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Maternal Lineage Structure and Chloroplast Diversity of South Sumatran Rice, Indonesia, Revealed by the trnH–psbA Intergenic Spacer

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Abstract. Despite the importance of comprehensive genetic characterization for the conservation and utilization of local rice germplasm in breeding programs, the maternal genetic diversity of South Sumatran local rice remains largely unexplored. In this study, we evaluated the genetic diversity, maternal lineage, and phylogenetic relationships of South Sumatran rice accessions using the chloroplast trnH–psbA intergenic spacer, a highly variable chloroplast marker widely used for assessing genetic diversity and phylogenetic relationships in rice and other plant species. A total of 18 South Sumatran rice accessions, along with reference sequences, were analyzed through sequence alignment, haplotype network construction, genetic distance clustering, and phylogenetic tree reconstruction using the Neighbor-Joining (NJ) and UPGMA methods. The trnH–psbA region showed a uniform sequence length of 415 bp and low nucleotide diversity (π = 0.096%), resulting in the identification of three haplotypes. Phylogenetic and genetic distance analyses consistently identified two accessions, Bone 1 and Pegagan 2, as genetically distinct, highlighting their potential value as candidates for future conservation and breeding initiatives. These findings provide a molecular basis for the preservation and strategic utilization of South Sumatran rice germplasm and emphasize the importance of conserving genetically divergent local accessions. Future studies integrating chloroplast and nuclear markers will enable a more comprehensive assessment of genetic diversity for rice breeding and conservation.

Keywords: chloroplast DNA; maternal lineage; phylogenetic analysis; rice diversity; trnH–psbA

1. Introduction

Global efforts to achieve sustainable development increasingly depend on the stability of agricultural production systems, particularly in staple crops such as rice, which play a central role in ensuring food security under SDG 2 (Saleem et al., 2025). In Indonesia, rice remains the cornerstone of national food policy, and its sustainable production is closely linked to the Asta Cita development framework, which prioritizes agricultural resilience and rural welfare (Ridwan & Suranto, 2025).

South Sumatra represents one of the important rice-producing regions in Indonesia due to its rice germplasm and heterogeneous agroecological conditions, including flood-prone wetlands, peat-influenced lowlands, and rain-fed upland areas (Paiman et al., 2020). These contrasting environments have facilitated the long-term maintenance of farmer-selected rice landraces that exhibit unique adaptive traits shaped by local ecological pressures (Li et al., 2025). Beyond their agronomic importance, such landraces constitute irreplaceable reservoirs of genetic variation that may contribute to future crop adaptation and resilience (Smith et al., 2021). However, despite the rich diversity of traditional rice cultivated in South Sumatra, comprehensive molecular characterization using DNA barcoding has not been widely conducted. Consequently, the genetic relationships, levels of genetic divergence, and evolutionary affinities among South Sumatran rice germplasm remain poorly understood. This lack of molecular evidence limits the identification of unique genetic resources and constrains their effective

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utilization in breeding, conservation, and germplasm management programs.

Genetic characterization is therefore fundamental for uncovering the structure and evolutionary relationships of traditional rice germplasm and for guiding strategic utilization in crop improvement (Sabar et al., 2024). Compared with morphological evaluation, molecular approaches allow more precise detection of genetic divergence and lineage patterns that are otherwise obscured by environmental plasticity (Samarina et al., 2025). Among these approaches, DNA barcoding offers a standardized and efficient framework for comparative analysis of plant genetic resources across regions and populations (Zhang et al., 2021).

Chloroplast markers are particularly informative for lineage-based studies because of their uniparental inheritance and evolutionary stability (Tong et al., 2015). Chloroplast markers are widely preferred because chloroplast genomes are predominantly maternally inherited, highly conserved, and exhibit low recombination, making them reliable for phylogenetic, phylogeographic, and species identification studies. In addition, chloroplast markers are relatively easy and cost-effective to amplify and analyze compared with SSRs, SNPs, or whole-genome approaches while still providing sufficient genetic variation for evolutionary analyses (Fu, 2021; Zhang et al., 2024).

The trnH–psbA intergenic spacer has been widely reported to provide reliable amplification and sufficient sequence variability for resolving genetic relationships among closely related plant taxa, including rice (Sánchez et al., 2020). Previous studies have confirmed its effectiveness in assessing genetic divergence and phylogenetic patterns in cultivated and wild rice relatives (Urtgam et al., 2026). In Indonesia, previous studies have successfully applied the rbcL DNA barcode to assess the genetic diversity of tidal swamp rice from South Kalimantan (Mursyidin et al., 2021) and Indonesian black rice (Basith et al., 2021).

Despite molecular studies on Indonesian rice germplasm, South Sumatran rice remains poorly characterized, particularly using the chloroplast trnH–psbA marker. This study addresses this gap by examining its genetic diversity, maternal lineage, and phylogenetic relationships. Therefore, this study aims to analyze the genetic diversity and phylogenetic relationships of South Sumatran indigenous rice using the chloroplast trnH–psbA marker. The findings provide the first comprehensive molecular assessment of this germplasm with this marker, offering insights into its genetic diversity, maternal lineage, and phylogenetic relationships. These results contribute to the molecular characterization of Indonesian rice germplasm and support its conservation and utilization in future rice breeding programs.

2. Materials and Methods

2.1 Plant materials

The study analyzed a total of 18 accessions collected to represent the geographic and agroecological diversity of traditional rice-growing regions in South Sumatra, Indonesia, including swampland, lowland, upland, peatland, and upland agroecosystems. Priority was given to indigenous landraces traditionally cultivated by local farmers and distinguished by unique local names and morphological characteristics. The complete list of the 18 accessions, two outgroup accessions, and their respective origins is presented in **Table 1**. The seeds were germinated following immersion in a fungicide solution and subsequent rinsing with warm water (30–35 °C). After transplantation, healthy and vigorous seedlings were selected for DNA isolation.

2.2 DNA assay

Genomic DNA was extracted from 14-day-old leaf tissues using the Wizard® Genomic DNA Purification Kit (Promega, USA), following a modified protocol based on (Hanum et al., 2018). DNA quantity and

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quality were assessed using a NanoDrop Lite spectrophotometer (Thermo Scientific, USA). PCR amplification was performed in a T-100 Thermal Cycler (Bio-Rad, USA) with a total reaction volume of 50 µL, consisting of 25 µL MyTaq™ HS Red Mix (2×; Bioline, USA), 0.4 µM of each primer (2 µL), 1 µL of template DNA, and 20 µL of nuclease-free water. The PCR conditions followed the protocol described by Adriansyah et al. (2022), comprising an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, with a final extension at 72 °C for 5 min. The trnH–psbA region was amplified using the primers trnH (5′-CGCGCATGGTGGATTCACAATCC-3′) and psbA (5′-GTTATGCATGAACGTAATGCTC-3′) (Gholave et al., 2017). PCR products were separated on 0.8% agarose gel electrophoresis in 1× TBE buffer, stained with GelRed (Biotium Inc., USA), and visualized using a GelDoc™ Go Imaging System (Bio-Rad, USA). Successfully amplified fragments were purified and subjected to bidirectional Sanger sequencing at 1st Base Ltd. (Malaysia).

Table 1. List of 19 South Sumatran rice accessions

No	Accession	Acc. Number	Origin (Category, District, Province)
1	Bone 1	PX849312	Swampland, Sungai Pinang, Ogan Ilir
2	Bone 2	PX849313	Swampland, Talang Dukun, Ogan Ilir
3	Bone 3	PX849314	Swampland, Sungai Pinang, Ogan Ilir
4	Koneng	PX849315	Swampland, Sungai Pinang, Ogan Ilir
5	Pegagan 1	PX849316	Swampland, Pemulutan, Ogan Ilir
6	Pegagan 2	PX849317	Swampland, Pemulutan, Ogan Ilir
7	Petek	PX849318	Swampland, Talang Dukun, Ogan Ilir
8	Si Putih 1	PX849319	Swampland, Muara Penimbung, Ogan Ilir
9	Si Putih 2	PX849320	Swampland, Talang Dukun, Ogan Ilir
10	Inpara 32	PX849321	Lowland, Indonesia
11	Jambat Tehas	PX849322	Upland, Semende, Muara Enim
12	Surya	PX849323	Upland, Semende, Lahat
13	Selibur Rimbe	PX849324	Upland, Semende, Muara Enim
14	Si Putih 3	PX849325	Swampland, Ogan Komering Ilir
15	Sanapi	PX849326	Swampland, Musi Banyuasin
16	Tarabas	PX849327	Lowland, Jawa Barat
17	Talang	PX849328	Peatland, Perigi, Ogan Komering Ilir
18	Kelara	PX849329	Upland, Pagar Alam
19	Jumbo	PX849330	Upland, Pagar Alam
20	Barokah	PX849331	Upland, Pagar Alam

2.3 Data analysis

Consensus sequences were generated by aligning and manually refining all nucleotide sequences using BioEdit v7.0.0 (Hall, 2011) and MEGA12 (Kumar et al., 2024). Multiple sequence alignment was subsequently performed with Multalin (http://multalin.toulouse.inra.fr/multalin/). Sequence homology was initially assessed using BLAST (NCBI). Haplotype network analysis was conducted using DnaSP v6 (Rozas et al., 2017) and PopART v1.7 (Leigh & Bryant, 2015). Heatmap visualization was generated in RStudio employing the Biostrings (Pagès et al., 2022), msa (Bodenhofer et al., 2015), ape (Paradis et al., 2004), pegas (Paradis, 2023), and ggplot2 (Wickham, 2017) packages. Phylogenetic relationships and genetic diversity were inferred using Neighbor-Joining and

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UPGMA methods. Node support was evaluated through bootstrap analysis with 1.000 replicates (Pattengale et al., 2010). All South Sumatran rice sequences were deposited in GenBank under accession numbers **PX849312-PX849331**.

3. Results and Discussions

3.1 Sequence characteristics of the trnH–psbA region in South Sumatran rice accessions

The trnH–psbA intergenic spacer is one of the most widely used chloroplast DNA regions for plant DNA barcoding and phylogeographic studies due to its high amplification success and moderate sequence variation across taxa (Li et al., 2025). Chloroplast DNA is maternally inherited and therefore reflects only maternal lineage variation. In a comprehensive meta-analysis of trnH–psbA sequences from a broad sampling of plant lineages, sequence lengths ranged from approximately 150 to over 900 bp, with most taxa (92%) within 340–660 bp, a range considered optimal for DNA barcoding and phylogenetic inference in angiosperms (Sánchez et al., 2020). trnH–psbA sequences in many plant groups, including grasses and other monocotyledons, typically exhibit lengths falling within the 268–614 bp range (Aygören Uluer, 2025). This range has also been observed in multiple barcoding studies where trnH–psbA served as a core locus for species identification and genetic comparison (Feng et al., 2021). In the present study, the uniform sequence length of 415 bp across 18 South Sumatran rice accessions, as presented in **Table 2** and **Figure 1**, falls well within the expected trnH–psbA size range for flowering plants and monocots in particular. This consistency suggests that the trnH–psbA region in *Oryza sativa* exhibits limited size variation among the tested accessions, reflecting a relatively conserved spacer length in this crop species (Song et al., 2017). Comparable findings have been reported where the trnH-psbA region in various plant taxa, including Poaceae members, presented similar sequence length distributions within this interval, facilitating reliable alignment and comparative analyses (Lee et al., 2022)

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Table 2. Genetic characteristics of the trnH–psbA region in South Sumatran rice accessions, Indonesia

Parameter	trnH–psbA
Range of sequence length (bp)	415
Parsimony informative sites	0
Number of polymorphic sites (S)	4
Indels sites	0
Bayesian information criterion (BIC)	1561.474
Akaike information criterion (AICc)	1271.574
Maximum likelihood value (InL)	-594.241
Transition/transversion bias value (R)	0.083
GC content (%)	37.133
Nucleotide diversity (π %)	0.096
Number of haplotypes, h	3
Haplotype (gene) diversity, Hd	0.195

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3.2 Genetic diversity of the trnH–psbA chloroplast region in South Sumatran rice accessions

The sequence dataset revealed only four polymorphic sites (S = 4) and no parsimony informative sites, which suggests a low level of nucleotide variation at this locus among the studied rice accessions is presented in **Table 2** and **Table 3**. Low polymorphism in trnH–psbA has been documented in some intraspecific studies where chloroplast markers tend to be highly conserved (Samarina et al., 2025), especially in cultivated species with narrow evolutionary divergence (Sayed et al., 2023).

Table 3. Maximum composite likelihood estimate of the pattern of nucleotide substitution

	A	T	C	G
A	-	9.33	6.72	12.33
T	8.77	-	3.6	7.51
C	8.77	5	-	7.51
G	14.41	9.33	6.72	-

The GC content (37.133%) observed in the trnH–psbA sequences, as presented in **Table 2** reflects the typical AT-rich nature of non-coding chloroplast spacers in angiosperms. High AT content is a common feature of intergenic regions such as trnH–psbA (Ho et al., 2023; Kassem, 2025), which can influence the overall substitution patterns and structural stability of chloroplast DNA (Dong et al., 2012).

As shown in Table 4, the low levels of genetic divergence observed among accessions further corroborate the limited genetic variation within the sample set. As shown in **Table 2,** the nucleotide diversity (π = 0.096%) was low, indicating minimal average pairwise differences among sequences. Nucleotide diversity values in trnH–psbA often show a wide range depending on taxonomic level and population structure (Urtgam et al., 2026), with higher values typically reported across interspecific comparisons but lower values within single species or closely related accessions (Sha et al., 2017).

The number of haplotypes (h = 3) and haplotype diversity (Hd = 0.195), as shown in **Table 2**, suggest that trnH–psbA haplotypes are not highly diversified among the South Sumatran rice accessions. These results are consistent with the general pattern of limited maternal lineage divergence in chloroplast regions, which is expected in cultivated species with shared ancestry or strong selection for agronomic traits (Brock et al., 2022). Similar patterns of limited chloroplast haplotype diversity have been reported in other studies where trnH–psbA yielded few haplotypes and low overall diversity within species (Urtgam et al., 2026). These findings highlight the usefulness of trnH–psbA for evaluating broad genetic relationships among accessions, while also emphasizing its limited resolution for detecting fine-scale intraspecific diversity in *Oryza sativa*.

3.3 Haplotype network analysis of trnH-psbA region in South Sumatran rice accessions

The median-joining network analysis of the chloroplastic trnH-psbA intergenic spacer reveals a distinct level of maternal genetic structuring among South Sumatran rice accessions is presented in **Figure 2**. The presence of three haplotypes (Hap_1, Hap_2, and Hap_3) suggests that while the germplasm shares a common evolutionary origin, specific mutational events have occurred over time. Hap_2 serves as the predominant ancestral hub, encompassing accessions from all surveyed locations, including Ogan Ilir, Muara Enim, and Jawa Barat. This wide distribution of a single haplotype across diverse geographical areas indicates a high rate of historical gene flow and seed exchange among farmers, a phenomenon frequently observed in Indonesian landraces where local wisdom and trade facilitate the movement of germplasm (Terryana et al., 2022; Thomson et al., 2009).

In contrast, Hap_1 and Hap_3 are separated from the ancestral Hap_2 by at least two mutational steps, suggesting a degree of

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genetic divergence within the Ogan Ilir population. The emergence of these satellite haplotypes exclusively in Ogan Ilir points to a potential micro-evolutionary refugium or a center of diversity for specific local cultivars. The use of the trnH-psbA region is particularly effective here, as its rapid evolutionary rate compared to other chloroplast genes allows for the detection of subtle variations in closely related rice accessions (Song et al., 2017). Furthermore, the integration of accessions from Jawa Barat into the central Hap_2 clade underscores the lack of significant phylogeographic barriers between Sumatra and Java, likely driven by anthropogenic movement rather than natural dispersal. Such high genetic connectivity is a critical consideration for in-situ conservation strategies, as it highlights the need to preserve specific regions (Imanbayeva et al., 2024; Kardos et al., 2021), like Ogan Ilir that harbor unique maternal lineages not found in other populations.

3.4 Heatmap cluster analysis of South Sumatran rice accessions

The clustered heatmap analysis reveals a high level of genetic uniformity among most South Sumatran rice accessions, reflected by near-zero genetic distances within a dominant cluster, as shown in Figure 3. This strong conservation in the trnH–psbA region is consistent with the uniparental inheritance and low mutation rate of chloroplast DNA, which makes it suitable for tracing maternal lineages and long-term evolutionary patterns (Li et al., 2025). In contrast, Bone 1 and Pegagan 2 appear as clear genetic outliers, forming a separate branch in the dendrogram and indicating distinct maternal lineages. Overall, the high similarity among the remaining accessions supports the influence of extensive farmer-mediated seed exchange across Sumatra, which has contributed to the homogenization of the maternal genetic structure among local rice populations.

3.5 Phylogenetic relationships

Comparative phylogenetic analysis using the Neighbor-Joining (NJ) and UPGMA methods with 1.000 bootstrap replicates revealed differences in the inferred genetic position of the Pegagan 2 accession. As shown in Figures 4A and 4B, the NJ and UPGMA trees are presented, respectively. In the NJ tree, Pegagan 2 displays a relatively long branch length, suggesting a higher level of nucleotide divergence in the trnH–psbA region compared with other local varieties. In contrast, the UPGMA dendrogram, which assumes a constant evolutionary rate, groups Pegagan 2 within a sub-cluster together with others accessions, supported by an 81% bootstrap value.

Despite this difference, both methods consistently separate the wild rice relatives (*Oryza rufipogon* and *Oryza punctata*) into a distinct clade with strong bootstrap support (100%). The limited chloroplast variation observed among the South Sumatran rice accessions may indicate a shared maternal ancestry resulting from rice domestication. This pattern is consistent with the predominantly maternal inheritance of chloroplast genomes and suggests that chloroplast markers provide insight into only part of the evolutionary history of cultivated rice.

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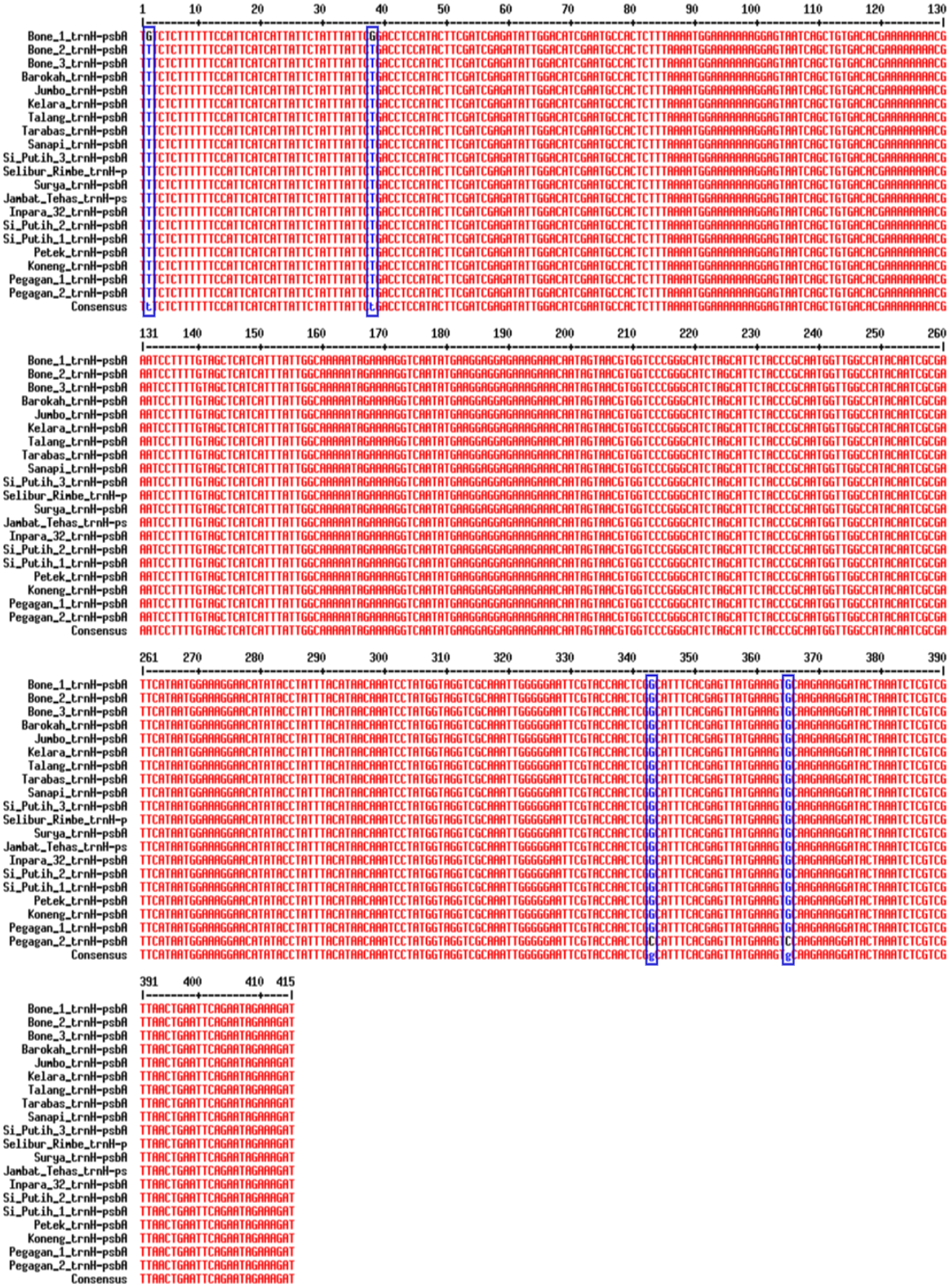

Figure 1. Multiple sequence alignment of the trnH–psbA region of South Sumatran rice accessions showing mutational events (indicated by filled rectangles)

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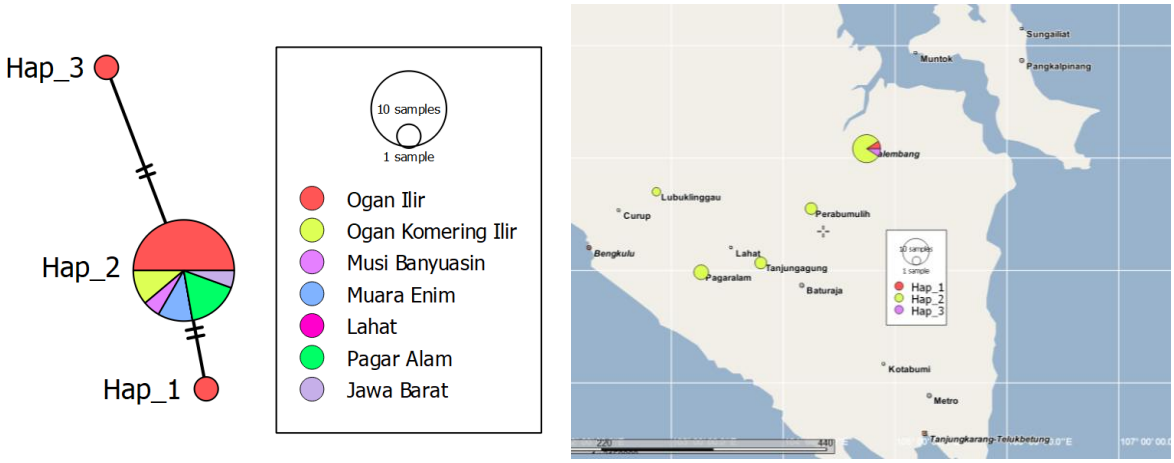

Figure 2. TCS haplotype network of the trnH–psbA region of South Sumatran rice accessions

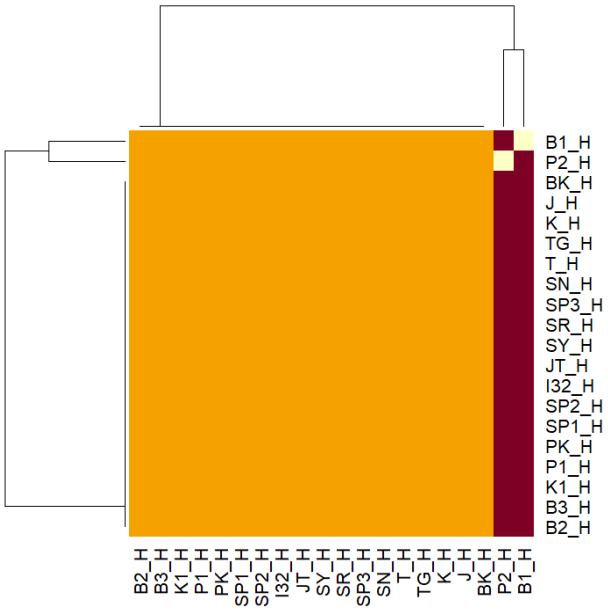

Figure 3. Heatmap analysis of trnH–psbA sequence variation among South Sumatran rice (*Oryza sativa* L.) accessions, Indonesia

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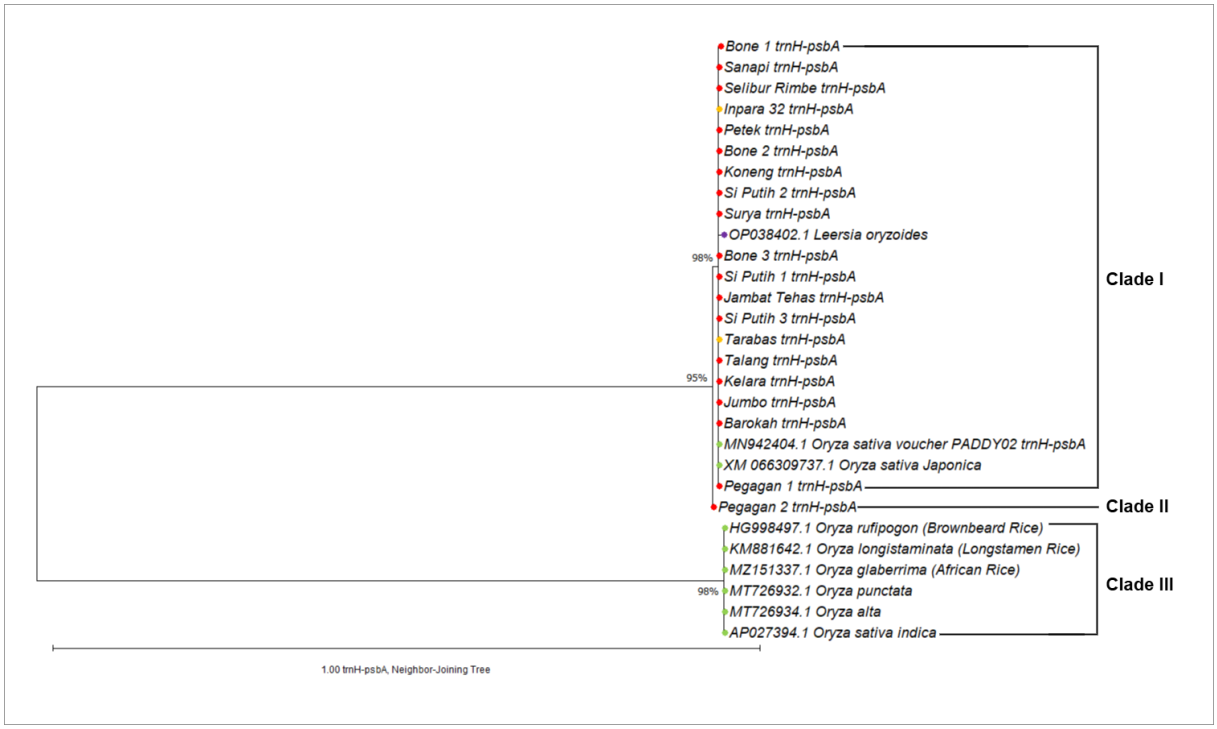
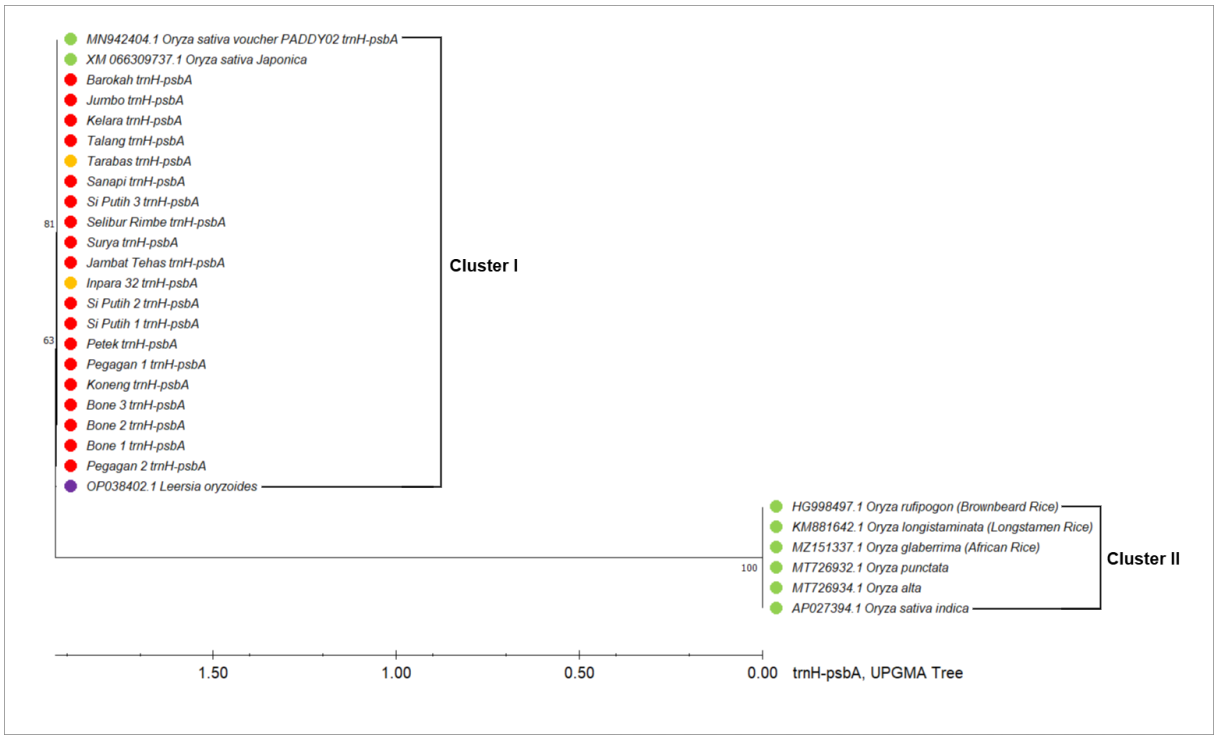


(A)



(B)

Figure 4. Genetic relationships among South Sumatran rice (*Oryza sativa* L.) accessions inferred from trnH–psbA sequences using the (A) Neighbor-joining, (B) UPGMA method with 1.000 bootstrap replicates

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Table 4. Genetic divergence of trnH–psbA sequences among South Sumatran rice accessions, Indonesia

	Code	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
Bone 1	A		0.005	0.005	0.005	0.005	0.019	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Bone 2	B	0.092		0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bone 3	C	**0.107**	1.000		0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Koneng	D	0.086	1.000	1.000		0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pegagan 1	E	0.087	1.000	1.000	1.000		0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pegagan 2	F	0.029	0.076	0.097	0.085	0.104		0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Petek	G	0.093	1.000	1.000	1.000	1.000	0.095		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Si Putih 1	H	0.078	1.000	1.000	1.000	1.000	0.097	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Si Putih 2	I	0.085	1.000	1.000	1.000	1.000	0.096	1.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Inpara 32	J	0.094	1.000	1.000	1.000	1.000	0.081	1.000	1.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Jambat Tehas	K	0.093	1.000	1.000	1.000	1.000	0.092	1.000	1.000	1.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Surya	L	0.099	1.000	1.000	1.000	1.000	0.092	1.000	1.000	1.000	1.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Selibur Rimbe	M	**0.106**	1.000	1.000	1.000	1.000	0.078	1.000	1.000	1.000	1.000	1.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000
Si Putih 3	N	0.078	1.000	1.000	1.000	1.000	0.081	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000
Sanapi	O	0.072	1.000	1.000	1.000	1.000	**0.104**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.000	0.000	0.000	0.000	0.000
Tarabas	P	0.097	1.000	1.000	1.000	1.000	0.092	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.000	0.000	0.000	0.000
Talang	Q	0.092	1.000	1.000	1.000	1.000	0.083	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.000	0.000	0.000
Kelara	R	0.089	1.000	1.000	1.000	1.000	0.099	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.000	0.000
Jumbo	S	0.086	1.000	1.000	1.000	1.000	**0.114**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.000
Barokah	T	0.070	1.000	1.000	1.000	1.000	0.084	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

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4. Limitations and Future Directions

This study was limited by the relatively small number of rice accessions, restricted geographic sampling within South Sumatra, and the use of a single chloroplast marker (trnH–psbA), which may not fully capture the genetic diversity of the germplasm. Because chloroplast DNA is maternally inherited, it cannot detect biparental genetic variation or fully resolve population structure. Future studies should include larger sample sizes, broader geographic coverage, and additional nuclear or genome-wide markers and phenotypic traits to provide a more comprehensive understanding of genetic diversity and phylogenetic relationships for rice germplasm conservation and breeding.

5. Conclusion

Although the trnH–psbA chloroplast region is useful for assessing maternal diversity, additional nuclear markers are required for a more comprehensive evaluation of overall genetic variation. The trnH–psbA chloroplast region revealed limited maternal genetic variation among South Sumatran rice accessions while identifying Bone 1 and Pegagan 2 as distinct maternal lineages. These findings provide preliminary insights into the maternal genetic structure of South Sumatran rice germplasm and establish a foundation for future germplasm characterization, conservation, and breeding studies.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author(s) used ChatGPT to proofread. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Authorship Contribution Statement

Fikri Adriansyah: Conceptualization, Methodology, Data analysis, and Manuscript drafting (100% contribution); Rujito Agus Suwignyo: Data analysis and Manuscript drafting (50% contribution); Andi Wijaya: Data analysis and Manuscript drafting (50% contribution); Upit Sarimana: Data analysis and Manuscript drafting (50% contribution); Imam Wibisiono: Review & Editing (30% contribution); Nabilah Amiros: Review & Editing (30% contribution).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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