

**MOLECULAR AND MORPHOLOGICAL
CHARACTERIZATION OF INDONESIAN FOXTAIL MILLET
(*Setaria italica*) GENOTYPES RELATED TO FEEDSTOCK
QUALITY IMPROVEMENT**

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**PLANT BREEDING AND BIOTECHNOLOGY STUDY PROGRAM
FACULTY OF AGRICULTURE
IPB UNIVERSITY
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RINGKASAN

M REZA PAHLEVI. Karakterisasi Molekuler dan Morfologi Genotipe Hotong (*Setaria italica*) Indonesia untuk Peningkatan Kualitas *Feedstock*. Dibimbing oleh SINTHO WAHYUNING ARDIE dan DAISUKE TSUGAMA.

Hotong memiliki potensi besar sebagai sumber bahan baku (*feedstock*), baik sebagai bahan bakar hayati berkelanjutan maupun pakan ternak. Walaupun belum dimanfaatkan secara luas, penelitian mengenai pemanfaatan hotong sebagai *feedstock* semakin meningkat. Studi pada level molekuler, baik genomik maupun transkriptomik, juga menyediakan informasi pada basis data publik yang dapat dimanfaatkan dalam pengembangan hotong sebagai *feedstock*.

Kandungan lignin merupakan karakter penting yang terkait langsung dengan kualitas *feedstock*. Rendahnya kandungan lignin dapat meningkatkan pencernaan pakan dalam sistem pencernaan hewan dan efisiensi hidrolisis enzimatis dalam produksi *biofuel*. Sebaliknya, kandungan lignin yang tinggi, bersama dengan kandungan selulosa yang tinggi, dapat meningkatkan ketahanan tanaman terhadap rebah. Perakitan varietas unggul hotong dengan kandungan lignin yang sesuai untuk *feedstock* dapat dicapai melalui penerapan bioteknologi dalam pemuliaan tanaman. Oleh karena itu, penelitian ini bertujuan untuk mengidentifikasi variasi genotipik pada salah satu gen kunci biosintesis lignin pada hotong, *Si4CL1* (*Setaria italica* 4-*coumarate-CoA ligase-1*), dan memperoleh variasi fenotipik pada genotipe hotong Indonesia terkait pemanfaatannya sebagai *feedstock*.

Penelitian ini terdiri atas dua percobaan. Percobaan pertama dilakukan pada Juli 2024 hingga Januari 2025 di Laboratorium Biologi Molekuler Tanaman 2, Departemen Agronomi dan Hortikultura, IPB, serta di Laboratorium Genomik Sumber Daya Tumbuhan, Asian Research Center for Bioresource and Environmental Sciences (ARC-BRES), Graduate School of Agricultural and Life Sciences, Tanashi Campus, The University of Tokyo. Analisis yang dilakukan mencakup analisis varian gen *Si4CL1* menggunakan data *short read archive* (SRA) pada basis data publik *whole genome sequencing* (WGS) dan *RNA sequencing* (RNA-seq), serta karakterisasi gen *Si4CL1* pada genotipe hotong Indonesia. Prediksi tingkat dampak putatif, serta efek varian terhadap pola *splicing* dan struktur protein dilakukan secara *in silico* menggunakan snpEff, prediktor situs *splicing*, dan Phyre2. Analisis *in silico* berhasil mengidentifikasi sebanyak 50 varian dari WGS dan 1.359 varian dari RNA-seq pada gen *Si4CL1*. Karakterisasi gen *Si4CL1* pada genotipe hotong Indonesia mengungkapkan adanya 17 varian berupa SNP dan INDEL yang terletak pada wilayah intronik, yang tidak terdapat pada set data SRA. Meskipun terdapat variasi sekuens *Si4CL1* antar genotipe hotong Indonesia, struktur protein yang diprediksi menunjukkan hanya sedikit perubahan, yang mengindikasikan adanya konservasi mekanisme fungsional pada protein Si4CL1.

Percobaan kedua dilakukan pada September 2024 hingga Januari 2025 di Kebun Percobaan Leuwikopo dan Laboratorium Pascapanen, Departemen Agronomi dan Hortikultura, Fakultas Pertanian, serta di Laboratorium Ilmu dan Teknologi Pakan, Fakultas Peternakan, IPB. Percobaan disusun dalam rancangan kelompok lengkap teracak (RKL) dengan delapan genotipe hotong sebagai faktor tunggal dan tiga ulangan. Hasil percobaan menunjukkan bahwa persentase *acid*



detergent fiber (ADF), kandungan selulosa, kandungan lignin, jumlah daun, dan tinggi tanaman merupakan karakter yang berpotensi menjadi kriteria seleksi dalam perakitan varietas unggul hotong sebagai *feedstock*. Karakter-karakter tersebut menunjukkan heritabilitas arti luas yang tinggi serta nilai koefisien keragaman genetik dan fenotipik (KKG dan KKF) yang sedang hingga tinggi, dengan selisih yang rendah antara keduanya, mengindikasikan kontrol genetik yang lebih kuat dibandingkan pengaruh lingkungan dalam menentukan fenotipe. Korelasi yang signifikan antar sejumlah karakter juga dapat mendukung strategi seleksi multi karakter dalam pemuliaan hotong sebagai *feedstock*. Hasil analisis kluster (*heatmap*) dan analisis komponen utama (PCA) menunjukkan adanya keragaman genetik antar genotipe hotong Indonesia, yang memungkinkan seleksi genotipe secara efektif berdasarkan karakter-karakter yang relevan dengan kualitas *feedstock*. Genotipe Hambapraing, Mauliru2, dan ICERI7 terpilih sebagai kandidat tetua yang menjanjikan, dengan kontribusi sifat-sifat unggul untuk program pemuliaan hotong yang ditujukan untuk peningkatan kualitas *feedstock*.

Hasil penelitian ini menunjukkan bahwa walaupun terdapat variasi kandungan lignin pada delapan genotipe hotong Indonesia, analisis *in silico* pada gen *Si4CLI* pada kedelapan genotipe tersebut mengindikasikan fungsi protein yang terkonservasi. Hal ini menunjukkan bahwa variasi kandungan lignin pada kedelapan genotipe hotong Indonesia tidak disebabkan oleh variasi pada daerah ekson dan intron gen *Si4CLI*.

Kata kunci: genotipe, *in silico*, keragaman, kriteria seleksi, lignin.

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SUMMARY

M REZA PAHLEVI. Molecular and Morphological Characterization of Indonesian Foxtail Millet (*Setaria Italica*) Genotypes Related to Feedstock Quality Improvement. Supervised by SINTHO WAHYUNING ARDIE and DAISUKE TSUGAMA.

Foxtail millet has enormous potential as a feedstock source for sustainable biofuel and animal feed. Research on the use of foxtail millet as a feedstock is growing, despite its limited utility. Information on a public database that can be utilized for foxtail millet enhancement as feedstock is also provided by molecular-level research, whether it be transcriptomic or genomic.

Lignin content is a critical trait that is directly related to feedstock quality. The lower the lignin content, the easier the feedstock is for being digested in animal digestive systems and the more efficient the enzymatic hydrolysis during biofuel production. The higher lignin content, along with the higher cellulose content, results in a plant that is more resistant to lodging. Foxtail millet variety development with lignin content depending on the intended application can be achieved either through biotechnology implementation or conventional plant breeding. Therefore, this research aims to identify genotypic variation in one of the rate-limiting enzymes in foxtail millet lignin biosynthesis, *Si4CL1* (*Setaria italica* 4-coumarate-CoA ligase-1), and obtain phenotypic variation in Indonesian foxtail millet genotypes related to their utilization as feedstock.

This study consisted of two experiments. The first experiment was conducted from July 2024 to January 2025 in the Plant Molecular Biology 2 Laboratory, Department of Agronomy and Horticulture, IPB University, and Lab of Genomics of Plant Resources, Asian Research Center for Bioresource and Environmental Sciences (ARC-BRES), Graduate School of Agricultural and Life Sciences, Tanashi Campus, The University of Tokyo. The analysis consisted of variant analysis of the *Si4CL1* gene utilizing whole genome sequence (WGS) and RNA sequencing (RNA-seq) datasets obtained from the public database and the *Si4CL1* gene in Indonesian foxtail millet genotypes. Potential putative impact level and effect of the variant on the splicing pattern and protein structure were also predicted using *in silico* approaches utilizing snpEff, splice site predictors, and Phyre2. *In silico* analysis of the *Si4CL1* gene using SRA datasets from WGS and RNA sequence successfully identified a total of 50 base variations from WGS and 1,359 variations from RNA-seq. Characterization of the *Si4CL1* gene in Indonesian foxtail millet genotypes revealed 17 unique SNPs and INDEL variants located within the intronic region. These variants may reflect geographical adaptation and evolutionary divergence of the *Si4CL1* gene within Indonesian populations. Despite the observed sequence variation among genotypes, the predicted protein structure exhibited only minor alterations, suggesting a conserved functional mechanism of the *Si4CL1* protein.

The second experiment was conducted from September 2024 to January 2025 in Leuwikopo Experimental Field and Post Harvest Laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, and the Feed Science and Technology Laboratory of the Faculty of Animal Science in IPB University. The experiment was arranged using a randomized complete block design with



genotypes as a single factor with three replications. The results indicated that acid detergent fiber (ADF) percentage, cellulose content, lignin content, number of leaves, and plant height have high potential for selection criteria for foxtail millet superior variety development for feedstock. These traits exhibited sufficient broad-sense heritability, along with moderate to high genotypic and phenotypic coefficients of variation (GCV and PCV), with a low difference between them, indicating strong genetic influence determining phenotype. The significant intercorrelations among these traits further support their integration into multi-trait selection strategies. Heatmap clustering and principal component analysis (PCA) showed the presence of genotypic variability, allowing for effective genotype selection based on traits relevant to feedstock quality. Among the genotypes evaluated, Hambapraing, Mauliru2, and ICERI7 emerged as promising parental candidates, offering favorable attributes for incorporating into breeding programs to improve feedstock quality.

This study demonstrated that although there were lignin content variations among eight Indonesian foxtail millet genotypes, the *in silico* analysis of the *Si4CL1* gene on those eight genotypes indicated conserved protein function. Therefore, lignin variations on those eight Indonesian foxtail millet genotypes were caused by neither exonic nor intronic nucleotide base variation in the *Si4CL1* gene.

Keywords: genotypes, *in silico*, lignin, selection criteria, variations

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MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF INDONESIAN FOXTAIL MILLET (*Setaria italica*) GENOTYPES RELATED TO FEEDSTOCK QUALITY IMPROVEMENT

M REZA PAHLEVI

Thesis
as part of the requirements to obtain a degree of
Magister Sains in
Plant Breeding and Biotechnology Study Program

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PREFACE

Praise to Allah SWT for His countless blessings, which enabled the completion of this thesis entitled “Molecular and Morphological Characterization of Indonesian Foxtail Millet (*Setaria Italica*) Genotypes Related to Feedstock Quality Improvement”. This research was conducted from March 2024 to February 2025. The author expresses gratitude to:

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Hopefully, this thesis will be useful for plant breeding and biotechnology development and all related fields.

Bogor, July 2025

M Reza Pahlevi

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