

Expression of signalling and defence-related genes mediated by over-expression of *JERF1*, and increased resistance to sheath blight in rice

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Sheath blight (SB) is one of the most destructive rice diseases that affect the yield and quality of rice. *Jasmonate and Ethylene Response Factor 1*, designated *JERF1*, can be induced by ethylene, methyl jasmonate (MeJA), abscisic acid (ABA) and salt stress. Previous research demonstrated that over-expression of *JERF1* enhanced drought tolerance by regulating the expression of stress-related genes in rice. The present study reports that *JERF1* could improve SB resistance of transgenic rice. At the molecular and physiological levels, *JERF1* could up-regulate the expression of signalling and defence-related genes and increase the activities of lipoxygenase (LOX) and phenylalanine ammonia lyase (PAL). Analysis of *cis*-elements showed that there were several DRE/CRT (dehydration-responsive element/C-repeat) and/or GCC boxes in the promoters of these candidate genes, indicating that *JERF1* might regulate the expression of defence-related genes via interaction with these *cis*-elements. These results suggest that *JERF1* might improve SB resistance by modulating the expression of defence-related genes. This may prove to be a useful strategy for genetic engineering to improve SB resistance using transcription factor genes in rice.

Keywords: Defence-related genes, expression, *JERF1*, rice, sheath blight, signalling

Introduction

Rice (*Oryza sativa*) is a staple food crop worldwide, but it experiences various abiotic and biotic stresses. Sheath blight (SB), caused by the soilborne fungus *Rhizoctonia solani*, is one of the most destructive rice diseases worldwide, resulting in severe loss of yield and quality in rice production (Tan *et al.*, 2007). Moreover, its damage has become more serious as a result of the widespread use of semi-dwarf, nitrogen-responsive and high-yielding varieties (Kagale *et al.*, 2011). Although chemical control is an effective method to cope with SB, it is unsustainable because of high cost and serious environmental pollution. In an attempt to breed resistant varieties, many rice germplasms have been identified as moderately resistant to SB; however, no completely resistant varieties have been obtained so far (Jia *et al.*, 2011). Moreover, increasingly more resistance quantitative trait loci (QTL) to SB have been mapped onto corresponding chromosomes (Xu *et al.*, 2011). Recently, numerous rice germplasm resources containing introgressed SB resistance alleles were created through marker-

assisted selection. However, these strategies are neither labour-saving nor reliable, because of strong associations between morphological traits and SB resistance (Willoquet *et al.*, 2012).

Transgenic technology is one of the most practical and effective ways to develop new rice varieties with better resistance to SB. Available gene resources can be divided into functional genes and regulatory genes. For instance, over-expression of endogenous functional genes encoding chitinase and ribosome-inactivating proteins conferred transgenic rice with enhanced resistance to SB (Kim *et al.*, 2003). However, the enhancement of disease resistance was limited depending on transgene source, plant species and pathogen sensitivity (van Loon *et al.*, 2006). Simultaneous expression of chitinase and LTPs (encoding lipid transfer proteins) in carrot rendered significantly higher resistance to pathogens compared to expression of one gene (Jayaraj & Punja, 2007). Because transcription factors can regulate a battery of downstream defence-related genes, the genetic engineering of key regulatory genes appears to be one of the most promising strategies to develop resistant varieties. For example, the transcription activator *BjNPR1* was reported to enhance resistance to *R. solani* in mungbean (Vijayan & Kirti, 2012).

Accumulated evidence has shown that ethylene response factor (ERF) proteins play an important role in regulating plant development processes and abiotic stress responses through interaction with multiple *cis*-elements and mediating the expression of a considerable number

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of genes (Mizoi *et al.*, 2012). For example, the ERF proteins SNORKEL1 and SNORKEL2 are involved in ethylene signalling and mediate rice adaptation to deep-water stress by triggering remarkable internode elongation (Hattori *et al.*, 2009). In addition, several ERF transcription factors play crucial roles in plant resistance to disease by regulating defence-related genes. For instance, over-expression of tomato genes *Pti4*, *Pti5* and *Pti6* increases *Arabidopsis* resistance to fungal and bacterial pathogens by regulating the expression of some pathogenesis-related (PR) genes (Gu *et al.*, 2002). Similarly, expression of *MtERF1-1* in *Medicago truncatula* and tomato *OPBP1* in rice enhances resistance to *R. solani* pathogens (Chen & Guo, 2008; Anderson *et al.*, 2010).

Jasmonate and Ethylene Response Factor 1 (*JERF1*) encodes an ERF transcription factor, identified from tomato, which can interact with the GCC-box and DRE (dehydration-responsive element) motif (Zhang *et al.*, 2004). Ectopic expression of *JERF1* could activate expression of stress-related genes and results in an increased tolerance to salinity and cold stresses in tobacco (Wu *et al.*, 2007). Similarly, over-expression of *JERF1* confers transgenic rice with improved drought tolerance by regulating the synthesis of ABA and the accumulation of proline (Zhang *et al.*, 2010). The present investigation reports that over-expression of *JERF1* could enhance rice resistance to SB by tuning the expression of defence-related genes associated with increasing the activities of lipoxygenase (LOX) and phenylalanine ammonia lyase (PAL).

Materials and methods

Plant materials and growth conditions

The seeds of wildtype (WT) Nipponbare and *JERF1*-over-expressing (OE) rice lines were germinated at 32°C for 2 days, and then grown in a growth chamber at 25°C with a 14 h light/10 h dark photoperiod. Quarter-strength Yoshida nutrition was used at the seedling stage (Yoshida *et al.*, 1971). The rice leaves were collected at the three-leaf stage for RNA extraction and enzyme activity assays.

Sheath blight inoculation

The controlled environment microchamber assay has proven to be a reliable method to evaluate varietal resistance to SB (Jia *et al.*, 2007). Here, both field inoculation and the microchamber assay were used to check the SB resistance of WT and OE plants. The *R. solani* isolate C30 with strong pathogenicity was used for inoculation. Active inoculums were produced by transferring mycelium to fresh potato dextrose agar (PDA) medium and culturing at 28°C for 3 days. Uniform mycelia discs (7 mm diameter) were excised from PDA plates using a pipette tip and were used for inoculation.

For the microchamber inoculation assay, 16 seedlings with similar vigour were selected and planted 2 cm apart in a plastic pot filled with sterilized soil. At the three-leaf stage, a colonized disc was fixed at the base of each rice seedling and the pot was covered with a 2.8 L clear plastic bottle with bottom and cap

previously removed, forming a microchamber. Cultivar Lemont was included in all experiments as the susceptible control. The greenhouse temperatures for day and night averaged 30 and 25°C, respectively. Disease lesions appeared at 3 days post-inoculation (dpi) and became more apparent *c.* 7–10 dpi. The disease severity was quantified as described by Jia *et al.* (2007). Nine diseased seedlings were cut at ground level and the lengths of the lesion and culm were measured using a ruler. A disease index was calculated as: $9 \times \text{lesion length/plant height}$ at 7 dpi. Each assay was then repeated three times as three replicates.

For field inoculations, rice plants were grown at the Changsha Lowland Experimental Station (28°11'N, 113°05'E) using a randomized complete block design with three replications. Thirty-day-old seedlings were transplanted in the experimental field. Three leaf sheaths per plant were used for inoculation at the late tillering stage. Using forceps, the inoculum was inserted into the collar of the third leaf from the flag leaf, to prevent it falling to the ground. To create ideal conditions for disease development, overhead sprinklers were used to maintain a high humidity level. Disease scores were measured at 30 dpi when plants were at late grain-filling stage. The lesion length around the sclerotium was measured and the disease index was rated as above, as $9 \times \text{total length of lesions on one plant/plant height}$.

RNA isolation and qPCR

In the microchamber inoculation assay, leaves were collected from uninoculated and inoculated seedlings at 2 dpi and frozen in liquid nitrogen. Total RNA was isolated from frozen samples using TRIzol (Invitrogen). After digestion with RNase-free DNase I (Invitrogen), *c.* 1 µg total RNA was used for cDNA synthesis in a 50 µL volume using M-MLV reverse transcriptase (Invitrogen) following the manufacturer's instructions. Subsequently, the cDNA was used to perform RT-PCR and quantitative PCR. For RT-PCR analysis, 1 µL cDNA was used as template in each PCR reaction (25 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72°C). *Actin* was used as a control for RT-PCR experiments. For qPCR, experiments were conducted on a four-colour real-time PCR system (IQ5, Bio-Rad) using SYBR Green real-time Master Mix (Takara) with gene-specific primers as given in Table 1. *Actin* was used as the reference gene. The relative gene expression was evaluated using the comparative cycle threshold method.

Determination of LOX and PAL activity

Leaves (0.5 g) of three-leaf stage seedlings were collected and homogenized in 5 mL 100 mM sodium phosphate buffer at pH 7.0, containing 2% (w/v) polyvinylpyrrolidone. After centrifugation at 10 000 g and 4°C for 15 min, the supernatant was used as a crude enzyme solution for measuring LOX and PAL activity. The concentration of total protein was determined using a NanoPhotometer (IMPLEN).

The determination of PAL activity was described by Reddy *et al.* (1994) with some modifications: the 2 mL reaction mixture contained 5 mM phenylalanine, 0.1 M sodium borate buffer at pH 8.7 and crude enzyme extract. The PAL activity was measured by monitoring the increase in OD₂₉₀ against a blank control without phenylalanine at 30°C. One unit of enzyme activity was defined as an increase in OD₂₉₀ of 0.01 min⁻¹. The assay of LOX activity was based on conjugated diene formation (Gökmen *et al.*, 2002). Substrate solution was prepared by dissolving linoleic acid (Sigma) in deionized water with 0.1%

Table 1 Primers for expression analysis of *JERF1* and defence-related genes

Gene	Accession number	Forward primer (5'–3')	Reverse primer (5'–3')	Size (bp)
<i>OsActin</i>	NM_001057621	CATTGGTGCTGAGCGTTTC	AGAAACAAGCAGGAGGACGG	445
<i>JERF1</i>	NM_001247584	ACTGCTCTGATTTTCGGTTGG	GCAAGAACTTCATCTGGGATTC	222
<i>OsGlu</i>	NM_001050587	CCCAATAAGTCGCCATCGT	ACACCACACGGCACTATGCT	135
<i>OsBBI</i>	NM_001185225	TCTGCCAGGACCAGTTCAC	TTGCTCTTGGTCACTGGCTA	293
<i>OsPR10</i>	NM_001056392	CGGACGCTTACAACATAATCG	GACAGCAACCAACCACACA	157
<i>OsLOX1</i>	NM_001057530	GGATGTCACCAAGGAGAAGG	CCTGAGACCCTGAACACGAA	197
<i>OsPAL1</i>	NM_001054017	CGGTGAAGAACTGCGTGA	TGGCGAAGATGGAGGTCT	249
<i>OsPAL2</i>	NM_001054018	CAACGCCCAACAGAATCA	CGAAACAACCAACACCTCT	274
<i>OsJAmyb</i>	NM_001074998	GAGGACCAGAGTGCAAAAGC	CATGGCATCCTTGAACCTCT	73
<i>OsWRKY45</i>	NM_001061727	AATTGCGTGGTCGTAAGAA	AAGTAGGCCTTTGGGTGCTT	95

Tween 20. The enzymatic reaction mixture consisted of 0.17 mM linoleic acid in 100 mM sodium phosphate buffer (pH 7.0) and crude enzyme solution to a total volume of 3 mL. A reaction mixture without substrate solution was used as the blank control. One enzyme unit was defined as the amount of enzyme required to increase the OD₂₃₄ of the reaction mixture by 0.001 min⁻¹.

Analysis of promoter sequences

The 2 kb upstream sequences of the selected signalling and defence-related genes were obtained from the NCBI database (<http://www.ncbi.nlm.nih.gov/>), and then submitted to the Plant *cis*-acting Regulatory DNA Elements Database for analysis (PLACE, <http://www.dna.affrc.go.jp/PLACE/signalscan.html>).

Results

Over-expression of *JERF1* and improved resistance to sheath blight of rice

Previous studies documented that the OE lines over-expressing *JERF1* showed no obvious differences in agronomic traits compared with WT (Zhang *et al.*, 2010). In this study, two OE lines (OE10 and OE11) were confirmed by RT-PCR amplifications (Fig. 1a) and used for the analysis of resistance to sheath blight.

Over-expression of *JERF1* was reported to enhance expression of PR genes in tobacco (Zhang *et al.*, 2004). To investigate whether *JERF1* plays a regulatory role in improving rice resistance to pathogen infection, sheath blight inoculation assays were performed in the controlled environment microchamber combined with field experiments. At 3 dpi in the microchamber, the susceptible control (cv. Lemont) and the wildtype plants exhibited initial water-soaked lesions at the base of the seedlings (Fig. 1b). Although the OE lines started to develop lesions, they showed a slower infection rate than WT and cv. Lemont. In the following days the lesions gradually turned dark brown and obvious, along with the extension of mycelia. The mycelium reached the top of stems and leaves at 7 dpi and resulted in the collapse of the WT and cv. Lemont seedlings, while the OE lines showed milder symptoms (Fig. S1). In order to quantify the infection of seedlings, the disease index of each line, WT and cv. Lemont was calculated. The disease index of

WT (5.1) was higher than those of the two OE lines (4.0–4.1; Fig. 1c), suggesting that the OE lines showed enhanced resistance to sheath blight during the seedling stage.

To determine whether this resistance in OE lines also occurred at a later stage of development, field inoculation was conducted at the later tillering stage. WT plants displayed more serious disease symptoms than the OE lines (Fig. 2a), consistent with the results of the microchamber assay. The disease indexes of WT, OE10 and OE11 were 2.8, 1.8 and 1.6, respectively (Fig. 2b). Together, these data suggest that over-expression of *JERF1* endowed the transgenic rice with enhanced resistance to sheath blight.

Over-expression of *JERF1* and expression of signalling and defence-related genes

Jasmonate (JA) and salicylic acid (SA) signalling pathways are key players in systemically induced defence against pathogens in plants (Bari & Jones, 2009). In order to explore the molecular mechanism of enhanced SB resistance in *JERF1* over-expressing rice, the expression of JA/SA synthesis and signalling genes including *OsLOX1*, *OsPAL1*, *OsPAL2*, *OsJAmyb* and *OsWRKY45* was analysed. Lipxygenase (LOX) and phenylalanine ammonia lyase (PAL) are two important enzymes involved in the synthesis of JA and SA, respectively. *OsJAmyb* and *OsWRKY45* play crucial roles in the JA- and SA-mediated signalling transduction and stress response (Lee *et al.*, 2001; Shimono *et al.*, 2012). As shown in Figure 3, the transcript level of *OsPAL1* was significantly higher in OE lines than in WT, in both untreated leaves and after SB infection, suggesting a direct activation of *OsPAL1* by *JERF1*. The expression levels of *OsLOX1*, *OsPAL2*, *OsWRKY45* and *OsJAmyb* showed no significant differences in untreated leaves. These candidate genes showed inducible expression in both wildtype and transgenic rice after SB infection, but their expression levels were significantly higher in OE lines than in WT after infection, implying that *JERF1* activated the expression of these genes indirectly.

Pathogenesis-related proteins and other defence components possess antimicrobial activities and protect plants against pathogen infection (van Loon *et al.*, 2006). The

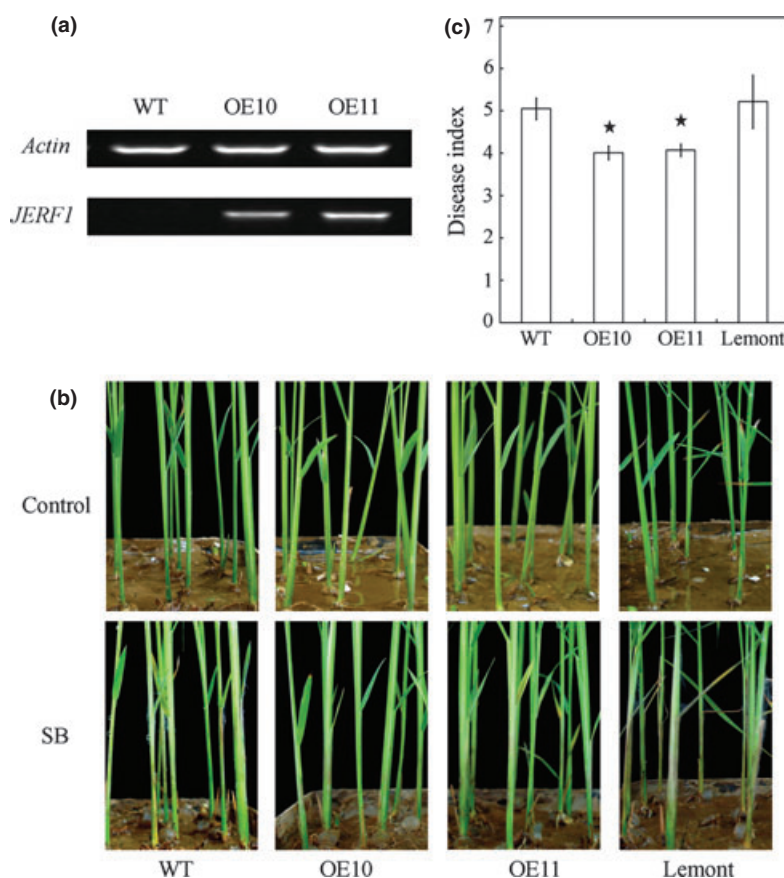


Figure 1 Over-expression of *JERF1* improves resistance of transgenic rice seedlings to sheath blight. (a) Analysis of the expression of *JERF1* in over-expression rice plants (OE10 and OE11) compared to wildtype (WT) through RT-PCR. (b) The phenotype of *JERF1*-over-expressing seedlings and wildtype plants at 3 days post-inoculation (dpi) with sheath blight. Control: three-leaf stage seedlings grown under normal growth conditions. SB: three-leaf stage seedlings were inoculated with sheath blight using the microchamber method and photographed at 3 dpi. Cultivar Lemont was included as the susceptible control. (c) The disease index of wildtype and *JERF1*-over-expressing seedlings, calculated as $9 \times \text{lesion length/plant height}$ at 7 dpi. These data are the average of three assays, and the error bars indicate SD. $P < 0.05$ was considered statistically significant by Student's *t*-test.

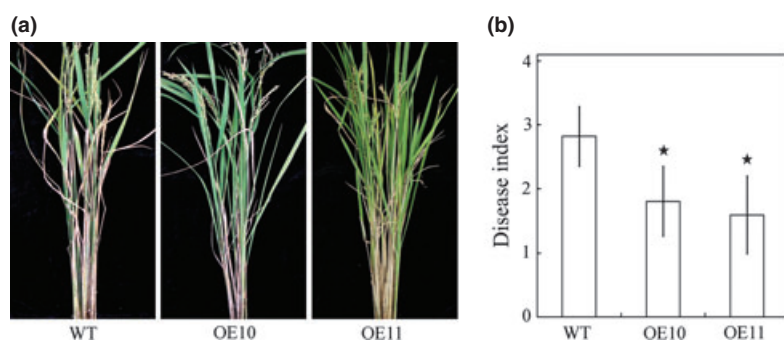


Figure 2 Over-expression of *JERF1* improves resistance of transgenic rice to sheath blight at later stages of development. (a) The phenotype of *JERF1*-over-expressing plants (OE10, OE11) and wildtype (WT) plants at 30 days post-inoculation in the field. (b) The disease index of wildtype and *JERF1*-over-expressing plants, calculated as for the microchamber inoculation assay. These data are the average of three assays, and the error bars indicate SD. $P < 0.05$ was considered statistically significant by Student's *t*-test.

expression of defence-related genes has been used as a marker of disease resistance response. The expression of several candidate genes in WT and OE lines was investi-

gated. As shown in Figure 4, the transcript levels of *OsGlu2* and *OsBBI*, which belong to *PR-5* and the Bowman-Birk inhibitor gene family, respectively, were

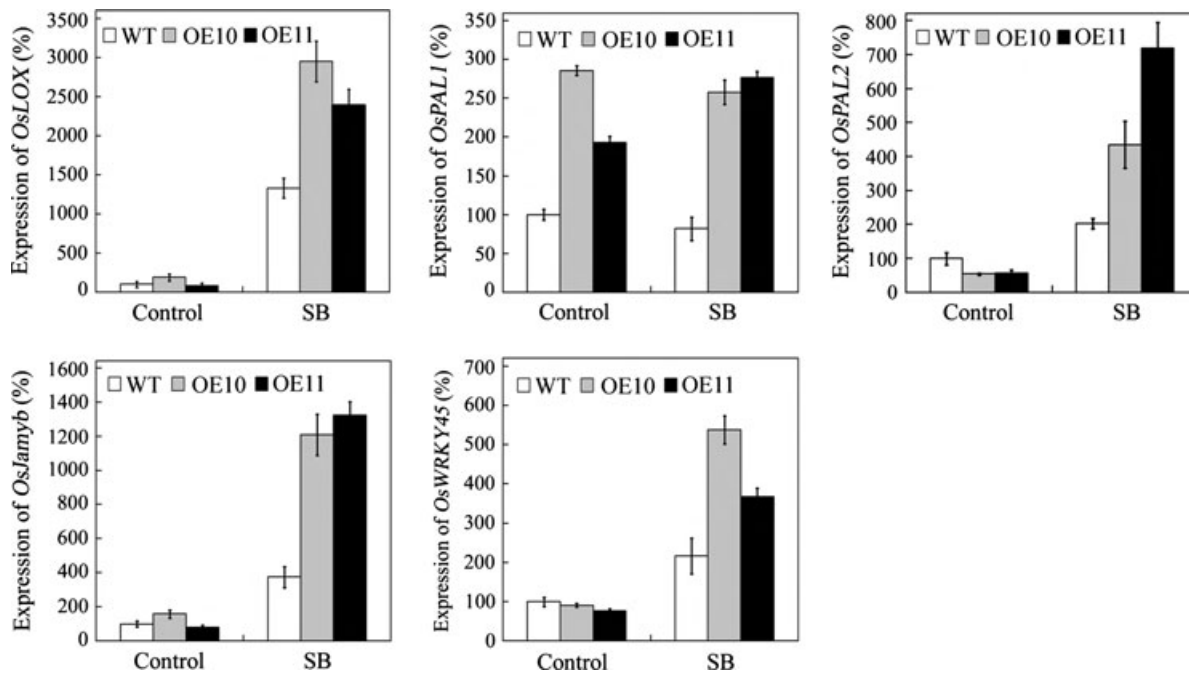


Figure 3 Over-expression of *JERF1* in rice modulates the expression of hormone biosynthesis and signalling genes. Control: the gene expression in three-leaf stage seedlings grown under normal growth conditions. SB: the gene expression in three-leaf stage seedlings at 2 days post-inoculation with sheath blight.

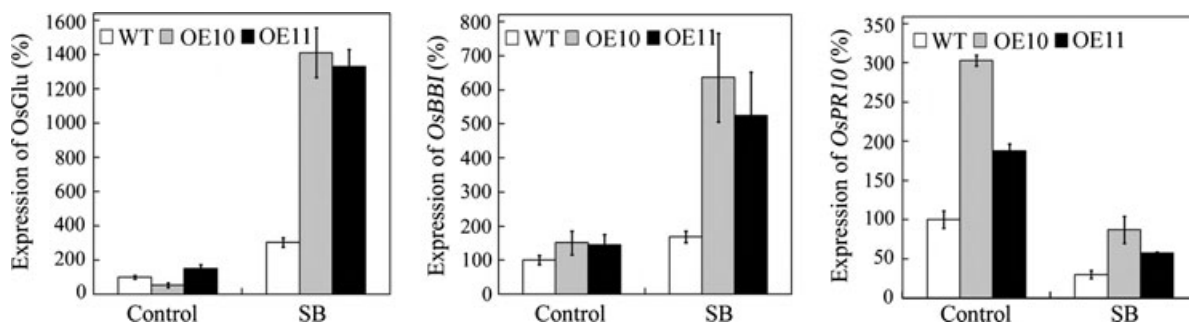


Figure 4 Over-expression of *JERF1* in rice regulates the expression of defence-related genes. Control: the transcript levels of defence-related genes in three-leaf stage seedlings grown under normal growth conditions. SB: the expression levels of defence-related genes in three-leaf stage seedlings at 2 days post-inoculation with sheath blight.

induced much higher in OE lines than in WT after SB inoculation. Although SB infection suppressed the expression of *OsPR10*, a relatively higher transcript level was maintained in the OE lines both without and after SB infection. Taken together, these results suggest that *JERF1* up-regulated the signalling and defence-related genes constitutively or inductively in transgenic rice.

Over-expression of *JERF1* and LOX and PAL activity

LOX and PAL are the key enzymes of JA and SA synthesis, respectively, which were reported to be closely correlated with plant resistance to various pathogens (Vanitha & Umesha, 2011). Because *JERF1* could up-regulate the expression of *OsLOX1*, *OsPAL1* and *OsPAL2*, LOX and PAL activity was measured in the leaves of seedlings

at the three-leaf stage. As shown in Figure 5a, the seedlings of WT showed significantly lower LOX activity ($16.8 \text{ U mg}^{-1} \text{ min}^{-1}$) than OE seedlings, which were 21.5 and $22.9 \text{ U mg}^{-1} \text{ min}^{-1}$ in OE10 and OE11, respectively. Similarly, the PAL activity of OE lines was greater than that of WT under normal growth conditions; 40.8 , 52.4 and $60.3 \text{ U mg}^{-1} \text{ h}^{-1}$ in WT, OE10 and OE11, respectively (Fig. 5b). These results imply that *JERF1* could up-regulate the activities of LOX and PAL in the leaves of transgenic rice.

JERF1 regulation of defence-related genes

Previous research showed that *JERF1* regulated its target genes by interacting with the ethylene-responsive elements such as DRE/CRT and GCC boxes in transgenic

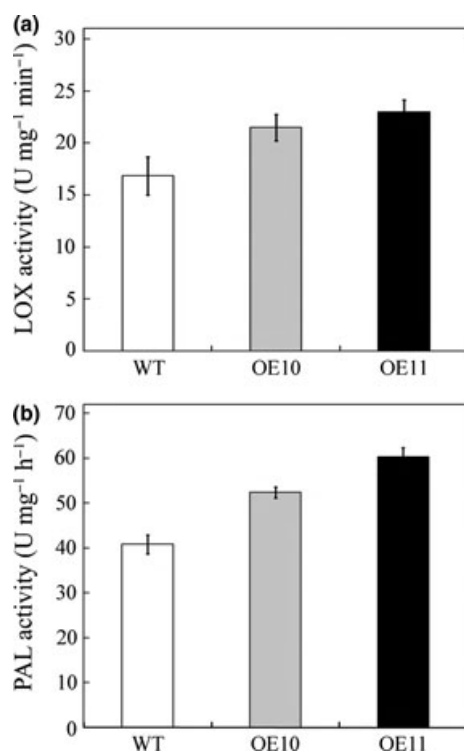


Figure 5 Over-expression of *JERF1* in rice increased the activities of (a) lipoxygenase (LOX) and (b) phenylalanine ammonia lyase (PAL). Leaves of rice seedlings at the three-leaf stage were collected for determination of LOX and PAL activities. The activities are the average of two replicates, and the error bars indicate SD.

tobacco (Zhang *et al.*, 2004). To understand the molecular regulatory mechanisms of *JERF1* in modulating the signalling and defence-related genes in transgenic rice, 2 kb nucleotides upstream to the start code of *JERF1* targeted genes were obtained through the rice genome database (<http://www.ncbi.nlm.nih.gov/>). Then, the potential *cis*-elements of these promoters were analysed

via the PLACE database (<http://www.dna.affrc.go.jp/PLACE/index.html>). The analyses showed that there were several DRE/CRT and/or GCC boxes in the candidate promoter of downstream genes (Table 2), suggesting that *JERF1* might regulate the expression of defence-related genes through binding to DRE/CRT and/or GCC boxes.

Discussion

Developing sheath blight resistant cultivars through conventional breeding has been slower than expected due to the lack of highly resistant rice donors, the polygenic nature of the resistance and the strong associations between morphological properties and disease development (Sharma *et al.*, 2009). Use of transgenic technology might be an economical and effective strategy for managing SB. An ERF gene (*JERF1*) isolated from tomato was found to play potential roles in regulating various abiotic stresses (Zhang *et al.*, 2004, 2010; Wu *et al.*, 2007). This report further demonstrates that *JERF1* activates the expression of the signalling and defence-related genes and results in the increased activity of defence-related enzymes associated with enhanced resistance to sheath blight in rice. Therefore, *JERF1* might be a useful gene for genetic engineering to improve SB resistance of rice plants.

Rice plants can activate a series of defence responses to cope with pathogen infection at the molecular, physiological and biochemical levels. JA and SA are the major defence signalling compounds mediating disease resistance in plants (Bari & Jones, 2009). As the key enzymes of JA and SA synthesis, the activities of LOX and PAL are two common physiological indicators for plant response to pathogens (Vanitha & Umesha, 2011). This report showed that the activities of LOX and PAL were significantly increased in transgenic rice compared to wildtype plants. Consistent with these results, OE lines exhibited higher expression of *OsLOX1*, *OsPAL1* and

Table 2 *cis*-element analysis of candidate promoter sequences of downstream genes

Gene	<i>cis</i> -element	Location sites	Core element
<i>OsGlu</i>	DRE/CRT	-764(-), -1444(-)	RCCGAC
	GCC-box	-423(+)	GCCGCC
<i>OsBBI</i>	DRE/CRT	-1280(+)	RCCGAC
	GCC-box	-1306(-), -1309(-), -1364(-), -1475(+)	GCCGCC
<i>OsPR10</i>	DRE/CRT	-226(-)	GCCGAC
<i>OsLOX1</i>	DRE/CRT	-1176(-), -1607(+)	RCCGAC
	GCC-box	-653(+), -656(+), -659(+), -662(+), -1088(+)	GCCGCC
<i>OsPAL1</i>	DRE/CRT	-589(+), -597(+), -605(+)	ACCGAC
	GCC-box	-411(+), -414(+)	GCCGCC
<i>OsPAL2</i>	DRE/CRT	-1883(-), -1113(-), -766(-)	RCCGAC
	GCC-box	-1703(+), -1734(+), -1737(+), -1756(+), -1778(+),	GCCGCC
		-1846(+), -1849(+), -1852(+), -1855(+), -1924(+),	
		-1986(+), -1989(+), -1992(+)	
<i>OsJAmyb</i>	GCC-box	-393(+)	GCCGCC
<i>OsWRKY45</i>	DRE/CRT	-142(+), -1292(+)	RCCGAC
	GCC-box	-400(+), -1429(+), -1432(+), -1436(-)	GCCGCC

OsPAL2 than WT, both in the non-treated control and after SB infection. Therefore, it is proposed that JERF1 modulates the activities of LOX and PAL through inducing the expression of these corresponding genes, which may result in the increase of JA/SA biosynthesis and activation of JA/SA-dependent defence responses. However, JA and SA pathways have been reported to suppress each other depending on *NPR1* (*non-expressor of pathogenesis-related genes1*; Li *et al.*, 2004). A possible explanation is that JERF1 activates the JA/SA-related defence response in an *NPR1*-independent manner. In addition, the expression of *OsJAmyb* and *OsWRKY45* has been reported to be closely associated with fungal resistance (Lee *et al.*, 2001; Shimono *et al.*, 2012). In this report, these two critical signalling genes were up-regulated by JERF1 after SB infection. However, it is reported that the constitutive expression of *OsWRKY45* in rice decreased the resistance to *R. solani* (Shimono *et al.*, 2012). One possible reason is due to the different pathogen strains used for inoculation in different studies. Alternatively, it is possible that the inductive expression of *OsWRKY45* might be more important to SB resistance. Moreover, the defence-related genes such as *OsBBI*, *OsPR10* and *OsGlu* were proven to be the downstream components of JA/SA signalling pathways and play crucial roles in plant defence against pathogens (Miché *et al.*, 2006; Hwang *et al.*, 2008). For example, application of the SA analogue benzothiadiazole (BTH) increased the resistance of rice to *Magnaporthe oryzae* by accelerating the induction of *PR* genes (Ahn *et al.*, 2005). The up-regulation of these defence-related genes in transgenic rice over-expressing *JERF1* might contribute to improving resistance to SB in transgenic rice by tuning SA and JA defence-related signalling pathways, which ultimately results in a slower infection and enhanced SB resistance in OE plants.

JERF1 has previously been reported to bind to the *cis*-elements of GCC and DRE/CRT boxes and regulate the expression of downstream genes (Zhang *et al.*, 2004). The analyses here showed that these *cis*-elements were present in the promoters of JERF1 targeted defence-related genes, implying that JERF1 might regulate their expressions via binding to GCC-box or/and DRE/CRT *cis*-elements. However, other than for *OsPAL1* and *OsPR10*, several targeted genes were not activated in untreated OE plants. It seems that JERF1 binds to the promoters of *OsPAL1* and *OsPR10* directly, but to those of other genes indirectly. Many ERF proteins contain several putative phosphorylation sites, and phosphorylation of these proteins was reported to enhance their binding to GCC boxes (Gu *et al.*, 2000). In addition, ERF proteins could interact with a basic leucine zipper protein ABF2 and activate the transcription of an ABA-responsive gene (Cheong *et al.*, 2003). Therefore, it is proposed that the regulatory function of JERF1 on downstream genes might require posttranscriptional modification such as phosphorylation or other cooperative proteins.

In order to survive under adverse environmental conditions, plants have evolved complicated mechanisms to

cope with various stresses. Recent studies have revealed that regulatory proteins including transcription factors, kinases and signal molecules might be involved in cross-talk between biotic and abiotic stress (Fujita *et al.*, 2006). For example, transgenic rice over-expressing *OsNAC6* exhibited enhanced tolerance to dehydration and high-salt stresses as well as to blast disease (Nakashima *et al.*, 2007). In addition, ectopic expression of *OsDREB1B* in tobacco increased tolerance to drought stress and resistance to streak virus infection (Gutha & Reddy, 2008). Consistent with these reports, this study showed that JERF1 decreased sensitivity of rice plants to sheath blight, in addition to previous studies showing improved tolerance to abiotic stresses such as drought and salt. However, a variety of regulatory proteins were found to play both positive and negative regulatory roles in mediating plant responses to biotic and abiotic stresses. For instance, *TSRF1* inversely regulated pathogen resistance and osmotic stresses in tobacco, implying a divergent regulatory mechanism between biotic and abiotic stresses (Zhang *et al.*, 2007). Based on these results, it is supposed that transcription factors modulated biotic and abiotic stresses by priming genes with different functions. JERF1 was reported previously to improve tolerance to various abiotic stresses by activating stress responsive genes and ABA biosynthesis (Zhang *et al.*, 2004, 2010; Wu *et al.*, 2007). In many cases, ABA interacts antagonistically with JA- and SA-signalling pathways and negatively regulates defence gene expression and disease resistance (Nahar *et al.*, 2012). However, ABA treatment also caused enhanced resistance to *Alternaria solani* in tomato seedlings through inducing defence responses (Song *et al.*, 2011). The ABA, JA and SA signalling pathways do not function independently. Instead, these pathways are interplayed via both positive and negative regulatory mechanisms (Mauch-Mani & Mauch, 2005). The present study showed that JERF1 effectively activated JA and SA pathways after SB infection by regulating defence and signalling genes. Therefore, it is proposed that the balance of ABA and JA/SA signalling might be an important determinant for the enhanced resistance of *JERF1* over-expression plants.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. The phenotype of wildtype and *JERF1*-over-expressing rice lines at 7 days post-inoculation with sheath blight using the microchamber method.