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Research Article

Expression of *TAD1 (Tillering & Dwarf1)* Gene in Hawara Bunar and IR64 Rice Cultivars

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ABSTRACT

Rice cv. Hawara Bunar is a local rice cultivar tolerant to aluminum (Al) and drought stress. However, the cultivar has inferior characteristics, such as a tall habitus and a small number of tillers, making the cultivar agronomically unattractive. Many genes control plant height and tiller number; one is the *TAD1* gene. Analysis of gene expression in two contrasting rice cultivars for both characters is a prerequisite for selecting certain genes for gene editing targets. This study aimed to analyze the gene expression of the *TAD1* in rice cv. IR64 and Hawara Bunar, and to construct a phylogenetic tree of genes that regulate rice plant height and tiller number. Gene expression analysis was conducted using the qRT-PCR technique, while the phylogenetic tree was constructed based on the Neighbor-Joining method using PAUP4 software. The results showed *TAD1* gene expression in the tillering phase of rice cv. Hawara Bunar is higher than the cv. IR64. The gene expression level in both cultivars corresponds to the plant height and tiller number characters in both rice cultivars. Phylogenetic analysis showed that the *TAD1* gene clustered with genes that cause the rice to have a tall habitus with few tillers. The results of a transcriptome meta-analysis reinforced the phylogenetic tree, which shows that the *TAD1* gene was found in a group of downregulated genes based on the volcano plots. Therefore, the *TAD1* gene can be selected as a target gene for editing in rice cv. Hawara Bunar to obtain superior characters.

Keywords: Gene expression, Hawara Bunar, IR64, Meta-analysis, Phylogenetics, RT-PCR, TAD1

Introduction

Rice (*Oryza sativa* L.) is one of the staple foods that fulfills 21% of the world's food needs [1]. Rice is the main food source in many countries worldwide, including Indonesia. The rice consumption of the population in Indonesia increases every year, especially during the COVID-19 pandemic, the average consumption per capita per week increased by 3.6% [2, 3]. The increasing consumption level correlates with the national rice demand, while the harvest area and rice production have decreased over the last five years. Land conversion is one of the causes of the decline in the harvest area, which reached 2.3% in 2021 [4]. The utilization of local rice cultivars that can tolerate non-optimal environmental conditions such as drought and critical soil conditions (marginal land) can increase the expansion of planting land and rice production in Indonesia. In 2018, the marginal land area in Indonesia was 14 million hectares [5]. Therefore, planting rice that can grow on marginal land can be an alternative solution to increase rice production in Indonesia.

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Hawara Bunar (HB) cultivar (cv.) is one of the local Tropical Japonica rice cultivars that have been widely studied regarding their tolerance to acid soil, aluminium (Al) stress, and drought [6, 7]. Based on those characteristics, it is known that rice cv. HB has an advantage intolerance to several abiotic stresses. However, the cultivar also has areas for improvement, such as plant height that reaches 150 cm, which is prone to lodging, and the low tiller number. The habitus character of rice cv. HB needs to be modified to be shorter to have an ideal architecture as a new type of rice character. Rice architecture is a major agronomic character that determines adaptability to the environment, harvest index, and yield potential [8].

The plant height and the number of tillers in rice are regulated by many genes (polygenic). The monoculm 1 (*MOC1*) gene is a member of the GRAS transcription factor (GAI, RGA, SCR) that regulates the number of tillers in rice. Degradation of *MOC1* protein interferes with the initiation of tiller buds, causing no-tiller formation, while the accumulation of *MOC1* protein causes more tiller formation. The *MOC1* gene may act as a regulator in controlling rice tillering and producing shorter rice [9]. The *TAD1/TE* gene directly regulates the degradation of *MOC1* protein in the cell cycle. Based on the *TAD1* roles, knock-out of the gene can be a solution to increase *MOC1* accumulation and produce a mutant with a shorter plant height and an ideal number of tillers. Besides the TAD1 gene, other genes regulate plant height in rice, i.e., the *D14*, *LAX1*, *LAX2*, *OSH1*, and *OSTB1* genes. D14 mutants show increased branching and decreased plant height [10]. *LAX1* and *LAX2* genes regulate axillary meristem formation and development [11]. The *OSH1* gene functions as a key meristem initiation and growth regulator, while the *OSTB1* gene regulates axillary bud growth [12]. When the expression of the *TAD1* gene in rice cv. HB is compared with its expression in rice cv. IR64, which has a short habitus and more tillers, is the role of the gene in rice cv. HB can be known, therefore it can be the basis for selecting the gene for editing to get an HB mutant with a shorter habitus and more tillers than the wild type.

The selected gene target is based on its relative expression level in rice cv. HB needs further analysis to compare its expression level with 15 other genes responsible for regulating the habitus and number of tillers rice through phylogenetic analysis and meta-analysis [10, 11]. Phylogenetic analysis is important to discover genes with the same expression profile as the gene will group in the same clade. Gene expression profiles can be compared among genes within a clade and gene expression profiles among clades. The expression levels of genes within a clade on the phylogenetic tree can be quantified using transcriptome meta-analysis [13, 14]. Meta-analysis is a quantitative study by processing data from previous studies using quantitative methods to obtain more comprehensive information [15, 16]. The results of the meta-analysis can be used to study the expression levels of genes that regulate the habitus and number of rice tillers. RNA-seq data related to genes controlling the rice plant height and tiller number are available and accessible in the NCBI database. Transcriptome data sets from plants used for meta-analysis can be generated from plant cells related to tissue origin and/or developmental stage. The transcriptome data from the developmental stage can be analyzed to obtain genes that dominantly regulate rice plant height and tiller number.

The objectives of the study were to analyze *TAD1* gene expression in rice cv. HB and IR64, to construct a phylogenetic tree of genes controlling plant height and tiller number in rice and to perform a transcriptome meta-analysis to quantify the level of expression of genes in the phylogenetic tree clade. A combination of gene expression, phylogenetic, meta-analyses data, and rice architecture characters can be used to identify potential gene candidates for genetic improvement of rice cv. HB. Knowledge of the relative gene expression in rice cv. HB is important for its application in rice genetic engineering, especially gene editing.

Material and Methods

Plant materials

Rice seeds for cv. HB and IR64 obtained from a collection of the Plant Physiology and Genetics Laboratory, Department of Biology IPB were used in the experiment. Gene expression analysis was conducted in the Plant Physiology and Genetics Laboratory and the Integrated Laboratory of the Department of Biology, IPB University.

Planting rice cv. Hawara Bunar and cv. IR64

Rice seeds cv. HB and IR64 were germinated and then planted on soil media. Fertilization of rice using urea at 14 and 21 days after planting (DAP), NPK at 42 DAP, and KCl at 70 DAP and 84 DAP. Each fertilizer was given as much as 10 g per pot. We used five pots per sample and three replications in the pre-tillering and tillering phases, respectively. We performed a Randomized Complete Block Design (RCBD) in this study. Plant watering to field capacity was done every three days. Leaf sampling was carried out at the pre-tillering phase (phase of tiller formation initiation) at 30 DAP and the tillering phase (phase of having tillers) at 50 DAP. The observed variables included plant height, number of tillers, number of nodes, number of productive tillers, the total number of grains per panicle, and panicle branching pattern.

Isolation of total RNA

Total RNA was isolated from young rice leaves using the Trizol method [17], particularly in rice cv. HB and IR64, respectively. RNA concentration was measured quantitatively using a spectrophotometer (MestroNano Pro, Taiwan) [18]. RNA quality was observed using 1% agarose gel electrophoresis in 1x TAE buffer at 100 volts for 30 min.

cDNA synthesis

Synthesis of cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific®, USA) following the protocol of the kit. A 5µg total RNA was added with 4 µl 5x reaction buffer, 1 µl ribolock RNase Inhibitor (20 U/µl), 2 µl 10 mM dNTP Mix, 1 µl oligo-DT18 primer (20 pmol) and 1 µl RivertAid M-Mul V reverse transcriptase (200 U/µl). The total reaction volume used was 10 µl. The mixture was incubated at 42℃ for 60 min, followed by reaction termination by heating at 70℃ for 5 min. cDNA obtained was then measured for concentration and purity using a spectrophotometer (MestroNano Pro, Taiwan). The quality of cDNA was observed using 1% agarose gel electrophoresis in 1x TAE buffer at a voltage of 100 for 30 min.

qRT-PCR

Gene expression was qPCR analyzed using the Sensi FAST™ SYBR® Hi-ROX kit (Bioline, USA) on a Quant Studio 5 instrument (Applied Biosystem, USA). DNA amplification uses primers designed based on the DNA sequences of the *TAD1* gene (gene of interest) and actin gene (housekeeping gene) (Table 1). The experiment was conducted with three biological replicates and two technical replicates in both cultivars. The amplification process (DNA thread doubling process) starts with a pre-denaturation process for 5 min at 95℃, followed by 30 cycles of denaturation at 95℃ for 10 sec, then an annealing 60℃ for 10 sec, and extension at 72℃ for 10 sec.

Table 1. Primers used for gene expression analysis

Gene Name	Primer Forward (5'-3')	Primer Reverse (5'-3')	Amplicons Lenght (bp)	Annealing Temperature (℃)
TAD1	CCGGCAACATATTCAGGTTC	CTGCATGCATTCCATAAGTA	216	60
Actin	GCCCATCTACGAGGGGATA	GGTGGTCGTGAAGGTGTAA	124	60

Phylogenetic tree construction

Genes encoding proteins that control plant height and the number of tillers were collected from the Gene Bank of the National Center for Biotechnology Information (NCBI). Eighteen gene sequences that consisted of 16 rice gene sequences [19] and two maize (*Zea mays* L.) gene sequences (outgroup) were aligned using Clustal W on UGENE software. The alignment results were saved as a Nexus file and then used to construct a phylogeny tree using the Neighbor-Joining method in PAUP4 software [20].

Meta-analysis of transcriptomes

Collection of transcriptome data from 2 studies [15, 21] on gene experiments affecting rice plant height and tiller number was obtained from NCBI. In total, we used 16 Sequence Read Archives (SRA) from Indica rice genotypes in the seedling phase. The range of read number in each SRA was 1,309,419 to 40,299,419, and the range of read size was 264,5 M to 12,200 M. On average, the read number and read size of each SRA were 12,342,384 and 3,501 M, respectively. RNA-seq with SRR accession codes were ac-

Table 2. RNA-seq datasets related to plant height and number of tillers in rice plants

Study and Accession Number	Total SRA	SRR Number	Total Read (Mb)	Total bases (Mb)	Genotype	Type of tissue	Stage of Development
Chen *et al*.[15]	10	SRR6638921	4.00	808.0	Indica	Seedling	No mention
PRJNA432063		SRR6638922	4.00	808.0			
		SRR6638923	4.00	808.0			
		SRR6638924	4.00	808.0			
		SRR6638925	1.92	388.4			
		SRR6638926	4.00	808.0			
		SRR6638927	4.00	808.0			
		SRR6638928	4.00	808.0			
		SRR6638929	4.00	808.0			
		SRR6638930	1.31	264.5			
Jha *et al*. [21]	6	SRR7231489	40.29	12,200.0	Indica	Seedling	35 DAP
PRJNA473574		SRR7231488	28.59	8,600.0	Pusa		
		SRR7231487	26.32	7,900.0	Basmati 1		
		SRR7231486	23.40	7,100.0			
		SRR7231485	23.55	7,100.0			
		SRR7231484	20.08	6,000.0			

cessed from NCBI's GEO DataSet (Table 2). Each accession was uploaded in fastq format. The quality of the sequences (reads) was evaluated using FastP software and then aligned and mapped with the rice reference genome cv. Nipponbare using RNA STAR software [22, 23]. The number of reads at each mapped gene locus is read using HTSeq-count software [24] that produces output in the form of a gene count. Data processing was done using cloud computing techniques on the Galaxy server [25].

The gene expression level in each sample was represented by the number of reads successfully mapped in gene count. Differential gene expression (DEG) analysis was performed simultaneously on all samples from both studies. Samples in each study were grouped based on wild-type and mutant strains. DEG analysis was performed with DESeq2 software. All samples from each study were normalized using the Relative Log Expression (RLE) method by considering study differences as a batch effect to reduce bias in the meta-analysis. Genes that had significantly altered expression levels compared to the wild type were determined based on the criterion of p-value <0.05.

Data analysis

Data from real-time PCR (RT-PCR) results were analyzed using a relative quantitative method to measure the gene's relative expression in rice cv. HB and IR64 with the formula $2^{-\Delta\Delta Ct}$ [26].

Results and Discussion

Quality and quantity of total RNA and cDNA

Rice starts the initiation of tiller formation from the fifth leaf at 30 DAP, and the tiller starts growing at 50 DAP. Samples of the youngest leaves were taken at both phases. The quality of the isolated RNA is quite good, as presented in the electrophoresis results (Figure 1).

Total RNA consists of 2 bands, 28S and 18S ribosomal RNA bands. In addition, the RNA was free from genomic DNA contamination because

it has been degraded with DNase. The total RNA that has been isolated has a fairly high quantity, with concentrations ranging from 993 to 1691 ng/µl and purity levels at the A260/A280 ratio ranging from 1.7 to 2.6 (Table 3).

The cDNA was synthesized from total RNA and yielded high concentrations with a good purity level of close to 2 based on wavelengths A260/A230 and A260/A280 (Table 4). The A260/A230 wavelength indicates the purity of phenol and carbohydrate, while the A260/A280 wavelength represents the protein purity. A good level of cDNA purity has an important effect on the success of the RT-PCR process.

Relative gene expression analysis

Gene expression analysis of *TAD1* gene in rice cv. HB is important to know the expression level of the gene, which can be used to predict the role of the gene in rice architecture regulation. Relative gene expression in rice cv. HB was compared with the expression of the gene in cv. IR64. The results showed *TAD1* relative expression of rice cv. HB in the pre-tillering phase (till-

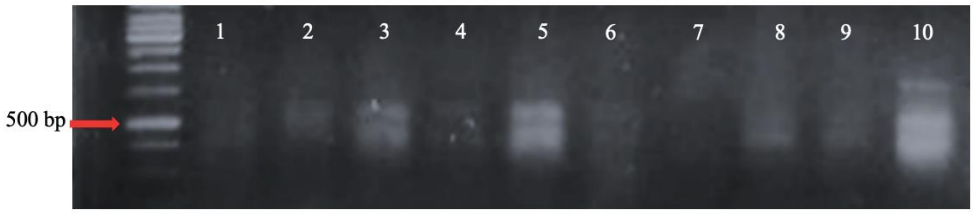

Figure 1. Total RNA profiles from rice leaves of cv. Hawara Bunar (1-5) and IR64 (6-10).

Table 3. Total leaf RNA concentration in cv. Hawara Bunar and IR64

Sample Name	Concentration (ng/µl)	A260/A230	A260/A280
Hawara Bunar 1	1600	1.9	2.4
Hawara Bunar 2	1658	1.9	2.3
Hawara Bunar 3	1651	1.8	1.7
Hawara Bunar 4	1658	1.8	1.8
Hawara Bunar 5	1470	1.9	2.4
IR64 1	1691	1.8	1.8
IR64 2	993	1.9	2.2
IR64 3	1662	1.9	2.0
IR64 4	1477	2.0	2.5
IR64 5	1560	2.0	2.6

Table 4. Concentration of cDNA samples from leaf RNA in cv. Hawara Bunar and IR64

Sample Name	Concentration (ng/µl)	A260/A230	A260/A280
Hawara Bunar 1	807	1.9	1.9
Hawara Bunar 2	904	1.9	1.9
Hawara Bunar 3	750	1.8	1.9
Hawara Bunar 4	965	1.9	1.9
Hawara Bunar 5	878	1.9	1.9
IR64 1	1021	2.0	1.9
IR64 2	1027	1.9	1.9
IR64 3	918	1.9	1.9
IR64 4	986	1.9	1.8
IR64 5	1075	1.8	1.9

er formation initiation phase) was 6.2 times higher than the relative expression of the gene in rice cv. IR64 at the same tillering phase. The gene expression of *TAD1* was increased at the tillering phase (with tillers) compared to the pre-tillering phase in both rice varieties. The relative expression of the *TAD1* gene in rice cv. HB in the tillering phase was 19.2 times higher than that of cv. IR64 in the same phase. The relative expression of the *TAD1* gene in cv. IR64 at the tillering phase increased 1.29 times from the expression of the same gene in the pre-tillering phase (Figure 2).

The vegetative character of rice cv. Hawara Bunar and IR64

The difference in the relative expression of the gene in cv. HB and IR64 correspond to the phenotype of both cultivars. Rice cv. HB has a plant height ranging from 112-150 cm with six nodes and an average of 2 tillers per plant, while IR64 rice has a height ranging from 51-85 cm with five nodes and has an average of 5 tillers per plant. The number of tillers of IR64 grown on paddy fields could reach 15 tillers per plant. The tillers on both cultivars grow at the base of the nodes. In addition to plant height differences, the leaf length differs between the two cultivars. Rice leaf length of cv. HB can reach up to 100 cm, while the longest leaf of IR64 rice is only 70 cm (Figure 3). The plant height character of rice cv. HB is closely related to the gene expression level ($r = 0.935$; *p-value* = 1.541e-9). Therefore, the gene is a potential target for editing to obtain shorter mutants with an ideal number of tillers. The difference in the plant height of rice cv. HB and IR64 were seen since seedlings (seedlings ready to be planted). Rice cv. HB grows taller faster and has the longest main roots, while the roots of seedling rice cv. IR64 appears to be smaller and shorter.

Reproductive characters of rice cv. Hawara Bunar and IR64

Rice reproductive characteristics determine yield potential. Rice cv. HB produces panicles at 85 DAP and harvested at 140 DAP, while rice cv. IR64 began to produce panicles at 70 DAP and harvested at 90 DAP. Rice cv. HB has 1-3 productive tillers, while rice cv. IR64 can produce 5-7 productive tillers. Each panicle of HB rice can produce 120-176 total grains in each panicle,

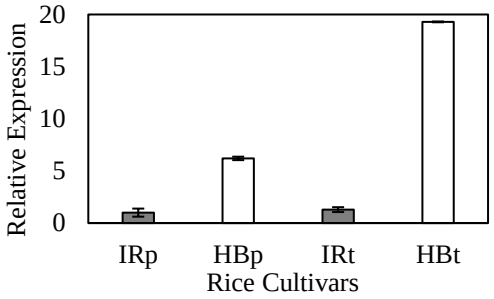

Figure 2. Relative expression of the *TAD1* gene in HB and IR64 rice. HBp: HB pre-tillering phase. HBt: HB tillering phase. IRp: IR64 pre-tillering phase. IRt: IR64 tillering phase. Bars indicate standard error.

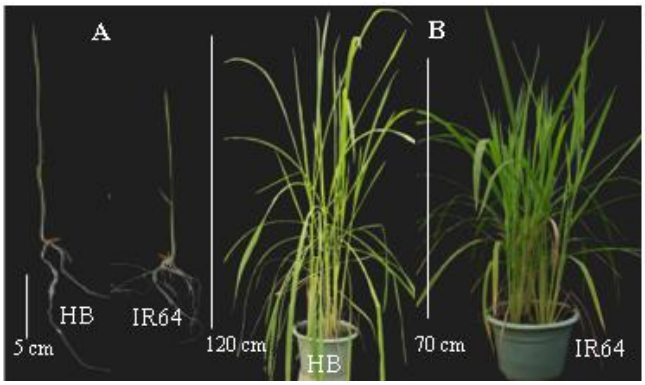

Figure 3. A. Seedling of rice cv. HB and IR64 at 2 weeks old, B. Phenotype of rice cv. Hawara Bunar and IR64 at 71 days after planting (DAP).

Figure 4. A.) Panicle of rice cv. Hawara Bunar, B.) Panicle of rice cv. IR64, C.) Comparison of seed and grain size of rice cv. Hawara Bunar and IR64, D.) Seeds and grains of rice cv. Hawara Bunar and IR64

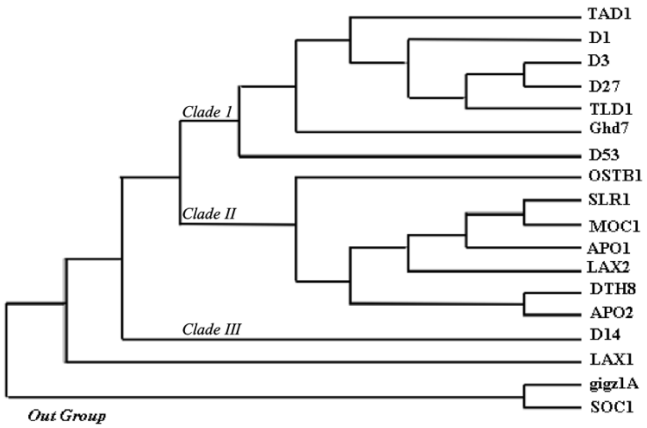

Figure 5. Phylogenetic tree of genes determining plant height and number of tillers in rice plants

with an average of 141 grains per panicle, while cv. IR64 can produce 78-145 grains per panicle, with an average of 126 grains per panicle. Both rice cultivars have different panicle branching, cv. HB has longer panicles but shorter branching than rice cv. IR64 (Figures 4A and 4B). Each panicle of rice cv. HB has 12-14 spikelets, while the number of spikelets in IR64 panicles is 9-14. The distance between the branching nodes of the panicles of rice cv. HB is longer than rice cv. IR64 with an average of 4 and 3 cm, respectively. Seeds of rice cv. HB has a reddish color, while the grains of rice cv. IR64 are white, besides the seeds of cv. HB has a larger and longer size than the seeds of cv. IR64 (Figure 4C and 4D).

Phylogenetic analysis of genes controlling plant height and number of tillers in rice

The results of the phylogenetic analysis showed that the genes controlling plant height and the number of tillers were grouped into three clades (Figure 5). Clade I consists of genes (*D3*, *d27*, and *d53*) related to strigolactone hormone biosynthesis. Clade II is a group of genes related to the regulation of initiation, growth, and development of axillary meristems in plants, while clade III consists of one gene. The gene IDs of the genes in the phylogenetic tree and their encoded proteins are presented in Table 5.

Differential gene expression

The meta-analysis study in this research was performed to support the *TAD1* gene expression analysis. The meta-analysis results showed that the two studies had different DEG characteristics. Genes that affect plant height and the number of rice tillers were found more in the study of [15] compared to the study of [21] (Figure 6). The red gene group represents genes with an upregulated expression mechanism, while the blue gene group has a downregulated expression mechanism for the expression of plant height and the number of rice tillers. A total of 16 in-group accessions (Table 5) in the phylogenetic tree were found at loci

that were successfully mapped through meta-analysis with the data sets of the two studies, especially the gene with locus Os03g0123300 (*TAD1* gene), which is in the downregulated group. This is in accordance with the results of gene expression. *TAD1* has a low expression level in the pre-tiller phase and its expression level increases significantly in the tiller phase, which is when rice cv. HB is actively growing tillers.

TAD1 is expressed to suppress tiller formation through the repression mechanism of *MOC1*. In both volcano plots, other genes that function for meristem growth were up-regulated (Table 6).

Table 5. Gene IDs, gene names, and proteins encoded by genes used in the phylogenetic analysis

Gene ID	Gene Bank Number	Gene name	Proteins
Os03g0123300	XM_026025199.1	*TAD1*	Activator of the anaphase promoting complex/cyclosome
Os05g0333200	AB028603.1	*D1*	Gibberellin signal transduction
Os06g0154200	CA765710.2	*D3*	Strigolactone (SL) signal perception
Os11g0587000	XM_026021535.1	*D27*	Strigolactone biosynthesis
Os11g0528700	XM_026027264.1	*TLD1*	Indole-3-acetic acid (IAA)-amido synthetase
Os07g0261200	OP030527.1	*Ghd7*	Regulator of growth, development, and stress-response
Os11g0104300	XM_015761976.2	*D53*	Repressor of strigolactone (SL) signaling
Os05g0591500	AB088343.1	*OSTB1*	TCP family transcription factor, Negative regulator for lateral branching
Os03g0707600	AB262980.1	*SLR1*	DELLA repressor protein, Gibberellin signaling
Os06g0610350	AY242058.1	*MOC1*	GRAS family nuclear protein, Control of tillering
Os06g0665400	XM_015786929.1	*APO1*	F-box protein, Lodging resistance and grain yield
Os04g0396500	AB669025.1	*LAX2*	Regulation of axillary meristem formation
Os08g0174500	KR815351.1	*DTH8*	Putative HAP3 subunit of the CCAAT box-binding
Os04g0598300	NM_001402417.1	*APO2*	Probable transcription factor, Control of inflorescence
Os04g0636900	XM_015775914.2	*D14*	Alpha/beta-hydrolase receptor Dwarf14 family protein
Os01g0831000	NM_001409099.1	*LAX1*	Basic helix-loop-helix (bHLH) transcription factor, Axillary meristem formation

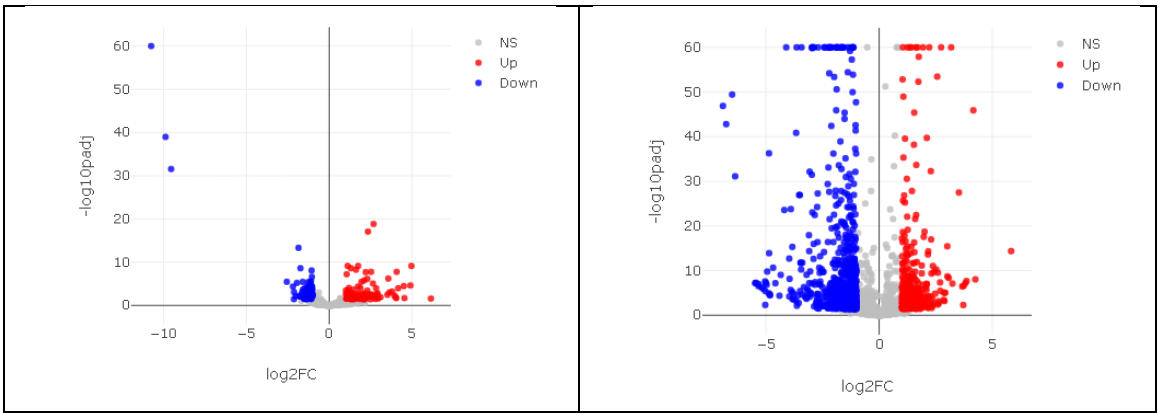

Figure 6. Differentially expressed genes at the individual study level. Volcano plots represent gene expression levels (log2 fold change) with significance based on the *p*-value. Red dots indicate increased expression (upregulation), and blue dots indicate decreased expression (downregulation). A. Study 1 [15] B. Study 2 [21].

Regulation of TAD1 in rice cv. Hawara Bunar

Plant height and the number of tillers are the main agronomic characteristics that are crucial in rice breeding to increase rice productivity [27]. In this study, we found that the *TAD1* gene is a gene that correlates with plant height (r = 0.935; *p-value* = 1.541e-9) and the number of tillers (r = -0.932; *p-value* = 2.375e-9) in rice cv. HB. The increase of *TAD1* gene expression in rice cv. HB and IR64 occurred as the vegetative phase changed to the reproductive phase. However, the relative expression of the *TAD1* gene in IR64 is lower than in rice cv. HB, which is in conjunction with short habitus and many tillers produced in the cv. IR64.

Three main factors determine rice productivity; the number of panicles produced by productive tillers per plant, the total grain in each panicle, and grain weight [28]. Rice cv. IR64 produces more panicles in each plant because of more productive tillers than that of cv. HB. Rice cv. IR64 has more productive tillers than rice cv. HB could be due to a low relative expression of the *TAD1* gene. Total grain in each panicle of cv. IR64 was less than that in cv. HB. Panicle characteristics of both rice cultivars were different. Panicle length and distance of panicle branching nodes of cv. IR64 was shorter than that of cv. HB. In addition, the number of panicle branches of cv. IR64 is less than cv. HB. Branching in rice is influenced by *APO1* and *APO2*, which affect the number of panicle branches and the number of rice tillers [21, 18]. Therefore, efforts should be made to shorten habitus and increase the number of productive tillers in rice cv. HB, through reducing the expression or eliminating the gene related to plant height and tiller number, will increase the rice harvest index of cv. HB.

In rice, the protein orthologous to Cdh1 functions as a co-activator of APC/C (Anaphase-promoting Complex). The APC/C, along with TE (Tillering Enhancer), a target site recognition, form a complex with a protein encoded by the *OsAPC10* gene. The interaction between both proteins degrades *MOC1* proteins in the G1 phase of the cell cycle, causing the *MOC1* protein to lose its function in controlling the initiation and growth of axillary meristems in the vegetative and generative phases. Mutation of the *TAD1* gene inhibits MOC1 protein degradation, resulting in the accumulation of the *MOC1*, causing the rice to have shorter habitus and more tillers [9, 28]. Expression of the *MOC1* gene has the potential to optimize branching in plants and balance height with branching [29]. The expression of *TAD1* gene was detected in vessels in the non-elongated part of the stem [9], while the *MOC1* protein is highly detected in axillary meristems. *MOC1* mutants only have one main stem without tillers because of the inhibition of tiller bud formation [12]. The *TAD1* gene interacts with *MOC1* directly in the nucleus at a 67-amino-acid N-terminal D-box that causes a degradation motif of *MOC1* protein by APC/C.

The genes that affect plant height and the number of rice tillers in this study have expression mechanisms involved in plant hormone biosynthesis and axillary bud development in rice plants. The *TAD1* gene has a downregulated mechanism against *MOC1* as it is downstream, so the *MOC1* protein is degraded as a result of *TAD1* expression. When *TAD1* expression drops, *MOC1* protein accumulates, which then increases the expression of *LAX1*, *LAX2*, and *OSH1*. Based on this, *MOC1* upregulates *LAX1*, *LAX2*, and *OSH1* genes. The gene expression modulated by the *MOC1* protein results in rice phenotypes with short habitus and more tillers [3, 18, 29].

Mutations that occur in the master regulator gene are predicted to cause *MOC1* protein to accumulate. Consequently, the rice mutant has a shorter habitus and more tillers than its wild type. This is because the regulation of tillering and plant height traits in rice is negatively correlated; namely, the more tillers produced, the shorter the habitus of the rice plant. This correlation is evidenced by the report on the rice phenotype with a single mutation TGG to TGA of the *LOC_Os03g03150* gene on chromosome 3, which shows a shorter habitus and an increased number of tillers compared to the wild type [9].

The selection of the gene from various genes that regulate the plant height and number of rice tillers is reinforced by the results of the phylogenetic analysis, which shows a strong position (clade I) with the mechanism of gene expression that occurs during the cell cycle (*TAD1* gene). The other genes in clade I (*D3, D27, D53,* and *TLD1* genes) affect the plant height with a small number of tillers through the pathway of strigolactone hormone biosynthesis. Strigolactone is a terpenoid group hormone that inhibits side branching in plants. This hormone is a downstream auxin hormone, suppressing (repressing)

branching [14, 16, 28, 30]. Based on the research results by [31], the hormone strigolactone inhibits the formation of rice tillering buds under inorganic phosphate (Pi) deficiency conditions. D27 and D3 mutants associated with the strigolactone biosynthesis pathway produce mutants with a bushy phenotype. The *TLD1* gene encodes the IAA hormone, an auxin derivative [32], while the *TAD1* gene acts as a master regulator that regulates plant height and number of tillers in rice [9].

The D14 gene, which is a member of clade III, is related to signaling in the biosynthesis pathway of the gibberellin (GA) hormone. Another gene, the *LAX1* gene, interacts with the *MOC1* and *LAX2* genes in the process of forming rice tillers [33,34]. The outgroups used in this study are *gigz1A* and *SOC1* genes that function to regulate inflorescence in maize plants [34].

Phytohormones such as auxin, GA, cytokinin, and strigolactone have important contributions to plant height growth and tiller number in rice through systemic signalling. Auxin stimulates the apical meristem growth and inhibits the growth of axillary buds, while cytokinin has the opposite role, stimulating the growth of axillary buds [35].

Giberellic acid controls plant height and tillering of rice by regulating cell division and elongation. Therefore, inhibition of GA synthesis can result in a shorter rice habitus [36]. Strigolactone contributes to the regulation of tiller growth and shoot branching [37]. Mutants that inhibit strigolactone biosynthesis show more branching than the wild type.

Mutation in the genes of the clade I member produced mutants with shorter habitus and more tillers [14, 30]. In contrast to clade I, clade II has gene members that show an opposite expression to the gene member of clade I. Expression of the genes in the clade II increases the number of rice tillers with a shorter habitus through regulation of the growth and development of axillary meristems. In other words, the expression of the genes promotes the formation of tillers in rice [33].

We analysed two sets of transcriptome data on gene experiments affecting plant height and the number of rice tillers from 2 independent studies [15, 21]. Study 1 examined *miR396-OsGRF7*, one of the plant-specific growth-regulating factors (GRFs) that have a central role in plant development. The study found that *OsGRF7* regulates auxin and gibberellin (GA) hormones in rice plants [14]. *OsGRF7* rice lines have rice phenotypes 20% shorter than the wild type. In the other study, study 2 characterized and analyzed the biochemical activity of *OsRDR3* and *OsRDR4* RNA-dependent RNA polymerases in rice development. Overexpression of the *OsRDR3* gene showed an increase in tillers, while knocked-down of the mock strains (miRNA) showed stunted growth and could not survive. Overexpression and knockdown of *OsRDR4* showed weaker and stunted growth compared to *OsRDR3* [21]. Research results from both studies show that genetic modification on genes affecting rice height and number of tillers can produce shorter rice with more tillers than the wild type. Both studies support the results of this study that shows the potential of the *TAD1* gene knocked down in rice cv. HB to produce superior characters compared to the wild type.

Conclusion

The expression of the *TAD1* gene in rice cv. Hawara Bunar is higher than that in rice cv. IR64, and its expression increases towards the tillering phase. This expression level correlates with the characters of plant height and the number of tillers. The *TAD1* gene is in one clade with genes that encode the traits of plant height and the number of tillers and is in a group of downregulated genes but shows high gene expression based on a meta-analysis. The meta-analysis results are consistent with the *TAD1* gene relative expression in rice cv. Hawara Bunar. The *TAD1* gene can be selected as a target gene for editing in rice cv. Hawara Bunar to obtain a mutant with a superior character, such as the ideal plant height and number of tillers.

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