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GENETIC DIVERSITY OF *KWENI* FRUIT (*Mangifera odorata* Griffith) FROM SUMATRA, INDONESIA, BASED ON MORPHOLOGICAL AND ISSR ANALYSES

E. JULIANTARI[1], N.R. DJUITA[1], FITMAWATI[2] and T. CHIKMAWATI[1*]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau, Indonesia
*Corresponding author email: tatikch@apps.ipb.ac.id
Email addresses of co-authors: erwinajuliantari24@gmail.com, nrdjuita@gmail.com, fitmawati2008@yahoo.com

SUMMARY

Sumatra is one of the largest islands. Its location on the equator between two major continents, namely, Asia and Australia, has an effect on its climate, which a type B 60 climate is with high rainfall of up to 130 mm/day. *Kweni* mango (*Mangifera odorata* Griff.), which is produced along the West Coast of Sumatra, has superior characteristics, including high productivity and the capability to produce fruits during the off-season. *Kweni* is distinguished from other commercial mangoes (*Mangifera indica* L.) by its unique aroma, sweet flavor, and soft fibers. This study aimed to determine the genetic diversity and classification of *kweni* fruit from Sumatra on the basis of morphological and molecular variations. Fruit samples were collected from the centers of diversity of *kweni*, including the Aceh, West Sumatra, Riau, and Bengkulu Provinces, in Indonesia. The data on 52 *kweni* accessions were recorded and analyzed by using 43 morphological characters, and 10 intersimple sequence repeat (ISSR) primers with 94 bands. The morphological characters that divided the *kweni* accessions into three major groups was the sweetness level of the pulp (°Brix). Group I had the sweetest flavor (16.7–21.0 °Brix), group II had a moderately sweet flavor (12.4–16.66 °Brix), and group III had a sour flavor (8–12.3 °Brix). The ISSR markers separated the *kweni* accessions into four main groups based on accession origin. Results further revealed that the *kweni* accessions were morphologically similar but genetically varied. The genetic resources of the *kweni* fruit used in this study and their classification could be used for the further improvement of mango in Sumatra through future breeding programs.

Keywords: Genetic diversity, morphological variations, intersimple sequence repeat analysis, germplasm, *kweni* fruit

Key findings: The genetic diverstiy of *kweni* mango accessions collected from different regions of Sumatra, Indonesia was studied. Morphological and ISSR analyses differentiated the *kweni* fruit accessions into three and four major groups based on sweetness level and origin, respectively. The results also revealed that specific ISSR bands could be used to identify superior mango genotypes for future breeding programs.

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INTRODUCTION

Kweni mango (*Mangifera odorata* Griffith), commonly known as *kwini*, *kuweni*, *kuwini*, or Saipan mango, is found along the West Coast of Sumatra, Indonesia. *Kweni* has a fragrant aroma, juicy pulp, and sweet flavor with a soft fibrous structure. Its fruit is usually eaten fresh directly or processed into *rujak* (fruit salad), *acar* (vegetable pickles), *asinan* (fruit pickles), porridge, or fresh drinks in Malaysian ethnic cuisine; it is also processed into ice sherbet (Bompard, 1992). *Kweni* has the potential to be used on a large scale as a natural flavor in the food industry (Wijaya *et al.,* 1999). *Kweni* is one of the underutilized plant species of the genus *Mangifera* and has tremendous medicinal potential as an antidiabetic (Lasano *et al.,* 2019), antioxidant, antimicrobial, and anticancer (Ismail *et al.,* 2019) plant. Studies have further revealed the potential promoting the diversity of *kweni* in society and increasing its potential for cultivation and health. Currently, *kweni* is has low economic value because it is less popular than other mango species in Indonesia. *Kweni* may disappear in the future due to its low competitiveness and development efforts. Therefore, large-scale cultivation and utilization are needed to prevent the extinction of *kweni*.

Borneo, Java, and Sumatra are the centers of diversity of *kweni* in Indonesia (Bompard, 1992). In Sumatra, *kweni* grows at an altitude of 0–800 masl, and its production may decrease at altitudes above 800 masl. Sumatra receives high rainfall, the main hindrance in cultivating the common mango (*Mangifera indica*), which generally prefers dry and hot weather conditions (Whitten *et al.,* 1997). In contrast to mango cultivars, *kweni* produces fruit well in Sumatra as a result of the wet climate with high temperature throughout the year and the short rainy season in the region. Furthermore, *kweni* flowers do not fall off easily, and their production is unaffected even under high rainfall (Fitmawati *et al.,* 2018). The peculiarity of the climate and the adaptation of *kweni* provide an opportunity to develop *kweni* cultivation in Sumatra.

Information on genetic diversity and variation are important for identifying desirable traits that could be integrated into breeding programs to develop superior *kweni* cultivars (Guliyev *et al.,* 2018). *Kweni* germplasm can be managed effectively by conducting a genetic diversity study based on morphological and molecular characteristics. Currently, many molecular markers are used to assess diversity below the species level. Among these markers, intersimple sequence repeats (ISSR) markers are highly efficient in providing accurate results. Past studies have revealed that ISSR markers can determine the genetic similarities among 28 *Mangifera* species and classify them on the basis of their origin (Ariffin *et al.,* 2015). Moreover, ISSR markers have been successfully used for the genetic diversity analysis, cultivar identification, and validation of mango genotypes (Luo *et al.,* 2011; Uddin *et al.,* 2014; Ho and Tu, 2019). The purpose of this study was to provide information on the genetic diversity and a classification system for *kweni* mango from Sumatra, Indonesia, on the basis of morphological and molecular characteristics.

MATERIALS AND METHODS

Plant material and procedure

In this study, 52 *kweni* mango (*M. odorata*) accessions were studied on the basis of morphological and molecular characteristics. All the *kweni* samples were collected from *kweni* centers of

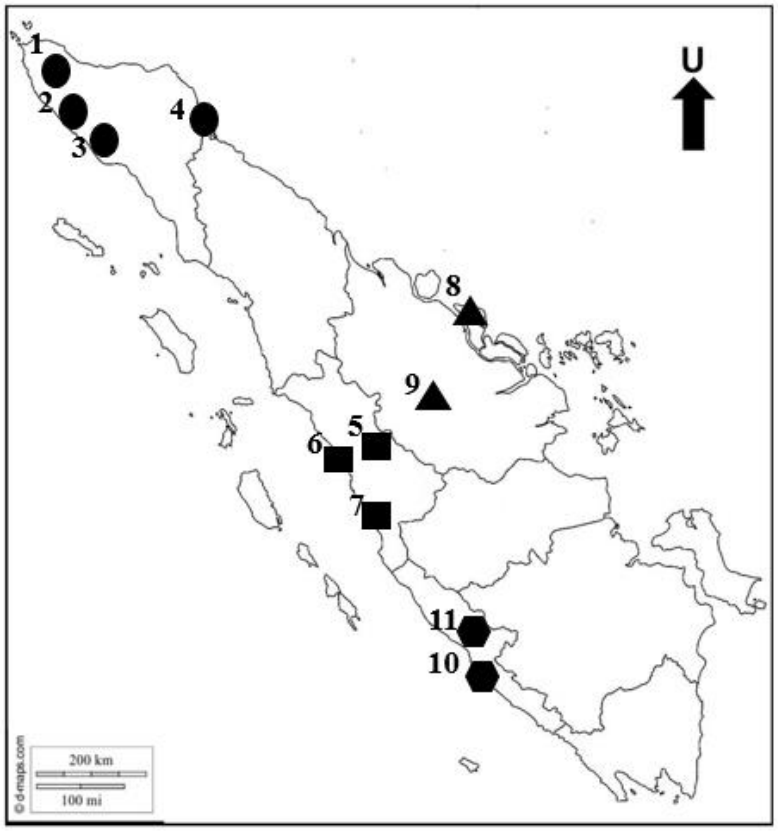

Figure 1. Collection sites of *M. odorata* accessions in Sumatra, Indonesia. (1) Aceh Besar, (2) Nagan Raya, (3) West Aceh, (4) Aceh Tamiang, (5) Solok, (6) Padang, (7) Pesisir Selatan, (8) Bengkalis, (9) Kuantan Singingi, (10) Bengkulu, and (11) Central Bengkulu Districts.

Table 1. Details of the *kweni* accessions used in this study.

No.	District/City	Location	Origin/Province	Number of Accessions
1	West Aceh	Meulaboh, West Aceh District	Aceh	2
2	Nagan Raya	Darul Makmur, Nagan Raya District Seunagan, Nagan Raya District	Aceh	2
3	Aceh Besar	Jantho, Kab, Aceh Besar District	Aceh	3
4	Aceh Tamiang	Tamiang Hulu, Aceh Tamiang District	Aceh	3
5	Solok	X Koto Singkarak, Solok District Kubung, Solok District	West Sumatra	9
6	Padang	Bungus Teluk Kabung, Padang City	West Sumatra	2
7	Pesisir Selatan	Tarusan, Pesisir Selatan District	West Sumatra	4
8	Bengkalis	Prapat Tunggal, Bengkalis District Bantan, Bengkalis District	Riau	8
9	Kuantan Singingi	Benai, Kuantan Singingi District Selebar, Bengkulu City	Riau	6
10	Bengkulu	Muara Bangkahulu, Bengkulu City Ratu Agung, Bengkulu City	Bengkulu	10
11	Central Bengkulu	Singaran Pati, Bengkulu City Pondok Kelapa, Central Bengkulu District	Bengkulu	3
	Total			52

diversity, which included the Aceh, West Sumatra, Riau, and Bengkulu Provinces, in Sumatra. The areas explored for sample collection included 11 Sumatran districts and cities, namely, West Aceh, Aceh Besar, Nagan Raya, Aceh Tamiang, Solok, Padang, Pesisir Selatan, Bengkalis, Kuantan Singingi, Bengkulu, and Central Bengkulu (Figure 1, Table 1).

Morphological analysis

The morphological characters observed in this study were 43 qualitative and quantitative characters of stems, leaves, fruits, and seeds in accordance with the mango descriptors given by the International Plant Genetic Resources Institute (IPGRI, 2006). The botanical terms used for various traits were based on Harris and Harris (2006) and Rifai and Puryadi (2008). Colors were determined in accordance with the Royal Horticultural Society color chart, fifth edition. Sweetness level was measured by using a Brix meter.

Molecular analysis

DNA was extracted by using a Geneaid Genomic DNA Mini Kit (Plant). Total DNA was separated through electrophoresis on 1% agarose gel in TBE 1× buffer and stained with ViSafe Green Gel Stain (10 000× in water). The results were observed under blue light with an Accuris Smart Blue Transilluminator and documented by using Smart Doc Enclosure with a smartphone.

DNA was amplified by using 10 ISSR primers (Table 2). Polymerase chain reaction (PCR) was carried out in a 15 µl volume that consisted of 1 µl of DNA (0.5–2.0 ng), 1 µl of the ISSR primer, 8 µl of DreamTaq™ Hot Start Green PCR Mix (DreamTaq Hot Start DNA polymerase, 2× DreamTaq Green Buffer, 0.4 mM dNTPs, and 4mM $MgCl_2$), and 5 µl of nuclease-free water.

Amplification was performed on a Windows-compatible mini PCR v1.6 thermal cycler. The PCR program consisted of an initial denaturation stage at 94 °C for 2 min followed by 35 cycles of denaturation at 93 °C for 30 s, annealing at 50 °C–54 °C for 30 s, extension at 72 °C for 30 s, and a final extension cycle at 72 °C for 5 min followed by cooling at 15 °C.

The amplified PCR products were separated by electrophoresis on 1% agarose gel in TBE 1× buffer and stained with ViSafe Green Gel Stain (10 000×in water). The results were recorded under blue light with an Accuris Smart Blue Transilluminator and were documented by using Smart Doc Enclosure with a smartphone.

Table 2. Details of the ISSR primers.

No.	Sequence	Annealing temperature (°C)	References	No.	Sequence	Annealing temperature (°C)	References
1	$VDV(CT)_7$	54.0	Ariffin et al., 2015	6	$(AC)_8G$	50.,0	Luo et al., 2011
2	$(GA)_8C$	54.0	Ariffin et al., 2015	7	$(AG)_8YT$	50.,0	Luo et al., 2011
3	$HVH(TG)_7$	54.0	Ariffin et al., 2015	8	$(GACA)_4$	50.,0	Mansour et al., 2008
4	$(AC)_8YT$	54.0	Ariffin et al., 2015	9	$(TG)_8RTRC$	52.,0	Mansour et al., 2008
5	$(CA)_8RC$	52.0	Luo et al., 2011	10	$(GT)_6CC$	50.,0	Mansour et al., 2008

Data analysis

Genetic relationships were recorded and analyzed by using 43 morphological characters and 10 ISSR primers. The morphological characters were scored in accordance with the mango descriptors given by the IPGRI (IPGRI, 2006). The banding pattern obtained with each ISSR primer was scored by using the Gel Pro Analyzer program.

The binary data were used to calculate the genetic similarity matrix with the similarity for quality data procedure. On the basis of the genetic similarity index, cluster analysis was conducted via the sequential agglomerative hierarchical and nested clustering procedure. The similarity coefficient was obtained via the simple matching method and clustering through the unweighted pair group method arithmetic average (UPGMA) method performed by using NTSYS pc version 2.01 (Numerical Taxonomy and Multivariate System) (Rohlf, 2000). GenAlex 6.5 was used for the analysis of molecular variance (AMOVA) and genetic diversity analysis (Peakall and Smouse, 2012).

RESULTS

***Kweni* mango classification based on morphological traits**

The 43 morphological characters of stems, leaves, fruits, and seeds of *kweni* accessions from the centers of diversity in Sumatra originated from the four provinces of Aceh, West Sumatra, Riau, and Bengkulu showed variations (Figure 2). The *kweni* accessions into three groups with a coefficient of similarity of 51% (Figure 3) in accordance with the sweetness level of the pulp (°Brix).

Group I consisted of 32 *kweni* accessions with the highest sweetness level (16.7–21.0 °Brix). Group II consisted of 13 accessions that had a medium sweetness level (12.4–16.66 °Brix). Group III consisted of seven accessions that with a sour flavor and low sweetness level (8–12.3 °Brix). Group I originated from the Provinces of Aceh (KA1-8 and KA10), Riau (KR1-14), and West Sumatra (KS1, KS3, KS5, KS8, KS9, KS10, KS11, KS13, and KS14). Group II originated from West Sumatra (KS6 and KS7) and Bengkulu (KB1-8 and KB11-13). Group III, which had the lowest number of *kweni* accessions (7 accessions) originated from the provinces of Aceh (KA9), West Sumatra (KS2, KS4, KS12, and KS15), and Bengkulu (KB9 and KB10).

All the *kweni* accessions in Group I had similar morphological traits, i.e., semiupright branches, warmed up thin leaf texture, low density of the areola reticulation on the upper surface of the leaves, yellowish-green ripe fruit skin, soft pulp texture, very juicy pulp, strong fruit aroma, low stone weight (2.47 g to 11.31 g), round fruit shape with a rounded base and apex, and no sinus (Figure 2). All of the accessions in Group II shared the following morphological traits: inclined fruit stalk insertion, oblong fruit shape with obtuse ends, flat surface of the fruit base, shallow sinuses, soft pulp texture, very watery pulp, strong fruit aroma, oblong fruit shape with an obtuse base and apex, and shallow sinus type. All of the accessions in group III had similar morphological characters, namely, slanted fruit stalk insertion, flat fruit base surface, absent fruit stalk cavity and fruit neck protrusions, rough fruit flesh texture, slightly watery flesh, no strong fruit aroma, round fruit shape with rounded obtuse base and apex, and no sinus. The fruit morphological characters of each group are presented in Figure 2.

The characteristic of ripe fruit flesh texture showed 100% correlation with fresh water content and ripe fruit aroma (Table 3). Perfectly ripe fruit exhibits the optimal sweetness. Fruit with a sweet taste generally has a strong ripe fruity aroma, fruit flesh texture, and flesh water content. The soft flesh texture of *kweni* can be reflected by the shallow depth of the fruit stalk cavity and a yellowish-green fruit skin. Correlated morphological characteristics could be used to detect the quality of *kweni* fruit. Consumers could

use the characteristics of shallow fruit stalk cavity depth, yellowish green skin color, and strong fruit aroma as markers for the selection of sweet *kweni* fruit.

Pearson correlation analysis on the 43 morphological characters provided 18 characters that had a strong correlation (65%–100%) and confidence level (Table 3). The morphological characters with high correlations were leaf texture and areola reticulation; fruit shape, diameter, thickness, and weight; fruit stalk cavity depth; fruit neck protrusion; fruit sinus type; ripe fruit skin color, flesh texture, aroma, and fiber quantity; highest-fiber length; pulp water content; fruit sweetness; and seed type and surface.

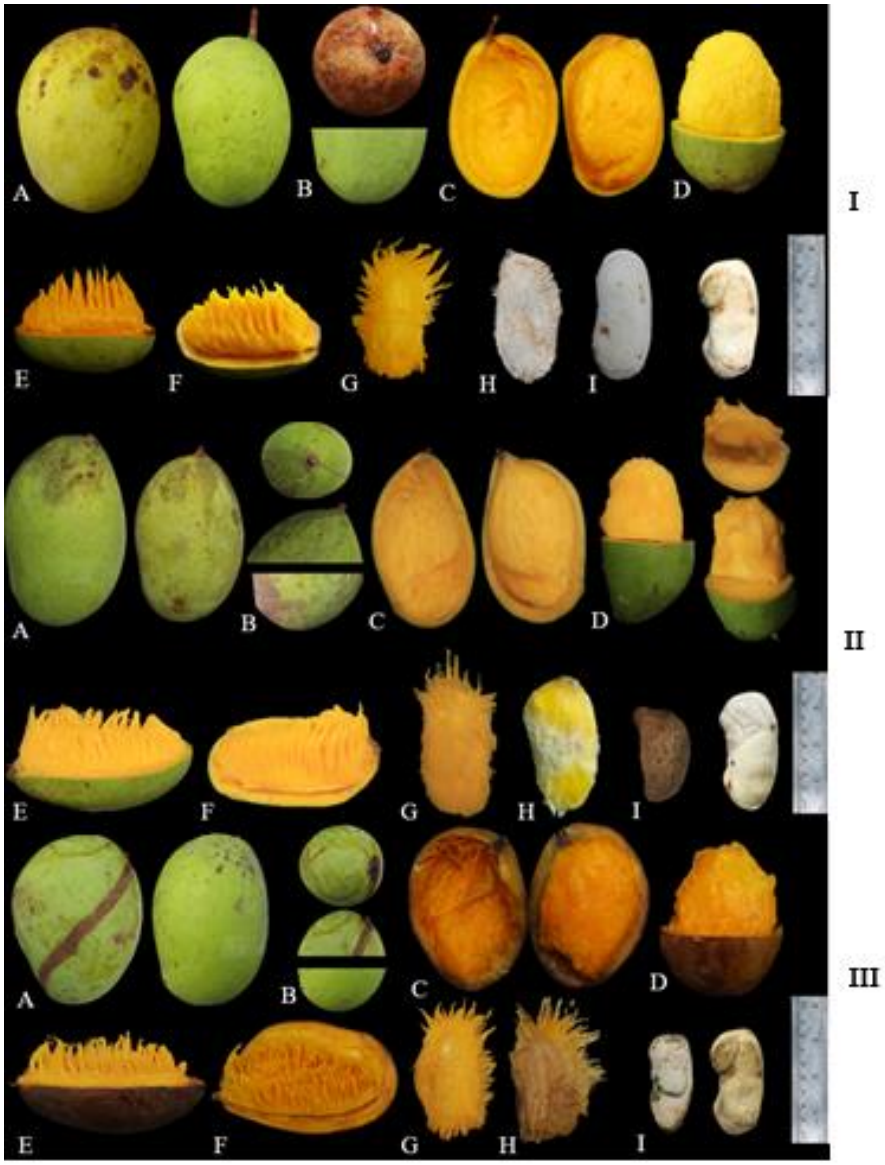

Figure 2. Morphological characters of Groups I, II, and III. (A) Fruit shape, (B) base and apex fruit shape, (C) mesocarp (flesh) color, (D) mesocarp thickness, (E) fiber length, (F) fiber quantity, (G) fiber adhesion to the endocarp, (H) endocarp shape, and (I) seed type.

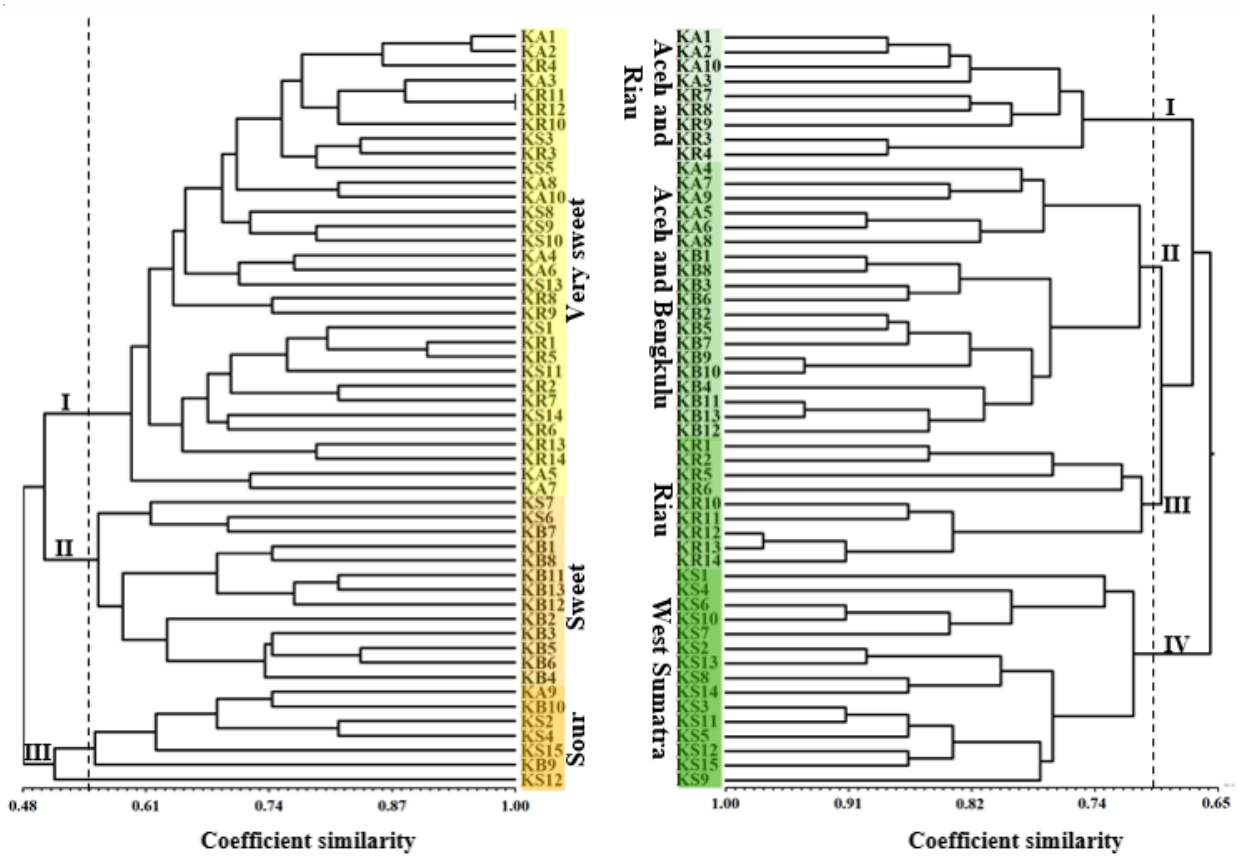

Figure 3. UPGMA dendrogram for 52 accessions of *M. odorata* Griff based on morphological characters (left) and ISSR markers (right). KA_{1-10} = Accessions from Aceh, KB_{1-13} = Accessions from Bengkulu, KR_{1-14} = Accessions from Riau, KS_{1-15} = Accessions from West Sumatra.

Table 3. Pearson correlation coefficients for the morphological characters of *kweni* fruit.

Characters	AR	FS	FD	FT	FSVD	RFSC	RFFT	RFFQ	KADB	ABM	SS
LT	1.00										
FD				0.80							
FW			0.71	0.74							
FNP					1.00						
FST		0.76									
RFFT						0.65					
FL								−0.77			
FWC							1.00				
RFA							1.00				
FSW							−0.75		0.75	−0.75	
ST											1.00

LT = leaf texture, RA = areola reticulation on the upper surface of the leaves, FS = fruit shape, FD = fruit diameter, FT = fruit thickness, FW = fruit weight, FSVD = fruit stalk cavity depth, FNP = fruit neck protrusion, FST = fruit sinus type, RFSC = ripe fruit skin color, RFFT = ripe fruit flesh texture, RFFQ = ripe fruit fiber quantity, FL = fiber length, FWC = flesh water content, RFA = ripe fruit aroma, FSW = fruit sweetness, ST = seed type, and SS = seed surface.

ISSR polymorphism and classification of *kweni* mango

Ten ISSR primers were used to identify the genetic diversity of 52 *kweni* accessions. The pattern, number, and size of bands varied between accessions and primers. The amplification results showed 94 bands, among which 68 were polymorphic. The percentage of polymorphism of each primer ranged from 42.85% to 100% with an average of 73.02%. The number of bands amplified for each primer ranged from 5 to 17, and the band size ranged from 100 bp to 2000 bp (Table 4). DNA amplification by using primers $VDV(CT)_7$ and $(AC)_8G$ yielded the largest band size (2000 bp), whereas that with primers $(GA)_8C$ and $(CA)_8RC$ produced the smallest band size (100 bp). Three common sizes of bands were found in *kweni* accessions, i.e., 300, 500, and 750 bp.

The number of bands produced by primers $(GT)_6CC$ and $(AG)_8YT$ was the lowest (5 bands), whereas the number of bands produced by primers $VDV(CT)_7$ was the highest (17 bands). The difference in the number of amplified bands for each primer was influenced by the presence of the complementary sequence. A primer that produces a high number of bands will have numerous complementary sequences (Rif'atunidaudina *et al.,* 2019). In the *kweni* mango genome, the bases in primers $(GT)_6CC$ and $(AG)_8YT$ had fewer complementary sequences than those in primers $VDV(CT)_7$. The number of complementary sequences in a primer was not correlated with the number of polymorphic bands. Although primer $(AG)_8YT$ provided the lowest number of bands, all of its bands were polymorphic.

The cluster analysis based on 94 ISSR bands classified the 52 accessions into four groups with a similarity coefficient of 65%–97% (Figure 3). All the *kweni* accessions tended to be grouped on the basis of their origin. A high value similarity coefficient between accessions in a population indicates that the group is derived from the same or nearby locations.

Group I consisted of four accessions collected from Aceh Province, i.e., two accessions from the West Aceh population; one accession from Nagan Raya; one accession from Aceh Tamiang; and five accessions from Riau Province, i.e., one accession from Kauntan Singingi and four accessions from the Bengkalis. Group II consisted of six accessions from Aceh Province, i.e., one accession from Nagan Raya population; three accessions from Aceh Besar; two accessions from Aceh Tamiang; and 13 accessions from Bengkulu Province, i.e., 11 accessions from the Bengkulu population and two accessions from Central Bengkulu. Group III consisted of nine accessions from Riau Province, i.e., four accessions from the Bengkalis population and five accessions from Kuantan Singingi. Group IV consisted of 15 accessions from West Sumatra, comprising nine accessions from the Solok population, two accessions from Padang, and four accessions from Pesisir Selatan.

This study also found two accessions (KR11 and KR12) that were morphologically the same with a similarity index value of 100% but were genetically different (Figure 3). This result showed that morphologically similar accessions have varied genetic diversity. The same findings have also been reported for *Cypella pusilla* (Pastori *et al.,* 2018) and *Avicennia* spp. (Sabdanawaty *et al.,* 2021).

Genetic diversity

The level of genetic diversity can be determined by analyzing the number of different alleles (Na), the number of effective alleles (Ne), the Shannon information index (I), and genetic diversity (h). I and h and diversity are important values that indicate the level of genetic diversity (Zhao *et al.,* 2015). The parameters of genetic diversity varied among the 11 populations of *kweni* (Table 5). The Na values ranged from 1.06 to 1.32, whereas the Ne values ranged from 0.59 to 1.28. I varied from 0.05 to 0.26, and h varied from 0.04 to 0.18. The percentage of polymorphic loci (%P)

Table 4. ISSR primer sequences and band profiles generated for *kweni* fruit accessions.

No.	Sequence (5′–3′)	Fragment size (bp) range	Total number of bands	Polymorphic bands	Percentage of polymorphic bands (%)
1	$VDV(CT)_7$	250–2000	17	13	76.47
2	$(GA)_8C$	100–1500	10	6	60.00
3	$HVH(TG)_7$	250–1000	12	6	50.00
4	$(TG)_8RTRC$	300–1250	7	3	42.85
5	$(CA)_8RC$	100–650	9	7	77.78
6	$(AC)_8G$	150–2000	10	8	80.00
7	$(GT)_6CC$	300–500	5	4	80.00
8	$(GACA)_4$	300–1500	12	11	91.67
9	$(AG)_8YT$	200–1000	5	5	100.00
10	$(AC)_8YT$	250–1500	7	5	71.42
Total			94	68	
Average			9.4		73.02

Y = pyrimidine (C,T); R = purine (A,G); V = A, G, C; B = T,G,C; D = A, T, G; H = A, T, C.

Table 5. Genetic diversity parameters of *kweni* populations based on ISSR markers.

Populations	Na	Ne	I	h	P (%)
West Aceh	1.06	0.59	0.05	0.04	8.51
Nagan Raya	1.13	0.79	0.11	0.07	18.09
Aceh Besar	1.15	0.91	0.12	0.08	19.15
Aceh Tamiang	1.17	0.94	0.14	0.10	25.53
Solok	1.32	1.23	0.26	0.18	44.68
Padang	1.13	0.83	0.11	0.07	18.09
Pesisir Selatan	1.11	0.85	0.10	0.06	18.09
Bengkalis	1.22	1.28	0.22	0.14	43.62
Kuantan Singingi	1.20	1.00	0.16	0.11	27.66
Bengkulu	1.19	1.18	0.18	0.12	38.30
Central Bengkulu	1.07	0.76	0.06	0.04	9.57
Mean	1.15	0.94	0.13	0.09	24.66

Na: Number of observed alleles, Ne: effective number of alleles, I: Shannon's information index, h: Diversity index, P: Percentage of polymorphic loci

varied from 8.51% to 44.68% with an average of 24.66%. The Solok population showed the highest level of genetic diversity (I = 0.26, h = 0.18), whereas the West Aceh population showed the lowest diversity (I = 0.05, h = 0.04). These results indicated that genetic diversity was relatively low in *kweni* fruits (Morris *et al.,* 2014, Silva *et al.,* 2015, Deng *et al.,* 2020).

Genetic diversity provides information regarding the extent of diversity that exists in the germplasm and the distribution of this diversity in various geographical populations (Tabin *et al.,* 2016). The *kweni* accessions originating from Solok had the highest diversity value because their site of origin has altitudes that varies between 390 masl to 560 masl and is located along Lake Singkarak. Environmental differences influence the plants to adapt to their habitat conditions, thus triggering genetic and physiological changes that can affect the diversity of the plant population. The percent value of the polymorphic locus in the Solok population was high (44.68%), indicating that the ISSR marker used to reveal the genetic diversity in *kweni* is an informative marker.

AMOVA showed that genetic variation within populations (68%) was higher than that among populations (32%), and the genetic differentiation among the *kweni* population was high (Fst = 0.32) (Table 6). This analysis indicated that *kweni* from Sumatra had a low level of diversity. These results were related to the low I and h obtained. High levels of population diversity are associated with low values of the differentiation coefficient (Nei and Kumar, 2000).

The level of differentiation among 11 populations of *kweni* mango was high due to the large geographical distance among populations. The Mantel test on the populations of *Michelia shiluensis* with SSR markers confirmed that genetic and geographic distances are significantly correlated, indicating that an increase in the geographic distance leads to increased genetic differentiation among populations (Deng *et al.,* 2020).

Specific bands for the detection of morphological characters

In addition to detecting genetic diversity, molecular markers can also be used to identify accessions on the basis of specific DNA fingerprint bands (Simi *et al.,* 2013, Patel *et al.,* 2015, Muazu *et al.,* 2016). When accessions are difficult to distinguish morphologically or a complete organ cannot be identified, then ISSR markers can be used for identification by using only using leaves.

Primer $(AC)_8YT$ provided the specific ISSR band pattern with the band size of 800 and 1000 bp that was able to detect fruit sweetness level (°Brix) (Figure 4). The accessions that had a low level of sweetness, such as KA9, KB9, KS2, and KS4, had sweetness values of 8 °Brix to 12 °Brix, whereas the *kweni* accessions that had a high level of sweetness, such as KA2, KA8, KS8, and KS11, had sweetness levels of 18 °Brix to 21 °Brix.

Table 6. AMOVA results for 52 *kweni* accessions in 11 populations.

Source of Variation	d.f.	SS	MS	Est. Var.	%	F_{st}	*P*-value
Among population	10	236.879	23.688	3.577	32%	0.323	0.001
Within population	41	307.871	7.509	7.509	68%	-	-
Total	51	544.750	-	11.086	100%	-	-

Figure 4. ISSR band profile obtained from primer $(AC)_8YT$ showing the specific bands for *kweni* fruit sweetness. The specific bands are shown in the box.
M = DNA Ladder 1 kb, KA_9 = Aceh Tamiang, KB_9 = Bengkulu, KS_2= Solok, KA_2 = West Aceh, KA_8 = Aceh Tamiang, and KS_8 = Solok.

Pearson correlation analysis on the 43 morphological characters and 94 ISSR bands revealed seven characters that were correlated with five ISSR bands with coefficients ranging from 0.511 to 0.677 (Table 7). The band with the size of 350 bp obtained with primer $(GA)_8C$ was strongly correlated with the characteristics of mature leaf color (2), leaf texture (3), and areola reticulation on the upper surface of the leaves (4). The band with the size of 300 bp of primer $(GA)_8C$ was strongly correlated with the morphological character of the sloping shoulder of the fruit (6). The band with the size of 750 bp obtained with primer $HVH(TG)_7$ was found to be correlated with stem bark color (1), and the band size of 350 bp was correlated with fruit stalk insertion (5). The band size of 300 bp of primer $(TG)_8$ RTRC was correlated with endocarp thickness (7).

Table 7. Pearson correlation coefficients between morphological characters and molecular markers.

Morphological characters	Primer bands				
	$(GA)_8C^{350}$	$(GA)_8C^{300}$	$HVH(TG)_7^{750}$	$HVH(TG)_7^{350}$	$(TG)_8RTRC^{300}$
1			0.526		
2	0.616				
3	0.677				
4	0.677				
5				-0.551	
6		0.537			
7					−0.511

1 = stem bark color, 2 = mature leaf color, 3 = leaf texture, 4 = areola reticulation on the upper surface of the leaves, 5 = the fruit stalk insertion, 6 = the sloping shoulder of the fruit, 7 = the endocarp thickness.

DISCUSSION

Fifty-two *kweni* accessions were collected from forest edges, roadsides, and house yards. Local communities utilize and harvest *kweni* fruit directly to be eaten or sold in traditional markets or along the roadside. Until now, no community has cultivated *kweni* on a large scale even though this fruit has the potential to be commercialized. The *kweni* mango is diverse and is distributed along the West Coast of Sumatra starting from Meulaboh, West Aceh District, to Bengkulu City and Bengkulu District in Indonesia.

The variations in *kweni* fruit shape from certain locations have been previously recorded. The accession originating from Bengkulu has an oval fruit shape with a rounded tip and base. By contrast, the *kweni* accessions obtained from other locations have rounded fruits with rounded tips and bases. The fruit shapes of the population from West Sumatra were more varied than those of other populations and ranged from rounded and oval because the accessions collected from West Sumatra originated from populations that were found at altitudes of 10–560 masl.

M. odorata Griff. is a polyembryonic mango (Kostermans and Bompard, 1993). However, some Sumatran accessions, including three accessions obtained from Aceh, five accessions collected from West Sumatra, and three accessions obtained from Bengkulu, had monoembryonic seed types. These data indicated that the *kweni* accessions from Sumatra varied widely in terms of the number of embryos in their seeds.

The cluster analysis of 52 *kweni* accessions from Sumatra could be used to create an intraspecific classification system because it clearly differentiated the accessions into three groups on the basis of fruit characteristics. Group I consisted of accessions with round fruit shapes and very sweet flavor. Group II

consisted of accessions with oblong fruit shapes and sweet flavor, and Group III comprised accessions with round fruit shape and sour flavor (Figures 2 and 3). *Kweni* fruit with a sweet flavor has a strong aroma and soft and very juicy pulp (Table 3).

Classification at the intraspecies level is closely correlated to the variations in the agronomic characters of the plant accessions. The diversity of cultivated plants needs to be classified with predictive values for future benefits. Clustering with UPGMA is very useful for classifying cultivated plants with temporary and artificial characteristics (Rifai, 2018). The interpretation of the relationship of *kweni* accessions is very useful for the characterization, selection, and improvement of seeds through a breeding program. The purpose of plant breeding is to produce cultivars with distinct, uniform, and stable (DUS) characteristics. Given that the classification of cultivated plants is crucial for cultivar registration purposes, a guaranteed DUS concept is necessary (Chisholm, 1998). Accession groups must be well described such that one or more characteristics can be used to distinguish one group from another and to establish a correct classification system as needed for various purposes.

A character that could differentiate the accession groups was selected in the cluster analysis based on 43 morphological characters. Clustering cultivated plants by selecting certain characteristics facilitates the selection of good-quality cultivated plants by users, such as farmers, breeders, and consumers. Three out of 43 characters can be used to detect *kweni* fruit with sweet flavor and soft flesh. These characters are the superior characters of *kweni* fruit and include the grooved base shape of a fruit, yellowish-green fruit skin color, and strong fruit aroma. Sweet *kweni* has a soft and very juicy pulp, indicating that the characteristics of flavor, pulp texture, fruit aroma, fruit base shape, and fruit skin color are the potential traits to be developed in *kweni* cultivation in Sumatra.

The cluster analysis results of *kweni* fruits based on ISSR characteristics were different from the classification based on morphological characteristics because the results of ISSR markers were unrelated to the observed morphological characters (Figure 3). The present results were in line with the findings for the classification of *belimbing dayak* (*Baccaurea angulata*) based on ISSR markers (Gunawan *et al.,* 2018, 2019). The classification of Indonesian *gandaria* (*Bouea macrophylla* and *Bouea oppositifolia*) based on ISSR markers was also different from that based on morphological characteristics, and some accessions were classified on the basis of population (Harsono *et al.,* 2016, 2018). These results showed that morphologically similar accessions were also genetically diverse.

The ISSR markers separated the *kweni* accessions into four groups that tended to be grouped on the basis of origin. On the basis of ISSR markers, the *kweni* accessions were grouped in accordance with the similarity of their habitats. High similarity characteristics within populations or between populations originating from different locations could be due to similarities in habitat and environmental conditions (Wang, 2020). The similarity in habitats can certainly cause high allele similarity, i.e., accessions that originate from different locations can group together (Ni *et al.,* 2018).

Genetic diversity among the *kweni* populations showed that variations were highly influenced by variation within the population because *Mangifera* is a cross-pollinated plant; this characteristic results in high gene flow between different individuals (Luo *et al.,* 2011). The same findings have also been reported for *M. indica* L. in India (Surapaneni *et al.,* 2013), and 113 cultivars of *M. indica* L. that originated from the global center of diversity of mango (Warschefsky and Von-Wettberg, 2019). High genetic diversity and variation occur in self-pollinating populations (Wright *et al.,* 2013). The AMOVA results could be used to classify

the genetic variations in a taxon on the basis of genetic distance to describe allele differences between loci (Harsono *et al.,* 2018). A large cross-breeding population is indicative of a large gene pool. A large gene pool provides extensive genetic variation and the capability to adapt easily (Ratnam, 2009).

Kweni accessions showed low genetic diversity based on their I and h values. The low value of h was influenced by the small number of accessions and environmental factors that can reduce variation (Pratami *et al.,* 2020). *Kweni* in Sumatra has a narrow environmental tolerance. Specifically, it can grow only at an altitude below 800 masl. This requirement limits its variety. In addition, *kweni* is a natural hybrid of *M. indica* and *Mangifera foetida* (Kiew 2002, Teo *et al*. 2002, Yonemori *et al*. 2002). Low *kweni* genetic diversity is related to the age of immature hybrids such that the variation is narrowed (Teixeira and Huber 2020). All the populations make important contributions to the viability and protection of *kweni* diversity.

Efforts need to be made such that the existing genetic resources remain available sustainably, and an *ex-situ* conservation approach must be developed. Germplasm resources can be propagated via grafting and artificial cross-pollination by using genetically different accessions to produce heterozygous seeds. *Kweni* conservation in Sumatra is important due to the superior characteristics of *kweni*, including high productivity and the capability to produce fruit during the off-season (Fitmawati *et al.,* 2018). Species with these desirable traits is an opportunity for the development of *kweni* mango cultivation in Sumatra.

ISSR-specific band patterns detected the morphological characteristics of fruit sweetness level (°Brix) in *kweni* (Figure 4). The present results were in line with the past findings of Kaleybar *et al.* (2015), who reported that ISSR markers with some specific bands have been able to distinguish two different accessions of rice (*Oryza sativa* L.) that have the same local name. Furthermore, specific bands resulting from the amplification of each ISSR primer can distinguish *tarum* (*Indigofera tictoria* L.) accessions from Java and Madura Islands on the basis of population origin (Hariri *et al.,* 2017). These findings are useful in the cultivation of *kweni*. The morphological characteristics of sweet taste, one of the characters for obtaining superior accessions, can be identified by using ISSR fingerprint markers, which can be detected by using extracted leaf DNA, and is also useful in mango breeding programs aiming to obtain superior cultivars. In this study, no correlation was found between site-specific characteristics and pulp Brix value. This result shows that sweetness is a genetically inherited trait and is not influenced by differences in environmental conditions.

This research proved that ISSR markers can be used for the genetic fingerprinting, identification, and accession classification of *kweni*. Its results were in line with the findings of Amom and Nongdam (2017), Dar *et al.* (2019) and Ali *et al.* (2020), who showed that ISSR markers could be used to study genetic diversity and to identify plant species and cultivars. Understanding the genetic diversity of germplasm allows breeders to utilize heterozygosity by crossing multiple accessions and preserving germplasm (Ab-Razak *et al.,* 2019).

CONCLUSIONS

This work identified three *M. odorata* accession groups. Group I had a round fruit shape with a very sweet flavor, Group II had an oblong fruit shape with a sweet flavor, and Group III had a round fruit shape with a sour flavor. Sweet-flavored *kweni* had a strong aroma and soft and very juicy pulp. The clustering of *kweni* based on ISSR markers was different from the classification based on morphological characters. The ISSR markers divided the *kweni* accessions into four groups on the basis of origin. The

results further revealed that the *kweni* accessions with similar morphologies had varied genetic diversity. Genetic variation within populations was higher than that among populations. The I and h values revealed *kweni* fruits from Sumatra have low genetic diversity, thus necessitating the conservation of *kweni* germplasm to maintain the existence of *kweni* in Sumatra. Two specific bands of the ISSR $(AC)_8YT$ primer with sizes of 800 and 1000 bp were found to be highly correlated with *kweni* pulp sweetness. These bands could be useful in the early identification of sweet *kweni* genotypes in breeding programs after validation.

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