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Genetic diversity of pigmented rice landraces (*Oryza sativa*) from South Sumatra, Indonesia revealed by SSR markers

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Abstract. *Hanum L, Wahyuni T, Oktariansyah Y. 2026. Genetic diversity of pigmented rice landraces* (Oryza sativa) *from South Sumatra, Indonesia revealed by SSR markers. Biodiversitas 27 (1): d270130. https://doi.org/10.13057/biodiv/d270130*. Pigmented rice landraces constitute an important component of Indonesia's rice genetic resources, yet information on their molecular diversity in South Sumatra remains limited. This study tested the hypothesis that pigmented rice landraces from South Sumatra exhibit SSR-detectable genetic variation among accessions, whereas genome-wide differentiation between pigmentation groups (red and black rice) is weak or non-significant. This study assessed the genetic diversity and relatedness of ten local pigmented rice (*Oryza sativa*) landraces, comprising seven red and three black rice accessions, using 11 Simple Sequence Repeat (SSR) markers. All markers produced clear and reproducible amplification profiles, generating fragments ranging from 127 to 480 bp, with six to twelve alleles per locus. Marker informativeness was consistently high, with PIC values of 0.790-0.917 (mean = 0.878), indicating strong discriminatory power for accession differentiation. SSR profiles were predominantly homozygous, reflecting the autogamous reproductive system of rice, although limited heterozygosity was detected at a few loci in specific accessions. Multilocus comparison revealed moderate divergence among landraces, supported by Jaccard similarity coefficients ranging from 0.769 to 1.000. UPGMA clustering resolved a structured pattern consisting of a closely related core group of accessions, an intermediate subgroup represented by Linggau and Tanjung Agung, and a more divergent subgroup comprising Sumber Jaya and Teluk Tenggirik. However, AMOVA indicated that molecular variation was predominantly distributed within populations (100%) rather than among populations (0%), with a non-significant differentiation between red and black rice groups (F_{st} = -0.038; p = 0.769). These findings provide baseline molecular evidence supporting conservation prioritization and highlight genetically distinct landraces as potential donors for future pigmented rice breeding programs. This study represents one of the few SSR-based molecular assessments focusing specifically on pigmented rice landraces from South Sumatra, Indonesia.

Keywords: Genetic diversity, landraces, pigmented rice, Simple Sequence Repeats (SSR) markers, South Sumatra

INTRODUCTION

Pigmented rice (*Oryza sativa* L.), particularly red and black rice, constitutes a distinctive and valuable component of traditional rice diversity across Asia. In Indonesia, including South Sumatra, pigmented rice has long been cultivated in localized agroecosystems and maintained through farmer-led selection and community-specific cultural practices (Paiman et al. 2020; Ratmini and Herwenita 2021). These landraces are highly valued not only for their characteristic grain coloration, which is associated with elevated levels of anthocyanins, flavonoids, and other bioactive compounds with antioxidant and potential health-promoting properties (Bhat et al. 2020; Chen et al. 2022), but also for their cultural and historical significance in traditional ceremonies, local cuisines, and customary seed-exchange systems (Mbanjo et al. 2019; Van Andel et al. 2019). Despite their importance, pigmented rice landraces are increasingly threatened by agricultural modernization, land-use change, and replacement by high-yielding modern cultivars, placing many local genotypes at risk of genetic erosion (Sivakumar et al. 2021; Joshi et al. 2023). This situation is particularly critical for locally maintained landraces, where erosion can occur rapidly when traditional cultivation practices decline, and seed systems shift toward uniform improved varieties. As a result, reliable molecular information is urgently needed to document the remaining genetic resources, identify unique landraces, and support evidence-based conservation and sustainable utilization strategies.

Understanding genetic diversity is fundamental to the effective management of rice genetic resources and the development of improved cultivars. Genetic variation underpins adaptation to biotic and abiotic stresses and determines the potential of landraces to contribute valuable traits to breeding programs (Hanum et al. 2025). In South Sumatra, pigmented rice cultivation spans diverse agroecological settings, ranging from lowland rainfed systems to upland traditional fields, where long-term farmer selection and environmental pressures may have shaped unique allelic combinations (Sitaresmi et al. 2023). However, despite this ecological and cultural heterogeneity, information on the genetic structure, evolutionary relationships, and degree of divergence among pigmented rice landraces from South Sumatra remains limited. Previous studies in Indonesia and neighboring regions have largely focused on agronomic performance, biochemical composition, or molecular diversity in other provinces and countries, such as Java, East Nusa Tenggara, and the Philippines (Mbanjo et al. 2019; Rana et

al. 2020; Sudan et al. 2023), leaving South Sumatra underrepresented in molecular diversity assessments.

Molecular markers provide powerful tools for elucidating genetic diversity and relationships among rice genotypes. Among these, Simple Sequence Repeat (SSR) markers are widely used due to their high polymorphism, codominant inheritance, reproducibility, and broad distribution across the rice genome (Salem et al. 2024). SSR markers are particularly effective for detecting allelic variation among closely related landraces, enabling fine-scale assessment of population structure and phylogenetic patterns (Choudhury et al. 2023). In rice genetic research, SSRs have been extensively applied for varietal identification, germplasm classification, evolutionary studies, and marker-assisted selection (Ramesh et al. 2020; Salem et al. 2024), making them well-suited for characterizing traditional pigmented rice varieties shaped by long-term localized domestication. Therefore, SSR-based profiling offers a practical and informative approach to generate baseline molecular evidence for South Sumatran pigmented rice landraces, which can guide conservation prioritization and identify genetically distinct accessions with potential breeding value.

In this study, we address the knowledge gap in South Sumatra by applying SSR markers to characterize multilocus variation across named pigmented rice landraces maintained by local farmers. This molecular approach is essential for documenting inter-accession genetic differentiation, clarifying patterns of genetic relatedness among accessions, and providing an initial framework for future studies incorporating larger sampling, phenotypic evaluation, and higher-resolution genomic tools. This study aimed to characterize the genetic diversity and genetic relatedness (clustering patterns) of pigmented rice landraces from South Sumatra using a set of highly polymorphic SSR markers. By analyzing multilocus genotypic profiles and inter-accession genetic distances, this research elucidates the extent and structure of genomic differentiation among local landraces, including accessions representing red and black rice. The resulting molecular framework provides a basis for identifying genetically distinct accessions and supports conservation planning, sustainable utilization, and the potential incorporation of pigmented rice landraces into future breeding programs. Ultimately, this study contributes to broader efforts to safeguard Indonesia's rice genetic heritage under changing agricultural and environmental conditions. Based on these considerations, this study tested the following hypothesis: Local rice landraces exhibit measurable genetic diversity that can be resolved using SSR markers, with genetic variation occurring predominantly within phenotypic groups rather than among them.

MATERIALS AND METHODS

Study materials

Leaf samples of pigmented rice were collected from ten local accessions representing traditional red and black rice landraces across multiple districts in South Sumatra, Indonesia (Table 1). The accessions were selected based on their recognition by local farmers as distinct traditional landraces, contrasting grain color (red and black rice), and continued cultivation in different agroecological settings. Sampling locations encompassed diverse lowland agricultural environments, including irrigated fields, upland farms, and community-managed traditional paddies. For each accession, leaf tissue was collected from a single healthy plant grown in a farmer's field, and genomic DNA was extracted from that individual plant without pooling. Each accession, therefore, represents one independent genotype corresponding to a named local landrace and was treated as a single operational taxonomic unit in all genetic diversity and phylogenetic analyses. Consequently, the analyses focus on genetic differentiation among accessions rather than on within-accession variability. In total, seven red rice and three black rice landraces were analyzed.

Field exploration and sample collection were initiated in July 2020. Laboratory analyses and data processing were conducted over an extended period as part of a longer-term research project at the Genetics and Biotechnology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indonesia, with all analyses completed by December 2025. All leaf samples were washed with distilled water to remove adhering debris, air-dried, wrapped in aluminum foil, labelled, and stored at -20°C prior to molecular analysis. Voucher specimens for all accessions were archived at the UNSRI Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, under the following codes: BT-1 (Bungin Tinggi), CT-1 (Cahya Tani), JM-1 (Jaya Mulya), KR-1 (Keli Rejo), L-1 (Linggau), SJ-1 (Sumber Jaya), SP-1 (Sirah Pulau Padang), C-1 (Cecar), TA-1 (Tanjung Agung), and TT-1 (Teluk Tenggirik).

Sampling design

Ten pigmented rice accessions were purposively selected as a baseline representation of farmer-recognized red and black landraces from multiple districts in South Sumatra (Table 1). This sampling enables SSR-based discrimination and inter-accession comparison, but it does not capture the full diversity of pigmented rice in the province. Each accession was represented by a single sampled individual, which was used as an operational proxy for the named landrace to support inter-accession discrimination, while acknowledging that additional sampling is required to quantify within-landrace genetic variation.

Procedures

DNA extraction

Genomic DNA was extracted from leaf tissues using the Plant Genomic DNA Kit DP305 (TIANGEN Biotech, Beijing, China) following the manufacturer's instructions. Approximately 100 mg of young leaf tissue was ground to a fine powder and subjected to cell lysis using the provided extraction buffer. After removal of cellular debris by centrifugation, DNA was bound to the silica membrane column, washed to eliminate contaminants, and finally eluted in 50 µL of elution buffer. The extracted DNA was stored at -20°C until further analysis.

Quantitative analyses of DNA extraction results

DNA concentration and purity were quantified using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific) set to the dsDNA mode. Before each reading, the instrument was calibrated with sterile distilled water, after which 2 µL of DNA extract was placed on the measurement surface. Purity was determined from the A260/A280 absorbance ratio, with values between 1.7 and 2.0 and concentrations above 100 ng μL^{-1} considered indicative of high-quality genomic DNA (Dewanata and Mushlih 2021; Utaminingsih and Sophian 2022; Versmessen et al. 2024). All measurements were conducted in duplicate, and only samples with stable purity and concentration values were selected for downstream PCR amplification.

Qualitative analyses of DNA extraction results

Electrophoresis of the extracted DNA and PCR amplicons was carried out on a 1% agarose gel prepared by dissolving 0.6 g of agarose in 60 mL of TAE buffer and heating the mixture until fully liquefied. Once cooled to a non-solidifying temperature, 2 µL of DNA stain was incorporated, and the gel was poured into a casting tray fitted with a comb to generate sample wells. After solidification, the gel was immersed in 1× TAE buffer, and 4 µL of DNA mixed with 2 µL of loading dye was loaded into each well, with a 1 kb λ HindIII ladder serving as the molecular size reference. Electrophoresis proceeded at 80 V for 45 minutes, followed by visualization of DNA fragments under a UV transilluminator (Accuris E3000, 115 VAC: Edison, NJ, USA). Only high-quality DNA exhibiting sharp, unsmeared bands was selected for subsequent analyses.

PCR amplification and electrophoresis

A total of 11 microsatellite (SSR) primer pairs were used to amplify targeted loci in pigmented rice. These primers—RM5742, RM6997, RM201, RM263, RM324, RM416, RM518, RM60, RM105, RM124, and RM223—were selected because they have been widely validated for rice genetic diversity studies. Primer sequences were obtained from previously published sources that collectively span multiple chromosomal regions and provide robust allelic discrimination, making them well-suited for assessing genetic diversity and phylogenetic relationships among pigmented rice accessions (Nugroho et al. 2017; Andarini and Nugroho 2023; Veeraghattapu et al. 2024). Each primer pair consists of a forward and reverse sequence designed to flank SSR regions with high polymorphism. The full nucleotide sequences are present in Table 2. PCR reactions were prepared in a final volume of 25 µL consisting of 12.5 µL GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA), 4.5 µL ddH_2O, 1 µL each of forward and reverse primers, and 6 µL of genomic DNA template. The DNA template had a typical concentration range of approximately 20-50 ng μL^{-1}, providing sufficient template for reliable SSR amplification without excessive DNA loading.

PCR amplification was performed using a Bio-Rad T100™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). Preliminary optimization of annealing temperatures for each SSR primer pair was carried out using a gradient PCR approach. Based on these optimization runs, primer-specific optimal annealing temperatures were determined and subsequently applied as fixed annealing temperatures in the final PCR reactions used for data analysis. The final PCR program consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, primer annealing at the optimized temperature specific to each SSR marker for 1 minute, and extension at 72°C for 2 minutes. A final extension was performed at 72°C for 7 minutes, after which reactions were held at 4°C (Utami et al. 2010; Veeraghattapu et al. 2024). Detailed annealing temperatures for each SSR primer are provided in Table 3.

Table 1. Pigmented rice samples sourced in this study

Code	Sample identity	Group	Region
BT	Bungin Tinggi	Red rice	Ogan Komering Ilir
CT	Cahya Tani	Red rice	Ogan Komering Ilir
JM	Jaya Mulya	Red rice	Belitang
KR	Keli Rejo	Red rice	Ogan Komering Ulu Timur
L	Linggau	Red rice	Lubuk Linggau
SJ	Sumber Jaya	Red rice	Ogan Komering Ulu Timur
SP	Sirah Pulau Padang	Red rice	Ogan Komering Ilir
C	Cecar	Black rice	Musi Rawas
TA	Tanjung Agung	Black rice	Karang Jaya
TT	Teluk Tenggirik	Black rice	Air Kumbang

Table 2. SSR primer sequences

SSR markers	Sequences
RM5742	Forward: 5′-GGCGAGCGATCCTCAAAC-3′
	Reverse: 5′-GTTTACTCACGCTCTGCCAG-3′
RM6997	Forward: 5′-CAACGCGGCAGTAAATTTGC-3′
	Reverse: 5′-GGCCTTGTCAGTCTACATGC-3′
RM201	Forward: 5′-CTCGTTTATTACCTACAGTACC-3′
	Reverse: 5′-CTACCTCCTTTCTAGACCGATA-3′
RM263	Forward: 5′-CCCAGGCTAGCTCATGAACC-3′
	Reverse: 5′-GCTACGTTTGAGCTACCACG-3′
RM324	Forward: 5′-CTGATTCCACACACTTGTGC-3′
	Reverse: 5′-GATTCCACGTCAGGATCTTC-3′
RM416	Forward: 5′-GGGAGTTAGGGTTTTGGAGC-3′
	Reverse: 5′-TCCAGTTTCACACTGCTTCG-3′
RM518	Forward: 5′-CTCTTCACTCACTCACCATGG-3′
	Reverse: 5′-ATCCATCTGGAGCAAGCAAC-3′
RM60	Forward: 5′-AGTCCCATGTTCCACTTCCG-3′
	Reverse: 5′-ATGGCTACTGCCTGTACTAC-3′
RM105	Forward: 5′-GTCGTCGACCCATCGGAGCCAC-3′
	Reverse: 5′-TGGTCGAGGTGGGGATCGGGTC-3′
RM124	Forward: 5′-ATCGTCTGCGTTGCGGCTGCTG-3′
	Reverse: 5′-CATGGATCACCGAGCTCCCCCC-3′
RM223	Forward: 5′-GAGTGAGCTTGGGCTGAAAC-3′
	Reverse: 5′-GAAGGCAAGTCTTGGCACTG-3′

Table 3. Optimized annealing temperatures (°C) of SSR primer pairs determined by gradient PCR

Accessions	SSR markers										
	RM5742	RM6997	RM201	RM263	RM324	RM416	RM518	RM60	RM105	RM124	RM223
BT	55.6°C	55.7°C	50.0°C	55.9°C	55.2°C	56.1°C	55.0°C	53.7°C	64.1°C	64.9°C	56.9°C
CT	58.3°C	56.2°C	50.0°C	56.0°C	52.7°C	54.9°C	55.0°C	53.7°C	64.7°C	60.8°C	55.9°C
JM	55.9°C	55.7°C	50.0°C	55.9°C	55.0°C	54.8°C	55.1°C	53.7°C	64.7°C	64.8°C	56.5°C
KR	56.4°C	55.7°C	50.4°C	55.9°C	52.5°C	56.1°C	55.0°C	54.4°C	64.9°C	62.8°C	55.8°C
L	55.9°C	55.8°C	52.5°C	55.9°C	53.6°C	54.8°C	55.1°C	53.7°C	64.9°C	62.8°C	55.8°C
SJ	59.1°C	56.7°C	52.5°C	57.8°C	54.2°C	54.8°C	55.4°C	53.7°C	65.1°C	64.9°C	55.9°C
SP	55.6°C	55.8°C	50.0°C	55.9°C	52.5°C	55.6°C	55.4°C	53.9°C	65.1°C	64.9°C	57.0°C
TA	55.6°C	55.7°C	49.8°C	55.9°C	52.7°C	55.6°C	55.0°C	53.7°C	64.2°C	55.0°C	55.8°C
TT	55.6°C	55.7°C	50.9°C	55.9°C	52.5°C	54.8°C	55.0°C	53.7°C	64.7°C	60.8°C	55.8°C
C	55.9°C	55.7°C	52.5°C	57.8°C	52.7°C	54.9°C	55.0°C	53.7°C	64.1°C	64.1°C	56.0°C

Note: BT: Bungin Tinggi, CT: Cahya Tani, JM: Jaya Mulya, KR: Keli Rejo, L: Linggau, SJ: Sumber Jaya, SP: Sirah Pulau Padang, TA: Tanjung Agung, TT: Teluk Tenggirik, C: Cecarz

PCR products were subsequently resolved on a 1.6% agarose gel prepared in 1× TAE buffer and stained with 2 µL DNA dye. The molten agarose was poured into a casting tray fitted with a comb to form wells and allowed to solidify before electrophoresis. Approximately 2 µL of each PCR product was mixed with 1 µL loading dye and loaded into the wells, alongside a 100 bp DNA ladder (A λ HindIII DNA ladder, Geneaid) used as a molecular size reference. Electrophoresis was conducted at 80 V for 45 minutes, after which banding profiles were visualized under a UV transilluminator (Accuris E3000, 115 VAC: Edison, NJ, USA). Clear, distinct bands corresponding to expected fragment sizes were documented for downstream genetic analyses.

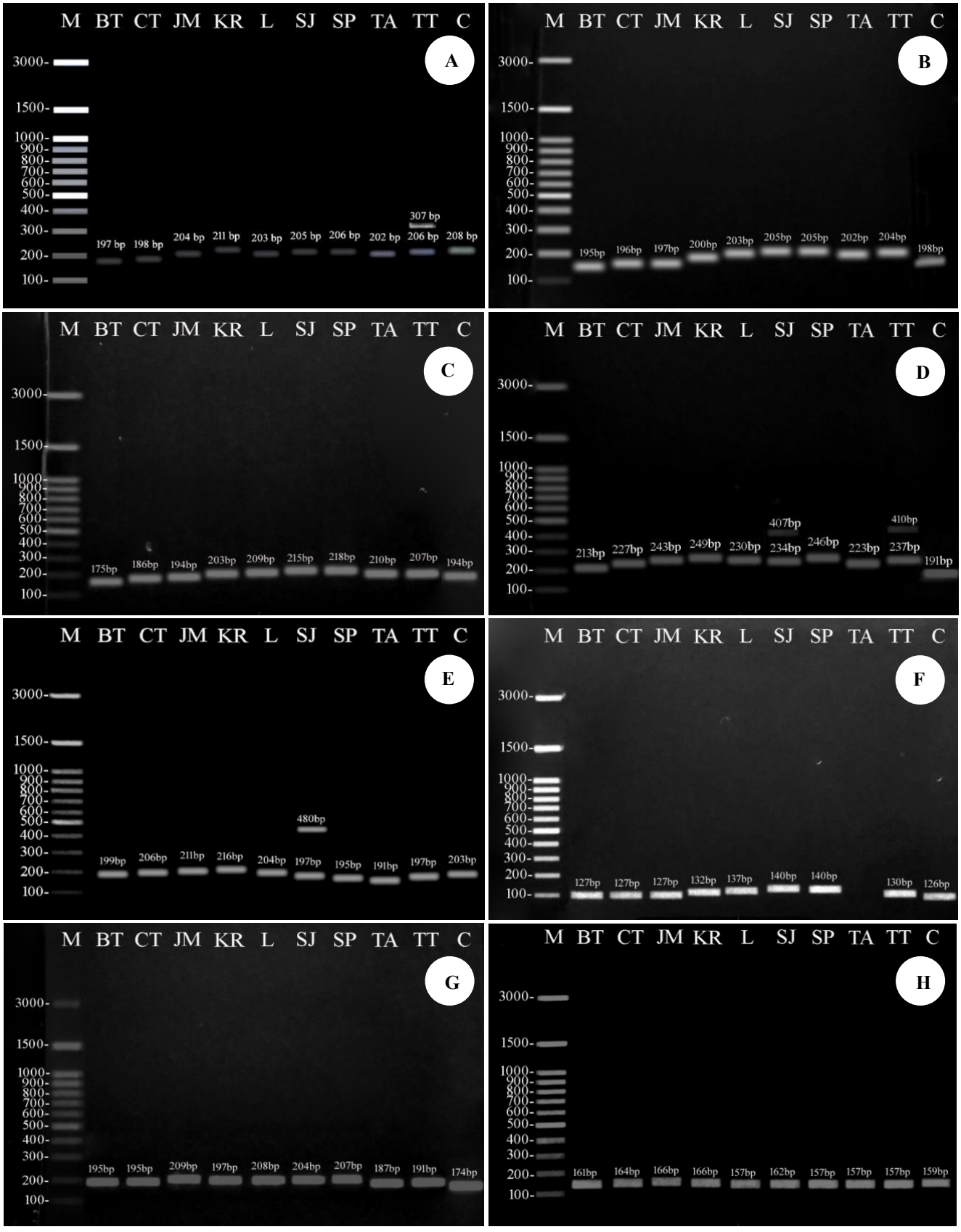
Data analysis

Genetic diversity, genetic distances, and phylogenetic relationships among the ten pigmented rice accessions were analyzed using multilocus SSR data. SSR alleles were scored from agarose gel electrophoresis based on fragment sizes (bp). Because agarose gels provide lower sizing resolution than capillary electrophoresis, allele calls and derived statistics were interpreted as gel-derived estimates suitable for accession discrimination rather than high-resolution allele-frequency inference. The number of alleles per locus (Na) was recorded as the total number of unique non-zero fragment sizes (bp) observed across accessions. Marker informativeness was assessed using Polymorphic Information Content (PIC), which was computed in RStudio (R version 4.1.3) using the *poppr* package (Kamvar et al. 2014) based on allele (band-state) frequency distributions across accessions. PIC values were interpreted following Botstein's criteria, where PIC>0.50 indicates highly informative markers (Yıldırım et al. 2024). PIC values in this study are interpreted as measures of gel-derived band/allele-state informativeness for accession discrimination, rather than high-resolution codominant allele-frequency estimates obtained from precise allele sizing. For polymorphism assessment and similarity-based clustering analyses, allele-size profiles were additionally converted into a binary band-state matrix (presence: 1, absence: 0), in which a band/allele state was considered polymorphic if it occurred in at least one accession but was absent in at least one other accession. These metrics were used to summarize the polymorphism patterns across loci based on band-state comparisons. Pairwise genetic similarity was calculated using the Jaccard coefficient in MVSP version 3.22 (Kovach 2011), and genetic distances were derived from non-shared band states between accession pairs. The resulting distance matrix was used for hierarchical clustering and phylogenetic reconstruction using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) (Segura-Alabart et al. 2022). Genetic partitioning between predefined phenotypic groups was assessed using Analysis of Molecular Variance (AMOVA) implemented in GenAlEx 6.5 (Peakall and Smouse 2005, 2012). Accessions were grouped into two populations based on grain pigmentation category (red rice and black rice). AMOVA quantified the proportion of molecular variance distributed among and within groups, and population differentiation was estimated using the Fixation Index (F_{st}). Statistical significance was evaluated by permutation testing in GenAlEx, and differences were considered significant at $p<0.05$. The combined interpretation of band polymorphism, PIC, Jaccard similarity, clustering patterns, and AMOVA enabled assessment of genetic structure and differentiation among pigmented rice landraces from South Sumatra.

RESULTS AND DISCUSSION

SSR amplification performance across pigmented rice accessions

Amplification using 11 SSR primers generated clear and scorable banding patterns across the ten pigmented rice accessions (Figure 1). Amplification with the 11 SSR primer pairs produced scorable banding profiles across the ten pigmented rice accessions. Fragment sizes ranged from 127 to 480 bp. Across markers, amplification was generally consistent, although missing bands were observed for RM416 in one accession and for RM223 in Linggau (L). Overall, the SSR panel generated informative multilocus band profiles for comparing genetic relatedness among accessions.


M BT CT JM KR L SJ SP TA TT C
A
3000-
1500-
1000-
900-
800-
700-
600-
500-
400-
300-
200-
100-
197 bp 198 bp 204 bp 211 bp 203 bp 205 bp 206 bp 202 bp 307 bp 206 bp 208 bp
M BT CT JM KR L SJ SP TA TT C
B
3000-
1500-
1000-
900-
800-
700-
600-
500-
400-
300-
200-
100-
195bp 196bp 197bp 200bp 203bp 205bp 205bp 202bp 204bp 198bp
M BT CT JM KR L SJ SP TA TT C
C
3000-
1500-
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700-
600-
500-
400-
300-
200-
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175bp 186bp 194bp 203bp 209bp 215bp 218bp 210bp 207bp 194bp
M BT CT JM KR L SJ SP TA TT C
D
3000-
1500-
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800-
700-
600-
500-
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300-
200-
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213bp 227bp 243bp 249bp 230bp 407bp 234bp 246bp 223bp 410bp 237bp 191bp
M BT CT JM KR L SJ SP TA TT C
E
3000-
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700-
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199bp 206bp 211bp 216bp 204bp 480bp 197bp 195bp 191bp 197bp 203bp
M BT CT JM KR L SJ SP TA TT C
F
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G
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H
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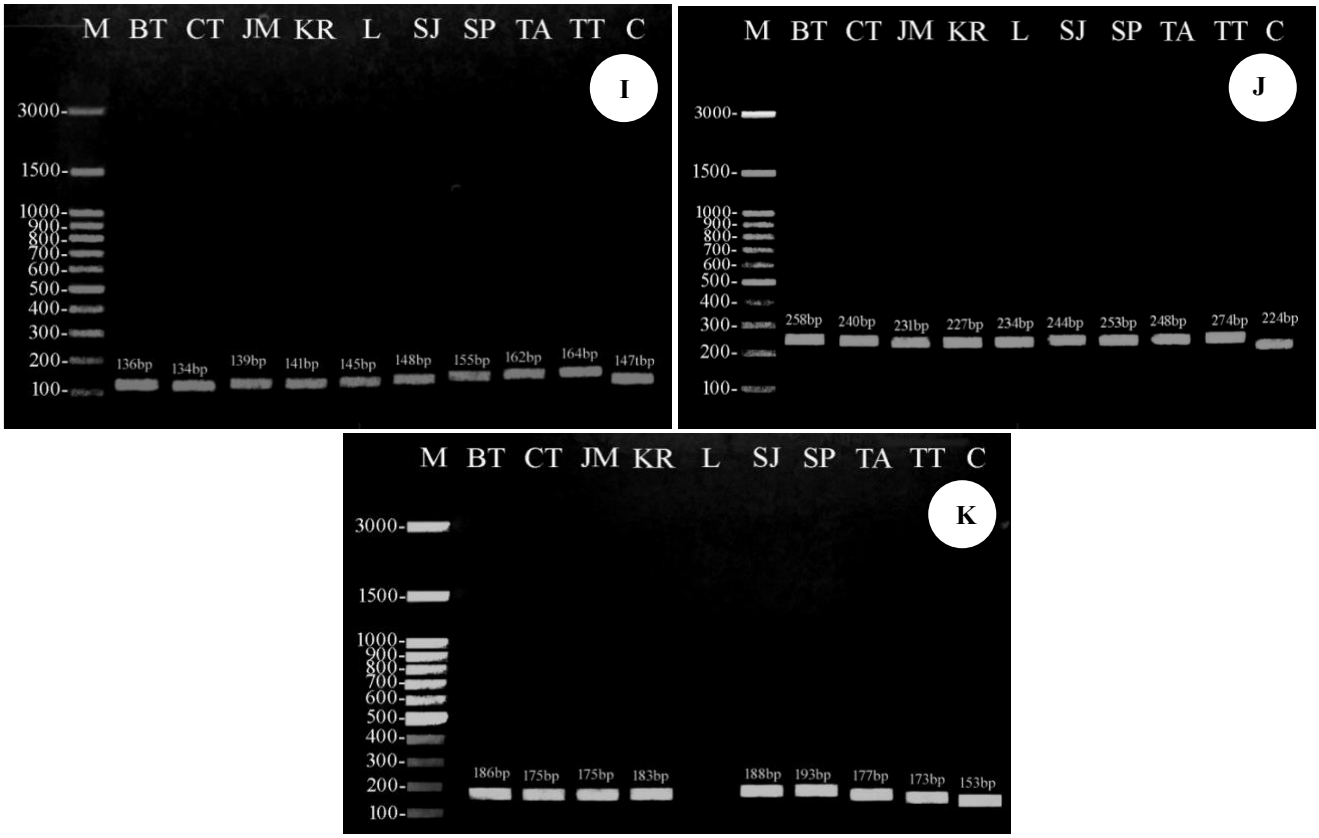

Figure 1. SSR amplification profiles (A-K) of pigmented rice accessions using primers: A. RM5742, B. RM6997, C. RM201, D. RM263, E. RM324, F. RM416, G. RM518, H. RM60, I. RM105, J. RM124, and K. RM223 (Accessions: BT: Bungin Tinggi, CT: Cahya Tani, JM: Jaya Mulya, KR: Keli Rejo, L: Linggau, SJ: Sumber Jaya, SP: Sirah Pulau Padang, TA: Tanjung Agung, TT: Teluk Tenggirik, C: Cecar)

The banding patterns generated by the 11 SSR markers showed that most accessions displayed Homozygous (HM) profiles across loci (Table 4), with only a few Heterozygous (HT) patterns detected at specific markers and in specific accessions. This predominance of HM profiles is consistent with the autogamous reproductive system of rice and reflects a high level of allelic fixation in the individual genotypes sampled. Because each named landrace accession was represented by a single plant, the observed homozygosity should be interpreted as genotype-level information and may not fully capture within-landrace heterogeneity that could exist across multiple plants maintained by farmers.

Notably, RM5742 produced a heterozygous genotype exclusively in TT (Teluk Tenggirik), while RM263 and RM324 generated heterozygous bands in SJ (Sumber Jaya) and TT, suggesting localized heterogeneity within these genotypes. The predominance of HM genotypes across all markers reflects a high degree of genetic fixation, which is typical of traditionally cultivated rice landraces maintained under farmer-managed selection. The few HT occurrences indicate loci where allelic variation persists, making these markers potentially informative for distinguishing closely related accessions. Overall, the genotype patterns in Table 4 support the broader finding that although pigmented rice in South Sumatra is generally genetically uniform within accessions, specific loci retain polymorphism that may contribute to differentiation among accessions.

Allelic variation and Polymorphic Information Content (PIC)

The number of alleles per locus (Na) ranged from 6 to 12, indicating that the selected SSR markers generated informative multilocus profiles across accessions (Table 5). Across the 11 SSR loci, five loci (45.450%) were polymorphic (RM5742, RM263, RM324, RM416, and RM223), whereas the remaining loci were monomorphic across all accessions. Although polymorphism was not uniformly detected across loci, all markers exhibited high PIC values (0.790-0.917; mean = 0.878), indicating strong informativeness for accession-level discrimination based on gel-derived allele (band) sizes.

Table 4. SSR banding patterns in ten pigmented rice accessions from South Sumatra, Indonesia

SSR markers	Pigmented rice accessions									
	BT	CT	JM	KR	L	SJ	SP	TA	TT	C
RM5742	HM	HM	HM	HM	HM	HM	HM	HM	HT	HM
RM6997	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM
RM201	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM
RM263	HM	HM	HM	HM	HM	HT	HM	HM	HT	HM
RM324	HM	HM	HM	HM	HM	HT	HM	HM	HM	HM
RM416	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM
RM518	HM	HM	HM	HM	HM	HM	HM	-	HM	HM
RM60	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM
RM105	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM
RM124	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM
RM223	HM	HM	HM	HM	-	HM	HM	HM	HM	HM

Note: HM: Homozygous; HT: Heterozygous. Accessions: BT: Bungin Tinggi, CT: Cahya Tani, JM: Jaya Mulya, KR: Keli Rejo, L: Linggau, SJ: Sumber Jaya, SP: Sirah Pulau Padang, TA: Tanjung Agung, TT: Teluk Tenggirik, C: Cecar. -: Missing band

Table 5. Allele number (Na), Polymorphic Information Content (PIC), and polymorphism summary of SSR loci in pigmented rice accessions from South Sumatra, Indonesia

SSR markers	Number of alleles (Na)	PIC value	Informativeness*	Polymorphic bands states (out of 2)**	Polymorphism (%)**
RM5742	10	0.893	High	1	50.000
RM6997	9	0.880	High	0	0.000
RM201	8	0.860	High	0	0.000
RM263	12	0.917	High	1	50.000
RM324	10	0.893	High	1	50.000
RM416	6	0.790	High	1	50.000
RM518	9	0.880	High	0	0.000
RM60	9	0.880	High	0	0.000
RM105	10	0.900	High	0	0.000
RM124	10	0.900	High	0	0.000
RM223	8	0.864	High	1	50.000

Note: *Follow Botstein's criteria (PIC>0.50: Highly informative), **Polymorphic band states were determined from the binary band-state matrix (presence/absence scoring), where each SSR locus contributed two band-state columns

Genetic diversity and distance patterns among accessions

Pairwise genetic distances calculated from multilocus SSR data revealed a moderately structured pattern of divergence among the ten pigmented rice accessions (Table 6). Distance values ranged from 8.000 to 11.000, indicating that while the accessions share a largely conserved genomic background, they also exhibit meaningful multilocus variability.

The lowest genetic distances were observed between BT and CT (8.000), between CT and JM (9.000), and between L and TA (9.000), reflecting a high degree of allelic similarity and suggesting closer genomic relationships within this subgroup. These accessions also displayed predominantly homozygous SSR profiles, consistent with long-term self-pollination and reduced genetic recombination characteristic of traditional rice landraces. In contrast, higher pairwise distances (10.000-11.000) were frequently associated with SJ and TT, positioning them as the most divergent accessions in the dataset. SJ exhibited elevated distances with nearly all accessions, while TT showed strong differentiation except with L and TA. This pattern highlights the presence of unique multilocus allele combinations in these two accessions, reinforcing their distinctiveness within the broader genetic network. Despite this variability, the overall distance range remains moderate, indicating that the germplasm is diverse yet genetically cohesive, as expected for regionally distributed landraces of a single species. To complement the distance matrix, a Jaccard similarity matrix was also generated (Table 7).

Similarity values ranged from 0.769 to 1.000, with the highest similarity (1.000) observed among BT, CT, JM, KR, SP, and C, indicating near-identical SSR profiles. In contrast, the lowest similarities (0.769) involved SJ and TT relative to several other accessions, reinforcing their divergent genetic position. These coefficients clearly separate SJ and TT from the remaining accessions, consistent with the genetic distance results. To assess the genetic diversity, Analysis of Molecular Variance (AMOVA) was performed (Table 8).

Analysis of Molecular Variance (AMOVA) revealed that genetic variation was entirely attributable to differences within populations (100.000%), while variation among populations was negligible (0%). Consistent with this pattern, the fixation index was low and negative (F_{st} = -0.038) and not statistically significant (p = 0.769), indicating an absence of detectable genetic differentiation between the defined population groups. In this analysis, "populations" represent phenotypic groupings based on

grain pigmentation (red vs black rice) rather than genetically inferred populations or geographic subpopulations. These findings suggest that the pigmented rice accessions share a largely overlapping multilocus SSR profile across the genome-wide markers used in this study.

Cluster and phylogenetic relationships

The UPGMA dendrogram derived from Jaccard similarity coefficients provided a clear hierarchical representation of the relationships inferred from the SSR dataset (Figure 2). Accessions BT, CT, JM, KR, SP, and C formed a cohesive cluster characterized by high internal similarity values (0.909-1.000). This grouping aligns closely with the low genetic distances observed among these accessions and underscores their shared allele patterns across the 11 SSR loci. L and TA emerged as an intermediate subgroup, clustering with the main red rice assemblage but positioned at slightly lower similarity levels (0.818-0.909). Their placement reflects moderate divergence yet maintains evidence of shared ancestry within the broader cluster. In contrast, SJ and TT formed a distinct branch, separated from the main cluster at similarity values of 0.769-0.857. This branching pattern confirms the distance matrix interpretation that these two accessions harbor unique genetic signatures relative to others. Their placement as late-joining nodes in the dendrogram indicates higher polymorphism and lower allele-sharing, which collectively contribute to the overall structure of the tree. The overall dendrogram topology reveals a germplasm structure composed of a primary cluster of closely related accessions, an intermediate subgroup (L and TA), and a divergent subgroup represented by SJ and TT. This configuration mirrors the multilocus distance gradients and illustrates the capacity of SSR markers to discriminate among pigmented rice accessions even within a relatively narrow geographic range.

Table 6. Genetic distance matrix among pigmented rice accessions

	BT	CT	JM	KR	L	SJ	SP	TA	TT	C
BT	0.000	8.000	10.000	11.000	10.000	11.000	11.000	10.000	11.000	11.000
CT	8.000	0.000	9.000	11.000	10.000	11.000	11.000	10.000	11.000	11.000
JM	10.000	9.000	0.000	11.000	10.000	11.000	11.000	10.000	11.000	10.000
KR	11.000	11.000	11.000	0.000	10.000	11.000	11.000	10.000	11.000	11.000
L	10.000	10.000	10.000	10.000	0.000	10.000	10.000	9.000	10.000	10.000
SJ	11.000	11.000	11.000	11.000	10.000	0.000	9.000	9.000	11.000	11.000
SP	11.000	11.000	11.000	11.000	10.000	9.000	0.000	10.000	10.000	11.000
TA	10.000	10.000	10.000	10.000	9.000	9.000	10.000	0.000	10.000	10.000
TT	11.000	11.000	11.000	11.000	10.000	11.000	10.000	10.000	0.000	11.000
C	11.000	11.000	10.000	11.000	10.000	11.000	11.000	10.000	11.000	0.000

Note: BT: Bungin Tinggi, CT: Cahya Tani, JM: Jaya Mulya, KR: Keli Rejo, L: Linggau, SJ: Sumber Jaya, SP: Sirah Pulau Padang, TA: Tanjung Agung, TT: Teluk Tenggirik, C: Cecar

Table 7. Jaccard similarity coefficients among pigmented rice accessions

	BT	CT	JM	KR	L	SJ	SP	TA	TT
BT	1.000								
CT	1.000	1.000							
JM	1.000	1.000	1.000						
KR	1.000	1.000	1.000	1.000					
L	0.909	0.909	0.909	0.909	1.000				
SJ	0.846	0.846	0.846	0.846	0.769	1.000			
SP	1.000	1.000	1.000	1.000	0.909	0.846	1.000		
TA	0.909	0.909	0.909	0.909	0.818	0.769	0.909	1.000	
TT	0.846	0.846	0.846	0.846	0.769	0.857	0.846	0.769	1.000
C	1.000	1.000	1.000	1.000	0.909	0.846	1.000	0.909	0.846

Note: BT: Bungin Tinggi, CT: Cahya Tani, JM: Jaya Mulya, KR: Keli Rejo, L: Linggau, SJ: Sumber Jaya, SP: Sirah Pulau Padang, TA: Tanjung Agung, TT: Teluk Tenggirik, C: Cecar

Table 8. Analysis of Molecular Variance (AMOVA) among pigmented rice accessions from South Sumatra, Indonesia

Source of variation	df	Sum of square	Mean square	Estimated variance	Variation (%)	F_{st}	*p*-value
Among populations	1.000	2.948	2.948	0.000	0.000	-0.038	0.769
Within populations	18.000	76.952	76.952	4.275	100.000	-	-
Total	19.000	79.900	-	4.275	100.000	-	-

Note: Significance was assessed by permutation testing ($\alpha = 0.05$). Negative F_{st} values are interpreted as no detectable population differentiation

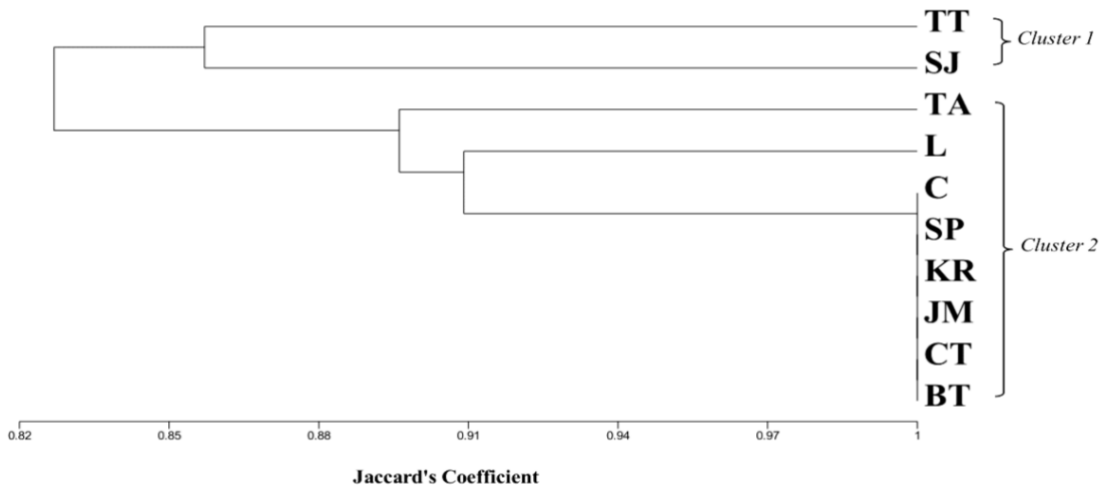

Figure 2. Phylogeny tree reconstruction on pigmented rice-based microsatellite markers. Note: BT: Bungin Tinggi, CT: Cahya Tani, JM: Jaya Mulya, KR: Keli Rejo, L: Linggau, SJ: Sumber Jaya, SP: Sirah Pulau Padang, TA: Tanjung Agung, TT: Teluk Tenggirik, C: Cecar

Discussion

The multilocus SSR dataset generated in this study provides a robust foundation for interpreting the genetic diversity, allelic structure, and phylogenetic relationships of pigmented rice landraces from South Sumatra. Consistent amplification across all eleven SSR markers, accompanied by clear and distinct banding patterns, reflects the high quality of the DNA templates and the compatibility of the primers with the genomic background of the accessions examined (Kuang et al. 2022; Chen et al. 2025). The amplicon size range of 127-480 bp falls within the expected profile for rice microsatellites reported in studies of Indonesian and Asian rice germplasm, as mentioned by Kristamtini et al. (2016), where fragment sizes commonly range between 100 and 500 bp. Comparable studies on Indonesian rice, including those by Terryana et al. (2022), also observed strong amplification consistency across SSR loci, underscoring the reliability of microsatellite markers in revealing genetic variability in traditional landraces. The predominance of sharp, non-smearing bands across loci further validates the dataset for downstream analyses such as genetic distance estimation and cluster reconstruction.

A striking feature of the SSR profiles is the predominance of homozygous genotypes across nearly all loci and accessions, a pattern consistent with the autogamous reproductive strategy of rice. Similar findings have been reported in studies of Javanese, Sumatran, and Bangladeshi rice landraces, where natural self-pollination and repeated farmer-managed propagation produce highly inbred populations with limited heterozygosity (Mursyidin 2022; Kurniawan et al. 2023; Islam et al. 2025). In the current study, only three markers—RM5742, RM263, and RM324—revealed heterozygous profiles in specific accessions (TT and SJ). Such rare heterozygous occurrences mirror observations by Ponsiva and Senthilkumar (2021), who noted that localized outcrossing or gene introgression may occasionally generate residual heterozygosity even in predominantly self-pollinating rice landraces. These polymorphic loci therefore represent genomic regions where evolutionary change persists, offering potential utility for distinguishing closely related accessions.

Allelic richness in this study ranged from 6 to 12 alleles per locus, aligning with previous reports that SSR markers typically yield high allelic diversity in rice. Studies by Gaballah et al. (2021) documented 2-11 (average about 7.2) alleles per SSR locus in diverse rice germplasm, highlighting the multilocus sensitivity of microsatellites. The high PIC values observed here (0.790-0.917, average ~0.878) further confirm the discriminatory capacity of the selected markers. Comparable PIC ranges were reported by Gaballah et al. (2021), who found that SSR markers with PIC>0.80 are highly informative for assessing genetic variation in landraces and breeding lines. The uniformly high PIC values in this study, therefore, affirm the usefulness of the marker panel for diversity assessment, varietal identification, and future genetic resource management.

The apparent contrast between relatively high PIC values and limited polymorphism arises because PIC reflects the informativeness of allele/band-state frequency distributions for discriminating among accessions, whereas the polymorphism metric summarizes the proportion of loci (or band states) that vary across the dataset. Similar patterns have been reported in SSR-based diversity studies, where markers showed high PIC values despite modest observed polymorphism, indicating strong discriminatory power among accessions rather than extensive within-accession variation (Gaballah et al. 2021).

Genetic distance analysis revealed moderate divergence among the ten accessions, with pairwise dissimilarity values ranging from 8 to 11. These values are consistent with findings from a prior study of regional rice germplasm, where genetic distances within landrace clusters are typically moderate due to shared ancestry but exhibit notable differentiation across geographically or culturally distinct groups (Cui et al. 2021). Similar distance ranges were observed in investigations of upland and lowland rice varieties in Sabah and Bangladesh, where farmer-driven seed

exchange, selection preferences, and ecological adaptation shape multilocus diversity (Alam et al. 2016; Simon et al. 2020). The high divergence observed in SJ and TT parallels findings from other studies that identified particular landraces with unique genetic signatures resulting from long-term isolation, localized adaptation, or introgression from uncharacterized parental genomes (Santos et al. 2019).

The AMOVA results further support the observed clustering patterns by indicating no significant partitioning of genetic variation between the predefined population groups. The estimate of among-population variation was 0%, whereas within-population variation accounted for 100% of the total molecular variance, and FST was close to zero (-0.038; $p = 0.769$). In practice, negative FST values are treated as zero and arise when within-group variation exceeds among-group variation due to sampling effects and limited divergence. This implies that, under the current sampling design and SSR band-based scoring approach, the red and black rice accessions do not form genetically distinct populations at the genome-wide marker level. The lack of strong population differentiation may reflect shared ancestry, farmer-mediated seed exchange, or the possibility that pigmentation traits are governed by a limited number of functional loci that are not directly captured by neutral SSR markers.

The UPGMA dendrogram derived from Jaccard similarity coefficients provides a clear hierarchical representation of relationships among the accessions. The formation of a cohesive cluster comprising BT, CT, JM, KR, SP, and C corresponds with the clustering patterns documented in a previous study of Indonesian pigmented rice, where red rice landraces commonly group together due to shared ancestry and selection history (Mau et al. 2017). Meanwhile, SJ and TT form a distinct branch, closely reflecting the structure reported in studies of specialty or geographically isolated landraces, which often harbor rare alleles or unique genetic signatures. These parallels reinforce the conclusion that the patterns observed in South Sumatra's pigmented rice are consistent with broader regional trends in rice evolution and domestication.

The genetic relatedness patterns revealed by SSR profiling can be interpreted in the context of local cultivation history and farmer management practices in South Sumatra. Farmers typically maintain pigmented rice landraces through repeated seed saving and selection for culturally and economically preferred traits such as grain pigmentation, cooking quality, aroma, and field performance under local agroecological conditions. Over time, such practices can maintain genetically cohesive core groups when seed exchange occurs across communities, while also allowing certain landraces to remain genetically distinct when selection is strong and seed flow is limited. In this context, the closely related core cluster identified in the UPGMA analysis may represent landraces sharing a common genetic background maintained through diffusion of planting materials, whereas the comparatively divergent accessions represent unique genetic resources likely shaped by localized selection histories. A study by Mbanjo et al. (2019) reveals that most variation of genetic background is within regions, with weak geographic structure, reflecting extensive local and long-distance seed exchange. Conversely, the greater divergence observed in accessions such as Sumber Jaya (SJ) and Teluk Tenggirik (TT) may indicate localized adaptation to specific agroecological conditions, long-term cultivation in relatively isolated communities, or restricted gene flow due to geographic and social boundaries. Together, these processes provide a plausible explanation for the coexistence of a genetically similar core group alongside a small number of distinct landraces, reinforcing the value of targeted conservation of divergent accessions and the need for broader sampling to capture the full spectrum of pigmented rice diversity in the region. Moreover, the lack of significant differentiation between red and black groups in AMOVA may further suggest that pigmentation types are maintained within shared genetic backgrounds through farmer selection on a limited number of traits rather than reflecting genome-wide divergence.

The implications of this study for breeding and conservation are substantial. The genetically uniform core cluster may serve as a stable base population for breeding programs seeking to enhance pigmentation traits, yield stability, or adaptation to South Sumatran agroecosystems. Divergent accessions, particularly SJ and TT, represent reservoirs of unique alleles that may contribute to improved stress tolerance, nutritional quality, or aromatic characteristics. Similar utilization of genetically distinct landraces has been reported in breeding programs in India and Thailand, where unique alleles from local varieties have been successfully introgressed into elite lines (Marone et al. 2021; Kongsil et al. 2024). Conservation efforts should prioritize these genetically distinct accessions, as their rare allelic compositions make them valuable components of regional biodiversity. The high informativeness of the SSR markers used here further supports the development of molecular authentication tools to protect traditional rice varieties from genetic erosion and unauthorized commercial exploitation.

Several limitations should be acknowledged. The sample size, while geographically representative, may not reflect the full breadth of rice diversity in South Sumatra. Similar limitations have been noted in other regional diversity evaluations, emphasizing the necessity for larger samples. SSR markers, while highly polymorphic, only cover a small portion of the genome and do not capture genome-wide variation that can be obtained using high-density SNP arrays or sequencing-based techniques (Zhang et al. 2022). The study also did not include phenotypic or environmental variables, limiting its ability to relate genetic variation to adaptive qualities. These limitations are similar to those discovered in other rice diversity studies, highlighting the significance of integrative techniques that incorporate genetic, phenotypic, and ecological data.

It should be noted that SSR alleles in this study were scored from agarose gel electrophoresis based on fragment size classes (bp). Because agarose gels provide lower sizing resolution than capillary electrophoresis, allelic resolution and heterozygosity detection may be underestimated, and allele-frequency-based inferences should be interpreted cautiously (Šarhanová et al 2018). Nevertheless, agarose-based SSR scoring remains suitable for comparative

assessments of genetic diversity, distance estimation, and clustering among landraces, particularly when the objective is to resolve broad genetic relationships rather than fine-scale population structure (Biswas et al. 2020).

Future studies should expand sampling to include a larger number of pigmented rice landraces across additional districts in South Sumatra and incorporate multiple individuals per landrace to capture within-landrace genetic variation. The use of higher-resolution genomic approaches, such as Single-Nucleotide Polymorphism (SNP) markers, Genotyping-By-Sequencing (GBS), or whole-genome sequencing, is recommended to overcome the limitations of binary SSR scoring and to provide finer resolution of allelic composition, heterozygosity, and population structure. Integrating molecular data with detailed morpho-agronomic traits, grain quality characteristics, and environmental variables would enable genotype-phenotype associations and improve understanding of local adaptation and farmer selection processes. In addition, future analyses employing more advanced population-genetic frameworks, including Bayesian clustering and landscape-genetic approaches, would help clarify gene flow patterns and potential differentiation between red and black rice groups. Such integrative and higher-resolution studies will strengthen conservation planning and enhance the effective utilization of pigmented rice landraces in breeding programs aimed at nutritional quality and agroecological resilience.

In conclusion, this study provides a baseline SSR-based assessment of genetic diversity and accession-level relatedness among ten pigmented rice landraces from South Sumatra. The results support our hypothesis that pigmented rice landraces exhibit SSR-detectable genetic variation among accessions, while genome-wide differentiation between pigmentation groups (red and black rice) is weak or non-significant. Marker informativeness was consistently high (PIC = 0.790-0.917, mean = 0.878), demonstrating strong discriminatory capacity for accession differentiation. Despite an overall close genetic similarity among several accessions, clustering analysis revealed a structured pattern of relatedness and identified Sumber Jaya (SJ) and Teluk Tenggirik (TT) as comparatively more divergent accessions. These genetically distinct landraces represent valuable local genetic resources that should be prioritized for germplasm conservation and further characterization. The generated molecular framework supports evidence-based management of pigmented rice diversity and highlights the potential use of distinct accessions as genetic donors in future breeding programs aimed at improving pigmented rice traits and adaptive performance under changing agroecological conditions. Analysis of Molecular Variance (AMOVA) showed that the majority of genetic variation was distributed within phenotypic groups (100%) rather than among them (0%), with no significant differentiation between red and black rice accessions ($F_{st} = -0.038$; $p = 0.769$), suggesting that observable traits alone do not fully capture underlying genetic structure. Ultimately, preserving and utilizing these genetically wide local varieties will be crucial for safeguarding Indonesia's rice heritage and for advancing sustainable rice improvement initiatives. Additionally, conserving these local landraces constitutes an important reservoir of genetic diversity with potential value for breeding and conservation programs.

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