mangosteen accessions at mangosteen production centers in four provinces in Java, Indonesia (Banten, West Java, Central Java, and East Java provinces) using RAPD-based primers.RAPD-based amplification revealed a total of 30 amplicons, 28 as polymorphicand two as monomorphic, with an average percentage of polymorphism of 72.22%.The results of PCR-RAPD visualization produce bands measuring 300bp-1500 bp. The dendrogram shows the grouping of all types of *G. mangostana*L.in general. The primer used can amplify the samples properly. Genetic variation occurs with coefficient values ranging from 25% to 100%. This study also explains the pattern of the spread of *G. mangostana*L. in Java for the first time. These results can be used as a reference for conserving*G. mangostana*L. and future breeding.

Keywords:*Garcinia mangostana*L.; Genetic distance; Mangosteen; Moleculardiversity; RAPD.

Deciphering the Analysis of the Genetic Diversity of Mangosteen (*Garcinia mangostana* L.) from Various Regions of Origin in Java, Indonesia

Introduction

The mangosteen plant (*Garcinia mangostana* L.) is one of the plants that produce tasty, delicious fruit and has high vitamins and xanthones. Mangosteenis well-known in the health sector as an anti-inflammatory, antibacterial, and antioxidant drug [1,2]. Mangosteen comes from Southeast Asia, especially Malaysia, Thailand, the Philippines, and Indonesia[2,3]. *G.mangostana* L.is a fruit commodity withpromising economic value to be developed. The mangosteen plant naturally breeds by breeding asexual apomictic[4]. The asexual way of breeding *G.mangostana* L.results in narrow genetic diversity and inheritance of *G.mangostana* L.traits through its female elders [5]. In reality,mangosteenhas variations in both phenotype and genetically [6]. Previous publications have described the presence of morphological variations in mangosteen in North Sumatra, Indonesia (Syahputra et al., 2021), and the Philippines [7].Moreover, genetic variations of some mangosteen accessions have been detected with microsatellite markers [8–10]. Indonesia has a high source of genetic diversity of *G.mangostana* L.[11–13]. The island of Java is the largest *G.mangostana*L.production center in Indonesia. Therefore, exploration, identification, and characterization of the diversity of *G.mangostana* L.are available in Indonesia.

Efforts to improve the genetics of *G.mangostana* L.are directed at obtaining high-yielding varieties. Superior approaches are existedto accelerate *G.mangostana* L.growth through the improved root system, fast production, high productivity, and good fruit quality[14]. Efforts to improve the approaches of *G.mangostana* L.have been carried out, including splicing techniques between mangosteen and its close relatives in other Garcinia genera, which aims to shorten the juvenile period[15]. Efforts to produce superior clones have also been made with gamma-ray irradiation [7]. A plant's genetic repair program to produce high-yielding varieties relies heavily on the availability of sources of genetic diversity.

The approach of the study of the genetic diversity of plants can be carried out by analysis of heredity and crossing. However, the mangosteen plant is a plant that has a long juvenile period, so it is less effective and efficient if performed by genetic and crossing analysis. The molecular analysis became an alternative to studying the*G.mangostana*L.garden diversity. Molecular markings with Random Amplified Polymorphic DNA (RAPD) are one of the proven molecular analyses in genetic diversity studies. The RAPD marker can detect the genetic diversity of plants with unknown genome sequences, such as the *G.mangostana* L.genitive in Java. This study aims to detect genetic variability in 50 accessions of *G.mangostana* L.on Java Island by using RAPD molecular marker.

Methods

Plant material collection

The mangosteen leaves (*Garcinia mangostana* L.) were taken from mangosteen production centers in six areas on the island of Java. Sample collection was carried out from February to April 2022.Samples were collected from trees growing in different locations representing four provinces on Jawan Island, Indonesia (Table 1). The total samples used were 40 accessions from Bogor, Sukabumi, Pandeglang, Purworejo, Banyuwangi, and ten from other genera, namely *G.*

malaccensis from Taman BuahMekarsari, Bogor (TBM) and *G. celebica* from the Bogor Botanical Garden (KRB). The authenticated specimens were deposited at Herbarium Bogoriensis, BRIN, Indonesia, under assigned voucher specimen numbers. Fresh leaf samples were selected, put in a plastic bag, and stored in a Cooling Boxforanalysisin the Center for Cultivation and Development of Biotechnology and Genetic Resources (BPTP), Bogor, Indonesia.

Table 1. Sample collection of *G.mangostana* L. from four provinces on Javaisland.

Provincial	Regency	CollectionCode	AccessionNumber	Location (latitude and longitude)
West Java	Bogor	BG1	1	6°36'36.1"S
	Bogor	BG2	2	106°46'36.0"E
	Bogor	BG3	3	6°34'41.2"S
	Bogor	BG4	4	106°44'20.2"E
	Bogor	BG5	5	6°34'37.4"S
	Sukabumi	SK1	6	106°44'15.0"E
	Sukabumi	SK2	7	6°33'32.5"S
	Sukabumi	SK3	8	106°36'02.9"E
	Sukabumi	SK4	9	6°34'40.0"S
	Sukabumi	SK5	10	106°45'06.1"E
				6°57'31.2"S
				106°49'19.0"E
				6°58'07.9"S
				106°49'09.1"E
				6°58'10.5"S
				106°49'06.0"E
				6°57'00.0"S
				106°46'51.5"E
				6°56'43.5"S
				106°46'52.6"E
Central Java	Cilacap	PW1	11	7°37'07.6"S
	Cilacap	PW2	12	109°02'29.9"E
	Cilacap	PW3	13	7°35'54.3"S
	Cilacap	PW4	14	109°01'50.2"E
	Cilacap	PW5	15	7°37'34.1"S
	Purworejo	PW6	16	108°59'50.3"E
	Purworejo	PW7	17	7°37'07.4"S
	Purworejo	PW8	18	108°57'44.9"E
	Purworejo	PW9	19	7°30'23.7"S
	Purworejo	PW10	20	109°07'03.9"E
				7°43'25.1"S
				109°59'41.8"E
				7°42'59.5"S
				109°59'46.0"E
				7°42'48.3"S
				110°01'15.1"E
				7°41'50.1"S

				110°00'51.0"E 7°41'43.6"S 110°00'53.1"E
East Java	Banyuwangi Banyuwangi Banyuwangi Banyuwangi Banyuwangi Banyuwangi Banyuwangi Banyuwangi Banyuwangi Banyuwangi	BY1 BY2 BY3 BY4 BY5 BY6 BY7 BY8 BY9 BY10	21 22 23 24 25 26 27 28 29 30	8°13'39.5"S 114°20'58.3"E 8°13'28.0"S 114°21'03.7"E 8°13'28.0"S 114°21'03.7"E 8°13'39.7"S 114°20'53.8"E 8°11'51.5"S 114°21'45.1"E 8°12'04.6"S 114°21'31.2"E 8°12'03.0"S 114°21'17.1"E 8°12'03.0"S 114°21'17.1"E 8°12'10.1"S 114°21'44.6"E 8°12'10.1"S 114°21'44.6"E
Banten	Pandeglang Pandeglang Pandeglang Pandeglang Pandeglang Pandeglang Pandeglang Pandeglang Pandeglang Pandeglang	PD1 PD2 PD3 PD4 PD5 PD6 PD7 PD8 PD9 PD10	31 32 33 34 35 36 37 38 39 40	6°24'23.5"S 106°03'23.6"E 6°24'23.7"S 106°03'01.5"E 6°24'32.9"S 106°02'43.5"E 6°24'32.9"S 106°02'43.5"E 6°29'40.0"S 105°56'05.3"E 6°29'42.6"S 105°55'47.2"E 6°40'08.7"S 105°53'30.1"E 6°40'24.7"S 105°53'30.8"E

				6°41'28.7"S 105°46'04.3"E 6°41'20.1"S 105°45'32.1"E
Collectiongarden	TBM TBM TBM TBM TBM KRB KRB KRB KRB KRB	TB1 TB2 TB3 TB4 TB5 KR1 KR2 KR3 KR4 KR5	41 42 43 44 45 46 47 48 49 50	6°24'52.9"S 106°59'11.7"E 6°24'52.9"S 106°59'11.7"E 6°24'52.9"S 106°59'11.7"E 6°24'52.4"S 106°59'10.4"E 6°24'52.4"S 106°59'10.4"E 6°36'00.2"S 106°48'04.5"E 6°36'00.2"S 106°48'04.5"E 6°36'01.4"S 106°48'00.0"E 6°36'01.4"S 106°48'00.0"E 6°35'44.6"S 106°48'04.7"E

Genomic DNA isolation andextraction

Total genomic DNA was extracted using a slightly modified method[16–18].The leaves of *G.mangostana*L.were cut in 1 cm^2 (without leaf bones) and inserted into a 2 mL Eppendorf tube. The leaves were soaked with liquid nitrogen, then grinded using chopsticks until they became powder. The DNA extraction method used the procedures listed in the Maxwell® RSC Plant DNA Kit byPromega Corporationwith modifications. DNA quality was tested by electrophoresis on a 1% agarose gel in a 1x TAE buffer solution at a voltage of 100 Volts for 60 minutes, then visualized using UV light (Biorad, USA). The DNA quantity was calculated using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA) based on light absorbance at λ 260 nm. The purity of DNA is known through the comparison of the value of light absorbance at λ 260 nm with the value of light absorbance at λ 280 nm (Å260/Å280). Pure DNA has a comparison value ranging from 1.8-2.0.

Polymerase Chain Reaction Amplification

DNA sequence process was carried out using the RAPD technique. Pure DNA from mangosteen leaves was taken and the DNA segments were amplified using a single primer. A total of ten RAPD primers from Operon Tech were used in this study (Table 2). Primers that provide firm and clear amplification tapes and produce polymorphic DNA bands were selected to amplify the DNA of the entire sample. PCR was performed with a volume of 10 µL containing 4 µL of 10 ng DNA, 1 µL of 2.5 pmol primer-F, and 5 µL of KAPA. The PCR reaction was carried out with 12 tubes for one primer by providing a regular annealing temperature range program on the Takara thermocycler machine. PCR conditions are initial denaturation of 96^0C for 5 minutes, denaturation of 94^0C for 45 seconds, annealing of 37^0C for 1 minute, elongation of 72^0C for 1 minute, and termination or final extension of 72^0C for 1 minute. PCR was performed for 35 cycles. Visualization of PCR results was carried out by horizontal electrophoresis with 2% (w/v) agarose gel in 1x TAE buffer at 50 Volts for 60 minutes.The goal is to determine how many different DNA fragments are present in a given sample [18]. The agarose gel was then immersed in Ethidium bromide and sprayed with Cerium (IV) Sulfate slowly to see the DNA bands on the UV-Geldoc (254 nm), then the observational data were generated from disclosing the agarose gel.

Data Analysis

The bands obtained from the RAPD technique were translated into binary data. The parameter observed was the appearance of bands on the agarose gel after UV irradiation. The bands that are present are assigned a value of "1" and "0" if absence. Clear and consistent polymorphic bands are used to create binary matrices for statistical analysis. Each occurrence of the ribbon represented one character. The binary data obtained were used to compile a genetic similarity matrix[19]. Analysis of molecular data similarity using the SIMQUAL procedure in the NTSYS program version 2.01 with the DICE coefficient method following Lamboy (1994). Cluster analysis based on genetic similarity values was carried out to see kinship relationships between accessions using the SAHN-UPGMA method displayed in the form of a tree diagram (dendrogram) through the NTSYS program version 2.01. Analysis of the main components using multivariate analysis on the Minitab program version 14.

Results and Discussion

The results of the total amplification of the DNA genome from 50 accessions of mangosteen from Java Island, Indonesia, on a spectrophotometer produced a PCR product that can be read and suspended so that the results can be analyzed. The results showed that 90 DNA fragments were obtained with 65 (72.22%) polymorphic and 26 (28.89%) monomorphic DNA fragments. Polymorphic bands belong to some sample individuals, while monomorphic bands belong to all sample individuals. The number of polymorphic DNA bands produced determines the diversity of a population since polymorphic DNA bands describe the state of the plant genome.

This study showed that the RAPD primer used had a high level of polymorphism (>50% polymorphic tape). Ten primers produce 3-11 detectable and discussional DNA bands. The highest number of polymorphic bands is found in the OPA-18 primer (Table 1). Observations of amplified DNA band patterns showed different DNA profiles. This difference was due to differences in nucleotide sequences in the four primers used, thus causing the primary attachment along the DNA of the sample genome also to differ.

Table 2. The primaryand number of amplified DNA bands and polymorphism rates at 50 accessions of *G.mangostana*L.

Primer	Sequence 5'-3'	Annealing (°C)	Bands		Qty.
			Polymorphic	Monomorphic	
OPA-16	AGCCAGCGAA	36	5	1	6
OPA-17	GACCGCTTGT	32	6	3	9
OPA-18	AGGTGACCGT	33	10	2	11
SBH-12	ACGCGCATGT	37	3	6	9
SBH-13	GACGCCACAC	36	4	1	5
OPB-1	GTTTCGCTCC	36	9	1	10
OPB-2	TGATCCCTGG	36	7	2	9
OPB-3	CATCCCTGG	36	6	4	10
OPA-4	AATCGGGCTG	36	9	1	10
OPA-5	AGGGGTCTTG	36	6	5	11
Amount			65	26	90
Percentages			72.22%	28.89%	100%

The RAPD of 50 accession fingerprint profiles of *G.mangostana*L. with ten primers selected shows the resulting tape ranges from 3 (SBH-12) to 10 (OPA-18) with the resulting band size ranging from 300 bp to 1,500 bp so that it can discourse (Fig. 1). The number and intensity of DNA bands produced after DNA amplification with PCR depend primarily on how the primer recognizes its complementary DNA sequences on the DNA prints (template DNA) used. DNA amplification results using the above ten random primers do not always obtain bands of the same intensity. The intensity of the amplified DNA bands in each primer is strongly influenced by the purity and concentration of DNA molds [20]. DNA prints containing polysaccharides, phenolic compounds, and too small concentrations of molded DNA often result in dim or obscure amplification of DNA bands [21].

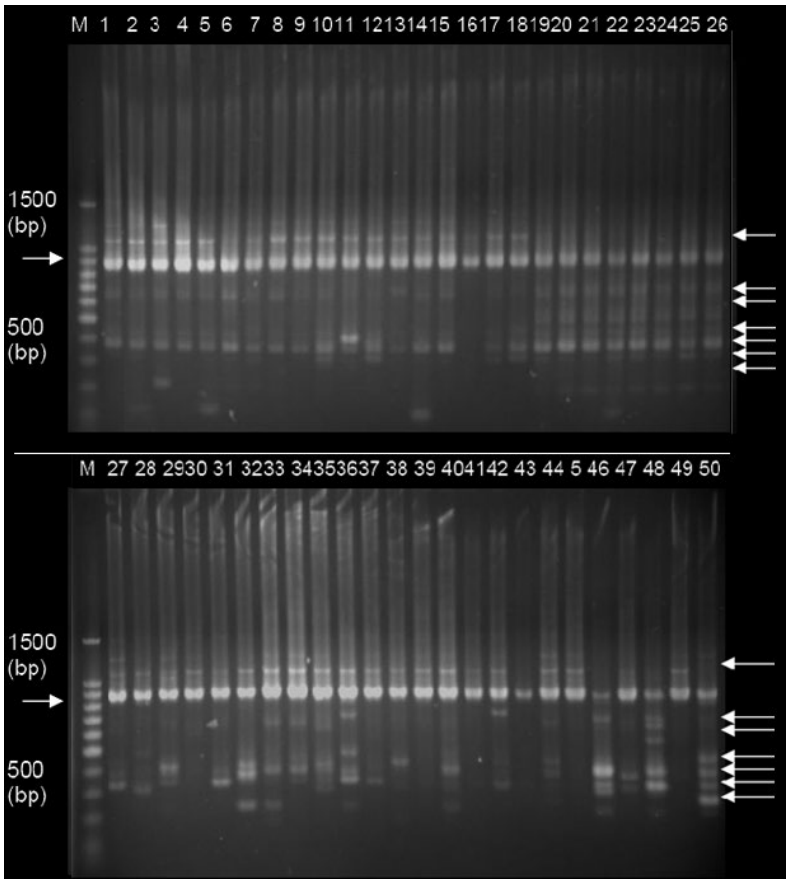

Fig. 1. PCR results of 50 samples of *G. mangostana* L. with OPA-18 primer. Description: M=DNA marker (Promega); arrow ⟶ = monomorphic, arrow ⟵ = polymorphic.

The distribution of primary pasting sites in DNA molds and the competition of primary attachment sites to DNA molds cause one fragment to be amplified in large quantities and the other fragments to be few [22]. The amplification process may be initiated in some places, but only a few sets can be detected as bands after Amplification [23]. The primary selection in RAPD analysis affects the resulting band polymorphism [24]. Each primer has its attachment site, resulting in the polymorphic DNA bands produced by each primer becoming different, both in the size of the number of base pairs and the number of DNA bands[18,23].

Cluster analysis showed the separation of 50 accessions of *G.mangostana*L.intotwo clustered clusters, namely, cluster A with a similarity coefficient of 0.87 and cluster B with a similarity coefficient of 0.82 (Fig.2). Cluster A consisted of two sub-clusters consisting of H (accession nos. 9 and 10 respectively from Sukabumi) and other sub-clusters that group into several small groups (C, D, E, F, and G). This was due to the group having the smallest or most different similarity value compared to the accession in sub-cluster H. Small similarity values can be possible because the accessions in that group were different or not found in groups in sub-cluster H. The absence of this locus can occur because the locus has been lost or does not exist, so the marker/primary used cannot recognize the locus contained in the group.

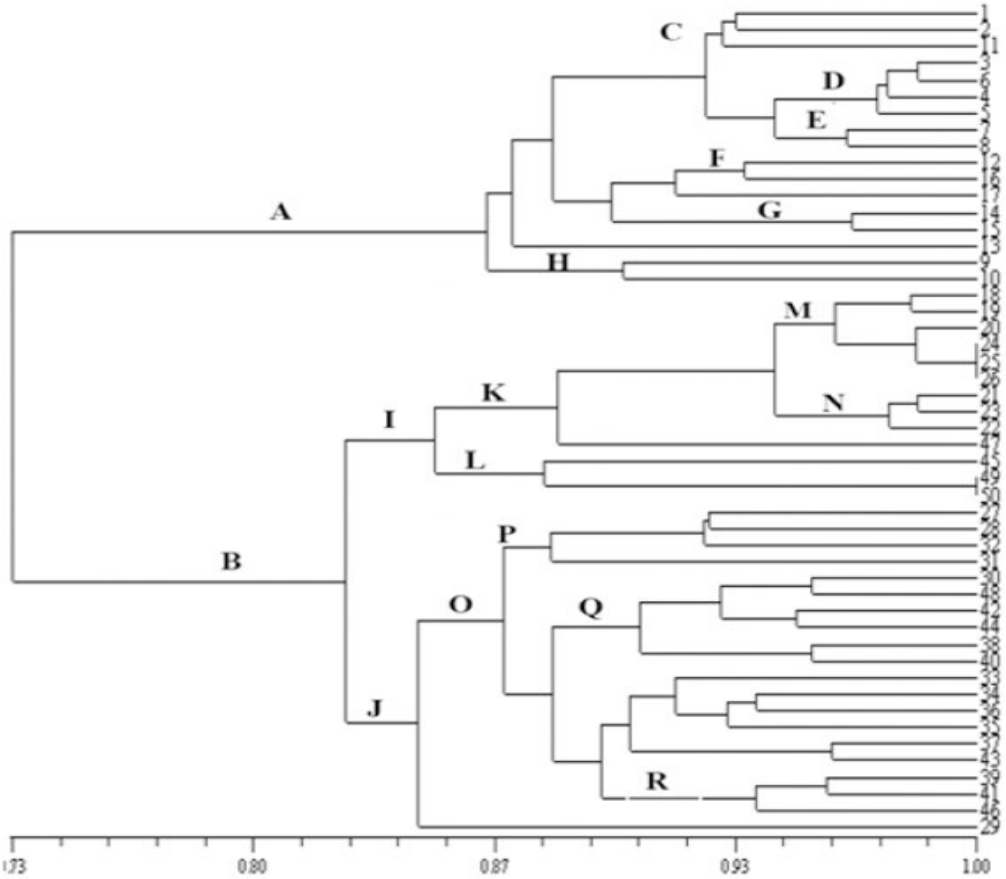

Fig.2. Dendrogram illustrating genetic relationship among 50 accessions of *G. mangostana*L. using UPGMA clustering.

Cluster B consisted of two sub-clusters, namely sub-cluster I, which was further divided into two small groups, L and K, which group again by population. The exciting thing is that the *G.mangostana*L.group from Banyuwangi is closer in kinship tothe *G.mangostana*L.from Bogor. Sub-cluster J is dominated by groups from the Pandeglang and TBM areas, although few are from the Banyuwangi area (accession number 29). An interesting phenomenon from the results of this cluster analysis is the grouping of individuals from different populations into one cluster. This indicates the presence of genetic diversity in *G.mangostana*L., which may be due to the presence of genetic recombination. This gives rise to the conjecture that the apomictic seeds of *G.mangostana*L.are not apomictic obligates.

Cluster analysis shows the separation of samples into clusters that cluster randomly and not based on clones. This was decided because the RAPD profile in the score was based on the similarity of DNA band sizes. Thus, if the clones analyzed group into one cluster, it indicates a high degree of similarity between the DNA loci. Molecular markers have become an effective way to obtain information on the existence of genetic diversity and to study population structures [20]. The diversity of phenotypes arises from the interaction between genetic and environmental factors. Diversity at the genetic level of *G.mangostana*L.as an apomixis plant in different growing environments on the island of Java needs to be identified. The characterization of germplasm is essential to utilize the potential for plant genetic diversity [21].

The RAPD marker used in this study has proven effective in providing information on the existence of genetic diversity of *G.mangostana*L. in Java.Although the results of DNA quality and quantity tests from this study showed less than optimal results. However, this research can be well amplified. Polymorphic DNA bands can be eluted, cloned, and sequenced to determine sequence uniqueness. Functional assignment requires interdisciplinary approaches such as functional genomics, bioinformatics, etc[20]. The emergence of mangosteen genetic clusters or diversity can be influenced by climatic conditions and soil conditions that are different in each location[25].

Although research on the genetic diversity of *G.mangostana*L. in Indonesia has been reported [26–28]. However, the results of this study complement the *G.mangostana*L.molecular data even better. These results can be used to add information to other researchers who focus on the development of *G.mangostana*L.cultivars. The presence of genetic variability and polymorphism among mangosteen accessions in Java indicates that the genetic richness of the *G.mangostana*L.in Indonesia is very high. Thus, extensive efforts need to be increased.

Conclusion

Molecular markers are an effective way to obtain information on the existence of genetic diversity. Interpretation of DNA banding patterns from 50 accessions of *G.mangostana* L. from five provinces in Java showed that there were various genetic variability and polymorphisms. The polymorphic bands of *G. mangostana* L. accessions obtained in this study can then be used to describe the nature of polymorphism between cultivars of *G. mangostana* L. in Indonesia and the world. Therefore, further investigation is needed for the possibility of developing molecular cultivation to find quality *G. mangostana* L. seeds.

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