

IDENTIFICATION AND CHARACTERIZATION OF GENES RELATED TO PLANT HEIGHT IN FOXTAIL MILLET (*Setaria italica* (L.) P. Beauv.)

SAFIRA AWALIYAH RAMADHANI



PLANT BREEDING AND BIOTECHNOLOGY STUDY PROGRAM
FACULTY OF AGRICULTURE
IPB UNIVERSITY
BOGOR
2026



@Hak cipta milik IPB University

Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.



STATEMENT ON SCIENTIFIC PAPER AND SOURCES OF INFORMATION AND COPYRIGHT TRANSFER

I declare that the thesis entitled “Identification and Characterization of Genes Related to Plant Height in Foxtail Millet (*Setaria italica* (L.) P. Beauv.)” is my work under the direction of the supervisory committee and has not been submitted in any form to any university. Sources of information originating from or quoted from published or unpublished works of other authors have been mentioned in the text and included in the Bibliography at the end of this thesis.

I hereby transfer the copyright from my writing to IPB University.

Bogor, August 2026

Safira Awaliyah Ramadhani
A2503241008

- Hak Cipta Dilindungi Undang-undang
1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
 2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.



@Hak cipta milik IPB University

Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.

RINGKASAN

SAFIRA AWALIYAH RAMADHANI. Identifikasi dan Karakterisasi Gen Terkait Tinggi Tanaman pada Hotong (*Setaria italica* (L.) P. Beauv.). Dibimbing oleh SINTHO WAHYUNING ARDIE dan WILLY BAYUARDI SUWARNO.

Hotong (*Setaria italica* (L.) P. Beauv.) merupakan tanaman pangan yang toleran terhadap perubahan iklim dan mampu beradaptasi pada berbagai kondisi cekaman lingkungan. Biji hotong memiliki kandungan nutrisi yang tinggi serta berbagai manfaat kesehatan, akan tetapi produktivitas hotong masih relatif rendah jika dibandingkan dengan tanaman pangan lainnya. Tinggi tanaman merupakan salah satu karakter agronomi yang menentukan produktivitas hotong. Tinggi tanaman dilaporkan memiliki korelasi positif dengan komponen hasil dan hasil gabah-biji, namun tanaman yang lebih tinggi cenderung lebih mudah rebah. Oleh karena itu, tinggi tanaman menjadi salah satu target utama dalam program pemuliaan hotong, dengan tujuan menghasilkan varietas dengan tinggi tanaman moderat yang memiliki ketahanan rebah yang lebih baik tanpa mengurangi potensi hasil.

Penelitian ini terdiri atas tiga percobaan. Percobaan pertama dilaksanakan pada Juli 2025 hingga Januari 2026 di Laboratorium Biologi Molekuler Tanaman 2, Departemen Agronomi dan Hortikultura, IPB. Analisis yang dilakukan meliputi asosiasi antara tinggi tanaman dan lokus SCoT, karakterisasi lokus SCoT yang berasosiasi dengan tinggi tanaman, serta analisis potensi *linkage* antara lokus SCoT dan gen yang berhubungan dengan tinggi tanaman. Hasil analisis asosiasi menunjukkan bahwa lokus SCoT23_800 berasosiasi secara signifikan dengan tinggi tanaman ($p = 0,0026$; $R^2 = 0,3588$). Karakterisasi terhadap lokus SCoT23_800 mengidentifikasi ORF4 yang berpotensi diterjemahkan menjadi mikroprotein dengan nilai probabilitas sebesar 71,89% serta memenuhi beberapa kriteria analisis *translation plausibility*. Analisis potensi *linkage* lebih lanjut menunjukkan bahwa lokus SCoT23_800 terletak berdekatan dengan gen homolog Peptidase S1 PA clan pada wilayah *linkage disequilibrium* (LD) *decay* di kromosom 2.

Percobaan kedua dilaksanakan secara paralel dengan percobaan pertama, yaitu pada periode Maret 2025 hingga April 2026. Percobaan ini bertujuan untuk mengidentifikasi gen atau *quantitative trait loci* (QTL) yang berhubungan dengan tinggi tanaman menggunakan metode *systematic literature review* (SLR). Penambahan data dilakukan pada basis data Scopus dan PubMed, sedangkan proses seleksi artikel mengikuti pedoman PRISMA 2020. Hasil proses identifikasi dan penyaringan menghasilkan 167 studi yang memenuhi kriteria inklusi dan digunakan dalam SLR. Metode yang digunakan dalam setiap studi kemudian divisualisasikan menggunakan diagram UpSet. Ekstraksi data dari seluruh artikel yang lolos tahap *full-text screening* mengidentifikasi enam kandidat gen yang dilaporkan pada sedikitnya dua studi. *OsGA20ox2* merupakan gen yang paling banyak diteliti ($n = 36$) pada padi dan diketahui memiliki ortolog, yaitu *SiGA20ox5*, pada hotong. Gen *SiGA20ox5* sebelumnya telah dikarakterisasi oleh Zhu *et al.* (2023) menggunakan metode *bulk segregant analysis* (BSA) dan penanda CAPS. Penelitian tersebut mengungkap adanya deleksi pada posisi 2.266 bp yang menyebabkan terbentuknya *premature stop codon*, sehingga menghasilkan fenotipe

kerdil pada varietas hotong 263A. Temuan tersebut menjadi dasar pemilihan *SiGA20ox5* sebagai gen kandidat yang dikaji lebih lanjut pada genotipe hotong lokal Indonesia dalam percobaan ketiga. Percobaan ketiga dilaksanakan dari Februari 2025 hingga April 2026 di Laboratorium Biologi Molekuler Tanaman 2, Departemen Agronomi dan Hortikultura, IPB. Percobaan ini bertujuan untuk mengarakterisasi gen *SiGA20ox5* yang teridentifikasi sebagai gen kandidat pada percobaan kedua. Analisis dilakukan melalui identifikasi *single nucleotide polymorphism* (SNP) pada genotipe hotong lokal Indonesia dengan menggunakan genom Yugul sebagai sekuens referensi. Hasil karakterisasi menunjukkan adanya dua SNP pada wilayah *coding sequence* (CDS) dan sepuluh SNP pada wilayah intron. Salah satu SNP pada wilayah CDS, yaitu pada posisi 626, ditemukan pada genotipe ICERI-5, ICERI-6, dan ICERI-7 serta menyebabkan mutasi *non-synonymous* yang mengubah asam amino Glu (*glutamic acid*) menjadi Asp (*aspartic acid*). Sementara itu, SNP pada posisi 749 merupakan mutasi *synonymous* sehingga tidak mengubah urutan asam amino. Analisis lebih lanjut menunjukkan bahwa substitusi asam amino pada posisi 626 tidak memengaruhi konformasi keseluruhan protein karena tidak berada pada wilayah *functional domain*. Perubahan tersebut kemudian divisualisasikan menggunakan pemodelan struktur protein tiga dimensi (3D).

Hasil penelitian ini menunjukkan bahwa terdapat kandidat ORF yang berpotensi untuk diterjemahkan menjadi mikroprotein, sehingga masih memerlukan validasi lebih lanjut melalui uji fungsional untuk memastikan apakah ORF4 benar-benar ditranslasikan dan berperan dalam mengendalikan tinggi tanaman. Selain itu, hasil karakterisasi gen *SiGA20ox5* menunjukkan bahwa variasi tinggi tanaman pada genotipe hotong lokal Indonesia tidak disebabkan oleh mutasi yang sama seperti yang dilaporkan pada varietas hotong 263A. Oleh karena itu, diperlukan penelitian lanjutan untuk memvalidasi apakah variasi tinggi tanaman pada genotipe hotong lokal Indonesia dipengaruhi oleh variasi basa pada CDS *SiGA20ox5* atau dikendalikan oleh variasi basa pada wilayah regulator gen tersebut.

Kata kunci: hotong, SNP, *systematic literature review*, tinggi tanaman

SUMMARY

SAFIRA AWALIYAH RAMADHANI. Identification and Characterization of Genes Related to Plant Height in Foxtail Millet (*Setaria italica* (L.) P. Beauv.). Supervised by SINTHO WAHYUNING ARDIE and WILLY BAYUARDI SUWARNO.

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is a climate-resilient cereal crop that is well adapted to a wide range of environmental stresses. It possesses high nutritional value and provides numerous health benefits through its grains. Plant height is one of the key agronomic traits influencing foxtail millet productivity. This trait has been reported to be positively correlated with yield components and grain yield. However, increased plant height is also associated with reduced lodging resistance, as taller plants are generally more susceptible to lodging. Therefore, plant height has become one of the primary targets in foxtail millet breeding programs, with the objective of developing moderately statured varieties that exhibit improved lodging resistance without compromising yield potential.

This study consisted of three experiments. The first experiment was conducted from July 2025 to January 2026 at Plant Molecular Biology Laboratory 2, Department of Agronomy and Horticulture, IPB University. The analyses included the association between plant height and SCoT loci, characterization of SCoT loci associated with plant height, and the analysis of the potential linkage between SCoT loci and genes related to plant height. The association analysis revealed that the SCoT23_800 locus was significantly associated with plant height ($p = 0.0026$; $R^2 = 0.3588$). Characterization of the SCoT23_800 locus identified ORF4, which has the potential to be translated into a microprotein with a predicted probability of 71.89% and fulfilled several criteria for translation plausibility. Furthermore, the potential linkage analysis indicated that the SCoT23_800 locus is located within the *linkage disequilibrium* (LD) decay region on chromosome 2, where homologs of the Peptidase S1 PA clan gene are present in both the upstream and downstream regions.

The second experiment was conducted in parallel with the first experiment, from March 2025 to April 2026. This experiment aimed to identify genes or *quantitative trait loci* (QTLs) associated with plant height using a *systematic literature review* (SLR) approach. Data mining was performed using the Scopus and PubMed databases, while article selection followed the PRISMA 2020 guidelines. The identification and screening process resulted in 167 studies that met the inclusion criteria and were included in the SLR. The methodologies employed across the included studies were subsequently visualized using an UpSet diagram. Data extraction from all studies that passed the *full-text screening* identified six candidate genes that were reported in at least two studies. Among these, *OsGA20ox2* was the most extensively investigated gene ($n = 36$) in rice and is known to have an ortholog, *SiGA20ox5*, in foxtail millet. The *SiGA20ox5* gene was previously characterized by Zhu *et al.* (2023) using *bulk segregant analysis* (BSA) and CAPS markers. Their study identified a deletion at the position of 2,266 bp that resulted in the formation of a *premature stop codon*, leading to a dwarf phenotype in the foxtail millet variety 263A. These findings provided the rationale for



selecting *SiGA20ox5* as the candidate gene for further characterization in Indonesian local foxtail millet genotypes in the third experiment. The third experiment was conducted from February 2025 to April 2026 at Plant Molecular Biology Laboratory 2, Department of Agronomy and Horticulture, IPB University. This experiment aimed to characterize the *SiGA20ox5* gene, which was identified as a candidate gene in the second experiment. The analysis was carried out by identifying *single-nucleotide polymorphisms* (SNPs) in Indonesian local foxtail millet genotypes using the Yugul genome as the reference sequence. The characterization identified two SNPs within the *coding sequence* (CDS) and ten SNPs within the intron regions. One SNP located at position 626 in the CDS was detected in the ICERI-5, ICERI-6, and ICERI-7 genotypes and resulted in a *non-synonymous* mutation that changed the amino acid Glu (glutamic acid) to Asp (aspartic acid). In contrast, the SNP at position 749 was a *synonymous* mutation and therefore did not alter the amino acid sequence. Further analysis indicated that the amino acid substitution at position 626 did not affect the overall protein conformation because it was located outside the *functional domain*. These structural differences were subsequently visualized using three-dimensional (3D) protein modeling.

The results of this study indicate the presence of a candidate ORF with potential to be translated into a microprotein, however, further validation through functional test is still required to determine whether ORF4 is indeed translated and plays a role in regulating plant height. In addition, the characterization of the *SiGA20ox5* gene revealed that variation in plant height among Indonesian local foxtail millet genotypes is not caused by the same mutation reported in the foxtail millet variety 263A. Therefore, further studies are needed to determine whether plant height variation in Indonesian local genotypes is influenced by base variation in the CDS of *SiGA20ox5* or caused by variations in the regulatory region of the gene.

Keywords: foxtail millet, plant height, SNP, systematic literature review



Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.

© Hak Cipta milik IPB, tahun 2026
Hak Cipta dilindungi Undang-Undang

Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan atau menyebutkan sumbernya. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik, atau tinjauan suatu masalah, dan pengutipan tersebut tidak merugikan kepentingan IPB.

Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apa pun tanpa izin IPB.



@Hak cipta milik IPB University

Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.

IDENTIFICATION AND CHARACTERIZATION OF GENES RELATED TO PLANT HEIGHT IN FOXTAIL MILLET (*Setaria italica* (L.) P. Beauv.)

SAFIRA AWALIYAH RAMADHANI

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

**PLANT BREEDING AND BIOTECHNOLOGY STUDY PROGRAM
FACULTY OF AGRICULTURE
IPB UNIVERSITY
BOGOR
2026**



@Hak cipta milik IPB University

Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.

Examiners on Thesis Examination:
1 Assoc. Prof. Daisuke Tsugama
2 Prof. Dr. Desta Wirnas, S.P., M.Si.



Thesis Title : Identification and Characterization of Genes Related to Plant Height in Foxtail Millet (*Setaria italica* (L.) P. Beauv.)
Name : Safira Awaliyah Ramadhani
Student ID : A2503241008

@Hak cipta milik IPB University

Approved by

Supervisor 1:
 Dr. Sintho Wahyuning Ardie, S.P., M.Si.

Supervisor 2:
 Dr. Willy Bayuardi Suwarno, S.P., M.Si.

Acknowledged

Head of Study Program:
 Prof. Dr. Desta Wirmas, S.P., M.Si.
 NIP 197012282000032001

Dean of the Faculty of Agriculture
 Prof. Dr. Ir. Suryo Wiyono, M.Sc. Agr.
 NIP 196902121992031003



Examination Date:
 10th July 2026

Graduation Date: 10 AUG 2026



@Hak cipta milik IPB University

Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.



PREFACE

Praise and gratitude are sincerely offered to Allah Subhanahu wa Ta'ala for His blessings, mercy, and guidance, which enabled the author to complete this thesis entitled “Identification and Characterization of Genes Related to Plant Height in Foxtail Millet (*Setaria italica* (L.) P. Beauv.)”. This research was conducted from February 2025 to April 2026.

The author would like to express sincere gratitude and appreciation to:

1. The Ministry of Finance of the Republic of Indonesia for providing scholarship and research funding through the Indonesia Endowment Fund for Education Agency (LPDP) during the author's study period (2024–2026)
2. Dr. Sintho Wahyuning Ardie, S.P., M.Si., and Dr. Willy Bayuardi Suwarno, S.P., M.Si., as the author's supervisors, and Assoc. Prof. Dr. Daisuke Tsugama, as thesis examiner, for their invaluable guidance, encouragement, constructive suggestions, and continuous support throughout this research and thesis preparation
3. Prof. Dr. Desta Wirnas, S.P., M.Si., Head of the Master's Program in Plant Breeding and Biotechnology, for her support and guidance during the author's study
4. The author's beloved parents, for their endless prayers, and support throughout the author's academic journey.
5. The laboratory technicians of the Plant Breeding and Molecular Biotechnology II Laboratory, especially Mr. Yudiansyah, S.Si., for his technical assistance and support during the research activities.
6. The author's best friends, Nida Yusrina and Muhammad Anthony Rizwandi, for their friendship, encouragement, and assistance throughout the research process
7. Ramadaniarto Rizqullah, M Reza Pahlevi, Marisa Vidya Luthfiany, and all members of the ASHIMA Research Group for their valuable support, cooperation, and the memorable experiences shared during this study
8. All admins and members of the IPB Book Party for the valuable moments and being my healing place

The author realizes that this thesis is not perfect and welcomes constructive suggestions for its improvement. It is hoped that this thesis will advance knowledge, particularly in plant breeding and molecular genetics.

Bogor, August 2026

Safira Awaliyah Ramadhani



TABLE OF CONTENT

LIST OF TABLES	xiii
LIST OF FIGURES	xiii
LIST OF APPENDICES	xiv
INTRODUCTION	1
1.1 Background	1
1.2 Objective	3
1.3 Research Hypothesis	3
1.4 Research Scope	3
LITERATURE REVIEW	5
2.1 Foxtail Millet (<i>Setaria italica</i> (L.) P. Beauv.)	5
2.2 Molecular Marker	6
2.3 Association Analysis	8
2.4 Systematic Literature Review	8
2.5 QTLs and Genes Related to Plant Height in Foxtail Millet	9
2.6 Gibberellin Biosynthesis	10
IDENTIFICATION AND CHARACTERIZATION OF SCoT23_800 LOCUS IN FOXTAIL MILLET	12
3.1 Abstract	12
3.2 Introduction	13
3.3 Research Method	13
3.4 Result and Discussion	16
3.5 Conclusion	24
IDENTIFICATION OF PLANT HEIGHT-RELATED GENES CANDIDATES THROUGH SYSTEMATIC LITERATURE REVIEW	25
4.1 Abstract	25
4.2 Introduction	26
4.3 Research Method	26
4.4 Result and Discussion	27
4.5 Conclusion	33
CHARACTERIZATION OF <i>SiGA20ox5</i> FROM INDONESIAN FOXTAIL MILLET GENOTYPES	34
5.1 Abstract	34
5.2 Introduction	35
5.3 Research Method	35
5.4 Result and Discussion	38
5.5 Conclusion	43
GENERAL DISCUSSION	44



VII	GENERAL CONCLUSION AND SUGGESTION	47
7.1	General Conclusion	47
7.2	General Suggestion	47
	REFERENCES	48
	APPENDICES	70
	BIBIOGRAPHY	59



LIST OF TABLES

1	Least squares mean of plant height of Indonesian foxtail millet genotypes and their Euclidean-based categorization	16
2	Characterization of SCoT23_800 locus	19
3	Translational initiation site motifs analysis for ORF4 of the SCoT23_800 locus	20
4	ORF integrity and codon usage bias in ORF4 of SCoT23_800 locus	21
5	Cross-study evidence for plant height candidate genes in rice and their foxtail millet homologs identified through Phytozome	32
6	Primers used for amplification of the <i>SiGA20ox5</i> gene	36
7	<i>SiGA20ox5</i> gene variant on chromosome 5 based on 1,844 foxtail millet genotypes in the SetariaDB database	41

LIST OF FIGURES

1	Research scope of identification and characterization of genes related to plant height in foxtail millet (<i>Setaria italica</i> (L.) P. Beauv.)	4
2	Gibberellic acid (GA) biosynthesis	11
3	QQ plot and Manhattan plot of SCoT marker associations for plant height	17
4	Re-amplification of the SCoT23_800 locus in (A) ten and (B) five foxtail millet genotypes	18
5	The position of ORF4 in chromosome 2	19
6	Predicted tertiary structure of ORF4 from the SCoT23_800 locus	22
7	ORF4 query to domain in BTB domain-containing protein (A0A4U6SY07)	23
8	Genomic position of the SCoT23_800 locus and neighboring genes on chromosome 2 based on the <i>Setaria italica</i> v2.2 genome from the Phytozome database	24
9	PRISMA 2020 flow diagram for article identification, screening, eligibility assessment, and study inclusion	28
10	UpSet diagram of methods included in SLR of research methods used for gene/QTL identification related to plant height in rice and foxtail millet	29
11	Forward and reverse primer positions at the <i>SiGA20ox5</i> gene	36
12	Visualization of the electrophoresis gels of four fragments of <i>SiGA20ox5</i>	38
13	Phylogenetic tree of <i>SiGA20ox5</i> in Indonesian foxtail millet and its homolog across representative species in the Poaceae family using the Neighbor-Joining (NJ) method	39
14	Protein structure of <i>SiGA20ox5</i> in Indonesian foxtail millet genotypes	42

15	Amino acid residue changes of (A) Botok4, NTB-1, Hambaprang, Mauliru-2, Buru, and Toraja genotypes, and (B) ICERI-5, ICERI-6, and ICERI-7 genotypes	43
16	Correlations among (A) plant height with yield, (B) plant height with lodging resistance, and (C) lodging resistance with yield	44
17	Chromosomal position of SCoT23_800 locus and genes related to plant height conducted from the SLR method	46

LIST OF APPENDICES

1	Sequence of the SCoT23_800 locus	71
2	Genes related to plant height validated by functional studies	72

