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Chloroplast Genetic Comparison of *Siam* and *Siputih* Swamp Rice (*Oryza sativa* L.) from South Sumatra Based on *rbcL* and *matK*

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ABSTRACT

Traditional swamp rice landraces are important components of South Sumatra's local rice genetic resources, yet their chloroplast sequence variation remains poorly documented. This study assessed chloroplast sequence similarity between two representative accessions of the traditional swamp rice landraces Siam and Siputih using the rbcL and matK markers. Both loci yielded reproducible amplification products of approximately 600 bp (rbcL) and 900 bp (matK), with high-quality bidirectional sequencing results. BLAST analysis revealed 99.66–100% sequence similarity to Oryza sativa and related Oryza references, including O. nivara, O. rufipogon, and O. glaberrima. Comparative analysis detected limited chloroplast variation: rbcL contained one single-nucleotide polymorphism (SNP) and two insertion–deletion (InDel) events, whereas matK showed no SNPs but three short InDel events. Nucleotide diversity was low (rbcL: $\pi = 0.00170$; matK: $\pi = 0.00000$), indicating minimal sequence divergence. Neighbour-Joining phylogenetic analysis using the Kimura 2-parameter model placed both accessions within the same major clade, with strong bootstrap support for rbcL and moderate support for matK. These results suggest closely related chloroplast sequences and a potentially shared maternal plastid lineage between the sampled accessions. This study provides baseline molecular evidence for chloroplast variation in traditional South Sumatran swamp rice and may inform future germplasm conservation and breeding research. However, because each landrace was represented by a single genotype, the findings are restricted to comparisons between the two sampled accessions. This study contributes to the limited chloroplast DNA-based molecular characterization of traditional swamp rice landraces from South Sumatra.

1. Introduction

Swamp rice (*Oryza sativa* L.) constitutes an important component of traditional rice diversity cultivated in wetland ecosystems across Southeast Asia (Mursyidin *et al.* 2022a). In Indonesia, particularly in South Sumatra, locally named swamp rice varieties such as *Siam* and *Siputih* have long been maintained in flood-prone and seasonally inundated agroecosystems (Paiman *et al.* 2020). These varieties are adapted to fluctuating water levels, prolonged submergence, and variable soil conditions, making them valuable genetic resources for rice production under hydrological stress (Mursyidin & Khairullah 2020). Beyond their agronomic importance, these landraces are embedded in local farming traditions, where farmer-led selection, seed exchange, and ecological adaptation have sustained them over generations (Paiman *et al.* 2020). However, these locally maintained varieties are increasingly threatened by the expansion of modern high-yielding cultivars, agricultural intensification, and land-use change, potentially accelerating genetic erosion and narrowing the genetic base of swamp rice in the region (Zapico *et al.* 2020; Mursyidin *et al.* 2022b; Hanum *et al.* 2025; Hanum *et al.* 2026).

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Effective conservation and sustainable utilization of traditional rice germplasm require molecular evidence to complement phenotypic characterization. Genetic variation underpins adaptive potential and determines the capacity of landraces to contribute beneficial traits such as stress tolerance and yield stability for breeding programs (Hidayatun *et al.* 2024; Subramanian *et al.* 2025). Despite the ecological and agricultural relevance of *Siam* and *Siputih*, molecular information describing their chloroplast-level sequence similarity remains limited. Previous studies in Indonesia have predominantly focused on agronomic performance, morphological characterization, or nuclear marker-based analyses conducted in other provinces (Hanum *et al.* 2020; Mursyidin *et al.* 2022a). Consequently, plastid-based sequence comparison of South Sumatran swamp rice, particularly using standardized DNA barcoding regions, remains scarce.

This gap is important because chloroplast genomes provide insight into maternal lineage relationships and plastid haplotype similarity among closely related accessions (Fu 2021; Brock *et al.* 2022). Standardized chloroplast markers also facilitate comparability across studies and regions, enabling integration of local data into broader phylogeographic and germplasm conservation frameworks (Lu *et al.* 2021; Samarina *et al.* 2025). Although chloroplast loci generally have limited intraspecific resolution, baseline plastid sequence data remain valuable for evaluating the discriminatory performance of commonly used barcoding loci in crops such as rice, where varietal differentiation may be subtle (Zhang *et al.* 2021; Ijaz *et al.* 2022).

Among chloroplast DNA markers, *rbcL* and *matK* are widely used in plant systematics and DNA barcoding because of their universality, reproducibility, and broad applicability across plant taxa (Ismail *et al.* 2020; Li *et al.* 2021). Although these loci are generally more conserved than nuclear markers, small sequence differences, including nucleotide substitutions and insertion–deletion (InDel) events, may still reveal haplotypic variation and provide baseline evidence for plastid comparison (Li *et al.* 2021; Magar *et al.* 2025). As internationally standardized plant DNA barcoding regions, these markers enable direct comparison with published reference sequences in public databases, supporting their use to establish initial plastid sequence baselines and detect accession-level haplotypic differences (Kress 2017; Lal *et al.* 2025). In cultivated crops, these markers have also been used to trace maternal inheritance and compare plastid haplotypes among landraces (López *et al.* 2021; Villanueva-Corrales *et al.* 2021; Brock *et al.* 2022). However, their application to swamp rice varieties from South Sumatra remains limited.

Therefore, this study aimed to compare chloroplast sequences between one *Siam* genotype and one *Siputih* genotype from South Sumatra using *rbcL* and *matK* sequence data. Specifically, we examined nucleotide polymorphisms and insertion–deletion (InDel) variation, estimated pairwise sequence divergence, and evaluated chloroplast haplotype similarity between the two analyzed accessions. This study serves as a pilot molecular baseline and is explicitly limited to comparison between the two sampled genotypes, without attempting population- or landrace-level diversity inference. The resulting plastid sequence data are intended to provide an initial molecular reference for future studies involving broader sampling, nuclear markers, and higher-resolution genomic approaches.

2. Materials and Methods

2.1. Study Material

Leaf samples of swamp rice were collected from two locally recognized varieties, *Siam* and *Siputih*, cultivated in South Sumatra Province, Indonesia. Table 1 provides the geographic coordinates of each sampling site, and Figure 1 illustrates their locations. Each named variety was represented by a single healthy plant collected from a farmer's field and treated as one operational taxonomic unit in all chloroplast sequence analyses. Accordingly, this sampling design aimed to assess chloroplast sequence variation between representative accessions of *Siam* and *Siputih* rather than within-landrace genetic diversity. We extracted genomic DNA from each individual separately without pooling, so each variety represented one independent genotype for the named local landrace. Both varieties are traditionally maintained in lowland swamp agroecosystems and are distinguished by local farmers based on morphological characteristics and agronomic performance under wetland conditions. Therefore, the scope of this study is restricted to inter-accession chloroplast sequence comparison and plastid haplotype similarity assessment between the two analyzed accessions.

Field exploration and sample collection were initiated in 2023 in South Sumatra Province, Indonesia. Laboratory analyses and data processing were conducted at the Genetics and Biotechnology

Table 1. Sampling locations and geographic coordinates of swamp rice varieties collected from South Sumatra, Indonesia

Code	Sample identity	Domestication status	Region	Habitat type	Altitude (m)	Coordinate	
						Latitude	Longitude
SP	*Siputih*	Cultivated	Ulak Segelung, Ogan Ilir, South Sumatra	Lowland swamp paddy	4.1	3° 15’ 13.3” S	104° 42’ 32.4” E
SM	*Siam*	Cultivated	Karya Jaya, Palembang, South Sumatra	Lowland swamp paddy	3.8	3° 3’ 4.0” S	104° 44’ 2.4” E

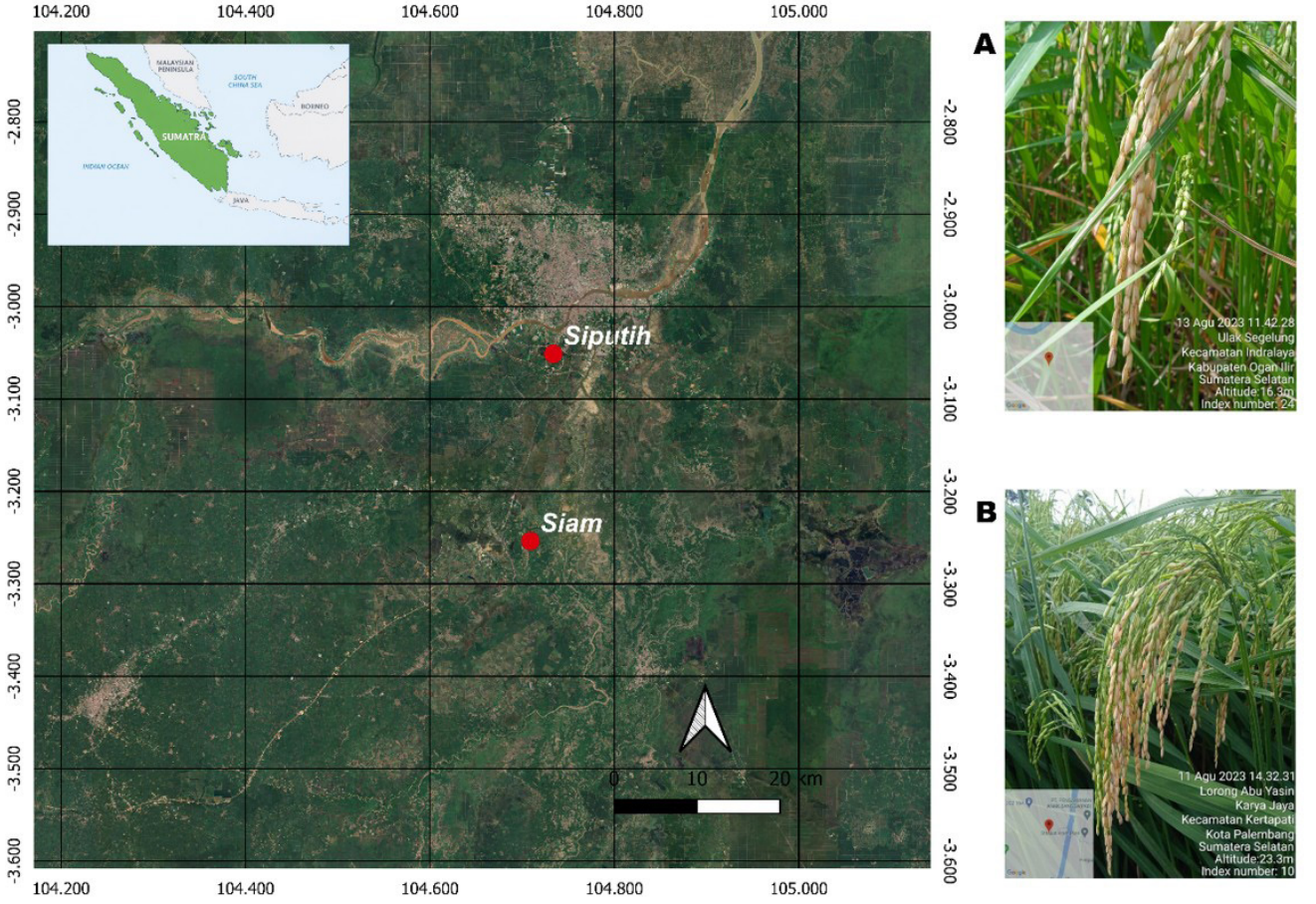


Figure 1. Geographic location of swamp rice sampling sites in South Sumatra, Indonesia. (A) *Siputih* (SP), (B) *Siam* (SM). Red circles indicate sampling points on the map of Sumatra Island

Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indonesia, as part of an institutional research program on rice genetic resources, and were completed by December 2025. All freshly collected leaf samples were washed with distilled water to remove adhering debris, air-dried, wrapped in aluminum foil, properly labeled, and stored at −20°C prior to DNA extraction and molecular analysis. Voucher specimens representing each sampled variety were deposited at the UNSRI Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. The archived voucher codes are SM–2 (*Siam*) and SP–2 (*Siputih*).

2.2. Study Design

Two locally recognized swamp rice varieties, *Siam* and *Siputih*, were purposively selected as farmer-recognized traditional landraces cultivated in South Sumatra (Table 1). The selection was based on local farmer recognition, continued cultivation in wetland agroecosystems, and their status as distinct named varieties within the local production system. This targeted sampling was specifically designed to assess inter-accession chloroplast sequence variation and plastid haplotype similarity between two sampled accessions, rather than to infer population genetic structure or capture within-landrace diversity across the

region. Each variety was represented by a single sampled individual, which served as an operational taxonomic unit corresponding to the named farmer-recognized landrace in chloroplast sequence analyses. Given the maternal inheritance and relatively conserved nature of plastid genomes, this design allows evaluation of sequence divergence and haplotypic differences between the two analyzed accessions.

2.3. Procedures

2.3.1. DNA Extraction and Quality Control

Genomic DNA was extracted from approximately 100 mg of fresh leaf tissue using the Plant Genomic DNA Mini Kit GP100 (Geneaid Biotech Ltd., Taiwan) following the manufacturer's protocol. After removal of cellular debris by centrifugation and filtration, the cleared lysate was mixed with binding buffer containing isopropanol and transferred to a GD silica membrane column for DNA adsorption. The column-bound DNA was washed to eliminate residual contaminants and finally eluted in 100 µL of pre-warmed elution buffer. The extracted genomic DNA was stored at −20°C until further analysis.

2.3.2. Quantitative Analyses of DNA Extraction Results

DNA concentration and purity were quantified using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific) operated in dsDNA measurement mode. Before each measurement, the instrument pedestal was cleaned with lint-free swabs and calibrated using 2 µL of nuclease-free water as a blank. Subsequently, 2 µL of each DNA extract was loaded onto the measurement pedestal, and absorbance values were recorded. DNA purity was evaluated based on the A_{260}/A_{280} absorbance ratio, with values ranging from 1.8 to 2.0 considered indicative of high-quality genomic DNA suitable for molecular analysis (Dewanata & Mushlih 2021; Utaminingsih & Sophian 2022; Versmessen *et al.* 2024). DNA concentration (ng μL^{-1}) was simultaneously recorded for each sample, with measured concentrations ranging from 28.4 to 45.2 ng μL^{-1} and A_{260}/A_{280} values of 1.85–1.98, indicating acceptable purity. All measurements were performed in duplicate to ensure consistency. DNA extracts within this concentration range were used directly as PCR templates without additional dilution or concentration prior to amplification.

2.3.3. Qualitative Analyses of DNA Extraction Results

The integrity of extracted genomic DNA and PCR amplicons was evaluated by electrophoresis on a 1% agarose gel prepared in 1× TBE buffer and stained with GelRed. Samples were separated at 80 V for 30 min alongside a λ HindIII DNA ladder as the molecular size reference. DNA bands were visualized under a UV transilluminator (Accuris E3000, 115 VAC: Edison, NJ, USA) and documented photographically. Only samples displaying clear, sharp, and unsmeared bands were considered suitable for subsequent molecular analyses.

2.3.4. PCR Amplification and Electrophoresis

A total of two chloroplast primer pairs were used to amplify the *rbcL* and *matK* regions in *Siam* and *Siputih* swamp rice. These loci were selected because they are widely applied in plant DNA barcoding and phylogenetic studies due to their universality, reproducibility, and taxonomic resolution. The *rbcL* primer pair consisted of *rbcL*-F (5′–ATGTCACCACAAACAGAGACTAAAGC–3′) and *rbcL*-R (5′–GTAAAATCAAGTCCACCRCG–3′), as reported by Mursyidin *et al.* (2021a, 2021b). For the *matK* region, amplification was performed using *matK* XF (forward: 5′–TAA TTT ACG ATC AAT TCA TTC–3′) and *matK*-MALP (reverse: 5′–ACA AGA AAG TCG AAG TAT–3′) following Putra *et al.* (2019). Each primer pair consists of forward and reverse sequences designed to flank conserved chloroplast coding regions suitable for DNA barcoding and comparative sequence analysis.

PCR reactions were prepared in a final volume of 25 µL containing 12.5 µL MyTaq™ HS Red Mix (Bioline, Meridian Bioscience, London, UK), 7.5 µL nuclease-free water, 1 µL of each forward and reverse primer, and 3 µL of genomic DNA template. The DNA template concentration ranged approximately between 20–50 ng μL^{-1}, ensuring sufficient template availability for consistent amplification. PCR amplification was performed using a Bio–Rad T100™ Thermal Cycler (Bio–Rad Laboratories, Hercules, CA, USA). The thermal cycling profile was adopted from the standard MyTaq™ HS Red Mix manufacturer protocol and applied consistently to both *rbcL* and *matK* primer sets. The profile consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s, and extension at 72°C

for 1 min, with a final extension at 72°C for 5 min. of used a single annealing temperature for both markers based on the validated amplification protocol, further supported by the production of clear, reproducible amplicons of the expected sizes in both loci, as confirmed by agarose gel electrophoresis. Furthermore, the annealing temperature of 48°C was selected based on preliminary optimization and consistently produced specific amplification for both markers without detectable non-specific products. PCR products were resolved on a 1% agarose gel prepared in 1× TBE buffer and stained with GelRed DNA dye. The molten agarose was poured into a casting tray fitted with a comb to form wells and allowed to solidify before electrophoresis. Load approximately 4–5 µL of each PCR product mixed with loading dye into the wells, alongside a λ HindIII DNA ladder (1 kb) as a molecular size reference. Electrophoresis was conducted at 80 V for 45–60 minutes. Amplification results were visualized under a UV transilluminator (Accuris E3000, 115 VAC: Edison, NJ, USA) and documented. Only clear and distinct bands corresponding to the expected fragment sizes were selected for downstream sequencing analysis.

2.3.5. DNA Sequencing

PCR products showing clear and specific amplification were purified and submitted to commercial sequencing facilities, PT Genetika Science Indonesia and 1st BASE Laboratories (Singapore), for bidirectional sequencing using the Sanger method. Both forward and reverse primers were used to ensure accurate base calling and reliable consensus sequence assembly.

2.4. Data Analysis

DNA sequence chromatograms obtained from bidirectional Sanger sequencing were manually inspected and edited using BioEdit (Hall 2021). Low-quality terminal regions were identified based on weak, overlapping, or unstable chromatogram peak signals at the sequence ends and were removed prior to downstream analysis. Ambiguous base calls were evaluated based on chromatogram peak quality and cross-checked against the complementary read to ensure accurate consensus sequence generation. Forward and reverse reads were aligned to generate consensus sequences, and unresolved sites, when present, were coded using standard IUPAC ambiguity notation or excluded from polymorphism analysis. The edited consensus sequences were exported in FASTA format for subsequent analyses. Sequence identity was verified using the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information platform (NCBI 2025). Reference sequences were selected based on the highest BLAST similarity scores and query coverage values. Specifically, the top one to two hits for each locus were considered, with preference given to full-length, high-coverage sequences from both cultivated *Oryza sativa* and closely related wild *Oryza* species to provide comparative phylogenetic context. Additional wild *Oryza* accessions were included to improve clade interpretation, whereas the outgroup was selected based on taxonomic relatedness and prior use in published *Oryza* phylogenetic studies. Selected reference sequences were downloaded and combined with the study sequences in FASTA format for further analysis. Multiple sequence alignment was performed using ClustalW 2.1 implemented in MEGA 11 (Tamura *et al.* 2021). The aligned dataset was examined to identify nucleotide substitutions and insertion–deletion (InDel) polymorphisms across the *rbcL* and *matK* regions. Phylogenetic relationships were reconstructed using the Neighbour-Joining (NJ) method under the Kimura 2-parameter substitution model in MEGA 11 (Tamura *et al.* 2021). Branch support was assessed using 1,000 bootstrap replicates. Given the highly conserved nature of the *rbcL* and *matK* loci and the limited number of informative polymorphic sites detected, the resulting tree topology was interpreted conservatively and used primarily as a descriptive framework for accession-level chloroplast sequence comparison rather than for broader evolutionary inference. Descriptive pairwise sequence-comparison analyses were conducted using DnaSP v6.12.03 (Rozas *et al.* 2017). The aligned chloroplast sequences were used to characterize nucleotide polymorphism and haplotypic differences between the *Siam* and *Siputih* accessions. The calculated parameters included the number of polymorphic sites (S), total mutations (Eta), haplotypes (h), nucleotide diversity (π), average nucleotide differences (k), and Watterson's theta (θ-W). InDel polymorphisms were additionally analyzed using the diallelic and non-overlapping model implemented in DnaSP v6.12.03. Because chloroplast genomes are maternally inherited and relatively conserved, all analyses were designed primarily to describe inter-accession sequence divergence rather than infer population-level genetic structure. Given that each variety was represented by a single representative accession was analyzed for each landrace, all DnaSP-derived parameters were treated

strictly as descriptive pairwise sequence-comparison statistics between the two analyzed accessions and were not interpreted as estimates of within-landrace or population-level diversity.

2.5. Data Availability

The newly generated chloroplast DNA sequences (*rbcL* and *matK*) from the *Siam* and *Siputih* swamp rice accessions have been deposited in the National Center for Biotechnology Information (NCBI) GenBank database. The accession numbers are as follows: *rbcL* sequences — PZ272584 (*Siam*) and PZ272585 (*Siputih*); *matK* sequences — PZ289792 (*Siam*) and PZ289791 (*Siputih*). All other data supporting the findings of this study, including aligned sequence datasets and analytical outputs, are available from the corresponding author upon reasonable request.

3. Results

3.1. DNA Amplification

PCR amplification of the chloroplast *rbcL* and *matK* genes from the *Siam* and *Siputih* swamp rice varieties produced clear amplification products. The electrophoretic profiles of the PCR products are shown in Figure 2.

Amplification of the *rbcL* and *matK* regions produced single bands of the expected sizes, approximately ~600 bp and ~900 bp, respectively, in both *Siam* and *Siputih* samples (Figure 2). No non-specific bands or smearing were observed, indicating successful and specific amplification of both chloroplast markers. The resulting PCR products were subsequently used for DNA sequencing analysis. Sequencing chromatograms exhibited clear and well-defined peaks for both accessions, enabling accurate nucleotide base calling and downstream sequence analyses (Table 2). Although the *matK* band intensity was lower in Siputih than in Siam, the amplicon passed sequencing quality requirements and generated high-quality sequence data for subsequent analyses.

3.2. Sequence Similarity Analysis Using BLAST

The edited consensus sequences obtained from bidirectional Sanger sequencing and used for BLAST analysis comprised 588 bp and 592 bp for the *rbcL* locus (*Siam* and *Siputih*, respectively) and 909 bp and 911 bp for the *matK* locus. These trimmed query regions were analyzed using the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information platform to confirm

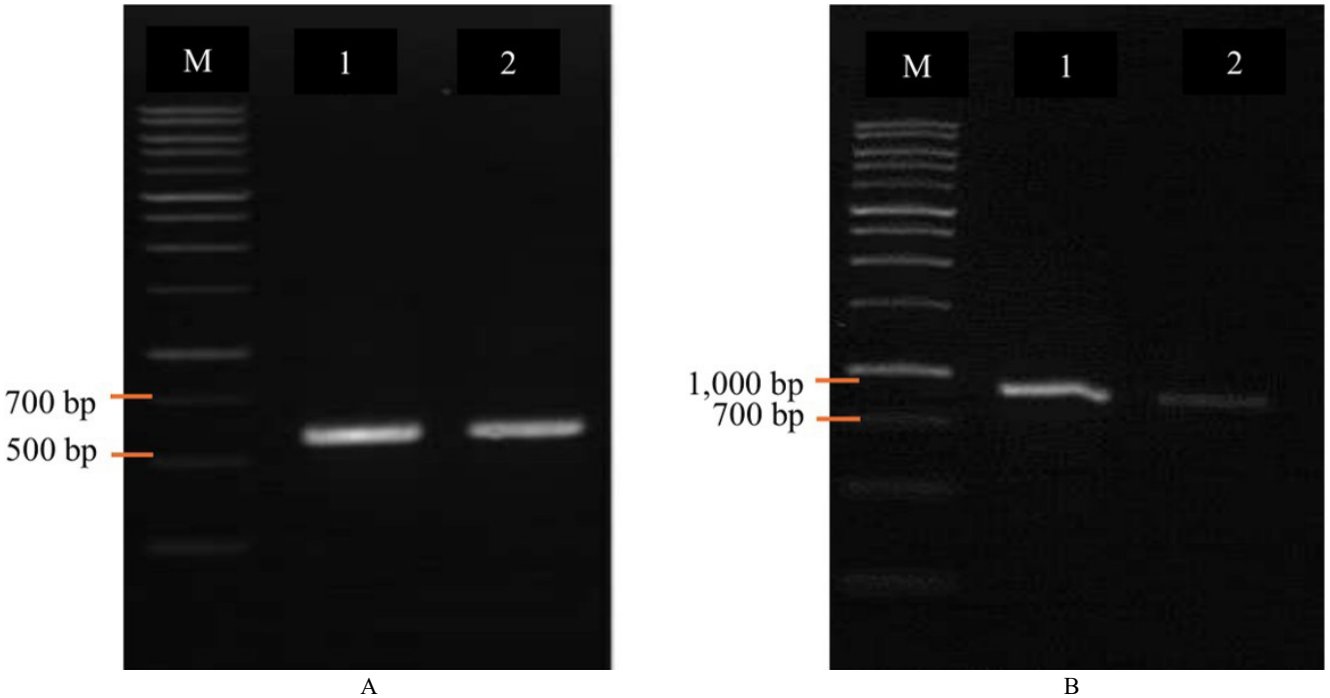


Figure 2. PCR amplification of chloroplast DNA markers in swamp rice. (A) *rbcL* gene, (B) *matK* gene. M: 1 kb DNA ladder; lane 1: *Siam*, lane 2: *Siputih*

sequence identity. For the *matK* marker, the *Siputih* sequence exhibited the highest similarity (99.89% identity) with *Oryza sativa* Japonica group Taichung 65 chloroplast DNA (accession LC739565.1) and *O. nivara* chloroplast DNA (accession LC666910.1). Meanwhile, the *Siam* sequence showed the highest similarity with the *O. rufipogon* chloroplast genome (accession OV050069.1) with 100% identity, followed by *O. glaberrima* chloroplast DNA (accession MW528842.1) with 99.89% identity. All alignments displayed query cover values of 99% and E-values of 0.0, indicating a strong sequence match with reference sequences deposited in GenBank (Table 3).

Similarly, BLAST analysis of the *rbcL* sequences revealed high similarity with previously reported *O. sativa* cultivars. The Siam sample showed 100% identity with *O. sativa* cultivar Karang Dukuh (accession MZ198250.1) and 99.83% identity with *O. sativa* cultivar Siam Mutiara (accession MZ198249.1). The *Siputih* sequence also exhibited 100% identity with *O. sativa* cultivar Karang Dukuh (accession MZ198250.1) and 99.66% identity with *O. sativa* cultivar NARC 17958 (accession KP827660.1). All alignments showed query cover values of 99% and E-values of 0.0, indicating strong sequence homology with rice sequences deposited in GenBank (Table 4).

Overall, BLAST results confirmed that the obtained sequences correspond to chloroplast regions of *Oryza* species, with very high similarity (99.66–100%) to reference sequences in the NCBI database.

Table 2. PCR amplification and sequencing quality of chloroplast markers in *Siam* and *Siputih* accessions

Markers	Sample	Band detected	Fragment size (Bp)	Band quality	Sequencing suitability
rbcL	SM	Present	~600	Clear, single band	Suitable
rbcL	SP	Present	~600	Clear, single band	Suitable
matK	SM	Present	~900	Thick bright band	Suitable
matK	SP	Present	~900	Thin band	Suitable

SM: *Siam*, SP: *Siputih*

Table 3. BLAST results for matK sequences

Sample	Closest species	Accession	% Query cover	E-value	% Identity
SP	*O. sativa* Japonica group taichung 65 chloroplast DNA, complete genome	LC739565.1	99.00	0.00	99.89
SP	*O. nivara* CN_HLJ_W_S163 chloroplast DNA, complete genome	LC666910.1	99.00	0.00	99.89
SM	*O. glaberrima* isolate P92 chloroplast, complete genome	MW528842.1	99.00	0.00	99.89
SM	*O. rufipogon* genome assembly, organelle: plastid:chloroplast	OV050069.1	99.00	0.00	100.00

SM: *Siam*, SP: *Siputih*

Table 4. BLAST results for *rbcL* sequences

Sample	Closest species	Accession	% Query cover	E-value	% Identity
SP	*O. sativa* cultivar Karang Dukuh ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene	MZ198250.1	99.00	0.00	100.00
SP	*O. sativa* cultivar NARC 17958 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene	KP827660.1	99.00	0.00	99.66
SM	*O. sativa* cultivar Karang Dukuh ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene	MZ198250.1	99.00	0.00	100.00
SM	*O. sativa* cultivar *Siam* Mutiara ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene	MZ198249.1	99.00	0.00	99.83

SM: *Siam*, SP: *Siputih*

3.3. Comparative Chloroplast Sequence Differences

Direct comparison of the chloroplast sequences between Siam and Siputih revealed limited but detectable differences in both loci (Table 5). The *rbcL* region contained one SNP and two InDel events, whereas *matK* showed no SNPs but contained three short InDel events. Both markers exhibited a total of three observed sequence differences, indicating very limited chloroplast divergence between the two accessions. For completeness, DnaSP-derived statistics are presented in Table 5 as descriptive pairwise sequence-comparison metrics and should not be interpreted as population-level estimates because only two sequences were analyzed.

Table 5. Pairwise chloroplast sequence-comparison statistics between *Siam* and *Siputih* accessions

Parameter	Marker	
	rbcL	*matK*
Alignment length (bp)	593	913
Effective sites (bp)	587	908
S	1	0
I	2	3
Total differences (S+I)	3	3
Total InDel sites	6	4
Avg. InDel length (bp)	3.000	1.330
π	0.00170	0.00000
θ-W	0.00170	0.00000
π (i)	0.00337	0.00329
θ(i)-W	2.000	3.000
k	1	0
k(i)	2	3
SNP haplotypes	2	1
InDel haplotype	2	2

π: nucleotide diversity (average number of nucleotide differences per site between sequences), π(i): InDel diversity per site, S: number of polymorphic (segregating) sites, I: number of insertion–deletion (InDel) events, k: average number of nucleotide differences between sequences, k(i): average number of InDel differences between sequences, θ-W: Watterson's theta estimated from segregating sites, θ(i)-W: Watterson's theta estimated from InDel polymorphism, bp: base pairs

3.4. Genetic Distance, Sequence Analysis, and Phylogenetic Tree Reconstruction

Genetic distances were calculated using the Kimura 2-parameter model implemented in MEGA 11 prior to phylogenetic reconstruction. For the *matK* dataset, genetic distances among the analyzed sequences ranged from 0.0000 to 0.9656 (Table 6). The smallest genetic distance was observed between the *Siam* sample and *O. sativa* japonica sequences (0.0000), indicating extremely high sequence similarity. The genetic distance between the *Siam* and *Siputih* samples was 0.0066, corresponding to approximately 99.34% sequence similarity, which suggests a very close genetic relationship between the two varieties.

A similar pattern was observed in the *rbcL* dataset (Table 7). The genetic distance between *Siam* and *Siputih* was 0.0017, indicating very low nucleotide sequence divergence within the analyzed *rbcL* region and supporting their close chloroplast genetic relationship. The overall

Table 6. Genetic distance matrix among *Siam* and *Siputih* swamp rice varieties and selected *Oryza* reference sequences based on chloroplast *matK* sequences

	1	2	3	4	5	6	7	8
Siputih								
Siam	0.0066							
O. sativa Japonica Group LC739565.1:1897-2805	0.0044	0.0000						
O. glaberrima MW528842.1	0.0044	0.0000	0.0000					
O. rufipogon OV050069.1	0.0066	0.0000	0.0000	0.0000				
O. nivara CN_HLJ_W_S163 LC666910.1	0.0066	0.0000	0.0000	0.0000	0.0000			
Sorghum bicolor AF164418.1	0.9643	0.9633	0.9623	0.9623	0.9656	0.9656		
Zea mays voucher 13CS8050 MN492739.1	0.9613	0.9633	0.9613	0.9613	0.9613	0.9613	0.0151	

Table 7. Genetic distance matrix among *Siam* and *Siputih* swamp rice varieties and selected *Oryza* reference sequences based on chloroplast *rbcL* sequences

	1	2	3	4	5	6	7	8	9
Siputih									
Siam	0.0017								
O. sativa cul. Karang Dukuh MZ198250.1	0.0000	0.0018							
O. sativa cul. Siam Mutiara MZ198249.1	0.0018	0.0000	0.0018						
O. sativa Tropical Japonica KT289404.1	0.0000	0.0018	0.0000	0.0018					
O. nivara voucher PADDY20 MN942410.1	0.0000	0.0018	0.0000	0.0018	0.0000				
O. glaberrima KM088021.1	0.0000	0.0018	0.0000	0.0018	0.0000	0.0000			
O. rufipogon OV050091.1	0.0000	0.0018	0.0000	0.0018	0.0000	0.0000	0.0000		
Z. mays voucher 13CS8050 MN205153.1	0.0464	0.0483	0.0464	0.0483	0.0464	0.0464	0.0464	0.0464	
T. aestivum voucher 06-JMS-0782 MG227483.1	0.0564	0.0544	0.0564	0.0544	0.0564	0.0564	0.0564	0.0564	0.0603

genetic distances among the analyzed taxa ranged from 0.0000 to 0.0603, with the largest values observed between the rice accessions and the outgroup species *Triticum aestivum*. In contrast, the genetic distances among the *Oryza* accessions remained very low, ranging from 0.0000 to 0.0018, indicating high sequence conservation within the genus.

These results indicate that the two analyzed accessions exhibit very limited chloroplast sequence divergence and cluster within the *Oryza* lineage. In contrast, the outgroup taxa are clearly separated from the rice clade.

Multiple sequence alignment of the chloroplast *rbcL* and *matK* sequences from the *Siam* and *Siputih* swamp rice varieties revealed a high degree of sequence conservation across both regions. Most nucleotide positions were identical between the two sequences, as indicated by continuous conserved sites throughout the alignment matrices. These conserved regions represent the majority of the analyzed sequences and reflect the relatively stable nature of chloroplast coding genes.

In the *matK* region, no single-nucleotide polymorphisms (SNPs) were detected between the two varieties. However, insertion–deletion (InDel) polymorphisms were identified at several positions in the alignment (1, 908, 912, and 913). A deletion was observed at the beginning of the *Siam* sequence relative to *Siputih*, while additional gap positions occurred near the terminal portion of the alignment. In total, three InDel events were detected across four InDel sites within the *matK* alignment (Table 8). These structural variations produced two distinct InDel haplotypes despite the absence of nucleotide substitutions.

In contrast, the *rbcL* alignment revealed limited nucleotide variation between the two accessions. A single SNP was detected at alignment position 3, where *Siputih* contained cytosine (C) and Siam contained thymine (T), representing a C→T transition (Table 9). In addition, two InDel events were detected, consisting of one leading gap at position 1 and a terminal gap block spanning positions 589–593 (Table 10). These observed differences generated two distinct SNP haplotypes and two InDel haplotypes across the two analyzed accessions.

Overall, the sequence alignment demonstrates that both chloroplast markers are highly conserved between the *Siam* and *Siputih* swamp rice varieties. Genetic differences were limited to a single nucleotide substitution in the *rbcL* region and several short insertion–deletion events detected in both markers, indicating extremely low chloroplast genetic divergence between the two varieties.

The phylogenetic trees reconstructed using the Neighbour-Joining (NJ) method under the Kimura 2-parameter substitution model provided a descriptive visualization of the chloroplast sequence relationships between the two analyzed accessions and selected reference sequences based on the *rbcL* and *matK* markers (Figure 3).

Table 8. InDel events in the sequence alignment of the chloroplast *matK* region

Species	Position			
	1	908	912	913
Siputih	G	-	T	G
Siam	-	A	-	-

–: InDel events detected in the alignment

Table 9. SNP positions identified in the *rbcL* sequence alignment

Species	Position
	3
Siputih	C
Siam	T

C→T transition detected at alignment position 3

Table 10. InDel events in the sequence alignment of the chloroplast *rbcL* region

Species	Position					
	1	589	590	591	592	593
Siputih	T	-	-	-	-	-
Siam	-	G	G	T	G	A

–: insertion–deletion (InDel) positions in the alignment

For the *rbcL*-based phylogeny, the *Siam* accession (PZ272584) and the *Siputih* accession (PZ272585) formed a closely associated subclade within Cluster I with strong bootstrap support (100), indicating very high chloroplast sequence similarity between the two analyzed accessions. This clustering pattern is consistent with the very low pairwise genetic distance observed in the *rbcL* dataset (0.0017) and with the minimal sequence variation detected across the aligned region. The Siam–Siputih subclade was positioned in close association with several *Oryza sativa* reference sequences, including cultivar Karang Dukuh (MZ198250.1), cultivar *Siam* Mutiara (MZ198249.1), and Tropical Japonica Group (KT289404.1), further supporting their placement within the broader *Oryza* clade. The short branch lengths observed within this cluster are consistent with the highly conserved nature of the *rbcL* locus.

Similarly, the *matK*-based phylogeny showed that the *Siam* (PZ289792) and *Siputih* (PZ289791) accessions formed a closely associated subclade within Cluster II with strong bootstrap support (99), indicating very high chloroplast sequence similarity between the two analyzed

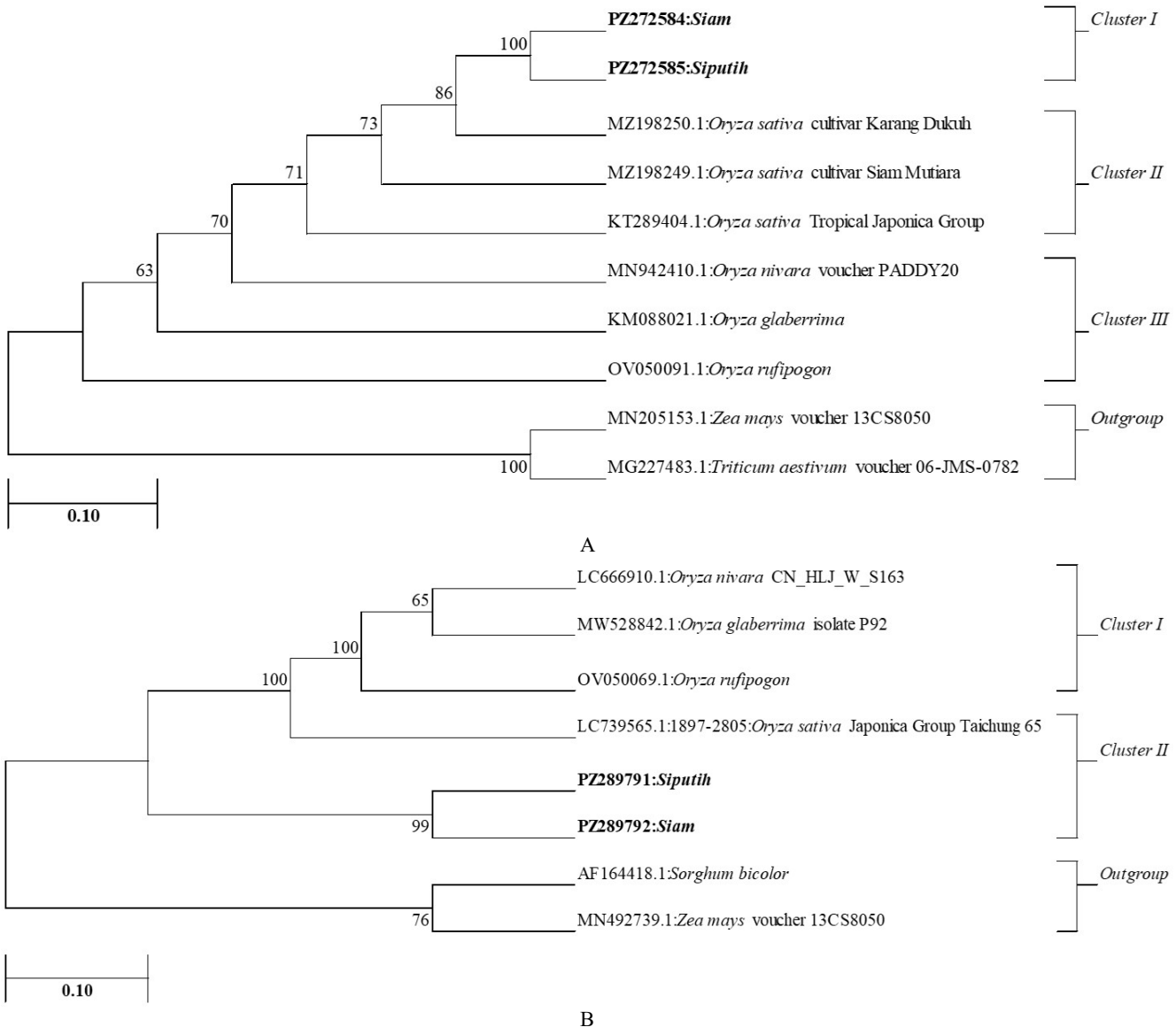


Figure 3. Phylogenetic tree reconstruction of *Siam* and *Siputih* rice accessions and reference sequences based on chloroplast DNA markers (*rbcL* (A) and *matK* (b)) using the Neighbor-Joining (NJ) method

accessions. This clustering pattern is consistent with the very low pairwise genetic distance observed in the *matK* dataset (0.0066) and limited sequence variation observed in the *matK* alignment, which was characterized primarily by short InDel events and no detected SNPs. The *Siam–Siputih* subclade was positioned in close association with several *Oryza* reference sequences, including *O. sativa* Japonica Group Taichung 65 (LC739565.1), *O. nivara* (LC666910.1), *O. glaberrima* (MW528842.1), and *O. rufipogon* (OV050069.1), further supporting their placement within the broader *Oryza* clade. The short branch lengths observed within this cluster are likewise consistent with the conserved nature of the *matK* locus.

Taken together, both phylogenetic trees consistently show that the *Siam* and *Siputih* accessions form a closely associated core subclade and are clearly separated from the outgroup taxa. The concordance between the *rbcL* and *matK* topologies supports the consistency of the observed chloroplast sequence similarity between the two sampled accessions. Given the highly conserved nature of both chloroplast loci and the limited number of informative sites, these trees are interpreted as descriptive visual summaries of pairwise chloroplast sequence similarity rather than evidence of broader phylogenetic separation or lineage history.

4. Discussion

The present findings should be interpreted strictly as evidence of chloroplast sequence similarity between the two sampled accessions, rather than as evidence of complete genomic similarity. This interpretative boundary is essential because chloroplast DNA reflects maternal inheritance only and therefore captures only the plastid component of ancestry. Consequently, phenotypic and agronomic differences between *Siam* and *Siputih* may be influenced more strongly by nuclear genomic variation, local adaptation, epigenetic regulation, and environmental effects than by the limited plastid sequence divergence detected in this study (Teske *et al.* 2020; Khade *et al.* 2025).

This interpretation is further supported by recent molecular studies on South Sumatran rice germplasm. Using 11 SSR loci, Hanum *et al.* (2026) reported high levels of nuclear genetic variation among local pigmented rice landraces (mean PIC = 0.878), despite their shared taxonomic identity. The contrast between high nuclear diversity and low chloroplast divergence observed in the present study reinforces the view that plastid markers primarily reflect conserved maternal ancestry. In contrast, much of the phenotypic and agronomic variation is structured within the nuclear genome.

The present study provides a chloroplast-based molecular comparison of two traditional swamp rice accessions from South Sumatra, *Siam* and *Siputih*, using the *rbcL* and *matK* loci. Both markers consistently revealed very low plastid-level sequence divergence, supported by high BLAST similarity values (99.66–100%), minimal observed sequence differences, low pairwise descriptive metrics, and congruent phylogenetic clustering. Taken together, these findings suggest that the two analyzed accessions may share a closely related maternal plastid haplotype, which is consistent with their placement within the broader *Oryza sativa* gene pool. The successful amplification of both chloroplast regions yielded clear and distinct bands at the expected sizes, ~600 bp for *rbcL* and ~900 bp for *matK*, accompanied by high-quality sequencing chromatograms. Within the present two-accession comparison, these results indicate that both primer pairs performed reliably and generated sequence data suitable for downstream comparative analysis. This observation is consistent with previous studies that have established *rbcL* and *matK* as widely used, reproducible chloroplast DNA barcoding loci across cultivated and wild plant taxa, including rice (Gostel *et al.* 2020; Nadiya *et al.* 2023; Sevindik *et al.* 2024; Corvalán *et al.* 2025).

BLAST analysis further confirmed that both analyzed accessions belong within the broader *Oryza* lineage, showing extremely high similarity to several *Oryza* reference accessions, including *O. sativa, O. nivara, O. rufipogon*, and *O. glaberrima*. Such high similarity is biologically expected because chloroplast-coding genes, particularly *rbcL* and *matK*, are generally conserved due to strong functional constraints associated with photosynthesis and plastid metabolism. The observed similarity to both cultivated and wild *Oryza* taxa is therefore consistent with the known domestication complexity of rice and the persistence of shared chloroplast haplotypes among related lineages (Brozynska *et al.* 2017; Cheng *et al.* 2019; Hanum *et al.* 2026).

The low chloroplast divergence observed in the present study is highly consistent with findings from other Indonesian swamp rice systems. Previous studies on traditional tidal swamp rice from South Kalimantan, Indonesia, using chloroplast barcoding markers, similarly reported low plastid diversity and close phylogenetic clustering among local landraces. These studies demonstrated that wetland-adapted rice accessions from different localities often share closely related chloroplast haplotypes, suggesting common maternal ancestry and long-term lineage conservation within Indonesian swamp agroecosystems. This cross-regional similarity is particularly important. Although South Sumatra and South Kalimantan represent different wetland environments, both regions are characterized by farmer-managed seed preservation, recurrent self-pollination, and long-term adaptation to flood-prone ecosystems (Mursyidin *et al.* 2018; Hanum *et al.* 2026). Such processes may promote the retention of stable maternal chloroplast lineages across generations (Moner *et al.* 2020). Therefore, the close genetic relationship observed between Siam and Siputih likely reflects a broader regional pattern in Indonesian swamp rice, where plastid markers reveal limited intraspecific divergence despite observable differences in local naming and morphology.

The high chloroplast similarity observed in the present study is also consistent with previous morphological observations of South Sumatran swamp rice. Earlier field-based assessments reported that the *Siputih* (Pegagan) landrace exhibited approximately 98% morphological homogeneity across multiple districts in South Sumatra, indicating a high degree of phenotypic stability (Kodir *et al.* 2018). Although morphological uniformity does not necessarily imply genetic uniformity, the combination of high field-level consistency and minimal chloroplast sequence divergence supports the interpretation that

conserved maternal lineages have been maintained within this traditional rice germplasm. Such concordance between morphological and chloroplast evidence provides additional support for the genetic stability of local swamp rice resources.

Direct sequence comparison revealed extremely limited differences between *Siam* and *Siputih*. The *rbcL* marker showed one SNP and two InDel events, whereas *matK* showed no SNPs but three short InDel events. Within the present two-accession comparison, the *matK* marker showed slightly greater detectable sequence variation than *rbcL*, particularly in the form of short InDel events and modest differences in phylogenetic support. This pattern is consistent with previous DNA barcoding studies indicating that *matK* often exhibits a somewhat higher evolutionary rate than the more conserved *rbcL* locus (Moner *et al.* 2020; Parida *et al.* 2023). These findings collectively suggest that the chloroplast genomes of both landraces are highly conserved at the nucleotide level, with only minor structural differences detectable through InDel polymorphisms (Urtgam *et al.* 2026). Such a pattern is consistent with the generally conserved nature of plastid coding regions and supports the inference of a close maternal evolutionary relationship between *Siam* and *Siputih*. The predominance of InDel-based variation in *matK* may also indicate that this locus captures subtle microstructural changes that are not reflected as nucleotide substitutions, thereby providing complementary resolution to *rbcL*.

An interesting observation in the present study is that *rbcL* contained a detectable SNP. In contrast, *matK* showed no nucleotide substitutions despite being generally regarded as the more rapidly evolving chloroplast barcode locus. This pattern differs from the commonly reported expectation that *matK* exhibits higher substitution rates and greater discriminatory power than *rbcL* in plants (Mishra *et al.* 2018; Matiz-Ceron *et al.* 2022). However, because the present analysis was restricted to only two closely related accessions, this departure from the expected trend likely reflects dataset-specific sequence variation rather than a contradiction of the broader evolutionary patterns documented for chloroplast barcoding markers. The presence of several short InDel polymorphisms in matK further suggests that sequence variation within this locus may be expressed primarily through small structural changes rather than nucleotide substitutions in the analyzed accessions.

From an evolutionary perspective, the limited number of polymorphic sites suggests that divergence between the two landraces may reflect relatively recent divergence at the chloroplast level or has occurred primarily in nuclear genomic regions rather than within the chloroplast genome. Within the present dataset, the *matK* marker showed slightly greater detectable sequence variation than *rbcL*, particularly in the form of short InDel events and modest differences in phylogenetic support. However, the limited sampling design precludes broader conclusions regarding marker discriminatory performance across South Sumatran swamp rice germplasm. Nevertheless, additional sampling is required before drawing broader conclusions regarding the relative performance of these markers.

The genetic distance analysis further supports this interpretation. The *rbcL* region showed a verified genetic distance of 0.0017 between *Siam* and *Siputih*. In contrast, the *matK* distance was 0.0066, indicating very low but detectable chloroplast sequence divergence between the two sampled accessions. The slightly higher divergence in *matK* is consistent with its generally higher evolutionary rate relative to the more conserved *rbcL* locus (Moner *et al.* 2020; Parida *et al.* 2023).

Similarly, the NJ trees derived from both loci should be interpreted as supportive visual summaries of pairwise chloroplast sequence similarity between the two analyzed accessions rather than as evidence of broader evolutionary separation among named landraces. In the phylogenetic reconstruction, both *rbcL* and *matK* consistently placed the *Siam* and *Siputih* accessions within closely associated subclades supported by high bootstrap values (100 and 99, respectively), indicating highly similar plastid sequences across the analyzed regions. The concordance of these topologies, together with their close association to selected *Oryza* reference sequences, supports the interpretation of very low chloroplast sequence divergence at the accession level. However, given the highly conserved nature of both loci and the limited number of informative differences, the tree topology remains primarily descriptive. It should not be extended to infer broader lineage history or landrace-level evolutionary separation. Several broader biological explanations, including long-term farmer seed preservation, retention of closely related plastid haplotypes, and adaptation within wetland agroecosystems, may plausibly contribute to the observed similarity (Kaewcheenchai *et al.* 2018; Zheng *et al.* 2022). However, these remain biologically reasonable hypotheses that are consistent with, but not directly demonstrated by, the present dataset.

However, several limitations should be acknowledged. Because only one individual per named landrace was analyzed, the present study cannot capture within-landrace

chloroplast variation. Therefore, all conclusions are restricted to pairwise chloroplast sequence comparison between the two sampled accessions. Future studies should include multiple individuals per landrace and integrate higher-resolution nuclear markers such as SSRs, SNPs, or genome-wide sequencing approaches to resolve better genetic structure, domestication history, and adaptive divergence in South Sumatran swamp rice germplasm.

Based on the analysis of two sampled accessions, each representing one genotype from the *Siam* and *Siputih* named varieties, the chloroplast loci *rbcL* and *matK* revealed very low plastid sequence divergence and close chloroplast similarity between the two analyzed accessions. Across the two loci, only minimal sequence differences were detected, consisting of one SNP and several short InDel events, indicating limited chloroplast variation within the present dataset. These findings should be interpreted strictly as pairwise chloroplast sequence comparison results between the two sampled accessions. They should not be extended to broader inference for the named landraces as a whole. Nevertheless, the present study provides an initial chloroplast molecular baseline that may support future investigations involving broader sampling and higher-resolution nuclear markers for swamp rice germplasm from South Sumatra.

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