

Phylogenetic relationship of superior durian (*Durio zibethinus*) cultivars native to South Kalimantan, Indonesia¹

Dindin Hidayatul Mursyidin²

ABSTRACT

Durian, especially *Durio zibethinus*, is an agricultural commodity with high economic value, both in local and global markets. This study aimed to determine the genetic diversity, relationships and correlation of superior cultivars of *D. zibethinus* ('Likol', 'Sahang' and 'Si Japang') native to South Kalimantan, Indonesia, using the *rbcL* marker, and compare them with other 48 cultivars from the GenBank database. All durian *rbcL* markers were analyzed using the MEGA-X software and phylogenetically reconstructed using two approaches: maximum likelihood (ML) and neighbor-joining (NJ). The durian phylogenetic tree was assessed by bootstrap analysis, and their relationships by Pearson's correlation and principal component analysis. The durian showed a low genetic diversity ($\pi\% = 0.056$); however, unique relationships were revealed. Following the *rbcL* region, this germplasm was grouped into five clades using ML and NJ. In this case, 'Si Japang' and 'Sahang' showed to be closely related to 'T16' from Malaysia, whereas 'Likol' was related to 'Monthong' from Thailand. However, based on the genetic divergence analysis, 'Sahang' had the farthest relationship with three durians from Thailand ('Metnai Kanyao', 'Chok Loi' and 'Malet Ar-Ri').

KEYWORDS: Breeding program, Borneo Island, genetic diversity, horticulture commodity.

INTRODUCTION

Durian, especially *Durio zibethinus*, is an agricultural commodity with high economic value, both in local and global markets (Mursyidin & Daryono 2016), which has become a promising export commodity. For example, in 2020, Indonesia succeeded in exporting this fruit to several other countries, e.g., Malaysia, Singapore, Saudi Arabia and Qatar, with a total transaction of 232 thousand USD (Rizaty 2021). In 2020, Indonesia, as one of the leading producers of durian in the world, produced

RESUMO

Relação filogenética de cultivares superiores de durian (*Durio zibethinus*) nativas de Kalimantan do Sul, Indonésia

O durian, especialmente *Durio zibethinus*, é uma commodity agrícola com alto valor econômico, tanto no mercado local quanto global. Objetivou-se determinar a diversidade genética, relações e correlação de cultivares superiores de *D. zibethinus* ('Likol', 'Sahang' e 'Si Japang') nativas de Kalimantan do Sul, Indonésia, usando o marcador *rbcL*, e compará-las com outras 48 cultivares do banco de dados GenBank. Todos os marcadores durian *rbcL* foram analisados usando o software MEGA-X e filogeneticamente reconstruídos utilizando-se duas abordagens: máxima verossimilhança (ML) e agrupamentos vizinhos (NJ). A árvore filogenética do durian foi avaliada por análise de bootstrap, e suas relações pela correlação de Pearson e análise de componentes principais. O durian apresentou baixa diversidade genética ($\pi\% = 0,056$); no entanto, relações únicas foram reveladas. Seguindo a região *rbcL*, esse germoplasma foi agrupado em cinco clados, utilizando-se ML e NJ. Nesse caso, 'Si Japang' e 'Sahang' mostraram-se intimamente relacionadas com 'T16' da Malásia, enquanto 'Likol' relacionou-se com 'Monthong' da Tailândia. No entanto, com base na análise de divergência genética, 'Sahang' apresentou o relacionamento mais distante com três durians da Tailândia ('Metnai Kanyao', 'Chok Loi' e 'Malet Ar-Ri').

PALAVRAS-CHAVE: Programa de melhoramento, Ilha de Bornéu, diversidade genética, commodity hortícola.

about 1.19 million metric tons of durian (Statista 2021). However, to meet the export market, the quality of this commodity is still relatively lower than a similar one from two neighboring countries, i.e., Malaysia and Thailand (DHI 2021). Thus, to improve the quality of durian, several strategic steps included in the breeding task, particularly the development of new superior cultivars, are necessary.

According to Acquaah (2007), germplasm collection is an urgent activity in helping the success of plant breeding programs (the development of new

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² University of Lambung Mangkurat, Faculty of Mathematics and Natural Sciences, Banjarbaru, South Kalimantan, Indonesia.
E-mail/ORCID: dindinhm@gmail.com/0000-0002-1200-0927.

superior cultivars). In Indonesia, particularly in the Kalimantan Island, approximately 18 of 27 durian world species have been found, including their wild relatives. While 16 are classified as endemic species, nine are edible, namely *D. zibethinus*, *D. kutejensis*, *D. dulcis*, *D. excelsus*, *D. lowianus*, *D. oxleyanus*, *D. grandiflorus*, *D. graveolens* and *D. testudinarium* (Mursyidin et al. 2022a). All durian species have fruit flesh with a distinctive taste, and other morphological characteristics include a high tolerance to environmental stress, such as acidic soil, and diseases, such as patch canker (Uji 2005). Thus, the germplasm is usable as a base population (parental) in breeding programs.

Genetic characterization is also urgent in assisting the durian breeding programs or developing new superior cultivars (Acquaah 2007). However, this activity is limited to morphological markers (Mursyidin & Daryono 2016). While these markers have several advantages, they often have limitations, such as multigenic inheritance, and they are strongly influenced by environmental variables (Jiang 2013, Wu et al. 2021). In addition, these morphological markers are less efficient, because they can only be applied to mature plants or wait for the flowering stage, so they require a longer observation time or are time-consuming (Mursyidin & Daryono 2016). Several molecular markers, such as RAPD, SSR and ISSR, have been applied in studying the genetic diversity and relationships of durian (Vanijajiva 2012, Mursyidin & Daryono 2016, Santoso et al. 2016). However, these markers are very subjective, and their analysis is less accurate (Lee et al. 2017). Wu et al. (2021) also reported that these markers have poor consistency, low reproducibility and a relatively complex operation. In this case, molecular characterization is not a substitute, but is complementary for morphological evaluation (Lima et al. 2018).

This study aimed to analyze and determine the nucleotide diversity, relationships and correlations of superior cultivars of durian (*D. zibethinus*) native to South Kalimantan, in Indonesia, using the *rbcL* marker - which is universal, generates a high sequence output and provides an unbiased alignment (Dong et al. 2013, Chesters et al. 2015) and is easy to analyze in most land plants (Hollingsworth et al. 2016) - and also compare them with other cultivars from the GenBank database.

MATERIAL AND METHODS

This study was conducted at the University of Lambung Mangkurat, Indonesia, from April to September 2021. Of the 51 superior cultivars of durian (*D. zibethinus*), three were collected from South Kalimantan, in Indonesia, and information on 48 cultivars was obtained from the GenBank database (USA 2022; Table 1).

The DNA was extracted and purified from the young leaves of durian samples using a plant genomic DNA mini kit from Geneaid Biotech Ltd., Xizhi, New Taipei, Taiwan (GP100). Leaves were collected from durian mother plants with more than 50 years old growing (cultivated) on farmers' plantations. The DNA was then measured quantitatively using UV-VIS spectrophotometry (GE Healthcare, Chicago, Illinois, United States). Subsequently, the DNA was amplified using the universal primer *rbcL* (Gholave et al. 2017), with a Thermocycler PCR machine (Labnet International Inc., Edison, New Jersey, United States). The primer flanks the *rbcL* gene partially by about 600 bp. PCR was employed with a total volume of 25 μ L [containing 22 μ L PCR mix from Bioline, UK (MyTaq HS Red), 2.0 μ L forward/reverse primers (10 μ M) and 1 μ L DNA template (10 ng)]. PCR conditions were set following Mursyidin et al. (2021): initial denaturation at 94 °C for 5 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s and extension at 72 °C for 45 s, and final extension at 72 °C for 7 min. The DNA target (*rbcL*) was then separated by electrophoresis using 2 % agarose gel, 1X TBE buffer solution and DNA staining (GelRed, SMOBiO Tech., Taiwan), and observed using a UV transilluminator. The DNA target was purified and sequenced bi-directionally using the Sanger method (1st Base Ltd., Selangor, Malaysia). All sequences were stored in the GenBank database (Table 1).

Data analysis was initiated by editing and assembling the *rbcL* sequence of durian using the MEGA-X software (Kumar et al. 2018). The *rbcL* sequences were then aligned by Clustal Omega (Sievers et al. 2020) and analyzed phylogenetically using a similar software (MEGA-X). In this stage, the nucleotide diversity was estimated following Nei & Kumar (2000). The phylogenetic trees were reconstructed by maximum likelihood (ML) and neighbor-joining (NJ) and evaluated using

Table 1. List of durian (*Durio zibethinus* Murr.) cultivars used in this study, with their *rbcL* sequence length, accession number and origin.

Number	Cultivar	Nucleotide length (bp)	GenBank accession number	Origin
1	'Likol'*	566	MZ479693	South Kalimantan, Indonesia
2	'Sahang'*	571	MZ479694	South Kalimantan, Indonesia
3	'Si Japang'*	529	MZ479695	South Kalimantan, Indonesia
4	'KR2618'	502	MG895967.1	Sumatra, Indonesia
5	'D2'	1,022	AF402949.1	Malaysia
6	'D13'	1,018	AF402950.1	Malaysia
7	'D24'	970	AF402956.1	Malaysia
8	'D178'	1,007	AF402951.1	Malaysia
9	'D194'	1,026	AF402952.1	Malaysia
10	'D197'	1,007	AF402953.1	Malaysia
11	'T16'	521	KX618222.1	Malaysia
12	'Chani'	642	MF139690.1	Thailand
13	'Chani Namtansai'	642	MF139691.1	Thailand
14	'Chat Sithong'	642	MF139693.1	Thailand
15	'Chompu Sri'	642	MF139697.1	Thailand
16	'Chomphu Phan'	642	MF139694.1	Thailand
17	'Chok-Loi'	642	MF139706.1	Thailand
18	'E-Lipnaitip'	642	MF139670.1	Thailand
19	'Haluk Maithuengphua'	642	MF139674.1	Thailand
20	'Kamoan Lueng'	642	MF139677.1	Thailand
21	'Kampan Doem'	642	MF139678.1	Thailand
22	'Kampan Phuang'	642	MF139698.1	Thailand
23	'Kansan'	642	MF139671.1	Thailand
24	'Kanyao'	642	MF139689.1	Thailand
25	'Kanyao Si-Nak'	642	MF139701.1	Thailand
26	'Kanyao Watsak'	642	MF139680.1	Thailand
27	'Kathoei Khuasan'	642	MF139709.1	Thailand
28	'Kathoei Nueakhao'	642	MF139696.1	Thailand
29	'Kop Chainam'	642	MF139681.1	Thailand
30	'Kop Choakhun'	642	MF139673.1	Thailand
31	'Kop Nasan'	642	MF139699.1	Thailand
32	'Kop Phikun'	642	MF139702.1	Thailand
33	'Kop Ratsami'	642	MF139704.1	Thailand
34	'Kop Suwan'	642	MF139708.1	Thailand
35	'Kop Watklual'	642	MF139672.1	Thailand
36	'Linlap-Lae'	642	MF139692.1	Thailand
37	'Longlap-Lae'	642	MF139684.1	Thailand
38	'Luang Thong'	642	MF139675.1	Thailand
39	'Malet Ar-Ri'	642	MF139707.1	Thailand
40	'Metnai Kanyao'	642	MF139705.1	Thailand
41	'Monthong'	1,455	MT321069.1	Thailand
42	'Nokyip'	642	MF139676.1	Thailand
43	'Phuang Mani'	642	MF139683.1	Thailand
44	'Salika'	642	MF139687.1	Thailand
45	'Taptim'	642	MF139703.1	Thailand
46	'Thongdaeng'	642	MF139679.1	Thailand
47	'Thongsuk'	642	MF139685.1	Thailand
48	'Thongtoichat'	642	MF139682.1	Thailand
49	'Thoraniwai'	642	MF139700.1	Thailand
50	'Tonyai'	642	MF139688.1	Thailand
51	'Yammawat'	642	MF139686.1	Thailand

* Collected directly by purposive sampling method.

bootstrapping for 1,000 replicates (Lemey et al. 2009). The relationships of durian cultivars were also assessed with the Pearson's correlation (Taylor 1990) and principal component analyses (PCA) (Mursyidin et al. 2022b). Finally, the coefficient differentiation or genetic divergence was measured using the Kimura 2-parameter model (Kimura 1980).

RESULTS AND DISCUSSION

Durian germplasm has unique *rbcL* sequences. In this study, the durian germplasm has different *rbcL* sequence lengths, recorded between 529 and 571 bp specifically for native South Kalimantan in Indonesia (Table 1), while all cultivars ranged from 502 to 1,455 bp (Table 2). The difference in the length of this gene sequence was typical, as it occurred in other germplasms, such as that of *Amorphophallus*. Mursyidin & Hernanda (2021) reported that this germplasm has a partial *rbcL* gene length of 543-1491 bp. For *D. zibethinus*, especially the 'Monthong' cultivar, the *rbcL* was recorded as being as long as 1,455 bp (Shearman et al. 2020). Following Clegg (1993), the *rbcL* gene includes 1,431 nucleotides coding for the large subunit protein, and the length varies among angiosperms or most flowering plants.

Conceptually, *rbcL* encodes ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), a bifunctional enzyme for plant photosynthesis. This gene is located in the large single-copy region of the plastid genome, with high homology among various genera (Bi et al. 2018). In this case, from

Table 2. Genetic information of *rbcL* sequences from durian (*Durio zibethinus* Murr.) germplasm was used in this study, including superior local cultivars from South Kalimantan, Indonesia.

Parameter	Entire population
Range of sequence length (bp)	502-1,455
Number of polymorphism (S)	14
Transition/transversion bias value (R)	4.067
Guanine-cytosine/GC content (%)	43.715
Nucleotide diversity (π %)	0.056
Coefficient of differentiation (d)	0.350
Bayesian information criterion (BIC)	5,335.634
Akaike information criterion (AICc)	4,489.221
Maximum likelihood value (<i>lnL</i>)	-2,144.323

the total length of the sequences found, only 14 loci showed mutations (Table 2). Therefore, the incidence of mutations was low or had high homology. Furthermore, all mutations found in the *rbcL* sequence of durians were substitutions, i.e., transitions and transversions (Figure 1).

Following Figure 1, the polymorphic sites or mutation events on the *rbcL* sequences of durian germplasm, especially from South Kalimantan, Indonesia, were found in six nucleotide positions (39, 603, 614, 615, 619 and 620) in the 'Sahang' cultivar. It was also present in durian germplasms from Thailand (comparisons), such as 'Kansan', 'Haluk Maithuenghua' and 'Metnai'.

In this case, the first mutation was dominant in the transversions (Table 3). According to Aloqalaa et al. (2019), the first type of mutation is more often present in this sequence than in transversions. Hence, it is familiar with molecular evolution (Stoltzfus & Norris 2016). However, mutations are an initial step in establishing the primary population for natural selection and are an integral part of either evolution or genetic diversity (Govindaraj et al. 2015). Consequently, this phenomenon is the main factor in rising genetic diversity (Frankham et al. 2004).

However, the durian showed a low nucleotide diversity (π %) of only 0.056 (Table 2). According to Gao et al. (2017), this is probably due to several factors, such as natural selection, genetic isolation, population decline, founder effect or inbreeding. In this case, inbreeding is the most probable, based on the low level of diversity (Mursyidin et al. 2017). According to Gao et al. (2017), inbreeding may reduce this diversity during domestication. Meanwhile, the latest conditions may correlate with disease resistance and disaster resilience to extreme conditions (Lloyd et al. 2016).

Table 3. Maximum likelihood estimates of the substitution matrix on the *rbcL* region of the durian (*Durio zibethinus* Murr.) germplasm¹.

From\To	A	T	C	G
A	-	2.4705 ^a	2.4705 ^a	20.0589 ^b
T	2.4705 ^a	-	20.0589 ^b	2.4705 ^a
C	2.4705 ^a	20.0589 ^b	-	2.4705 ^a
G	20.0589 ^b	2.4705 ^a	2.4705 ^a	-

¹ Under the Kimura 2-parameter model; ^a transversions; ^b transitions.

Cultivar	Nucleotide_Position							
	40	50	60	70	460	601	610	620
Si_Japang	ACAGAGACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC			
Sahang	ACAGAGACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGCGGTGGACTTGATTTTA		
E-Lipnathip	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Luang_Thong	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Nokyip	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kampan_Lueang	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kampan_Doen	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Thongdeang	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kanyao_Matsak	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kop_Chainam	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Thongyoichat	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Phuang_Mani	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kanyao	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Chani	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Chani_Namansai	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Linlap-Lae	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Chat_Sithong	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Chonphu_Phan	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kathoei_Nueakhao	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Chompu_Sri	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kanpan_Phuang	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kop_Nasan	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Thoraniwai	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Longlap-Lae	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Thongsuk	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Yannawat	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Salika	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Tonyai	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kanyao_Si-Mak	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kop_Phikun	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Taptin	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kop_Ratsami	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kop_Suwan	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kop_Choakhan	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kop_MatkLual	ACAGAACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kansan	ACAGAACTAAAGCAAGGGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Kathoei_Khuasan	ACAGAACTAAAGCAAGGGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Haluk_Maithuengphua	ACAGAACTAAAGCAAGGGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Chok-Loi	ACAGAACTAAAGCAAGGGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Malet_Ar-Ri	ACAGAACTAAAGCAAGGGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Metnai	ACAGAACTAAAGCAAGGGTGGATTCAAAGGGGGGT				CTAAAC	CGTGGTGGACTTGATTTAC		
Likol	ACAGAGACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CG		
T16					CTAAAC	CGCGGTGGACTTG		
KR2618					CTAAAC	CGTG		
Monthong	ACAGAGACTAAAGCAAGTGTGGATTCAAAGCTGGTGT				CTAAAC	CGTGGTGGACTTGATTTACC		
D194					CTAAAC	CGTGGTGGACTTGATTTACC		
D2					CTAAAC	CGTGGTGGACTTGATTTACC		
D13					CTAAAC	CGTGGTGGACTTGATTTACC		
D178					CTAAAC	CGTGGTGGACTTGATTTACC		
D197					CTAAAC	CGTGGTGGACTTGATTTACC		
D24					CTAAAC	CGTGGTGGACTTGATTTACC		
Consensus	acagaaactaaagcaagtgttggattcaaagctggtgt				CTAAAC	CGTGGTGGACTTGatTTacc		

Figure 1. Nucleotide position of polymorphic sites on the *rbcL* region of durian (*Durio zibethinus* Murr.) cultivars.

This diversity is valuable for generating a primary population for natural selection and evolutionary forces (Govindaraj et al. 2015). According to Monteiro et al. (2017), nucleotide diversity represents the average proportion of nucleotide differences between all possible pairs of sequences obtained for that population. As a result, it is a critical parameter in the evolution of future adaptive changes, or a requirement for future adaptive changes. In other words, nucleotide

diversity is a parameter used to measure the degree of polymorphism or genetic diversity within a population (Nei & Li 1979). Thus, it has a crucial impact on conservation tasks (Lloyd et al. 2016).

In this context, understanding genetic diversity is necessary to improve the effectiveness and efficiency of this endeavor. Some variables of conservation, such as genetic diversity loss, can only be managed through extensive population genetic investigations (Luan et al. 2006). However,

inferring genetic diversity from nucleotide diversity using a single plastid locus has several limitations, as the data are not comprehensive. Therefore, two or more molecular markers are suitable for inferring this goal (CBOL Plant Working Group 2009).

Furthermore, information on nucleotide diversity is also valuable for plant breeding purposes. In this situation, breeders use all aspects of plant genetic resources or diversity to develop new superior cultivars with attractive traits, mainly linked to various abiotic and biotic stress adaptations (Swarup et al. 2021). Additionally, broadening the genetic diversity of the next-generation population enables it to face future changes in environmental conditions. However, only the base population needs a high genetic diversity to adapt rapidly to environmental changes (Lloyd et al. 2016). As a result, these efforts are urgent and may be employed in several ways, such as hybridization, introgression and mutagenesis (Allier et al. 2020).

In addition to nucleotide diversity, the durian showed unique relationships. Following the *rbcL* region, this germplasm was grouped into five clades for ML and NJ (Figures 2 and 3, respectively). Generally, the superior local cultivars of durian from South Kalimantan were separated from the cultivars. For ML (Figure 2), 'Si Japang' and 'Sahang' were grouped into the same clade with 'T16' (comparison cultivar from Malaysia), whereas 'Likol' was grouped with 'Monthong' (from Thailand). For NJ (Figure 3), durian from this region was included in the same clade (V) with 'T16'.

The phylogenetic trees reflected the monophyletic divergence of this germplasm. Slobodian & Pastana (2020) defined a monophyletic group as a set of taxa descended from a single taxon or common ancestor. A taxon set is described by sharing apomorphic ("derived") conditions, and members within the set are considered more closely related to each other than to any taxon classified outside of this group (Slobodian & Pastana 2020), for example, vascular plants (Kadereit et al. 2016), the *Psammolestes* genus (Oliveira et al. 2018) and dinoflagellates (Orr et al. 2012).

The different features were shown by PCA (Figure 4), where 'Likol' was near 'Si Japang' and 'Sahang' was near 'KR2618' and 'D194'. A close relationship was also revealed between 'Monthong' (a superior cultivar of Thailand) and 'D197' and

'D13' from Malaysia. A similar grouping was reported by Siew et al. (2018) using SSR markers. According to Teh et al. (2017), the difference between 'Monthong' and 'D197' (also known as 'Musang King') lies in fruit weight, fruit flesh color, fragrance and sweetness level. In this case, 'Monthong' has heavier fruits with fragrant and sweet fruit flesh flavors, while 'D197', although smaller, has a strong aroma and sweet fruit flesh taste (Teh et al. 2017).

Based on genetic divergence analysis (Figure 5), most durian germplasms have a close relationship, except for 'Sahang' and three durian cultivars of Thailand ('Metnai Kanyao', 'Chok Loi' and 'Malet Ar-Ri'), which have the farthest evolutionary divergence of 18.89. The results were also supported by a Pearson's correlation, where most of the durian germplasms had strong relationships (Figure 6). According to Flint-Garcia (2013), this phylogenetic information is valuable for conservation and breeding efforts. In other words, these results may be applied to analyze species delineation, genetic divergence and gene flow, also inferring species and their evolutionary history (Fernández-García 2017).

For plant breeding, this information could be applied to forecast the genetic diversity of progeny (Acquaah 2007). In concept, when individuals with distant relationships cross, progeny may vary widely. Conversely, when there is a closely related cross, the genetic diversity is narrow (Acquaah 2007, Turner-Hissong et al. 2020). In this case, the durian 'Likol' of South Kalimantan might be crossed with 'Monthong' from Thailand.

Based on agro-morphological characteristics, although 'Likol' has a sweet and savory fruit flesh taste, its thickness is lacking. However, 'Monthong' from Thailand has a thick fruit flesh. In addition, the 'Likol' fruit flesh is less fragrant, unlike 'Monthong'. Based on the fruit performance, 'Likol' has a relatively thin fruit skin, while 'Monthong' is thick. According to Lara et al. (2019), the thickness of the fruit skin correlates with storage potential or shelf life. Although this cross has not yet been carried out, an example of the Malaysian durian, namely 'D190', is the hybrid of 'D24' and 'D10' (Siew et al. 2018).

The results of this study are important for supporting future breeding and conservation programs of durian germplasm, both locally and globally.

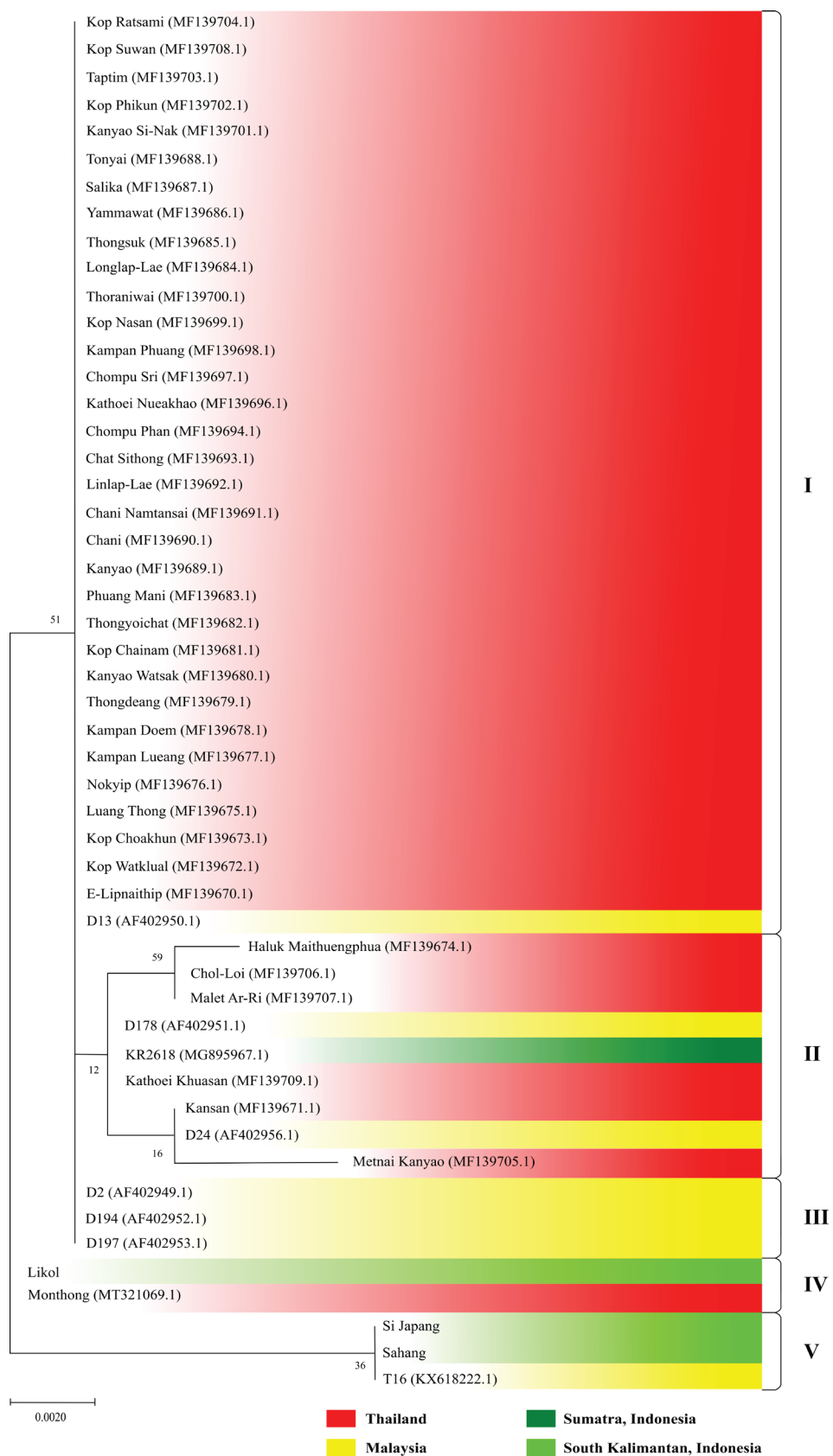


Figure 2. Phylogenetic relationship of durian (*Durio zibethinus* Murr.) cultivars from South Kalimantan, Indonesia, with others inferred by maximum likelihood (ML).

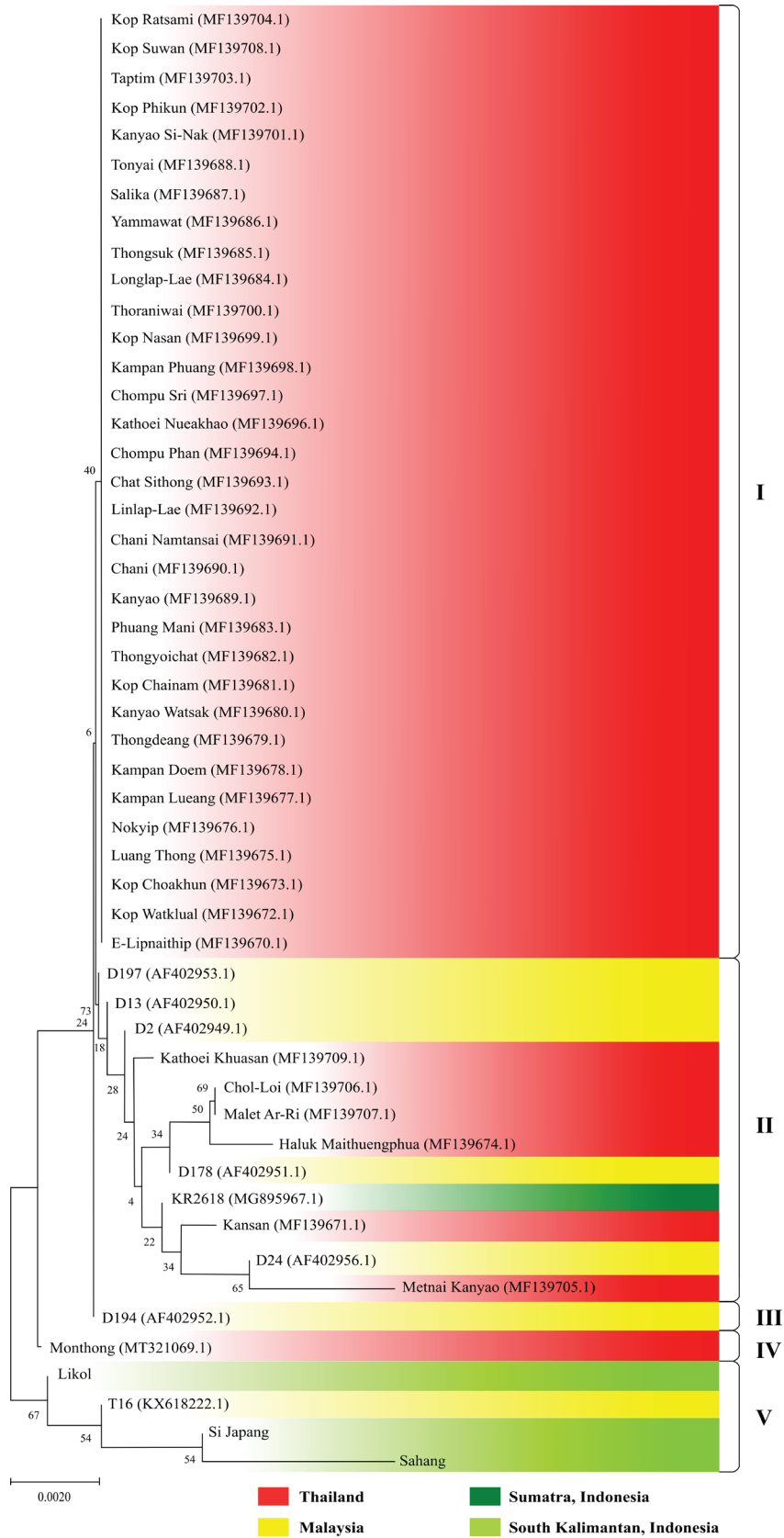


Figure 3. Phylogenetic relationship of durian (*Durio zibethinus* Murr.) cultivars from South Kalimantan, Indonesia, with others inferred by the neighbor-joining (NJ) method.

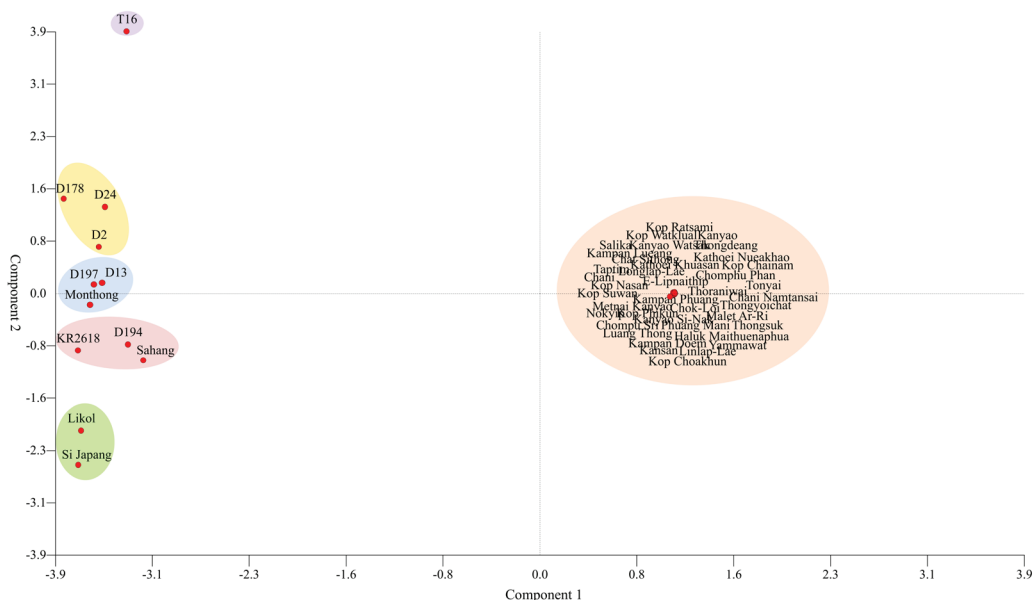


Figure 4. Grouping of durian (*Durio zibethinus* Murr.) cultivars from South Kalimantan, Indonesia, with other cultivars inferred by principal component analysis (PCA).

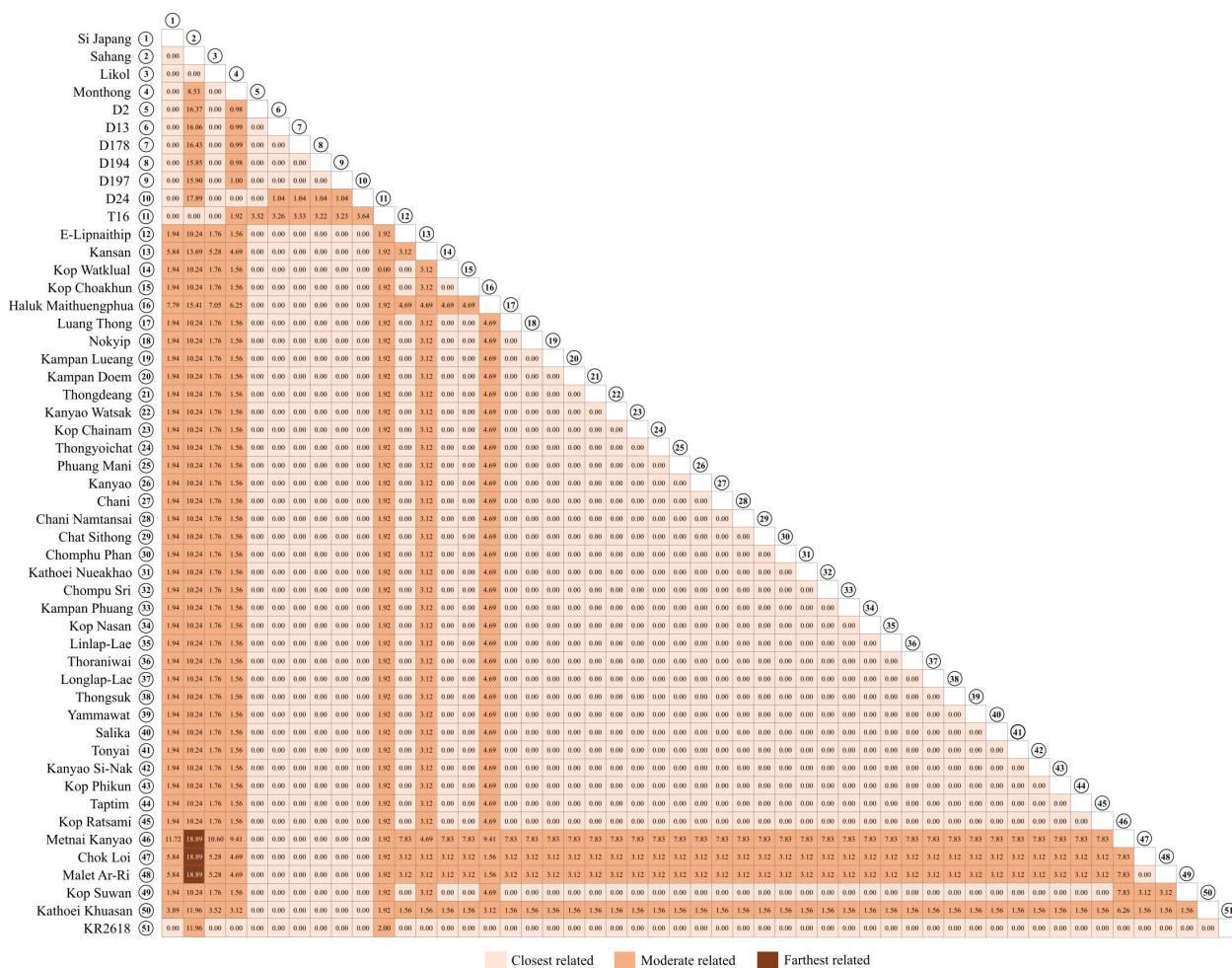


Figure 5. Genetic divergence among durian (*Durio zibethinus* Murr.) cultivars from South Kalimantan, Indonesia, and other cultivars.

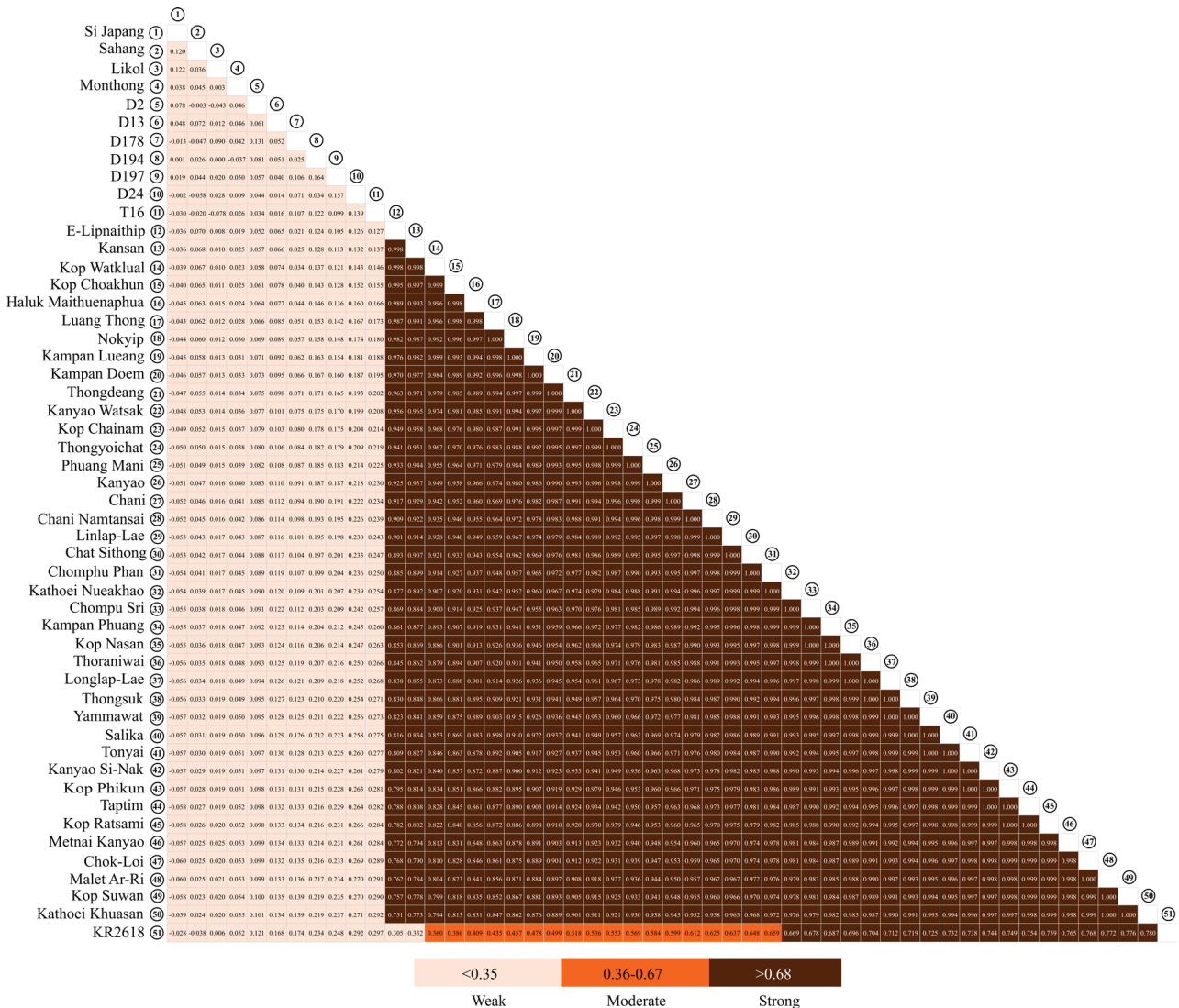


Figure 6. Pearson's correlation among durian (*Durio zibethinus* Murr.) cultivars from South Kalimantan, Indonesia, and other cultivars.

CONCLUSIONS

1. While the durian cultivars from South Kalimantan, in Indonesia, had a low nucleotide diversity ($\pi\% = 0.056$), they presented unique relationships;
2. 'Si Japang' and 'Sahang' are cultivars closely related to 'T16' from Malaysia, whereas 'Likol' was related to 'Monthong' from Thailand. The two latest cultivars might be crossed to obtain new superior characteristics;
3. Based on the genetic divergence analysis, 'Sahang' had the farthest relationship to the three durian cultivars from Thailand, namely 'Metnai Kanyao', 'Chok Loi' and 'Malet Ar-Ri'.

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