Research Article

Genetic Diversity of Selected *Mangifera* Species Revealed by Inter Simple Sequence Repeats Markers

Zulhairil Ariffin,¹ Muhammad Shafie Md Sah,² Salma Idris,² and Nuradni Hashim¹

¹Agrobiodiversity and Environment Research Centre, Malaysian Agriculture Research and Development Institute (MARDI),
Persiaran MARDI-UPM, 43400 Serdang, Selangor Darul Ehsan, Malaysia

²Genebank and Seed Centre, Malaysian Agriculture Research and Development Institute (MARDI), Persiaran MARDI-UPM,
43400 Serdang, Selangor Darul Ehsan, Malaysia

Correspondence should be addressed to Zulhairil Ariffin; hairi@mardi.gov.my

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ISSR markers were employed to reveal genetic diversity and genetic relatedness among 28 *Mangifera* accessions collected from Yan (Kedah), Bukit Gantang (Perak), Sibuti (Sarawak), and Papar (Sabah). A total of 198 markers were generated using nine anchored primers and one nonanchored primer. Genetic variation among the 28 accessions of *Mangifera* species including wild relatives, landraces, and clonal varieties is high, with an average degree of polymorphism of 98% and mean Shannon index, $H_0 = 7.50$. Analysis on 18 *Mangifera indica* accessions also showed high degree of polymorphism of 99% and mean Shannon index, $H_0 = 5.74$. Dice index of genetic similarity ranged from 0.0938 to 0.8046 among the *Mangifera* species. The dendrogram showed that the *Mangifera* species were grouped into three main divergent clusters. Cluster I comprised 14 accessions from Kedah and Perak. Cluster II and cluster III comprised 14 accessions from Sarawak and Sabah. Meanwhile, the Dice index of genetic similarity for 18 accessions of *Mangifera indica* ranged from 0.2588 to 0.7742. The dendrogram also showed the 18 accessions of *Mangifera indica* were grouped into three main clusters. Cluster I comprised 10 landraces of *Mangifera indica* from Kedah. Cluster II comprised 7 landraces of *Mangifera indica* followed by Chokanan to form Cluster III.

1. Introduction

The genus *Mangifera* belongs to the family of Anacardiaceae. Kochummen [1, 2] recorded 40 *Mangifera* species and they are distributed from India and Sri Lanka throughout Malaysia to the Philippines and Papua New Guinea in the east. Bompard [3] have listed Malayan Peninsula, Borneo, and Sumatra as the highest diversity that can be found in distribution area for this species worldwide. Thirty species have been reported to be recorded in Malaysia. Most of the *Mangifera* species are domesticated and cultivated in garden orchard or home garden [4]. Example are *Mangifera indica* (mangga), *Mangifera caesia* (binjai), *Mangifera foetida* (bacang), and *Mangifera odorata* (kuini). *Mangifera indica* (mango) is the only species being cultivated as a commercial crop. However, at intraspecific level, there are many mango cultivars, clones, and landraces found in the home gardens and orchards. Some of the cultivars, clones, and landraces are difficult to

identify using morphological characters. Presently, molecular markers based on polymerase chain reaction (PCR) have been widely used. Molecular markers or also known as DNA markers are known for their rapid and widely used to study the genetic diversity, identify redundancies in germplasm collections, test accession stability and integrity, and resolve taxonomic relationships [5]. Molecular markers for assessment of genetic variation in plants have shown many advantages. They are neutral, not related to age and tissue type, and not influenced by the environmental conditions, have feasibility and lower costs, and are more informative than morphological markers [6]. Thus, molecular markers can be considered to be more effective approach compared to morphological markers to identify plant genotypes in a germplasm or fruit trees collection.

Intersimple Sequence Repeats or ISSR have been proven useful for detecting genetic polymorphisms among accessions by generating a large number of markers that target

TABLE 1: List of *Mangifera* species.

Site	Species	Local name (Landrace/cultivar/clone)	Name of sample
Yan, Kedah	<i>Mangifera indica</i> L.	Pelam Harumanis	HarumanisY
	<i>Mangifera indica</i> L.	Pelam Pedal Ayam	PedalAyam
	<i>Mangifera indica</i> L.	Pelam Sala	PelamSala
	<i>Mangifera indica</i> L.	Pelam Air	PelamAir
	<i>Mangifera indica</i> L.	Mangga Telur	MangTel
	<i>Mangifera indica</i> L.	Pelam Ciku	PelamCiku
	<i>Mangifera indica</i> L.	Pelam Sawa	PelamSaw
	<i>Mangifera indica</i> L.	Pelam Pipi Merah	PelPipMer
	<i>Mangifera indica</i> L.	Pelam 6 Kati	P6Kati
	<i>Mangifera indica</i> L.	Harumanis	PHarmanis
	<i>Mangifera foetida</i> Lour	Bacang	Bac1Yan
	<i>Mangifera foetida</i> Lour	Bacang	Bac2Yan
Bukit Gantang, Perak	<i>Mangifera foetida</i> Lour	Bacang	BacPerak
	<i>Mangifera pentandra</i> Hook f.	Mempelam Bemban	MBemban
Sibuti, Sarawak	<i>Mangifera indica</i> L.	Mahathir	MahathirS1
	<i>Mangifera indica</i> L.	Harumanis	HarumanisS
	<i>Mangifera indica</i> L.	Chokanan	ChokananS
	<i>Mangifera indica</i> L.	Mangga Apel	MangApelS
	<i>Mangifera indica</i> L.	Mahathir	MahathirS2
	<i>Mangifera odorata</i> Griff.	Kuini	KuiniS
	<i>Mangifera pentandra</i> Hook f.	Mempelam	MempelamS
Papar, Sabah	<i>Mangifera indica</i> L.	Mangga Manila	MangManila
	<i>Mangifera indica</i> L.	Mangga Apel	MApelSbh
	<i>Mangifera indica</i> L.	Alor Manis	AlorManis
	<i>Mangifera laurina</i> Blume	Mangga Air 1	MangAir1
	<i>Mangifera laurina</i> Blume	Mangga Air 2	MangAir2
	<i>Mangifera odorata</i> Griff.	Wani	WaniSbh
	<i>Mangifera caesia</i> Jack	Belunu Manis	BelunuManis

multiple microsatellite loci distributed across the genome. ISSR are often used because of their capability of reproducibility, with no gene sequence information, and prior genetic studies are required for the analysis. ISSR markers have been reported to undertake the assessment of genetic diversity of mango cultivars [7], cashew germplasm [8], and “uba” mango tree in Brazil [9], identification of mango cultivars of Thailand [10], and also the genetic diversity analysis of Asian bitter melon (*Momordica charantia* L.) in India [11].

This study was undertaken to assess the genetic diversity and genetic relationship of *Mangifera* species and cultivars/landraces/clones in four sites using ISSR in order to provide basic information for future biodiversity conservation and management program of the *Mangifera* genetic resources.

2. Materials and Methods

2.1. Plant Materials. Leaf samples of 28 accessions from six *Mangifera* species were collected from Yan (Kedah), Bukit

Gantang (Perak), Sibuti (Sarawak), and Papar (Sabah) as listed in Table 1.

2.2. DNA Extraction. Total genomic DNA was isolated from *Mangifera* leaves (2-3 g) using CTAB method as described by Murray and Thompson [12] with slight modifications. The DNA solution was quantified using biospectrophotometer to check the quality and quantity of DNA extracted. DNA samples with ratio absorbance 260/280 of more than 1.8 will be considered good to proceed for the next steps. DNA bands were also checked on 1% agarose gel electrophoresis. The DNA was then diluted with sterile distilled water into a concentration of 60 ng/ μ L.

2.3. ISSR Analysis. A total of 15 sets of primers were tested for ISSR amplification. The 15 ISSR primers are UBC-808, UBC-809, UBC-811, UBC-826, UBC-834, UBC841, UBC-854, UBC-855, UBC-868, UBC-873, UBC-876, UBC-881, UBC-886, UBC-891 and UBC-895. The final volume of PCR reaction was 25 μ L which contains 2 mM $MgCl_2$, 0.2 mM

TABLE 2: Profiles of ISSR primers used for ISSR analysis.

Number	Name of primer	Primer sequence 5'-3'	Ta (°C)	% GC
1	UBC-808	(AG) ₈ C	54.2	52.9
2	UBC-811	(GA) ₈ C	54.2	52.9
3	UBC-826	(AC) ₈ C	54.0	46.7
4	UBC-841	(GA) ₈ YC	55.0	50.0
5	UBC-855	(AC) ₈ YT	59.0	44.4
6	UBC-873	(GACA) ₄	50.2	50.0
7	UBC-876	(GATA) ₂ (GACA) ₂	45.0	37.5
8	UBC-881	GGG(TGGGG) ₂ TG	59.0	82.4
9	UBC-886	VDV(CT) ₇	54.0	41.2
10	UBC-891	HVH(TG) ₇	54.0	41.2

dNTP, 2.5 μ L buffer (10x), 0.2 μ L Taq DNA polymerase, 0.2 μ M primer, and 60 ng DNA template. The thermal profile of the PCR reaction was programmed as follows: initial denaturation at 92°C for 1 minute, followed by 40 cycles at 92°C for a min, annealing at 50°C–60°C for 2 minutes, and extension at 72°C for 2 minutes. The final extension was at 72°C for 5 minutes followed by cooling to 4°C. The annealing temperature, Ta (°C), and % GC content were given in Table 2. The amplified products were separated on 1.8% agarose gels with 1x TAE buffer at 60 V for 150 minutes. The gels and the bands were visualized and acquired under UV light using GelDoc2000 documentation system. The size of the amplified products was estimated using 2-log DNA bp ladder.

2.4. Data Analysis. Shannon's diversity indices (H) were calculated [13]. Amplified products which appeared reproducible and distinct were scored as present (1) or absent (0). The performance of ISSR markers used was measured using three parameters: polymorphic information content (PIC), marker index (MI), and resolving power (RP). PIC was calculated using the following formula; $PIC_i = 2fi(1 - fi)$, where PIC_i is the polymorphic information content of the locus i , fi is the frequency of the amplified fragments, and $(1 - fi)$ is the frequency of nonamplified fragments. The PIC for each primer was calculated using the average PIC value from all loci of each primer [14, 15]. Marker index was calculated as product of polymorphic information content and effective multiplex ratio; $MI = EMR \times PIC$ [16]. The ability of a primer to differentiate between genotypes or also known as resolving power (RP) was also calculated; $RP = \sum Ib$. Ib represents the informative fragments by the following formula: $Ib = 1 - (2 \times |0.5 - pi|)$, where pi is the proportion of the species containing the i th band [17].

The binary data was used for calculating Dice coefficient and a similarity coefficient matrix was constructed. The cluster analysis was used based on the methods of Unweighted Pair-Group Method for Arithmetic Averages Analysis (UPGMA) [18] using computer programme Ntsys-pc version 2.11 [19]. Bootstrap analysis was performed using WinBoot [20] software to test the consistency or the statistical significance of the genetic relationships between species shown in the dendrograms.

3. Results and Discussion

Out of 15 ISSR primers screened, only ten ISSR primers were chosen. The primers consist of nine anchored primers (dinucleotide, tetranucleotide, and pentanucleotide repeats) named as UBC-808, UBC-811, UBC-826, UBC-841, UBC-855, UBC-873, UBC-886, UBC-881, and UBC-891 including one nonanchored primer (tetranucleotide repeats) named as UBC-876. The chosen primers were able to produce polymorphic banding patterns and each primer had different annealing temperatures that ranged from 50.0°C to 60.0°C (Table 2).

A total of 198 markers were generated with polymorphism in 197 bands (99.4%). The number of polymorphic markers varied from 11 (UBC-811) to 25 (UBC-855) with an average of 19.7 polymorphic bands per primer. The size of the amplified products ranges from 250 bp to 6000 bp (Table 3). Another set of data also were calculated using only 18 *Mangifera indica* accessions (Table 4). Of these, a total of 163 polymorphic markers were obtained which showed 98% polymorphism.

In this study, high PIC value was observed for primer UBC-841 at 0.52 and low PIC value of 0.12 for primer UBC-826, with an average of PIC value per primer 0.27, was obtained for 28 *Mangifera* accessions. The highest effective multiplex ratio (EMR) was observed with the primer UBC-891 (6.46) and the lowest was observed with the primer UBC-876 (2.50), with an average EMR of 4.32 per primer. The highest MI was observed with the primer UBC-891 (2.13) and the lowest in the primer UBC-873 (0.58) with an average MI of 1.19 per primer was obtained. Meanwhile, the highest RP value was observed with the primer UBC-811 (22.18) and the lowest with the primer UBC-873 (7.0) with an average RP of 12.94 per primer was obtained. On the other hand, PIC value of 0.34 for primer UBC-891 and low PIC value of 0.21 for primer UBC-873, with an average PIC value of 0.27 per primer, were obtained for 18 *Mangifera indica* accessions. The highest effective multiplex ratio (EMR) was observed with the primer UBC-886 (7.67) and the lowest was observed with the primer UBC-873 (2.67), with an average EMR of 4.49 per primer. The highest MI was observed with the primer UBC-891 (2.34) and the lowest in the primer UBC-873 (0.56) with an average MI of 1.25 per primer was obtained. However, the highest RP value was observed with the primer UBC-811 (18.0) and the lowest with the primer UBC-873 (6.4) with an average RP of 10.33 per primer was obtained. Both ISSR analyses showed that the primers, UBC-811 and UBC-891, were considered as good markers based on the high value of MI and RP, respectively. On the contrary, PIC values indicated that the primers used were different for both analyses, UBC-841 for 28 *Mangifera* accessions and UBC-891 for 18 *Mangifera indica* accessions. Gajera et al. [21] reported low PIC values that ranged from 0.138 for primer UBC-807 to 0.393 for primer UBC-825 using 21 ISSR primers on 20 *Mangifera indica* genotypes of Indian Gir forest region. Srivastava et al. [22] also reported PIC value of 0.217 with 12 ISSR markers which was lower compared to RAPD (0.245) and DAMD (0.298) markers systems used but indicated higher value of MI (marker index) which showed the efficiency of ISSR markers. High PIC value may

TABLE 3: List of 10 ISSR primers used for polymorphism detection on 28 *Mangifera* accessions.

Primer	TB	PB	PPB (%)	Amplicon band size (bp)	PIC	EMR	MI	RP
UBC-808	18	17	94.4	450–2500	0.23	4.12	0.95	13.56
UBC-811	11	11	100	430–2500	0.29	4.36	1.26	22.18
UBC-826	25	25	100	500–3200	0.12	3.89	0.47	8.72
UBC-841	20	20	100	250–3000	0.52	3.93	2.04	12.5
UBC-855	25	25	100	300–6000	0.23	4.07	0.94	9.12
UBC-873	20	20	100	900–5000	0.17	3.43	0.58	7.00
UBC-876	18	18	100	700–5000	0.25	2.50	0.63	10.67
UBC-881	18	18	100	700–5000	0.24	3.11	0.75	9.67
UBC-886	21	21	100	350–2800	0.29	7.32	2.12	19.52
UBC-891	22	22	100	350–3000	0.33	6.46	2.13	16.45
Total	198	197						
Mean	19.8	19.7	99.44		0.27	4.32	1.19	12.94

TB: total band, PB: polymorphic band, PPB (%): percentage of polymorphic band (%), PIC: polymorphic information content, EMR: effective multiplex ratio, MI: marker index, and RP: resolving power of primer.

TABLE 4: List of 10 ISSR primers used for polymorphism detection on 18 *Mangifera indica* accessions.

Primer	TB	PB	PPB (%)	Amplicon band size (bp)	PIC	EMR	MI	RP
UBC-808	13	12	92.3	450–2500	0.29	4.21	1.22	12.62
UBC-811	10	9	90	430–2500	0.25	4.50	1.13	18.00
UBC-826	19	19	100	500–3200	0.26	4.44	1.15	8.42
UBC-841	16	16	100	300–3000	0.28	4.11	1.15	9.25
UBC-855	22	22	100	300–6000	0.26	4.22	1.10	6.91
UBC-873	15	15	100	900–5000	0.21	2.67	0.56	6.40
UBC-876	16	16	100	700–5000	0.25	3.22	0.81	7.25
UBC-881	16	16	100	700–5000	0.26	3.00	0.78	6.75
UBC-886	18	18	100	350–2800	0.29	7.67	2.22	15.33
UBC-891	20	20	100	450–3000	0.34	6.89	2.34	12.40
Total	165	163						
Mean	16.6	16.3	98.23		0.27	4.49	1.25	10.33

TB: total band, PB: polymorphic band, PPB (%): percentage of polymorphic band (%), PIC: polymorphic information content, EMR: effective multiplex ratio, MI: marker index, and RP: resolving power of primer.

be contributed by tetranucleotide repeats GAGA and GACA contained in the primers as discussed by He et al. [23].

3.1. ISSR Analysis Using 28 *Mangifera* Accessions. The degree of genetic variation among the 28 *Mangifera* accessions calculated from the data of each primer showed the highest value (Shannon index, H_0 : 9.51) for UBC-886 and the lowest (H_0 : 5.84) for UBC-873. The mean value for H_0 is 7.50, indicating a high level of genetic diversity among the 28 *Mangifera* accessions. The high of Shannon index for 28 *Mangifera* accessions may be influenced by different cultivation locations where the samples were collected, from Yan (Kedah), Bukit Gantang (Perak), Sibuti (Sarawak), and Papar (Sabah), due to the dynamic agrogeographical conditions, respectively. The different level of species occurring in the 28 *Mangifera* accessions may also contribute to the higher diversity value.

The genetic relationship of 28 *Mangifera* accessions was obtained from the markers data using Dice similarity coefficient. Dice's similarity matrix (table not shown) indicated the pairwise comparison of the relative genetic similarity between 28 *Mangifera* accessions ranging from a maximum of 0.8046 (between Mangga Air 1 and MempelamS) to a minimum of 0.0938 (between Pelam 6 Kati and Belunu Manis). The genetic relatedness of 28 *Mangifera* accessions was shown in the dendrogram constructed using Dice's similarity coefficient values. The 28 *Mangifera* accessions were distributed into three main divergent clusters, I, II, and III, at coefficient value of 0.53 (Figure 1).

Cluster I consists of three subclusters (A, B, and C). Subcluster A consists of ten landraces, cultivars, and clones of *Mangifera indica* originated from Yan, Kedah. They were Harumanis, Pelam Harumanis, Pelam Pedal Ayam, Pelam

FIGURE 1: Cluster of 28 *Mangifera* accessions based on Dice similarity coefficient with bootstrap significance values.

Sala, Pelam 6 Kati, Mangga Telur, Pelam Ciku, Pelam Sawa, Pelam Air, and Pelam Pipi Merah. Both Harumanis and Pelam Harumanis were separated from the other landraces due to their clonal type. Only Mangga Telur and Pelam Ciku were found very closely related with a bootstrap significance value of 55%. Subcluster B composed of only one species of *Mangifera pentandra* named as Mempelam Bemban originated from Bukit Gantang, Perak, while subcluster C consisted of three landraces of *Mangifera foetida* (bacang) originated from Yan, Kedah, and Bukit Gantang, Perak, with a very high bootstrap significance value of 95%. Based on the cluster formed, it was clearly shown that different species of *Mangifera* were distributed into specific clusters, in which subcluster A has all the landraces of *Mangifera indica* including two clones, while subcluster B has only *Mangifera pentandra* and subcluster C has *Mangifera foetida*.

Cluster II consisted of three subclusters (D, E, F, G, and H) with a total of twelve landraces of *Mangifera* species including one clone (Harumanis) originated from Sibuti, Sarawak, and Papar, Sabah. Subcluster H consisted of only one clone of *Mangifera indica* named as Chokanan. While subcluster D consisted of four landraces of *Mangifera indica*, two landraces named Mahathir, two landraces named Mangga Apel, and one clone, Harumanis. Landraces Mahathir together with Mangga Apel were found very closely related with bootstrap of significance value of 89% and 98%, respectively. Two wild relatives (*Mangifera odorata*) named as Kuini and Wani were grouped in subcluster E, having the highest bootstrap significance value of 99%. However, Mangga Manila and Alor Manis were separated from the other *Mangifera* landraces to form subcluster F. Two wild relatives of *Mangifera*, named as Mangga Air (*Mangifera laurina*) and Mempelam (*Mangifera pentandra*), were grouped in subcluster G. The Mempelam from Sibuti, Sarawak, was found to be closely related to Mangga Air 1 originated from Papar, Sabah, with a bootstrap

significance value of 68%. A report also shared similar finding with one accession of *Mangifera indica* found closely related to a group containing *Mangifera odorata* accessions. Nevertheless, Yamanaka et al. [24] have succeeded to classify 35 accessions comprising 11 *M. indica* L., 11 *M. odorata* Griff., 7 *M. foetida* Lour., and 6 *M. caesia* Jack accessions into 4 groups corresponding to the 4 *Mangifera* species using AFLP.

Finally, cluster III also known as subcluster I showed only one landrace of *Mangifera caesia* named as Belunu Manis originated from Papar, Sabah. Belunu Manis showed more divergent relationship from the other groups of *Mangifera* species. In addition, the morphology characteristics of this species are quite different from the other groups. It can be seen that *Mangifera caesia* is quite distant from the other species based on the dendrogram generated by AFLP [24]. Obviously, the *Mangifera* species were divided into two major clusters; one cluster has all the Peninsular of Malaysia's species and the other cluster has eastern of Malaysia's species based on the bootstrap significance value of 67% and 99%, respectively.

3.2. ISSR Analysis Using 18 *Mangifera indica*. The degree of genetic variation among the 18 *Mangifera indica* accessions calculated from the data of each primer showed the highest value (Shannon index, H_0 : 6.45) for UBC-891 and the lowest (H_0 : 4.85) for UBC-873. The mean value for H_0 is 5.74, indicating a high level of genetic diversity among the *Mangifera indica* compared to the value of 6.00 for 211 *Nephelium lappaceum* accessions [25] and 2.32 for 85 *Lansium domesticum* accessions [26]. The high of Shannon index for 18 *Mangifera indica* accessions may be influenced by different cultivation locations where the samples were collected, from Yan (Kedah), Sibuti (Sarawak), and Papar (Sabah), due to the dynamic agrogeographical conditions, respectively.

FIGURE 2: Cluster of 18 accessions (*Mangifera indica*) based on Dice similarity coefficient with bootstrap significance values.

The genetic relationship of *Mangifera indica* was obtained from the markers data using Dice similarity coefficient. Dice's similarity matrix (table not shown) indicated the pairwise comparison of the relative genetic similarity between 18 *Mangifera indica* accessions ranging from a maximum of 0.7742 (between Mangga Apel Sabah and Mangga Apel Sarawak) to a minimum of 0.2588 (between Pelam Pipi Merah and Mangga Manila). The genetic relatedness of *Mangifera indica* was shown in the dendrogram constructed using Dice's similarity coefficient values.

In comparison to Figure 1, the 18 accessions were distributed into three main divergent clusters, I, II, and III, at coefficient value of 0.61 (Figure 2). Cluster I consisted of three subclusters (A, B, and C). Subcluster A consists of only two accessions named Harumanis and Pelam Harumanis. Subcluster B composed of 6 landraces named Pelam Pedal Ayam, Pelam Sala, Pelam 6 Kati, Mangga Telur, and Pelam Ciku. Mangga Telur and Pelam Ciku were closely related with a bootstrap significance of more than 55%. Pelam Air and Pelam Pipi Merah were grouped in subcluster C. All accessions in subclusters A, B, and C were originated from Yan, Kedah.

Cluster II consisted of four subclusters (D, E, F, and G). Subcluster D was represented by clone Mahathir with a bootstrap significance of 90%. Harumanis originated from Sibuti, Sarawak, was the only clone in subcluster E. However, each of Mangga Apel originated from Sibuti, Sarawak, and Papar, Sabah, together with Mangga Manila and Alor Manis also originated from Papar, Sabah, was grouped in subcluster F and subcluster G, respectively. Mangga Apel was found very closely related with a bootstrap significance of 98% which was the highest value followed by Mahathir in subcluster D.

Cluster III consisted of one accession named Chokanan, the only clone representing subcluster H. All *Mangifera indica*

in clusters II and III were collected from Sibuti, Sarawak, and Papar, Sabah.

Based on the clustering analysis, it was obvious that the 18 *Mangifera indica* accessions were divided into 2 major clusters; one cluster has all the Peninsular of Malaysia's accessions and the other cluster has east of Malaysia's accessions based on the bootstrap significance value of 98% and 85%, respectively.

Many studies were conducted to determine the genetic variation of *Mangifera* species [27–30]. For *Mangifera* species, morphological characters such as fruit and leaves characters have been used in the early years to show differences among the accessions. The leaf and fruit characters have been observed to be highly heritable and used to classify the Thai mango [27]. Salma et al. [29] have identified 26 mango clones into four groups based on the flower and fruit characters. The high degree of variation may depend on the methods used. PCR based method has become a very useful tool to aid the classification works more easily. It is also very effective method to improve the management of germplasm collections in terms of duplicate identification, estimation of diversity, and enhancement utilization of the existing collections. Various studies on genetic variation using RAPD, ISSR, SSR, and AFLP on the *Mangifera* species have been reported [7, 10, 31–35].

The study reveals high value of genetic diversity among the 28 *Mangifera* accessions. Usually, the fixation of new alleles in specific population along with recombination and segregation of alleles by open-pollination of genetically different in mangoes in varied agroclimates may contribute to the high genetic diversity index [36]. The broad range of Dice similarity matrix for both analyses indicated that the level genetic variability for 28 *Mangifera* accessions which comprised of landrace/cultivar/clone was influenced

by the environmental changes that occurred in the four sites. Furthermore, the clustering analysis showed that there were significant dissimilarities among the 28 *Mangifera* accessions originated from the Peninsular of Malaysia compared to Sarawak and Sabah regions. Karihaloo et al. [34] and Ravishankar et al. [35] reported that there is isolation within mango cultivars between North and South India using RAPD. This is in contrast to a report which stated the otherwise which claimed no separation between the mango cultivars from North India and South India using ISSR [7]. However, in another report, cumulative data using RAPD, ISSR, and DAMD methods have succeeded to cluster the mango cultivars into two different regions of India, eastern and northern regions [22].

The information on genetic diversity and genetic relationship of the *Mangifera* species is very important to be documented for proper identification of *Mangifera* species. This knowledge can be used as a tool for plant breeders to improve the strategies in breeding programmes as well as an initial effort to establish the conservation programme of the *Mangifera* species. Thus, an adequate study of local collections must be conducted before the accessions are lost, especially the wild relatives of *Mangifera* species. In order to prevent the loss, the farmers should be educated to maintain the valuable materials they possessed in their home garden or orchards. Therefore, consideration should be made to keep these accessions available in the germplasm collections for the future.

4. Conclusion

In summary, this study showed that ISSR are useful markers in genetic diversity studies based on the very high polymorphism level detected by the primers in the 28 *Mangifera* accessions collected. The ISSR markers are also suitable for determining the genetic similarity among the *Mangifera* species which are able to group them accordingly based on their origins, Peninsular and eastern of Malaysia. It is also recommended that these *Mangifera* accessions need to be conserved on farm and also collected for germplasm collection for conservation programmes. Perhaps these accessions may become useful as a genetic material in the breeding research in the future, especially the wild relatives of *Mangifera* species which are capable of developing resistant factors to disease and pest of mango cultivation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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