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Research Article

Genetic diversity and relationship of mango and its wild relatives (*Mangifera* spp.) based on morphological and molecular markers[1]

Dindin Hidayatul Mursyidin[2]

ABSTRACT

Mango and its wild relatives (*Mangifera* spp.) are essential for future mango breeding, including preservation programs, because they provide many beneficial genes (agronomic traits), particularly those related to resistance to biotic and abiotic stressors. However, there is a limited understanding of the genetic diversity and relationships of this germplasm. This study aimed to determine the diversity and relationship between endemic mango and its wild relatives (*Mangifera* spp.) from Borneo Island, Indonesia, using leaf morphology and the internal transcribed spacer (ITS) region. Fifteen samples of *Mangifera*, covering 12 species, were used. Morphologically, the endemic *Mangifera* had a low diversity of only 0.22. Based on the ITS sequence, *Mangifera* endemic to Borneo had a high level of genetic diversity (0.069). In addition, this sequence had a total variable number of 215 bp, of which 110 bp were singleton sites, 89 informative parsimony and 41 indels. Phylogenetic analysis showed that *Mangifera* was grouped into three clusters for leaf morphological traits and four clades for the ITS region. In this case, the furthest relationship was pointed out by 'Hampalam' (*M. laurina*) and 'Tambusui' (*M. macrocarpa*), as well as by 'Rawa-Rawa' (*M. similis*) and 'Samputar' (*M. torquenda*). In contrast, the closest relationship was shown by 'Hambawang Damar' (*M. foetida*) and 'Hambawang Puntara' (*M. foetida*), including 'Samputar' (*M. torquenda*) and 'Pauh' (*M. quadrifida*). In particular, the common mango (*M. indica*) was closely related to 'Asam Buluh' and 'Hampalam' (*M. laurina*) and distantly related to 'Pauh' (*M. quadrifida*) and 'Rawa-Rawa' (*M. similis*).

KEYWORDS: Edible fruit, nuclear DNA, phylogenetic relationship.

RESUMO

Diversidade genética e relação entre a manga e seus parentes silvestres (*Mangifera* spp.) com base em marcadores morfológicos e moleculares

A manga e seus parentes silvestres (*Mangifera* spp.) são essenciais para o futuro melhoramento da fruta, incluindo programas de preservação, pois fornecem muitos genes benéficos (características agronômicas), particularmente aqueles relacionados à resistência a estressores bióticos e abióticos. No entanto, há uma compreensão limitada da diversidade genética e das relações desse germoplasma. Objetivou-se determinar a diversidade e a relação entre a manga endêmica e seus parentes silvestres (*Mangifera* spp.) da Ilha de Bornéu, Indonésia, utilizando-se morfologia foliar e a região do espaçador interno transcrito (ITS). Quinze amostras de *Mangifera*, abrangendo 12 espécies, foram utilizadas. Morfologicamente, a *Mangifera* endêmica apresentou uma baixa diversidade de apenas 0,22. Com base na sequência ITS, a *Mangifera* endêmica de Bornéu apresentou um alto nível de diversidade genética (0,069). Além disso, essa sequência tinha um número variável total de 215 pb, dos quais 110 pb eram sítios singleton, 89 informativos para parcimônia e 41 indels. A análise filogenética mostrou que a *Mangifera* foi aglomerada em três grupos para características morfológicas foliares e quatro clados para a região ITS. Neste caso, a relação mais distante foi apontada por 'Hampalam' (*M. laurina*) e 'Tambusui' (*M. macrocarpa*), bem como por 'Rawa-Rawa' (*M. similis*) e 'Samputar' (*M. torquenda*). Em contraste, a relação mais próxima foi mostrada por 'Hambawang Damar' (*M. foetida*) e 'Hambawang Puntara' (*M. foetida*), incluindo 'Samputar' (*M. torquenda*) e 'Pauh' (*M. quadrifida*). Particularmente, a manga comum (*M. indica*) estava intimamente relacionada com 'Asam Buluh' e 'Hampalam' (*M. laurina*) e distantemente relacionada com 'Pauh' (*M. quadrifida*) e 'Rawa-Rawa' (*M. similis*).

PALAVRAS-CHAVE: Fruta comestível, DNA nuclear, relação filogenética.

INTRODUCTION

Mango (*Mangifera indica* L.), belonging to the Anacardiaceae family, is one of the most important fruit crops in the world (Azim et al. 2014, Jena & Chand 2021). According to FAO (2022), mango continued to be the most significantly traded commodity, in terms of exported quantities, in 2021, with an increase of approximately 3 %, or 75,000 tons from the previous year. Currently, mango

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is commercially grown in over 100 tropical and subtropical countries, e.g., India, Indonesia, China, Pakistan, Mexico, Brazil and Nigeria (Jena & Chand 2021, WPR 2023).

Indonesia is the second-leading mango producer in the world today, with a production of about 3.6 million tons (WPR 2023). This success is inseparable from the ideal climate and ample farmland to cultivate and harvest the crops (WPR 2023), including the variety of cultivars and the presence of wild relatives of mangoes with their unique characteristics (Anggraheni & Mulyaningsih 2021). Examples include *M. caesia* ('Binjai'), *M. foetida* ('Hambawang'), *M. odorata* ('Kueni') and *M. casturi* ('Kasturi') (Ariffin et al. 2015). Some species have been cultivated by local farmers, although not intensively, whereas others are wild in the forest (Ariffin et al. 2015).

The *Mangifera* genus has approximately 69 species globally, with 30 endemic to Indonesia (Hidayat et al. 2011, Anggraheni & Mulyaningsih 2021). According to Fitmawati et al. (2017), the high diversity of *Mangifera* is essential for future mango breeding programs. This is because wild mango relatives provide many beneficial genes (agronomic traits) for breeding, such as having good resistance to biotic and abiotic stressors (Fitmawati et al. 2017), as reported by Ledesma et al. (2017). However, their economic importance, genetic diversity and phylogenetic relationships are poorly understood (Salma et al. 2010) due to the complexity of vegetative and reproductive organs (Hidayat et al. 2011, Ariffin et al. 2015).

The key to success in mango breeding is determining the genetic diversity within and among species (Anggraheni & Mulyaningsih 2021). For years, morphological traits have been applied in many phylogenetic studies, including mango (Majumder et al. 2013, Mohamed et al. 2015, Toili et al. 2016, Fitmawati et al. 2020, Zhang et al. 2020). However, these traits have certain limitations due to being time consuming and strongly influenced by environmental conditions. Presently, molecular marker utilization is more comprehensive to support and strengthen the morphological data or facilitate the phylogenetic resolution of the germplasm (Fitmawati et al. 2017).

In general, molecular marker applications are fast, neutral and not influenced by environmental factors, unlike morphological traits (Ariffin et al. 2015). During the last few years, several molecular markers have been applied to address the genetic diversity and relationship between mango and wild relatives, e.g., random amplified polymorphic DNA or RAPD (Fitmawati et al. 2010, Anggraheni & Mulyaningsih 2021), microsatellite or inter simple sequence repeat (ISSR) (Ravishankar et al. 2011, Shamili et al. 2012, Nazish et al. 2017) and chloroplast DNA (cpDNA) markers (Fitmawati & Hartana 2010, Azim et al. 2014, Fitmawati et al. 2017, Rafidah et al. 2019).

This study aimed to determine the genetic diversity and relationship of endemic mango and its wild relatives (*Mangifera* spp.) from Borneo Island, Indonesia, using leaf morphology and internal transcribed spacer (ITS) region. According to Senavirathna et al. (2020), the ITS is the nuclear molecular marker that is useful in determining the genetic diversity and phylogeny of germplasm. This is due to the high mutation rate in this region (Lee et al. 2017). In addition, ITS provides universality and simplicity in its application and has been successfully applied in some plants, e.g., *Acanthopanacis* (Zhao et al. 2015), *Anoectochilus* (Chen & Shiau 2015, Thinh et al. 2020), *Dioscorea* (Purnomo et al. 2017), *Uncaria* (Zhu et al. 2018) and *Zanthoxylum* (Zhao et al. 2018, Suriani et al. 2021).

MATERIAL AND METHODS

The study was conducted at the University of Lambung Mangkurat, Indonesia, from November 2021 to May 2022. Covering 12 *Mangifera* species, 15 plant samples were used (Table 1). The samples were collected from eight locations, being six in South Borneo and two in Central Borneo, Indonesia (Figure 1). All leaf samples were taken to the laboratory to be morphologically and molecularly prepared. Morphological characterization was performed using leaf characteristics only (IPGRI 2006, Hasim et al. 2016).

Molecular characterization begins with DNA extraction using leaf samples and the Geneaid commercial kit (GP100, UK). In this stage, 50 g of leaf samples were crushed and prepared according to the manufacturer's instructions until pure DNA was obtained. Before amplification, DNA (genome) samples were quantified using spectrophotometric methods. DNA amplification was performed using a PCR machine (MultiGene Optimax, Labnet

Table 1. Samples of the *Mangifera* used in this study, including local names and origins.

Local name	Species	Code	Origin
'Rawa-Rawa'	*Mangifera similis*	M1	Balangan, South Kalimantan
'Tambusui'	*Mangifera macrocarpa*	M2	Balangan, South Kalimantan
'Kasturi'	*Mangifera casturi*	M3	Hulu Sungai Selatan, South Kalimantan
'Samputar'	*Mangifera torquenda*	M4	Muara Teweh, Central Kalimantan
'Asam Tungku'	*Mangifera pajang*	M5	Palangka Raya, Central Kalimantan
'Kueni'	*Mangifera odorata*	M6	Hulu Sungai Tengah, South Kalimantan
'Tandui'	*Mangifera rufocostata*	M7	Balangan, South Kalimantan
'Asam Buluh'	*Mangifera laurina*	M8	Hulu Sungai Selatan, South Kalimantan
'Pauh'	*Mangifera quadrifida*	M9	Hulu Sungai Selatan, South Kalimantan
'Binjai'	*Mangifera caesia*	M10	Tabalong, South Kalimantan
'Kasturi Mawar'	*Mangifera casturi*	M11	Banjar, South Kalimantan
'Hambawang Damar'	*Mangifera foetida*	M12	Hulu Sungai Tengah, South Kalimantan
'Mangga Madu'	*Mangifera indica*	M13	Banjarbaru, South Kalimantan
'Hambawang Puntara'	*Mangifera foetida*	M14	Banjar, South Kalimantan
'Hampalam'	*Mangifera laurina*	M15	Banjarbaru, South Kalimantan

International Inc., Nort Carolina, USA) with a total volume of 25 µL and the following reaction conditions: initial denaturation (94 ºC for 5 min); denaturation (94 ºC for 30 s); annealing (48 ºC for 30 s); extension (72 ºC for 45 s); and final extension (72 ºC for 7 min) (Mursyidin et al. 2021). The PCR reaction consisted of 22.0 µL PCR mix [containing DNA polymerase, dNTP and $MgCl_2$ (Bioline, USA), DNA template (2 µL) and primary DNA (1 µL; 10 µM)]. The primers used in the study were for ITS: forward (5'-TCGTAACAAGGTTTCCGTAGGTG-3) and reverse (5'-TCCTCCGCTTATTGATATGC-3'). The amplification results were visualized using agarose gel electrophoresis (2 %) and a UV transilluminator, and then documented with a digital camera. Sequencing was carried out at 1st Base Ltd. (Malaysia), using the Sanger method, bi-directionally, with an ABI PRISM 377 DNA sequencer (Applied Biosystems, Massachusetts, USA).

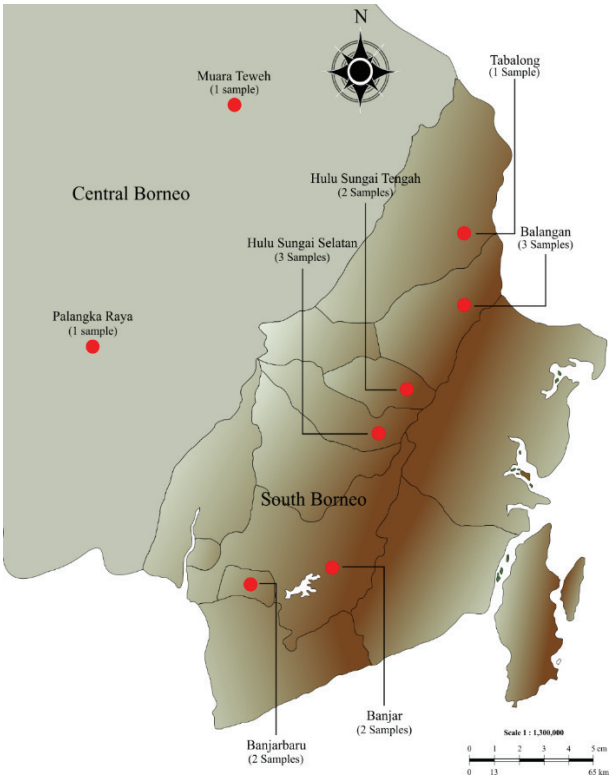

Figure 1. Map of sampling location, where 15 samples of *Mangifera* were collected, including South and Central Kalimantan (Borneo), Indonesia. See Table 1 for detailed information on sample identity.

The leaf morphological data were analyzed using a multivariate approach in MVSP version 3.1 (Kovach 2007). The Shannon diversity index (*H'*) was applied to determine the genetic diversity of this germplasm, with high ($H' > 0.60$), moderate ($0.40 \leq H' \leq 0.60$) and low ($H' < 0.40$) criteria (Mursyidin & Khairullah 2020). For the molecular analysis, the ITS regions were aligned and analyzed using the MEGA 11 software (Tamura et al. 2021), to determine the genetic diversity, GC content, variable sites (including informative parsimony and singleton sites) and phylogenetic relationships. In this case, genetic diversity was carried out using the nucleotide diversity index (π) method (Nei & Li 1979). Meanwhile, the genetic relationship was reconstructed using the unweighted pair group method with arithmetic average (UPGMA) and

maximum likelihood (ML) methods (Lemey et al. 2009). The dendrogram and phylogram were then evaluated with bootstrap statistics (1,000 replicates) and confirmed using principal component analysis or PCA (Mursyidin et al. 2022). Tree diagrams were evaluated visually (Baum 2008).

RESULTS AND DISCUSSION

Morphologically, endemic *Mangifera* has a unique leaf shape. In general, this germplasm showed six different leaf shapes: elliptical, lanceolate, linear, oblong-lanceolate, oblong and ovate (Figure 2). In this case, unique leaves were shown by 'Tambusui' (*M. macrocarpa*) with the linear form and 'Binjai' (*M. caesia*) with the oblong-lanceolate form (see samples M2 and M10, respectively, in Figure 2). Based on leaf texture, most *Mangifera* leaves were coriaceous, except for membranous for 'Tambusui' (*M. macrocarpa*). In addition, five *Mangifera* samples, including 'Tambusui' (*M. macrocarpa*), 'Kasturi' and 'Kasturi Mawar' (*M. casturi*), 'Samputar' (*M. torquenada*) and 'Pauh' (*M. quadrifida*), had leaf pubescence or a glabrous character (Table 2). Other

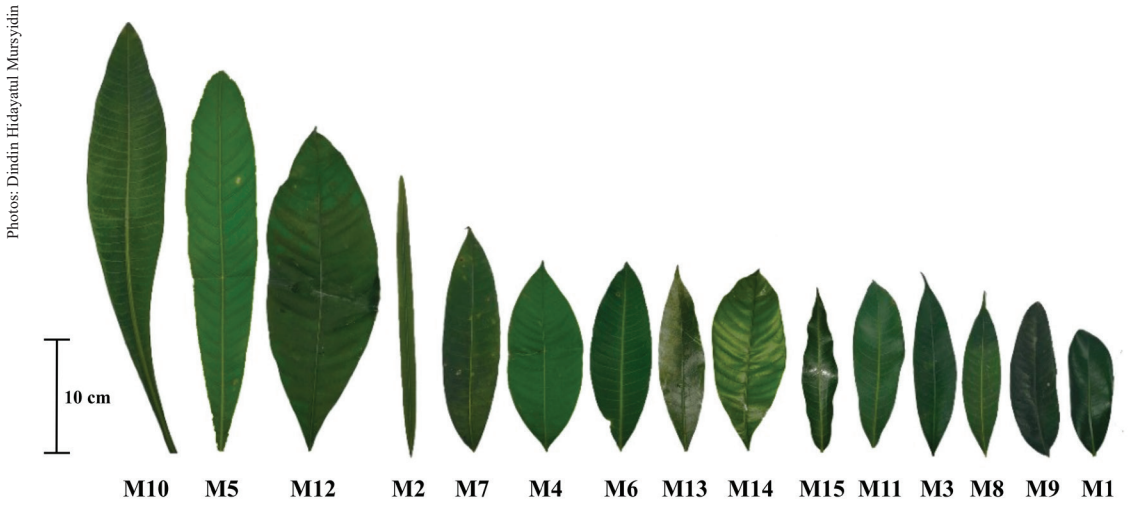

Figure 2. Leaf morphology of endemic mango and wild relatives (*Mangifera* spp.) from Borneo, Indonesia, showing unique difference forms, from oblong-lanceolate (M10), linear (M2) to elliptical (M1). Detailed information on these leaf characteristics is provided in Table 2.

Table 2. Morphological leaf characteristics of *Mangifera* endemic to Borneo, Indonesia.

Local name	Species	Code	Leaf characters						
			Leaf blade shape	Leaf venation	Leaf texture	Leaf apex shape	Leaf base shape	Leaf margin	Leaf pubescence
'Rawa-Rawa'	*M. similis*	M1	Elliptical	Medium	Coriaceous	Obtuse	Round	Wavy	Absent
'Tambusui'	*M. macrocarpa*	M2	Linear	Wide	Membranous	Obtuse	Acute	Entire	Present
'Kasturi'	*M. casturi*	M3	Lanceolate	Medium	Coriaceous	Acuminate	Acute	Entire	Present
'Samputar'	*M. torquenda*	M4	Ovate	Medium	Coriaceous	Acuminate	Obtuse	Entire	Present
'Asam Tungku'	*M. pajang*	M5	Oblong	Medium	Coriaceous	Obtuse	Acute	Entire	Absent
'Kueni'	*M. odorata*	M6	Ovate	Wide	Coriaceous	Acute	Acute	Entire	Absent
'Tandui'	*M. rufocostata*	M7	Lanceolate	Wide	Coriaceous	Acuminate	Obtuse	Entire	Absent
'Asam Buluh'	*M. laurina*	M8	Lanceolate	Medium	Coriaceous	Acuminate	Acute	Entire	Absent
'Pauh'	*M. quadrifida*	M9	Elliptical	Medium	Coriaceous	Obtuse	Round	Wavy	Present
'Binjai'	*M. caesia*	M10	Oblong-lanceolate	Wide	Coriaceous	Acute	Acute	Entire	Absent
'Kasturi Mawar'	*M. casturi*	M11	Elliptical	Medium	Coriaceous	Acute	Acute	Wavy	Present
'Hambawang Damar'	*M. foetida*	M12	Ovate	Medium	Coriaceous	Acute	Acute	Wavy	Absent
'Mangga Madu'	*M. indica*	M13	Lanceolate	Medium	Coriaceous	Acuminate	Acute	Entire	Absent
'Hambawang Puntara'	*M. foetida*	M14	Ovate	Medium	Coriaceous	Acute	Acute	Wavy	Absent
'Hampalam'	*M. laurina*	M15	Lanceolate	Medium	Coriaceous	Acuminate	Obtuse	Wavy	Absent

leaf characteristics of this germplasm are provided in Table 2.

According to Kole (2021), most *Mangifera* leaves are oblong, as in *M. indica*, *M. laurina* and *M. foetida*. According to Zumajo-Cardona et al. (2019), the leaf morphology may correspond to evolution through independent processes, such as branching, planation, webbing and overtopping. Based on these traits, this germplasm has low diversity (Table 3).

Based on the ITS sequence, *Mangifera* endemic to Borneo had a genetic diversity of 0.069 (Table 4). According to Jagadeesh et al. (2018), the value of such diversity is relatively high. The genetic diversity of *Mangifera* was also higher, if compared to other studies with similar markers, such as Soumnya & Nair (2017), who showed a genetic diversity of 0.035 in *Averrhoa*, and Haque et al. (2009), who found a genetic diversity of 0.039 in *Commiphora wightii*. A similar value of 0.068 was reported by Jagadeesh et al. (2018) for *Magnaporthe oryzae*. According to Mursyidin et al. (2021), a high genetic diversity in germplasm is closely related to mutations that occur in the sequences studied.

Table 3. Shannon diversity index of *Mangifera* leaf characteristics.

Character	H' Index*	Criteria**
Leaf blade shape	0.15	Low
Leaf venation	0.22	Low
Leaf texture	0.34	Low
Leaf apex shape	0.35	Low
Leaf base shape	0.14	Low
Leaf margin	0.32	Low
Leaf pubescence	n/a	Low
Average	0.22	Low

* Referred to the Shannon diversity index; ** high ($H' > 0.60$), moderate ($0.40 \leq H' \leq 0.60$) and low ($H' < 0.40$); n/a: not applicable.

Table 4. Genetic information of the internal transcribed space (ITS) sequence of *Mangifera* endemic to Borneo, Indonesia, using Kimura 2-parameter model.

Parameter	ITS
Range of sequence length (bp)	640-727
Total number of bases analyzed (*n*)	760
Bayesian information criteria (BIC)	5992.844
Akaike information criteria (AICc)	5807.212
Maximum likelihood value (ln*L*)	-2877.531
Nucleotide diversity (π)	0.069
Variable sites (bp)	215
Singleton sites (bp)	110
Parsimony informative sites (bp)	89
Indels (bp)	41
Transition/transversion bias values (*R*)	1.70
Transition/transversion ratio	1.84
GC content (%)	58.64

In this case, the ITS sequence of *Mangifera* had a total variable number of 215 bp, of which 110 bp were singleton sites, 89 were informative parsimony and 41 were indels (Table 4). Figure 3 shows more clearly the mutations that occurred. Based on this figure, substitutions were higher than indels. Furthermore, this sequence had a GC content of 58.64 %, with bias values and ratios of transition/transversion of 1.70 and 1.84, respectively.

According to Lee et al. (2017), the ITS region shows a high mutation rate. Compared to other studies, this number was higher than the results of Soumnya & Nair (2017) in the ITS *Averrhoa* region, with as many as 54 variable characteristics and 33 parsimony informative sites. Even in *Trichogramma*, the ITS region has only 14 variable sites (Viana et al. 2021). According to Drábková et al. (2009), the high mutation event on ITS is linked to hybridization. In this context, differences in ITS sequences may be met after hybridization and become homogenized after a time, but the latter may not be consistent among descendant lineages (Soltis & Soltis 2009).

Related to indels, this gene mutation is essential, since it determines which part of the protein is affected, and not all amino acids are necessary for a proper protein function (Rodriguez-Murillo & Salem 2013). According to Ludwig (2016), several mechanisms generate indels, e.g., complete and partial chromosomal duplication, proliferation of transposable elements, replication errors and unequal crossover. These diverse mutational mechanisms of indel production contribute to single locus and total DNA size variation in the genome. In this case, however, deletions were more frequent than insertions. Furthermore, the genomic prevalence of indels declines with length, and this decline is faster for insertions than deletions (Ludwig 2016).

Apart from mutation, genetic diversity is necessary for breeding and conservation programs. For plant breeding, genetic diversity is essential to promote the adaptability of populations to environmental changes and to preserve large gene pools for future genetic breeding (Govindaraj et al. 2015). In other words, knowledge on the genetic and population diversity of germplasm collections serves

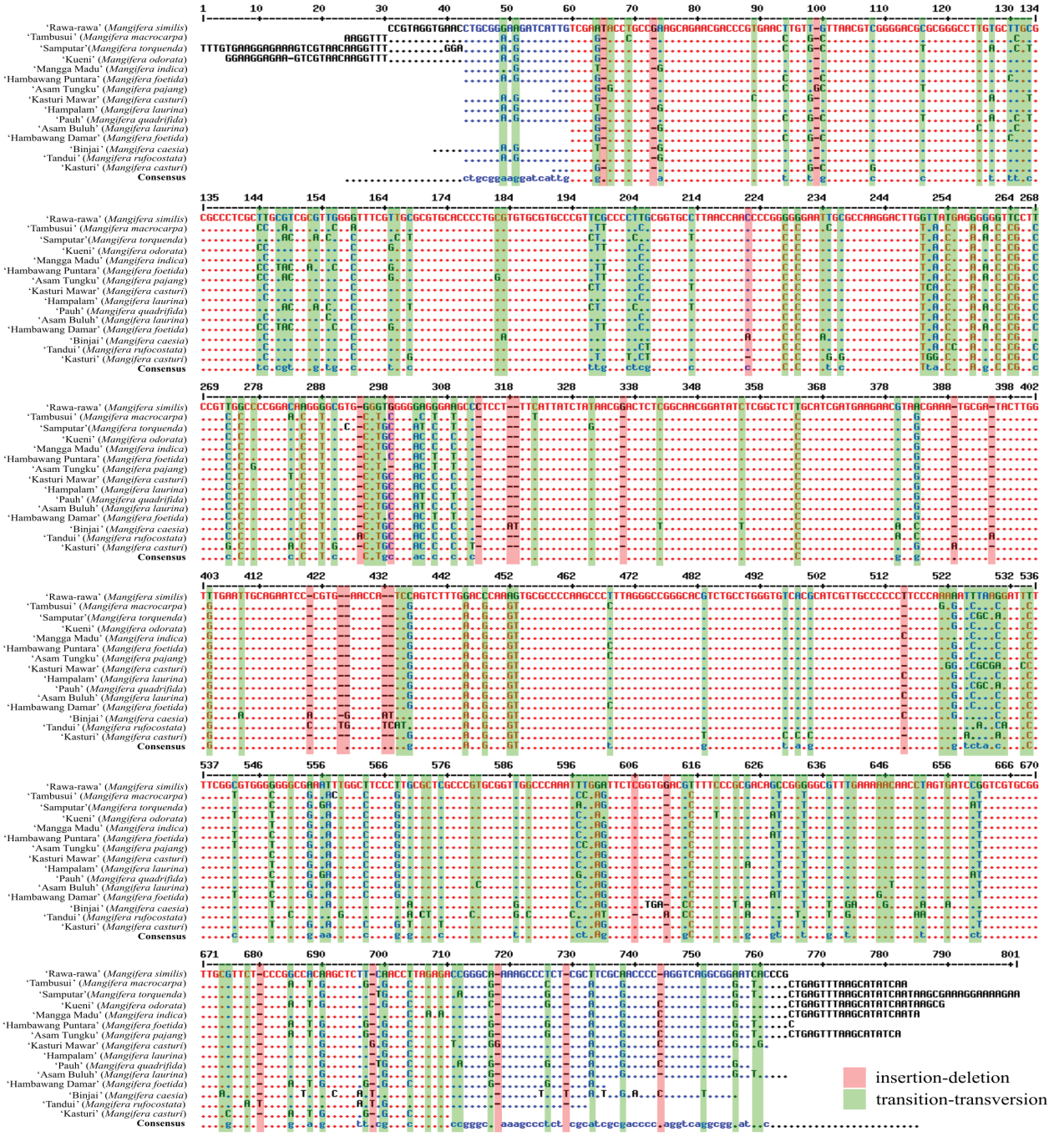

Figure 3. Multiple sequence alignment showing some mutational events on the internal transcribed spacer (ITS) region in *Mangifera* endemic to Borneo, Indonesia.

as a solid foundation for crop breeding (Dwivedi et al. 2017). In this case, defining the population diversity within the germplasm is beneficial to avoid false associations while performing association mapping studies (Jena & Chand 2021). Furthermore, knowledge on the genetic background of parents is a necessary start to developing new varieties endowed with high-yielding features of fruit and more adapted to constantly changing climatic conditions (Govindaraj et al. 2015).

For conservation, genetic diversity is critical to long-term survival, sustainable productivity and genetic enhancement of commercially profitable genotypes (Rachmat et al. 2016). In this context,

the assessment of the level of genetic diversity of a species or population helps to ascertain its current status and threat (Graudal et al. 2014). Thus, the information can provide a basis for adopting appropriate scientific management policies and devising effective conservation strategies (Jena & Chand 2021). In other words, information on this parameter is essential for the effective and efficient management of conservation and the prospective utilization of biodiversity in any crop species (Gavin et al. 2018). In particular, genetic diversity is an essential precursor in studying a species (Wu et al. 2020). This is because the range and magnitude of heterogeneity in the species or population greatly influence its evolutionary potential (Jena & Chand 2021).

In addition to genetic diversity, determining the phylogenetic relationship among cultivated varieties, including their wild relatives, is also necessary (Skuza et al. 2019). In this study, *Mangifera* was grouped into three clusters for morphological traits (Figure 4) and four clades for ITS (Figure 5). Different groupings were shown by the PCA analysis, where six groups were for morphological (Figure 6A) and seven for molecular (Figure 6B) traits. Following the similarity coefficient (Figure 7A), the furthest relationship is shown by 'Hampalam' (*M. laurina*) and 'Tambusui' (*M. macrocarpa*) at 0.095. The nearest is by popular mango (*M. indica*) and 'Asam Buluh' (*M. laurina*).

Based on its coefficient of divergence (Figure 7B), the furthest genetic relationship was shown by 'Rawa-Rawa' (*M. similis*) with 'Samputar' (*M. torquenda*), while the closest relationship was shown by 'Samputar' (*M. torquenda*) with 'Pauh' (*M. quadrifida*). In particular, the popular mango (*M. indica*) was closely related to *M. laurina*, i.e., 'Hampalam' and 'Asam Buluh', at divergence

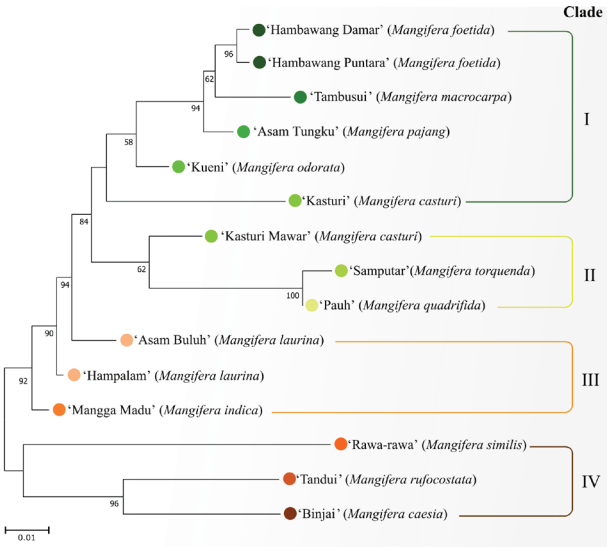

Figure 5. Phylogram showing the grouping of *Mangifera* endemic to Borneo, Indonesia, into four clades. The number on the node is a bootstrap value of 1,000 times (above 50).

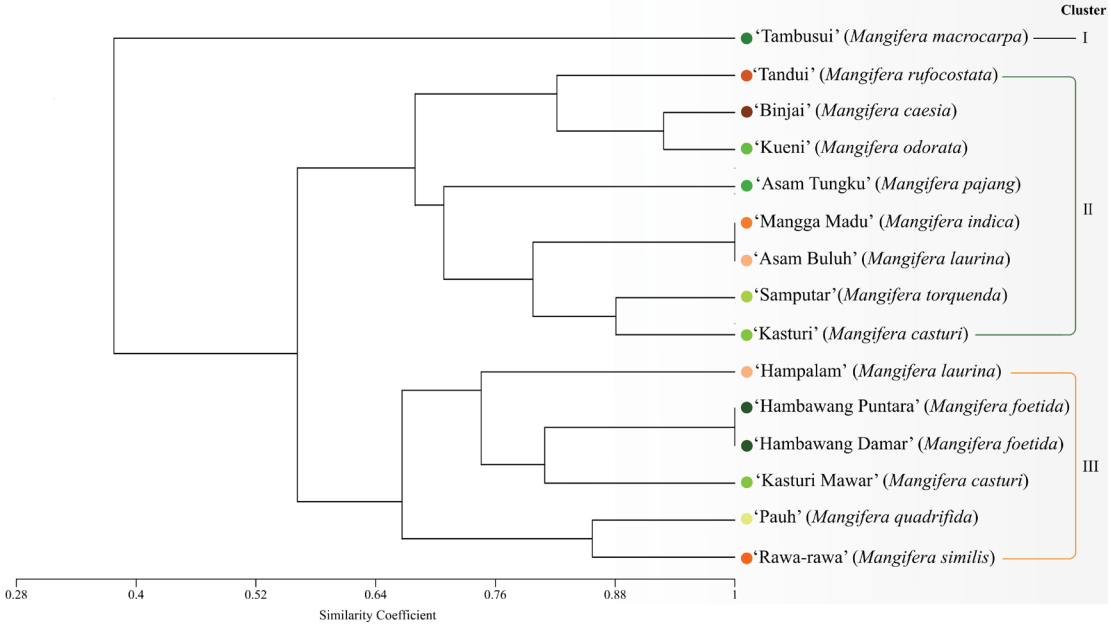

Figure 4. Dendrogram showing the grouping of *Mangifera* endemic to Borneo, Indonesia, into three clusters.

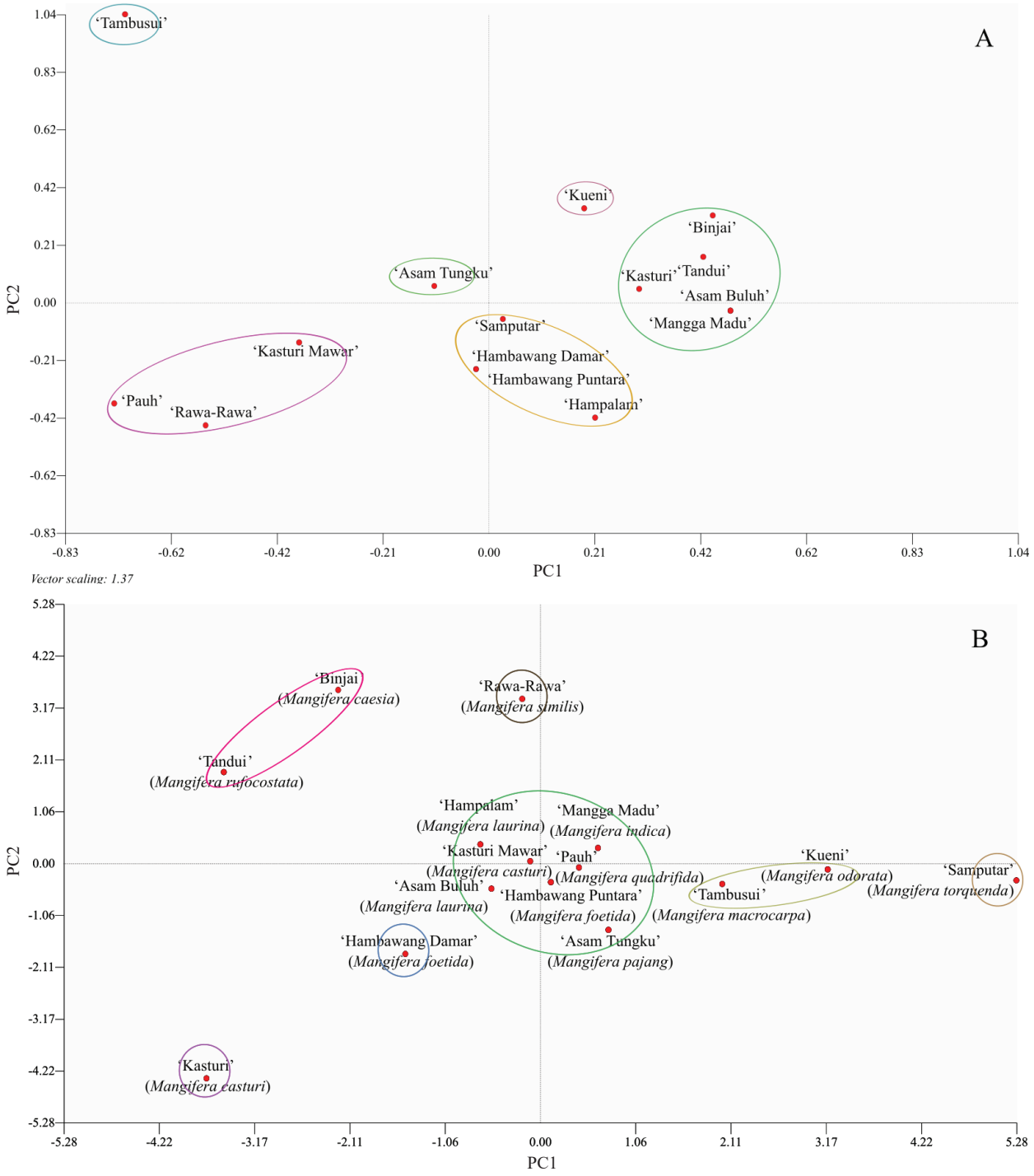

Figure 6. Principal component analysis (PCA) showing the grouping of *Mangifera* endemic to Borneo, Indonesia, into six groups for morphological traits (A) and seven for molecular traits (B). In this case, the total variability was 35.024 % for PC1 and 64.066 % for PC2.

coefficients of 0.010 and 0.022, respectively. However, this mango was distantly related to *M. similis* ('Rawa-Rawa'), with a divergence coefficient of 0.084 (Figure 7B).

Following the literature, *M. similis* has elliptical fruits about 75 mm long and 65 mm wide, sometimes even up to 100 mm in diameter (Atmoko et al. 2016). When ripe, the taste of this fruit tends to be

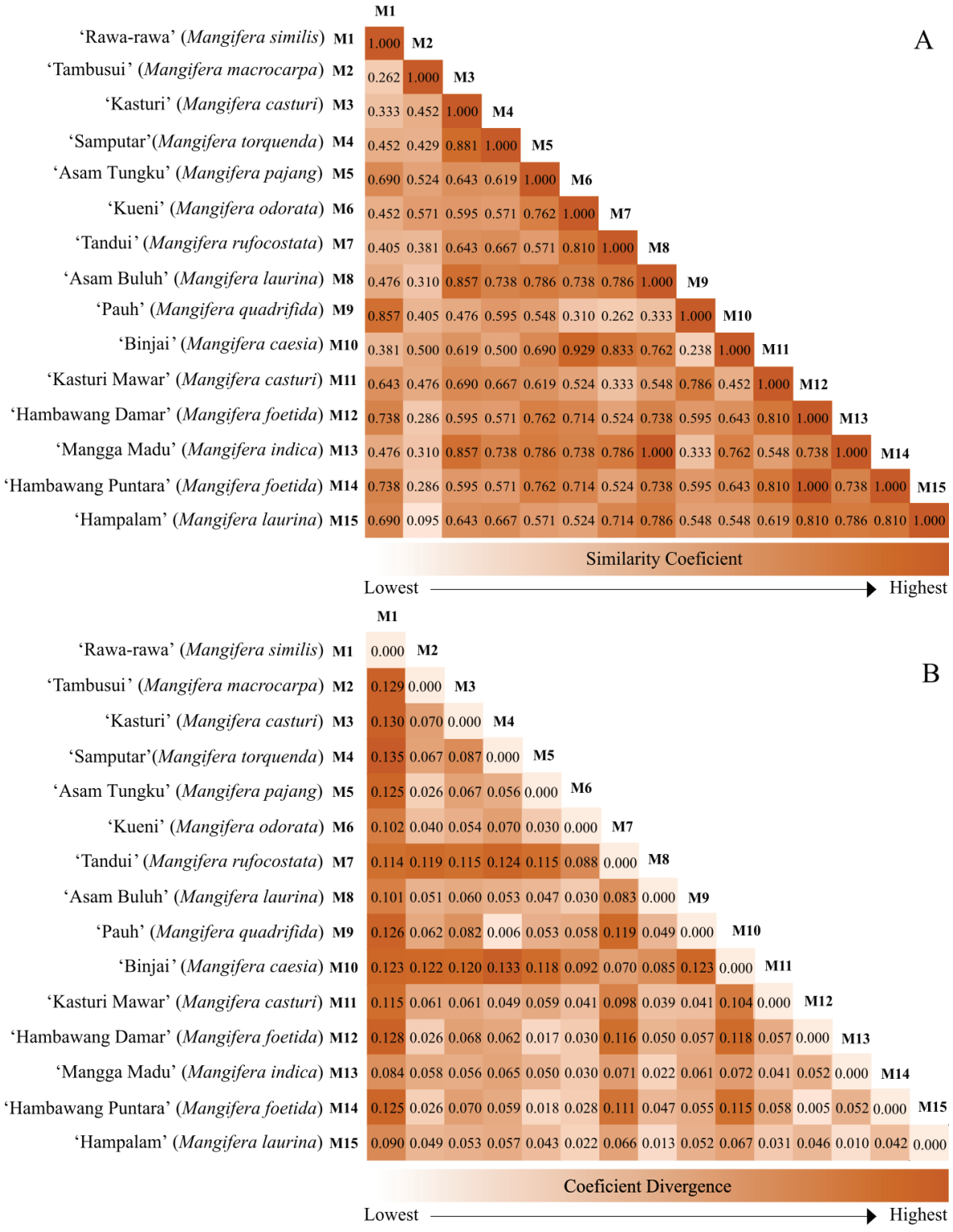

Figure 7. Heatmap showing the similarity coefficient (A) and genetic divergence (B), representing the genetic relationship for *Mangifera* endemic to Borneo, Indonesia.

sweet, with a slight sensation of refreshing sourness (Lim 2012a). Meanwhile, *M. laurina* has a drupe-like small mango, obliquely oblong, and a small fruit (Lim 2012b). While the fruit is less delicious because it is very sour, this species has a potential as a genetic resource, because it is resistant to the anthracnose

disease caused by *Colletotrichum gloeosporioides* (Verheij & Coronel 1991). In this case, interspecific hybridization between a popular mango (*M. indica*) and its wild relatives, e.g., *M. rubrapetala* ('Raba'), *M. casturi* ('Kasturi') and *M. lalijiwa* (honey mango), has been reported by Ledesma et al. (2017).

In short, wild relatives provide many essential genes that are beneficial in breeding programs, such as resistance to pests and diseases (Migicovsky & Myles 2017). Thus, well-to-type multiplication for the preservation of endemic mango populations is essential to maintain the diversity present in local landraces, to prevent the extinction of elite genotypes available in these areas, and to reduce the risk of loss of desired characteristics (such as fruit quality) due to uncontrolled depression of natural inbreeding (Jena & Chand 2021).

CONCLUSIONS

Based on leaf morphology, endemic mango (*Mangifera* spp.) from Borneo, Indonesia, had a low diversity. In contrast, following the internal transcribed spacer (ITS) region, this germplasm had a high level of genetic diversity. The phylogenetic analysis showed that *Mangifera* was grouped into three clusters for morphological traits and four clades for molecular traits (ITS). In this case, the furthest relationships were between 'Hampalam' (*M. laurina*) and 'Tambusui' (*M. macrocarpa*) and between 'Rawa-Rawa' (*M. similis*) and 'Samputar' (*M. torquenda*). In contrast, the closest relationships were shown for 'Hambawang Damar' and 'Hambawang Puntara' (*M. foetida*), and for 'Samputar' (*M. torquenda*) and 'Pauh' (*M. quadrifida*).

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