

Article

The Development of a Core SSR Marker Set for DNA Fingerprinting and Accession Discrimination in Durian (*Durio zibethinus*)

Yikai Wang ^{1,2} , Jiaying Sheng ^{1,2}, Chonghao Zhong ^{1,2}, Chaofan Zheng ^{1,2}, Jiaquan Huang ^{1,2,3} , Dongdong Li ^{1,2}, Fuwang Yang ⁴, Zhifu Cui ⁵, Guozhu Zhang ⁶, Meng Wang ^{1,2,*} and Hua Tang ^{1,2,3,*}

¹ School of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, China; yikaiwang@163.com (Y.W.); shengjiaying@163.com (J.S.); zchycheng@163.com (C.Z.); zheng13103028307@163.com (C.Z.); jqhuang@163.com (J.H.); lidd@hainu.edu.cn (D.L.)

² Institute of Sanya Breeding and Multiplication, Hainan University, Sanya 572025, China

³ Institute of Baoting, Hainan University, Baoting 572300, China

⁴ Hainan Youqi Agriculture Co., Ltd., Sanya 572031, China; fuwang@163.com

⁵ Hainan State Farms Academy of Sciences Group Co., Ltd., Sanya 572025, China; zhifu555@163.com

⁶ Hainan Baoting Yunding Durian Planting Co., Ltd., Baoting 572313, China; zhangguozhu999@126.com

* Correspondence: wm159357zdx@163.com (M.W.); tanghua@hainanu.edu.cn (H.T.)

Highlights

What are the main findings?

- From 160 SSR primer pairs, 18 polymorphic markers were developed in durian.
- A minimum six-locus core SSR marker set generated unique multilocus genotypes for all 58 accessions.

What are the implications of the main findings?

- The core SSR marker set provides a compact and cost-effective tool for routine durian DNA fingerprinting and accession identification. The marker set can facilitate cultivar authentication, germplasm management, and the efficient utilization of durian genetic resources.

Abstract

Durian (*Durio zibethinus*) germplasm may be incorrectly named, mislabeled, or genetically misidentified during introduction, exchange, and nursery stock distribution. Morphological identification is also affected by environmental conditions and plant developmental stages. A core simple sequence repeat (SSR) marker set was developed for the identification of durian germplasm. Genome-wide mining identified 106,776 SSR loci in the durian reference genome, and 18 stable and polymorphic markers were selected from 160 candidate primer pairs through agarose gel screening and M13-tailed fluorescent capillary electrophoresis. A total of 142 alleles were detected at the 18 SSR loci across the 58 durian accessions, with 3–15 alleles per locus and PIC values ranging from 0.422 to 0.849. Exhaustive evaluation of all marker combinations identified a minimum six-locus core SSR marker set comprising Y6, Y12, Y15, Y75, Y91, and Y108, which generated unique multilocus genotypes for all 58 accessions in the current validation panel, with a cumulative probability of identity (PID) of 7.99×10^{-8} . STRUCTURE analysis, with K representing the number of inferred genetic clusters, supported K = 8 and revealed admixture in several accessions. The core SSR marker set developed in this study can distinguish the 58 durian accessions and be used to construct their DNA fingerprints. Validation in larger and more genetically diverse germplasm collections is required before routine commercial deployment.



Academic Editor: Jia-Long Yao

Received: 4 August 2026

Revised: 4 September 2026

Accepted: 7 September 2026

Published: 9 September 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

Keywords: SSR markers; DNA fingerprinting; germplasm identification; genetic diversity

1. Introduction

Durian (*Durio zibethinus*) is a tropical fruit tree of the family Malvaceae and is widely cultivated in Southeast Asia. Owing to its distinctive flavor and high economic and nutritional value, durian is often referred to as the “king of tropical fruits” [1]. In recent years, durian trade and market demand have expanded rapidly across Southeast Asia, driven in large part by growing demand from China, which has become the world’s largest durian import market [2]. Durian cultivars differ considerably in market value, fruit quality, and breeding potential. Accurate cultivar identification is therefore essential for germplasm conservation, varietal authenticity testing, nursery stock traceability, and genetic improvement.

Conventional identification of durian germplasm is based mainly on morphological traits, which can be influenced by environmental conditions, cultivation practices, and plant developmental stage [3]. During long-term germplasm introduction, exchange, and nursery stock distribution, labeling errors, homonymy, and synonymy may also occur. Similar nomenclatural problems have been reported in other fruit crops, indicating that cultivar names do not always correspond to the actual genetic identity of the material [4]. Accurate identification of durian is further complicated by the fact that fruit traits cannot be evaluated until plants reach reproductive maturity, which limits reliable identification at the seedling stage. A practical and reproducible molecular marker system is therefore required for durian germplasm management and varietal authenticity testing. DNA fingerprinting and associated profile databases are increasingly used to support intellectual property protection, nursery stock authentication, cultivar identification, and germplasm management [5,6].

Advances in genome sequencing and molecular marker techniques have made DNA-based methods increasingly useful for the characterization of fruit tree germplasm. The availability of a high-quality durian reference genome has facilitated the development of molecular markers [7]. Although single-nucleotide polymorphism (SNP) markers provide higher marker density and are well suited to high-throughput genotyping, SSR markers remain attractive for routine fingerprinting because of their codominant inheritance, high polymorphism, relatively low cost, and ease of implementation in routine laboratories [5,8]. Compared with EST-SSRs, genomic SSRs are more widely distributed across the genome and are therefore well suited to germplasm identification and genetic diversity analysis [9,10]. RAPD, ISSR, and SSR markers have previously been used to assess genetic diversity in durian [11–13], but the systematic development of a core SSR marker set and DNA fingerprints for durian remains limited.

Previous studies in potato and grapevine have demonstrated that compact core SSR marker sets can provide efficient and reliable cultivar identification [14,15]. A small set of stable and highly polymorphic SSR markers can reduce genotyping costs while maintaining sufficient discriminatory power among accessions. Such markers are useful for routine germplasm management, identity verification, and nursery stock traceability. However, few studies have developed core SSR marker sets, standardized genotyping protocols, or DNA fingerprints for durian. Existing marker systems also require further validation in larger germplasm collections and across different genotyping platforms.

Conventional agarose gel electrophoresis has limited ability to resolve SSR alleles that differ by only a few base pairs, which may result in underestimation of allele number and marker polymorphism. Capillary electrophoresis provides higher resolution and allows

for more accurate allele calling and consistent genotyping. The M13-tailed primer method further reduces fluorescent-labeling costs because the same labeled M13 primer can be used with different locus-specific primers [16].

Therefore, this study aimed to develop and validate genome-derived SSR markers for durian using capillary electrophoresis, identify a compact core SSR marker set capable of discriminating the 58 accessions, construct DNA fingerprints and molecular identity codes, and assess the genetic diversity and population structure of the analyzed accession panel.

2. Materials and Methods

2.1. Plant Materials

Fifty-eight durian accessions were obtained from the durian germplasm repository of Hainan Youqi Agriculture Co., Ltd., Sanya 572031, China (18.4° N, 109.3° E). Of these, 52 were recorded as originating from Malaysia, 5 from Thailand, and 1 from Vietnam. All accessions were maintained in the same open-field germplasm repository under the natural climatic conditions of Sanya and were managed uniformly using routine irrigation, fertilization, and pest and disease control practices. Their accession codes, cultivar names, geographic origins, and available background information are provided in Table S1. Young leaves were collected from each accession in October 2025 for genomic DNA extraction and SSR genotyping.

2.2. DNA Extraction

Genomic DNA was extracted from 0.15 g of young durian leaf tissue using a modified CTAB method suitable for polysaccharide- and polyphenol-rich plant materials [17]. DNA integrity was checked on 1% agarose gels, and purity and concentration were measured using an ND-100C ultra-micro UV-visible spectrophotometer (MIULAB, Hangzhou, China). Only samples with A260/A280 ratios of 1.8–2.0, A260/A230 ratios > 2.0, and DNA concentrations > 100 ng/μL were retained for further analyses. Samples that did not initially meet these criteria were re-extracted and reassessed until DNA meeting all specified quality criteria was obtained for subsequent analyses. Prior to PCR, all DNA samples were diluted to 50 ng/μL and stored at −20 °C.

2.3. SSR Identification and Primer Design

SSR loci were identified from the durian reference genome available in the Genome Warehouse database under accession number GWHESET000000000. Genome-wide SSR identification was performed using the MISA web platform [18]. The minimum repeat numbers were set at 10 for mononucleotide repeats, 6 for dinucleotide repeats, and 5 for trinucleotide to hexanucleotide repeats. Primers were designed from sequences flanking the identified SSR loci using Primer Premier 5.0. Primer length was set at 18–24 bp, melting temperature at 55–60 °C, GC content at 40–60%, and expected amplicon size at 100–400 bp.

Candidate primer pairs were designed based on SSR motif type, repeat number, flanking-sequence quality, and chromosomal location. The primers were first screened by PCR and 2.5% agarose gel electrophoresis for amplification specificity, stability, and polymorphism. Primer pairs retained for capillary electrophoresis were modified by adding an 18 bp M13 universal sequence to the 5' end of each forward primer. A fluorescently labeled M13 universal primer was then included in the PCR mixture. All primers were synthesized by (Beijing Tsingke Biotech Co., Ltd., Beijing, China).

2.4. SSR Amplification and Capillary Electrophoresis Genotyping

Genomic DNA from 15 randomly selected durian accessions was used for the preliminary screening of candidate primer pairs by 2.5% agarose gel electrophoresis. PCR was

performed in a total volume of 12 μL containing 6 μL of $2\times$ Taq PCR Master Mix (Vazyme, Nanjing, China), 0.5 μL each of the forward and reverse primers (10 $\mu\text{mol/L}$), 0.5 μL of template DNA (50 ng/ μL), and 4.5 μL of ddH₂O. The PCR program consisted of initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 15 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. PCR products were separated on 2.5% agarose gels in $0.5\times$ TBE buffer at 105 V for 35 min. Gel images were obtained using a Gel Doc Go imaging system (Bio-Rad, Hercules, CA, USA). Primer pairs were retained after the initial screening when they produced clear, reproducible, and specific amplification products without obvious non-specific bands or smearing. Primer pairs that failed to meet these criteria were excluded.

Among the primer pairs retained after the initial screening, those showing preliminary polymorphism and appropriate fragment sizes were selected for subsequent validation by capillary electrophoresis. Primer pairs retained after the second round of screening were used for PCR amplification and capillary electrophoresis (CE) genotyping of all 58 durian accessions. Each 15 μL PCR mixture contained 7.5 μL of $2\times$ Taq PCR Master Mix, 0.15 μL of M13-tailed forward primer (10 $\mu\text{mol/L}$), 0.6 μL of reverse primer (10 $\mu\text{mol/L}$), 0.6 μL of FAM-labeled M13 universal primer (10 $\mu\text{mol/L}$), 0.5 μL of template DNA (50 ng/ μL), and 5.65 μL of ddH₂O. The PCR cycling conditions were the same as those used in the preliminary screening. The sequence of the M13 universal primer was 5'-TGTAACGACGGCCAGT-3'.

After fluorescent PCR amplification, 2 μL of each product was examined by 1% agarose gel electrophoresis. The products were diluted as needed according to band specificity and intensity to obtain approximately uniform concentrations for capillary electrophoresis. Each 10 μL loading mixture contained 1 μL of diluted fluorescent PCR product, 0.5 μL of GeneScan™ 500 LIZ size standard, and 8.5 μL of Hi-Di™ Formamide. After centrifugation, the mixtures were denatured at 95 °C for 3 min and immediately cooled. Capillary electrophoresis was performed on a 96-capillary ABI 3730xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Fragment separation was carried out using POP-7™ Polymer and 3730 Running Buffer (10 $\times$). Fragment sizes and genotypes were scored in GeneMarker 3.0.1 (SoftGenetics, State College, PA, USA) using GeneScan™ 500 LIZ as the size standard and the Fragment (Plant) analysis mode. Genotype data were exported in Excel format, and electropherograms were exported as PDF files. The allele sizes reported in this study are the binned apparent fragment sizes generated by GeneMarker and include the 18 bp M13 tail attached to the 5' end of the forward primer.

As a preliminary assessment of genotyping reproducibility, five accessions were randomly selected for reanalysis using independently extracted DNA. Allele sizes and multilocus genotypes obtained from the repeated PCR amplifications and CE runs were compared with those obtained in the initial analysis. The CE results were also compared with the amplification specificity and major polymorphic banding patterns observed during the earlier agarose gel electrophoresis screening to evaluate consistency between the two stages of marker analysis.

2.5. Construction of DNA Fingerprints and Molecular Identity Codes

Using the allele fragment sizes obtained by capillary electrophoresis at the 18 SSR loci in the 58 durian accessions, all possible combinations of the loci were examined in order of increasing number of loci. The core SSR marker set was defined as the combination containing the fewest loci that could uniquely distinguish all 58 accessions. The probability of identity (PID) for each SSR locus was calculated in Microsoft Excel based on the allele frequencies observed in the 58 accessions [19]. The PID of a single locus represents the theoretical probability that two unrelated accessions randomly selected from the population

have the same genotype at that locus. Assuming independence among loci, the cumulative multilocus PID was calculated as the product of the locus-specific PID values and expressed as $-\log_{10}(\text{PID})$. A lower raw PID, or equivalently a higher $-\log_{10}(\text{PID})$, indicates greater theoretical discriminatory power.

At each core SSR locus, the detected alleles were arranged in ascending order of fragment size and assigned fixed binary positions. The presence of an allele in an accession was scored as “1”, and its absence as “0”. For a homozygous genotype, the position corresponding to the allele was scored as “1”; for a heterozygous genotype, the positions corresponding to both alleles were scored as “1”. In the DNA fingerprint profiles, “1” was represented by a black square and “0” by a white square. For each accession, genotypes at the loci constituting the final core SSR marker set were recorded in a fixed locus order as “smaller allele/larger allele”. The locus-specific genotype entries were then joined with hyphens to generate a multilocus molecular identity code for each accession.

To provide a more intuitive visualization of fragment-size variation among durian accessions at different SSR loci, synthetic gel profiles were reconstructed in Excel using the final binned allele sizes and corresponding fluorescence peak heights obtained by capillary electrophoresis. Band positions were determined by allele fragment size, whereas band darkness was scaled according to the log-transformed fluorescence peak height. Homozygous genotypes were displayed as a single band, whereas heterozygous genotypes were displayed as two bands.

2.6. Genetic Diversity and Population Structure Analysis

Allele frequencies at each locus were calculated from the capillary electrophoresis genotype data obtained at the SSR loci retained after screening for the 58 durian accessions. The observed number of alleles (N_a), the effective number of alleles (N_e), Nei's gene diversity index (H), and Shannon's information index (I) were calculated using PopGene32 v. 1.32. The polymorphism information content (PIC) of each SSR locus was calculated in Microsoft Excel from the allele frequencies [20].

To compare SSR allele composition and fingerprint patterns among the accessions, each allele detected at the retained SSR loci was treated as an independent binary character. The presence of an allele was scored as “1” and its absence as “0”, generating an allele presence-absence matrix. Dice similarity coefficients between accessions were calculated using NTSYS-pc 2.10. Cluster analysis was performed based on the similarity matrix using the unweighted pair-group method with arithmetic mean (UPGMA). The Dice coefficients were also converted to genetic distances as $1 - \text{SDice}$ for principal coordinate analysis (PCoA).

2.7. Analysis of Molecular Variance

Analysis of molecular variance (AMOVA) was performed in GenAlEx 6.5 using codominant genotype data from the retained SSR loci to examine the distribution of genetic variation under different grouping schemes.

Two grouping schemes were used. The first grouping scheme was based on the clusters defined by the UPGMA dendrogram. The second was based on the recorded geographic origins of the accessions. Because only one accession was recorded as originating from Vietnam, it was not treated as a separate group, as among-individual variation within that group could not be estimated. The Vietnamese accession was therefore excluded, and the remaining 57 accessions were assigned to two groups according to their recorded origins: Malaysia and Thailand. AMOVA was used to estimate variance components among groups, among individuals within groups, and within individuals, together with the proportion of total genetic variation explained at each level. Genetic differentiation statistics were also calculated. Statistical significance was assessed using 9999 random permutations.

2.8. Population Structure Analysis Using STRUCTURE

Bayesian clustering analysis was performed in STRUCTURE 2.3.4 using the codominant SSR genotype data to examine the genetic structure of the 58 durian accessions. The number of inferred genetic clusters (K) was evaluated [21]. Analyses were conducted under the admixture model with correlated allele frequencies, with K ranging from 2 to 10. For each value of K, 10 independent runs were performed, each with a burn-in period of 100,000 iterations followed by 100,000 Markov chain Monte Carlo iterations. The most likely value of K was determined by considering the mean log probability of the data for each K [$\ln P(K)$], the Evanno ΔK statistic, which reflects the second-order rate of change in $\ln P(K)$ among successive K values, and the consistency among replicate runs.

3. Results

3.1. Genome-Wide SSR Identification and Primer Development

A total of 106,776 SSR loci were identified in the 735.19 Mb durian reference genome, with an average density of 145.24 loci/Mb. Dinucleotide repeats were the most abundant, accounting for 54.66% of all SSRs, followed by trinucleotide repeats (29.53%), whereas the other repeat types were less frequent (Figure 1A). In addition, 12,940 compound SSRs were detected, representing 12.12% of the total SSR loci (Figure 1B). The frequencies of di-, tri-, and tetranucleotide SSRs declined as the number of repeat units increased (Figure 1C). Based on motif type, repeat number, flanking-sequence quality, and chromosomal location, 160 candidate primer pairs were designed.

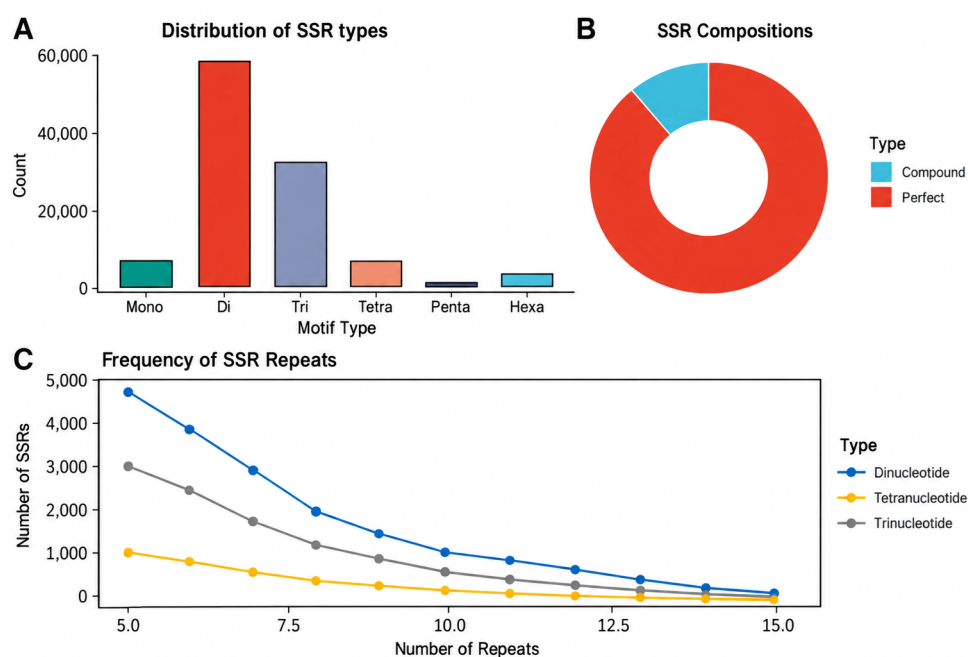


Figure 1. Genome-wide characterization of SSR loci in durian reference genome. (A) Distribution of SSR types. (B) Proportions of perfect and compound SSRs. (C) Frequency distribution of di-, tri-, and tetranucleotide SSRs by number of repeat units.

3.2. SSR Marker Screening and Polymorphism Evaluation

Of the 160 candidate primer pairs, 56 produced stable and specific amplification products during the initial screening. Based on band clarity, preliminary polymorphism, and expected fragment size, 18 primer pairs were selected for capillary electrophoresis genotyping of the 58 durian accessions (Table 1). A total of 142 alleles were detected across the 18 SSR loci. The observed number of alleles per locus (N_a) ranged from 3 to 15, with a mean of 7.89. Y6 had the largest number of alleles (15), whereas Y18 had the fewest (3).

The effective number of alleles (N_e) ranged from 2.13 to 7.26 and averaged 3.90. Nei's gene diversity index (H) ranged from 0.5299 to 0.8622, with a mean of 0.7070, whereas Shannon's information index (I) ranged from 0.8179 to 2.2297, with a mean of 1.5055. The polymorphism information content (PIC) ranged from 0.4222 to 0.8485 and averaged 0.6639. Y6 had the highest PIC value, whereas Y18 had the lowest. Fifteen of the 18 loci had PIC values greater than 0.50, indicating that most of the selected loci were highly polymorphic and informative for genetic diversity analysis and DNA fingerprint construction in durian. The primer sequences of the 18 SSR markers are provided in Table S2.

Table 1. Polymorphism parameters and allele fragment size ranges of the 18 SSR loci.

Primer Name	Chromosome	Na	Ne	H	I	PIC	Fragment Size Range (bp)
Y6	3	15	7.26	0.86	2.23	0.85	201–235
Y12	8	5	3.73	0.73	1.40	0.69	113–136
Y15	11	9	4.01	0.75	1.64	0.72	183–232
Y18	15	3	2.13	0.53	0.82	0.42	136–147
Y20	11	6	2.56	0.61	1.21	0.57	256–273
Y25	26	8	4.33	0.77	1.64	0.73	205–248
Y36	20	5	2.13	0.53	1.03	0.49	238–262
Y56	18	11	3.65	0.73	1.63	0.69	205–233
Y60	1	4	2.58	0.61	1.15	0.57	180–195
Y75	14	10	4.94	0.80	1.89	0.78	271–300
Y91	22	8	4.50	0.78	1.69	0.75	256–303
Y103	1	4	2.50	0.60	1.06	0.52	226–236
Y108	7	10	6.21	0.84	2.04	0.82	183–215
Y110	12	7	2.99	0.67	1.27	0.61	237–269
Y122	9	7	2.30	0.57	1.03	0.48	196–218
Y139	23	7	3.76	0.73	1.49	0.69	189–257
Y148	27	11	4.85	0.79	1.86	0.77	183–231
Y153	17	12	5.82	0.83	2.03	0.81	231–257
Average		7.89	3.90	0.71	1.51	0.66	

Note: Na, observed number of alleles; Ne, effective number of alleles; H, Nei's gene diversity index; I, Shannon's information index; PIC, polymorphism information content.

As a preliminary assessment of genotyping reproducibility, five randomly selected accessions were re-genotyped using independently extracted DNA. The allele profiles at the six core SSR loci were identical to those obtained in the initial analysis, resulting in 100% concordance. The allele patterns obtained by capillary electrophoresis were also generally consistent with the major polymorphic banding patterns observed during the preliminary agarose gel screening, supporting consistency between the two screening stages.

3.3. Discriminatory Power of Core SSR Marker Set

As the number of loci increased, the discriminatory power of the marker combinations also increased. The $-\log_{10}$ -transformed cumulative PID increased from 1.50 for one locus to 7.10 for six loci. The cumulative PID of the six-locus combination was 7.99×10^{-8} , indicating a very low probability that two unrelated accessions would have the same genotype at all six loci (Figure 2A).

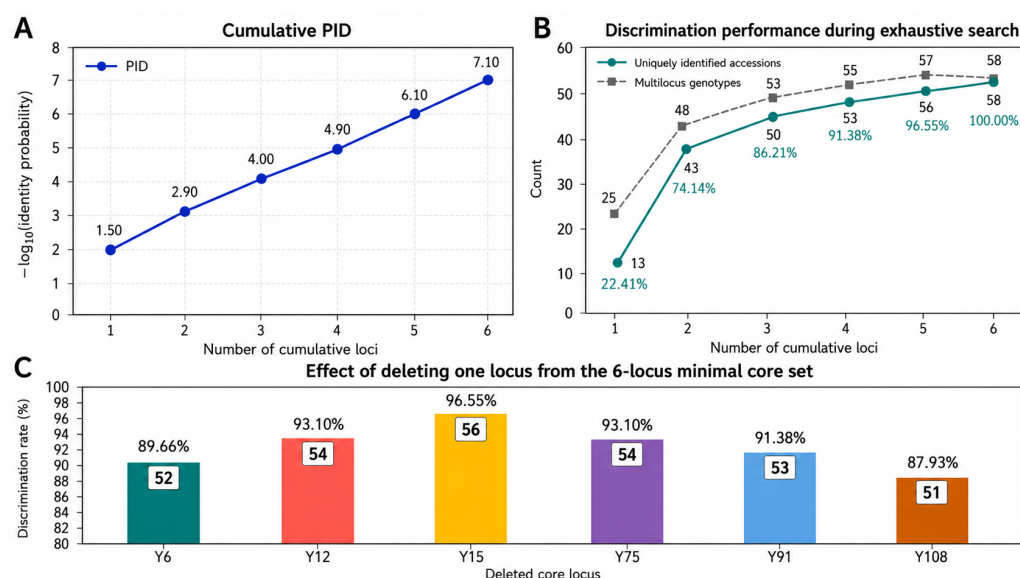


Figure 2. The selection and discriminatory power of the core SSR marker set. **(A)** Changes in the cumulative probability of identity (PID) as SSR loci were added sequentially. **(B)** The number of multilocus genotypes, number of uniquely identified accessions, and cumulative discrimination rate of the best-performing marker combination for each number of loci. **(C)** Changes in discriminatory power after the removal of Y6, Y12, Y15, Y75, Y91, or Y108 from the core marker set. Values above the bars indicate discrimination rates, and values within the bars indicate the numbers of uniquely identified accessions. PID is the probability that two randomly selected unrelated accessions have the same multilocus genotype.

To identify the smallest SSR marker set capable of distinguishing all accessions, all 262,143 possible combinations of the 18 loci were examined. For combinations containing one to five loci, the best-performing combinations produced 25, 48, 53, 55, and 57 multilocus genotypes, respectively, of which 13, 43, 50, 53, and 56 accessions had unique genotypes. The corresponding discrimination rates were 22.41%, 74.14%, 86.21%, 91.38%, and 96.55%. Thus, no combination containing five or fewer loci could distinguish all 58 accessions. The six-locus combination Y6 + Y12 + Y15 + Y75 + Y91 + Y108 produced a unique multilocus genotype for each of the 58 accessions, giving a discrimination rate of 100% (Figure 2B). This combination was therefore identified as the core SSR marker set.

Removal of any one locus from the core SSR marker set reduced the number of accessions with unique genotypes to 51–56, corresponding to discrimination rates of 87.93–96.55% (Figure 2C). Therefore, all six loci were required to distinguish the 58 accessions.

3.4. DNA Fingerprint Database and Molecular Identity Code Construction

3.4.1. Construction of the DNA Fingerprint Database

An SSR genotype database was established for the 58 durian accessions using the capillary electrophoresis data obtained at the 18 SSR loci (Table S3). The core SSR marker set, comprising Y6, Y12, Y15, Y75, Y91, and Y108, was used to construct the DNA fingerprints. A total of 57 alleles were detected across these six loci. At each locus, the alleles were arranged in ascending order of fragment size and assigned fixed binary positions. The presence of an allele was scored as “1” and its absence as “0”, generating a standardized binary fingerprint profile for each accession.

In the DNA fingerprint matrix, the 58 accessions are shown as rows, and the allele positions at the six core loci are shown as columns (Figure 3). Each accession had a unique six-locus genotype, allowing all 58 accessions in the study panel to be distinguished.

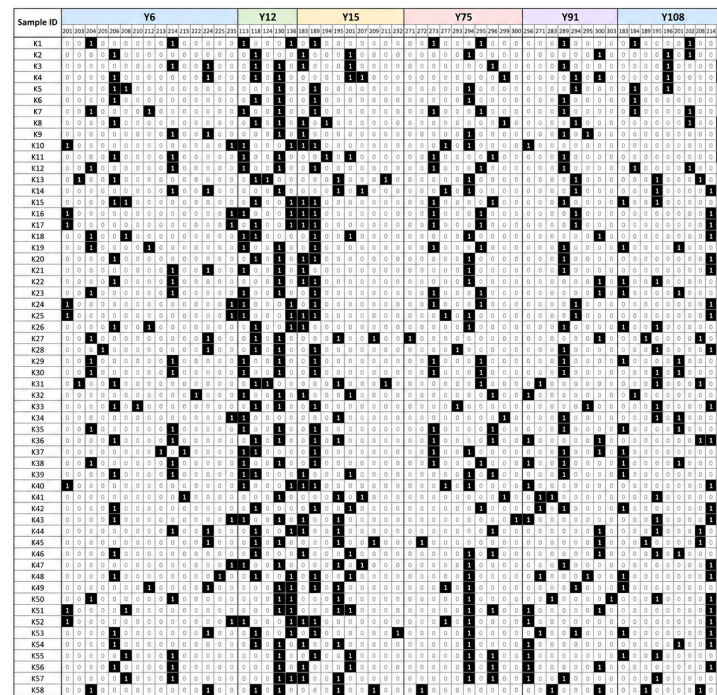


Figure 3. DNA fingerprint profiles of 58 durian accessions based on six core SSR loci. The 58 durian accessions are shown on the y-axis, and the allele fragment sizes detected at each SSR locus are shown on the x-axis. Black cells indicate the presence of the corresponding allele and are coded as “1”, whereas white cells indicate its absence and are coded as “0”.

To visualize the multilocus genotypes generated by the six core SSR markers, synthetic gel profiles were reconstructed for five selected accessions (K1–K5) from the final binned allele sizes and corresponding fluorescence peak heights (Figure 4). Distinct banding patterns were observed among the five accessions across Y6, Y12, Y15, Y75, Y91, and Y108. Homozygous genotypes were represented by one band, whereas heterozygous genotypes were represented by two bands. Complete synthetic gel profiles for all 58 accessions are provided in Figure S1.

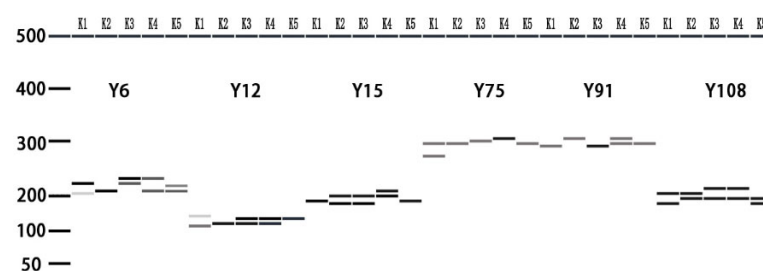


Figure 4. Synthetic gel profiles of five durian accessions at six core SSR loci. Band positions indicate allele sizes, with one band representing homozygous genotypes and two bands representing heterozygous genotypes.

3.4.2. Construction of Molecular Identity Codes

For each accession, the genotype at each of the six core loci was recorded as “smaller allele size/larger allele size” in the fixed order Y6, Y12, Y15, Y75, Y91, and Y108. For homozygous genotypes, the same allele size was recorded twice, whereas two different allele sizes were recorded for heterozygous genotypes. The genotype entries for the six loci were joined by hyphens to generate a six-locus molecular identity code for each accession. The 58 accessions had 58 distinct codes (Table 2), allowing all accessions in the study panel to be uniquely identified.

Table 2. Six-locus SSR fingerprints and molecular identity codes of the 58 durian accessions.

Sample ID	Molecular ID (Y6-Y12-Y15-Y75-Y91-Y108)	Sample ID	Molecular ID (Y6-Y12-Y15-Y75-Y91-Y108)
K1	204/214-113/136-189/189-273/295-289/289-184/202	K30	204/214-113/130-189/189-273/295-289/289-195/201
K2	206/206-118/118-183/201-294/294-300/300-196/202	K31	203/206-118/124-195/211-295/295-271/271-195/208
K3	214/224-118/130-183/201-296/296-289/289-196/215	K32	222/222-113/130-183/201-296/296-256/256-184/184
K4	206/224-118/130-201/207-299/299-294/300-196/215	K33	206/210-118/130-189/189-293/293-295/295-195/214
K5	206/208-130/130-189/189-294/294-294/294-184/196	K34	235/235-113/113-195/195-299/299-289/289-195/201
K6	206/206-118/130-189/189-294/294-289/289-184/184	K35	204/214-113/130-189/189-273/296-289/289-183/201
K7	204/212-113/130-189/189-273/295-289/289-184/202	K36	206/214-118/130-189/195-273/296-256/300-208/214
K8	206/206-118/130-183/194-299/299-294/294-202/215	K37	213/215-113/118-189/189-273/294-289/300-183/183
K9	214/224-130/130-183/183-294/294-289/295-215/215	K38	204/214-113/130-189/189-273/295-256/289-183/201
K10	201/235-113/136-183/189-277/294-256/256-215/215	K39	206/214-113/118-183/201-294/296-289/289-183/183
K11	206/214-113/130-194/201-273/296-289/289-215/215	K40	201/201-113/136-183/189-277/294-256/256-214/214
K12	204/214-113/130-189/189-273/295-289/289-184/202	K41	215/215-130/130-195/207-299/299-271/283-195/195
K13	203/206-118/124-195/211-294/294-294/294-195/208	K42	206/206-118/118-189/201-295/295-271/289-183/214
K14	214/224-130/130-195/207-277/294-294/294-195/214	K43	206/235-113/130-183/195-300/300-256/256-195/214
K15	206/208-118/136-183/189-273/296-289/289-183/195	K44	214/224-118/136-183/195-296/296-300/300-195/208
K16	201/235-113/136-183/189-273/295-294/294-214/214	K45	224/224-118/130-195/209-272/272-300/300-189/208
K17	201/235-118/136-183/189-273/295-294/294-214/214	K46	206/206-118/118-183/201-294/296-300/300-195/201
K18	204/208-113/118-189/201-294/294-300/300-214/214	K47	235/235-113/130-195/207-294/294-289/289-214/214
K19	204/212-113/130-189/189-273/295-289/289-183/201	K48	206/225-118/136-189/201-294/294-271/295-183/214
K20	206/206-118/130-183/189-294/294-289/289-214/214	K49	212/224-130/136-189/195-277/294-294/294-183/183
K21	214/224-113/130-183/183-294/294-289/289-214/214	K50	204/214-130/136-195/195-294/294-283/303-195/214
K22	206/214-130/130-183/189-294/294-300/300-183/195	K51	201/208-130/136-195/201-294/296-256/300-214/214
K23	204/214-113/130-189/189-273/295-300/300-183/201	K52	201/235-113/136-183/189-277/294-256/256-214/214
K24	201/235-113/136-189/189-273/295-294/294-214/214	K53	206/224-118/136-189/232-294/294-271/294-183/214
K25	201/235-113/136-183/189-277/294-294/294-214/214	K54	206/206-118/130-183/195-294/294-256/256-201/214
K26	206/212-118/136-183/183-294/294-289/289-183/195	K55	208/214-130/130-189/201-294/296-256/256-183/195

Table 2. Cont.

Sample ID	Molecular ID (Y6-Y12-Y15-Y75-Y91-Y108)	Sample ID	Molecular ID (Y6-Y12-Y15-Y75-Y91-Y108)
K27	204/224-118/130-195/209-271/271-300/300-189/208	K56	206/214-130/130-183/201-294/296-256/300-214/214
K28	205/224-118/130-189/189-293/293-289/289-195/214	K57	208/214-130/136-183/195-294/296-256/289-195/195
K29	204/214-113/130-189/189-273/295-289/289-183/201	K58	204/224-118/130-195/209-272/272-283/300-189/208

3.5. Cluster Analysis and Analysis of Molecular Variance

3.5.1. Cluster Analysis

Dice similarity coefficients were calculated from the allele presence–absence matrix of the 18 SSR loci, and UPGMA cluster analysis was performed for the 58 durian accessions. The accessions showed substantial variation in their SSR allelic profiles. At a Dice similarity coefficient of approximately 0.42, the accessions were divided into six clusters for descriptive purposes (Figure 5). Cluster I comprised two accessions (K31 and K13); Cluster II comprised three accessions (K27, K58, and K45); Cluster III comprised two accessions (K41 and K34); Cluster IV comprised 19 accessions (K37, K26, K49, K10, K52, K40, K25, K17, K24, K16, K7, K12, K1, K23, K38, K35, K30, K29, and K19); Cluster V comprised nine accessions (K8, K4, K46, K2, K42, K15, K33, K6, and K5); and Cluster VI comprised 23 accessions (K44, K32, K11, K39, K3, K18, K53, K48, K28, K20, K21, K9, K14, K50, K51, K47, K54, K43, K36, K22, K57, K56, and K55).

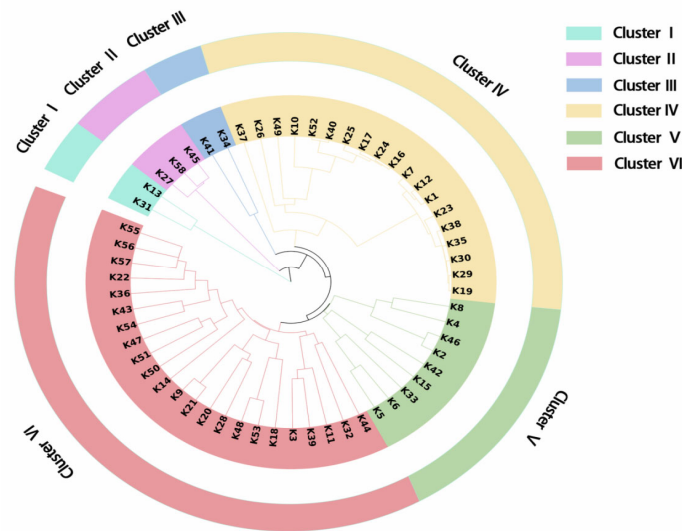


Figure 5. A UPGMA dendrogram of 58 durian accessions based on Dice similarity coefficients calculated from the allele presence–absence matrix of the 18 SSR loci. At a Dice similarity coefficient of approximately 0.42, the accessions were divided into six clusters for descriptive purposes (Clusters I–VI, indicated by different colors).

3.5.2. Partitioning of Genetic Variation by AMOVA

Based on the descriptive partitioning of the UPGMA dendrogram, the 58 durian accessions were divided into six clusters. AMOVA revealed that genetic variation among clusters accounted for 2% of the total variation, whereas variation among individuals within clusters and within individuals accounted for 17% and 81%, respectively. The genetic differentiation coefficient (F_{ST}) among clusters was 0.016 (permutation $p = 0.029$; Table 3). When grouped by recorded origin, the single Vietnamese accession was excluded,

and the remaining 57 accessions were divided into two groups according to their origins: Malaysia and Thailand. AMOVA results for this grouping showed that variation between origin groups accounted for 5% of the total variation, while variation among individuals within groups and within individuals accounted for 17% and 78%, respectively. The F_{ST} between the two groups was 0.049 (permutation $p = 0.008$).

Table 3. Analysis of molecular variance (AMOVA) based on UPGMA clustering and geographic origin grouping.

Grouping Criterion	Source	df	SS	MS	Estimated Variance	Percentage of Variation	Stat	Value	<i>p</i>
UPGMA-based	Among populations	5	45.932	9.186	0.106	2%			
	Among individuals within populations	52	387.654	7.455	1.102	17%			
	Within individuals	58	304.500	5.250	5.250	81%	F_{ST}	0.016	0.029
Geographic origin grouping	Among populations	1	13.463	13.463	0.328	5%			
	Among individuals within populations	55	411.317	7.478	1.125	17%			
	Within individuals	57	298.000	5.228	5.228	78%	F_{ST}	0.049	0.008

Note: df, degrees of freedom; SS, sum of squares; MS, mean square; F_{ST} , coefficient of genetic differentiation among populations. *p*-values were obtained from 9999 random permutations.

3.6. Population Structure Inferred by STRUCTURE

Mean $\text{LnP}(K)$ generally increased with increasing K , although some fluctuations were observed. According to the Evanno method, ΔK reached its maximum at $K = 8$, supporting eight as the most likely number of genetic clusters for the 58 accessions based on the 18 SSR loci. At $K = 8$, most accessions were assigned predominantly to a single genetic cluster, whereas several accessions showed membership in two or more clusters, indicating varying levels of admixture (Figure 6).

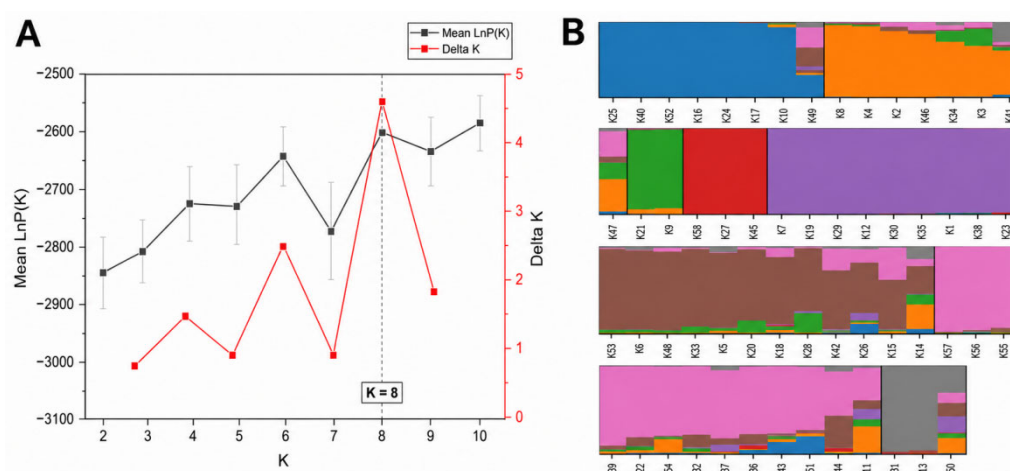


Figure 6. Population structure analysis of 58 durian accessions using STRUCTURE. (A) Mean $\text{LnP}(K)$ and ΔK values across K values ranging from 2 to 10. (B) Population structure at $K = 8$. Each vertical bar represents an accession, with eight inferred genetic groups indicated by different colors. Accessions are ordered by their predominant ancestral component and arranged in four rows.

3.7. Principal Coordinate Analysis

Principal coordinate analysis (PCoA) was performed using the Dice distance matrix derived from the allele presence–absence matrix of the 18 SSR loci. The first two coordinates explained 19.74% and 13.17% of the SSR allelic variation, respectively, cumulatively accounting for 32.91% of the total variation. To visualize the distribution of the STRUCTURE-inferred genetic groups in the PCoA space, accessions were colored according to their predominant ancestral component assignments at $K = 8$ (Figure 7).

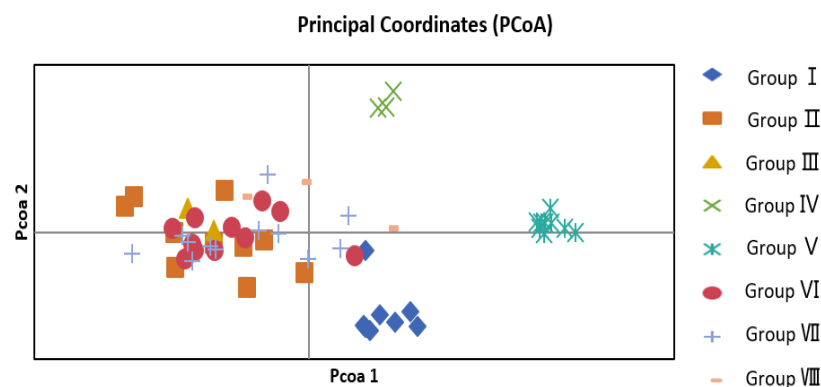


Figure 7. Principal coordinate analysis (PCoA) of 58 durian accessions based on the Dice distance matrix derived from the allele presence–absence data at 18 SSR loci. Different colors and symbols denote Groups I–VIII inferred from STRUCTURE at $K = 8$.

Group I is mainly distributed in the lower part of the plot, Group IV in the upper part, and Group V in the right region, with these three groups showing some separation from one another. Groups II, III, VI, and VII are mostly located in the left and central regions, with considerable overlap among them. Group III, consisting of only two accessions, clusters close to Group II. Groups VI and VII have relatively broad distributions and overlap with some accessions of Group II. The three accessions of Group VIII are scattered throughout the plot.

4. Discussion

Simple sequence repeat (SSR) markers are codominant, highly polymorphic, reproducible, and widely distributed throughout plant genomes. They have therefore been widely used for genetic diversity assessment, population structure analysis, cultivar identification, and germplasm management [22–24]. SSR-based identification systems have been successfully applied in citrus, grapevine, apple, and other horticultural crops [25–27]. In contrast, molecular marker studies in durian remain relatively limited and have generally focused on genetic diversity or small germplasm panels, restricting their application in systematic germplasm evaluation, varietal authenticity testing, and molecular breeding [28].

The SSR density identified in the durian genome (145.24 loci/Mb) was higher than the values of 41.2, 49.1, and 74.8 loci/Mb reported for *Gossypium hirsutum*, *G. arboreum*, and *G. raimondii*, respectively [29]. However, SSR density is influenced by genome assembly quality, detection thresholds, and the definition of compound SSRs. Therefore, this comparison should be interpreted descriptively rather than as evidence of a strict interspecific difference. Nevertheless, the large number of loci identified indicates that the durian genome contains abundant SSR resources for marker development.

The mean PIC of the selected SSR markers (0.6639) was higher than the value of 0.39 reported previously [30]. The relatively high PIC values likely reflect both the marker-selection process and the allele frequency distribution in the present accession panel.

Because the 18 loci were selected from 160 candidates based partly on preliminary polymorphism, the final marker panel was intentionally enriched for informative loci. In addition, capillary electrophoresis provided substantially higher allele size resolution than the preliminary agarose gel screening. Whereas agarose electrophoresis may not reliably distinguish SSR fragments differing by only a few base pairs, capillary electrophoresis enables more precise fragment sizing and can resolve closely spaced alleles that might otherwise be scored as the same band. Thus, capillary electrophoresis does not increase the underlying biological polymorphism, but can provide a more accurate estimate of allele number and marker informativeness. In addition, the M13-tailed fluorescent labeling strategy reduced genotyping costs and improved the suitability of the system for large scale analysis [31]. These results show that the present study converted genome-wide SSR resources into a set of experimentally validated and informative markers for durian germplasm identification and genetic analysis.

Previous durian SSR systems were developed mainly for genetic diversity analysis and preliminary fingerprinting. One study developed 67 SSR primer pairs from an SSR-enriched genomic library and subsequently evaluated 11 of these loci in 17 durian accessions, identifying 40 alleles with a mean PIC value of 0.390 [30]. Another study developed seven primer pairs from GenBank sequences, of which five were polymorphic, and obtained unique fingerprints for 21 of 27 Malaysian durian types [32]. In addition, nine SSR loci were used to analyze 24 Thai cultivars, detecting 21 alleles with PIC values of 0.258–0.535, although two cultivar pairs remained indistinguishable [13]. In contrast, the present study employed genome-wide SSR mining and capillary electrophoresis to validate 18 polymorphic loci and further identified the core SSR marker set that generated unique molecular identity codes for all 58 accessions in the current panel.

Exhaustive evaluation of all combinations of the 18 polymorphic SSR loci identified Y6, Y12, Y15, Y75, Y91 and Y108 as the core SSR marker set capable of distinguishing all 58 accessions. The six loci generated 58 unique multilocus genotypes, with a cumulative PID of 7.99×10^{-8} . The compact size and high discriminatory power observed in the current panel indicate that this set may provide a cost-effective starting point for routine genotyping, subject to validation in broader germplasm collections.

Core SSR sets have also been developed for cultivar identification in grapevine, potato, kernel-used apricot, and mandarin [14,15,33,34]. However, the number of markers required varies with the genetic relatedness of the materials, the information content of the loci, and genotyping resolution. Closely related accessions may therefore require more markers than genetically diverse collections.

The distribution of the six core loci across different chromosomes and the reduction in discriminatory power after removal of any single locus suggest that these markers provide complementary information. Further validation in a broader germplasm collection will be needed before the marker set is applied beyond the present accession panel.

UPGMA analysis based on the 18 SSR loci divided the 58 accessions into six clusters at a descriptive Dice similarity threshold of approximately 0.42. AMOVA showed that variation among clusters accounted for only 2% of the total genetic variation, whereas variation among individuals within clusters and within individuals accounted for 17% and 81%, respectively. Although the among cluster differentiation was statistically significant, the low F_{ST} value of 0.016 indicates that the clusters represent differences in SSR profiles rather than strongly differentiated genetic populations.

When grouped by recorded geographic origin, variation between the Malaysian and Thai accessions accounted for 5% of the total variation, with an F_{ST} of 0.049. Most variation remained within origin groups and within individuals, suggesting that geographic origin explained only a limited proportion of the genetic differences. Because the two groups

contained 52 and 5 accessions, respectively, this result should not be generalized to the broader durian germplasm of Malaysia and Thailand.

STRUCTURE analysis supported $K = 8$, whereas UPGMA produced six descriptive clusters. This difference is not contradictory because UPGMA groups accessions according to pairwise similarity, while STRUCTURE estimates ancestry components and allows for admixed membership [21,35]. Together with the low among-group variation and the overlap among groups in the PCoA, these results indicate weak genetic substructure with no clear boundaries among all inferred groups. Given the limited and geographically unbalanced sampling, the $K = 8$ solution should therefore be interpreted as a statistical partition of the analyzed accession panel and does not necessarily correspond to eight biologically distinct populations.

Previous SSR studies also found incomplete correspondence among durian cultivar names, geographic origins, and molecular groupings [13,32]. Genome-wide studies identified different numbers of genetic groups, including three major groups among 114 accessions analyzed using more than 39 million SNPs [36]. Such differences likely reflect variation in sample composition, marker density, and analytical resolution. The admixed ancestry observed in some accessions may be associated with shared parentage, germplasm exchange, or similar breeding backgrounds, but these possibilities require validation using pedigree records and genome-wide data.

The core SSR marker set generated unique genotypes for all 58 accessions and showed concordant genotyping results across independent DNA extractions in the five accessions included in the preliminary reproducibility assessment. It can support germplasm verification, DNA fingerprint database construction, varietal authenticity testing, and nursery stock management. Compared with morphology-based identification, this molecular system provides objective and standardized records that are less affected by environmental conditions and plant developmental stages. Compared with the full 18-locus panel, the core SSR marker set reduces genotyping effort and cost.

However, the core SSR marker set was developed and evaluated using the current panel of 58 accessions. Consequently, the 100% discrimination observed in this study should be considered specific to the current panel and may not be retained when additional or more diverse germplasm is included. Moreover, the panel was geographically unbalanced, comprising 52 Malaysian accessions, 5 Thai accessions, and 1 Vietnamese accession, and therefore cannot be considered representative of the broader genetic diversity of durian. Accordingly, the core SSR marker set comprising Y6, Y12, Y15, Y75, Y91, and Y108 should be regarded as a candidate fingerprinting system for the current panel, and its discriminatory performance should be validated using larger, geographically broader, and more balanced germplasm collections before broader application.

In addition, the possible occurrence of null alleles was not explicitly evaluated in this study. Null alleles may result in allele dropout and apparent homozygosity, potentially affecting allele frequency estimates and genetic analyses. Although the selected SSR loci showed stable amplification, future validation should include specific assessment of null alleles.

5. Conclusions

This study identified 106,776 SSR loci in the durian reference genome and screened 18 stable and polymorphic SSR primer pairs from 160 candidates. Across 58 durian accessions, these loci detected 142 alleles, with a mean PIC of 0.6639, demonstrating their high informativeness for genetic diversity analysis. Exhaustive combination analysis identified the core SSR marker set comprising Y6, Y12, Y15, Y75, Y91 and Y108. This marker set generated a unique multilocus genotype for each accession and enabled the construction of DNA fingerprints and molecular identity codes. Genetic analyses showed that most varia-

tion occurred within individuals and among individuals within groups, whereas recorded geographic origin explained only a small proportion of the variation in the present panel. These results provide a promising and compact marker framework for durian germplasm identification, fingerprint database construction, and accession management within the analyzed accession panel, warranting validation in larger, geographically broader, and more balanced germplasm collections before broader application.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12091145/s1>. Figure S1: Complete synthetic gel profiles of 58 durian accessions at the six core SSR loci; Table S1: Information of the 58 durian accessions, including accession codes, cultivar names, and geographic origins. Table S2: Nucleotide sequences of the 18 SSR primers used for capillary electrophoresis; Table S3: SSR allele genotypes of the 58 durian accessions.

Author Contributions: Conceptualization, H.T. and Y.W.; methodology, H.T., M.W. and Y.W.; software, Y.W. and J.S.; validation, Y.W., J.S., M.W., D.L., C.Z. (Chonghao Zhong), C.Z. (Chaofan Zheng) and H.T.; formal analysis, Y.W. and M.W.; investigation, Y.W., J.H., C.Z. (Chonghao Zhong) and C.Z. (Chaofan Zheng); resources, H.T., F.Y., G.Z. and Z.C.; data curation, Y.W. and J.S.; writing—original draft preparation, Y.W.; writing—review and editing, H.T. and M.W.; visualization, Y.W., J.S. and M.W.; supervision, H.T.; project administration, H.T. and M.W.; funding acquisition, H.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Hainan Province Science and Technology Special Fund (ZDYF2026SXLH028); the earmarked fund for HNARS (HNARS-11-Z01); Science and Technology Courtyard Project of Hainan State Farms Holding Group (HKKJ2025KJXY07); Special Fund for Tropical High-efficiency Agriculture Industrial Technology System of Hainan University (THAITS-5).

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: Fuwang Yang was employed by the company Hainan Youqi Agriculture Co., Ltd. Zhifu Cui was employed by the company Hainan State Farms Academy of Sciences Group Co., Ltd. Guozhu Zhang was employed by the company Hainan Baoting Yunding Durian Planting Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Husin, N.A.; Rahman, S.; Karunakaran, R.; Bhore, S.J. A review on the nutritional, medicinal, molecular and genome attributes of Durian (*Durio zibethinus* L.), the King of fruits in Malaysia. *Bioinformation* **2018**, *14*, 265. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Chomdej, O.; Tongyoo, P.; Nualsri, C.; Duangsodsri, T.; Yamjabok, J.; Attavanich, W.; Poonpolgul, S.; Poovarodom, S.; Sahaya, S.; Nakkanong, K. Durian Varieties, Their Breeding and Intellectual Property Perspectives in Southeast Asia with Special References to Thailand. *Horticulturae* **2026**, *12*, 831. [\[CrossRef\]](#)
3. Vanijajiva, O. Genetic variability among durian (*Durio zibethinus* Murr.) cultivars in the Nonthaburi province, Thailand detected by RAPD analysis. *J. Agric. Technol.* **2011**, *7*, 1107–1116.
4. Cabezas, J.A.; Ibáñez, J.; Lijavetzky, D.; Vélez, D.; Bravo, G.; Rodríguez, V.; Carreño, I.; Jermakow, A.M.; Carreño, J.; Ruiz-García, L. A 48 SNP set for grapevine cultivar identification. *BMC Plant Biol.* **2011**, *11*, 153. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Testolin, R.; Messina, R.; Cipriani, G.; De Mori, G. SSR-based DNA fingerprinting of fruit crops. *Crop Sci.* **2023**, *63*, 390–459. [\[CrossRef\]](#)
6. Zhang, C.; Lin, P.; Yao, X.; Ren, H.; Wang, K. Genome-wide development of genomic SSR markers, polymorphism analysis, and dosage-aware SSR fingerprinting in hexaploid *Camellia oleifera*. *BMC Plant Biol.* **2025**, *25*, 1627. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Li, W.; Chen, X.; Yu, J.; Zhu, Y. Upgraded durian genome reveals the role of chromosome reshuffling during ancestral karyotype evolution, lignin biosynthesis regulation, and stress tolerance. *Sci. China Life Sci.* **2024**, *67*, 1266–1279. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Liu, Z.; Zhang, J.; Wang, Y.; Wang, H.; Wang, L.; Zhang, L.; Xiong, M.; He, W.; Yang, S.; Chen, Q. Development and cross-species transferability of novel genomic-SSR markers and their utility in hybrid identification and trait association analysis in Chinese cherry. *Horticulturae* **2022**, *8*, 222. [\[CrossRef\]](#)

9. Hu, J.; Wang, L.; Li, J. Comparison of genomic SSR and EST-SSR markers for estimating genetic diversity in cucumber. *Biol. Plant.* **2011**, *55*, 577–580. [[CrossRef](#)]
10. Manco, R.; Chiaiese, P.; Basile, B.; Corrado, G. Comparative analysis of genomic- and EST-SSRs in European plum (*Prunus domestica* L.): Implications for the diversity analysis of polyploids. *3 Biotech* **2020**, *10*, 543. [[CrossRef](#)] [[PubMed](#)]
11. Hannum, S.; Wahyuningsih, H.; Sinaga, R.; Pasaribu, N.; Hartanto, A. Genetic diversity among durian (*Durio zibethinus* Murr.) populations from Nias Island, Indonesia using RAPD markers. *Appl. Ecol. Environ. Res.* **2020**, *18*, 7337. [[CrossRef](#)]
12. Ngo, T.T.T.; Nguyen, N.T.; Thong, N.H.T.; Vo, V.C.T.; Le, A.Q.T.; Nguyen, P.V. Assessing genetic distinctiveness of Vietnamese *Durio zibethinus* genotypes using inter-simple sequence repeat (ISSR) markers and chloroplast DNA barcoding. *Genet. Resour. Crop Evol.* **2025**, *72*, 9809–9820. [[CrossRef](#)]
13. Songnuan, W.; Pichakum, A.; Traiperm, P.; Rungjangsuwan, E.-O.; Siriwananakul, U.; Leeratsuwan, N.; Chareonsap, P.P.; Kulpradit, K.; Somsri, S.; Swangpol, S.C. Diversity of durian (*Durio zibethinus* L.) from Nonthaburi, Thailand based on morpho-palatability characteristics and simple sequence repeat markers. *Agric. Nat. Resour.* **2019**, *53*, 218–227. [[CrossRef](#)]
14. Kishine, M.; Tsutsumi, K.; Kitta, K. A set of tetra-nucleotide core motif SSR markers for efficient identification of potato (*Solanum tuberosum*) cultivars. *Breed. Sci.* **2017**, *67*, 544–547. [[CrossRef](#)] [[PubMed](#)]
15. Migliaro, D.; De Nardi, B.; Vezzulli, S.; Crespan, M. An upgraded core set of 11 SSR markers for grapevine cultivar identification: The case of berry-color mutants. *Am. J. Enol. Vitic.* **2017**, *68*, 496–498. [[CrossRef](#)]
16. Schuelke, M. An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol.* **2000**, *18*, 233–234. [[CrossRef](#)] [[PubMed](#)]
17. Porebski, S.; Bailey, L.G.; Baum, B.R. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Report.* **1997**, *15*, 8–15. [[CrossRef](#)]
18. Beier, S.; Thiel, T.; Münch, T.; Scholz, U.; Mascher, M. MISA-web: A web server for microsatellite prediction. *Bioinformatics* **2017**, *33*, 2583–2585. [[CrossRef](#)] [[PubMed](#)]
19. Waits, L.P.; Luikart, G.; Taberlet, P. Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Mol. Ecol.* **2001**, *10*, 249–256. [[CrossRef](#)] [[PubMed](#)]
20. Botstein, D.; White, R.L.; Skolnick, M.; Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **1980**, *32*, 314. [[PubMed](#)]
21. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [[CrossRef](#)] [[PubMed](#)]
22. Castellana, S.; Ranzino, L.; Beritognolo, I.; Cherubini, M.; Luneia, R.; Villani, F.; Mattioni, C. Genetic characterization and molecular fingerprint of traditional Umbrian tomato (*Solanum lycopersicum* L.) landraces through SSR markers and application for varietal identification. *Genet. Resour. Crop Evol.* **2020**, *67*, 1807–1820. [[CrossRef](#)]
23. Özkan, G.; Haliloğlu, K.; Türkoğlu, A.; Öztürk, H.I.; Elkoca, E.; Pocza, P. Determining genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from Türkiye using SSR markers. *Genes* **2022**, *13*, 1410. [[CrossRef](#)] [[PubMed](#)]
24. Singh, J.; Sharma, A.; Sharma, V.; Gaikwad, P.N.; Sidhu, G.S.; Kaur, G.; Kaur, N.; Jindal, T.; Chhuneja, P.; Rattanpal, H. Comprehensive genome-wide identification and transferability of chromosome-specific highly variable microsatellite markers from citrus species. *Sci. Rep.* **2023**, *13*, 10919. [[CrossRef](#)] [[PubMed](#)]
25. Barkley, N.A.; Roose, M.L.; Krueger, R.R.; Federici, C.T. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). *Theor. Appl. Genet.* **2006**, *112*, 1519–1531. [[CrossRef](#)] [[PubMed](#)]
26. Emanuelli, F.; Lorenzi, S.; Grzeskowiak, L.; Catalano, V.; Stefanini, M.; Troggio, M.; Myles, S.; Martinez-Zapater, J.M.; Zyprian, E.; Moreira, F.M. Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. *BMC Plant Biol.* **2013**, *13*, 39. [[CrossRef](#)] [[PubMed](#)]
27. Lassois, L.; Denancé, C.; Ravon, E.; Guyader, A.; Guisnel, R.; Hibrand-Saint-Oyant, L.; Poncet, C.; Lasserre-Zuber, P.; Feugey, L.; Durel, C.-E. Genetic diversity, population structure, parentage analysis, and construction of core collections in the French apple germplasm based on SSR markers. *Plant Mol. Biol. Report.* **2016**, *34*, 827–844. [[CrossRef](#)]
28. Prihatini, R.; Anggraeni, L.; Hadiati, S.; Pramanik, D.; Nugroho, K.; Sukartini, S.; Budiyaniti, T.; Budiyaniti, E.; Baswarsati, B.; Purnama, S. Genomic research on the king of fruit (*Durio* spp.): A systematic literature review. *Genet. Resour. Crop Evol.* **2025**, *72*, 7619–7638. [[CrossRef](#)]
29. Wang, Q.; Fang, L.; Chen, J.; Hu, Y.; Si, Z.; Wang, S.; Chang, L.; Guo, W.; Zhang, T. Genome-wide mining, characterization and development of microsatellite markers in *Gossypium* species. *Sci. Rep.* **2015**, *5*, 10638. [[CrossRef](#)] [[PubMed](#)]
30. Santoso, P.J.; Pancoro, A.; Suhandono, S.; Aryantha, I.N.P. Development of simple-sequence repeats markers from Durian (*Durio zibethinus* Murr. cultv. Matahari) genomic library. *Agrivita J. Agric. Sci.* **2017**, *39*, 257–265. [[CrossRef](#)]
31. Hayden, M.J.; Nguyen, T.M.; Waterman, A.; Chalmers, K.J. Multiplex-ready PCR: A new method for multiplexed SSR and SNP genotyping. *BMC Genom.* **2008**, *9*, 80. [[CrossRef](#)] [[PubMed](#)]
32. Siew, G.Y.; Ng, W.L.; Tan, S.W.; Alitheen, N.B.; Tan, S.G.; Yeap, S.K. Genetic variation and DNA fingerprinting of durian types in Malaysia using simple sequence repeat (SSR) markers. *PeerJ* **2018**, *6*, e4266. [[CrossRef](#)] [[PubMed](#)]

33. Sun, Y.; Chen, J.; Zhang, Y.; Liu, R.; Wang, S.; Dong, S. Establishment and application of DNA molecular fingerprint of kernel-used apricots using capillary electrophoresis with fluorescence-labeled simple sequence repeats (SSR). *Genet. Resour. Crop Evol.* **2025**, *72*, 657–672. [[CrossRef](#)]
34. Wu, X.; Wu, S.; Fang, H.; Huang, D.; Chen, C.; Lou, B.; Liu, P.; Tang, Y.; Feng, J.; Deng, C. Fluorescent SSR-Based DNA Fingerprinting and Molecular Identity Card Development for 69 Mandarin Accessions. *Horticulturae* **2026**, *12*, 445. [[CrossRef](#)]
35. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [[CrossRef](#)] [[PubMed](#)]
36. Zhong, Y.; Feng, L.; Deng, H.; Ji, X.; Zhang, J.; Sun, Y.; Lin, P.; Qiao, Y.; Xie, S.; Wang, H. Genomic resequencing reveals genetic diversity, population structure, and core collection of durian germplasm. *Commun. Biol.* **2025**, *8*, 1273. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.