Research Article

Genetic diversity of cultivated mangosteen and its wild relatives (*Garcinia* spp.) based on leaf morphology and molecular markers[1]

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ABSTRACT

The mangosteen (*Garcinia mangostana* L.) germplasm still has limitations in fruit quality, drought tolerance and susceptibility to pests or diseases. This study investigated the genetic diversity and relationships of mangosteen with its wild relatives (*Garcinia* spp.) based on leaf morphology and the internal transcribed spacer (ITS) region, including its secondary structure. Based on leaf morphology, the mangosteen and its wild relatives generally showed a low genetic diversity. However, the leaf texture and pubescence had a high genetic diversity (0.71 and 0.77, respectively). Furthermore, based on the ITS markers, the genetic diversity of *Garcinia* at the interspecies level was much higher than that at the intraspecies one (0.043 and 0.005, respectively). The unweighted pair group method with the arithmetic average (UPGMA) revealed that mangosteen is grouped into four main clusters, with 'Manggis Banjar' and 'Palembang' in the same cluster. Similarly, the ITS positioned *Garcinia* into several clades, with 'Manggis Banjar', 'Kandangan' and 'Palembang' grouped into a similar clade. The biochemical reconstruction showed that *Garcinia* has unique ITS secondary structures, i.e., ring and four-helix models. Even though the cultivated mangosteen and its wild relatives had low diversity based on leaf morphology, the ITS markers showed a high genetic diversity. Furthermore, the reconstruction of the ITS secondary structure has supported this germplasm's phylogenetic tree.

KEYWORDS: *Garcinia mangostana* L., internal transcribed spacer, phylogenetic analysis.

RESUMO

Diversidade genética de mangostão cultivado e seus parentes silvestres (*Garcinia* spp.) com base na morfologia foliar e marcadores moleculares

O germoplasma do mangostão (*Garcinia mangostana* L.) ainda apresenta limitações à qualidade dos frutos, tolerância à seca e suscetibilidade a pragas ou doenças. Objetivou-se investigar a diversidade genética e as relações do mangostão com seus parentes silvestres (*Garcinia* spp.), com base na morfologia foliar e na região do espaçador interno transcrito (ITS), incluindo sua estrutura secundária. Segundo a morfologia foliar, o mangostão e seus parentes silvestres geralmente apresentaram baixa diversidade genética. Entretanto, a textura e a pubescência foliar mostraram alta diversidade genética (0,71 e 0,77, respectivamente). Além disso, com base nos marcadores ITS, a diversidade genética de *Garcinia* no nível interespécies foi muito maior do que no intraespécies (0,043 e 0,005, respectivamente). O método de grupos de pares não ponderados com a média aritmética (UPGMA) revelou que o mangostão é agrupado em quatro grupos principais, com 'Manggis Banjar' e 'Palembang' no mesmo cluster. Da mesma forma, o ITS posicionou *Garcinia* em vários clados, com 'Manggis Banjar', 'Kandangan' e 'Palembang' agrupados em um clado semelhante. A reconstrução bioquímica mostrou que *Garcinia* dispõe de estruturas secundárias únicas de ITS, ou seja, modelos de anel e quatro hélices. Embora o mangostão cultivado e seus parentes silvestres tenham apresentado baixa diversidade com base na morfologia foliar, os marcadores ITS mostraram alta diversidade genética. Além disso, a reconstrução da estrutura secundária do ITS deu suporte à árvore filogenética deste germoplasma.

PALAVRAS-CHAVE: *Garcinia mangostana* L., espaçador interno transcrito, análise filogenética.

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is a flowering plant whose fruit is edible and favored by most people worldwide (Seethapathy et al. 2018). This is because apart from the taste of the fruit, some parts of the plant can also be used for other needs, especially for medicine or as a source of medicinal raw materials. For example, the rind of mangosteen is rich in xanthones and can be an anticancer, antibacterial, anti-inflammatory, antioxidant and antiviral agent (Hazarika &

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Lalnunsangi 2019, Wee et al. 2022). Mangosteen has traditionally been used by Asian society to treat various diseases, such as diabetes, jaundice, obesity and liver (Gogoi et al. 2021).

Concerning its potential, it is unsurprising that mangosteen has high economic value and has even become a potential export commodity. In 2020, Indonesia was the largest mangosteen-producing country in the world, with a production of 270,110 tons (Maps of World 2023) and an export transaction value of up to 75.67 million U.S. dollars (SRD 2023). The export destinations for this fruit include four neighboring countries (Malaysia, Thailand, Vietnam and Hong Kong), the Middle East (Kuwait, Oman, Qatar, United Arab Emirates and Bahrain) and Europe (Denmark and France).

However, when examined more closely, the quality of Indonesian mangosteen fruit and plants still have limitations, including low fruit quality, unattractive tree characteristics, lack of drought tolerance and rootstock susceptibility to pests and diseases (Murthy et al. 2018). Moreover, because this fruit is apomictic and agamospermic (the development of fruit and seeds occurs without going through gamete fusion), the mangosteen shows a narrow genetic diversity. Therefore, the main essential activities are exploring and characterizing mangosteen germplasm and its wild relatives (Mursyidin & Maulana 2020).

According to Hazarika & Lalnunsangi (2019), among the various species of *Garcinia* around the world, there are about 40 species of mangosteen relatives whose fruits are edible and have superior genes to support genetic expansion or mangosteen breeding. These include *Garcinia atroviridis*, *G. hombroniana*, *G. indica*, *G. multiflora* and *G. pedunculata* (Hazarika & Lalnunsangi 2019). Specifically, in Indonesia, as many as 64 of 400 *Garcinia* spp. worldwide can be used in breeding programs (Seethapathy et al. 2018). Based on this number, 25 *Garcinia* spp. are found in Kalimantan; 22 species each are found in Sumatra and Sulawesi. The remainder occur in other islands, such as Java, Nusa Tenggara, Maluku and Papua (Mursyidin & Maulana 2020).

According to Acquaah (2015), analysis of genetic diversity and relationships is urgent to support plant genetic expansion (breeding) programs. These parameters can be estimated based on morphological, cytological, biochemical and even molecular (DNA sequence) approaches (Yu et al. 2022). This study aimed to investigate the genetic diversity and relationships of mangosteen and its wild relatives based on leaf morphology, internal transcribed spacer (ITS) regions and secondary structure.

Senavirathna et al. (2020) stated that the internal transcribed spacer (ITS) is valuable in determining the germplasm's genetic diversity and phylogeny. This is due to the region's high mutation rate (Lee et al. 2017). In addition, the ITS provides simplicity and universality in its application to some plants, e.g., *Acanthopanacis* (Zhao et al. 2015), *Anoectochilus* (Thinh et al. 2020), *Dioscorea* (Purnomo et al. 2017), *Uncaria* (Zhu et al. 2018) and *Zanthoxylum* (Zhao et al. 2018). Thus, the results provide beneficial information supporting the future preservation, cultivation and utilization of mangosteen in breeding programs.

MATERIAL AND METHODS

In total, 44 samples of mangosteen (*Garcinia mangostana*) and 33 of its wild relatives (*Garcinia* spp.) were used in this study. Twelve germplasm samples, covering ten *Garcinia* spp. (Table 1), were collected directly using the purposive sampling method in two regencies of South Kalimantan, Indonesia (Figure 1), in August 2023 or during the rainy season. The remaining samples were collected from GenBank. Morphological and molecular analyses were performed in this study. For the morphological analysis, seven leaf traits were observed based on the guidance of IPGRI (2003) and Hasim et al. (2016) (see Table 2 for details).

The leaf samples were prepared and extracted for molecular analysis using the commercial DNA extraction kit from Geneaid, UK (GP100), following the manufacturer's instructions. The DNA was then quantified spectrophotometrically and amplified using a PCR machine from Labnet International Inc., Madison, New Jersey, USA (MultiGene Optimax), with a total volume of 25 µL, consisting of 22.0 µL of PCR mix (Bioline, Memphis, Tennessee, USA), 2 µL of DNA template and 1 µL of primary DNA (10 µM). The PCR reaction was performed in three stages, as it follows: first, initial denaturation (94 ºC; 5 min); second, 35 cycles of denaturation (94 ºC; 30 s), annealing (48 ºC; 30 s) and extension (72 ºC; 45 s); third, final extension (72 ºC; 7 min)

Table 1. Samples of *Garcinia* used in this study, including local names, origins, internal transcribed spacer (ITS) sequence length and their genetic status.

Local name	Species	Sample code	Origin (regency)	ITS length (bp)	Genetic status
'Manggis Pantai'	*G. celebica*	G11	Hulu Sungai Selatan, South Kalimantan	716	Native
'Mundu'	*G. dulcis*	G14	Hulu Sungai Selatan, South Kalimantan	693	Native
'Mundar'	*G. forbesii*	G13	Hulu Sungai Selatan, South Kalimantan	708	Native
'Manggis Waku'	*G. latissima*	G12	Sulawesi	690	Introduction
'Tevakun'	*G. maingayi*	G3	Hulu Sungai Selatan, South Kalimantan	724	Native
'Manggis Banjar'	*G. mangostana*	G4	Balangan, South Kalimantan	729	Native
'Manggis Kandangan'	*G. mangostana*	G7	Hulu Sungai Selatan, South Kalimantan	729	Native
'Manggis Palembang'	*G. mangostana*	G9	Palembang, South Sumatra	709	Introduction
'Manggis Burung'	*G. porrecta*	G5	Hulu Sungai Selatan, South Kalimantan	709	Native
'Manggis Kancing'	*G. prainiana*	G6	Balangan, South Kalimantan	710	Native
'Manggis Pir'	*G. nervosa*	G10	Balangan, South Kalimantan	710	Native
'Asam Kandis'	*G. xantochymus*	G2	Balangan, South Kalimantan	707	Native

(Mursyidin et al. 2021). The following ITS primer sequences were used in this study: forward (5'-TCGTAACAAGGTTTCCGTGTG-3) and reverse (5'-TCCTCCGCTTATTGATATGC-3') (Liu et al. 2021). The DNA targets were visualized using 2 % agarose gel electrophoresis under a UV transilluminator. Finally, DNA targets were sequenced bi-directionally using the ABI PRISM 377 DNA sequencer from Applied Biosystems (Waltham, Massachusetts, USA) at Apical Scientific Sdn. Bhd. (Seri Kembangan, Selangor, Malaysia).

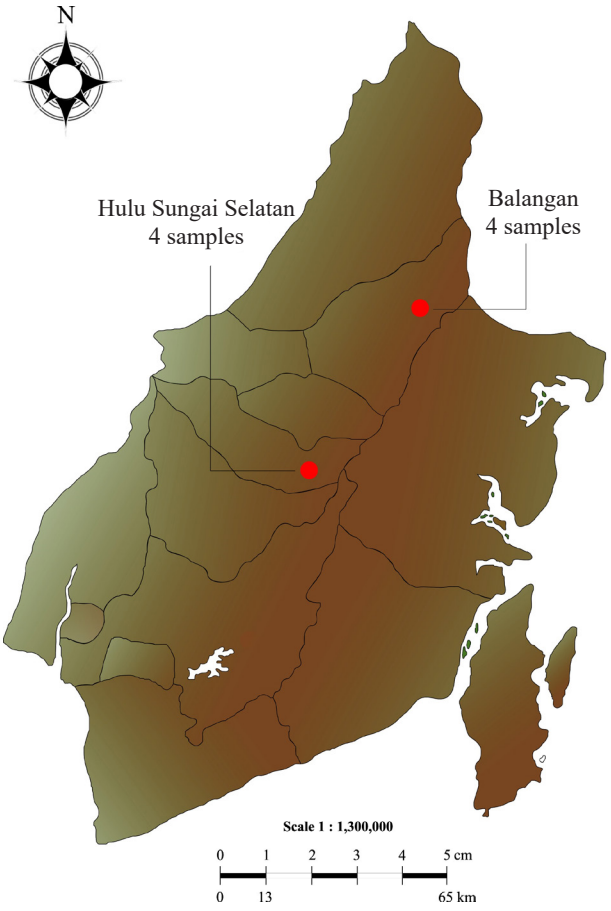

Figure 1. Map of South Kalimantan, Indonesia, where 12 samples of *Garcinia* were collected. For each sample, see Table 1 for details.

Analysis was carried out on the morphological and molecular data obtained. The analysis of morphological data began by tabulating leaf characteristic data (see Table 2) and then converting it into multivariate numbers. With the assistance of the MVSP ver. 3.1, the data were then standardized to determine the genetic diversity and relationships (Kovach 2007). In these cases, genetic diversity was determined based on the Shannon diversity index (*H'*), whereas the relationships were determined by the unweighted pair group method with the arithmetic average (UPGMA) (Mursyidin et al. 2022a). The sequences of ITS regions were first aligned for molecular data, and the resemblance was analyzed using the MEGA 11 software (Tamura et al. 2021). Subsequently, the genetic diversity, GC content and variable sites (including informative parsimony and singleton sites) were specified with the same software using the nucleotide diversity index (π) method (Nei & Li 1979). Phylogenetic analysis was carried out using the maximum likelihood (ML) method (Lemey et

al. 2009). The phylogram was then evaluated using bootstrap statistics (1,000 replicates) (Mursyidin et al. 2022a).

RESULTS AND DISCUSSION

Mangosteen and its wild relatives showed differences in leaf shape (Figure 2). Three mangosteen cultivars showed three leaf shapes: oblanceolate ('Manggis Kandangan'), elliptical ('Manggis Banjar') and cordate ('Manggis Palembang'). Meanwhile, the wild relatives of mangosteen showed two other leaf forms, namely oblong and lanceolate. Similarly, striking differences in the morphological characteristics of these leaves were shown by leaf apex shapes: acute, acuminate, obtuse and bristle-tipped. More details about some of these characteristics are shown in Table 2.

Based on leaf morphological characteristics, the mangosteen and its wild relatives generally showed low genetic diversity (Table 3). However, the leaf texture and leaf pubescence had a high genetic diversity (0.71 and 0.77, respectively). At the intraspecies level (Table 3), all characters showed a low level of diversity. In contrast, at the interspecies level, the leaf margin showed a high level of diversity, with an index value of 0.96 (Table 3). Furthermore, based on ITS markers, the genetic diversity of mangosteen at the intraspecies level was much lower than that of its wild relatives (interspecies) (0.005 and 0.043, respectively; Table 4).

Table 2. Leaf morphological characteristics of cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.).

Local name	Species	Code	Leaf characters						
			Leaf blade shape	Leaf venation	Leaf texture	Leaf apex shape	Leaf base shape	Leaf margin	Leaf pubescence
'Manggis Kandangan'	*G. mangostana*	G7	Oblanceolate	Medium	Membranous	Acute	Cuneate	Entire	Absent
'Manggis Banjar'	*G. mangostana*	G4	Elliptical	Medium	Coriaceus	Obtuse	Obtuse	Entire	Absent
'Manggis Palembang'	*G. mangostana*	G9	Cordate	Medium	Coriaceus	Bristle-tipped	Cordate	Entire	Absent
'Manggis Pir'	*G. nervosa*	G10	Oblong	Wide	Coriaceus	Bristle-tipped	Cordate	Entire	Absent
'Manggis Kancing'	*G. Prainiana*	G6	Elliptical	Medium	Coriaceus	Acute	Oblique	Entire	Absent
'Manggis Burung'	*G. porrecta*	G5	Oblong	Medium	Coriaceus	Acute	Cuneate	Entire	Absent
'Manggis Waku'	*G. latissima*	G12	Elliptical	Wide	Coriaceus	Obtuse	Cuneate	Undulate	Present
'Asam Kandis'	*G. xantochymus*	G2	Elliptical	Medium	Coriaceus	Bristle-tipped	Cuneate	Entire	Absent
'Mundar'	*G. forbesii*	G13	Elliptical	Narrow	Coriaceus	Acute	Cuneate	Entire	Absent
'Mundu'	*G. dulcis*	G14	Lanceolate	Narrow	Coriaceus	Acuminate	Cuneate	Entire	Absent
'Manggis Pantai'	*G. celebica*	G11	Oblanceolate	Medium	Coriaceus	Acute	Cuneate	Entire	Absent
'Tevakun'	*G. maingayi*	G3	Lanceolate	Medium	Coriaceus	Acute	Cuneate	Entire	Absent

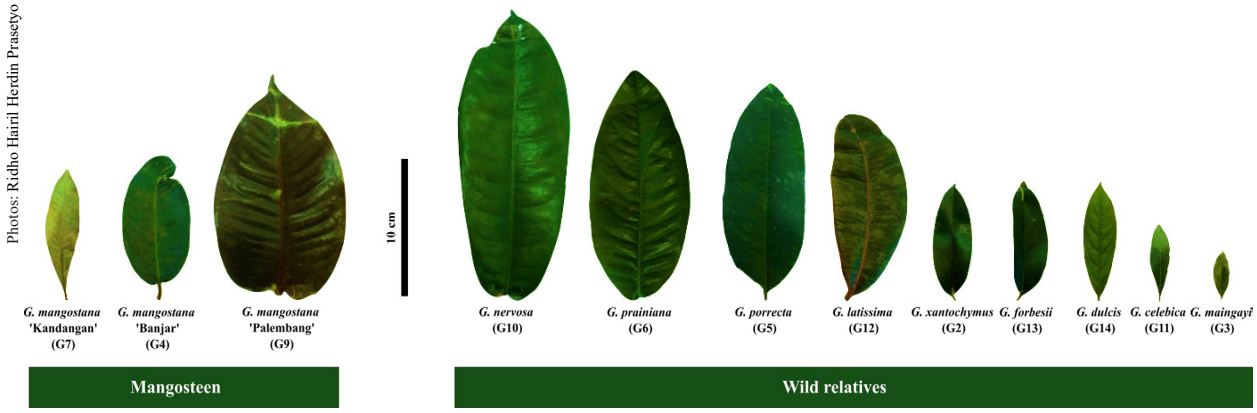

Figure 2. Leaf morphological differentiation of cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.).

According to Jackson et al. (2014), a high genetic diversity is necessary for environmental change. In this context, germplasm with a high genetic diversity is more resilient and adaptive to environmental changes than that with narrow or low genetic diversity. In other words, the high genetic diversity becomes an evolutionary signal for resolving relationships among plant germplasm at all taxonomic levels (Mursyidin et al. 2022b). Hence, this parameter is essential in evolution, especially in generating future founder populations (Dizkirici et al. 2010).

Conceptually, the emergence of potential genetic diversity is closely related to mutations in the target gene sequence or germplasm genome (Yusop et al. 2022). In this study, transition and transversion were the most common mutations in the *Garcinia* ITS sequences, including indels (Figure 3). The ITS region generally has a higher nucleotide substitution rate. Still, this gene often shows several insertions or deletions, which can directly or indirectly affect the stability of the structure and function of the protein produced (Mursyidin & Setiawan 2023).

Apart from the mutations occuring in the ITS region of *Garcinia*, it is important to broaden genetic diversity to improve agricultural quality and achieve various goals such as pest and disease resistance, drought, salinity and other abiotic stress tolerances, and higher quality and yield. Migicovsky & Myles (2017) stated that expanding the breeding pool to include wild relatives can provide a crucial new source of desirable traits in perennial crops. Witherup et al. (2019) stated that most wild relatives provide several unique genes for improving the genetic diversity of baseline populations before a bottleneck is present (Yan 2021).

Technically, various efforts can be made to increase the genetic diversity of mangosteen, including hybridization or crossing with wild relatives. For example, *G. celebica* has a male functionality gene for refining the mangosteen species (*G. mangostana* L.) (Sutthinon et al. 2018). In addition, the genetic expansion of mangosteen can also be done through introgression or mutagenesis (Allier et al. 2020). Introgression, or introgressive hybridization, is a long-term process in which genetic material is transferred from one species to another by the repeated backcrossing of an interspecific hybrid (Neale & Wheeler 2019).

Meanwhile, mutagenesis is a process by which the genetic information of an organism is changed

Table 3. Genetic diversity of mangosteen and its wild relatives (*Garcinia* spp.) based on leaf morphological characteristics.

Characteristics	Shannon index		
	Intraspecies (*G. mangostana*)	Interspecies (*Garcinia* spp.)	Entire
Leaf blade shape	0.00[a]	0.38[b]	0.12[b]
Leaf venation	0.16[b]	0.49[c]	0.17[b]
Leaf texture	0.27[b]	0.32[b]	0.71[d]
Leaf apex shape	0.25[b]	0.00[a]	0.16[b]
Leaf base shape	0.00[a]	0.16[b]	0.35[b]
Leaf margin	0.16[b]	0.96[d]	0.22[b]
Leaf pubescence	0.00[a]	0.30[b]	0.77[d]
Average	0.12	0.37	0.36

[a] No variation; [b] low; [c] moderate; [d] high.

Table 4. Molecular characteristics of the ITS sequences of mangosteen (*Garcinia mangostana*) and its wild relatives[1].

Parameter	Intraspecies (*G. mangostana*)	Interspecies (*Garcinia* spp.)
Sequence length (bp)	709-729	670-724
Polymorphic sites (*S*)	77	319
Akaike information criterion (AICc)	3,293.113	8,668.035
Bayesian information criterion (BIC)	4,010.445	10,019.492
Maximum likelihood value (ln*L*)	-1,560.316	-4,181.586
Transition/transversion bias value (*R*)	3.677	2.009
Guanine-cytosine/GC content (%)	52.25	51.94
Nucleotide diversity (π)	0.005	0.043
Tajima test of neutrality (D)	-2.831	-1.702

[1] Following the Kimura 2-parameter model.

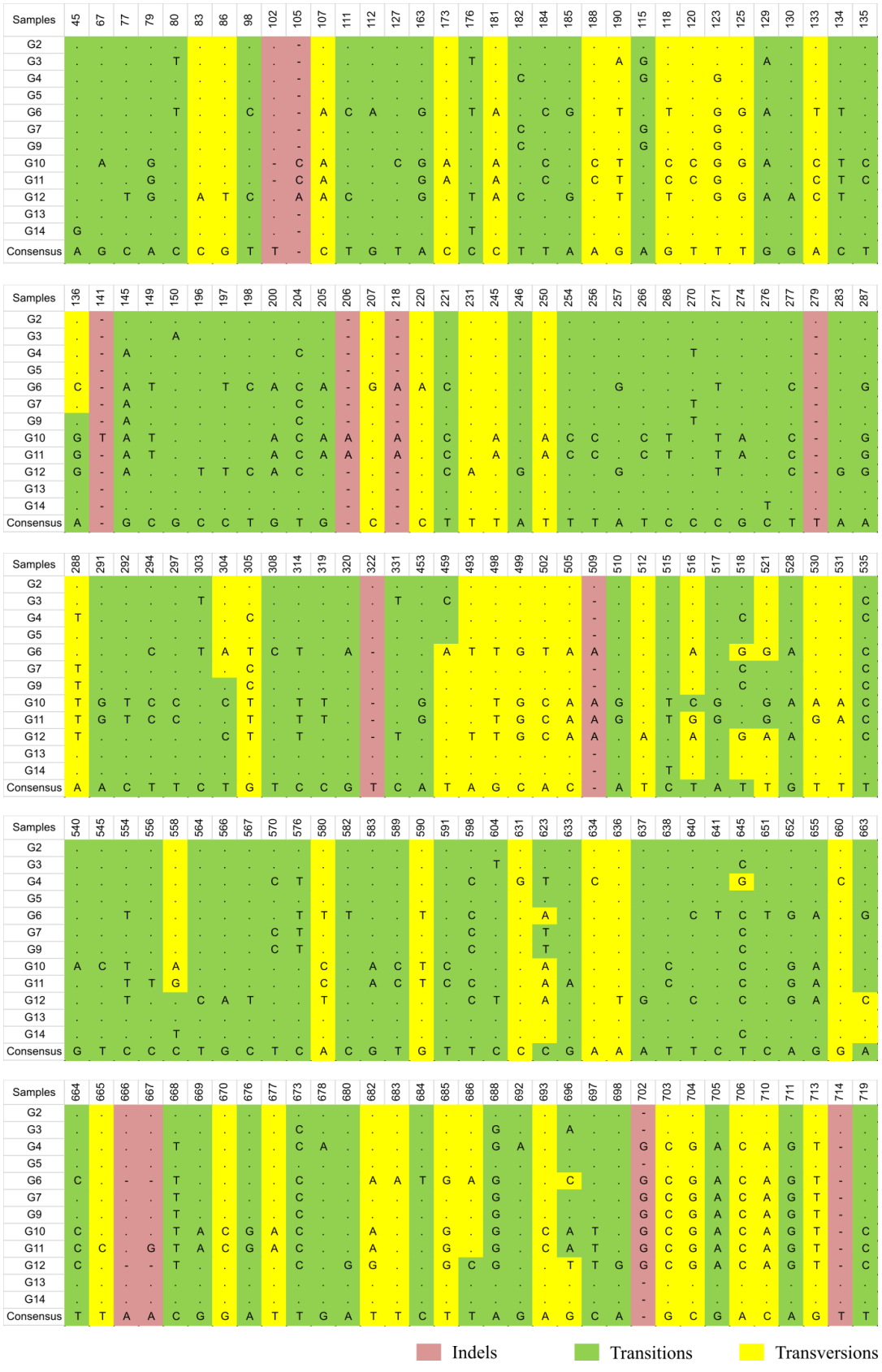

Figure 3. Polymorphism in the ITS region of cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.) from South Kalimantan, Indonesia, showing three mutation events: indels, transition and transversion.

by the production of a mutation, both spontaneously in nature or artificially, by mutation-causing agents (Zhang & Vijg 2018). This *Garcinia* germplasm has been bred to improve fruit quality, tree characteristics, drought tolerance and rootstock management. In this case, selections were made for 'Julu' (a mangosteen cultivar from the Philippines), which has larger fruits, seeds and more acidic pulp (Murthy et al. 2018). Similarly, the commercially available Malaysian cultivar 'Mesta', with smaller fruit size and seedless, was also developed for this goal (Murthy et al. 2018).

Based on leaf morphology, mangosteen is grouped into four main clusters, with 'Manggis Banjar' and 'Palembang' in the same cluster (III). 'Manggis Kandangan' was separated into cluster IV (Figure 4). 'Manggis Waku' (*G. latissima*) and 'Manggis Pir' (*G. nervosa*) were separated relatively far from other *Garcinia* samples, forming a cluster. As shown in Figure 5, the closest genetic relationship was shown by 'Tevakun' (*G. maingayi*) and 'Manggis Pantai' (*G. celebica*) and the farthest between 'Manggis Waku' (*G. latissima*) and 'Manggis Kandangan' (*G. mangostana*).

In terms of leaf morphology, ITS markers positioned the genetic relationship of *Garcinia* into several clades (Figure 6). As shown in Figure 6, all cultivated mangosteen (*G. mangostana*), namely 'Manggis Banjar', 'Kandangan' and 'Palembang', were grouped into one clade. Its wild relatives were spread among other clades. In this case, five mangosteen relatives, namely 'Tevakun' (*G. maingayi*), 'Mundu' (*G. dulcis*), 'Asam Kandis' (*G. xantochymus*), 'Manggis Gunung' (*G. porrecta*) and 'Mundar' (*G. forbesii*), were in the same clade. The other four *Garcinia* spp. were separated into two clades: 'Manggis Kancing' (*G. prainiana*) with 'Manggis Waku' (*G. latissimi*) and 'Manggis Pantai' (*G. celebica*) with 'Manggis Pir' (*G. nervosa*). Figure 7 shows more clearly the genetic distance between *Garcinia* samples based on ITS markers.

According to Mursyidin & Khairullah (2020), information on genetic relationships is indispensable in plant conservation and breeding programs. In this case, information about the furthest genetic relationship between parents is strongly considered to produce offspring with high or wide genetic diversity. Conversely, crossing parents with close genetic relationships tends to be avoided, as it can produce offspring with low or narrow genetic diversity (Acquaah 2015). In practice, based on the characteristics of male flowers (especially the petals color and the pistil presence and shape), fruit shape, color of the leaves and pattern of the lines on the glandular, *G. mangostana* was most similar to *G. malaccensis* (Nazre et al. 2018). This result was confirmed by Sobir et al. (2009) using isozyme and amplified fragment length polymorphism (AFLP) markers.

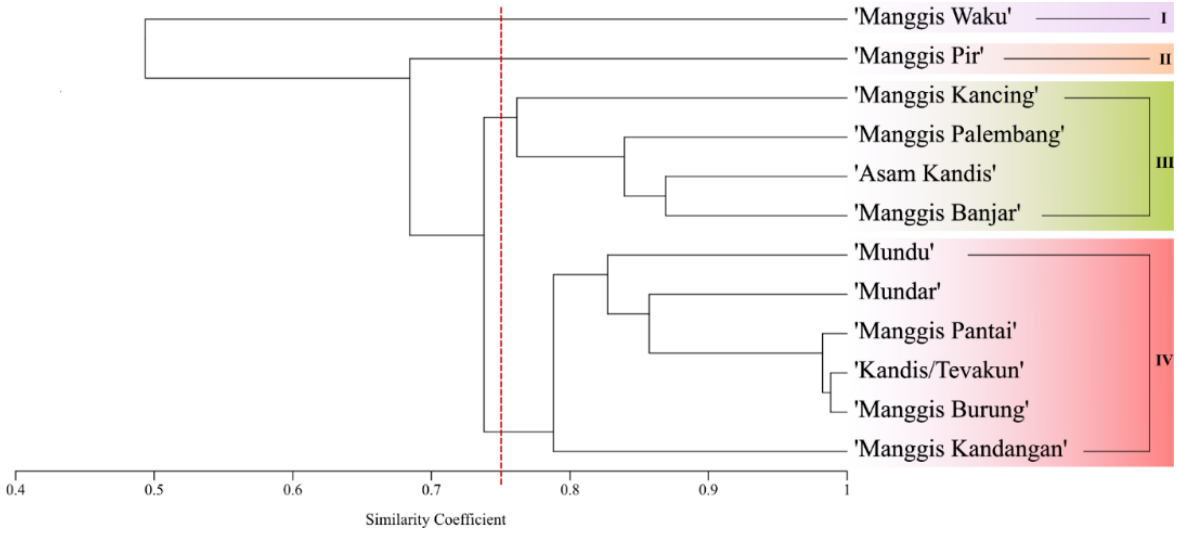

Figure 4. Dendrogram showing the genetic relationship between cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.) from South Kalimantan, Indonesia.

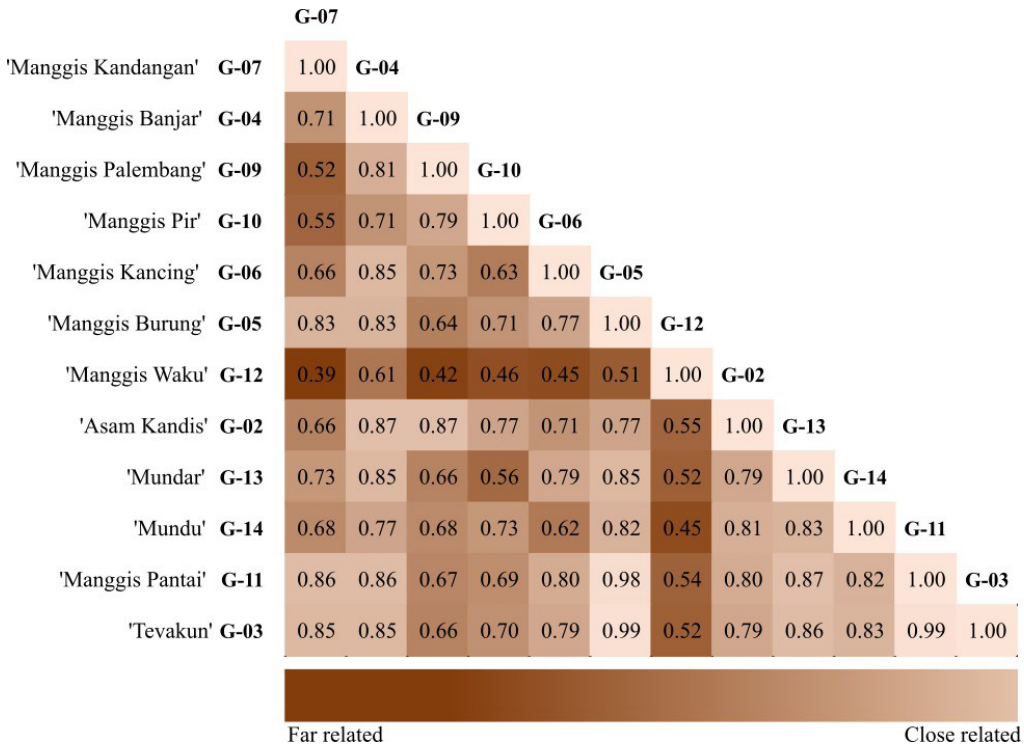

Figure 5. Genetic distance among cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.) from South Kalimantan, Indonesia, based on morphological markers.

However, based on biochemical markers, Mursyidin & Maulana (2020) reported closeness between mangosteen (*G. mangostana*) and *G. wightii* and the furthest relationship with *G. cylindrocarpa*. Based on isozyme markers, Sinaga et al. (2010) reported close relationships among *G. mangostana*, *G. malaccensis* and *G. hombroniana*. According to Sobir et al. (2009), *G. hombroniana* is the ancestor (progenitor) of *G. mangostana*.

Using inter simple sequence repeat (ISSR) markers, Sobir et al. (2011) reported the possibility of *G. malaccensis* as an allopolyploid derivative of *G. mangostana*. The results of Sulassih et al. (2013) also revealed the grouping of these three species based on morphological markers and ISSRs. They predicted that *G. malaccensis* and *G. celebia* were the ancestors of *G. mangostana*. Based on ITS markers, *G. mangostana* had the closest relationship with *G. penangiana* (Nazre 2014), *G. xanthochymus* (Parthasarathy et al. 2016) and *G. intermedia* (Liu et al. 2016).

Despite the importance of genetic diversity and relationships, the ITS region of the *Garcinia* sequence forms a unique secondary structure. In this study, *Garcinia* had a secondary structure in the ITS region with a four-helix or four-fingered hand pattern and ring models (Figure 8). The ring model is characterized by a radiating central and internal loop interconnected with unpaired nucleotides in the helices (Figure 8B). Meanwhile, the second model is characterized by the most extended stem of the ring model. Its two neighboring stems merge into a much longer stem (Figure 8A).

In the literature, the first model is mainly found in eukaryotic (Zhang et al. 2020) and angiosperm plant groups in general (Özgişi 2020), while the second model is found in vertebrates (Zhang et al. 2020). According to Xian et al. (2023), although the ITS region has a high nucleotide sequence variation, the secondary structure pattern of this region is constantly maintained (conserved). Thus, the results of its reconstruction can be used to strengthen the results of phylogenetic analysis (Nafisi et al. 2023). Jiménez-Gaona et al. (2023) stated that secondary structure results can improve the phylogenetic resolution obtained from the primary sequence and thus provide a tool for species delimitation.

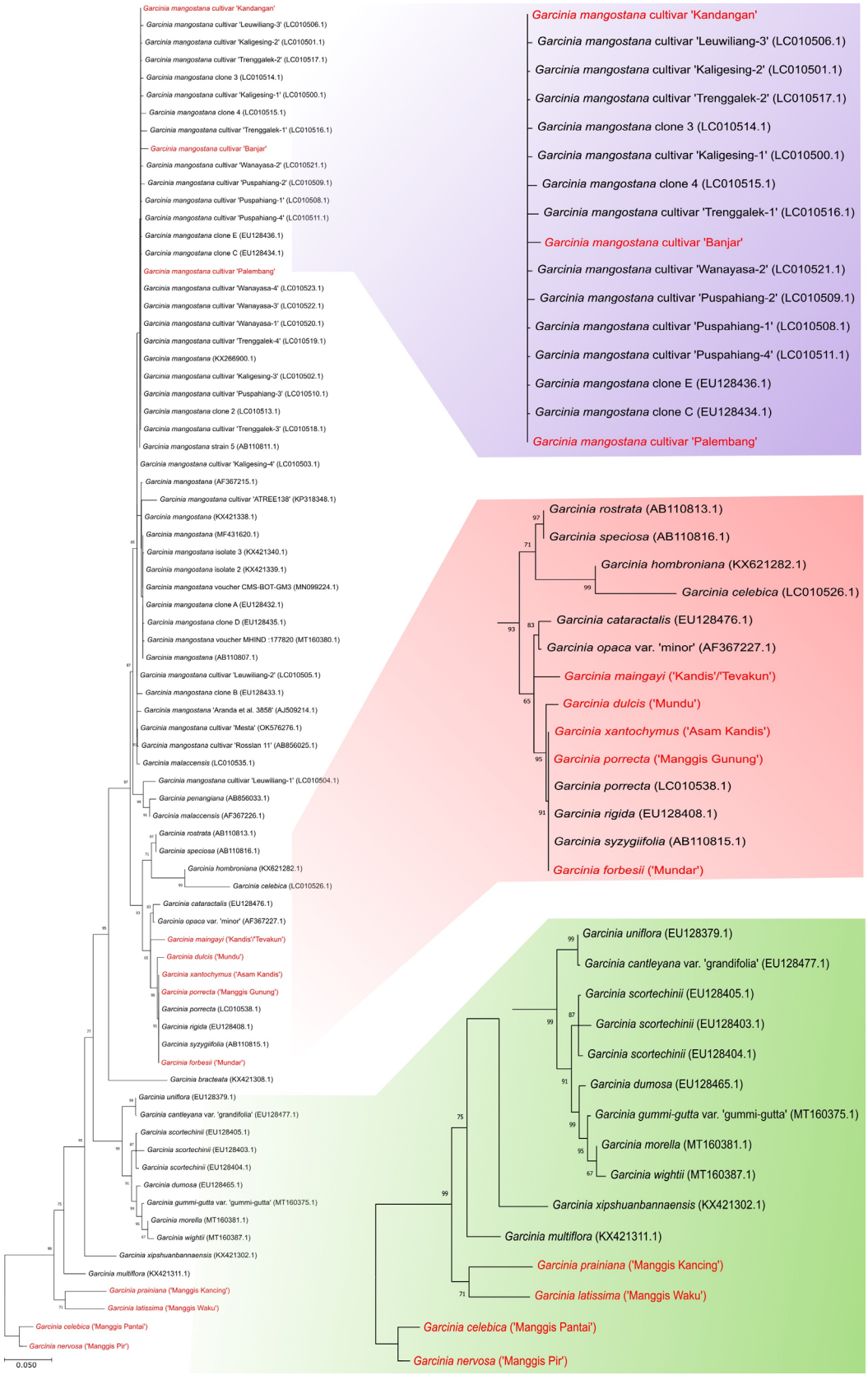

Figure 6. Phylogenetic position of cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.) from South Kalimantan, Indonesia, based on the ITS region.

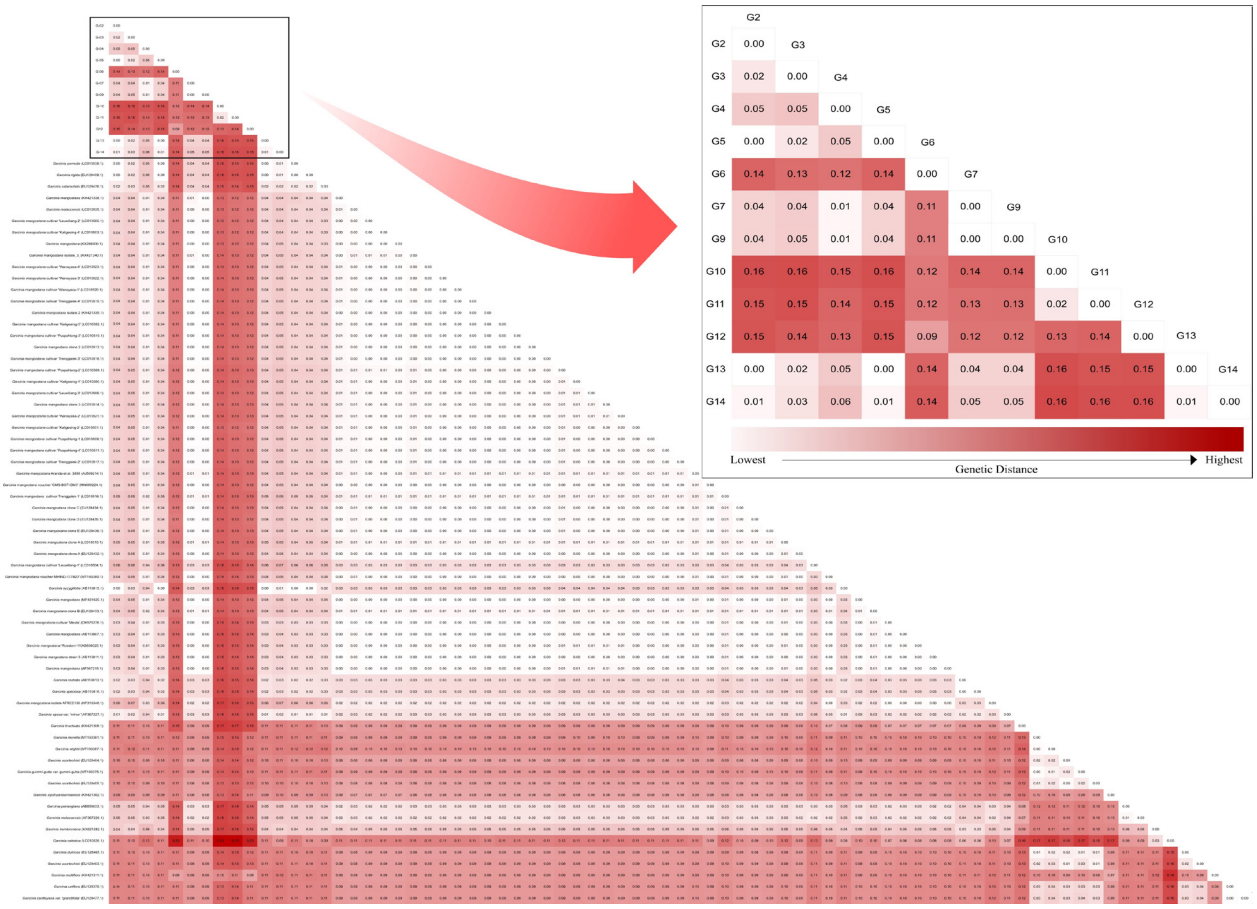

Figure 7. ITS region genetic distance of cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.) from South Kalimantan, Indonesia.

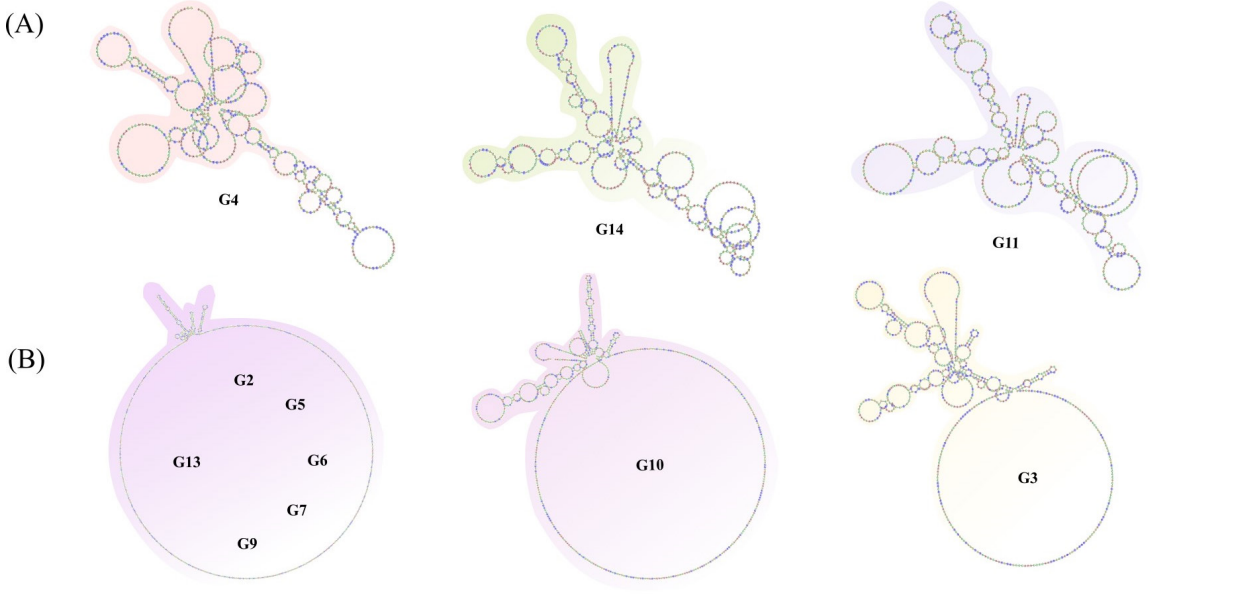

Figure 8. Model showing differences in the secondary structure of the ITS region from cultivated mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.) from South Kalimantan, Indonesia: A) four-helix model; B) ring model. G2-G14: *Garcinia* samples (see Table 1 for details).

CONCLUSIONS

1. Cultivated mangosteen and its wild relatives show a low diversity based on leaf morphology, but internal transcribed spacer (ITS) markers provide a high genetic diversity;
2. The reconstruction of the ITS secondary structure supports this germplasm's phylogenetic tree.

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REFERENCES

ACQUAAH, G. Conventional plant breeding principles and techniques. *In*: KHAYRI, J. M. (ed.). *Advances in plant breeding strategies*: breeding, biotechnology and molecular tools. Cham: Springer, 2015. p. 115-158.

ALLIER, A.; TEYSSÈDRE, S.; LEHERMEIER, C.; MOREAU, L.; CHARCOSSET, A. Optimized breeding strategies to harness genetic resources with different performance levels. *BMC Genomics*, v. 21, e349, 2020.

DIZKIRICI, A.; KAYA, Z.; CABI, E.; DOĞAN, M. Phylogenetic relationships of *Elymus* L. and related genera (Poaceae) based on the nuclear ribosomal internal transcribed spacer sequences. *Turkish Journal of Botany*, v. 34, n. 6, p. 467-478, 2010.

GOGOI, N.; GOGOI, A.; NEOG, B.; BARUAH, D.; SAIKIA, P. Phylogenetic analysis and genetic diversity of *Garcinia* species using ITS region and ISSR markers. *Proceedings of the National Academy of Sciences, India Section B - Biological Sciences*, v. 91, n. 2, p. 343-351, 2021.

HASIM, A.; HERDIYENI, Y.; DOUADY, S. Leaf shape recognition using centroid contour distance. *IOP Conference Series: Earth and Environmental Science*, v. 31, e012002, 2016.

HAZARIKA, T. K.; LALNUNSANGI, C. Exploring genetic diversity of *Garcinia lanceifolia* Roxb. (Clusiaceae), a highly medicinal and endangered fruit of north-east India. *Genetic Resources and Crop Evolution*, v. 66, n. 1, p. 61-69, 2019.

INTERNATIONAL PLANT GENETIC RESOURCES INSTITUTE (IPGRI). *Descriptors for mangosteen (Garcinia mangostana)*. Rome: IPGRI, 2003.

JACKSON, M.; FORD-LLOYD, B.; PARRY, M. *Plant genetic resources and climate change*. Oxfordshire: CAB International, 2014.

JIMÉNEZ-GAONA, Y.; VIVANCO-GALVÁN, O.; CRUZ, D.; ARMIJOS-CARRIÓN, A.; SUÁREZ, J. P. Compensatory base changes in ITS2 secondary structure alignment, modelling, and molecular phylogeny: an integrated approach to improve species delimitation in *Tulasnella* (Basidiomycota). *Journal of Fungi*, v. 9, n. 9, e894, 2023.

KOVACH, W. *MVSP-multi-variate statistical package*. Wales: Kovach Computing Services, 2007.

LEE, S. Y.; MOHAMED, R.; FARIDAH-HANUM, I.; LAMASUDIN, D. U. Utilization of the internal transcribed spacer (ITS) DNA sequence to trace the geographical sources of *Aquilaria malaccensis* Lam. populations. *Plant Genetic Resources: Characterisation and Utilisation*, v. 16, n. 2, p. 103-111, 2017.

LEMEY, P.; SALEMI, M.; VANDAMME, A. M. *The phylogenetic handbook*: a practical approach to phylogenetic analysis and hypothesis testing. Cambridge: Cambridge University Press, 2009.

LIU, S. H.; HUANG, C. C.; LIAO, C. K. Rediscovery of an 'extinct' species *Scleria sumatrensis* Retz. in Taiwan using both morphological and molecular authentications. *Taiwania*, v. 66, n. 3, p. 398-407, 2021.

LIU, Z.; NI, Y.; LIU, B. O. Genetic relationships of several *Garcinia* species (Clusiaceae) revealed by ITS sequence data. *International Educational Scientific Research Journal*, v. 2, n. 3, p. 11-15, 2016.

MAPS OF WORLD. *Top 10 mangosteen producing countries*. 2023. Available at: https://www.mapsofworld.com/world-top-ten/mangosteen-producing-countries.html. Access on: Dec. 12, 2023.

MIGICOVSKY, Z.; MYLES, S. Exploiting wild relatives for genomics-assisted breeding of perennial crops. *Frontiers in Plant Science*, v. 8, e460, 2017.

MURSYIDIN, D. H.; KHAIRULLAH, I. Genetic evaluation of tidal swamp rice from South Kalimantan, Indonesia based on the agro-morphological markers. *Biodiversitas*, v. 21, n. 10, p. 4795-4803, 2020.

MURSYIDIN, D. H.; KHAIRULLAH, I.; SYAMSUDIN, R. Genetic diversity and relationship of Indonesian swamp rice (*Oryza sativa* L.) germplasm based on agro-morphological markers. *Agriculture and Natural Resources*, v. 56, n. 1, p. 95-104, 2022a.

MURSYIDIN, D. H.; MAULANA, F. N. Genetic diversity and relationship of *Garcinia* based on bioactive compounds and their biological activities: in silico study. *Berita Biologi*, v. 19, n. 3A, p. 269-280, 2020.

MURSYIDIN, D. H.; NAZARI, Y. A.; AHYAR, G. M. Z.; MAKRUF, M. I. Molecular identity of native coconut (*Cocos nucifera* L.) germplasm from South Kalimantan, Indonesia. *Australian Journal of Crop Science*, v. 16, n. 3, p. 424-430, 2022b.

MURSYIDIN, D. H.; NAZARI, Y. A.; BADRUZSAUFARI, E.; MASMITRA, M. R. D. DNA barcoding of the tidal swamp rice (*Oryza sativa*) landraces from South Kalimantan, Indonesia. *Biodiversitas*, v. 22, n. 4, p. 1593-1599, 2021.

MURSYIDIN, D. H.; SETIAWAN, A. Assessing diversity and phylogeny of Indonesian breadfruit (*Artocarpus* spp.) using internal transcribed spacer (ITS) region and leaf morphology. *Journal of Genetic Engineering and Biotechnology*, v. 21, e15, 2023.

MURTHY, H. N.; DANDIN, V. S.; DALAWAI, D.; PARK, S. Y.; PAEK, K. Y. Breeding of *Garcinia* spp. *In*: AL-KHAYRI, J.; JAIN, S.; JOHNSON, D. (ed.). *Advances in plant breeding strategies*: fruits. Cham: Springer, 2018. p. 773-809.

NAFISI, H.; KAVEH, A.; KAZEMPOUR-OSALOO, S. Characterizing nrDNA ITS1, 5.8S and ITS2 secondary structures and their phylogenetic utility in the legume tribe Hedysareae with special reference to *Hedysarum*. *PLoS ONE*, v. 18, n. 4, e0283847, 2023.

NAZRE, M. New evidence on the origin of mangosteen (*Garcinia mangostana* L.) based on morphology and ITS sequence. *Genetic Resources and Crop Evolution*, v. 61, n. 6, p. 1147-1158, 2014.

NAZRE, M.; NEWMAN, M. F.; PENNINGTON, R. T.; MIDDLETON, D. J. Taxonomic revision of Garcinia section *Garcinia* (Clusiaceae). *Phytotaxa*, v. 373, n. 1, p. 1-52, 2018.

NEALE, D. B.; WHEELER, N. C. Hybridization and introgression. *In*: NEALE, D. B.; WHEELER, N. C. *The conifers*: genomes, variation and evolution. Cham: Springer, 2019. p. 387-429.

NEI, M.; LI, W. H. Mathematical model for studying genetic variation in terms of restriction endonucleases (molecular evolution/mitochondrial DNA/nucleotide diversity). *PNAS*, v. 76, n. 10, p. 5269-5273, 1979.

ÖZGIŞI, K. Structural characterization of ITS2 and CBC species concept applications in the tribe Coluteocarpeae (Brassicaceae). *Turkish Journal of Botany*, v. 44, n. 3, p. 295-308, 2020.

PARTHASARATHY, U.; NANDAKISHORE, O. P.; ROSANA, O. B.; NIRMAL BABU, K.; SENTHIL KUMAR, R.; PARTHASARATHY, V. A. Identification of molecular markers to study the *Garcinia* spp. diversity. *Indian Journal of Experimental Biology*, v. 54, n. 6, p. 400-405, 2016.

PURNOMO, P.; DARYONO, B. S.; SHIWACHI, H. Phylogenetic relationship of Indonesian water yam (*Dioscorea alata* L.) cultivars based on DNA marker using ITS-rDNA analysis. *Journal of Agricultural Science*, v. 9, n. 2, p. 154-161, 2017.

SEETHAPATHY, G. S.; TADESSE, M.; URUMARUDAPPA, S. K. J.; GUNAGA, S. V.; VASUDEVA, R.; MALTERUD, K. E.; SHAANKER, R. U.; BOER, H. J. de; RAVIKANTH, G.; WANGENSTEEN, H. Authentication of *Garcinia* fruits and food supplements using DNA barcoding and NMR spectroscopy. *Scientific Reports*, v. 8, e10561, 2018.

SENAVIRATHNA, H. M. T. N.; RANAWEERA, L. T.; MUDANNAYAKE, M. M. A. W. P.; NAWANJANA, P. W. I.; WIJESUNDARA, W. M. D. A.; JAYARATHNE, H. S. M.; RATNASURIYA, M. A. P.; WEEBADDE, C. K.; SOORIYAPATHIRANA, S. D. S. S. Assessment of the taxonomic status of the members of genus *Artocarpus* (Moraceae) in Sri Lanka. *Genetic Resources and Crop Evolution*, v. 67, n. 5, p. 1163-1179, 2020.

SINAGA, S.; SOBIR, S.; POERWANTO, R.; ASWIDINNOOR, H.; DURYADI, D. Genetic diversity and the relationship between the Indonesian mangosteen (*Garcinia mangostana*) and the related species using isozyme markers. *Jurnal Natur Indonesia*, v. 13, n. 1, p. 53-58, 2010.

SOBIR, S.; POERWANTO, R.; SANTOSA, E.; SINAGA, S.; MANSYAH, E. Genetic variability in apomictic mangosteen (*Garcinia mangostana*) and its close relatives (*Garcinia* spp.) based on ISSR markers. *Biodiversitas Journal of Biological Diversity*, v. 12, n. 2, p. 59-63, 2011.

SOBIR, S.; SINAGA, S.; POERWANTO, R.; RISMITASARI, R.; LUKMAN, R. Comparison analysis of genetic diversity of Indonesian mangosteens (*Garcinia mangostana* L.) and related species by means isozymes and AFLP markers. *Biodiversitas Journal of Biological Diversity*, v. 10, n. 4, p. 163-167, 2009.

STATISTA RESEARCH DEPARTMENT (SRD). *Export value of mangosteen in Indonesia 2016-2022*. New York: Statista Inc., 2023.

SULASSIH, S.; SOBIR, S.; SANTOSA, E. Phylogenetic analysis of mangosteen (*Garcinia mangostana* L.) and its relatives based on morphological and inter simple sequence repeat (ISSR) markers. *SABRAO Journal of Breeding and Genetics*, v. 45, n. 3, p. 478-490, 2013.

SUTTHINON, P.; SAMUELS, L.; MEESAWAT, U. Male functionality in *Garcinia celebica* L.: a candidate ancestor species of mangosteen (*G. mangostana* L.). *Botany*, v. 96, n. 10, p. 685-693, 2018.

TAMURA, K.; STECHER, G.; KUMAR, S. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, v. 38, n. 7, p. 3022-3027, 2021.

THINH, B. B.; CHAC, L. D.; THU, L. T. M. Application of internal transcribed spacer (ITS) sequences for identifying *Anoectochilus setaceus* Blume in Thanh Hoa, Vietnam. *Proceedings on Applied Botany, Genetics and Breeding*, v. 181, n. 2, p. 108-116, 2020.

WEE, C. C.; NOR MUHAMMAD, N. A.; SUBBIAH, V. K.; ARITA, M.; NAKAMURA, Y.; GOH, H. H. Mitochondrial genome of *Garcinia mangostana* L. variety Mesta. *Scientific Reports*, v. 12, n. 1, e9480, 2022.

WITHERUP, C.; ZUBERI, M. I.; HOSSAIN, S.; ZEREGA, N. J. C. Genetic diversity of Bangladeshi jackfruit (*Artocarpus heterophyllus*) over time and across seedling sources. *Economic Botany*, v. 73, n. 2, p. 233-248, 2019.

XIAN, Q.; WANG, S.; LIU, Y.; KAN, S.; ZHANG, W. Structure-based GC investigation sheds new light on ITS2 evolution in *Corydalis* species. *International Journal of Molecular Sciences*, v. 24, n. 9, e7716, 2023.

YAN, W. A systematic narration of some key concepts and procedures in plant breeding. *Frontiers in Plant Science*, v. 12, e24517, 2021.

YU, S.; ZHAO, X.; WANG, Y.; JIANG, D.; ZHANG, Y.; HU, L.; LIU, Y. Morphological, cytological, and molecular-based genetic stability analysis of *in vitro*-propagated plants from newly induced aneuploids in *Caladium*. *Agriculture*, v. 12, e1708, 2022.

YUSOP, M. S. M.; MOHAMED-HUSSEIN, Z. A.; RAMZI, A. B.; BUNAWAN, H. Cymbidium mosaic virus infecting orchids: what, how, and what next? *Iranian Journal of Biotechnology*, v. 20, e3020, 2022.

ZHANG, L.; VIJG, J. Somatic mutagenesis in mammals and its implications for human disease and aging. *Annual Review of Genetics*, v. 52, n. 78, p. 397-419, 2018.

ZHANG, W.; TIAN, W.; GAO, Z.; WANG, G.; ZHAO, H. Phylogenetic utility of rRNA ITS2 sequence-structure under functional constraint. *International Journal of Molecular Sciences*, v. 21, n. 17, e6395, 2020.

ZHAO, L. L.; FENG, S. J.; TIAN, J. Y.; WEI, A. Z.; YANG, T. X. Internal transcribed spacer 2 (ITS2) barcodes: a useful tool for identifying Chinese *Zanthoxylum*. *Applications in Plant Sciences*, v. 6, n. 6, e01157, 2018.

ZHAO, S.; CHEN, X.; SONG, J.; PANG, X.; CHEN, S. Internal transcribed spacer 2 barcode: a good tool for identifying *Acanthopanacis cortex*. *Frontiers in Plant Science*, v. 6, e840, 2015.

ZHU, S.; LI, Q.; CHEN, S.; WANG, Y.; ZHOU, L.; ZENG, C.; DONG, J. Phylogenetic analysis of *Uncaria* species based on internal transcribed spacer (ITS) region and ITS2 secondary structure. *Pharmaceutical Biology*, v. 56, n. 1, p. 548-558, 2018.