



**PLANT BREEDING AND BIOTECHNOLOGY STUDY PROGRAM
FACULTY OF AGRICULTURE
IPB UNIVERSITY
BOGOR
2025**



**THE MORPHOLOGY AND MOLECULAR STUDIES OF
INDONESIAN FOXTAIL MILLET (*Setaria italica* [L.] P. Beauv.)
ACCESSIONS RELATED TO STEM LODGING RESISTANCE**

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RINGKASAN

SUWASDI. Studi Morfologi dan Molekuler Akses Hotong Indonesia (*Setaria italica* [L.] P. Beauv) terkait Sifat Ketahanan Cekaman Rebah Batang. Dibimbing oleh SINTHO WAHYUNING ARDIE dan WILLY BAYUARDI SUWARNO.

Tanaman hotong merupakan tanaman sereal potensial dengan potensi hasil tertinggi kedua pada kelompok *millets*. Salah satu faktor pembatas budidaya tanaman hotong adalah cekaman abiotik. Cekaman rebah batang merupakan kelompok cekaman abiotik yang cukup sulit untuk diprediksi dan diukur di lapangan budidaya. Cekaman rebah batang dipengaruhi oleh kompleks cekaman seperti angin, kekeringan, curah hujan ekstrem, dan faktor genetik tanaman. Cekaman rebah batang pada tanaman hotong telah dilaporkan berpengaruh terhadap penurunan potensi hasil. Salah satu upaya untuk meminimalisasi kerugian akibat cekaman rebah batang adalah identifikasi akses yang memiliki idiotipe ideal terkait ketahanan terhadap cekaman rebah batang. Karakter-karakter kunci yang terkait berupa karakter morfologi, biokimia, maupun asosiasi penanda genetik. Idiotipe ideal yang diinginkan adalah tanaman yang memiliki kekuatan batang dan hasil yang tinggi, serta memiliki tingkat kereahan batang yang rendah. Tujuan dari penelitian ini adalah mengidentifikasi dan memperoleh akses hotong yang memiliki ketahanan tinggi terhadap cekaman rebah batang.

Penelitian ini terdiri atas dua percobaan. Percobaan pertama dilakukan pada bulan Maret hingga September 2023 di Kebun Percobaan Cikabayan Bawah, Departemen Agronomi dan Hortikultura, Fakultas Pertanian, IPB University. Percobaan pertama bertujuan untuk mengevaluasi sifat ketahanan terhadap cekaman rebah batang dari lima akses hotong Indonesia dan mengidentifikasi sifat penting yang mempengaruhinya. Percobaan disusun berdasarkan *split plot* rancangan kelompok lengkap teracak (RKLT) dengan empat ulangan. Petak utama percobaan adalah tipe cekaman rebah batang yang terdiri atas tanpa cekaman rebah, tipe cekaman rebah batang temporal (24 jam pada 10 HSA), dan tipe cekaman rebah batang permanen (10 HSA sampai panen). Anak petak percobaan adalah lima akses hotong yang terdiri atas Botok-10, Botok-4, NTB-1, Mauliru-2, dan Hambapraing. Pengamatan dilakukan pada tiga kelas karakter utama yaitu karakter malai, karakter kekuatan batang, dan karakter kandungan selulosa-lignin batang. Analisis yang digunakan antara lain indeks toleransi, korelasi Pearson, dan analisis dendrogram. Hasil penelitian menunjukkan bahwa akses Botok-10 dan Botok-4 memiliki tingkat ketahanan yang lebih tinggi terhadap rebah batang dibandingkan akses lain dengan idiotipe tinggi tanaman, kekuatan mekanik batang, kandungan selulosa, dan *yield index* yang lebih tinggi dibandingkan akses NTB-1, Mauliru-2, dan Hambapraing. Akses Botok-10 dan Botok-4 diduga memiliki daya adaptasi dan ketahanan yang lebih baik terhadap cekaman rebah batang berdasarkan indeks TOL, SSI, STI, SSPI dan YI. Analisis korelasi Pearson menunjukkan indeks cekaman rebah batang berkorelasi negatif signifikan dengan kekuatan patah batang, kekuatan mekanik batang, dan kandungan selulosa. Hasil analisis dendrogram membagi kelima akses ke dalam kelompok tahan (Botok-10 dan Botok-4) dan kelompok rentan (NTB-1, Mauliru, dan Hambapraing) berdasarkan nilai indeks TOL dan persentase penurunan hasil.



Percobaan kedua dilakukan pada September 2023 hingga Februari 2024 di Laboratorium Bioteknologi Molekuler Tanaman (PMB 2), Departemen Agronomi dan Hortikultura, Fakultas Pertanian, IPB University. Tujuan percobaan adalah karakterisasi gen penyandi biosintesis selulosa dan lignin pada sembilan aksesi tanaman hotong. Materi genetik yang digunakan adalah aksesi Botok-10, Botok-4, NTB-1, Mauliru-2, Hambapraing, ICERI-5, ICERI-6, Buru, dan Toraja. Karakterisasi dilakukan pada gen *SiCesA4* (4.775 pb) yang diamplifikasi dalam tujuh fragmen dan gen *SiCCR1* (2.447 pb) yang diamplifikasi dalam dua fragmen. Karakterisasi yang dilakukan meliputi tingkat similaritas gen *SiCesA4* dan *SiCCR1* pada sembilan aksesi hotong dengan dua aksesi referensi (IC-403579 dan Yugu1), karakterisasi variasi basa nukleotida untuk situs SNP, analisis pohon filogenik, dan prediksi domain terkonservasi yang terbentuk. Hasil karakterisasi gen *SiCesA4* menunjukkan tingkat similaritas sembilan aksesi hotong dengan dua aksesi referensi dengan nilai 100%, sedangkan pada spesies lain berkisar 70-86%. Sekuens gen *SiCesA4* yang disejajarkan dengan aksesi referensi tidak menunjukkan variasi basa nukleotida. Hanya empat dari tujuh fragmen gen *SiCesA4* yang berhasil dibaca sekuennya dengan metode Sanger pada penelitian ini. Oleh karena itu, diduga masih terdapat potensi variasi basa nukleotida pada fragmen yang belum berhasil diisolasi. Analisis pohon filogenik menunjukkan gen *SiCesA4* dari sembilan aksesi berada dalam satu kelompok yang sama dengan dua sekuens referensi. Tingkat kemiripan terdekat diperoleh pada gen *CesA4* dari gandum (*TaCesA4*) dan barley (*HvCesA4*). Prediksi domain terkonservasi gen *SiCesA4* menunjukkan domain terkonservasi *cellulose synthase* yang berada pada domain PLN02189 *superfamily*.

Karakterisasi gen *SiCCR1* pada sembilan aksesi hotong menunjukkan tingkat similaritas 98-99% dengan aksesi referensi dan 85-97% dengan spesies lain dalam famili *Poaceae*. Sekuens gen *SiCCR1* pada sembilan aksesi yang disejajarkan dengan aksesi referensi menunjukkan beberapa variasi basa nukleotida. Keragaman basa nukleotida (SNP) yang teridentifikasi antara urutan basa nukleotida gen *SiCCR1* dari sembilan aksesi hotong yaitu *synonymous* SNP di intron 1 (basa 40, 41, dan 42) dan intron 4 (posisi basa 1.398), serta *non-synonymous* SNP di ekson 6 (posisi basa 2.029). Variasi basa nukleotida pada posisi 2.029 merubah translasi asam amino valin menjadi leusin pada aksesi NTB-1. Analisis pohon filogenik menunjukkan gen *SiCCR1* dari sembilan aksesi hotong berada dalam satu kelompok yang sama dengan aksesi referensi, kecuali aksesi Botok-10. Hal ini diduga karena Botok-10 memiliki sekuens terpendek yang berhasil diisolasi dibandingkan dengan aksesi lainnya. Gen *SiCCR1* memiliki kedekatan dengan gen *CCR1* dari *Panicum virgatum*, *Panicum hallii*, *Zea mays*, dan *Sorghum bicolor*. Prediksi domain terkonservasi gen *SiCCR1* menunjukkan domain terkonservasi NADB Rossman *superfamily*. Domain NADB sering ditemukan pada berbagai protein yang terlibat dalam reaksi redoks dan berbagai enzim yang berperan dalam jalur metabolisme.

Kata kunci: kekuatan batang, kloning gen, lignin, selulosa



SUMMARY

SUWASDI. The Morphology and Molecular Studies of Indonesian Foxtail Millet (*Setaria italica* [L.] P. Beauv.) Accessions Related to Stem Lodging Resistance.
Supervised by SINTHO WAHYUNING ARDIE and WILLY BAYUARDI SUWARNO.

Foxtail millet is a potential cereal crop with the second-highest yield potential among millets. However, abiotic stress may lead to potential yield loss in foxtail millet production. Stem lodging is one of the abiotic stresses that is difficult to predict and measure in the field. Various stress factors such as wind, drought, extreme rainfall, and plant genetic factors determine the degree of stem lodging. Stem lodging in foxtail millet has been reported to decrease yield potential. One approach to minimizing losses due to stem lodging was identifying accessions with an ideal idioype related to resistance to stem lodging. Key characteristics associated with stem lodging include morphological traits, biochemical traits, or genetic marker associations. The ideal idioype was a plant with strong stems and high yield but a low tendency for stem lodging. The general objective of this study was to identify and obtain foxtail millet accessions resistant to stem lodging stress.

This study consisted of two experiments. The first experiment was conducted from March to September 2023 at the Cikabayan Bawah Experimental Farm, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. The objective of this experiment was to determine the lodging resistance of five Indonesian foxtail millet accessions and identify important traits determining the stem lodging resistance of those accessions. The experiment used a split-plot randomized complete block design with four replications. The main plot was the type of stem lodging stress, which included no lodging stress, temporal stem lodging stress (24 hours at 10 days after anthesis), and permanent stem lodging stress (10 days after anthesis until harvest). The subplot factor was five accessions of foxtail millet, namely Botok-10, Botok-4, NTB-1, Mauliru-2, and Hambapraing. Observations were made on three main character classes: panicle traits, stem strength, and cellulose-lignin content in stems. The analyses used included tolerance indices, Pearson correlation, and dendrograms. The results indicated that accessions Botok-10 and Botok-4 had higher resistance levels compared to other accessions, with an ideal idioype of plant height, mechanical stem strength, cellulose content, and yield index. Tolerance index analysis also suggested that Botok-10 and Botok-4 had higher adaptability and resistance to stem lodging based on TOL, SSI, STI, SSPI, and YI indices. The Pearson correlation analysis revealed a significant negative correlation between the stem lodging index and stem breaking strength, stem mechanical strength, and cellulose content. Dendrogram analysis classified the resistant group (Botok-10 and Botok-4) and the susceptible group (NTB-1, Mauliru, and Hambapraing) based on TOL indices and potential yield loss.

The second experiment was conducted from September 2023 to February 2024 at the Plant Molecular Biology Laboratory (PMB 2), Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. The aim was to characterize genes responsible for cellulose and lignin biosynthesis in nine Indonesian foxtail millet accessions. The genetic material used included accessions Botok-10, Botok-4, NTB-1, Mauliru-2, Hambapraing, ICERI-5, ICERI-6, Buru,



and Toraja. Characterization was carried out on the *SiCesA4* gene with a sequence length of 4,775 bp amplified in seven fragments and the *SiCCR1* gene with a sequence length of 2,447 bp amplified in two fragments. Characterization included similarity levels of the *SiCesA4* and *SiCCR1* genes among the nine foxtail millet accessions with two reference accessions (IC-403579 and Yugu1), nucleotide variation for SNP sites, phylogenetic tree analysis, and prediction of conserved domains. The characterization of the *SiCesA4* gene revealed 100% similarity between the nine foxtail millet accessions and the two reference accessions, whereas similarity with other species ranged from 70-86%. No nucleotide base variation was found in the *SiCesA4* gene sequences aligned with the reference accessions. Only four out of seven *SiCesA4* gene fragments were successfully sequenced using the Sanger method in this study. Thus, it is suspected that there may still be potential base variations in the fragments that were not successfully isolated. Phylogenetic tree analysis showed that the *SiCesA4* gene from the nine accessions clustered in the same group as the two reference sequences, with the closest similarity found to the *Ces4* gene from wheat (*TaCesA4*) and barley (*HvCesA4*). Prediction of conserved domains for the *SiCesA4* gene identified the cellulose synthase conserved domain within the PLN02189 superfamily.

Characterization of the *SiCCR1* gene in the nine foxtail millet accessions showed a 98-99% similarity with the reference accessions and 85-97% with other species in the *Poaceae* family. Alignment of *SiCCR1* gene sequences from the nine accessions with the reference accessions revealed several nucleotide base variations. Identified nucleotide base variations (SNPs) in the *SiCCR1* gene included synonymous SNPs in intron 1 (nucleotide 40, 41, and 42) and intron 4 (nucleotide 1,398), as well as non-synonymous SNPs in exon 6 (nucleotide 2,029). The nucleotide variation at position 2,029 changes the amino acid translation from valine to leucine in the NTB-1 accession. Phylogenetic tree analysis showed that the *SiCCR1* gene from the nine foxtail millet accessions clustered in the same group as the reference accessions, except for the Botok-10 accession. This is suspected to be due to Botok-10 having the shortest isolated sequence length compared to other accessions. The *SiCCR1* gene showed closeness to the *CCR1* genes of *Panicum virgatum*, *Panicum hallii*, *Zea mays*, and *Sorghum bicolor*. Prediction of conserved domains for the *SiCCR1* gene identified the NADB Rossman superfamily domain. The NADB domain is frequently found in various proteins involved in redox reactions and various enzymes participating in metabolic pathways.

Keywords: cellulose, gene cloning, lignin, stem strength



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SUWASDI

Thesis

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

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Praise God Almighty for all the blessings so that the author can complete this thesis entitled “The Morphology and Molecular Studies of Indonesian Foxtail Millet (*Setaria italica* [L.] P. Beauv.) Related to Stem Lodging Resistance”. This research was conducted from March 2023 to March 2024. This thesis was made to obtain a Magister Sains degree in the Plant Breeding and Biotechnology study program, Faculty of Agriculture, IPB University.

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Bogor, January 2025
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