Plant Omics Journal POJ

POJ 7(6):540-548 (2014) ISSN:1836-3644

Identification and network construction of zinc finger protein (*ZFP*) genes involved in the rice-*Magnaporthe oryzae* interaction

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Abstract

Previous studies have shown that some rice *ZFP* genes from the WRKY, RING, C2H2 and LSD1 families are associated with defense against *Magnaporthe oryzae* (*M. oryzae*). However, it remains unknown whether other ZFP families are involved in the rice-*M. oryzae* interaction. Here, we reported the global characterization of rice *ZFP* genes involved in the rice-*M. oryzae* interaction based on bioinfromatics analysis of important rice databases. By analyzing the data obtained from the microarray database, we found that 241 *ZFP* genes belonging to 27 families were expression-responsive to *M. oryzae*. Among these ZFP families, a total of 23 ZFP families were newly identified to be involved into the rice-*M. oryzae* interaction. The expression patterns of the *ZFP* genes with expression responsiveness to *M. oryzae* in each family were similar, suggesting that each family might play similar roles in the rice-*M. oryzae* interaction. The result of co-expression gene network analysis revealed that the *M. oryzae*-responsive *ZFP* genes were able to be co-expressed with those genes regulating some biological processes. These biological processes mainly included methylation modification, protein kinase activity, transcription activity, posttranscriptional modification and protein transfer. The result suggested that the regulated network of the rice-*M. oryzae* interaction was well organized, although it was complicated. Moreover, we identified four *ZFP* genes that might play important roles for regulating blast disease resistance.

Keywords: rice, *Magnaporthe oryzae*, zinc finger protein, network, interaction.
Abbreviations: ZFP_Zinc finger protein, Cys_Cysteine, His_Histidine, DG_Digu, LTH_Lijangxintuanheigu, qRT-PCR_quantitative RT-PCR, LLS_log likelihood score.

Introduction

The transcription factor IIIA from *Xenopus oocytes* is the first Zinc finger protein (ZFP) identified as it contains a repeated motif of "zinc finger" (Miller et al., 1985). The term "zinc finger" refers to a sequence of protein motif that is able to bind to a zinc ion by Cysteine (Cys) and Histidine (His) in order to stabilize its three-dimensional structure (Takatsuji, 1998). There are no consolidated standard for naming ZFP families. Different names of ZFP families are based on different standards of classification. To clearly distinguish ZFP families from each other, the MSU Rice Genome Annotation database classified ZFPs into different families based on annotation, and a large number of ZFPs do not confusedly belong to two or more families simultaneously.

Most ZFPs are transcription factors and play critical roles in diverse biological processes in plants (Li et al., 2013; Takatsuji, 1998). For instance, the ZFPs from the C3H family, such as SOMNUS from Arabidopsis and OsDOS from rice, contribute to light-dependent seed germination and delaying leaf senescence, respectively (Kim et al., 2008; Kong et al., 2006). A PHD-finger protein, PERSISTENT TAPETAL CELL1, regulates tapetal cell death and pollen development in Arabidopsis (Li et al., 2011). Recent studies show that several ZFP families, such as GATA (Dangl et al., 1996), Dof (Yanagisawa, 2002), C2H2 (Gourcilleau et al., 2011), RING (Lim et al., 2010; Ma et al., 2009), C3H (Wang et al., 2008), FYVE (Whitham et al., 1994) and WRKY (Peng et al., 2008), are associated with biotic resistance. For example, AT4G20380 from Arabidopsis GATA family is associated with hypersensitive response (Dangl et al., 1996). AT3G50410 from Arabidopsis Dof family is involved in defense response (Yanagisawa, 2002). *OsDOS* from rice C3H family is associated with jasmonate signaling pathway for defense response (Kong et al., 2006). *OsWRKY3* and *OsWRKY13* from rice WRKY family regulate the expression of defense-related genes directly (Liu et al., 2005; Qiu et al., 2008). *Q40392* from tobacco FYVE family regulates tobacco mosaic virus resistance positively (Whitham et al., 1994). The above studies show that ZFPs are closely associated with biotic stress.

Rice is one of the most important food crops worldwide. Blast caused by the fungus *M. oryzae* is one of the most devastating diseases of rice (Fukuoka et al., 2009). The *M. oryzae* pathogens can infect all aerial rice tissues including leaves, stem and panicle (Ribot et al., 2008). It also can infect rice roots and xylem vessels (Sesma and Osbourn, 2004). Some genes from four ZFP families in rice, such as RING (Lim et al., 2010; Liu et al., 2008; Zhou et al., 2008), WRKY (Liu et al., 2005; Ryu et al., 2006; Wang et al., 2007) LSD1 (Wang et al., 2005) and C2H2 (Agarwal et al., 2007; Ham et al., 2006) have been found to function in defending rice blast disease. For examples, *OsBIRF1*(LOC_Os02g50930), *OsLSD1* (LOC_ Os08g06280) and *OsRFP1* (LOC_Os01g52110) from RING, *OsWRKY53* (LOC_Os05g27730) and *OsWRKY71* (LOC_

Os02g08440) from WRKY, *Tsip1* from C2H2 families, respectively, are able to enhance rice resistance to blast disease (Chujo et al., 2007; Ham et al., 2006; Liu et al., 2007; Zhou et al., 2008). Two rice WRKY genes, *OsWRKY76* and *OsWRKY28*, are reported as negative regulators for rice blast defense (Chujo et al., 2013; Yokotani et al., 2013). However, it remains largely unknown whether other *ZFP* genes are involved in the rice-*M. oryzae* interaction.

In this study, we identified a total of 1258 *ZFP* genes from RGAP database. After expression profiling analysis, we report that 241 *ZFP* genes, belonging to 27 families, are involved in the rice-*M. oryzae* interaction. Among these ZFP families, we found that these 23 families, such as GATA, FYVE, TDDP, RBPO, A20, TFIIB, Dof, C3H, CDGSH, PADPP, LYAR, ZK, TAZ, MSRING, Ubox, Bbox, NFX1, AN, UBR, C5HC2, DHHC, PHD and CHY were newly identified for their functions involved in the rice-*M. oryzae* interaction. We also constructed the co-expression network using the *M. oryzae*-responsive *ZFP* genes as core sets and found that many genes regulating some biological processes were co-expressed with these *ZFP* genes. Moreover, we found that four *ZFP* genes, *LOC_Os04g57600*, *LOC_Os09g01640*, *LOC_Os04g53720* and *LOC_Os06g14190*, from C3H, ZK, RING and NFX1 families, respectively, showed differential expression patterns between the compatible and incompatible interactions of rice-*M. oryzae*.

Results

Identification of rice ZFP genes and expression analysis after blast inoculation

We totally identified 1,258 genes that encoded proteins containing ZFP domain from 55,986 genes released from RGAP database. These *ZFP* genes belonged to 53 ZFP families (Table 1) and were distributed on all 12 chromosomes of rice whole genome. The number of *ZFP* genes (163) distributed on Chromosome 2 was the largest, whereas the number of *ZFP* genes (47) distributed on Chromosome 10 was the smallest.

To identify the *ZFP* genes that were responsive on the inoculation with the pathogen of *M. oryzae*, we collected the data from the microarray available at the website (http://www.ricearray.org/expression/expression.php) and performed comparative expression analysis between the *M. oryzae*-inoculation and mock-inoculation (Cao et al., 2012). The genes, of which the expression is significantly changed (fold change $\geq$2 or fold $\leq$ 0.5) post *M. oryzae*-inoculation, were selected. We found that 241 genes belonging to 27 ZFP families were responsive on the inoculation with the compatible blast pathogen of *M. oryzae*. Among them, 155 were expression-induced, whereas 81 were expression-repressed (Fig. 1, Supplemental Table 1). These results suggested that these *ZFP* genes were involved in the rice-*M. oryzae* interaction. All the *ZFP* genes from the CDGSH and NF-Xl families and less than 50 percent of the genes from each of other 25 ZFP families were significantly responsive upon *M. oryzae* infection (Fig. 2). Among the 27 families, the number of the genes belonging to the RING families was the largest (66), and that belonging to the WRKY families was the second (45). These results suggest that, compared with other ZFP families, the genes from the CDGSH, NF-X1, RING and WRKY families more likely play key roles in the rice-*M. oryzae* interaction.

Identification of new ZFP families involved in the rice-M. oryzae interaction

The *ZFP* genes with expression changed by *M. oryzae* were from 27 families (Table 2). Among these ZFP families, only the WRKY, RING, LSD1 and C2H2 had previously been reported as regulators for rice blast resistance (Chujo et al., 2007; Ham et al., 2006; Liu et al., 2008; Liu et al., 2005; Qiu et al., 2008; Qiu et al., 2007; Ryu et al., 2006; Wang et al., 2008; Wang et al., 2007; Wang et al., 2005; Zeng et al., 2004; Zhang et al., 2008). Thus, our results suggested that the GATA, FYVE, TDDP, RBPO, A20, TFIIB, Dof, C3H, CDGSH, PADPP, LYAR, ZK, TAZ, MSRING, Ubox, Bbox, NFX1, AN, UBR, C5HC2, DHHC, PHD and CHY families were also involved in the rice-*M. oryzae* interaction. The genes from eight ZFP families, such as AN, A20, TFIIB, C3H, C5HC2, LYAR, NF-X1 and TDDP, with expression changed were induced, whereas the genes from seven ZFP families, such as CHY, DHHC, CDGSH, LSD1, MSRING, PADPP and UBP, with expression changed were repressed by *M. oryzae* (Table 2). Additionally, the expression of most *ZFP* genes from the WRKY, RING, C2H2, RBPO, Dof, C3H, TAZ, Ubox and Bbox families were induced, whereas those from the GATA and PHD families were mainly repressed by *M. oryzae*. These results suggested that the expression patterns of the *ZFP* genes in each family were similar in the rice-*M. oryzae* interaction and these *ZFP* genes in each family involved in the rice-*M. oryzae* might play similar roles during defending against *M. oryzae*.

Construction of the expression network of the ZFP genes with expression responsive on the inoculation with blast fungus M. oryzae

To further understand the biological functions of these *ZFP* genes, we constructed the co-expressed gene network using the *ZFP* genes with expression significantly changed as the core points via the online RiceNet tool (http://www.functionalnet.org/ricenet/search.html). The co-expressed network usually provides the likelihood (designed log likelihood score, LLS) that the genes participate in the same process conditioned on each dataset. The bootstrapping of 0.632 is used for all LLS evaluations (Lee et al., 2011). The result showed that 608 genes were co-expressed with 87 core point *ZFP* genes from 17 families (Table 2, Supplemental Table 2). The functions of the genes that were co-expressed with the *ZFP* genes regulated by *M. oryzae*, were mainly involved in transcription process, methylation modification, protein kinase activity, transcription activity, posttranscriptional modification and protein transfer (Fig. 3 and Supplemental Table 2). This suggested that these *ZFP* genes played important functions in the rice-*M. oryzae* interaction through their respective biological roles in rice.

Among the *ZFP* genes associated with the rice-*M. oryzae* interaction in the co-expressed gene network, we found that ten *ZFP* genes could construct a small co-expressed gene network in the rice-*M. oryzae* interaction. The ten *ZFP* genes included LOC_Os10g31850 from CHY, LOC_Os09g37720, LOC_Os09g01640, and LOC_Os05g07000 from ZK, LOC_Os06g14190 from NFX1, LOC_Os04g53720 from RING, LOC_Os02g19804, LOC_Os01g61830, LOC_Os04g57600 and LOC_Os08g06330 from C3H. To further confirm the co-expressed network result, we analyzed the Pearson correlation of transcriptional expression among the co-expressed *ZFP* genes in Nipponbare at 72 and 96 hour post inoculation with the pathogen of *M. oryzae*. Except for LOC_Os08g06330, the expression of all of the other nine *ZFP* genes were significantly correlated post inoculation with the pathogen of *M. oryzae* (Supplemental Table 3). This result also suggested that the co-expressed gene network was reliable.

Table 1. The ZFP families in rice. The detail included family name, abbreviation, Pfam, number of genes.

Family	Abbreviation	Pfam	Number of gene
C2H2 type	C2H2	PF00096	96
XS zinc finger domain	XS	PF03470	7
SWIM zinc finger	SWIM	PF04434	10
U1 zinc finger	U1	PF06220	3
GATA zinc finger	GATA	PF00320	27
FYVE zinc finger	FYVE	PF01363	19
Topoisomerase DNA binding C4 zinc finger	Topi	PF01396	2
Tim10/DDP family zinc finger	TDDP	PF02953	9
HIT zinc finger	HIT	PF04438	6
Sec23/Sec24 zinc finger	SS	PF04810	9
CSL zinc finger	CSL	PF05207	3
Zinc finger found in FPG and IleRS	FPGIle	PF06827	1
GRF zinc finger	GRF	PF06839	30
LSD1 zinc finger	LSD1	PF06943	7
CW-type Zinc Finger	CW	PF07496	12
DNA Polymerase alpha zinc finger	DPACS	PF08996	1
Zn-finger in Ran binding protein and others	RBPO	PF00641	19
ZPR1 zinc-finger domain	ZPR1	PF03367	1
A20-like zinc finger	A20	PF01754	12
Disease resistance - zinc finger -chromosome condensation-like region	DR	PF08381	16
TFIIB zinc-binding	TFIIB	PF08271	6
Dof domain, zinc finger	Dof	PF02701	30
DNL zinc finger	DNL	PF05180	3
Zinc finger C-x8-C-x5-C-x3-H type	C3H	PF00642	69
Primase zinc finger	Primase	PF09329	1
Putative zinc-finger domain	PZFD	PF10650	1
Iron-binding zinc finger CDGSH type	CDGSH	PF09360	1
BED zinc finger	BED	PF02892	10
Poly(ADP-ribose) polymerase and DNA-Ligase Zn-finger region	PADPP	PF00645	2
LYAR-type C2HC zinc finger	LYAR	PF08790	2
Zinc knuckle	ZK	PF00098	51
Yippee putative zinc-binding protein	YipP	PF03226	6
Plant zinc cluster domain(WRKY)	WRKY	PF10533	102
C3HC zinc finger-like	C3HC	PF07967	3
Zinc-finger domain	ZFD	PF10276	1
TAZ zinc finger	TAZ	PF02135	4
transporter	H2C2	PF09337	9
ZZ type	ZZ	PF00569	8
RING finger	RING	PF00097	391
MIZ/SP-RING zinc finger	MSRING	PF02891	5
U-box domain	Ubox	PF04564	77
B-box zinc finger	Bbox	PF00643	30
NF-X1 type zinc finger	NFX1	PF01422	2
AN1-like Zinc finger	AN	PF01428	16
MYND finger	MYND	PF01753	12
Putative zinc finger in N-recognin (UBR box)	UBR	PF02207	2
C5HC2 zinc finger	C5HC2	PF02928	4
TRAF-type zinc finger	TRAF	PF02176	5
C2HC5-type	C2HC5	PF06221	1
DHHC zinc finger domain	DHHC	PF01529	30
PHD-finger	PHD	PF00628	73
CHY zinc finger	CHY	PF05495	7
Zn-finger in ubiquitin-hydrolases and other protein	UHOP	PF02148	4

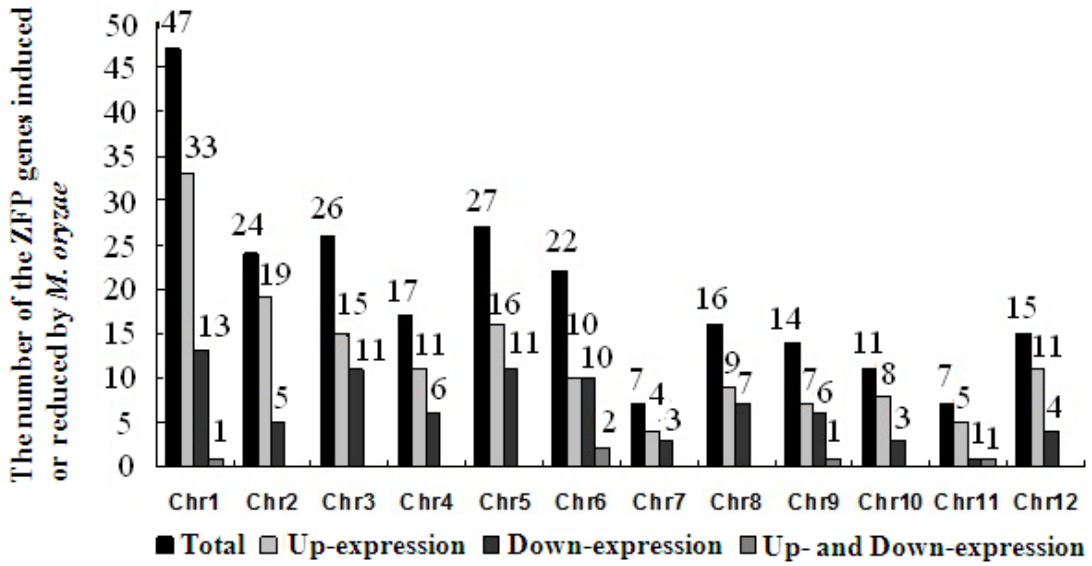

Fig 1. The chromosomal distributions of the ZFP genes with expression responsiveness to M. oryzae. The number of ZFP genes with regulation on chromosome 1 is the largest, whereas that on chromosome 7 is smallest.

The expression patterns of several ZFP genes in compatible and incompatible varieties

To determine whether the expression patterns of the *ZFP* genes in *M. oryzae*- incompatible rice variety were different from that in *M. oryzae*-compatible rice variety, we examined the transcriptional expression of eight co-expressed *ZFP* genes, except for LOC_Os01g61830, of which no good primers available for quantitative RT-PCR (qRT-PCR), in the blast-resistance variety Digu (DG) and blast-susceptible variety Lijiangxintuanheigu (LTH) post inoculated with *M. oryzae*. We found that the expression patterns of LOC_Os04g57600, LOC_Os09g01640, LOC_Os04g53720 and LOC_Os06g14190 were similar between DG and LTH post inoculated with *M. oryzae* (Fig. 4A). This result suggested that the four *ZFP* genes, LOC_Os04g57600, LOC_Os09g01640, LOC_Os04g53720, and LOC_Os06g14190, might positively regulate the basal resistance in both the resistant and susceptible variety. However, the expression patterns of another four *ZFP* genes, LOC_Os05g07000, LOC_Os02g19804, LOC_Os09g37720, and LOC_Os10g31850, were respectively different between DG and LTH post inoculated with *M. oryzae* (Fig. 4B). These results indicate that the four *ZFP* genes in DG might be involved in the defense response against *M. oryzae*.

Discussion

A lot of ZFP genes were involved in the rice-M. oryzae interaction

In this study, among 1258 *ZFP* genes, expressions of 241 *ZFP* genes were pathogen-responsive at 72h and/or 96h post *M. oryzae* inoculation. Previous studies suggested that about 20 *ZFP* genes are involved in the rice-*M. oryzae* interaction (Li et al., 2013; Liu et al., 2008; Ryu et al., 2006; Xu and He, 2007; Zeng et al., 2004; Zhou et al., 2008). Thus, our study revealed that a lot of *ZFP* genes with their transcriptional reprogram were involved in the pathogen-responsiveness and might play important roles for defending rice blast disease.

Newly identified ZFP families were involved in the rice-M. oryzae interaction

These induced and repressed 241 *ZFP* genes belonged to 27 ZFP families. Among these families, some *ZFP* genes from WRKY, RING, LSD1 and C2H2 have previously been identified as associated with defending blast disease (Chujo et al., 2007; Ham et al., 2006; Liu et al., 2007; Wang et al., 2005; Zhou et al., 2008). Thus, we found a total of 23 families as the newly identified *M. oryzae*-related ZFP families. Among these 23 families, the *ZFP* genes from eight families with expression changed were all induced, whereas those from seven families with expression changed were all repressed by *M. oryzae*, indicating that these *ZFP* genes from same family likely co-positively or co-negatively regulate plant defense response during the rice-*M. oryzae* interaction. Interestingly, among the newly identified ZFP families, some with same name of ZFP family of human and other life species were also associated with disease resistance. For examples, NF-X1 protein from human NFX1 family is a transcriptional repressor for regulating the duration of an inflammatory response (Song et al., 1994). ZFAR1 from Arabidopsis C3H family increases local susceptibility to Botrytis (AbuQamar et al., 2006). Znf216 from human A20/AN1 family negatively regulates inflammatory response by inhibiting NFkappaB activation (Huang et al., 2004), and Q40392 from tobacco FYVE family can enhance tobacco mosaic virus resistance (Whitham et al., 1994). Thus, in addition to the WRKY, RING, LSD1 and C2H2 families reported previously, many other ZFP families were very likely involved in the rice-*M. oryzae* interaction.

The regulated network of the rice-M. oryzae interaction is well organized although it is complicated

The molecular mechanism of the rice-*M. oryzae* interaction is complicated. Co-expressed gene network analyses reveal that 601 genes were co-expressed with 87 *M. oryzae*-responsive *ZFP* genes. The genes co-expressed with *ZFP* genes were mainly associated with six biological functions, such as transcription process, methylation modification, protein kinase activities, transcription activities, posttranscriptional modification

Table 2. The *ZFP* genes with up- and down-regulation in each ZFP family. The ZFP family names in bold meant new identified ZFP families involved in the rice-*M. oryzae* interaction.

ZFP Family	Total Number	up-regulated genes	down-regulated genes	up- and down-regulated genes[#]
C2H2*	96	12	3	0
WRKY*	102	41	3	2
RING*	391	39	26	1
LSD1	7	0	2	0
GATA*	27	2	6	0
FYVE	19	2	2	0
TDDP	9	1	0	0
RBPO*	19	4	2	0
A20	12	2	0	0
TFIIB	6	1	0	0
Dof*	30	6	3	1
C3H*	69	6	3	1
CDGSH*	1	0	1	0
PADPP*	2	0	1	0
LYAR	2	0	1	0
ZK*	51	3	1	1
TAZ	4	1	1	0
MSRING*	5	0	1	0
Ubox*	77	22	5	1
Bbox	30	7	5	0
NFX1*	2	2	0	0
AN	16	3	0	0
UBR	2	0	1	0
C5HC2*	4	1	0	0
DHHC*	30	0	2	0
PHD*	73	1	10	0
CHY*	7	0	2	0

Note: *means that some *ZFP* genes in these families were involved in the co-expressed gene network.
#means that the expressions of these *ZFP* genes were up-regulated or down-regulated at 72 hour post inoculation, but down-regulated or up-regulated at 96 hour post inoculation.

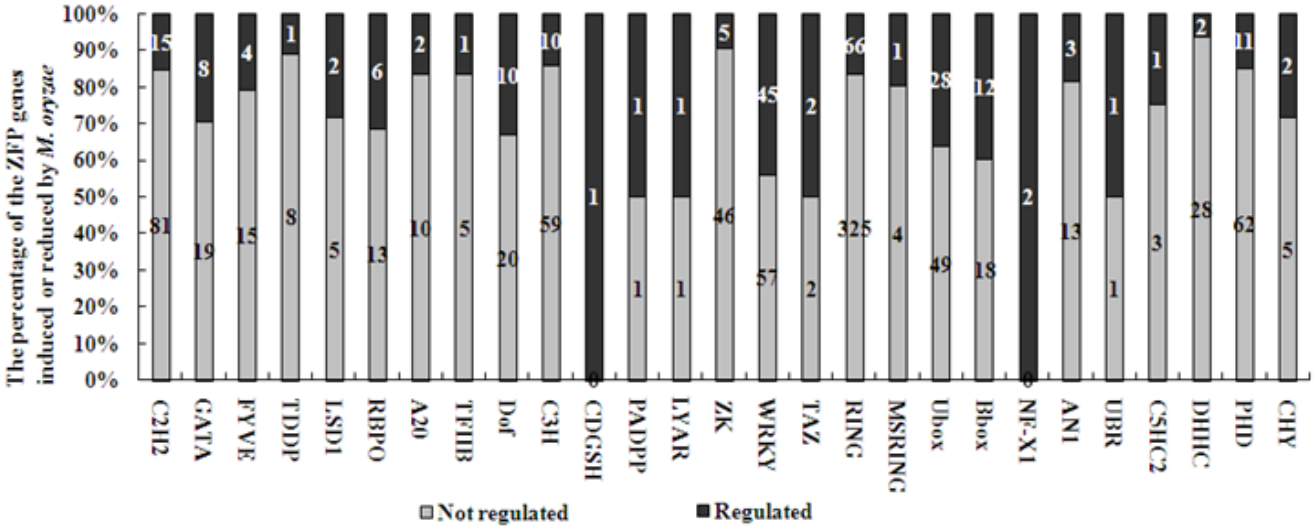

Fig 2. The percentage of the genes from different ZFP families with expression responsiveness to *M. oryzae*. The *ZFP* genes in 27 families were significantly regulated by *M. oryzae*. All the genes from CDGSH and NF-Xl ZFP families and less than 50% from each of the other 25 ZFP families are expression responsiveness to *M. oryzae.* The columns in black color represent the number of the *ZFP* genes with expression responsiveness and the columns in gray color represent the number of the *ZFP* genes without expression responsiveness to *M. oryzae*.

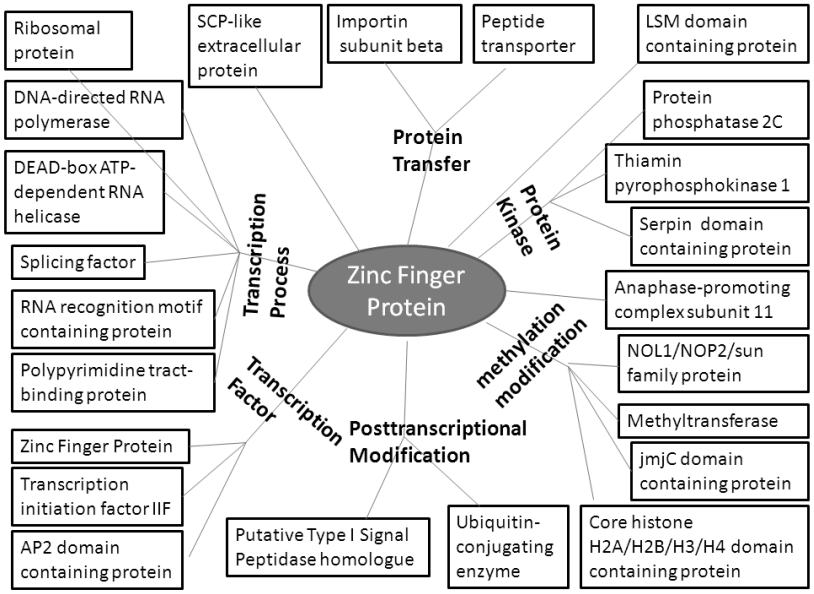

Fig 3. Construction of the co-expression network of the *ZFP* genes with expression responsiveness to *M. oryzae*. The functions of these genes mainly included transcription process, methylation modification, protein kinase, transcription factor, posttranscriptional modification and protein transfer.

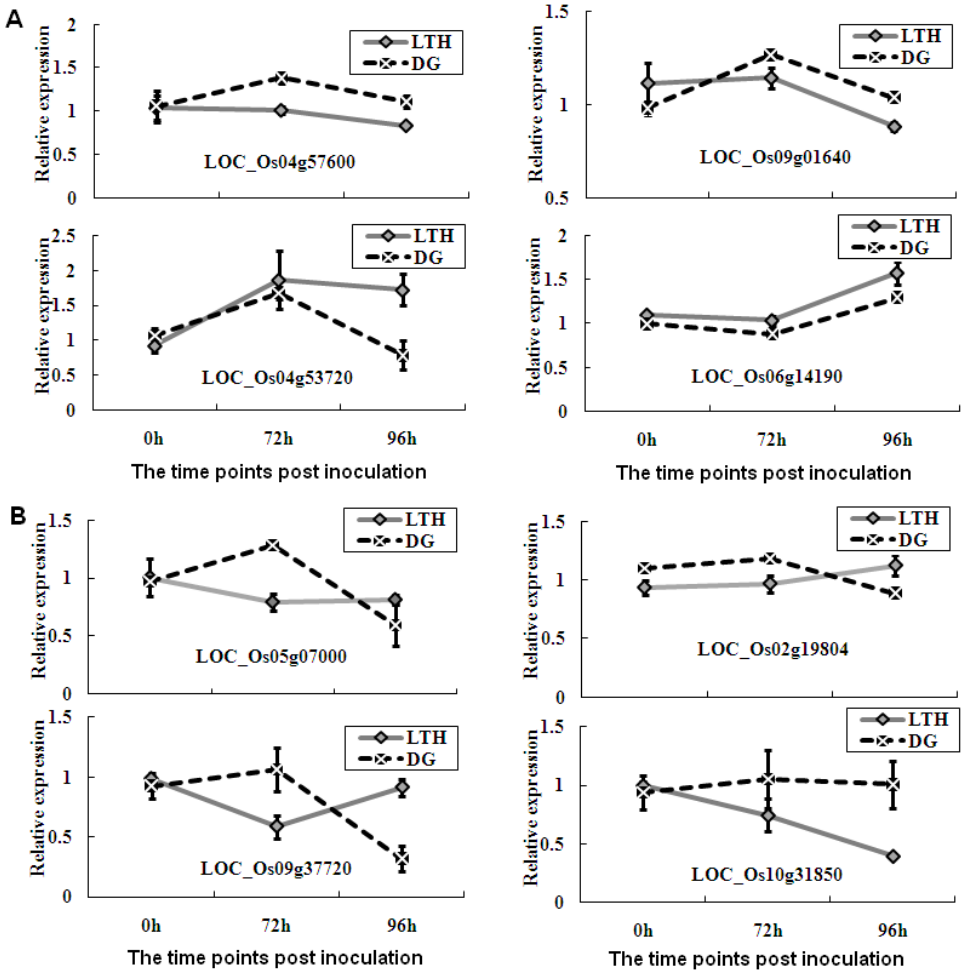

Fig 4. The expression patterns of eight *ZFP* genes from DG and LTH at time points post inoculation with *M. oryzae*. A: The expression analyses on four *ZFP* genes with similar expression patterns. B: The expression analyses on four *ZFP* genes with different expression patterns.

and protein transferring, suggesting that the above biological functions were also involved in the rice-*M. oryzae* interaction. Meanwhile, previous studies have proved that these biological functions are also associated with pathogen defense, such as methylation modification in Arabidopsis (Zhang, 2012), receptor-like kinases in plant species (Yang et al., 2012), post-translational modification in plant species (Stulemeijer and Joosten, 2008) and peptide transporter in Arabidopsis (Karim et al., 2007). These results also suggested that the regulation of *ZFP* genes on blast resistance passes through these important biological processes.

The different expression patterns of some ZFP genes in different varieties might result into different resistance to M. oryzae

The expression patterns of the same gene in the varieties with different tolerance or resistance are usually different under the same stress, suggesting that these genes play important roles in response to biotic or abiotic stress (Li et al., 2010; Xiang et al., 2013). In this study, we found that expressions of *OsLSD1* (LOC_Os08g06280) and *OsRFP1* (LOC_Os01g52110) from the rice variety Nipponbare were significantly down-regulated during the rice-*M. oryzae* interaction. Overexpression of these two genes can enhance resistance to rice blast (Wang et al., 2005; Zhou et al., 2008), suggesting that they positively regulate blast defense response. *OsWRKY28* (LOC_Os01g51690) and *OsWRKY76* (LOC_Os09g25060) negatively regulate rice blast defense as reported previously (Chujo et al., 2013; Yokotani et al., 2013). Interestingly, we found that expressions of the two genes from Nipponbare were significantly up-regulated in the rice-*M. oryzae* interaction. Thus, these results suggested that expression patterns of some *ZFP* genes were directly associated with blast defense. In our study, among screened eight *ZFP* genes, we found that four *ZFP* genes, LOC_Os05g07000, LOC_Os02g19804, LOC_Os09g37720 and LOC_Os10g31850, exhibited their different expression patterns between *M. oryzae*-incompatible rice DG and *M. oryzae*-compatible rice LTH. As the genes with different expression patterns in compatible and incompatible varieties upon the inoculation with pathogen might play different roles in plant disease resistance response (Bagnaresi et al., 2012; Tao et al., 2003; Wei et al., 2013), it will be of great interest to characterize the functions of the four *ZFP* genes during the rice-*M. oryzae* interaction in the future studies.

Materials and Methods

Identification of rice ZFP genes

The proteins that potentially contain zinc finger domain were retrieved from the MSU Rice Genome Annotation database 7.0 (RGAP, http://rice.plantbiology.msu.edu) (Ouyang et al., 2007) using 'Zinc' and 'Zinc finger' as 'Search Text' against the non-redundant proteins. The retrieved proteins containing zinc finger domain were designed as ZFP. Declaring a gene belonging to the ZFP family was based on the annotation of RGAP (Table 1).

Collection of the expression data of ZFP genes and construction of co-expressed ZFP gene network

The expression profiles of *ZFP* genes were downloaded manually from the Rice oligonucleotide array database (http://www.ricearray.org/) (Cao et al., 2012). The series accession of GED dataset is GSE7256. Normalization and expression data analysis are conducted as described by Cao et al., (2012). The co-expressed *ZFP* gene network was established on the basis of database of probabilistic functional gene network of *Oryza sativa* (http://www.functionalnet.org/ricenet/search.html) (Lee et al., 2011).

Plant materials

The seeds from the blast disease resistant rice variety DG (Chen et al., 2006; Chen et al., 2004; Shang et al., 2009) and the susceptible rice variety LTH (Chen et al., 2004) were germinated on moisturized papers and grown in soil in a growth chamber with a 12h light/12h dark photoperiod. Two-week-old rice seedlings of DG and LTH were inoculated with the *M. oryzae* spore suspension of 5×10^5 conidia mL^{-1} with 0.2% Tween-20 (blast isolate ZB15) using spraying method. For a mock-inoculation, the rice seedlings of the same growth stage were inoculated with 0.2% Tween-20. The spore- and mock-inoculated rice seedlings were kept in dark inoculation chambers with 95% humidity at 28 °C. After 24 hpi, the plants were maintained in the growth chamber at 28ºC in 12h light/12h dark photoperiod with 95% humidity. The leaves harvested respectively from 0h, 72h and 96h post inoculation were ground with liquid nitrogen, and immediately stored at -80 °C until RNA extraction. The remaining seedlings were left for disease evaluation at 8 days post inoculation to confirm that the inoculation was successfully performed.

Quantitative RT-PCR experiment

Total RNA was extracted using TRIzol_reagent (Invitrogen Life Technologies, Shanghai, China) in accordance with the manufacturer's protocols. cDNA was synthesized using cDNA reverse transcription Kit (Invitrogen Life Technologies, Shanghai, China). The Real Time-PCR program was run by BIO-RAD CFX Manager Software with three experimental replicates (see Supplemental Table 4 for gene-specific primer sequences, and annealing temp). The reference gene *UBQ5* (the forward primer: AACCAGCTGAGGCCCAAGA; the reverse primer: ACGATTGATTTAACCAGTCCATGA) was used as control for qRT-PCR experiments (Jain et al., 2006). The $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels with three experimental repeats (Livak and Schmittgen, 2001)

Conclusions

On the basis of rice genome, we found that 1,258 *ZFP* genes in 53 families were distributed on all 12 chromosomes of rice whole genome. Two hundred and forty-one *ZFP* genes from 27 families were likely involved in the rice-*M. oryzae* interaction. Among the 27 families, the GATA, FYVE, TDDP, RBPO, A20, TFIIB, Dof, C3H, CDGSH, PADPP, LYAR, ZK, TAZ, MSRING, Ubox, Bbox, NFX1, AN, UBR, C5HC2, DHHC, PHD and CHY families were newly identified for their functions in the rice-*M. oryzae* interaction. We constructed the network of the induced and reduced *ZFP* genes post inoculated with *M. oryzae* and found that 36 percent of these rice-*M. oryzae* interaction related *ZFP* genes were able to be co-expressed with those genes possessing various biological functions, such as methylation modification, protein kinase activity, transcription activity, posttranscriptional modification and protein transfer. Four *ZFP* genes showed different expression patterns between the compatible and incompatible interactions of rice-*M.oryzae*. It provided candidate *ZFP* genes for the further research on blast defense.

Acknowledgements

This work was supported by the grants from National Natural Science Foundation of China (NSFC 31171622; 31371705), Sichuan "Hundred Talents Plan" and "High Talents" start-up fund of Sichuan Agricultural University in China to X. W. Chen and the grants from Specialized Research Fund for the Doctoral Program of Higher Education (20125103120011).

References

AbuQamar S, Chen X, Dhawan R, Bluhm B, Salmeron J, Lam S, Dietrich RA, Mengiste T (2006) Expression profiling and mutant analysis reveals complex regulatory networks involved in arabidopsis response to Botrytis infection. Plant J. 48: 28-44.

Agarwal P, Arora R, Ray S, Singh AK, Singh VP, Takatsuji H, Kapoor S, Tyagi AK (2007) Genome-wide identification of C2H2 zinc-finger gene family in rice and their phylogeny and expression analysis. Plant Mol Biol. 65: 467-485.

Bagnaresi P, Biselli C, Orru L, Urso S, Crispino L, Abbruscato P, Piffanelli P, Lupotto E, Cattivelli L, Vale G (2012) Comparative transcriptome profiling of the early response to *Magnaporthe oryzae* in durable resistant vs susceptible rice (Oryza sativa L.) genotypes. PLoS One. 7: e51609.

Cao P, Jung KH, Choi D, Hwang D, Zhu J, Ronald PC (2012) The rice oligonucleotide array database: an atlas of rice gene expression. Rice. 5: 17.

Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, Liu G, Xu J, Ling Z, Cao G, Ma B, Wang Y, Zhao X, Li S, Zhu L (2006). A B-lectin receptor kinase gene conferring rice blast resistance. Plant J. 46: 794-804.

Chen XW, Li SG, Xu JC, Zhai WX, Ling ZZ, Ma BT, Wang YP, Wang WM, Cao G, Ma YQ, Shang JJ, Zhao XF, Zhou KD, Zhu LH (2004) Identification of two blast resistance genes in a rice variety, Digu. J Phytopathology. 152: 77-85.

Chujo T, Miyamoto K, Shimogawa T, Shimizu T, Otake Y, Yokotani N, Nishizawa Y, Shibuya N, Nojiri H, Yamane H, Minami E, Okada K (2013) OsWRKY28, a PAMP-responsive transrepressor, negatively regulates innate immune responses in rice against rice blast fungus. Plant Mol Biol. 82: 23-37.

Chujo T, Takai R, Akimoto-Tomiyama C, Ando S, Minami E, Nagamura Y, Kaku H, Shibuya N, Yasuda M, Nakashita H, Umemura K, Okada A, Okada K, Nojiri H, Yamane H (2007) Involvement of the elicitor-induced gene OsWRKY53 in the expression of defense-related genes in rice. Biochim Biophys Acta. 1769: 497-505.

Dangl JL, Dietrich RA, Richberg MH (1996). Death Don't Have No Mercy: Cell death programs in plant-microbe interactions. Plant Cell. 8: 1793-1807.

Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K, Yano M (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. Science. 325: 998-1001.

Gourcilleau D, Lenne C, Armenise C, Moulia B, Julien JL, Bronner G, Leblanc-Fournier N (2011) Phylogenetic study of plant Q-type C2H2 zinc finger proteins and expression analysis of poplar genes in response to osmotic, cold and mechanical stresses. DNA Res. 18: 77-92.

Ham BK, Park JM, Lee SB, Kim MJ, Lee IJ, Kim KJ, Kwon CS, Paek KH (2006) Tobacco Tsip1, a DnaJ-type Zn finger protein, is recruited to and potentiates Tsi1-mediated transcriptional activation. Plant Cell. 18: 2005-2020.

Huang J, Teng L, Li L, Liu T, Chen D, Xu L G, Zhai Z, Shu HB (2004) ZNF216 is an A20-like and IkappaB kinase gamma-interacting inhibitor of NFkappaB activation. J Biol Chem. 279: 16847-16853.

Jain M, Nijhawan A, Tyagi AK, Khurana JP (2006) Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. Biochem Biophys Res Commun. 345: 646-651.

Karim S, Holmstrom KO, Mandal A, Dahl P, Hohmann S, Brader G, Palva ET, Pirhonen M (2007) AtPTR3, a wound-induced peptide transporter needed for defence against virulent bacterial pathogens in Arabidopsis. Planta. 225: 1431-1445.

Kim DH, Yamaguchi S, Lim S, Oh E, Park J, Hanada A, Kamiya Y, Choi G (2008) SOMNUS, a CCCH-type zinc finger protein in Arabidopsis, negatively regulates light-dependent seed germination downstream of PIL5. Plant Cell. 20: 1260-1277.

Kong Z, Li M, Yang W, Xu W, Xue Y (2006) A novel nuclear-localized CCCH-type zinc finger protein, OsDOS, is involved in delaying leaf senescence in rice. Plant Physiol. 141: 1376-1388.

Lee I, Seo YS, Coltrane D, Hwang S, Oh T, Marcotte EM, Ronald PC (2011) Genetic dissection of the biotic stress response using a genome-scale gene network for rice. Proc Natl Acad Sci U S A. 108: 18548-18553.

Li H, Yuan Z, Vizcay-Barrena G, Yang C, Liang W, Zong J, Wilson ZA, Zhang D (2011) PERSISTENT TAPETAL CELL1 encodes a PHD-finger protein that is required for tapetal cell death and pollen development in rice. Plant Physiol. 156: 615-630.

Li WT, He M, Wang J, Wang YP (2013) Zinc finger protein (ZFP) in plant-A review. Plant Omics. 6: 474-480.

Li WT, Wei YM, Wang JR, Liu CJ, Lan XJ, Jiang QT, Pu ZE, Zheng YL (2010) Identification, localization, and characterization of putative USP genes in barley. Theor Appl Genet. 121: 907-917.

Lim SD, Yim WC, Moon JC, Kim DS, Lee BM, Jang CS (2010) A gene family encoding RING finger proteins in rice: their expansion, expression diversity, and co-expressed genes. Plant Mol Biol. 72: 369-380.

Liu H, Zhang H, Yang Y, Li G, Wang X, Basnayake BM, Li D, Song F (2008) Functional analysis reveals pleiotropic effects of rice RING-H2 finger protein gene OsBIRF1 on regulation of growth and defense responses against abiotic and biotic stresses. Plant Mol Biol. 68: 17-30.

Liu X, Bai X, Wang X, Chu C (2007) OsWRKY71, a rice transcription factor, is involved in rice defense response. J Plant Physiol. 164: 969-979.

Liu XQ, Bai XQ, Qian Q, Wang XJ, Chen MS, Chu CC (2005) OsWRKY03, a rice transcriptional activator that functions in defense signaling pathway upstream of OsNPR1. Cell Res. 15: 593-603.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 25: 402-408.

Ma K, Xiao J, Li X, Zhang Q, Lian X (2009) Sequence and expression analysis of the C3HC4-type RING finger gene family in rice. Gene. 444: 33-45.

Miller J, McLachlan AD, Klug A (1985) Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus oocytes*. EMBO J. 4: 1609-1614.

Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K, Thibaud-Nissen F, Malek RL, Lee Y, Zheng L, Orvis J, Haas B, Wortman J, Buell CR (2007) The TIGR rice genome annotation resource: improvements and new features. NAR 35 Database Issue: D846-851.

Peng Y, Bartley LE, Chen X, Dardick C, Chern M, Ruan R, Canlas PE, Ronald PC (2008) OsWRKY62 is a negative regulator of basal and Xa21-mediated defense against *Xanthomonas oryzae* pv. *oryzae* in rice. Mol Plant. 1: 446-458.

Qiu D, Xiao J, Xie W, Liu H, Li X, Xiong L, Wang S (2008) Rice gene network inferred from expression profiling of plants overexpressing OsWRKY13, a positive regulator of disease resistance. Mol Plant. 1: 538-551.

Qiu D, Xiao J, Ding X, Xiong M, Cai M, Cao Y, Li X, Xu C, Wang S (2007) OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. Mol Plant Microbe In. 20: 492-499.

Ribot C, Hirsch J, Balzergue S, Tharreau D, Notteghem JL, Lebrun MH, Morel JB (2008) Susceptibility of rice to the blast fungus, *Magnaporthe grisea*. J Plant Physiol. 165: 114-124.

Ryu HS, Han M, Lee SK, Cho JI, Ryoo N, Heu S, Lee YH, Bhoo SH, Wang GL, Hahn TR, Jeon JS (2006) A comprehensive expression analysis of the WRKY gene superfamily in rice plants during defense response. Plant Cell Rep. 25: 836-847.

Sesma A, Osbourn AE (2004) The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. Nature. 431: 582-586.

Shang J, Tao Y, Chen X, Zou Y, Lei C, Wang J, Li X, Zhao X, Zhang M, Lu Z, Xu J, Cheng Z, Wan J, Zhu L (2009) Identification of a new rice blast resistance gene, Pid3, by genomewide comparison of paired nucleotide-binding site--leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. Genetics. 182: 1303-1311.

Song Z, Krishna S, Thanos D, Strominger JL, Ono SJ (1994) A novel cysteine-rich sequence-specific DNA-binding protein interacts with the conserved X-box motif of the human major histocompatibility complex class II genes via a repeated Cys-His domain and functions as a transcriptional repressor. J Exp Med. 180: 1763-1774.

Stulemeijer IJ, Joosten MH (2008) Post-translational modification of host proteins in pathogen-triggered defence signalling in plants. Mol Plant Pathol. 9: 545-560.

Takatsuji H (1998). Zinc-finger transcription factors in plants. Cell Mol Life Sci. 54: 582-596.

Tao Y, Xie Z, Chen W, Glazebrook J, Chang H S, Han B, Zhu T, Zou G, Katagiri F (2003) Quantitative nature of arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell. 15: 317-330.

Wang D, Guo Y, Wu C, Yang G, Li Y, Zheng C (2008) Genome-wide analysis of CCCH zinc finger family in arabidopsis and rice. BMC Genomics. 9: 44.

Wang H, Hao J, Chen X, Hao Z, Wang X, Lou Y, Peng Y, Guo Z (2007) Overexpression of rice WRKY89 enhances ultraviolet B tolerance and disease resistance in rice plants. Plant Mol Biol. 65: 799-815.

Wang L, Pei Z, Tian Y, He C (2005) OsLSD1, a rice zinc finger protein, regulates programmed cell death and callus differentiation. Mol Plant Microbe In. 18: 375-384.

Wei T, Ou B, Li J, Zhao Y, Guo D, Zhu Y, Chen Z, Gu H, Li C, Qin G, Qu LJ (2013) Transcriptional profiling of rice early response to Magnaporthe oryzae identified OsWRKYs as important regulators in rice blast resistance. PLoS ONE. 8: e59720.

Whitham S, Dinesh-Kumar S P, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin-1 receptor. Cell. 78: 1101-1115.

Xiang K, Li WT, Chen XW, Yuan GS, Chen WL, Zhang ZM, Shen YO, Lin HJ, Pan GT (2013) Clustering and expression analysis of chitinases in maize and rice. J Life Sci. 7: 244-251.

Xu C, He C (2007) The rice *OsLOL2* gene encodes a zinc finger protein involved in rice growth and disease resistance. Mol Genet Genomics. 278: 85-94.

Yanagisawa S (2002) The Dof family of plant transcription factors. Trends Plant Sci 7: 555-560.

Yang X, Deng F, Ramonell KM (2012) Receptor-like kinases and receptor-like proteins: keys to pathogen recognition and defense signaling in plant innate immunity. FiB. 7: 155-166.

Yokotani N, Sato Y, Tanabe S, Chujo T, Shimizu T, Okada K, Yamane H, Shimono M, Sugano S, Takatsuji H, Kaku H, Minami E, Nishizawa Y (2013) OsWRKY76 is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance. J Exp Bot. 64(16): 5085-5097.

Zeng LR, Qu S, Bordeos A, Yang C, Baraoidan M, Yan H, Xie Q, Nahm BH, Leung H, Wang GL (2004) Spotted leaf11, a negative regulator of plant cell death and defense, encodes a U-box/armadillo repeat protein endowed with E3 ubiquitin ligase activity. Plant Cell. 16: 2795-2808.

Zhang J, Peng Y, Guo Z (2008) Constitutive expression of pathogen-inducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. Cell Res. 18: 508-521.

Zhang X (2012) Dynamic differential methylation facilitates pathogen stress response in arabidopsis. Proc Natl Acad Sci U S A. 109: 12842-12843.

Zhou S, Zhao W, Li H, Guo Z, Peng YL (2008) Characterization of a novel RING finger Gene *OsRFP1*, which is induce by ethylene, salicylic acid and blast fungus infection in rice. J Phytopathology. 156: 396-402.