Isolation of a Rice *WRKY* Gene *OsWRKY52*, Whose Expression Is Induced by *Magnaporthe grisea**

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Abstract WRKY proteins, a big family of transcription factors, are involved in regulation diverse developmental and physiological processes in plants. Here, a novel *WRKY* gene, *OsWRKY52*, was isolated from a rice cDNA library. This gene included an open reading frame of 1 719 bp in length, and the deduced polypeptide contained 572 amino acids, sharing 54% identity with a WRKY1 protein from *Avena sativa*. Expression of *OsWRKY52* gene was induced rapidly by *Magnaporthe grisea* in the incompatible interaction with rice plant. OsWRKY52 protein, expressed prokaryotically bound specifically to W box *cis* elements derived from the promoter of a rice *PR1a*. Transcriptional activation assay was performed by a yeast one- hybrid method. Regions of transactivation were identified to be the N-terminal serine- and threonine-rich islands and the C-terminal acidic domain of OsWRKY52. These results suggest that OsWRKY52, as a transcription activator, may be involved in defense responses against *Magnaporthe grisea* in rice plants.

Key words DNA-binding, *Magnaporthe grisea*, *Oryza sativa*, transcription activation, transcription factor, WRKY

Being sessile, plants have evolved a variety of elaborate regulatory mechanisms to fine-tune their metabolisms for survival under various environmental stresses, such as pathogens, during growth and development. This kind of physiological flexibility is largely mediated by altering the expression states of genes. Increasing evidences reveal that transcription factors play crucial roles in regulation of gene expression. WRKY proteins constitute a large group of transcription factor family in plants. There are over 70 members in *Arabidopsis*[1,2] and 102 members in rice[3]. A common characteristic is that WRKY proteins possess one or two highly conserved WRKY domains, which comprises approximately 60 amino acids in length with invariant WRKY sequence in its N-terminus followed by a novel zinc finger motif. Based on the phylogenetic analysis of the WRKY domains and their structure, WRKY can be classified into three groups with several subgroup[1,3,4]. Generally, which contains two WRKY domains is categorized into the group Ⅰ, while those containing only one WRKY domain are classified into group Ⅱ or Ⅲ with difference in the zinc finger pattern. However, the zinc finger pattern of subgroup Ⅲb of rice exhibits significant variations, suggesting diversifications of WRKY proteins in rice[3,4].

High conservation of WRKY domain in WRKY proteins is mirrored by their cognate binding W box *cis* element with core sequence of TGAC[5-8], albeit other binding sequences were also found[9]. W box elements are present within the promoters of many pathogen defense-related, development-, metabolism-associated genes and *WRKY* themselves[5,6,9,10], implying that WRKY proteins might regulate expression of a variety of genes in plants.

The strong and rapid induction of transcription of *WRKY* genes responsive to pathogen infection or treatments with elicitors, salicylic acid in many plant species suggest they might mediate defense responses against diseases in plants[11] (see review). For example, 49 out of 72 members of *WRKY* genes in *Arabidopsis* are shown to be induced by pathogen infection or by salicylic acid[2]. Recently, the involvement of WRKY proteins in pathogen defense signaling has been demonstrated. For example, the expression of *NPR1*, as a transcription coactivator of genes involved in the resistance signaling pathways, is positively regulated

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by certain WRKY proteins, such as AtWRKY18 [12], AtWRKY70 [13]. Furthermore, WRKY proteins functions in other physiological or developmental processes, such as senescence [6,14], trichome development [15], sesquiterpene syntheses [16], carbohydrate metabolism [9], and abiotic stress responses, including wound, drought, cold, and freezing [8,11]. These observations demonstrate that WRKY proteins play diverse regulatory roles in plants.

Sequencing of the whole rice genome [17,18] and abundant information available in the GenBank provide an ideal platform for comparative analysis of *WRKY* superfamily within the rice genome, and also greatly facilitate gene isolation. Previously, we found 102 members of *WRKY* in the rice genome by data-mining and annotation [3]. Furthermore, in an endeavor for investigating the potential involvement of *WRKY* genes in defense responses for rice blast, a disastrous disease of rice caused by *Magnaporthe grisea*, we isolated and characterized several rice *WRKY* genes from a cDNA library constructed from suspension rice cells (*Oryza sativa*, cultivar IR72) treated with elicitors from *M. grisea* cell walls [7] (and unpublished data). Herein, we report one of these *WRKY* genes, designated as *OsWRKY52*, which was induced by *M. grisea* inoculation.

1 Materials and methods

1.1 Reagents and kits

LATaq DNA polymerase and restriction endonucleases were purchased from TakaRa Biotechnology (Dalian). PCR gel extract kit was purchased from Sangon Biotechnology (Shanghai). Isopropyl β-D-thiogalactopyranoside (IPTG) was purchased from Sigma (USA). Yeast transformation vectors were from Clontech (USA). Hybond-N^+ nylon membrane was from Amersham Pharmacia (UK).

1.2 Isolation of *OsWRKY52* gene and sequence analysis

All primers used in this study are listed in Table 1. According to deposited genome sequence (AAAA0001000357), primers were designed as w52F and w52R for amplification of *OsWRKY52* cDNA. The PCR templates used were plasmids of a cDNA library from rice suspension-cultured cells elicitated by cell wall components of *M. grisea*. PCRs were performed with LATaq polymerase at 94℃ for 2 min, followed by 30 cycles at 94℃ denaturation for 1 min, at 59℃ annealing for 1 min, at 72℃ extension for 2 min, and finished by an extension at 72℃ for 10 min. The purified PCR products were subcloned into a pUCm-T, and the resulting plasmid (pUCmT-OsWRKY52) was sequenced.

Table 1 Sequences of primers used in this study

Name	Sequence (restriction enzyme site)	Nucleotide position[1)]
w52F	5′ AAGGATCCTCCGTCTCCGATGACC 3′ (*Bam*H Ⅰ)	−10 ~ 6
w52R	5′ TCATCAAAACAGCAGCGGCTG 3′	1 702 ~ 1 719
52F	5′ AACCCGGGGATGACCGCCGCGCC 3′ (*Sma* Ⅰ)	−1 ~ 14
C1R	5′ TCA GTCGACCGTACTTGCGCCAGTTGT 3′ (*Sal* Ⅰ)	664 ~ 681
C2R	5′ TCA GTCGACCTGCACAACCACCCTC 3′ (*Sal* Ⅰ)	1 134 ~ 1 149
C3R	5′ TCAGTCGACCAGGTAGCTCGAGGGC 3′ (*Sal* Ⅰ)	1 443 ~ 1 458
N1F	5′ AACCCGGGGACGGGCGCATTGTTCAG 3′ (*Sma* Ⅰ)	246 ~ 263
N2F	5′ AACCCGGGCTACAACTGGCGCAAGTAC 3′ (*Sma* Ⅰ)	663 ~ 681
C3F	5′ AACCCGGGGCAGGGCGGCGGC 3′ (*Sma* Ⅰ)	1 458 ~ 1 470
52R	5′ TCAGTCGACAAACAGCAGCGGCTGCTC 3′ (*Sal* Ⅰ)	1 699 ~ 1 716

[1)]Numbers are defined as nt positions relative to the "A" of the start codon of *OsWRKY52* gene.

Database searches were carried out with the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI). Protein sequence alignment was conducted with ClustalW program and BOXSHADE (http://bioweb.pasteur.fr) [19]. Determination of chloroplast transfer peptide was performed using ChloroP (http://www.cbs.dtu.dk/services/ ChloroP) [20].

1.3 Expression of OsWRKY52 in *Escherichia coli* and electrophoretic mobility shift assay

For construction of expression vector, the encoding region of *OsWRKY52* was amplified by PCR method using the forward primer w52F paired with a reverse primer 52R with a *Sal* Ⅰ site just before the stop codon. The PCR products digested with *Bam*H Ⅰ and *Sal* Ⅰ were ligated in frame with the His tag of pET-29b (+) linearized with the same enzyme digestion. The resulting pET-OsWRKY52 was transformed into *E. coli* BL21 (DE3) strain. For expression of OsWRKY52, BL21 strains carrying either recombined expression plasmids or pET-29b were cultured in 5 ml LB containing 100 mg/L kanamycin at 37℃ overnight, and re-inoculated 1 ml of the culture into 100 ml new LB containing 100 mg /L kanamycin for continuing incubation. When the *A* value reached approximate 0.6, 0.4 mmol/L IPTG was added into the culture for protein induction. The cells were lysed by sonication (3×45 s, 150 W) with 1 min intervals on ice. Pellets and supernatants were pooled respectively for SDS-PAGE analysis. The clear supernatants were used for electrophoretic mobility shift assay (EMSA).

EMSA was carried out as described previously[7].

1.4 Transactivation activity assays for OsWRKY52

The primer pair of w52F and 52 R was used to amplify the coding sequence of *OsWRKY52*. The products were inserted in frame into the C-terminus of Gal4 binding domain (BD) in pGBKT vector to generate pBD-WRKY. Other deletion mutants were constructed in the same way. Fragments of ΔC1, ΔC2, and ΔC3 were amplified by using primer C1R, C2R, and C3R paired with 52F, respectively. Similarly, primer N1F, N2F, and C3F paired with primer 52R were utilized to amplify the deletion derivatives ΔN1, ΔN2, and ΔN3, respectively. Finally, N1F primer plus C1R, N2F plus C2R, and N1F plus C3R were applied to amplify the sequences for the truncated ΔN1C1, ΔN2C2, and ΔC3, respectively. The different Gal4 BD fusion plasmids and the vacant vector pBDKT were transformed into yeast strain AH109, and incubated on SD-Trp or SD-Trp-Ade-His selective medium at 28℃ for 3 days. Assay of α-galactosidase activity was performed with transformed cell lines grown in liquid SD-Trp medium using *p*-nitrophenyl α-D-galactopyranoside as a substrate according to the manufacturer's instruction. pCL-1 (possessing Gal BD and AD in fusion) was used as positive control in liquid assay.

1.5 Rice growth and *Magnaporthe grisea* inoculation

Rice (*Oryzae sativa* L. var. Xiushui 11) seeds were imbibed in water at 37℃ for 2 days, and then grown in vermiculite with the addition of water and mineral nutrient solution in a greenhouse. Relative humidity was maintained at 70%. Twenty five- day-old plants were inoculated with conidia of *M. grisea* (97-220E3, compatible strain; 93-217F1, incompatible strain), according to the procedures reported by He *et al*[21]. Rice plants sprayed with sterilized water were set as a control.

1.6 RNA gel blot

RNA extraction was performed as described by [22]. Briefly, total RNA (20 μg, unless otherwise stated) was fractionated in a 1.2% agarose containing formaldehyde, transferred to Hybond-N^+ nylon membrane and hybridized with ^{32}P-labeled probe (nt 1 442~1 719) specific for *OsWRKY52*. After washing with 2× SSC plus 0.1% SDS at 65℃ for 5 min and with 1× SSC plus 0.1% SDS at 65℃ for 5 min, the membrane was autoradiographed using a Phosphoimaging system (Amersham Pharmacia, UK).

2 Results

2.1 Isolation of *OsWRKY52* cDNA and sequence analysis

To get a clue on the involvement of rice *WRKY* genes in defense response to pathogens, we employed a cDNA library from rice suspension cultured cells elicited by *M. grisea* cell wall for amplification. A fragment of 1 729 bp in length was amplified. Sequencing revealed that there were several nucleotide polyphenisms between the amplified fragment (from IR72 cultivar) and the predicted gene of the *japonica* cultivar Nipponbare used in the genome sequence project. This gene, named as *OsWRKY52*[3] had an open reading frame of 1 719 bp, and encoded a predicted polypeptide of 572 amino acids with a molecular mass of 59.7 ku and a p*I* of 5.5 (Figure 1). Comparison with the genomic and cDNA sequences indicated that three introns existed in the gene. The deduced OsWRKY52 harbors two WRKY domains and is classified into the group Ⅰ[3]. Interestingly, an alteration exists in the C2H2 zinc finger motif of the N-terminal WRKY domain, in which a serine instead of an asparagine residue took place between the two histidines (Figure 1). Additionally, OsWRKY52 protein was found to contain a serine-rich island (13 ~ 63 amino acid), a threonine-rich (81~140 amino acid) island, an acidic region located on the C-terminus, and

a consecutive glutamine stretch (538~542 amino acid) inside the acidic region. These regions suggest that OsWRKY52 may function as a transcription activator. Moreover, OsWRKY52 possesses a predicted chloroplast transit signal peptide located in its N-terminus (1~20 amino acid), indicating a putative chloroplast or plastid resident protein.

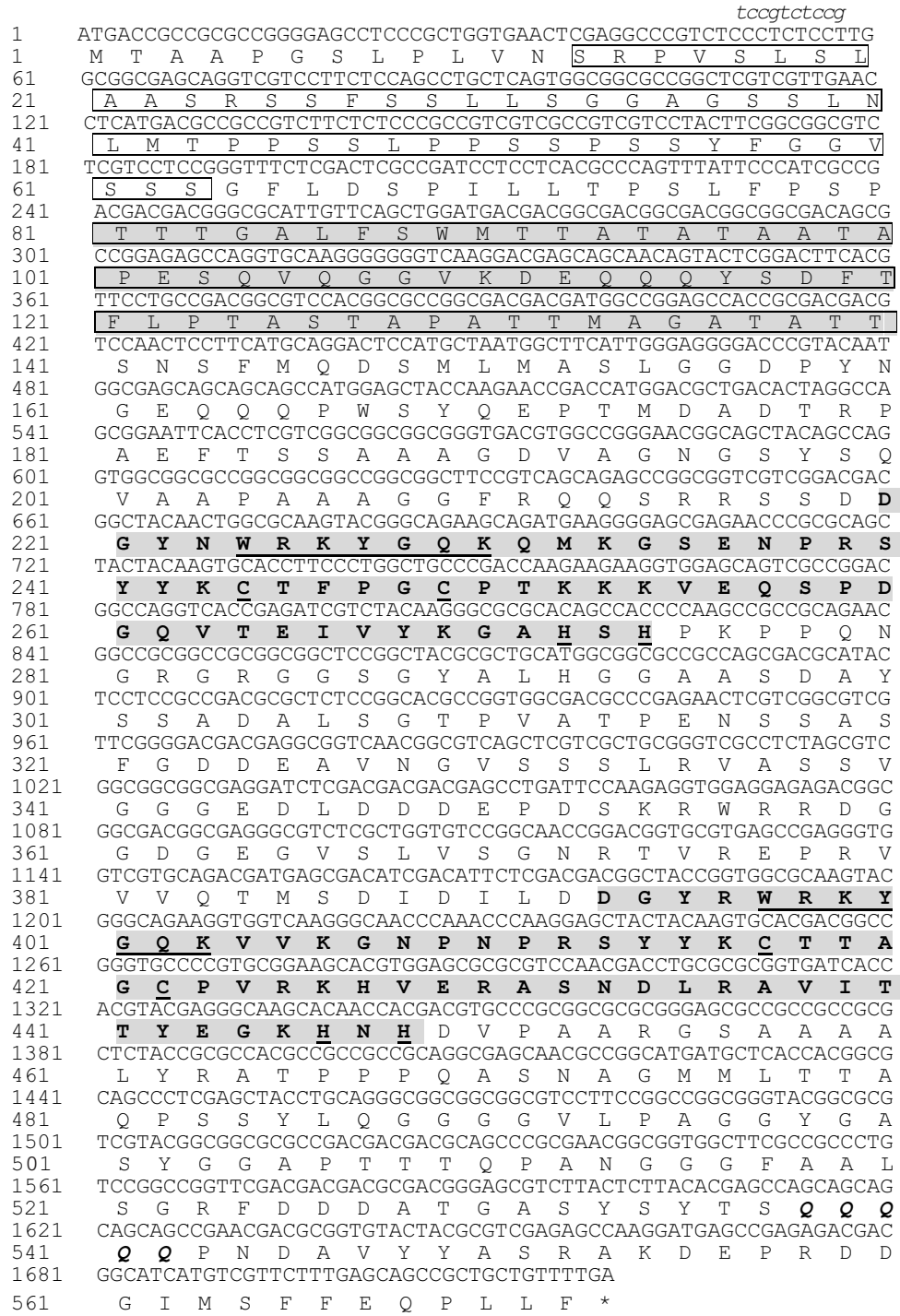

Fig.1 Nucleotide and deduced amino acid sequence of the *OsWRKY52* cDNA

The 5′ untranslated region is italicized. Two WRKY domains are in bold and shaded, in which the invariant WRKYGQK and the cysteine and histidine residues forming the potential zinc-finger motif are underlined. The serine island is boxed. The threonine island is boxed and shaded. The consecutive 5 glutamines (Q) within the C-terminal acidic region is in bold and italicized.

Alignment of OsWRKY52 with other WRKY proteins from different species was performed, and the region covering the two WRKY domains was shown in Figure 2. OsWRKY52 displays the highest degree (54%) of identity with AsWRKY1 from *Avena sativa*, and with 51% and 49% identity to ABF1 from *Ipomoea batatas* and WRKY24 from rice, respectively. This close relationship was further addressed by the phylogenetic analysis, using the subgroup Ⅰa members available, in which the four members mentioned above were claded into one subgroup with high bootstrap values (data not shown).

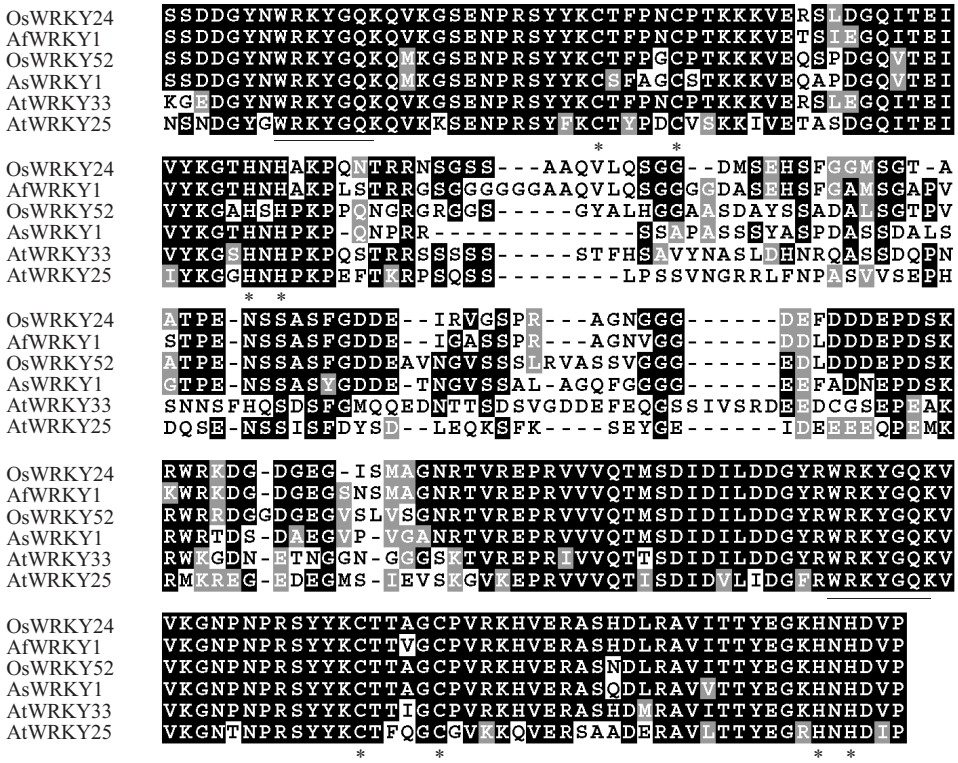

Fig.2 Alignment of the OsWRKY52 deduced protein sequence with closely related WRKY proteins covering the two WRKY domains

The deduced amino acid sequence of OsWRKY52 was aligned, by using ClustalW program and BOXSHADE (http://bioweb.pasteur.fr), with *Avena fatua* AfWRKY1 (ABF1) (S61413); *Avena sativa* AsWRKY1 (AAD32677); *Arabidopsis thaliana* AtWRKY25 (AAL13040), AtWRKY33 (AAM34736); *Oryza sativa* OsWRKY24 (AAT84156). Identical amino acids are shown in black boxes, and similar amino acids are shown in gray boxes. The WRKYGQK peptide stretch is underlined. The cysteine and histidine residues forming the potential zinc-finger motif are marked underneath with ∗.

2.2 Recombinant OsWRKY52 binds with W box element

To investigate the affinity of OsWRKY52 with *cis* elements, we firstly expressed the protein in *E. coli* BL21 (DE3). *E. coli* harboring pET29-WRKY plasmid was induced with 0.4 mmol/L IPTG, and the protein expression was shown in Figure 3a. OsWRKY52 protein of expected size was mainly expressed in the pellets and also a small fraction in the supernatant (lanes *3* and *4*). This amount of native recombinant protein in the supernatant was used in the eletrophoretic mobility shift assay.

Next, we tested the capability of OsWRKY52 binding to W box *cis* elements by employing EMSA method. The W box containing oligonucleotide (P22) used was from the promoter of a rice *PR-1a* gene. As shown in Figure 3c, the crude OsWRKY52 containing proteins bound to ^{32}P-labbeled P22 probe, whereas the mutated probe (mP22, with mutation of the core T*GA*C sequence to T*CT*C or the complementary GTCA to G*A*G*A*) did not show any binding activity. To determine the specificity of the binding, we used

cold (unlabeled P22) and the mutated probes as competitors in the assays. The binding to ^{32}P-labbeled P22 was significantly reduced by addition of 10-fold excess of the cold probes, and almost abolished by 100-fold excess of addition. By contrast, addition of 100-fold of the mutated probes was not able to exert competitive effects on the formation of DNA-protein complex. To exclude the possibility that bacterium-derived proteins may bind to the selected DNA fragment as well, we used the similar lysate of *E. coli* harboring pET-29b empty vector induced by the same concentration of IPTG as a control. No any retarded band was observed. These results indicate that OsWRKY52 bound specifically to W box containing DNA fragment.

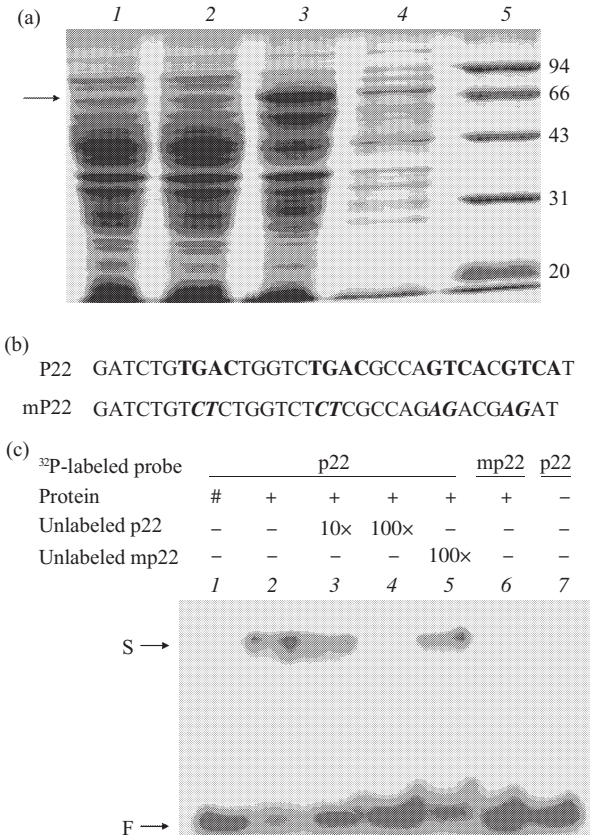

Fig.3 Expression of recombinant OsWRKY52 (a) and its specific binding to W box elements (b, c)

(a) *1*: Bacteria carrying pET-29b with induction; *2* and *3*: Bacteria carrying pET-OsWRKY52 without or with induction, respectively; *4*: Supernatant of bacteria carrying pET-OsWRKY52 with induction; *5*: Marker standard. Arrow indicates the position of the fusion protein. (b) Nucleotide sequences used in the electrophoretic mobility shift assays. The TGAC or its complementary GTCA sequences are in bold. The nucleotides in italic are mutated in mP22 probe, compared with those in P22 probe. (c) Electrophoretic mobility shift assays were performed using crude OsWRKY52-His fusion protein mixed with ^{32}P-labeled p22 or mp22. Notations of 10× and 100× indicate that the amount of unlabeled competitive probes added was 10- and 100-fold excess of the labeled one, respectively. "+" and "−" signs indicate presence or absence of the reagent in the reaction. The positions arrowed by "S" and "F" indicate respectively, the DNA-protein complex, the free probes. "#" represents lysate protein from *E. coli* transformed with pET-29b as a control.

2.3 Analysis of transcription activating ability in yeast

OsWRKY52 has an acidic region (p*I*=3.8) in its C-terminal end, a serine-rich and a threonine-rich regions (Figure 1), giving a hint that it may act as a transcription activator. To confirm this probability, we generated pBD-WRKY containing the whole coding sequence of *OsWRKY52* fused in frame to Gal4 binding domain in pGBKT (pBD), and transformed into yeast strain AH109. Cell lines transformed with pBD-WRKY grew normally on synthetic SD-Trp-Ade-His medium, revealing that OsWRKY52 had transcription activation capability in yeast. To narrow down the minimal transcriptional activation region, a series of deletion mutants were constructed. As shown in Figure 4, all the yeast cells carrying the deletion mutant plasmids, except for that of ΔN2C2 (amino acid 221~383) in the region of the beginnings of both N- and C-terminal WRKY domains, were able to grow on the selective medium, indicating that the N-terminal WRKY domains were not responsible for the transcription activation of OsWRKY52. Also, the C- terminal WRKY domain was not involved in the transcriptional activation by comparing the α-galactosidase activity of ΔC1 and ΔC2 constructs. Deletion of the C-terminal acidic region (86 amino acids) of OsWRKY52 resulted in a decrease of α-galactosidase activity to 56.3% of that obtained with the full length sequence, indicating that this acidic region is one of ADs for OsWRKY52. This notion was further supported by the fact that relative high α-galactosidase activity was remained in the yeast transformed with pBD-C3 plasmid, containing only the C-terminal acidic region. Furthermore, deletion of the serine-rich region (ΔN1, amino acid 82~572), or the threonine-rich region (ΔN1C3, amino acid 82~227) was shown to decrease the α-galactosidase activity to 26.5% and 42.6% of that activated by OsWRKY52, respectively. These results indicate that OsWRKY52 is a transcriptional activator, in which the C-terminal acidic region, the serine- and the threonine-rich islands may be involved.

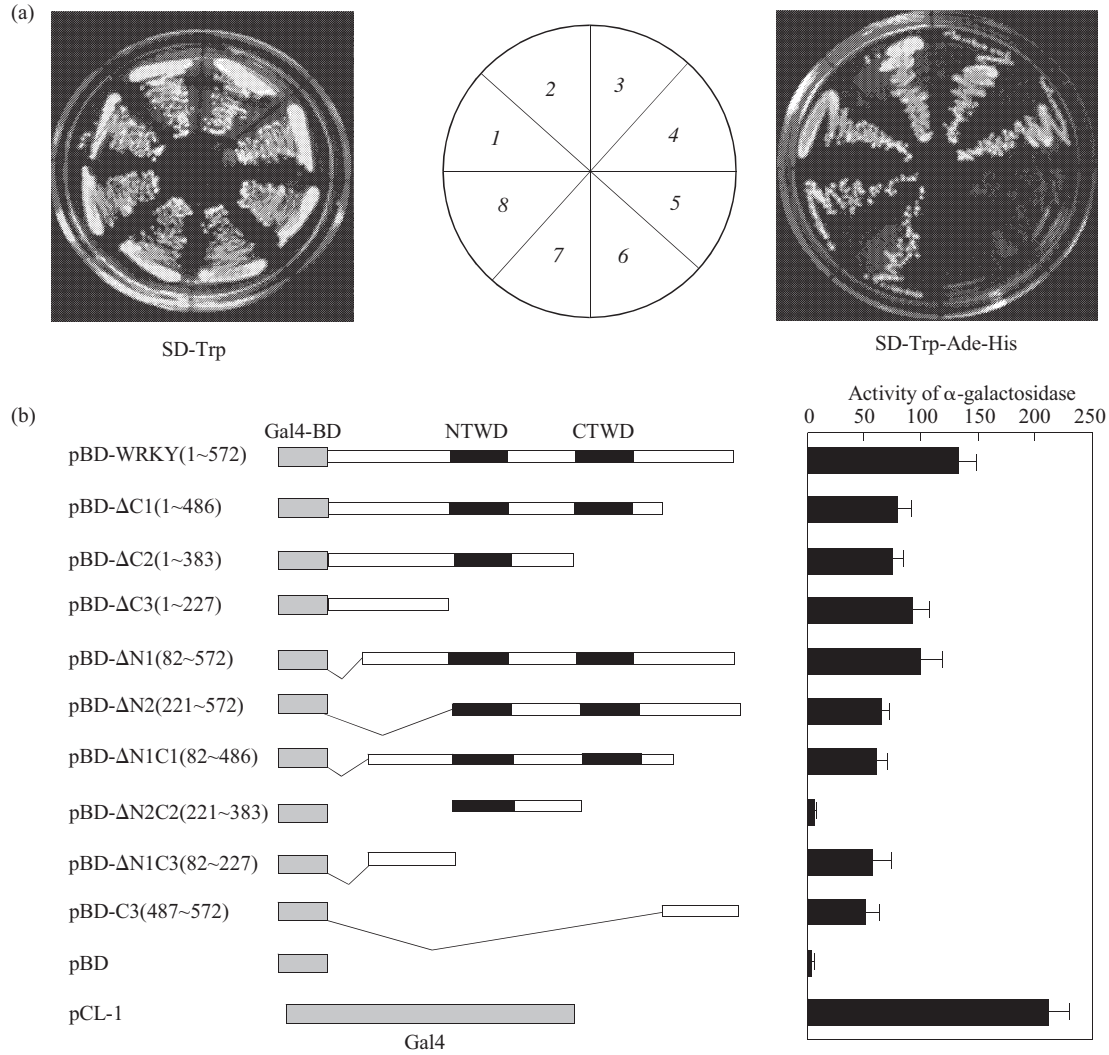

Fig.4 Analyses of transactivation activity and determination of transcription activating domains for OsWRKY52 in yeast
(a) The whole *OsWRKY52* coding sequence and different deletion mutants were inserted into the GAL4 binding-domain vector (pBD) to generate various transformation constructs, and introduced into yeast strain AH109. The yeast cells harboring various fusion plasmids or control vector (pBD) were streaked on the SD-Trp or SD-Trp-Ade-His selective medium and incubated at 30℃ for 3 days. *1*: pBD-WRKY; *2*: pBD-ΔN1; *3*: pBD-ΔC1; *4*: pBD-ΔN2; *5*: pBD-ΔN2C2; *6*, 2: pBD; *7*: pBD-C3; pBD-ΔN1C1. (b) The transformants were cultured on SD-Trp at 30℃ for 3 days. The liquid culture of yeast was used to assay its translation product using *p*-nitrophenyl α-galactopyranoside. The yeast cells carrying pCL-1 (cultured on SD-Leu) was used as the positive control. The numbers in brackets represent retained amino acid residues of each construct. NTWD and CTWD represent N-terminal and C-terminal WRKY domains, respectively. Values are indicated as $\bar{x} \pm s$ of three independent experiments.

2.4 Induction of *OsWRKY52* gene by *Magnaporthe grisea* treatment

To investigate its organ-specific expression, total RNA were extracted from various organs of rice plants, and hybridized with the specific probe for *OsWRKY52*. Expression of *OsWRKY52* gene was observed in roots, stems, and leaves of rice plants, whereas the level of the expression in flowers was much lower comparing with that in other organs (Figure 5a).

Our primary aim was to explore potential functions of rice *WRKY* genes in plant disease defense. Because the cDNA library employed in this study was constructed from cells treated with elicitors of *M. grisea*, it is rational to investigate the changes of *OsWRKY52* expression upon *M. grisea* inoculation. As shown in Figure 5b, the mRNA accumulation of *OsWRKY52* was increased rapidly 3 h after treatment with 93-217F1, an incompatible strain of *M. grisea* to this rice cultivar. However, the induction of *OsWRKY52* expression by inoculation with the compatible strain 97-220E3 was much less, compared with that induced by incompatible strain at the same time point. The result suggests that *OsWRKY52* gene might be implicated in defense responses against pathogens in rice.

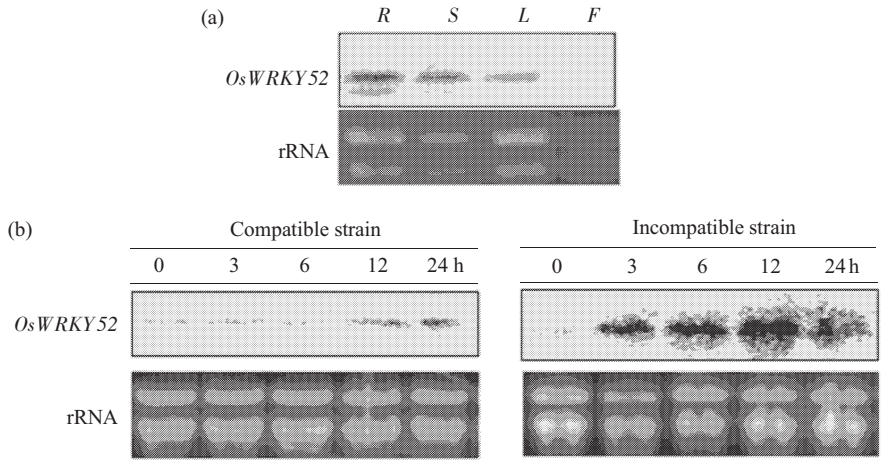

Fig.5 RNA gel blot analysis of *OsWRKY52* expression

Total RNA was isolated from roots (*R*), stems (*S*), leaves (*L*) and flowers (*F*) of 25-d-old rice plants (a), or from leaves of 25-d-old rice plants inoculated with compatible or incompatible *Magnaporthe grisea* strain and sampled at the indicated time (b). Hybridization was performed with a specific probe of *OsWRKY52*, as indicated in "**Materials and methods**". Equal loading of 20 μg (a) or 10 μg (b) total RNA to each lane was checked by ethidium bromide staining.

3 Discussion

In this paper, we describe a rice *WRKY* gene, *OsWRKY52* isolated from a cDNA library. Analyses of primary structure indicate that it was a novel *WRKY* gene in rice, belonging to the group Ⅰ subfamily. Phylogenetically, OsWRKY52 was most close to WRKY proteins from Poaceae, indicating the convergence of the member in the evolution.

With progress of the genomic sequencing, more and more entire genome sequences of different model organisms or organisms of economic value are accessible. Taking rice for example, genes can be cloned by using the convenient PCR method, basing on gene-specific primers designed on available rice genome sequences[17,18]. Recently, a rice *WRKY* gene, *OsWRKY71* encoding a transcriptional repressor of gibberrelin signaling in aleurone cells, and the full-length cDNAs of 13 rice *WRKY* genes were isolated by this strategy[23,24]. These examples indicate that PCR methodology based on bioinformatics is feasible for cloning of rice *WRKY* genes.

As demonstrated by substantial investigations, binding specifically to *cis* elements is one of prominent biochemical characteristics of transcription factors. Likely, OsWRKY52 exhibited a specific binding activity to W box *cis* elements derived from the promoter of rice *PR1a*, suggesting that OsWRKY52 might interact directly with this kind of *cis* elements in *planta* to regulate gene expression. Since the expression of *OsWRKY52* gene was induced by *M. grisea*, an expectation was OsWRKY52 involving in rice defense responses. Another biochemical feature of transcription factors is, if not all, acting as transcription activators or/and repressors of many physiological processes[25~28]. For instance, PcWRKY1 protein, a pathogen inducible parsley *WRKY* gene, is capable of transactivating through interaction with W box within the promoters of parsley *PR1* and its own[26]. Also, we demonstrated OsWRKY52 might be a transcription activator at least in yeast and narrowed down the possible transcription activation domains of the protein. These results, together with the result obtained by RNA blot analysis, suggest that OsWRKY52 may mediate defense responses to rice blast through activating its target genes *via* binding to W box elements.

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稻瘟菌诱导的水稻 *WRKY* 基因 *OsWRKY52* 的分离和鉴定 *

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摘要 WRKY 蛋白参与植物对生物或非生物胁迫反应和一些发育、代谢过程，在植物中组成一个转录因子大家族. 从水稻 cDNA 文库中分离到一个新的 *WRKY* 基因——*OsWRKY52* cDNA，包括一个 1 719 bp 的开放读码框，推测编码一个由 572 个氨基酸组成的蛋白质，与燕麦 (*Avena sativa*) AsWRKY1 具有 54%的氨基酸一致性. 该基因被非亲和性稻瘟菌快速诱导. 凝胶阻滞实验结果表明，原核表达的 OsWRKY52 能与水稻 *PR1a* 启动子上的 W 盒元件特异结合. 采用酵母单杂交体系的方法证明了 OsWRKY52 具有转录激活活性,其丝氨酸岛、苏氨酸岛和 C 端的富酸性氨基酸区是负责转录激活的区域. 这些结果提示 OsWRKY52 作为一个转录激活子，可能参与植物对稻瘟菌的应答反应.

关键词 DNA 结合，稻瘟菌(*Magnaporthe grisea*)，水稻(*Oryza sativa*)，转录激活，转录因子，WRKY

学科分类号 Q5, Q7

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