

# Overexpression of an F-box Protein Gene Reduces Abiotic Stress Tolerance and Promotes Root Growth in Rice

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**ABSTRACT** As one of the largest gene families, F-box domain proteins have important roles in regulating various developmental processes and stress responses. In this study, we have investigated a rice F-box domain gene, *MAIF1*. The *MAIF1* protein is mainly localized in the plasma membrane and nucleus. *MAIF1* expression is induced rapidly and strongly by abscisic acid (ABA) and abiotic stresses. *MAIF1* expression is also induced in root tips by sucrose, independent of its hydrolytic hexose products, glucose and fructose, and the plant hormones auxin and cytokinin. Overexpression of *MAIF1* reduces rice ABA sensitivity and abiotic stress tolerance and promotes rice root growth. These results suggest that *MAIF1* is involved in multiple signaling pathways in regulating root growth. Growth restraint in plants is an acclimatization strategy against abiotic stress. Our results also suggest that *MAIF1* plays the negative role in response to abiotic stress possibly by regulating root growth.

**Key words:** Rice (*Oryza sativa* L.); F-box domain; abscisic acid; abiotic stress; tolerance; root growth.

## INTRODUCTION

Plants are sessile organisms that adapt themselves to the changing environment through a series of molecular responses. The physiological basis for these molecular responses is the integration of many transduced events into a comprehensive network of signaling pathways, including perception of environmental stress signals, generation of secondary messengers, and transduction of stress signals. In addition to uncovering an increasing number of transcription factors that have important regulatory roles in these signaling pathways, previous studies have revealed multiple signal transduction pathways, such as protein kinase signaling, calcium signaling, ABA signaling, and sugar signaling, that occupy a central place in these transduction networks (Himmelbach et al., 2003; Rolland et al., 2006; Yamaguchi-Shinozaki and Shinozaki, 2006; Nakashima et al., 2009).

F-box proteins, characterized by a conserved F-box motif of approximately 40 amino acids, represents one of the largest protein families, with about 700 members in *Arabidopsis* and rice (Gagne et al., 2002; Jain et al., 2007). As a component

of Skp1-Cullin-F-box (SCF) E3 ubiquitin ligase, the F-box protein has a crucial role in conferring specificity to the complex for appropriate targets (Smalle and Vierstra, 2004). In plants, only a small portion of F-box proteins have been studied and they have been shown to play important roles in regulating various developmental processes and stress responses by integrating almost all phytohormone signaling pathways (Lechner et al., 2006; Dreher and Callis, 2007; Zhang et al., 2008). In rice, two F-box proteins have been characterized. Gibberellin-insensitive dwarf2 acts as a positive regulator of gibberellic acid signaling, and dwarf3 (D3) controls tiller bud activity (Sasaki et al., 2003; Gomi et al., 2004; Ishikawa et al., 2005).

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The monocot cereal rice (*Oryza sativa*) develops an embryonic and post-embryonic root system displaying complex root structures with several types, including primary, lateral, and adventitious roots (Hochholdinger et al., 2004). It is known that the plant hormones, auxin and cytokinin, play important roles in regulating root development. The genes that affect auxin or cytokinin biosynthesis, homeostasis, transport, or signal transduction also affect root development (Aloni et al., 2006; Dello Ioio et al., 2007; De Smet and Jurgens, 2007; McSteen, 2010). In rice, the auxin-regulated genes, *CRL1* and *WOX11*, are crucial regulators of root development (Inukai et al., 2005; Zhao et al., 2009). Besides, ABA and sugars also regulate root growth and development.

Here, we investigated the expression and function of a rice F-box gene (Os02g44990). It has been reported that this F-box gene is induced by abiotic stress and is a potential target of microRNAs (miRNAs) (Jain et al., 2007; Sunkar et al., 2005); therefore, we named it *MAIF1* (miRNAs regulated and abiotic stress induced F-box gene).

## RESULTS

### *MAIF1* Encodes an F-box Domain Protein

*MAIF1* has an uninterrupted ORF of 1131 bp, encoding a 377-amino acid protein. The N-terminal region of *MAIF1* contains an F-box domain, which presents conserved amino acids with those of well known F-box proteins from *Arabidopsis*, yeast, and humans, suggesting that *MAIF1* also functions in conjunction with the E3 ubiquitin ligase complex (Figure 1). The C-terminal domain of F-box proteins, such as Leu-rich repeat, WD-40, or Kelch regions, has been shown to play a role in substrate recognition (Smalle and Vierstra, 2004). In contrast, the C-terminal region of *MAIF1* contains a plant-specific domain (DUF295) with unknown function.

### Expression Patterns of *MAIF1* and Sub-Cellular Localization of *MAIF1*

RNA gel blots could not detect *MAIF1* mRNA expression (data not shown), but reverse transcription–polymerase chain reaction (RT–PCR) analysis demonstrated low levels of *MAIF1* ex-

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MAIF1(27-67)  LPPPELLPLICKKLPDSADFVRFRTVCSAMRDAAPLSDVPPQ
SKP2(100-137) LPPDELLLGIFSCCLCP-ELLKVSQVCKRMVRLASDESLW--
CDC4(278-316) LPPFETSLKIFNNLQFE-DIINSLGVSONMKNKIRKSTSLW-
Cyclin F(35-73) LPEDEVLFHILKULSVE-DILAVRAVHSQKDLVDNHASVW-
UFO(41-80)    LPPPLDRVIAFLPPP-AFRTROVCKRFYSLLFNTFLET
COI1(16-56)  TVDDVIEQVMITYITDPKDRDSASLVCRRFKIDSETREHVT
TIR1(9-47)   FPPPEVLEHVFSFIQLDKDRNSVSLVCKSWYETERWCRRK--

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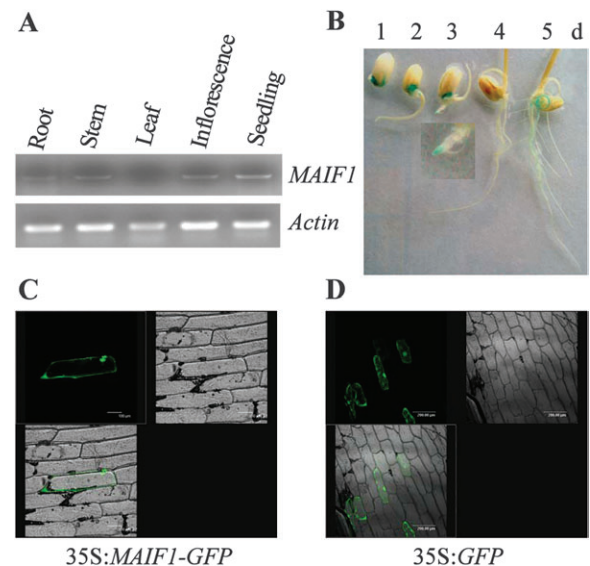
**Figure 1.** *MAIF1* Contains a Conserved F-box Domain.

Approximately 40 amino acids that constitute the F-box motif in *MAIF1* were aligned with comparable regions from F-box-containing proteins in humans (SKP2 and Cyclin F), yeast (CDC4), and *Arabidopsis* (UFO, and COI1, TIR1). Black blocks indicate residues identical to the *MAIF1* sequence, and gray blocks indicate similar amino acids.

pression in roots, stems, inflorescences, and seedlings (Figure 2A). To determine the expression patterns in detail, a 1987-bp DNA fragment upstream of the *MAIF1* ATG start codon was isolated and fused to  $\beta$ -glucuronidase (*GUS*) gene to generate promoter–*GUS* transgenic rice. In *MAIF1* promoter–*GUS* transgenic plants, histochemical staining revealed that *GUS* activity in germinating seeds in the first 3 d. *GUS* activity was also detected at the root tips of 2- and 3-day-old seedlings. At day 4, *GUS* activity was hardly detected in any tissues. At day 5, weak *GUS* activity was detected in primary and adventitious roots (Figure 2B). To examine the sub-cellular localization of *MAIF1*, a green fluorescent protein (GFP) reporter gene was fused in frame to the last codon of the *MAIF1* coding region to produce an *MAIF1*–GFP fusion protein. Epidermal cell transient expression assays in onion (*Allium cepa*) revealed that *MAIF1*–GFP was mainly expressed in the nucleus and plasma membrane comparing to the GFP expression in whole cell (Figure 2C and 2D).

### ABA and Abiotic Stress Induce *MAIF1* mRNA Accumulation

Previous microarray results have shown that *MAIF1* was induced following 3 h of drought, high-salinity, or low-temperature treatment (Jain et al., 2007). To verify the microarray *MAIF1*



**Figure 2.** Expression Patterns of *MAIF1* and Sub-Cellular Localization of *MAIF1*.

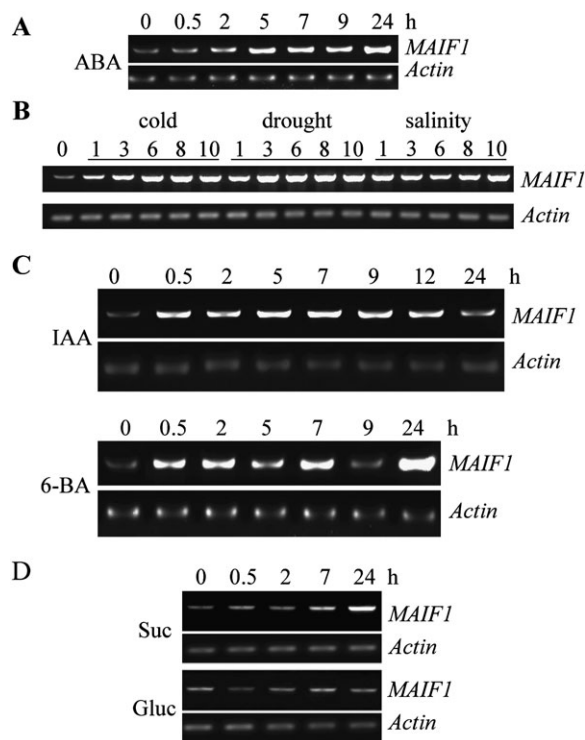
(A) Expression of the *MAIF1* gene in different tissues. Total RNA was isolated from various tissues (root, leaf, stem, inflorescence, and seedling). RT–PCR was performed with either *MAIF1*- or actin-specific primers.

(B) *MAIF1* promoter–*GUS* expression pattern in transgenic rice seeds or seedlings at 1–5 d after germination. The magnified image of the 3-day-old root is under the image of the 3-day-old seedling. (C, D) Sub-cellular localization of *MAIF1* protein. Fluorescent microscopy of transiently transformed epidermal onion cells expressing the 35S promoter-driven *MAIF1*–GFP fusion protein (C) or 35S promoter-driven GFP protein (D).

expression results and check whether *MAIF1* expression was regulated by ABA, 7-day-old rice seedlings were subjected to different stress and ABA treatments. The seedlings were harvested for RNA extraction at different time points during the treatments and the *MAIF1* transcripts were quantified by RT-PCR. The results demonstrated that the transcript level of *MAIF1* was rapidly and strongly induced by these treatments (Figure 3A and 3B). Once we determined that *MAIF1* was up-regulated by ABA and abiotic stress, we tested whether the *MAIF1* promoter was responsible for this up-regulation. We found that GUS activity and *GUS* mRNA was not increased in *MAIF1* promoter-*GUS* transgenic rice after ABA and abiotic stress treatments (data not shown), implying post-transcriptional regulation of *MAIF1* mRNA accumulation, consistent with the hypothesis that *MAIF1* is a potential target for miRNAs (Sunkar et al., 2005).

#### Auxin and Cytokinin Induce *MAIF1* Expression in Root Tips

The transcript levels of *MAIF1* were also checked after treatment with 20  $\mu$ M indole-3-acetic acid (IAA) or 6-benzylaminopurine (6-BA). Analysis by RT-PCR revealed that *MAIF1* expression could be rapidly induced by IAA within 30 min, attaining



**Figure 3.** ABA, Abiotic Stressors, Auxin, Cytokinin, and Sucrose Induce *MAIF1* Expression.

The wild-type rice seedlings at 7 d old were transferred to liquid media containing 20  $\mu$ M ABA (A), IAA, 6-BA (C), or 6% sucrose or glucose (D), or subjected to a cold temperature (4°C), drought conditions, or salt (200 mM NaCl) (B). The seedlings were harvested for RNA extraction at different time points during the treatments, and the *MAIF1* transcripts were quantified by RT-PCR. *Actin* RNA served as an internal control.

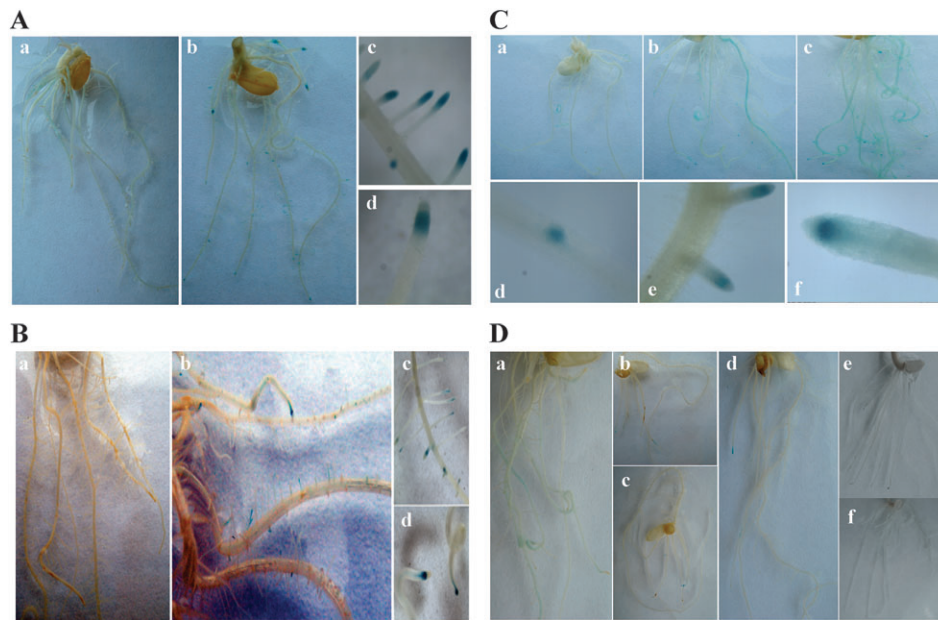
a maximum level after 7 h (Figure 3C). This suggests that *MAIF1* is an early response gene of auxin. By 6-BA treatment, *MAIF1* mRNA accumulation was strongly induced from 30 min to 7 h, recovering to the untreated level after 9 h, and then increasing at 24 h (Figure 3C). To determine the *MAIF1* expression patterns to these signaling molecules in detail, 4-day-old seedlings of *MAIF1* promoter-*GUS* transgenic rice were treated with 1  $\mu$ M naphthalene acetic acid (NAA) or 6-BA. The results showed that after a 3-h NAA treatment, a substantial increase in GUS activity was detected at sites of lateral root formation and the cell division zones of root tips in primary, adventitious, and lateral roots (Figure 4A). To a lesser extent, GUS activity was also detected at the same sites after a 3-h 6-BA treatment (Figure 4B).

#### Sucrose Induces *MAIF1* Expression in Root Tips

Sugars have pivotal roles as signaling molecules in modulating plant growth, development, and stress responses (Rolland et al., 2006). We investigated whether *MAIF1* was a sugar responsive gene. To do this, 7-day-old seedlings of wild-type rice were transferred to liquid media containing 6% sucrose or glucose. The seedlings were harvested for RNA extraction at different time points during the treatments, and *MAIF1* transcripts were quantified by RT-PCR. The analysis showed that glucose hardly affected the *MAIF1* mRNA accumulation, while sucrose treatment increased *MAIF1* mRNA accumulation slightly at 24 h (Figure 3D). To determine *MAIF1* expression patterns to sucrose in detail, 4-day-old seedlings of *MAIF1* promoter-*GUS* transgenic rice were subjected to 3 or 6% sucrose treatment. The results showed that after 2 d of sucrose treatment, a substantial increase in GUS activity was detected at sites of lateral root formation and cell division zones of root tips in primary, adventitious, and lateral roots (Figure 4C), exhibiting the same expression patterns as those induced by NAA and 6-BA. Sucrose signaling effects can be attributed to the action of its hydrolytic hexose products, glucose and fructose (Koch, 2004; Rolland et al., 2006). However, more and more evidence indicates that sucrose regulates specific responses that are not affected by hexose (Smeekens, 2000; Rolland et al., 2006; Ramon et al., 2008). In order to test whether the *MAIF1* response was specific to sucrose, 4-day-old seedlings of *MAIF1* promoter-*GUS* transgenic rice were treated with 3% glucose, 3% fructose, 1.5% glucose plus 1.5% fructose, 3% maltose or 100 mM mannitol; the results showed that *MAIF1* expression hardly responded to these treatments (Figure 4D). Taken together, these observations indicate that sucrose-inducible *MAIF1* expression in root tips was sucrose-specific and not because of glucose, fructose, or osmotic stress.

#### Overexpression of *MAIF1* Promotes Rice Root Growth

The *MAIF1* ORF was inserted into an expression plasmid under the control of the 35S promoter to further investigate the function of *MAIF1*. The plasmid construct was transformed into wild-type rice. Through RT-PCR analysis, it was determined



**Figure 4.** Auxin, Cytokinin, and Sucrose Induce *MAIF1* Promoter–*GUS* Expression in Root Tips.

**(A)** The 4-day-old seedlings of *MAIF1* promoter–*GUS* transgenic rice were subjected to 1  $\mu\text{M}$  NAA. After 3 h of NAA treatment, the seedlings were stained for *GUS* activity (b) and untreated seedlings were used as the control (a). The magnified images demonstrate the lateral root formation site and lateral root tips (c) and primary root tips (d) of treated seedlings.

**(B)** The 4-day-old seedlings of *MAIF1* promoter–*GUS* transgenic rice were subjected to 1  $\mu\text{M}$  6-BA. After 3 h of the 6-BA treatment, the seedlings were stained for *GUS* activity (b), with untreated seedlings used as the control (a). The magnified images show the lateral root formation site and lateral root tips (c) and primary root tips (d) of treated seedlings.

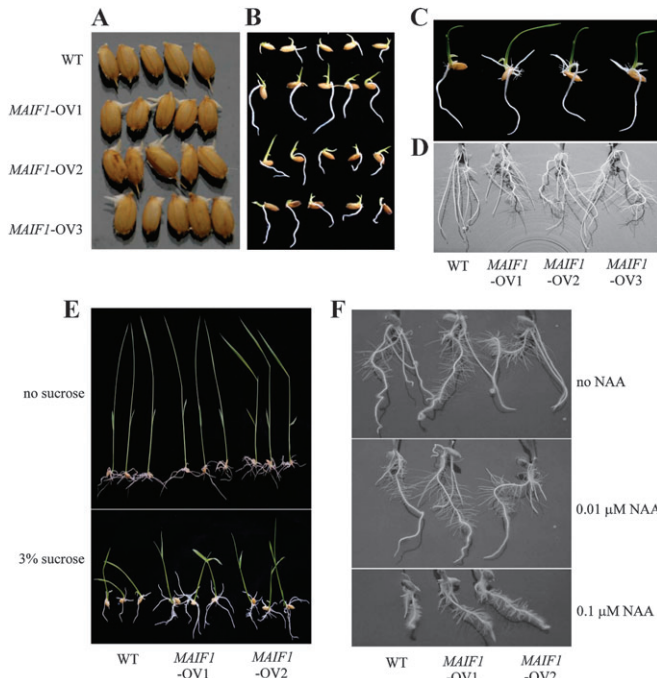
**(C)** The 4-day-old seedlings of *MAIF1* promoter–*GUS* transgenic rice were subjected to 3% (b) or 6% (c) sucrose treatment. Following 2 d of treatment, the seedlings were stained for *GUS* activity with untreated seedlings as a control (a). The magnified images display the lateral root formation site (d), lateral root tips (e), and primary root tips (f) of treated seedlings.

**(D)** The 4-day-old seedlings of *MAIF1* promoter–*GUS* transgenic rice were subjected to 3% glucose (b), 3% fructose (c), 1.5% glucose and 1.5% fructose (d), 3% maltose (e), or 100 mM mannitol (f) treatments. After 2 d of treatment, the seedlings were stained for *GUS* activity with untreated seedlings as the control (a).

that *MAIF1* mRNA accumulation increased in *MAIF1* transgenic plants (data not shown). Homozygous transgenic lines of the T2 generation were used to measure the growth phenotypes. The results indicated that overexpression of *MAIF1* promoted rice root growth. When seeds were soaked in water in the dark for 2 d, coleoptiles of *MAIF1*-overexpressed seeds emerged earlier compared with the wild-type (Figure 5A). Seeds were then transferred to a nutrient medium to investigate the growth under light/dark cycle conditions. After 2 d of growth, *MAIF1*-overexpressed lines appeared to grow faster than wild-types and had longer primary roots (Figure 5B). After 4 d of growth, in contrast to the wild-type, *MAIF1*-overexpressed lines formed lateral and adventitious roots earlier (Figure 5C). After 7 d of growth, *MAIF1*-overexpressed lines demonstrated more and longer lateral roots than the wild-type (Figure 5D). These observations indicate that an increase in *MAIF1* expression promotes root growth. We also assessed the effects of *MAIF1* overexpression in plants treated with sucrose and auxin. The results indicated that overexpression of *MAIF1* reduced the inhibitory effects of sucrose and auxin on root growth (Figure 5E and 5F).

#### Overexpression of *MAIF1* Reduces Rice ABA Sensitivity

We have shown that ABA strongly induces *MAIF1* expression, providing clues for the regulatory role of *MAIF1* in ABA signaling. The results indicated that transgenic plants overexpressing *MAIF1* reduced ABA sensitivity during seed germination. In the ABA-free medium, the *MAIF1* transgenic plants exhibited normal seed germination rates, similar to wild-type rice plants. However, in the medium containing 3  $\mu\text{M}$  ABA, about 60% of seeds from *MAIF1* overexpressing transgenic plants germinated, whereas the germination of wild-type rice seeds was severely inhibited, with about 30% of seeds germinating (Figure 6A). We also investigated their response to ABA treatment during seedling growth. As shown in Figure 6B, differences between them and wild-type plants were observed mainly in the root morpha. In the ABA-free medium, roots of transgenic seedlings overexpressing *MAIF1* grew slightly faster than those of the wild-type plants. In the presence of 3  $\mu\text{M}$  ABA, the growth of roots, especially lateral roots of wild-type plants, was severely inhibited, whereas root growth in transgenic plants was hardly affected. In the medium



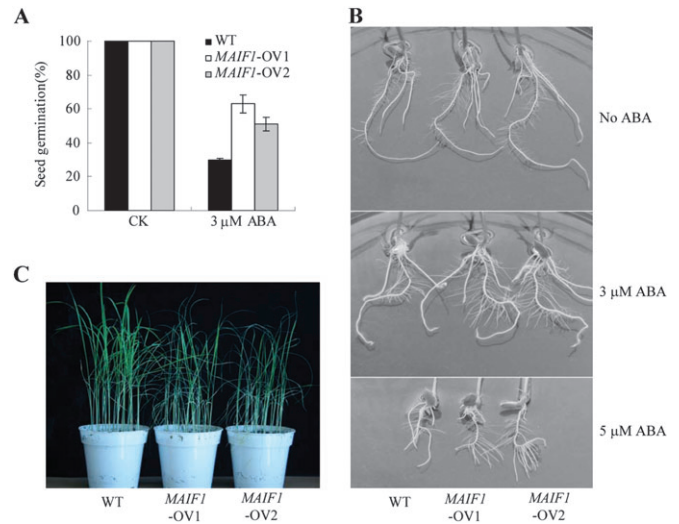
**Figure 5.** Phenotypes of Overexpressing *MAIF1* Rice Plants.

Seeds of the wild-type and transgenic lines were soaked in water in the dark for 2 d (A), then transferred to nutrition medium for growth over 2 (B), 5 (C), and 7 d (D), or into nutrition medium containing 3% sucrose (E), or 0.1 or 0.01  $\mu\text{M}$  NAA (F). Phenotypes of seedling growth were observed.

containing 5  $\mu\text{M}$  ABA, growth of adventitious and lateral roots in wild-type plants was totally inhibited but the transgenic plants still exhibited short adventitious and lateral roots. These results indicated that transgenic seedlings overexpressing *MAIF1* were hyposensitive to ABA during root growth when compared to the wild-type rice seedlings. The above observations indicate that *MAIF1* has a negative role in ABA signaling during rice seed germination and root growth.

#### Overexpression of *MAIF1* Reduces Rice Tolerance to Abiotic Stresses

The strong induction of *MAIF1* expression by abiotic stress suggested that this gene might be involved in stress tolerance. We assessed the response of the transgenic plants to abiotic stressors. The results showed that the transgenic plants were more sensitive than the wild-types to drought stress. Following 12 d of water-withholding conditions, the majority of transgenic plants were withered, but wild-type plants grew continuously. After 1 d of re-watering, only a small fraction of the transgenic plants survived whereas almost all of the wild-type plants recovered (Figure 6C). Similarly, the transgenic plants exhibited more sensitive phenotypes than wild-type plants subjected to salt or cold treatment (data not shown). These results indicate that *MAIF1* has a negative role in rice tolerance to abiotic stressors.



**Figure 6.** Overexpression of *MAIF1* Reduces the Sensitivity of Rice to ABA and Drought Tolerance.

(A) Plants overexpressing *MAIF1* are less sensitive to ABA than wild-type plants during seed germination. Following 1 d of soaking, rice seeds were transferred to ABA-free medium (CK) or 3  $\mu\text{M}$  ABA-containing medium for germination. Germination rate was measured 3 d after transfer. Error bars represent the standard error (triplicate measurements;  $n = 60$ ).

(B) Root growth of seedlings overexpressing *MAIF1* and wild-type seedlings in ABA-containing medium at the post-germination stage. The 2-day-old seedlings were kept on or transferred from ABA-free medium to medium with 3 or 5  $\mu\text{M}$  ABA to grow for 5 d. (C) Drought response of seedlings overexpressing *MAIF1* and wild-type seedlings. Seedlings of each genotype were planted in barrels, in triplicate, with each barrel containing 25 seedlings. Drought stress was initiated at the four-leaf stage and conducted by withholding water for 12 d followed by 1 d of re-watering.

## DISCUSSION

### *MAIF1* Is a Sucrose-Responsive Gene

Sugars not only fuel cellular carbon and energy metabolism, but also have pivotal roles as signaling molecules for the modulation of plant growth, development, and stress responses (Rolland et al., 2006). It has been shown that the glucose sensor, hexokinase (HXK1), and nuclear HXK1 complex have important regulatory functions in governing gene expression and plant stress responses in *Arabidopsis* (Moore et al., 2003; Cho et al., 2006). Sucrose signaling effects on growth and metabolism can be attributed to the action of its hydrolytic hexose products, glucose and fructose (Koch, 2004; Rolland et al., 2006). However, increasing evidence indicates that sucrose regulates specific responses that are not affected by hexose (Smeekens, 2000; Rolland et al., 2006; Ramon et al., 2008). A sucrose transporter like protein, SUT2, has been suggested to be a sucrose sensor (Barker et al., 2000). In a number of genes whose expression is regulated by sucrose, but not by glucose or fructose, and several sucrose-responsive *cis*-elements, including the sucrose-responsive element (SURE), A- and B-boxes and the TGGACGG element, have been

identified (Vaughn et al., 2002; Rolland et al., 2002). High level of sucrose specifically represses translation of the *Arabidopsis* S-class bZIP, which is dependent on the unusually long 5' UTR of the ATB2/bZIP11 transcript (Rook et al., 1998). However, the actual sucrose signaling mechanism is still largely unknown, especially in rice. *MAIF1* expression is induced by sucrose, but not by glucose and fructose (Figures 3D, 4C, and 4D), suggesting that *MAIF1* is a new component of the signaling pathway mediated by sucrose.

### ***MAIF1* Is Involved in Multiple Signaling Pathways in Regulating Root Growth**

Signal molecules, such as plant hormones and sugars, have crucial roles in controlling plant growth and development, and adapting to stress. It has also been shown that there is widespread cooperation of different signaling molecules, which is very important for plants in regulating these physiological and environmental processes precisely. Cytokinin and auxin are known to act antagonistically in controlling meristem activities. Interactions between auxin- and ABA-dependent responses have been described in regulating root growth, embryogenesis, and seed germination (Ni et al., 2001; Brady et al., 2003; Rock and Sun, 2005; Liu et al., 2007; Belin et al., 2009). Genetic and molecular studies revealed many sugar signaling mutants overlapped with ABA or ethylene signaling components (Smeekens, 2000; Gazzarrini and McCourt, 2001; León and Sheen, 2003; Ramon et al., 2008). Cross-talk of sugar with auxin and cytokinin in regulating the plant cycle has also been shown (Hartig and Beck, 2006). However, the important cross-talk points of these signaling pathways are largely unknown, especially in rice. We have demonstrated in this study that ABA, cytokinin, auxin, and sucrose all induced *MAIF1* expression (Figure 3). Cytokinin, auxin, and sucrose all induced *MAIF1* expression in the same tissue of root tips (Figure 4). Furthermore, overexpression of *MAIF1* reduced the inhibition of these signal molecules on rice root growth. These results indicated that *MAIF1* is involved in multiple signaling pathways in regulating root growth.

### **Overexpression of *MAIF1* Reduces Rice Tolerance to Abiotic Stress Possibly by Increasing Cell Division in Roots**

Plants have developed an extensive array of defensive responses, which are linked to an array of morphological, physiological, and biochemical responses that decrease stress exposure, limit damage, or facilitate repair of damaged systems (Xiong et al., 2002; Mahajan and Tuteja, 2005). Relatively little attention has been paid to abiotic stressors inducing morphogenic responses that comprise a mixture of growth inhibition and activation (Potters et al., 2007). It has been suggested that morphogenic responses are an acclimatization strategy to abiotic stress (Potters et al., 2007). For example, it has been described that the growth restraint conferred by DELLA proteins is beneficial and promotes survival under abiotic stress conditions (Achard et al., 2006). *MAIF1* expression is induced during cell division of root tips (Figure 4), and overexpression of *MAIF1* promotes root growth and reduces the inhibition of

abiotic stressors (Figure 5). This implies that under abiotic stress conditions, *MAIF1*-overexpressing plants lose the growth restraint. Indeed, *MAIF1*-overexpressing plants showed reduced inhibition of root growth and tolerance under salt stress condition (data not shown). So, we propose that reduced growth restraint by increasing cell division in roots is a possible reason leading to reduce the tolerance of *MAIF1*-overexpressing rice, though more data are needed to confirm this hypothesis.

## **METHODS**

### **Plant Materials and Growth Conditions**

Rice (*Oryza sativa*) subsp. *japonica* (Nipponbare) was used as the wild-type and the genetic background for transgenic plants. Plants were grown in half-strength MS nutrient solution at 25°C with a 16/8-h light/dark photoperiod and 70–80% relative humidity.

### **Plasmid Constructs and Generation of Transgenic Plants**

The ORF fragment of *MAIF1* was amplified directly from genomic DNA because of no intron in this gene and was cloned into pCAMBIA1300 containing the 35S promoter. For construction of the *MAIF1* promoter–*GUS* construct, a 1987-bp promoter region upstream of the *MAIF1* ATG start codon was amplified from genomic DNA. This fragment was cloned into pCAMBIA1300 and fused with the coding sequence of *GUS*. Constructs were electroporated into *Agrobacterium tumefaciens* EHA105, which was used to transform wild-type rice.

### **RT-PCR Analysis**

Total RNA was isolated from different tissues to examine the expression of *MAIF1*. In order to test *MAIF1* response to sugars and hormones, 7-day-old seedlings were treated with 6% glucose, 6% sucrose, 20 μM IAA, or 20 μM 6-BA. Seedlings were harvested at different times for RNA isolation and untreated seedlings were used as controls. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). For the RT-PCR, RNase free DNase I-treated total RNA (3 μg) was subjected to a RT reaction using SuperScript III following the manufacturer's instructions (Invitrogen). Input RNA was normalized for each reaction using actin primers and *MAIF1* amplification was performed with specific primers (5'-CCCCGGATTGTGCCCTCTG-3' and 5'-TGGCCTTCTGTGTTCTCTAGT-3').

### **Sub-Cellular Localization**

For transient expression in onion (*Allium cepa*) epidermal cells, a *MAIF1*–*GFP* fusion construct was made with the pBI121 vector, which utilized a 35S promoter. After bombardment, the onion epidermis was incubated in MS medium for 16 h in the dark and visualized using a fluorescence microscope.

### **Histochemical Detection of GUS Activity**

Seeds and young seedlings at different developmental stages were collected from *MAIF1* promoter–*GUS* transgenic plants to

determine *MAIF1* expression patterns. Transgenic seedlings were treated as described in the results section to test the induction of GUS expression by sugars and hormones. Untreated transgenic seedlings were used as a control. For GUS staining, materials were incubated overnight at 37°C in 0.5 mg ml<sup>-1</sup> 5-bromo-4-chloro-3-indolyl-glucuronic acid, 1 mM potassium ferricyanide, 1 mM potassium ferrocyanide, 0.1% Triton X-100, 20% methanol, and 0.1 M sodium phosphate buffer (pH 7.0). Samples were then cleared with 70% ethanol.

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No conflict of interest declared.

## REFERENCES

- Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J., and Harberd, N.P. (2006). Integration of plant responses to environmentally activated phytohormonal signals. *Science*, **311**, 91–94.
- Aloni, R., Aloni, E., Langhans, M., and Ullrich, C.C. (2006). Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Ann. Bot.* **97**, 883–893.
- Barker, L., Kühn, C., Weise, A., Schulz, A., Gebhardt, C., Hirner, B., Hellmann, H., Schulze, W., Ward, J.M., and Frommer, W.B. (2000). SUT2, a putative sucrose sensor in sieve elements. *Plant Cell*, **12**, 1153–1164.
- Belin, C., Megies, C., Hauserová, E., and Lopez-Molina, L. (2009). Abscisic acid represses growth of the *Arabidopsis* embryonic axis after germination by enhancing auxin signaling. *Plant Cell*, **21**, 2253–2268.
- Brady, S.M., Sarkar, S.F., Bonetta, D., and McCourt, P. (2003). The ABCISIC ACID INSENSITIVE 3 (ABI3) gene is modulated by farne-sylation and is involved in auxin signaling and lateral root development in *Arabidopsis*. *Plant J.* **34**, 67–75.
- Cho, Y.H., Yoo, S.D., and Sheen, J. (2006). Regulatory functions of nuclear hexokinase1 complex in glucose signaling. *Cell*, **127**, 579–589.
- De Smet, I., and Jurgens, G. (2007). Patterning the axis in plants—auxin in control. *Curr. Opin. Genet. Dev.* **17**, 337–343.
- Dello Ioio, R., Linhares, F.S., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Costantino, P., and Sabatini, S. (2007). Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Curr. Biol.* **17**, 678–682.
- Dreher, K., and Callis, J. (2007). Ubiquitin, hormones and biotic stress in plants. *Ann. Bot. (Lond.)*, **99**, 787–822.
- Gagne, J.M., Downes, B.P., Shiu, S.H., Durski, A.M., and Vierstra, R.D. (2002). The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in *Arabidopsis*. *Proc. Natl Acad. Sci. U S A.* **99**, 11519–11524.
- Gazzarrini, S., and McCourt, P. (2001). Genetic interactions between ABA, ethylene and sugar signaling pathways. *Curr. Opin. Plant Biol.* **4**, 387–391.
- Gomi, K., Sasaki, A., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., Kitano, H., and Matsuoka, M. (2004). *GID2*, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. *Plant J.* **37**, 626–634.
- Hartig, K., and Beck, E. (2006). Crosstalk between auxin, cytokinins, and sugars in the plant cell cycle. *Plant Biol.* **8**, 389–396.
- Himmelbach, A., Yang, Y., and Grill, E. (2003). Relay and control of abscisic acid signaling. *Curr. Opin. Plant Biol.* **6**, 470–479.
- Hochholdinger, F., Park, W.J., Sauer, M., and Woll, K. (2004). From weeds to crops: genetic analysis of root development in cereals. *Trends Plant Sci.* **9**, 42–48.
- Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Shibata, Y., Gomi, K., Umemura, I., Hasegawa, Y., Ashikari, M., Kitano, H., and Matsuoka, M. (2005). Crown rootless1, which is essential for crown root formation in rice, is a target of an AUXIN RESPONSE FACTOR in auxin signaling. *Plant Cell*, **17**, 1387–1396.
- Ishikawa, S., Maekawa, M., Arite, T., Onishi, K., Takamura, I., and Kyo-zuka, J. (2005). Suppression of tiller bud activity in tillering dwarf mutants of rice. *Plant Cell Physiol.* **46**, 79–86.
- Jain, M., Nijhawan, A., Arora, R., Agarwal, P., Ray, S., Sharma, P., Kapoor, S., Tyagi, A.K., and Khurana, J.P. (2007). F-Box proteins in rice: genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. *Plant Physiol.* **143**, 1467–1483.
- Koch, K. (2004). Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr. Opin. Plant Biol.* **7**, 235–246.
- Lechner, E., Achard, P., Vansiri, A., Potuschak, T., and Genschik, P. (2006). F-box proteins everywhere. *Curr. Opin. Plant Biol.* **9**, 631–638.
- León, P., and Sheen, J. (2003). Sugar and hormone connections. *Trends Plant Sci.* **8**, 110–116.
- Liu, P.P., Montgomery, T.A., Fahlgren, N., Kasschau, K.D., Nonogaki, H., and Carrington, J.C. (2007). Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and postgermination stages. *Plant J.* **52**, 133–146.
- Mahajan, S., and Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics*, **444**, 139–158.
- McSteen, P. (2010). Auxin and monocot development. *Cold Spring Harb. Perspect. Biol.* **2**, a001479.
- Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.H., Liu, Y.X., Hwang, I., Jones, T., and Sheen, J. (2003). Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science*, **300**, 332–336.
- Nakashima, K., Ito, Y., and Yamaguchi-Shinozaki, K. (2009). Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. *Plant Physiol.* **149**, 88–95.
- Ni, D.A., Wang, L.J., Ding, C.H., and Xu, Z.H. (2001). Auxin distribution and transport during embryogenesis and seed germination of *Arabidopsis*. *Cell Res.* **11**, 273–278.
- Potters, G., Pasternak, T.P., Guisez, Y., Palme, K.J., and Jansen, M.A.K. (2007). Stress-induced morphogenic responses: growing out of trouble? *Trends Plant Sci.* **12**, 98–105.

- Ramon, M., Rolland, F., and Sheen, J. (2008). Sugar sensing and signaling. *The Arabidopsis Book* (: TAB). 1543–8120 (American Society of Plant Biologists), pp. 1–22.
- Rock, C.D., and Sun, X. (2005). Crosstalk between ABA and auxin signaling pathways in roots of *Arabidopsis thaliana* (L.) Heynh. *Planta*. **222**, 98–106.
- Rolland, F., Baena-Gonzalez, E., and Sheen, J. (2006). Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu. Rev. Plant Biol.* **57**, 675–709.
- Rolland, F., Moore, B., and Sheen, J. (2002). Sugar sensing and signaling in plants. *Plant Cell*. **14** (Suppl.), S185–S205.
- Rook, F., et al. (1998). Sucrose-specific signalling represses translation of the *Arabidopsis* ATB2 bZIP transcription factor gene. *Plant J.* **15**, 253–263.
- Sasaki, A., et al. (2003). Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. *Science*. **299**, 1896–1898.
- Smalle, J., and Vierstra, R.D. (2004). The ubiquitin 26S proteasome proteolytic pathway. *Annu. Rev. Plant Biol.* **55**, 555–590.
- Smeekens, S. (2000). Sugar-induced signal transduction in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**, 49–81.
- Sunkar, R., Girke, T., Jain, P.K., and Zhu, J.K. (2005). Cloning and characterization of microRNAs from rice. *Plant Cell*. **17**, 1397–1411.
- Vaughn, M.W., Harrington, G.N., and Bush, D.R. (2002). Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem. *Proc. Natl Acad. Sci. U S A*. **99**, 10876–10880.
- Xiong, L., Schumaker, K.S., and Zhu, J.K. (2002). Cell signaling during cold, drought, and salt stress. *Plant Cell*. **14**, S165–S183.
- Yamaguchi-Shinozaki, K., and Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* **57**, 781–803.
- Zhang, Y., Xu, W., Li, Z., Deng, X., Wu, W., and Xue, Y. (2008). F-box protein DOR functions as a novel inhibitory factor for ABA-induced stomatal closure under drought stress in *Arabidopsis thaliana*. *Plant Physiol.* **148**, 2121–2133.
- Zhao, Y., Hu, Y., Dai, M., Huang, L., and Zhou, D.X. (2009). The WUSCHEL-related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. *Plant Cell*. **21**, 736–748.