# Overexpression of TIFY genes promotes plant growth in rice through jasmonate signaling

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**6) Abbreviations:**

FOX hunting system, full-length cDNA overexpressing gene hunting system; JA, jasmonate; QTL, quantitative trait locus


## Abstract

Because environmental stress can reduce crop growth and yield, the identification of genes that enhance agronomic traits is increasingly important. Previous screening of full-length cDNA overexpressing (FOX) rice lines revealed that OsTIFY11b, one of 20 TIFY proteins in rice, affects plant size, grain weight, and grain size. Therefore, we analyzed the effect of *OsTIFY11b* and nine other TIFY genes on the growth and yield of corresponding TIFY-FOX lines. Regardless of temperature, grain weight and culm length were enhanced in lines overexpressing TIFY11 subfamily genes, except *OsTIFY11e.* The TIFY-FOX plants exhibited increased floret number and reduced days to flowering, as well as reduced spikelet fertility, and *OsTIFY10b*, in particular, enhanced grain yield by minimizing decreases in fertility. We suggest that the enhanced growth of TIFY-transgenic rice is related to regulation of the jasmonate signaling pathway, as in *Arabidopsis.* Moreover, we discuss the potential application of TIFY overexpression for improving crop yield.



## Key words

grain weight, floret number, growth promotion, rice (*Oryza sativa* L.), TIFY/JAZ proteins

## Introduction

High yield is anticipated to resolve the serious global shortage of cereals, especially rice (*Oryza sativa*), which is the most important staple food crop, feeding half of the world's population. However, the growth of this crop is vulnerable to environmental stressors such as high temperature, especially during the grain-filling stage, when high temperatures can cause reduced both yield and product quality by decreasing the size and number of grains [1,2] and by impairing the deposition of storage materials such as starch and protein, respectively [3,4]. Furthermore, of the yield components affected by such environmental stresses, grain size is one of the most important.

Several genes that influence grain size have been identified. For example, the quantitative trait locus (QTL) *GS3* encodes a transmembrane protein and is increased for grain length and weight [5]. Another QTL of grain width and weight, *GW2*, encodes a RING-type ubiquitin E3 ligase [6], whereas the QTL *GW5* encodes a novel nuclear protein [7]. The QTL *qSW5* has also been reported to influence grain width [8], and deletions in *qSW5* significantly increase sink size by increasing the number of cells in the lemmas of rice flowers. Meanwhile, the gene *GIF1*, which encodes cell-wall invertase, has been reported to regulate grain-filling and yield [9].

For other yield components, genes that increase the sink ability of rice plants, such as grain number, panicle size, and flowering time, have also been identified by QTL analysis, including *Gn1a* [10] and *Ghd7* [11]. In addition, the gene *OsSPL14*, which promotes panicle branching and higher grain yield for effect by microRNA excision [12,13] also contributes to rice sink ability.

To identify genes that regulate growth and grain yield in rice, we have employed the

full-length cDNA overexpressing gene (FOX) hunting system in rice [14–17]. Among ~14,200 FOX-rice lines expressing ~7400 independent full-length cDNAs, five lines that harbored *OsTIFY11b*-cDNA exhibited enhanced plant growth and increased grain size and weight [14,18], and the tissue enlargement of the *OsTIFY11b*-FOX lines was attributed to enhanced cell division, not increased cell expansion. Since the *OsTIFY11b*-FOX line was less sensitive to the jasmonate (JA) treatment, which typically causes growth retardation in plants [19,20], we hypothesized that *OsTIFY11b* promotes growth by enhancing cell division *via* reduced JA sensitivity [18].

The TIFY protein family includes two groups, which are characterized by the presence (group I) or absence (group II) of a GATA zinc-finger domain, and a total of 18 and 20 members have been identified in *Arabidopsis* and rice, respectively [21,22]. Almost all members of group II, including *OsTIFY11b*, harbor two characteristic conserved domains, TIFY and Jas, and several members, including JAZ1, JAZ3/JAI3, and JAZ10/JAS1, act as negative regulators of the response to JA in *Arabidopsis* [19,23,24]. The TIFY domain, which is characterized by the common motif TIF[F/Y]XG [21], is necessary for homo- and heteromeric dimerization between the TIFY family members [25,26], and the Jas domain is required for interaction with other proteins, including COI1, a key component of the JA receptor; MYC2, a transcriptional activator of JA-responsive genes that functions downstream of the JA receptor; and another transcriptional repressor, NINJA, in *Arabidopsis* [24,27–31]. In addition, other members have been reported as novel interactors of JAZ proteins, including MYC3, MYC4 [32–34], MYB21, MYB24 [35], and the WD-repeat/bHLH/MYB transcriptional complexes such as MYB75 [36], bHLH003,

bHLH013, bHLH017 [37] and EIN3/EIL1 [38]. Meanwhile, DELLA proteins, the key repressors in GA-signaling, also interact with JAZ proteins [39] and in rice, the bHLH protein OsbHLH148 was reported to interact with OsTIFY/JAZ proteins and confer drought tolerance [40], whereas OsJAZ9 (also known as OsTIFY11a) interacts with OsbHLH062 and OsNINJA and is involved in salt stress tolerance [41]. Additionally, all rice JAZ proteins except for OsJAZ14 interact with OsMYC2 [42], and OsJAZ8 (also known as OsTIFY10c) interacts with OsNINJAs and one of the OsCOI1 proteins and is involved in bacterial blight tolerance [43]. However, little is known about physiological roles that the respective TIFY/JAZ proteins play in rice grain production.

Therefore, in the present study, we profiled the expression of nine TIFY genes that were similar to *OsTIFY11b* in plants exposed to JA treatment and wounding, and we conducted a molecular phylogenetic analysis. We also analyzed the yield-related traits of 10 TIFY-FOX plants that expressed 10 respective rice TIFY genes, including *OsTIFY11b*, and found that all the analyzed *OsTIFY* genes promoted plant growth, both under normal- and high-temperature conditions. Finally, we discuss the growth- and heat tolerance-promoting characteristics of each of the 10 TIFY genes.

## Materials and Methods

*Plant materials and growing conditions*

Seeds of wild-type (WT) and transgenic rice (*Oryza sativa* L. 'Nipponbare') were sterilized in an antiformin solution that contained ~2.5% available chlorine for 30 min and were then germinated on Murashige and Skoog (MS) agar medium with or without 50 mg $L^{-1}$ hygromycin. After 2 weeks, the resulting plants were transferred to soil and

grown in air-conditioned greenhouses under natural light and 12-h light and 12-h dark cycles at 27°C and 24°C, respectively (normal temperature), or 32°C and 27°C (high temperature). Six plants each were grown in plastic containers (15×10×6 cm) that were filled with 700 mL of rice nursery culture soil (0.16 g each of nitrogen, phosphate, and potassium), and each plant was restricted to the main culm by the removal of the tillers. Approximately 15 d before heading, each container was amended with 3 g fertilizer (0.18 g nitrogen, 0.24 g phosphate, 0.18 g potassium, and 0.06 g magnesium); and ~45 d after flowering, agronomic traits were measured, and the caryopses were harvested, counted and weighed. Specifically, plant height was measured as the location of the plant organ (leaf tip or panicle tip) that was furthest from the ground.

*Plasmid construction*

The overexpression vectors of the 10 OsTIFY genes (*OsTIFY 3, 9, 10a, 10b, 10c, 11a, 11b, 11c, 11d,* and *11e*) [22] were constructed by inserting full-length TIFY cDNA fragments, which were excised from pFLCI vectors with *Sfi*I [44], into the compatible *Sfi*I sites of pRiceFOX [14] in the forward orientation. For the overexpression vectors of *OsTIFY9, 10c, 11d* and *11e,* full-length cDNA fragments were amplified from the vectors of pME18SFL3 or pCMVFL3 by PCR primers with *Sfi*I recognition sites (Supplemental Table 1), and the *Sfi*I-digested PCR products were inserted into the compatible *Sfi*I sites of pRiceFOX.

*Rice transformation*

The TIFY-overexpression vectors and empty vectors were introduced into

*Agrobacterium tumefaciens* strain EHA105 [45] by electroporation, and rice transformation was performed as described previously [46].

*Quantitative RT-PCR and semi-quantitative RT-PCR analysis*

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. First-strand cDNAs were synthesized from each RNA preparation (1 μg/reaction) using an oligo (dT) primer and a PrimeScript RT Reagent Kit (Takara Bio, Ohtsu, Japan) in 20-μL reactions.

TIFY gene-specific primers (see Supplemental Table 2) were used in quantitative RT-PCR, and the *UBQ5* primer pair (Supplemental Table 2) was used as an internal control for normalization of the quantitative RT-PCR reaction. Quantitative RT-PCR was performed with 1 μL of cDNA template per 25-μL reaction and a Thermal Cycler Dice Real Time system TP850 (Takara Bio), using SYBR Premix Ex Taq (Takara Bio). The reaction conditions were as follows: 10 s at 95°C, followed by 40 cycles of 5 s at 95°C and 30 s at 60°C. The threshold cycle (Ct) was auto-calculated by the accompanying analysis software, and the normalized expression level of each gene was calculated by the ΔΔCt method.

Semi-quantitative RT-PCR was performed in a total volume of 20 μL with 1 μL of template cDNA from the above-mentioned 20-μL reaction and PrimeSTAR HS DNA polymerase with GC Buffer (Takara Bio) with amplification for 30 s at 94°C, which was followed by an appropriate number of cycles of 10 s at 98°C, 5 s at 50°C, and 30 s at 72°C (Fig. 1). The resulting RT-PCR products were resolved by electrophoresis.

*Treatment with methyl jasmonate (MeJA)*

Rice seeds were sterilized in a solution of sodium hypochlorite that contained ~2.5% available chlorine for 20 min and were allowed to imbibe sterilized water for 2 d at 4°C in darkness. The seeds were germinated on a plastic mesh that was floated in a culture vessel on aseptic and deionized water in the absence or presence of various concentrations of methyl jasmonate (0 to 100 μM), and the resulting seedlings were grown in a growth chamber, at 28°C in continuous darkness for 5 d. Then, the lengths of the coleoptiles were measured [47].

For gene expression analysis, the seeds were germinated on a plastic mesh floating in a culture bottle containing deionized water at 28°C under continuous darkness in a growth chamber for 5 d. To examine the effect of MeJA, root sections of the resulting seedlings were soaked in a 450 μM MeJA solution for 1 or 5 h. After treatment, the samples were immediately frozen in liquid nitrogen and kept at -80°C until the RNA was isolated.

*Treatment with mechanical wounding*

Rice seeds were sterilized using the same methodology as for the MeJA treatments. The seeds were germinated in a culture bottle with Murashige and Skoog medium and incubated in a growth chamber at 28°C under continuous white light. After 5 d, the proximal regions of the young shoots were cut three times each, using a surgical blade, and at various intervals after wounding, the shoots were harvested, immediately frozen in liquid nitrogen, and stored at -80°C until the total RNA was isolated.

## Results

*Identification of growth-promoting TIFY genes in rice*

Our previous screening identified several independent rice FOX lines that overexpressed *OsTIFY11b*, a TIFY gene that improves plant growth by enhancing grain size [18]. In order to identify additional growth-promoting genes, we planned to conduct a functional survey of other TIFY gene members by observing the growth of rice plants in which the individual genes were overexpressed. Twenty members of TIFY genes have been identified so far in rice [22] and a molecular phylogenetic tree was constructed by comparing the peptide sequences and conserved motif locations of the 20 genes, using the ClustalW method. This analysis grouped *OsTIFY11b* with three TIFY10 members and six other TIFY11 members (Supplemental Fig. 1).

*Expression of TIFY genes in plants exposed to jasmonate and wounding*

Since *Arabidopsis* has TIFY homologs, JAZ proteins act as negative regulators in JA signaling [19, 23,24], and their expression is responsive to JA and wounding. With this knowledge, we observed the effects of JA treatment and wounding on the expression of rice TIFY genes. Treatment of seedlings with 450 μM MeJA or wounding extensively upregulated the expression of *OsTIFY10* and *11* members, and moderately upregulated other group II TIFY genes, such as *OsTIFY3* and *OsTIFY9*. However, no change was observed in the expression of group I TIFY genes, namely *OsTIFY1a* and *OsTIFY2a* (Fig. 1A and B).

TIFY11 *subfamily overexpression increases grain weight and culm length*

*OsTIFY11b* and nine other similar members (*OsTIFY3, 9, 10a*, *10b*, *10c*, *11a, 11c*, *11d* and *11e*) were selected for overexpression in transgenic rice plants (Supplemental Fig. 1). Approximately 9–14 independent transgenic lines were obtained for each gene, and $T_0$ plants from each of the lines exhibited enhanced-growth phenotypes, including increased grain weight, plant height, and culm length (Supplemental Fig. 2). Among the transgenic lines, two lines with a single transgene were introduced, and the four *OsTIFY11b*-transgenic lines were used for further phenotypic analyses. The expression level of the introduced gene in the leaves of the transgenic plants was elevated to from 9.1 (*OsTIFY10a*-overexpressor) to 4139 (*OsTIFY11a*-overexpressor) times that of the corresponding gene in WT plants (Fig. 2). Grain weight was increased by constitutive overexpression of the TIFY11 subfamily genes, except for *OsTIFY11e*, and moderately increased by the overexpression of *OsTIFY9*, *OsTIFY10a*, *OsTIFY10b*, and *OsTIFY3*. Conversely, a modest decrease in grain weight was observed for overexpression of *OsTIFY10c* under the normal-temperature condition (Fig. 3A). Similarly, under the high-temperature condition, grain enlargement was also apparent for the *OsTIFY11a*-, *OsTIFY11b*-, *OsTIFY11c*-, and *OsTIFY3*-transgenic plants (Fig. 3B), and the overexpression of TIFY11 subfamily genes, except for that of *OsTIFY11e*, resulted in the greatest growth increase. In fact, grain weight increased 1.16-fold on average under the normal-temperature condition and 1.11-fold under the high-temperature condition, when compared to WT plants. Furthermore, the overexpression of TIFY11 subfamily genes, other than *OsTIFY11e*, also increased culm lengths by 1.21- and 1.29-fold on average under the normal- and high-temperature conditions, respectively, when compared to WT plants, and the culm lengths of *OsTIFY10a-*, *OsTIFY10b-*, *OsTIFY9-*,

and *OsTIFY3*-transgenic plants were increased significantly under both temperatures, as well (Fig. 3C and D).

TIFY *overexpression increases flower number and shortens the period to flowering*

Overexpression of the TIFY genes analyzed in the present study increased the number of florets to various extents, with average increases of 1.42- and 1.45-fold under the normal- and high-temperature conditions, respectively (Fig. 3E and F). However, more caryopses were aborted in the transgenic lines than in WT plants (Fig. 3G and H), which resulted in an overall reduction in total grain yield per plant for many of the TIFY-transgenic lines, especially when grown under the high-temperature condition (Supplemental Tables 3 and 4). Meanwhile, the *OsTIFY10b*-overexpressing lines exhibited relatively high and stable ratios of seed sets under both the normal- and high-temperature conditions (Fig. 3), and when considering other agronomically important traits, almost all the transgenic lines exhibited enhanced-growth phenotypes, such as early flowering and increased plant height, panicle length, and leaf length, under both normal- and high-temperature conditions (Fig. 3I and J, Supplemental Tables 3 and 4).

TIFY*-overexpressing transgenic plants are less sensitive to jasmonate*

In order to determine the physiological involvement of TIFY in the perception of the growth-inhibiting hormone JA, the response of coleoptile growth to MeJA was examined for transgenic rice seedlings that expressed either *OsTIFY3*, *9*, *10b*, *11a*, *11b*, or *11d*. Coleoptile elongation in both transgenic and WT plants was inhibited by MeJA

application in a dose-dependent manner. However, the TIFY-overexpressing plants exhibited less inhibition than the WT plants (Fig. 4), which indicated that they were less sensitive to JA.

## Discussion

*Rice TIFY-overexpressing lines exhibit enhanced-growth phenotypes*

Twenty TIFY domain-containing genes have been identified in rice so far [22], and these genes are separated into two groups based on the presence (group I) or absence (group II) of a zinc-finger domain [21]. Among the group II members, OsTIFY11a has been reported to confer salt tolerance [22], whereas OsTIFY11b promotes plant growth, such as grain size, by enhancing the accumulation of transient starch and sucrose in culms and leaf sheaths prior to heading [18]. Alignment analysis of the TIFY peptide sequences revealed an additional N-terminal domain (Supplemental Fig. 1) that is conserved in TIFY10 and TIFY11 members. However, relatively low similarity was detected in the coding regions overall (<30%; Supplemental Table 5), which suggested that each member possesses diversified physiological roles. That observation prompted the systematic determination of growth-related traits for transgenic rice plants that overexpressed individual TIFY genes that either contained the N-terminal domain (*OsTIFY10a*, *OsTIFY10b*, *OsTIFY10c*, *OsTIFY11a*, *OsTIFY11b*, *OsTIFY11c*, *OsTIFY11d*, and *OsTIFY11e*) or did not (*OsTIFY3* and *OsTIFY9*) (Supplemental Fig. 1). According to the Rice Expression Profile Database (http://ricexpro.dna.affrc.go.jp/), the expression levels of innate TIFY genes in WT leaves are variable in a range of approximately 1000-fold, and *OsTIFY3, 10a* and *10b* genes show relatively

high-expression levels among the TIFY genes. Therefore, the three genes would be the major TIFY members expressed in young seedlings (Fig. 1). Unexpectedly, almost all the examined TIFY-overexpressing lines, including those expressing genes without N-terminal domains, exhibited similar enhanced-growth phenotypes, in terms of increased caryopsis number, plant height, and grain weight, as well as a similar contraction in flowering time (Fig. 3 and Supplemental Table 3). TIFY11 members in particular, except for *OsTIFY11e*, had strong effects on grain weight and culm length; however, the TIFY11-transgenic lines also exhibited an increase in caryopsis abortion, which resulted in similar or less total grain yield per plant, when compared to WT plants. Conversely, *OsTIFY10b*-transgenic plants exhibited higher total grain yield than WT plants, owing to fewer aborted caryopses, although its effect on grain enlargement was relatively weak.

Therefore, taken together, it appears that TIFY genes have growth-promoting effects to varying extents, and in the present study, these effects were observed irrespective of the presence of an N-terminal domain, which could be attributed to diverse specific activity and/or the expression level of the products. As such, TIFY11 members and *OsTIFY10b* could be useful for improving crop yield *via* increased grain size and number, respectively. Notably, the TIFY-transgenic lines produced increased grain sizes, even under the high-temperature condition, when compared to WT plants (Fig. 3 and Supplemental Table 4). Ordinarily, high-temperature stress results in decreased grain size [1,2], which then decreases the grain value. The overexpression of TIFY genes may be a useful strategy for preventing decreases in grain production from high-temperature stress.

The increases in grain size and caryopsis number that result from the ectopic expression of TIFY genes is a unique phenomenon observed in rice. In *Arabidopsis*, the loss-of-function mutants of *PPD1* and *PPD2*, which encode TIFY proteins with different motifs than the rice TIFY members examined in the present study, exhibited enhanced-growth phenotypes, including increased leaf number and silique width [48]. Similarly, the overexpression of the *ZIM* gene, a member of the group I TIFY gene family that contains a GATA zinc-finger domain, resulted in elongated hypocotyls and petioles [49]. These observations in *Arabidopsis* support the involvement of TIFY family proteins in the regulation of plant growth, and in the future, additional TIFY-FOX lines will be used to explore the phenotypes and the different functions of TIFY genes among both rice and *Arabidopsis*.

*TIFY genes may promote growth by desensitizing plants to JA*

The leaf elongation caused by the overexpression of *OsTIFY11b* was previously attributed to an increase in cell number, not cell size [18], which suggests that the TIFY protein accelerated cell division. Indeed, TIFY proteins have been shown to regulate cell division, whereas the JA inhibits progression of the cell cycle [50,51], and repeated wounding retards the growth of plants *via* wound-induced JA. A mutant plant that expressed a deletion form of JAI3/JAZ3, which was resistant to JA-induced destruction by the F box protein COI1, failed to exhibit such wounding-induced growth retardation [52], which suggested that the deletion form of JAI3/JAZ3 interrupted the action of JA.

Expression of *OsTIFY3*, *9*, *10b*, *11a*, *11b*, and *11d* genes was responsive to JA (Fig. 1). Transgenic plants overexpressing those cDNAs exhibited reduced sensitivity to

MeJA, in terms of coleoptile growth inhibition (Fig. 4). Therefore, rice TIFY genes, such as *OsTIFY3*, *9*, *10b*, *11a*, *11b*, and *11d* might accelerate cell division in transgenic plants by desensitizing plants to JA and disrupting its negative effect on the progression of the cell cycle, thus allowing the expansion of various organs, including leaves, culms, and grains.

In the present study, the ectopic overexpression of TIFY genes promoted the growth of rice plants, specifically in caryopsis number, grain weight, culm length, and period to flowering, and this effect was demonstrated under various temperatures, which indicates the potential of the genes to be used for improving grain yield and biomass, while also increasing tolerance to environmental stressors, such as high temperature. However, the low floret fertility of the TIFY-transgenic lines used in the present study directly affects grain number, which will need to be improved through further modification, such as the optimization of promoters, in future studies. We propose that plant growth-regulating TIFY genes can be utilized as novel and valuable tools for breeding high-yield and heat-tolerant crops.

## Author contribution

Conceived and designed the experiments: MH, HY, HI. Performed the experiments: MH, MM, HN, M. Kishimoto, KI-O, M. Kajikawa, NI-T. Analyzed the data: MH, MM, HY, HI. Contributed reagents/materials/analysis tools: NH, ST, YN. Contributed to the writing of the manuscript: MH, HY, HI.

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**Figure Legends**

**Fig. 1. Expression of TIFY genes in rice plants exposed to methyl jasmonate (MeJA) and mechanical wounding. (**A) Five-day-old plants were treated with (+) or without (−) 450 μM MeJA. At the indicated time, total RNA was isolated from the shoots and subjected to semi-quantitative RT-PCR, using the gene-specific primers listed in Supplemental Table 2. *UBQ5* was used as an internal control. The number of PCR cycles for detection of individual OsTIFY genes is indicated to the right of the panels. (B) Five-day-old plants were wounded three times using a surgical blade, and total RNA was extracted from the shoot at the indicated times and subjected to RT-PCR analysis.

**Fig. 2. Relative transcript abundance of introduced genes in TIFY FOX lines.** Leaf blades were analyzed at two weeks after sowing, using quantitative RT-PCR.

**Fig. 3. Agronomic traits for TIFY FOX lines grown under normal- or high-temperature conditions.** (A-B) Grain weight, (C-D) culm length, (E-F) floret number, (G-H) spikelet fertility, and (I-J) period until heading at normal [27°C (12-h) / 24°C (12-h)] and high [32°C (12-h) / 27°C (12-h)] temperature conditions, respectively. WT, wild-type plants (); 11b to 3, *OsTIFY11b* to *3* FOX lines ($T_{2\text{-}3}$, n=3-5, see Supplemental Tables 3 and 4 for details). Error bars indicate standard deviations. Asterisks indicate values that are significantly different from those of the WT plants, as determined by *t*-tests: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

**Fig. 4. Response of TIFY FOX lines to methyl jasmonate (MeJA).** $T_3$ seedlings of OsTIFY FOX lines and WT plants were treated with 0, 1, or 10 μM MeJA for 5 d, and their relative coleoptile lengths (%) were calculated by comparing the values to the corresponding control treatments ($H_2O$) of each line. Data and error bars indicate the means ± SE (WT, n=22-24; OsTIFY FOX lines, n=13-25). Two independent FOX lines were examined for *OsTIFY11a*, *10b* and *3*.

Figure 1

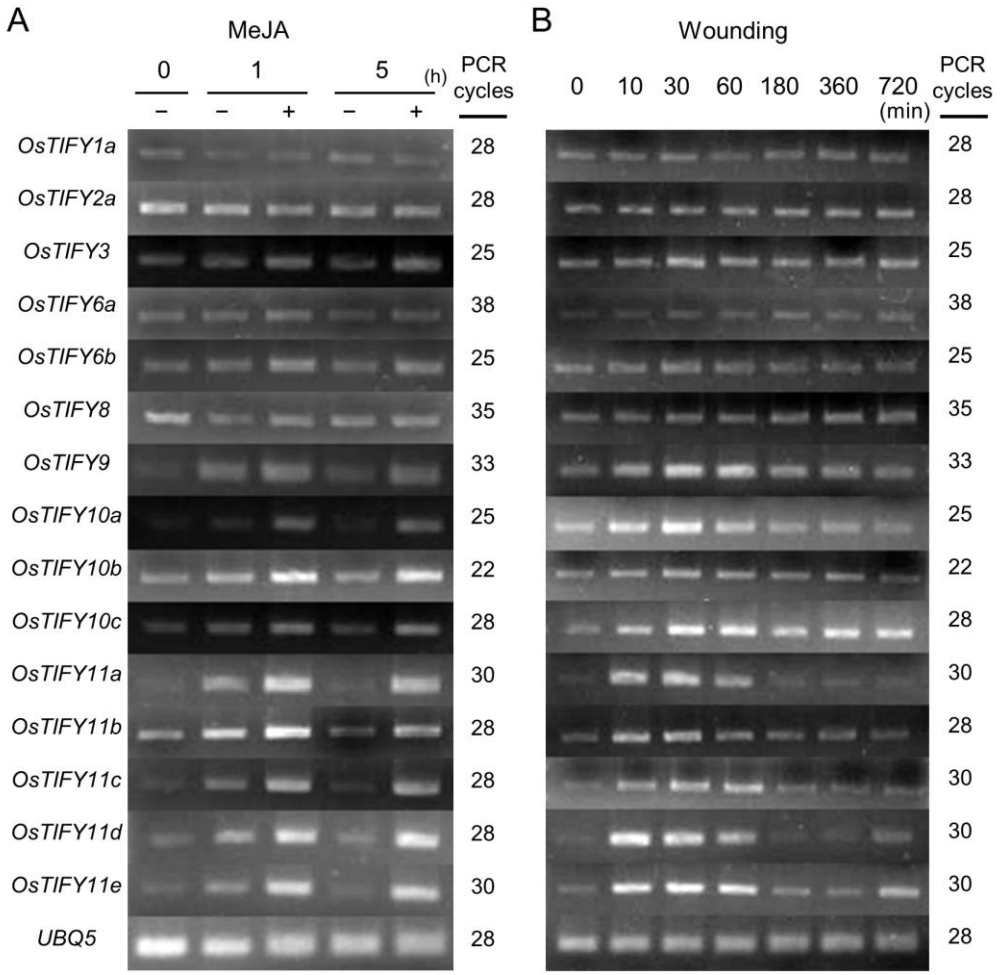

A
MeJA
0
1
5
(h)
PCR cycles
−
−
+
−
+
OsTIFY1a
OsTIFY2a
OsTIFY3
OsTIFY6a
OsTIFY6b
OsTIFY8
OsTIFY9
OsTIFY10a
OsTIFY10b
OsTIFY10c
OsTIFY11a
OsTIFY11b
OsTIFY11c
OsTIFY11d
OsTIFY11e
UBQ5
28
28
25
38
25
35
33
25
22
28
30
28
28
28
30
28
B
Wounding
0
10
30
60
180
360
720
(min)
PCR cycles
28
28
25
38
25
35
33
25
22
28
30
28
30
30
28

Figure 2

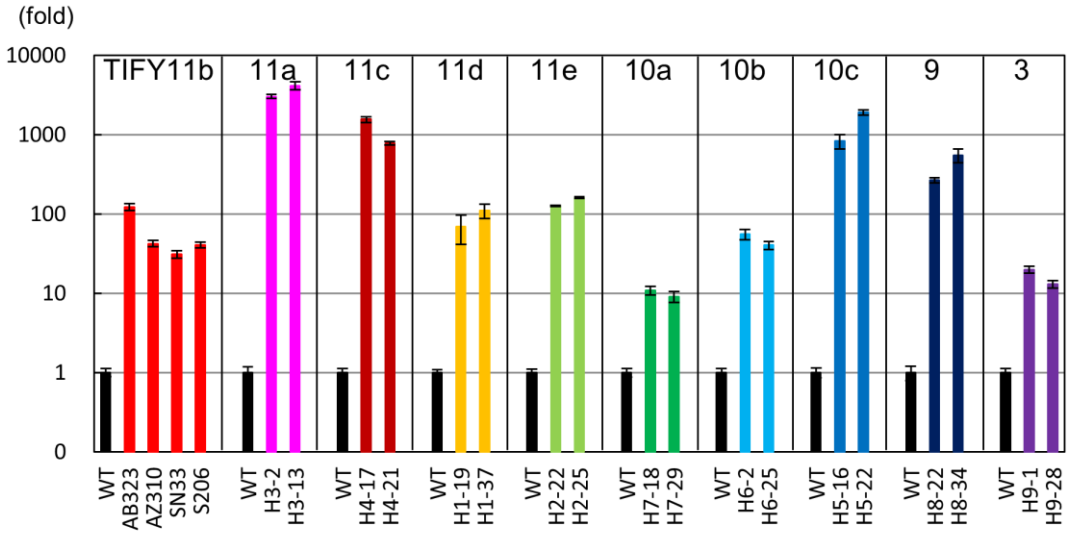

(fold)
10000
1000
100
10
1
0
TIFY11b
11a
11c
11d
11e
10a
10b
10c
9
3
WT
AB323
AZ310
SN33
S206
WT
H3-2
H3-13
WT
H4-17
H4-21
WT
H1-19
H1-37
WT
H2-22
H2-25
WT
H7-18
H7-29
WT
H6-2
H6-25
WT
H5-16
H5-22
WT
H8-22
H8-34
WT
H9-1
H9-28

Figure 3

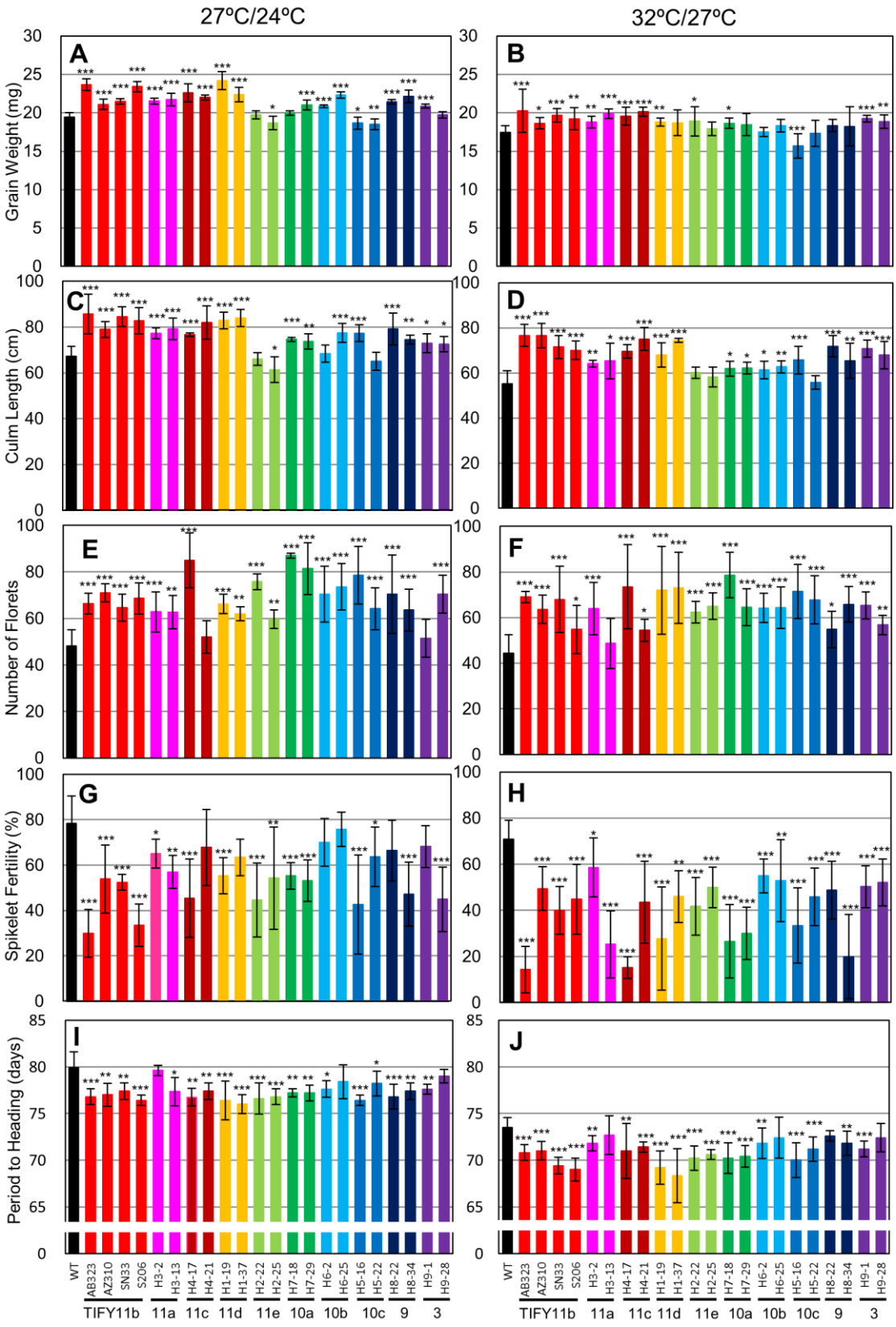
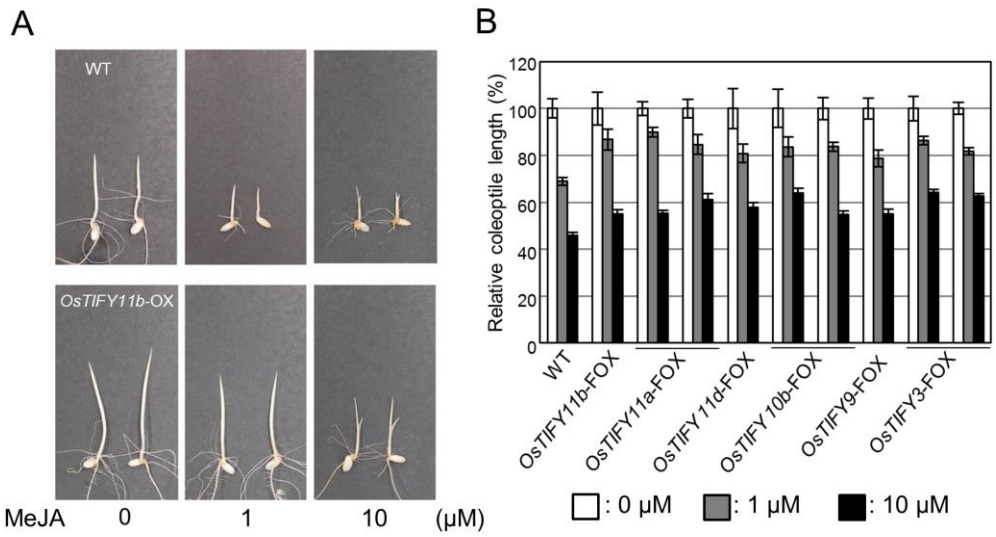
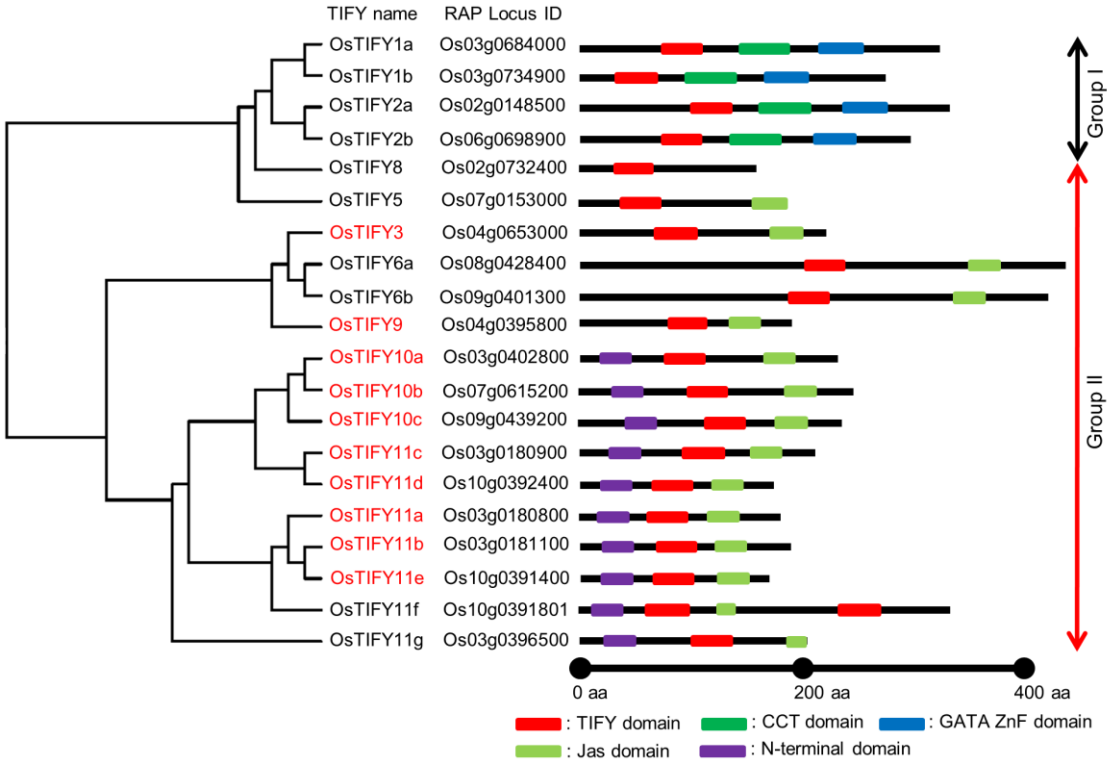

27ºC/24ºC
32ºC/27ºC
A
B
C
D
E
F
G
H
I
J
Grain Weight (mg)
Culm Length (cm)
Number of Florets
Spikelet Fertility (%)
Period to Heading (days)
WT
AB323
AZ310
SN33
S206
H3-2
H3-13
H4-17
H4-21
H1-19
H1-37
H2-22
H2-25
H7-18
H7-29
H6-2
H6-25
H5-16
H5-22
H8-22
H8-34
H9-1
H9-28
TIFY11b
11a
11c
11d
11e
10a
10b
10c
9
3

Figure 4


A
WT
OsTIFY11b-OX
MeJA
0
1
10
(μM)
B
Relative coleoptile length (%)
120
100
80
60
40
20
0
WT
OsTIFY11b-FOX
OsTIFY11a-FOX
OsTIFY11d-FOX
OsTIFY10b-FOX
OsTIFY9-FOX
OsTIFY3-FOX
: 0 μM
: 1 μM
: 10 μM

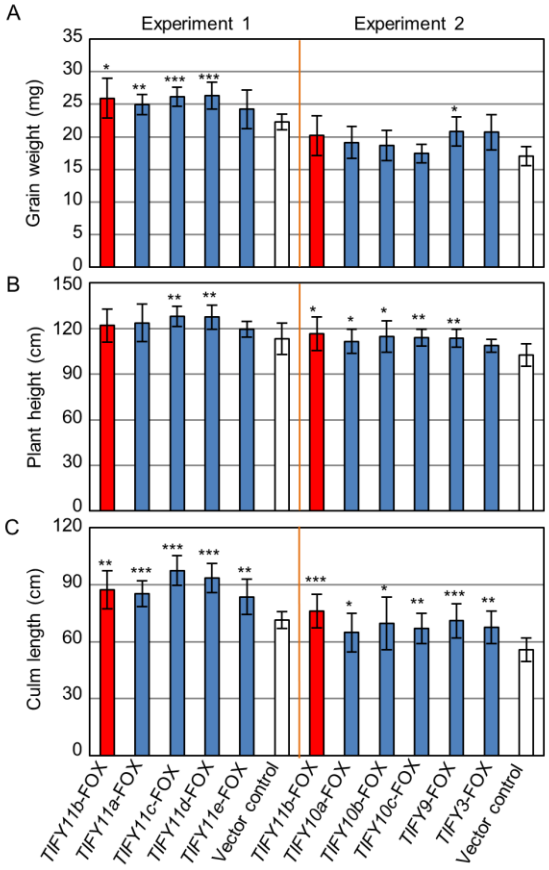
Supplemental Fig. 1




**Supplemental Fig. 1. Phylogenetic tree of rice TIFY proteins, constructed using the ClustalW method.**

The gene names of the 20 TIFY proteins are indicated with Rice Annotation Project locus IDs and highly conserved domains. The 10 TIFY genes analyzed in the present study are indicated with red text.

Supplemental Fig. 2




**Supplemental Fig. 2. Growth characteristics of TIFY-overexpressing lines.**
Rice transformation was performed twice. Experiment 1: *OsTIFY11a* lines, n=12; *OsTIFY11c* lines, n=9; *OsTIFY11d* lines, n=10; *OsTIFY11e* lines, n=12; *OsTIFY11b* lines, n=7; vector control lines, n=6. Experiment 2: *OsTIFY10a* lines, n=14; *OsTIFY10b* lines, n=12; *OsTIFY10c* lines, n=14; *OsTIFY9* lines, n=14; *OsTIFY3* lines, n=14; *OsTIFY11b* lines, n=9; vector control lines, n=8. TIFY11b and vector control lines were used as controls for plant growth. (A) Average single grain weight of $T_1$ seeds in TIFY-overexpressing lines, (B) Average plant height in TIFY-overexpressing lines ($T_0$), (C) Average culm length in TIFY-overexpressing lines ($T_0$). Columns and error bars indicate the means $\pm$ SD. Asterisks indicate values that are significantly different than those of the vector control lines, as indicated by *t*-tests: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

**Supplemental Table 1. Primers used for the construction of TIFY overexpression vectors.**

| | | |
|---|---|---|
| *OsTIFY9* | Forward primer | 5'-cacggccaaatcggccATACGTGTTCTAGCTAAGCTAGCTAGCTAG -3' |
| | Reverse primer | 5'-cacggcccttatggccCTGGCCCCTGCCTGGTCTGTACCCTCTAC -3' |
| *OsTIFY10c* | Forward primer | 5'-cacggccaaatcggccAGAGAGAGACAACCAGACGGGAGCTTGTCG -3' |
| | Reverse primer | 5'-cacggcccttatggccAGCCAAAATAGTAACAATGTAATTTATACGGCG -3' |
| *OsTIFY11d* | Forward primer | 5'-cacggccaaatcggccACACACACATCCCACCTCCCCACACGCAAGC -3' |
| | Reverse primer | 5'-cacggcccttatggccGACAACAGTCTTTCCAGTCTTTTGAGTAAT -3' |
| *OsTIFY11e* | Forward primer | 5'- cacggccaaatcggccAAGAAGAAATTAACCCGAGATTTTCTCCGAAC -3' |
| | Reverse primer | 5'-cacggcccttatggccAACATAGAGATGTTTGAATTCCATCATCGTTC -3' |

Underlined text indicates *Sfi*I recognition sites.

**Supplemental Table 2. Primers used for RT-PCR and semi-quantitative RT-PCR analysis of both TIFY and housekeeping genes.**

| Gene name | Forward primer (5'−3') | Reverse primer (5'−3') | Region |
|---|---|---|---|
| *OsTIFY1a* | ctggattctttgcctacagg | cttcagaagcagctccaaag | 3'UTR |
| *OsTIFY2a* | ctaggattcatgtggttctgc | cagacaatgcactgatgctg | 3'UTR |
| *OsTIFY3* | ctgtcaacttcgaatggtgg | gcagtttgctgttcgagtaaac | 3'UTR |
| *OsTIFY6a* | gccacttagtagtacgagag | ggccatggcaatggcatattg | 3'UTR |
| *OsTIFY6b* | ccatagattcggccattctg | cagctagaccagcattaagc | 3'UTR |
| *OsTIFY8* | ggcactagataccgagttg | cgagtgaacatcactactgc | 3'UTR |
| *OsTIFY9* | gcacacggcgtcctagctag | actggcccctgcctggtctg | 3'UTR |
| *OsTIFY10a* | gtgttaggagatgatccgatg | gaggtttcttgggttgtactg | 3'UTR |
| *OsTIFY10b* | ggttgcatggaaacttcacc | gtcaccatgtgttaacatgcc | 3'UTR |
| *OsTIFY10c* | ctggttgctagtgctagtg | cagcagcaaacaatgtggag | 3'UTR |
| *OsTIFY11a* | gagagctcgatcgattgatc | gcgataactagggtaactgc | 3'UTR |
| *OsTIFY11b* | gagaagaagacgcccacctc | ggcacaaggggtttcccatc | ORF~3'UTR |
| *OsTIFY11c* | cacgtgtctgtggaaagtg | catcaatccgtgtgcatgg | 3'UTR |
| *OsTIFY11d* | ctccactagagcatatacag | gacaacagtctttccagtc | 3'UTR |
| *OsTIFY11e* | gtgaggatgcttatgcttgc | gatccctagcatatgtactac | 3'UTR |
| *UBQ5* | accacttcgaccgccactact | acgcctaagcctgctggtt | ORF |

**Supplemental Table 3. Growth characteristics of TIFY FOX lines grown under normal-temperature condition.**

| Line name | Plant height | | Panicle length | | Flag leaf length | | Grain number | | Total grain weight | |
|---|---|---|---|---|---|---|---|---|---|---|
| | cm | | cm | | cm | | | | mg | |
| *OsTIFI11b*-FOX | | | | | | | | | | |
| AB323 (T3, n=5) | 107.9 ± 3.9 | *** | 16.2 ± 0.7 | ** | 22.9 ± 3.1 | | 19.8 ± 7.3 | *** | 466.5 ± 161.9 | ** |
| | 106% | | 113% | | 105% | | 52% | | 64% | |
| AZ310 (T3, n=5) | 112.7 ± 4.6 | *** | 14.9 ± 0.5 | | 21.2 ± 1.9 | | 38.2 ± 10.8 | | 778.1 ± 210.4 | |
| | 111% | | 104% | | 97% | | 101% | | 107% | |
| SN33 (T3, n=5) | 114.5 ± 3.5 | *** | 14.7 ± 0.7 | | 21.7 ± 2.4 | | 33.8 ± 3.3 | | 708.4 ± 50.3 | |
| | 112% | | 102% | | 100% | | 89% | | 98% | |
| S206 (T2, n=5) | 108.4 ± 4.0 | *** | 15.8 ± 0.4 | * | 23.8 ± 2.6 | | 23.0 ± 7.1 | ** | 499.5 ± 164.3 | * |
| | 106% | | 110% | | 109% | | 61% | | 69% | |
| *OsTIFY11a*-FOX | | | | | | | | | | |
| H3-2 (T2, n=5) | 108.4 ± 3.3 | *** | 15.1 ± 1.7 | | 21.9 ± 5.5 | | 40.8 ± 6.5 | | 874.3 ± 143.2 | |
| | 106% | | 105% | | 101% | | 108% | | 121% | |
| H3-13 (T2, n=3) | 111.8 ± 3.8 | *** | 15.5 ± 0.8 | | 22.8 ± 4.6 | | 35.7 ± 5.9 | | 763.4 ± 138.6 | |
| | 110% | | 108% | | 105% | | 94% | | 105% | |
| *OsTIFY11c*-FOX | | | | | | | | | | |
| H4-17 (T2, n=4) | 112.6 ± 1.7 | *** | 17.2 ± 0.9 | *** | 26.3 ± 2.7 | * | 37.3 ± 10.7 | | 797.7 ± 239.8 | |
| | 110% | | 120% | | 121% | | 99% | | 110% | |
| H4-21 (T2, n=5) | 102.4 ± 5.8 | | 14.0 ± 0.7 | | 19.1 ± 3.6 | | 35.0 ± 8.1 | | 776.6 ± 154.9 | |
| | 100% | | 97% | | 88% | | 93% | | 107% | |
| *OsTIFY11d*-FOX | | | | | | | | | | |
| H1-19 (T2, n=5) | 109.6 ± 2.4 | *** | 16.0 ± 1.1 | * | 24.1 ± 3.6 | | 36.4 ± 4.5 | | 878.6 ± 107.2 | |
| | 108% | | 111% | | 111% | | 96% | | 121% | |
| H1-37 (T2, n=3) | 109.4 ± 4.8 | *** | 16.8 ± 0.4 | ** | 29.8 ± 0.9 | ** | 39.3 ± 5.5 | | 872.7 ± 136.0 | |
| | 107% | | 117% | | 137% | | 104% | | 120% | |
| *OsTIFY11e*-FOX | | | | | | | | | | |
| H2-22 (T2, n=5) | 101.4 ± 5.1 | | 15.1 ± 0.3 | | 22.5 ± 1.4 | | 33.8 ± 12.4 | | 661.3 ± 249.3 | |
| | 100% | | 105% | | 103% | | 89% | | 91% | |
| H2-25 (T2, n=5) | 96.9 ± 3.5 | ** | 15.2 ± 1.5 | | 24.4 ± 2.6 | | 32.6 ± 13.8 | | 610.8 ± 271.5 | |
| | 95% | | 106% | | 112% | | 86% | | 84% | |
| *OsTIFY10a*-FOX | | | | | | | | | | |
| H7-18 (T2, n=5) | 105.8 ± 0.9 | ** | 16.4 ± 0.3 | ** | 25.4 ± 1.2 | | 48.0 ± 4.7 | * | 946.7 ± 92.3 | ** |
| | 104% | | 115% | | 117% | | 127% | | 131% | |
| H7-29 (T2, n=5) | 109.3 ± 1.9 | *** | 16.2 ± 1.0 | ** | 25.7 ± 5.4 | | 43.4 ± 11.5 | | 901.8 ± 213.2 | |
| | 107% | | 113% | | 118% | | 115% | | 124% | |
| *OsTIFY10b*-FOX | | | | | | | | | | |
| H6-2 (T2, n=5) | 107.7 ± 3.8 | *** | 15.2 ± 0.6 | | 22.6 ± 3.1 | | 48.6 ± 6.3 | * | 979.5 ± 140.9 | ** |
| | 106% | | 106% | | 104% | | 129% | | 135% | |
| H6-25 (T2, n=5) | 104.8 ± 2.8 | | 15.9 ± 1.0 | * | 22.4 ± 4.3 | | 55.2 ± 2.8 | *** | 1227.9 ± 59.6 | *** |
| | 103% | | 111% | | 103% | | 146% | | 169% | |
| *OsTIFY10c*-FOX | | | | | | | | | | |
| H5-16 (T2, n=5) | 110.5 ± 1.4 | *** | 16.0 ± 0.6 | ** | 24.4 ± 2.7 | | 33.4 ± 15.9 | | 617.3 ± 322.4 | |
| | 108% | | 112% | | 112% | | 88% | | 85% | |
| H5-22 (T2, n=5) | 97.8 ± 2.6 | ** | 14.9 ± 0.5 | | 24.1 ± 2.2 | | 41.4 ± 11.4 | | 742.4 ± 198.0 | |
| | 96% | | 104% | | 111% | | 110% | | 102% | |
| *OsTIFY9*-FOX | | | | | | | | | | |
| H8-22 (T2, n=5) | 103.4 ± 5.3 | | 16.4 ± 0.9 | ** | 25.4 ± 2.7 | | 46.6 ± 14.8 | | 984.6 ± 320.1 | * |
| | 101% | | 114% | | 116% | | 123% | | 136% | |
| H8-34 (T2, n=5) | 104.8 ± 2.4 | | 14.9 ± 0.8 | | 23.4 ± 4.4 | | 30.0 ± 10.8 | | 659.9 ± 239.1 | |
| | 103% | | 104% | | 107% | | 79% | | 91% | |
| *OsTIFY3*-FOX | | | | | | | | | | |
| H9-1 (T2, n=5) | 100.7 ± 2.9 | | 15.5 ± 0.7 | | 24.3 ± 2.7 | | 35.0 ± 7.3 | | 716.0 ± 136.4 | |
| | 99% | | 108% | | 111% | | 93% | | 99% | |
| H9-28 (T2, n=5) | 105.7 ± 3.2 | * | 15.1 ± 1.0 | | 23.6 ± 4.0 | | 31.2 ± 9.1 | | 608.3 ± 181.7 | |
| | 104% | | 105% | | 108% | | 83% | | 84% | |
| WT | | | | | | | | | | |
| Nipponbare (n=22) | 101.9 ± 2.9 | | 14.4 ± 1.2 | | 21.8 ± 3.9 | | 37.8 ± 8.6 | | 725.0 ± 167.3 | |
| | 100% | | 100% | | 100% | | 100% | | 100% | |

Values and error bars indicate means ± SD (n=3-5). Percentages indicate relative values, compared to those of wild-type plants. Asterisks indicate values that are significantly different than those of the wild-type plants, as indicated by *t*-tests: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

**Supplemental Table 4. Growth characteristics of TIFY FOX lines grown under high-temperature condition.**

| Line name | Plant height | | Panicle length | | Flag leaf length | | Grain number | | Total grain weight | |
|---|---|---|---|---|---|---|---|---|---|---|
| | cm | | cm | | cm | | | | mg | |
| *OsTIFI11b*-FOX | | | | | | | | | | |
| AB323 (T3, n=5) | 98.9 ± 2.8 | *** | 14.0 ± 0.9 | | 19.5 ± 1.8 | | 10.0 ± 7.3 | *** | 209.6 ± 161.3 | *** |
| | 114% | | 98% | | 101% | | 32% | | 42% | |
| AZ310 (T3, n=5) | 106.8 ± 4.4 | *** | 14.6 ± 1.1 | | 23.9 ± 4.9 | ** | 31.4 ± 6.7 | | 562.4 ± 146.3 | |
| | 123% | | 103% | | 124% | | 100% | | 113% | |
| SN33 (T3, n=5) | 96.9 ± 7.1 | ** | 15.9 ± 1.0 | *** | 29.7 ± 4.4 | *** | 26.8 ± 7.3 | | 513.8 ± 148.0 | |
| | 112% | | 112% | | 154% | | 86% | | 103% | |
| S206 (T2, n=5) | 89.3 ± 6.6 | | 14.4 ± 1.3 | | 24.4 ± 4.3 | ** | 24.6 ± 9.5 | * | 438.7 ± 204.1 | |
| | 103% | | 101% | | 127% | | 79% | | 88% | |
| *OsTIFY11a*-FOX | | | | | | | | | | |
| H3-2 (T2, n=5) | 94.4 ± 4.6 | * | 15.2 ± 1.3 | * | 19.8 ± 3.5 | | 36.6 ± 5.5 | | 634.6 ± 100.6 | * |
| | 109% | | 107% | | 103% | | 117% | | 128% | |
| H3-13 (T2, n=3) | 96.2 ± 1.1 | * | 14.1 ± 0.6 | | 19.6 ± 3.7 | | 13.3 ± 9.0 | | 259.5 ± 171.4 | ** |
| | 111% | | 99% | | 102% | | 43% | | 52% | |
| *OsTIFY11c*-FOX | | | | | | | | | | |
| H4-17 (T2, n=4) | 98.6 ± 3.3 | *** | 15.9 ± 2.6 | * | 26.6 ± 7.0 | *** | 11.5 ± 6.4 | *** | 224.6 ± 111.6 | *** |
| | 113% | | 112% | | 138% | | 37% | | 45% | |
| H4-21 (T2, n=5) | 98.9 ± 2.5 | *** | 13.9 ± 0.6 | | 18.5 ± 1.4 | | 23.4 ± 9.0 | * | 439.1 ± 145.4 | |
| | 114% | | 98% | | 96% | | 75% | | 88% | |
| *OsTIFY11d*-FOX | | | | | | | | | | |
| H1-19 (T2, n=5) | 91.9 ± 9.9 | | 15.7 ± 1.6 | ** | 24.2 ± 7.1 | * | 17.0 ± 12.4 | *** | 297.6 ± 219.9 | ** |
| | 106% | | 110% | | 125% | | 54% | | 60% | |
| H1-37 (T2, n=3) | 103.0 ± 4.4 | *** | 17.2 ± 1.8 | *** | 30.4 ± 8.5 | *** | 32.3 ± 2.1 | | 591.4 ± 60.6 | |
| | 118% | | 121% | | 158% | | 103% | | 119% | |
| *OsTIFY11e*-FOX | | | | | | | | | | |
| H2-22 (T2, n=5) | 87.8 ± 5.4 | | 15.0 ± 0.6 | * | 19.6 ± 1.8 | | 25.8 ± 7.2 | | 475.7 ± 120.4 | |
| | 101% | | 105% | | 102% | | 82% | | 96% | |
| H2-25 (T2, n=5) | 87.4 ± 3.1 | | 16.1 ± 0.9 | *** | 24.6 ± 3.0 | *** | 32.6 ± 7.3 | | 583.7 ± 147.4 | |
| | 101% | | 114% | | 127% | | 104% | | 117% | |
| *OsTIFY10a*-FOX | | | | | | | | | | |
| H7-18 (T2, n=5) | 92.8 ± 5.2 | * | 16.5 ± 1.4 | *** | 25.5 ± 9.4 | * | 21.4 ± 13.7 | * | 372.6 ± 228.5 | |
| | 107% | | 116% | | 132% | | 68% | | 75% | |
| H7-29 (T2, n=5) | 92.2 ± 5.4 | | 15.0 ± 1.1 | | 23.5 ± 3.6 | ** | 19.6 ± 8.2 | *** | 322.2 ± 147.8 | ** |
| | 106% | | 105% | | 122% | | 63% | | 65% | |
| *OsTIFY10b*-FOX | | | | | | | | | | |
| H6-2 (T2, n=5) | 97.8 ± 4.8 | *** | 14.9 ± 1.4 | | 22.5 ± 4.3 | * | 35.2 ± 5.8 | | 550.6 ± 107.0 | |
| | 113% | | 105% | | 116% | | 112% | | 111% | |
| H6-25 (T2, n=5) | 97.4 ± 5.7 | ** | 15.4 ± 1.4 | * | 23.7 ± 3.8 | ** | 32.8 ± 8.3 | | 583.3 ± 155.5 | |
| | 112% | | 108% | | 123% | | 105% | | 117% | |
| *OsTIFY10c*-FOX | | | | | | | | | | |
| H5-16 (T2, n=5) | 95.0 ± 5.6 | ** | 16.4 ± 1.3 | *** | 28.9 ± 8.0 | *** | 23.8 ± 12.4 | * | 362.3 ± 216.3 | |
| | 109% | | 116% | | 150% | | 76% | | 73% | |
| H5-22 (T2, n=5) | 89.1 ± 8.0 | | 15.9 ± 1.5 | *** | 25.6 ± 4.1 | *** | 31.0 ± 8.7 | | 480.3 ± 155.5 | |
| | 103% | | 112% | | 132% | | 99% | | 97% | |
| *OsTIFY9*-FOX | | | | | | | | | | |
| H8-22 (T2, n=5) | 99.2 ± 1.4 | *** | 14.5 ± 1.1 | | 19.4 ± 4.2 | | 26.2 ± 5.1 | | 440.4 ± 80.5 | |
| | 114% | | 102% | | 100% | | 84% | | 89% | |
| H8-34 (T2, n=5) | 93.4 ± 4.6 | * | 15.2 ± 1.0 | * | 23.4 ± 1.4 | ** | 12.4 ± 11.1 | *** | 223.1 ± 201.6 | *** |
| | 107% | | 107% | | 121% | | 40% | | 45% | |
| *OsTIFY3*-FOX | | | | | | | | | | |
| H9-1 (T2, n=5) | 94.4 ± 3.8 | * | 15.5 ± 0.6 | ** | 21.6 ± 1.3 | | 33.0 ± 8.0 | | 593.0 ± 151.1 | |
| | 109% | | 109% | | 112% | | 105% | | 119% | |
| H9-28 (T2, n=5) | 98.2 ± 4.8 | *** | 14.8 ± 1.1 | | 21.5 ± 4.4 | | 29.4 ± 5.4 | | 480.4 ± 95.7 | |
| | 113% | | 104% | | 111% | | 94% | | 97% | |
| WT | | | | | | | | | | |
| Nipponbare (n=23) | 86.9 ± 5.7 | | 14.2 ± 0.7 | | 19.3 ± 2.7 | | 31.3 ± 5.7 | | 497.1 ± 113.2 | |
| | 100% | | 100% | | 100% | | 100% | | 100% | |

Values and error bars indicate means ± SD (n=3-5). Percentages indicate relative values, compared to those of wild-type plants. Asterisks indicate values that are significantly different than those of the wild-type plants, as indicated by *t*-tests: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

**Supplemental Table 5. Amino acid sequence identities of 15 TIFY proteins from rice.**

| | OsTIFY11b | OsTIFY11e | OsTIFY11a | OsTIFY11d | OsTIFY11c | OsTIFY10c | OsTIFY10b | OsTIFY10a | OsTIFY9 | OsTIFY3 | OsTIFY6b | OsTIFY6a | OsTIFY8 | OsTIFY1a | OsTIFY2a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OsTIFY11b | 100 | | | | | | | | | | | | | | |
| OsTIFY11e | 25.7 | 100 | | | | | | | | | | | | | |
| OsTIFY11a | 18.5 | 23.8 | 100 | | | | | | | | | | | | |
| OsTIFY11d | 24.5 | 26.1 | 25.9 | 100 | | | | | | | | | | | |
| OsTIFY11c | 25.8 | 23.8 | 16.0 | 19.3 | 100 | | | | | | | | | | |
| OsTIFY10c | 22.0 | 20.0 | 21.9 | 17.6 | 19.4 | 100 | | | | | | | | | |
| OsTIFY10b | 25.6 | 25.0 | 17.2 | 26.1 | 20.3 | 27.1 | 100 | | | | | | | | |
| OsTIFY10a | 26.2 | 14.9 | 12.4 | 18.3 | 17.1 | 23.4 | 44.4 | 100 | | | | | | | |
| OsTIFY9 | 25.8 | 22.1 | 20.2 | 17.7 | 17.8 | 15.0 | 18.9 | 14.8 | 100 | | | | | | |
| OsTIFY3 | 22.9 | 22.3 | 21.2 | 22.3 | 18.9 | 21.9 | 18.7 | 19.1 | 17.3 | 100 | | | | | |
| OsTIFY6b | 18.5 | 23.8 | 16.5 | 16.7 | 16.4 | 13.4 | 21.5 | 16.2 | 15.4 | 20.4 | 100 | | | | |
| OsTIFY6a | 14.4 | 20.2 | 19.0 | 17.0 | 18.0 | 19.2 | 14.1 | 18.3 | 14.3 | 23.7 | 36.6 | 100 | | | |
| OsTIFY8 | 8.7 | 13.9 | 14.2 | 12.3 | 20.0 | 13.3 | 13.8 | 13.6 | 15.5 | 21.6 | 15.4 | 8.9 | 100 | | |
| OsTIFY1a | 15.6 | 19.6 | 18.7 | 16.2 | 11.9 | 13.1 | 15.8 | 12.0 | 16.9 | 17.7 | 19.8 | 15.6 | 9.7 | 100 | |
| OsTIFY2a | 18.6 | 25.3 | 19.5 | 20.9 | 18.7 | 14.6 | 18.9 | 16.1 | 21.5 | 22.9 | 17.0 | 14.3 | 15.6 | 32.2 | 100 |

Identity was calculated using GENETYX Version 7.0, with predicted amino acid sequences deduced from each OsTIFY cDNA.