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Genetic diversity of mangoes (*Mangifera indica* L.) and its relatives in Seliu Island, Belitung District, Indonesia based on Inter-Simple Sequence Repeat markers

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Abstract. *Resmi DDC, Chikmawati T, Djuita NR, Fendiyanto MH, Rahmawati D. 2024. Genetic diversity of mango* (Mangifera indica *L.*) *and its relatives in Seliu Island, Belitung District, Indonesia based on Inter-Simple Sequence Repeat markers. Biodiversitas 25: 4253-4264.* Seliu Island is known as a mango island, but information on their identity, taxonomic status, and abundance of mango variety on this island has yet to be recorded. This study aimed to identify the genetic diversity of mangoes on Seliu Island. Fifteen Inter-Simple Sequence Repeat (ISSR) primers were used to profile the genetic diversity of 49 mango accessions from Seliu Island. The selected primers produced 193 polymorphic bands out of 222 (86.17%). All mango accessions on Seliu Island were identified and can be classified into eight species: *Mangifera indica*, *Mangifera laurina*, *Mangifera zeylanica*, *Mangifera magnifica*, *Mangifera quadrifida*, *Mangifera caesia*, *Mangifera odorata*, and *Mangifera foetida*. The genetic diversity among species was high, with a Shannon's information index of 0.334 and expected heterozygosity of 0.210. The unweighted pair group method with arithmetic average (UPGMA) dendrogram was created using a simple matching method, and all accessions were grouped into two main clusters according to their subgenus, *Limus* (Marchand) Kosterm and *Mangifera*. All accessions are also grouped according to their species. This study shows that the accessions from Seliu Island and West Java tend to cluster based on geographical origin. The study of mango genetic diversity using ISSR markers provided valuable information on the genetic relationships and variability among local mango cultivars on Seliu Island. This information can be used for breeding programs, conservation, and developing mango varieties.

Keywords: Local cultivar, molecular identification, polymorphism, small island

Abbreviations: Na: Number of observed alleles; Ne: Number of effective alleles; I: Shannon information index; He: Expected heterozygosity; PIC: Polymorphic information content; PPB: Percentage of polymorphic band; ISSR: Inter-simple sequence repeat; PCA: Principal component analysis

INTRODUCTION

Mango (*Mangifera indica* L.) is a member of the Anacardiaceae family, the world's fifth most productive fruit crop (FAO 2021). Mango is not native to Indonesia. This species was introduced from India (Mukherjee and Litz 2009), but it is a major agricultural crop in Indonesia (Sulistyowati et al. 2015). The genetic diversity analysis of *Mangifera*, particularly in Indonesia, has been extensively studied using various molecular markers (Anggraheni and Mulyaningsih 2021; Fitmawati et al. 2010, 2017; Mursyidin 2023). A study that analyzed the diversity of Indonesian mango cultivars based on morphological and Random Amplified Polymorphic DNA (RAPD) markers successfully identified 82 cultivars based on morphology and 76 based on DNA (Fitmawati et al. 2010). The study also reported high mango genetic diversity in Indonesia (Fitmawati et al. 2010). Other genetic diversity studies conducted based on RAPD, Inter Simple Sequence Repeat (ISSR), and microsatellites indicated that mangoes have a high level of genetic diversity at both interspecies and intraspecies levels (Archak et al. 2014; Mansour et al. 2014; Hussein et al. 2023). Mango varieties are frequently designated with distinctive names based on their physical form, taste, aroma, place of origin, effect, color, or specific cultural symbols, particularly in various regions of Indonesia (Gajanana et al. 2015; Rahman 2020).

Previous studies have identified genetic diversity in mango varieties across several locations in Indonesia, including Java Island (Fitmawati et al. 2009; Anggraheni and Mulyaningsih 2021), West and South Borneo (Kostermans and Bompard 1993), and Sulawesi (Fitmawati 2010). Since 2012, research on the diversity of *Mangifera* has been conducted in many provinces in mainland Sumatra, including Aceh, North Sumatra, West Sumatra, Riau, Jambi, Bengkulu, South Sumatra, and Lampung (Fitmawati and Hayati 2018). Most studies were conducted in the big islands of Indonesia, Jawa, Borneo, and Sumatra (Fitmawati et al.

2017; Hidayat et al. 2021; Mursyidin 2023). However, the lack of information on mango germplasm in small islands, particularly on Seliu Island, known as "the mango paradise", represents a significant issue. Seliu Island is located within the Membalong Sub-district in the southwestern region of Belitung District in Bangka Belitung Province. Visitors to Seliu Island will find themselves surrounded by a mango tree-lined road. The mango trees on Seliu Island are numerous, yet the identity, taxonomic status, and number of these trees have never been reported. This deficiency of data highlights the need for more comprehensive research and awareness in this area. The objective of the study related to the genetic diversity of mangoes in Seliu Island was to ensure the identity of each local mango cultivar. Definitive identity is crucial for the future development of each accession, as well as for the efficient management of mango germplasm and as a genetic resource for mango breeding programs (Sherman et al. 2015) in small islands.

Mango breeders and consumers typically utilize agronomic characteristics to compare mango cultivars (Puspita et al. 2021). However, the availability of agronomic characters is influenced by environmental factors. It is available during the year, imposing limitations on using the data to characterize genetic diversity. Therefore, molecular identification is needed to overcome this problem (Sutrisno 2018). Several DNA markers are available to estimate genetic diversity in fruit crops (Nwosisi et al. 2019). The ISSR marker is notable for its high level of polymorphism consistency, qualifying it as a marker that can be utilized in the analysis of genetic profiles (Ariffin et al. 2015). ISSR markers use microsatellite sequences as primers to produce highly polymorphic multilocus markers widely used in genetic diversity, phylogeny, and evolution studies (Gemmill and Grierson 2021). In previous study, the molecular relationships of 15 mango cultivars from Pakistan were analyzed based on ISSR markers (Toili et al. 2017). Furthermore, the genetic diversity of several Indian mango cultivars was identified using ISSR and SCoT markers, along with other genetic markers (Jena and Chand 2021). Another study also used SCoT and ISSR data, and showed that the four new mango types, called 'Aya,' 'Kasturi,' 'Maya', and 'Omer,' have different genetic profiles and represent novel genetic resources. These can help to develop new mango varieties in Egypt. The study also showed that SCoT and ISSR markers can be used to identify different mango genotypes (Ghounim et al. 2022).

The utility of ISSR in identifying the genetic diversity of an organism has been widely recognized, with applications spanning the identification of plant cultivars, medicinal plants, and invasive plants; taxonomic identification of interspecies and intraspecies; genetic mapping; and the assessment of genetic variation in plants and populations, both in-situ and ex-situ, for conservation and restoration management (Wu et al. 2011). ISSR, with its proven safety, efficiency, and cost-effectiveness, can provide population genetic structure with adequate resolution compared to DNA barcoding by involving more ISSR primers in an analysis (Kumar et al. 2016). ISSR is a reliable and robust method to infer plant genetic diversity (Gemmill and Grierson 2021). Additionally, these markers can be utilized to distinguish cultivars and validate mango genotypes (Uddin et al. 2014), thereby identifying differences in mango varieties on Seliu Island. This study aims to identify, classify, and describe the genetic diversity of mango on Seliu Island based on molecular data using ISSR markers.

MATERIALS AND METHODS

Study area

The research project was conducted from March 2023 to March 2024. Plant samples were collected on Seliu Island, Bangka Belitung Islands, Indonesia (Figure 1). Molecular data were analyzed at the Biotechnology Laboratory, SEAMEO Biotrop, and the Plant Physiology and Genetics Research Laboratory, Department of Biology, Institut Pertanian Bogor.

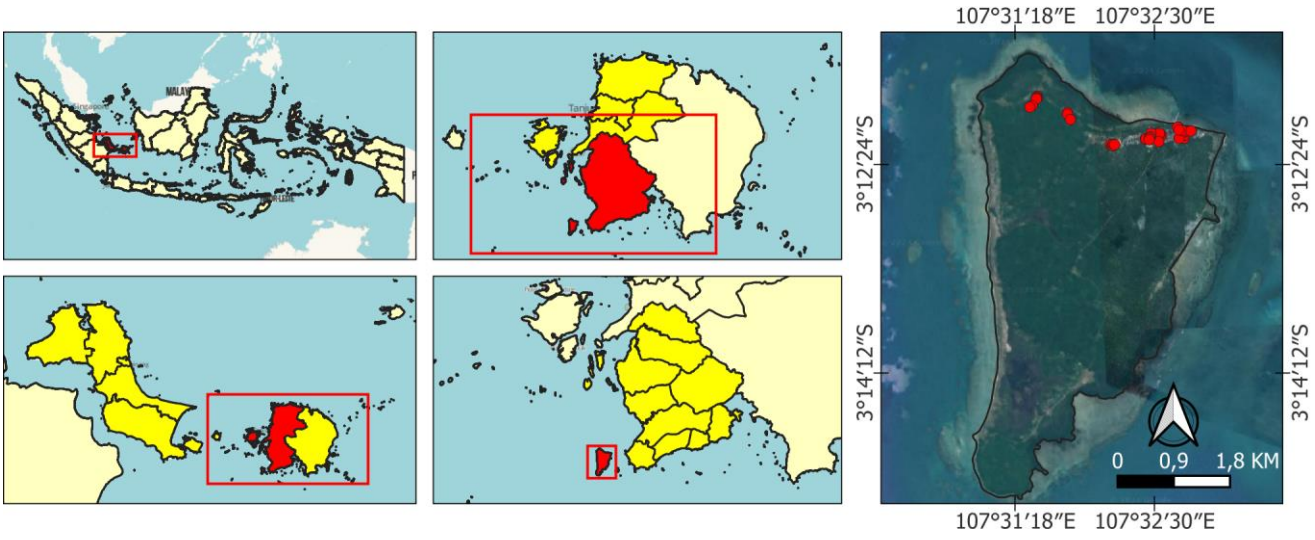

Figure 1. Location of Seliu Island, Membalong, Belitung District (3°12'09.7"S 107°32'37.8"E), Bangka Belitung Islands, Indonesia, indicating the sampling sites of mangoes (*Mangifera indica* L.) and its relatives

Procedures

Mango sample collection

Forty-nine mango accessions were collected from Seliu Island, while 13 were collected from Majalengka and Bogor, West Java, for comparative purposes (Table 1). The sample locations were determined using the roaming method, a sampling method designed to search for a specific sample at specific locations (Rugayah et al. 2004). The technique was carried out by walking around the observation area, and then the plant discoveries during the observation were recorded. The observation area included people's home yards and gardens. Each accession with similar agronomic characteristics (fruit shape, size, and color) was considered a replicate derived from a different individual. Leaf samples were collected, stored in ziplock plastic bags, treated with silica gel, and labeled with the accession number, location, cultivar name, and replicate number. Sampling locations were marked using a Global Positioning System (GPS).

Species identification

Each mango accession collected from Seliu Island was identified according to the local names of each species in The Mangoes: Their Botany, Nomenclature, Horticulture, and Utilization (Kostermans and Bompard 1993) and *Mangifera* of Sumatra contains information about *Mangifera* based on morphological, anatomical and molecular identification (Fitmawati and Hayati 2018).

Molecular analysis

DNA isolation

DNA was isolated using the Geneaid Plant Genomic DNA Mini Kit. The quantity of the isolated DNA was tested using the nanodrop procedure, and the quality of the isolated DNA was tested through an electrophoresis procedure in 1% agarose gel containing 3 µL FloroSafe DNA Stain, which was carried out in 1x Tris Acetate-EDTA (TAE) for 45 minutes at 100V. The electrophoresis results were observed using Kodak Gel Logic 200. The digital images of the agarose gel were obtained using Kodak 1D 3.6 Image Analysis software.

DNA amplification

Before the initiation of the amplification process, a selection of 22 ISSR primers was conducted on eight samples. DNA was amplified using 22 ISSR primers (Table 2), with only 15 producing polymorphic bands. Furthermore, DNA amplification from all mango accessions was conducted using the 15 ISSR primers following the procedure developed for mango (Juliantari et al. 2021). The DNA was amplified in a 20-µL PCR mixture, which included 2 µL of DNA, 2 µL of ISSR primers, 10 µL of DreamTaq™ Hot Start Green PCR Master Mix, and 6 µL of nuclease-free water.

Table 1. Collection of local mango cultivars in Seliu Island and West Java

Seliu Island				West Java (Majalengka and Bogor)	
Accession number	**Local cultivar name**	**Accession number**	**Local cultivar name**	**Accession number**	**Local cultivar name**
DC006	Asam Bacang	DC005 - U2	Mangga Indramayu	DC025 - U1	Mangga Cengkir
DC019	Asam Burut	DC008 - U1	Mangga Manalagi	DC025 - U2	Mangga Cengkir
DC003	Asam Kumbang	DC008 - U2	Mangga Manalagi	DC029 - U1	Mangga Arumanis
DC004 - U1*	Asam Limus	DC008 - U3	Mangga Manalagi	DC029 - U2	Mangga Arumanis
DC004 - U2**	Asam Limus	DC009 - U1	Mangga Udang	DC027 - U1	Mangga Manalagi
DC015	Kemang	DC009 - U2	Mangga Udang	DC027 - U2	Mangga Manalagi
DC018	Kuini Cabul	DC009 - U3	Mangga Udang	DC030 - U1	Mangga Gedong Apel
DC016	Kuini Lipar	DC010 - U1	Mangga Telor	DC030 - U2	Mangga Gedong Apel
DC017	Mangga A	DC010 - U2	Mangga Telor	DC031 - U1	Mangga Gedong Gincu
DC022 - U1	Mangga Apel	DC002 - U1	Pelam Dade Punai	DC031 - U2	Mangga Gedong Gincu
DC022 - U2	Mangga Apel	DC002 - U2	Pelam Dade Punai	DC035	Mangga Gedong
DC022 - U3***	Mangga Apel	DC014 - U1	Pelam Dimdim	DC040	Kemang
DC007 - U1	Mangga Arumanis	DC014 - U2	Pelam Dimdim	DC038	Limus
DC007 - U2	Mangga Arumanis	DC014 - U3	Pelam Dimdim		
DC007 - U3	Mangga Arumanis	DC013 - U1	Pelam Dudol		
DC020 - U1	Mangga Betawi	DC013 - U2	Pelam Dudol		
DC020 - U2	Mangga Betawi	DC013 - U3	Pelam Dudol		
DC001 - U1	Mangga Damar	DC011 - U1	Pelam Jawe		
DC001 - U2	Mangga Damar	DC011 - U2	Pelam Jawe		
DC001 - U3	Mangga Damar	DC011 - U3	Pelam Jawe		
DC023 - U1	Mangga Gedong Gincu	DC012 - U1	Pelam Panjang		
DC023 - U2	Mangga Gedong Gincu	DC012 - U3	Pelam Panjang		
DC023 - U3	Mangga Gedong Gincu	DC021 - U1	Pelam Pao		
DC005 - U1	Mangga Indramayu	DC024	Pelam Sabot		

Note: *U1: 1st repetition; **U2: 2nd repetition; ***U3: 3rd repetition

Table 2. Profile of 15 selected ISSR primers

ISSR primer	Sequence (5'-3') of primer	Primer length	Annealing temperature (°C)	Amplicon band size (bp)		Total band	Polymorphic band	Percentage of polymorphic band (%)
				Min	Max			
UBC-808[3]	$(AG)_8C$	17	54.2	226	1382	15	13	86,67
UBC-826[3]	$(AC)_8C$	17	52	339	1895	20	18	90,00
UBC-873[3]	$(GACA)_4$	16	50.2	356	2240	19	18	94,74
UBC-876[3]	$(GATA)_2(GACA)_2$	16	45	380	1561	17	16	94,12
UBC-881[3]	$GGG(TGGGG)_2TG$	15	59	526	1782	14	12	85,71
UBC-886[3]	$VDV(CT)_7$	17	54	259	1164	14	12	85,71
UBC-841[3][1]	$(GA)_8YC$	18	48	230	1411	17	16	94,12
UBC-811[3][1]	$(GA)_8C$	17	53	250	1528	12	9	75,00
UBC-810[1]	$(GA)_8T$	17	46.5	388	1983	16	14	87,50
UBC-825[1]	$(AC)_8T$	17	52	293	1976	16	14	87,50
UBC-880[1]	$(GGAGA)_3$	15	48	310	1149	12	10	83,33
HB10[2]	$(GA)_6CC$	14	44	244	1488	13	10	76,92
HB-19B1[2]	$(GT)_6CC$	14	50	192	1706	13	11	84,62
HB-12[2]	$(CAC)_3GC$	11	37	412	1500	12	10	83,33
HB-13[2]	$(GAG)_3C$	10	38	148	1418	12	10	83,33
		Total				222	193	
		Mean				15	13	86,17

Note: [a]Ariffin et al. (2015), [b]Ho and Tu (2019), [c]Ghounim et al. (2022)

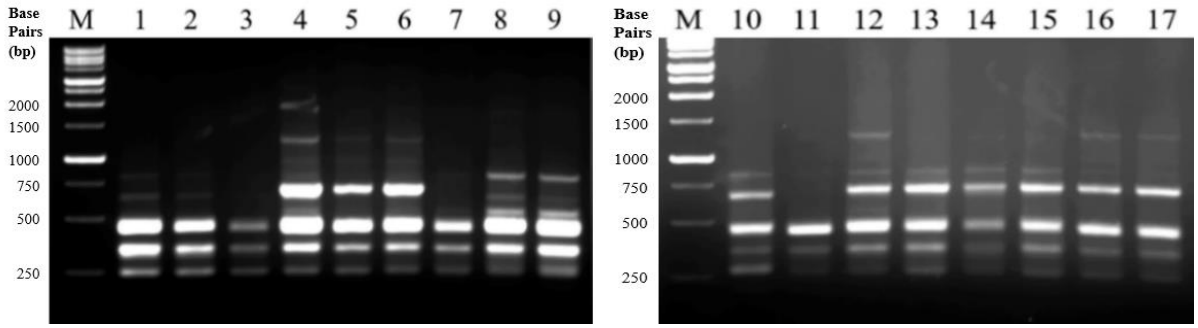

Figure 2. UBC-808 primer polymorphic bands; M: 1 Kb DNA Ladder, 1-17: sample

Visualization

The DNA amplification results and 1 kb DNA ladder were electrophoresed at 100 V for 65 minutes using a 1% agarose gel in 1x TAE buffer, adding 3 µL FloroSafe DNA Stain. The resulting banding pattern was observed and documented using the Kodak Gel Logic 200 Imaging System.

Data analysis

The documentation of DNA banding patterns was analyzed using CLIQS 1D, which is used to create binary data. This data was comprised of 0 (zero) data for no bands and unclear bands (Pratama et al. 2022) and 1 (one) for clear bands at the same position (Nurhasanah et al. 2023). Genetic analysis was performed, including the number of observed alleles (Na), number of effective alleles (Ne), Shannon information index (I), expected heterozygosity (He), and Polymorphic Information Content (PIC) was performed using the GenAlex 6.501 program. Nei's genetic distance among species was calculated using the population genetic analysis software version 1.32 (PopGene32). Cluster analysis was then conducted based on a binary data matrix, with the simple matching coefficient calculated and a dendrogram constructed using the unweighted pair group method with arithmetic average (UPGMA) through the NTSys-PC 2.1.1a program. Principal Component Analysis (PCA) using the Paleontological Statistics (PAST) program was used to analyze the grouping pattern of mango accessions based on band similarity.

RESULTS AND DISCUSSION

The study of genetic diversity is a scientific basis in plant breeding programs to effectively optimize the selection of parents so that they can reproduce in specific agroecological conditions and situations (Bohra et al. 2022). Diversity analysis at the molecular level using PCR-based markers is an efficient and rapid method to identify relationships and differences among genotypes (Amiteye 2021). As many as 15 out of 22 ISSR primers were selected as primers capable of generating polymorphic banding patterns (Figure 2) and subsequently used to calculate the genetic diversity of mangoes on Seliu Island. Amplification of mango DNA using 15 ISSR primers successfully identified 222 alleles, of which 193 (86.17%) were polymorphic (Table 2). Amplicons range in size from 148 to 2240 bp. Two primers yielded a high number of observational and polymorphic bands (PB = 18), namely UBC-826 ($(AC)_8C$) and UBC-873 ($(GACA)_4$) (Table 2).

Genetic diversity analysis

In Seliu Island, out of 49 mango accessions, 24 local cultivars were identified and can be classified into eight species, namely *M. indica* (18 accessions), *Mangifera laurina* (20 accessions), *Mangifera zeylanica* (3 accessions), *Mangifera magnifica* (1 accession), *Mangifera quadrifida* (1 accession), *Mangifera caesia* (1 accession), *Mangifera odorata* (2 accessions), and *Mangifera foetida* (3 accessions). The genetic diversity analysis of 49 *Mangifera* accessions was calculated based on the number of observed alleles (Na), number of effective alleles (Ne), Shannon information index (I), expected heterozygosity (He), and polymorphic information content (PIC) (Table 3). The Na value for each ISSR primer ranged from 1.583 to 1.947, averaging 1.783. The maximum Ne value was found in primer HB10 (Ne=1.502), and the minimum number of effective alleles was found in primer UBC-811 (Ne=1.169) with an average of 1.334.

Shannon information index values ranged from 0.218 to 0.405, with the highest value in primer HB10 and the lowest value in primer UBC-811, with an average value of 0.334. The highest expected heterozygosity value was found in primer HB10 (He=0.276), and the lowest value was found in primer UBC-811 (He=0.124) with an average value of 0.210. The PIC values for the 15 evaluated ISSR primers were relatively high for 49 *Mangifera* accessions, ranging from 0.155 (HB-13) to 0.314 (UBC-826), with an average value of 0.259 (Table 3). Genetic diversity analysis of 18 *M. indica* accessions was also carried out. The highest PIC value of 0.281 for primer HB-19B1 and the lowest value is 0.119 for primer UBC-880, with an average of 0.192 were obtained for 18 accessions of *M. indica* in Seliu Island (Table 4), and has lower values when compared to the value of *Mangifera* genetic diversity (Table 5).

Table 3. Parameters of the genetic diversity among the 15 ISSR primers for 49 *Mangifera* accessions

Primer	Na[1]	Ne[2]	I[3]	He[4]	PIC[5]	PPB[6] (%)
UBC-808[(a)]	1.800	1.396	0.376	0.245	0.271	86.67
UBC-826[(a)]	1.850	1.397	0.368	0.237	0.314	90.00
UBC-873[(a)]	1.947	1.434	0.381	0.250	0.271	94.74
UBC-876[(a)]	1.941	1.367	0.332	0.215	0.203	94.12
UBC-881[(a)]	1.786	1.219	0.278	0.160	0.256	85.71
UBC-886[(a)]	1.786	1.351	0.350	0.222	0.280	85.71
UBC-841[(a)]	1.882	1.371	0.374	0.236	0.286	94.12
UBC-811[(a)]	1.583	1.169	0.218	0.124	0.195	75.00
UBC-810[(b)]	1.813	1.293	0.328	0.200	0.282	87.50
UBC-825[(b)]	1.813	1.340	0.362	0.226	0.306	87.50
UBC-880[(b)]	1.750	1.269	0.299	0.181	0.253	83.33
HB10[(c)]	1.615	1.502	0.405	0.276	0.279	76.92
HB-19B1[(c)]	1.769	1.275	0.297	0.180	0.251	84.62
HB-12[(c)]	1.750	1.330	0.332	0.208	0.277	83.33
HB-13[(c)]	1.667	1.294	0.311	0.194	0.155	83.33
Mean	1.783	1.334	0.334	0.210	0.259	86.17

Note: [a]Ariffin et al. (2015), [b]Ho and Tu (2019), [c]Ghounim et al. (2022). [1]Na: Number of observed alleles; [2]Ne: Number of effective alleles; [3]I: Shannon information index; [4]He: Expected heterozygosity; [5]PIC: Polymorphic Information Content; [6]PPB: Percentage of Polymorphic Band

The total genetic diversity (H_T) in *Mangifera* showed 0.256, while the coefficient of genetic differentiation (G_{ST}) revealed 0.746 (Table 6). It indicated that 74.6% of the total genetic variation of *Mangifera* in Seliu Island occurs among the species, and the remaining 25.4% occurs within the species. Nei's genetic distance displayed a correlation with varietal differences. *M. caesia* and *M. zeylanica* have the highest value of the genetic distance (0.429), while *M. indica* and *M. laurina* show the lowest value (0.068) (Table 7). Species that exhibit a greater distance from the group may be attributed to genetic variation resulting from differences in morphological or genetic characteristics of the elders (Li et al. 2018; Juliantari et al. 2021).

Table 4. Parameters of the genetic diversity among the 15 ISSR primers for 18 *Mangifera indica* accessions

Primer	Na[1]	Ne[2]	I[3]	He[4]	PIC[5]	PPB[6] (%)
UBC-808[(a)]	1.200	1.228	0.220	0.142	0.193	46.67
UBC-826[(a)]	1.400	1.345	0.300	0.199	0.250	65.00
UBC-873[(a)]	1.316	1.380	0.315	0.212	0.238	63.16
UBC-876[(a)]	1.118	1.317	0.262	0.177	0.161	52.94
UBC-881[(a)]	1.643	1.209	0.256	0.150	0.239	78.57
UBC-886[(a)]	1.214	1.222	0.214	0.137	0.188	50.00
UBC-841[(a)]	1.412	1.405	0.353	0.236	0.208	70.59
UBC-811[(a)]	1.083	1.110	0.141	0.079	0.123	50.00
UBC-810[(b)]	1.313	1.211	0.224	0.137	0.159	62.50
UBC-825[(b)]	1.375	1.228	0.239	0.148	0.182	62.50
UBC-880[(b)]	1.083	1.145	0.169	0.102	0.119	50.00
HB10[(c)]	1.462	1.427	0.359	0.243	0.190	69.23
HB-19B1[(c)]	1.769	1.339	0.340	0.214	0.281	84.62
HB-12[(c)]	1.250	1.305	0.268	0.177	0.206	58.33
HB-13[(c)]	1.500	1.347	0.339	0.219	0.149	75.00
Mean	1.342	1.281	0.267	0.172	0.192	62.61

Note: [a]Ariffin et al. (2015), [b]Ho and Tu (2019), [c]Ghounim et al. (2022). [1]Na: Number of observed alleles; [2]Ne: Number of effective alleles; [3]I: Shannon information index; [4]He: Expected heterozygosity; [5]PIC: Polymorphic Information Content; [6]PPB: Percentage of Polymorphic Band

Table 5. Average genetic diversity parameters of *Mangifera* and *Mangifera indica*

Population	Parameter ($\bar{x}$)					
	Na[1]	Ne[2]	I[3]	He[4]	PIC[5]	PPB[6] (%)
Mangifera	1.783	1.334	0.334	0.210	0.259	86.17
Mangifera indica	1.342	1.281	0.267	0.172	0.192	62.61

Note: [1]Na: Number of observed alleles; [2]Ne: Number of effective alleles; [3]I: Shannon information index; [4]He: Expected heterozygosity; [5]PIC: Polymorphic Information Content; [6]PPB: Percentage of Polymorphic Band

Table 6. Total genetic diversity of *Mangifera* in Seliu Island

Sample numbers	H_T[1]	H_S[2]	G_{ST}[3]
49	0.2564	0.0651	0.7460

Note: [1]H_T: Total genetic diversity; [2]H_S: Genetic diversity within populations; [3]G_{ST}: Coefficient of genetic differentiation

Table 7. Nei's genetic distance among species of *Mangifera* in Seliu Island

POP	*M. foetida*	*M. magnifica*	*M. quadrifida*	*M. caesia*	*M. odorata*	*M. zeylanica*	*M. indica*	*M. laurina*
M. foetida	****							
M. magnifica	0.3417	****						
M. quadrifida	0.2694	0.1452	****					
M. caesia	0.1874	0.3657	0.3788	****				
M. odorata	0.1647	0.3531	0.2658	0.2702	****			
M. zeylanica	0.3434	0.4008	0.3392	0.4285	0.3495	****		
M. indica	0.1952	0.2291	0.1716	0.2534	0.1859	0.2345	****	
M. laurina	0.2473	0.2669	0.1973	0.3074	0.2102	0.2724	0.0675	****

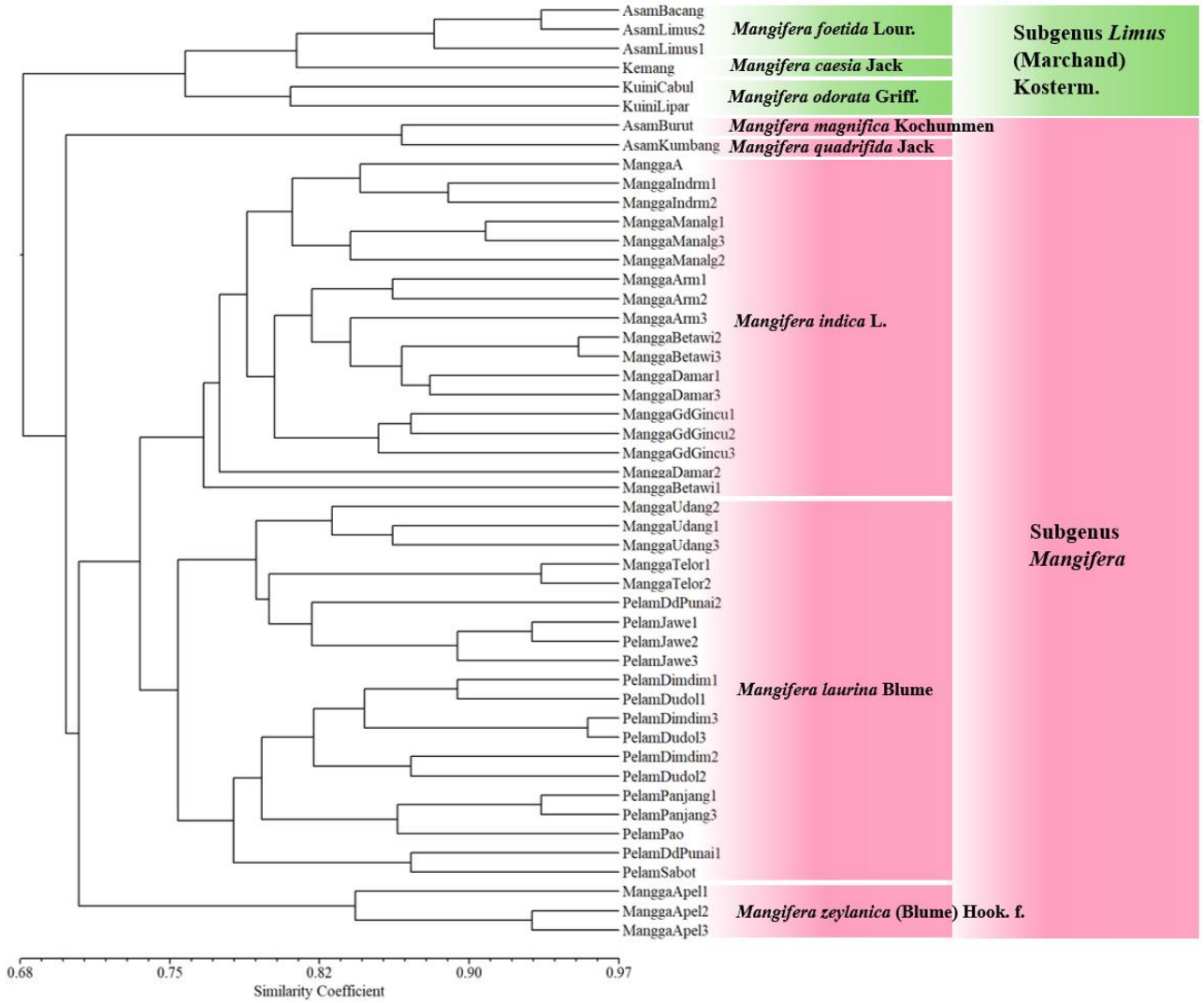

Figure 3. A dendrogram of *Mangifera* from Seliu Island based on ISSR markers classified the 49 accessions with a similarity coefficient of 68% - 95%

Cluster analysis

Dendrogram analysis of the molecular data comprising 49 mango accessions on Seliu Island revealed the existence of two main clusters at a similarity coefficient of 68%, ranging from 0.68 to 0.95, namely Cluster I and II. Cluster I belongs to the subgenus *Limus* (Marchand) Kosterm and Cluster II is part of the subgenus *Mangifera* (Figure 3). In each cluster, the varieties of *Mangifera* also formed small groups of each species. Cluster I comprised three species at a similarity coefficient of 76%, namely *M. foetida*, *M. odorata*, and *M. caesia*. Cluster II comprised five species at a similarity coefficient of 86%, namely *M. indica* L, *M. laurina*, *M. zeylanica*, *M. magnifica*, and *M. quadrifida*. For comparison purposes, dendrograms of 49 accessions from Seliu Island and 13 accessions from West Java were constructed using the same method, and the resulting dendrograms were found to exhibit similar groupings to those observed in the dendrogram of accessions on Seliu Island, with similarity coefficient values ranging from 0.69 to 0.95 (Figure 4).

The cluster analysis results were corroborated by PCA (Alam et al. 2015). Two PCA tests were conducted based on the band similarity of all samples from Seliu Island and *M. indica* samples from Seliu Island. The PCA of all samples on Seliu Island yielded a two-dimensional plot with groupings indicating consistency with the clustering analysis, including two main groups: the subgenus *Limus* (Marchand) Kosterm. and the subgenus *Mangifera*. Each accession was grouped according to species (Figure 5). The principal component values of all samples from Seliu Island included PC 1 (12.68%) and PC 2 (9.11%) for 21.79%. The PCA of *M. indica* samples on Seliu Island demonstrated a tendency for accessions to cluster based on each local cultivar. The PC values of *M. indica*, including PC 1 (14.97%) and PC 2 (11.47%), yielded a total of 26.44% (Figure 6).

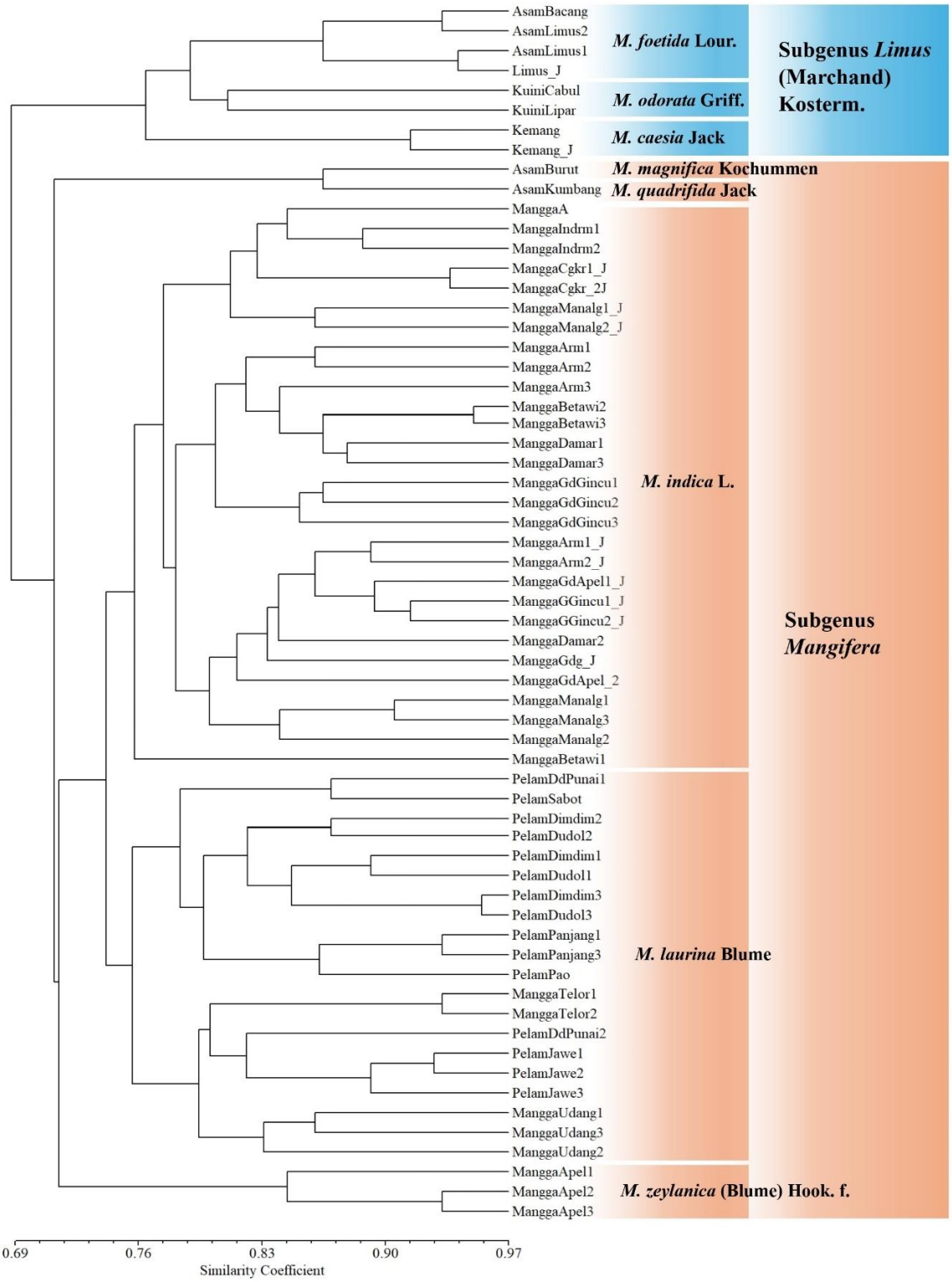

Figure 4. A dendrogram of *Mangifera* based on ISSR markers classified the 49 accessions from Seliu Island and 13 accessions from West Jawa (Majalengka and Bogor) with a similarity coefficient of 69% - 95%

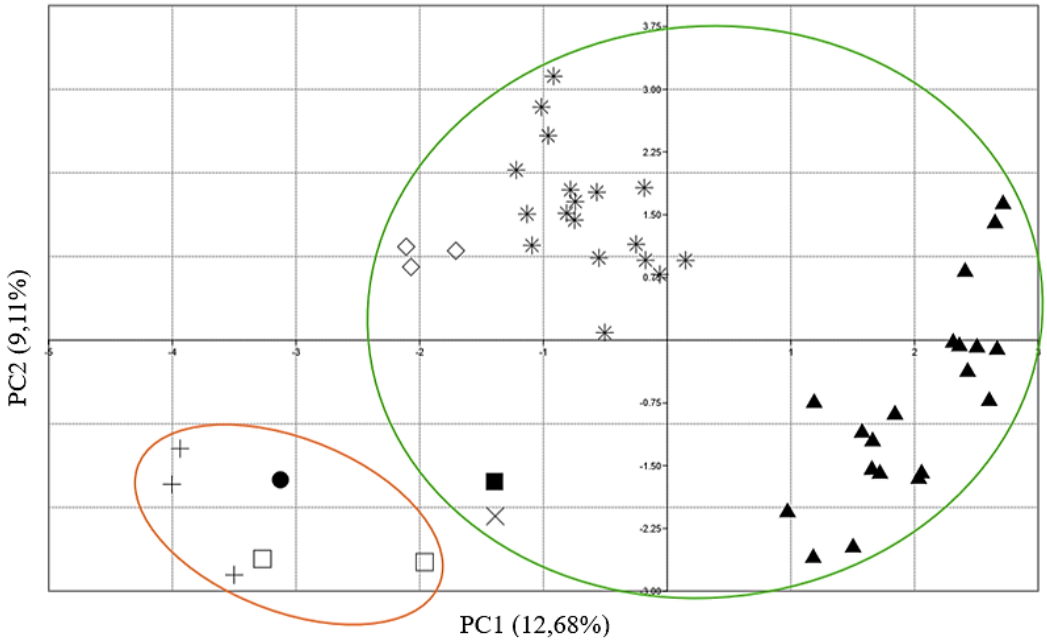

Figure 5. The two-dimensional of PCA plot illustrates the correlation among *Mangifera* accessions from Seliu Island as determined by ISSR markers; PC 1 (12.68%) and PC 2 (9.11%) for 21.79%. The symbol represents: *M. laurina* Bl. (▲), *M. magnifica* Kochumen. (▯), *M. odorata* Griff. (▯), *M. zeylanica* (Bl.) Hooker f. (◇), *M. foetida* Lour. (✚), *M. quadrifida* Griff. (✕), *M. indica* L. (✱), *M. caesia* Bl. (●). The color represents the grouping of *Mangifera*: Subgenus *Mangifera* (🟢), Subgenus *Limus* (Marchand) Kosterm. (🟠)

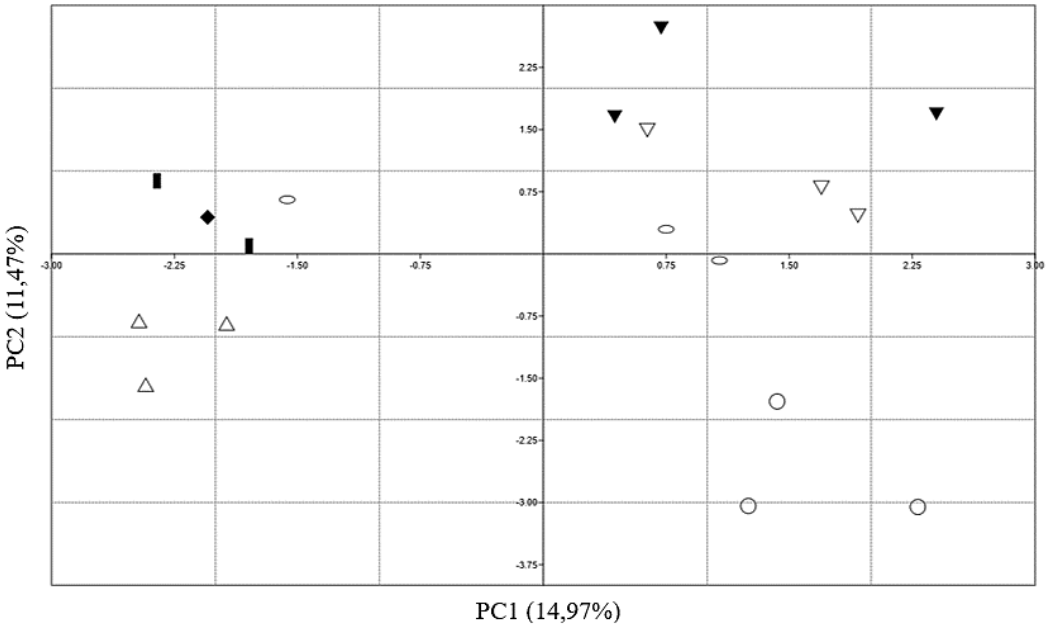

Figure 6. The two-dimensional of PCA plot illustrates the correlation among *Mangifera indica* accessions from Seliu Island as determined by ISSR markers; PC 1 (14.97%) and PC 2 (11.47%) yielded a total of 26.44%. The symbol represents : Mangga Manalagi (△), Mangga Arumanis (▼), Mangga Indramayu (▮), Mangga Betawi (▽), Mangga A (◆), Mangga Damar (◯), Mangga Gedong Gincu (◯)

Discussion

The PIC values indicated that the primers used differed for both analyses: primer UBC-826 for 49 *Mangifera* accessions and primer HB-19B1 for 18 *M. indica* accessions. The PIC value of *Mangifera* in Seliu Island is 0.259 on average, which indicates that the ISSR primers utilized in this study are moderately informative primers for analyzing the genetic diversity of *Mangifera* in Seliu

Island. The PIC value indicates the level of allelic variation (Serrote et al. 2020). This PIC value is defined as a value that informs the level of polymorphism of a marker used. The PIC value is classified into three categories based on the level of information it provides. PIC values >0.5 are considered highly informative, those between 0.5>PIC>0.25 provide moderate information, and PIC values <0.25 are less informative (Botstein et al. 1980). Ariffin et al. (2015) also reported moderate PIC values for ISSR markers in *Mangifera*, with an average PIC value per primer of 0.270 using 10 ISSR primers on 28 *Mangifera* accessions. Meanwhile, The ISSR primers used in this study are less informative ($\bar{x}$PIC=0.192) for the analysis of genetic diversity within *M. indica* on Seliu Island. The low PIC values that ranged from 0.138 (UBC-807) to 0.393 (UBC-825) using 21 ISSR primers on 20 *M. indica* genotypes of the Indian Gir forest region were also reported (Gajera et al. 2011). The PIC value is higher than that observed in previous studies conducted by Azam et al. (2019) (PIC = 0.180). However, the value is lower than that obtained by Razak et al. (2019) (PIC=0.459) and Hidayat et al. (2021) (PIC = 0.539).

The He value will be directly proportional to the PIC value. The more polymorphic the locus, the more heterozygous individuals are found, which indicates genetic variation. High heterozygosity suggests the richness of genetic variation (Lathifah 2016). The PPB, He, and I are three important parameters used to measure genetic variation at the species level (Ma et al. 2021; Riastiwi et al. 2022). The results of this study demonstrate a high level of genetic polymorphism of *Mangifera* (PPB = 86.17%), whereas a moderate level of genetic polymorphism of *M. indica* (PPB = 62.61%). The previous research also reported the genetic diversity of *Mangifera* accessions using ten primers (UBC-808, UBC-811, UBC-826, UBC-841, UBC-855, UBC-873, UBC-876, UBC-881, UBC-886, UBC-891) was very high values (99.44% polymorphic of *Mangifera*; 98.23% polymorphic of *M. indica*) (Ariffin et al. 2015). High polymorphic levels were also found in the genetic characterization of mango accessions in Vietnam using ISSR (98.1%), revealing a high polymorphism information content (PIC) of 0.91 and suggesting that these methods are effective for determining the genetic variation of mango (Ho and Tu 2019). Genetic variability of Alphonso mango (*M. indica*) from different locations of South Konkan, India, was conducted using ISSR markers, and the average polymorphism among ten locations was 46.62% (Patil et al. 2019). The percentage of polymorphic bands indicates genetic diversity among individuals within or between populations. The higher the percentage of polymorphic bands, the more informative the marker is because it can detect more genetic differences between individuals or populations (Abdelaziz et al. 2020).

Based on genetic diversity analysis, this study revealed a high genetic diversity between mango accessions in the Seliu Island, a small island part of Bangka Belitung Province, and consistent with previous research that has identified a high level of diversity within mango populations in Sumatra, including the provinces of Aceh, North Sumatra, Riau, West Sumatra, Jambi, Bengkulu, Lampung, South Sumatra, Bangka Belitung (no reference is made for Seliu Island), and Riau Archipelago (Kostermans and Bompard 1993; Fitmawati et al. 2018). The I and He values can vary between zero and one; a closer value to zero indicates a lower level of genetic diversity (Silva et al. 2015). The He value is lower than the research of Jena and Chand (2021), which is He = 0.28. However, based on the standard value of I and He, the value of *Mangifera* (He = 0.210) is more than 0.20, so it is classified as high genetic diversity (Ismail et al. 2019; Luo et al. 2019). Meanwhile, the He value of *M. indica* is below the threshold of 0.20, indicating low genetic diversity. The total genetic diversity (H_T) among species of *Mangifera* in Seliu Island was more significant than the genetic diversity within a species (H_S). The high discrimination ability of ISSR markers, as well as the process of recombination and segregation of alleles through cross-pollination of mangoes, appear to contribute to high genetic variability in mangoes (Azam et al. 2019; Hidayat et al. 2021), and also there were various factors cause low genetic diversity of a species, including the small population size and geographical isolation (Li et al. 2018), and the tendency for the species to have vegetative reproduction or self-pollinate, with low mutation rate (Xu et al. 2015). Vegetative reproduction in *M. indica* occurs more frequently and faster in the propagation and distribution of commercial mangoes. Many members of *M. indica* have been cultivated, and people usually grow mangoes with vegetative reproduction because of the opportunity to propagate plants faster in this way than from seeds.

The recent study on *Mangifera* in Seliu Island demonstrated that Nei's genetic distance, dendrogram clustering patterns, and PCA were correlated with variety differences. Based on differences in varieties, the analysis successfully distinguished two main clusters: Cluster I and Cluster II. Cluster I comprised three species from subgenus *Limus*, while Cluster II comprised five from subgenus *Mangifera*. Based on the differences in variety, the two main groups have different floral disc morphologies. Cluster I, subgenus *Limus*, has a flower disc narrower than the base of the ovary, stalk-like or even lacking, and the basal parts of the filaments often united into an annulus. Meanwhile, Cluster II, subgenus *Mangifera*, has a flower disc that is broader than the base of the ovary, is cushion-like, and the filament bases are not fused (Kostermans and Bompard 1993). Furthermore, the cluster analysis demonstrated that *Mangifera* accessions are grouped according to their species.

Accessions from West Java were used as comparison accessions to improve the clarity and informativeness of the clustering results by confirming the clustering position of each cultivar from Seliu Island. The comparison cultivars, including mango *cengkir*, *arumanis*, *manalagi*, *gedong apel*, *gedong gincu*, *gedong*, *kemang*, and *limus*, are local cultivars from West Java that have been cultivated (Handayani 2023; Triani and Ariffin 2019). The dendrogram indicates that the Seliu Island and West Java accessions have been correctly assigned to a single species group. However, in the *M. indica* cluster, each accession from Seliu Island and West Java tends to cluster based on geographical origin. The clustering analysis results also indicate the potential influence of geographical location and differences in

microclimate on the grouping of an accession within the observed species. Microclimate differences between populations of *Alpinia malaccensis* (Burm.f.) Roscoe did not cause any character differences, and even some characters overlap (Setiawan et al. 2022). However, the microclimate conditions may affect their behavior (Nassar et al. 2018); as a result, they established their groups based on geographical factors.

In addition, the cluster analysis results in this study are consistent with those of Teo et al. (2002), which indicated that *M. odorata* is a hybrid of *M. indica* and *M. foetida*. Furthermore, the analysis demonstrated that *M. odorata* is more closely related to *M. foetida* than *M. indica*. The grouping of *M. indica*, *M. laurina*, *M. zeylanica*, and *M. quadrifida* in one cluster is consistent with the grouping observed in the previous research based on the *trnL-F* gene sequence (Fitmawati et al. (2017). However, the relationship between *M. magnifica* and *M. quadrifida* must still be better understood. *M. indica* is genetically closely related to *M. laurina*. *M. laurina* is often mistaken for *M. indica* and merged into *M. indica* because tree habits and leaves are very similar. These findings are consistent with earlier research based on the *trnL-F* gene sequence (Fitmawati and Hartana 2010) and a combination of E-RAPD and morphological markers (Fitmawati 2006). *M. laurina* is a suitable rootstock for cultivars of *M. indica* grown on periodically inundated river banks in Kalimantan. The loose glabrous inflorescences show no sign of anthracnose (*Colletotrichum gloeosporioides*, whereas those of the mango are severely damaged. Crossing *M. laurina*'s resistance to anthracnose into *M. indica* would be a breakthrough (Bompard 1992).

Seliu Island is currently experiencing issues with the susceptibility of several mango varieties to pests and diseases, namely mango weevil (*Sternochetus mangiferae*), that infest and damage the fruit, ultimately leading to crop failure. These mango varieties are typically cultivated in residential gardens and are commercial mangoes (*M. indica*). In contrast, the mango species observed in abandoned gardens (kelekak) are not susceptible to pests and diseases. The mango species permitted to grow wild in gardens belong to the subgenus *Limus*. It is essential to analyze genetic diversity, as this can inform breeding and conservation programs. Genetic diversity is critical in plant breeding, enhancing populations adaptive capacity to environmental changes and preserving large gene pools for future genetic breeding. The subgenus *Limus* members on Seliu Island are a prime example of the value of genetic and population diversity in germplasm collections for plant breeding. Understanding genetic and population diversity in germplasm collections is the base of plant breeding. For conservation, preserving genetic diversity is crucial for long-term survival and, importantly, the sustained productivity of commercially favorable genotypes (Rachmat et al. 2016). Wild relatives, with their essential genes, play a key role in breeding programs, providing resistance to pests and diseases (Migicovsky and Myles 2017), preventing the extinction of superior genotypes endemic to these areas, and reducing the risk of loss of desirable characteristics (such as fruit quality) due to uncontrolled depression of natural inbreeding (Jena and Chand 2021).

In conclusion, 24 local mango cultivars on Seliu Island were identified and can be classified into eight species of *Mangifera*. This study demonstrated that ISSRs are valuable markers in genetic diversity studies, as evidenced by the high polymorphism level observed in the 49 accessions of *Mangifera* on Seliu Island. Furthermore, the ISSR markers are suitable for determining the genetic similarity among the *Mangifera* species, which can be used to group them according to their subgenera, *Limus* and *Mangifera*. The markers can also group accessions according to their species. The findings of this study indicate that each accession from Seliu Island and West Java tends to cluster based on its geographical origin. It is also recommended that these *Mangifera* accessions be conserved on farms and collected for germplasm collection for conservation programs. These accessions may become helpful as genetic material in breeding research in the future, particularly the wild relatives of *Mangifera* species, which can develop resistant factors, such as fruit resilience, to diseases and pests of mango cultivation.

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