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ORIGINAL PAPER

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The rice *Rim2* transcript accumulates in response to *Magnaporthe grisea* and its predicted protein product shares similarity with TNP2-like proteins encoded by *CACTA* transposons

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Abstract A rice transcript, *Rim2*, was identified that accumulated in both incompatible and compatible interactions between rice and *Magnaporthe grisea*. The *Rim2* transcript also accumulated in response to treatment with a cell wall elicitor derived from *M. grisea*. A 3.3-kb *RIM2* cDNA clone was isolated and is predicted to encode a protein of 653 amino acids, which shares 32–55% identity with TNP2-like proteins encoded by *CACTA* transposons of other plants. A 1.05-kb segment of the *Rim2* sequence shows 82% nucleotide sequence identity with sequences flanking the *A1* and *C* members of the rice *Xa21* disease resistance gene family. The 5′-upstream region of *Rim2* was cloned and the transcriptional start sites were identified. The 5′ and 3′ noncoding termini of *Rim2* are AT-rich. A c*is*-element showing similarity to a sequence that mediates defense-associated transcriptional activation of the tobacco retrotransposon *Tnt1*, and four motifs that fit the consensus sequence of the elicitor-responsive elements in the promoters of the parsley *PR-1* genes were found in the 5′-upstream region. Four imperfect tandem repeats were identified in the 3′ noncoding terminus. Southern analysis with genomic DNA from different rice species indicated that *Rim2* is present in 3–4 copies per genome. These results suggest that *Rim2* may be one component of a large *CACTA*-like element, whose transcript accumulates in response to attack by pathogens.

Key words Rice · *Magnaporthe grisea* · *Rim2* · TNP2-like protein · Induction

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Introduction

Plants have developed sophisticated systems for responding to environmental stresses. For example, some plant transposable elements (TEs) are activated in response to environmental alterations (McClintock 1984; Burr and Burr 1988; Gierl et al. 1989; Arnault and Dufornel 1994). Upon gamma irradiation, the maize TE *Mu* excises from the *bronze*-2 locus, resulting in purple-spotted kernels (Walbot 1988). *Mu* elements can also be activated during cell culture (James and Stadler 1989). In tobacco, the copy number of the retrotransposons *Tto1*, *Tto2* and *Tnt1* increases during tissue culture, whereas no difference in copy number was observed among individuals of the same cultivars or different cultivars, suggesting that the three transposons are not transpositionally active in normally propagated plants (Hirochika 1993). Similarly, the carrot transposon *Tdc1*, a *CACTA* or *En*/*Spm*-like transposon, was found to be activated during long-term culture of cell suspensions, and caused somatic variation (Ozeki et al. 1997). In rice, the retrotransposons *Tos10*, *Tos17* and *Tos19* appear to be inactive under normal growth conditions; however, they were found to transpose under tissue culture conditions (Hirochika et al. 1996). Some 5–30 transposed *Tos17* copies were detected in plants regenerated from tissue culture, and the activation of *Tos17* induced mutation. Thus, it is thought that activation of transposable elements under stress provides plants with genomic plasticity in the face of diverse environment conditions (Arnault and Dufournel 1994; McDonald 1995).

In addition to activation of transposition, some retrotransposons show increased transcript accumulation when plants are subjected to environmental stresses. For example, the transcript of the tobacco *Tto1* accumulates after wounding or following treatment with methyl jasmonate – an inducer of wounding-responsive genes in plants (Takeda et al. 1998). The transcript of the rice *Tos17* was only detectable under tissue culture

conditions (Hirochika et al. 1996). Challenging plants with pathogens can also induce the transcription of some retrotransposons. For example, the tobacco *Tnt1* transcript accumulates in response to microbial elicitors from the incompatible fungi *Trichoderma viride* and *Phytophthora*, and the bacterium *Erwinia chysanthemi* (Pouteau et al. 1994). These pathogens have in common the ability to initiate a hypersensitive response (HR) in tobacco. *Tnt1* transcription is also induced by viral infection (Moreau-Mhiri et al. 1996). These results indicate that *Tnt1* activation might be a local and early plant response to microbial stress (Pouteau et al. 1994). In addition, wounding, freezing and other abiotic factors, such as sodium salicylate, cupric chloride and oxidative stress, induce transcription of *Tnt1* (Mhiri et al. 1997). A tandemly repeated sequence named BII located upstream of the *Tnt1* transcription start site in the 5′ long terminal repeat (called the LTR U3 region) mediates its activation in association with the plant defense response. The sequence of the BII motif (CCAACC-N_7-CT) is similar to a well-characterized motif, named the H-box (CCTACC-N_7-CT) involved in the transcriptional activation of the chalcone synthase promoter. An element named BI with the core sequence GGCATGTGC, upstream of the BII elements, resembles G-box related sequences (Casacuberta and Grandbastien 1993; Vernhettes et al. 1997). These results suggest that transcriptional activation of plant retrotransposons is activated by stress, and activation is under the control of *cis*-regulatory sequences similar to those of plant defense genes (Grandbastien 1998).

Many laboratories are interested in how rice (*Oryza sativa* L.) responds to challenge by the fungal pathogen *Magnaporthe grisea*, the causal agent of rice blast disease, one of the most destructive diseases in rice (Zeigler et al. 1994). Several rice defense genes whose transcripts accumulate in response to pathogen challenge have been isolated. These genes include those for 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGR*) (Nelson et al. 1994), lipoxygenase (Peng et al. 1994), phenylalanine ammonia-lyase (*PAL*) (Zhu et al. 1995) and chitinase (Xu et al. 1996). Transcripts of some other rice genes, such as *PBZ1* (Midoh and Iwata 1996), *RMa1* (He et al. 1997) and *RMa3* (Dong et al. 1997), accumulate in response to infection by *M. grisea*. Likewise, transcription of rice genes for HMGR, chitinase and GDP-dissociation inhibitors (GDIs) is induced by elicitors derived from *M. grisea* cell wall (Nelson et al. 1994; Xu et al. 1996; Kim et al. 1999). Here we describe a rice transcript, *Rim2* (rice gene induced by *M. grisea*), which accumulates in response to infection by *M. grisea* and treatment with a cell wall elicitor from *M. grisea*. The amino acid sequence of the predicted RIM2 protein is similar to those of TNP2-like proteins encoded by the *En*/*Spm* transposon family [also known as *CACTA* elements due to the presence of the conserved sequence CACTA in their terminal inverted repeats (TIRs)]. In addition, *Rim2* shares 82% nucleotide sequence identity with DNA sequences flanking members A1 and C of the rice *Xa21*disease resistance gene family (Song et al. 1995, 1997).

Materials and methods

Plant materials

For inoculation with *M. grisea*, and RNA extraction, two previously developed near-isogenic rice (*O. sativa* L. subsp. *indica*) lines, H7R and H7S, were used (He and Shen 1990; He et al. 1992). H7R carries the disease resistance gene *Pi-r1(t)* and is resistant to the Chinese blast race ZB15, H7S lacks this gene and is therefore susceptible. The rice IR72 (susceptible) cell suspension line was used for elicitor treatment. Eight rice lines were used for Southern analysis: IR24, IR72, C039, H7R (*O. sativa* L. subsp. *indica*), Taipei 309, Moroberekan (*O. sativa* L. subsp. *japonica*), *O. nivara* and *O. glumaepatula* (wild rice).

Inoculation with *M. grisea*, wounding, elicitor treatment and RNA preparation

Twenty-day-old H7R and H7S seedlings were spray-inoculated with the blast race ZB15 at concentration of 2×10^4 spores per ml in 0.01% Tween-20. The mock inoculation control consisted only of 0.01% Tween-20. The inoculated plants were grown in the dark for the first 24 h, and then grown on a day/night cycle of 12/12 h at 28 °C with a minimum of 90% humidity in a growth chamber (Percival Scientific, Boone, Iowa). For wounding, leaf disks (1 cm^2) from fully expanded young leaves of H7R were incubated for up to 24 h in 50 mM sodium phosphate buffer (pH 7.0) at 26 °C in the dark (Xu et al. 1996). The IR72 cell line was treated for 1–4 h with the cell wall elicitor, a high-molecular-weight polysaccharide fraction from the mycelium cell wall of *M. grisea*, as previously described (Xu et al. 1996). Total RNA was isolated from whole leaves, leaf disks and cell cultures at different time points after inoculation, wounding or elicitor treatment, using the TRIzol reagent as per manufacturer's protocol (GIBCO-BRL Life Technologies, Gaithersburg, Md.).

Differential mRNA display and cDNA cloning

Differential mRNA display PCR (DD-PCR) was carried out using total RNA extracted from H7R and H7S plants 12 h after inoculation as described (Liang and Pardee 1993; He et al. 1996). A 786-bp DD-PCR fragment obtained with a combination of T_{12}CA as the anchored primer and 5′-CAGCGAATAG-3′ as the arbitrary primer was excised from the sequencing gel, re-amplified and cloned into the TA-easy cloning vector (Promega, Madison, Wis.). The resulting clone, named Rim19-8, was used as probe to screen a λ ZAP cDNA library made from rice IR24 seedlings (a generous gift from Dr. S. McCouch, Cornell University, Ithaca, N.Y.). One cDNA clone, *Rim2*, was identified and the plasmid was excised from λ phage as described (Ausubel et al. 1991).

DNA and protein sequence analysis

For DNA sequencing, all templates were prepared using the Wizard Plus DNA purification kit (Promega). Sequencing was performed on an ABI 373A DNA sequencer using the Ready Reaction Dye Deoxy Terminator Cycle sequencing kit (Perkin-Elmer, Foster City, Calif.). Genbank searches for homologs were performed with the BLAST program at the National Center for Biotechnology Information. The GCG sequence analysis program Pileup was used for multiple alignment of protein sequences (Genetics Computer Group, Madison, Wis.). Putative promoter regions were identified using the BCM Search Launcher program NNPP/Eukaryotic (Baylor College of Medicine, Houston, Tex.).

Northern and Southern hybridizations

Aliquots (30 μg) of total RNA were loaded on 1% formaldehyde-agarose gels for Northern analysis. For Southern blots 5-μg samples of genomic DNA were digested with *Eco*RI, and loaded on 0.8% agarose gels (Ausubel et al. 1991). After electrophoresis, RNA and DNA were blotted to Hybond-N^+ membranes (Amersham, Piscataway, N.J.) and fixed to the membranes by UV cross-linking. The *Rim19-8* cDNA was used as a probe for both Northern and Southern analysis, and labeled with [α-^{32}P]dCTP by the random primer method (Ausubel et al. 1991). The blots were hybridized for 16–24 h in Church's buffer (Church and Gilbert 1984), washed twice for 20 min each at room temperature in 2 × SSC and 0.1% SDS, and then twice for 20 min at 65 °C in 1 × SSC and 0.1% SDS. The blots were then autoradiographed using X-ray films (Kodak). The *Rim2* expression level was quantified using a Phosphor Image System (Molecular Dynamics, Sunnyvale, Calif.), its expression level at time 0 was set at 1. The Northern blots were reprobed with the 18S *Arabidopsis* rDNA (Chory et al. 1989) to normalize for variations in RNA loading.

5′-RACE and primer extension

5′-terminal rapid amplification of cDNA ends (5′-RACE) was performed using the 5′-RACE system as described by the manufacturer (GIBCO BRL Life Technologies). Two gene-specific (GSP) antisense oligonucleotides were used: RIM2-1 (5′-TGAACAAACGTCTTAGCCG-3′) and RIM2-2 (5′-ATCTTCTTTGTTAGCTGCCCGTCA-3′), corresponding to nucleotide sequences 195–177 and 136–113 of the cloned *RIM2* cDNA (see Fig. 3).

Primer extension analysis was conducted by the method of Ausubel et al. (1991). An antisense 21mer oligonucleotide RIM2-3 (5′-TTCACATGGATCGTCTCGTCT-3′), corresponding to residues 110–90 of the *Rim2* cDNA, was end-labeled with [γ-^{32}P]dATP, and RNA from H7R inoculated for 12 h with *M. grisea* was used for the extension reaction. The 1.3-kb 5′-upstream genomic DNA clone *Rimsph13* (see below) was sequenced to determine the corresponding transcription start site.

Cloning of the 5′-upstream genomic DNA

The short 5′-upstream DNA region of *Rim2* was amplified by PCR from genomic DNA using the primer RIM2-2 and 10mer random primers (Operon, Alameda, Calif.) for 60 cycles of 94 °C for 1 min; 60 °C for 1 min; 72 °C for 1 min; 94 °C for 1 min; 42 °C for 1 min; and 72 °C for 1 min. A 0.3-kb PCR product (*Rim2-531*) was excised from the agarose gel, cloned into the TA-easy cloning vector (Promega) and sequenced. The PCR product *Rim2-531* was used as probe to screen a rice bacterial artificial chromosome (BAC) library (Wang et al. 1995), and a BAC clone, *N22H17*, was identified. From this BAC clone, a 1.3-kb *Sph*I fragment (*Rimsph13*) carrying the *Rim2* 5′-upstream region was subcloned into the vector pGEM-7 (Promega) and sequenced.

Results

Identification and cloning of the *Rim2* cDNA fragment

A 786-bp DD-PCR fragment, *Rim19-8*, was identified by DD-PCR using RNA from H7R and H7S inoculated with the blast race ZB15, together with the anchored and arbitrary primers. This cDNA was detected only after inoculation with *M. grisea* (data not shown). The cDNA fragment was cloned and sequenced, and the corresponding gene was named *Rim2*.

The *Rim2* transcript accumulates during the interaction between rice and *M. grisea*, and in elicitor-treated cell suspensions

Total RNA was extracted from the H7R and H7S leaves inoculated with the *M. grisea* race ZB15 at different time points. Northern analysis showed that *Rim2* transcripts accumulated in both incompatible and compatible interactions from 8 h until at least 36 h after inoculation (Fig. 1A). *Rim2* transcripts were undetectable in control plants (time 0). Mock inoculation slightly induced accumulation of the *Rim2* transcript (Fig. 1B), suggesting that *Rim2* might be slightly induced by the inoculation conditions. *Rim2* transcript levels had increased approximately 40-fold by 36 h after inoculation (Fig. 1C). All Northern blots revealed two transcripts of about 4 kb and 3.3 kb, indicating that *Rim2* may exist in different copies or have two ORFs. No difference in transcript accumulation was observed between the incompatible and compatible interactions, indicating that *Rim2* induction is not a resistance gene-related response to *M. grisea* attack.

A polysaccharide fraction from the cell wall of *M. grisea*, known to induce transcription of a rice

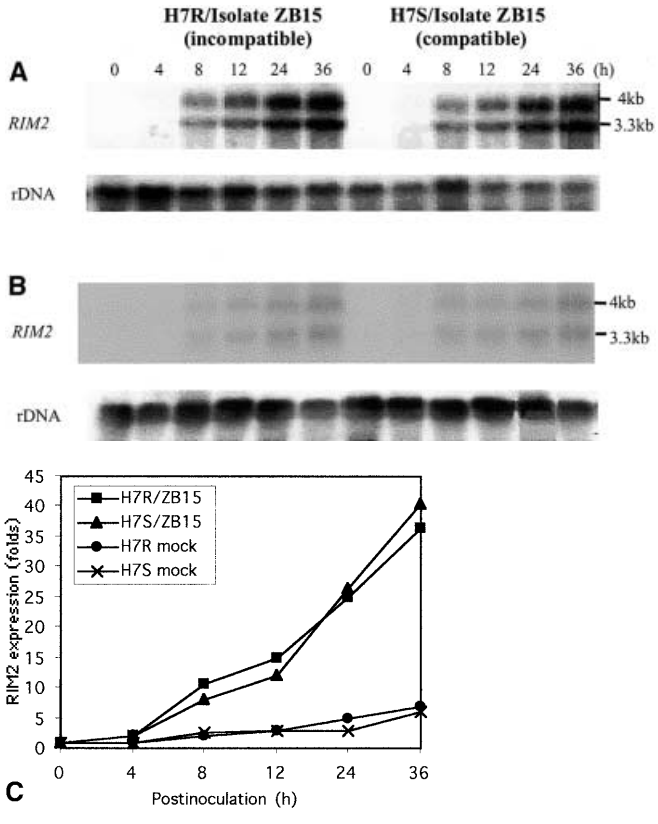

Fig. 1A–C Accumulation of *Rim2* transcripts in incompatible and compatible interactions between the rice line H7R or H7S and *M. grisea* race ZB15. The Northern blots show the accumulation of *Rim2* transcripts over a period from 0 h to 36 h after inoculation with the *M. grisea* race ZB15 (**A**) or the Tween-20 control (**B**). (**C**) Quantitative analysis of *Rim2* expression levels by phosphor imaging. The expression level at 0 h was set to 1

chitinase gene (Xu et al. 1996), also induced *Rim2* transcription in a rice IR72 cell suspension line, 1 and 2 h after treatment with elicitor (Fig. 2A). Wounding slightly induced *Rim2* transcription 4 h after wounding (Fig. 2B). The results indicate that *Rim2* is strongly induced both by *M. grisea* and an elicitor derived from *M. grisea* cell wall.

Isolation and characterization of the full-length *Rim2* cDNA and 5′-upstream genomic region

Using the 786-bp DD-PCR clone *Rim19-8* as a probe to screen 5×10^5 clones of a rice cDNA library, a 3.3-kb cDNA clone was isolated (Fig. 3). The 3.3-kb cDNA consists of an ORF of 1959 bp and a long AT-rich, noncoding 3′ tail. 5′-RACE did not amplify product beyond the 5′ terminus of the cloned *Rim2* cDNA. This result indicates that the isolated 3.3-kb cDNA fragment is a full-length clone, the same size as the 3.3-kb transcript detected on Northern blots. We have not yet isolated the 4-kb cDNA observed on Northern blots. Using the gene-specific primer RIM2-2 (5′-ATCTTCTTTGTTAGCTGCCCGTCA-3′) and a 10mer random primer (5′-AACCTCTCTG-3′), we amplified a 289-bp fragment including a short 5′-region DNA sequence from genomic DNA by PCR, and the fragment was named *Rim2-531*. The sequence of *Rim2-531* showed that it represented the 5′ region of the *Rim2* cDNA from −176 to 113 (Fig. 3). *Rim2-531* was used as a probe to screen a rice BAC library of 1.07×10^4 clones (Wang et al. 1995). A 1.3-kb *Sph*I fragment carrying the 5′-flanking region of *Rim2* was cloned from BAC *N22H17*, and the resulting clone was named *Rimsph13*. *Rimsph13* was AT-rich and contained sequences that are co-linear with the *Rim2* cDNA sequence from 1–59, confirming that *Rimsph13* carries the 5′-upstream region of *Rim2* (Fig. 3).

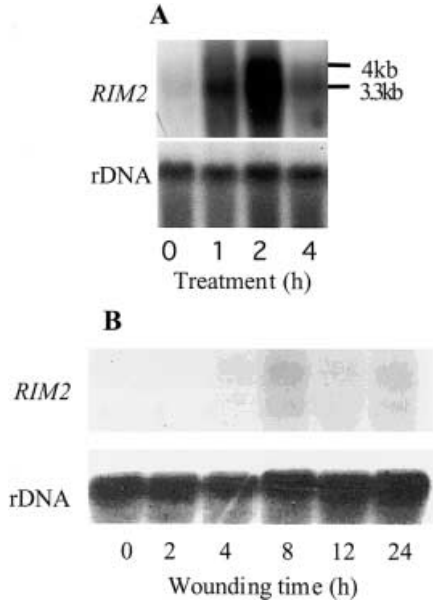

Fig. 2A, B Northern analysis of *Rim2* expression in cell cultures treated with a cell wall elicitor from *M. grisea* for 0–4 h (**A**) or in leaves subjected to wounding and assayed 0–24 h later (**B**)

The transcription start sites were determined by primer extension analysis. A major band was detected among the primer extension products, corresponding to the A residue in the sequence GTCCCGCATGTCC (Fig. 3). An additional primer extension product 1 nucleotide shorter than the major product was also detected that corresponded to the T immediately adjacent to that A. The major site was designated as position +1 for transcription. The putative translation initiation codon was 149 bp downstream from the major transcription start site. No typical TATA box consensus sequence was found in this 5′-upsteam region. However, a 50-bp putative promoter sequence that shares 50.4% identity with the minimal CaMV 35S promoter (Garner et al. 1981) was found in the region −76 to −27 . The sequence from −214 to −129 upstream of the putative promoter sequence shares 46% identity with the LTR U3 region of *Tnt1*, which contains the BII tandem repeats. The sequence CCACTC-N_8-CT found in this BII-like region is similar to the BII motif CCAACC-N_7-CT, although no tandem repeats were identified (Fig. 3). The sequence GGCATTTGGC found from position −125 to −116 is very similar to the BI core sequence found in *Tnt1* (Casacuberta and Grandbastien 1993), although the BI-like sequence is located downstream of the BII-like region instead of upstream as observed in *Tnt1* (Fig. 3; Casacuberta and Grandbastien 1993). Four motifs with the sequences (T)TGAC(C) that are identical to the consensus of the elicitor-responsive elements found in the promoters of the parsley *PR-1* genes (Rushton et al. 1996) and the maize *PRms* gene (Raventós et al. 1995) were found in the distal upstream region from −683 to −426 (Fig. 3). A similar motif was also found in the BII element of *Tnt1* (Vernhettes et al. 1997).

Four imperfect tandem repeats of a 106-bp sequence were found (repeats 1–4) in the 3′ noncoding region from position 2766 to 3152 of *Rim2* (Fig. 3). Of the four repeats, repeats 1 and 2 are almost identical to each other. Repeats 3 and 4 show some nucleotide changes relative to repeats 1 and 2. Repeat 4 is truncated by a 35-bp deletion at the 3′ end.

The predicted RIM2 protein shares 32–55% identity with TNP2-like proteins encoded by *CACTA*-like elements

Rim2 has an ORF of 1959 bp, which encodes a predicted protein of 653 amino acids (Fig. 4A). Database searches showed that the RIM2 protein is similar to TNP2-like proteins encoded by transposons in other plants (Table 1). As previously reported, these TNP2-like proteins are encoded by members of the *En*/*Spm* family or *CACTA* class of transposable elements, which have the consensus sequence CACTA in their TIRs (Pereira et al. 1986; Nacken et al. 1991). Multiple alignment of the amino acid sequences of RIM2, TNP2, TNP2-like,

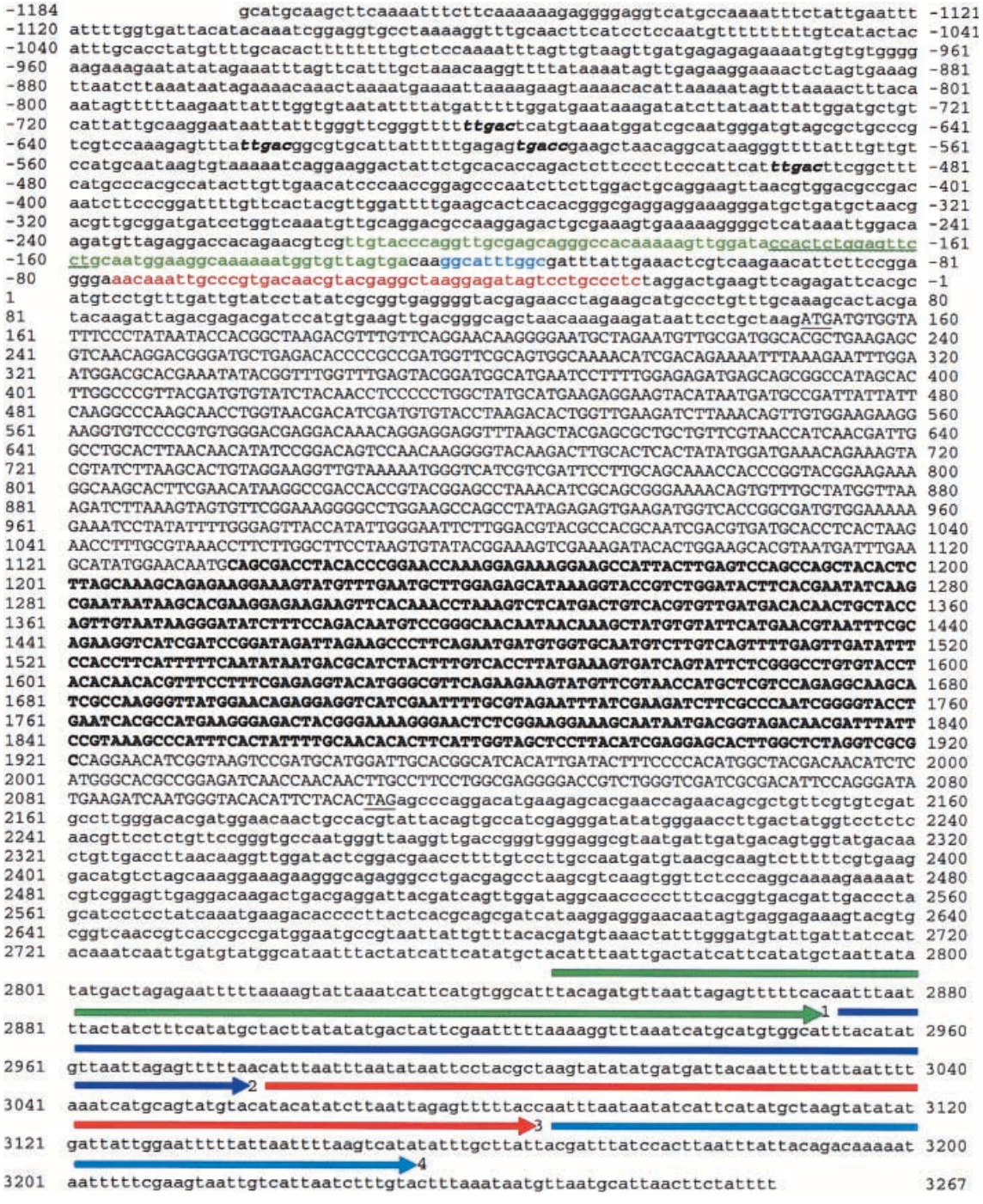

Fig. 3 Nucleotide sequence of the *Rim2* cDNA and its 5′-flanking region. Transcriptional initiation sites were determined by primer extension analysis. The first transcriptional initiation site (residue A) is referred to as +1. The 786-bp DD-PCR fragment *Rim19-8* is indicated in *bold*. The BAC clone *RimSphI* includes the sequence from −1184 to 59. Translation initiation and termination codons are *underlined*, and the ORF is indicated in *upper case*. A putative promoter sequence is marked in *red*, the BII-like sequence in *green*, with the BII-like motif indicated by the *wavy underline* and the BI-like sequence in *turquoise*. Four putative elicitor-responsive motifs are indicated in *bold italics*. Four tandem repeats are indicated by *arrows* in *green*, *blue*, *red* and *turquoise*. The Genbank Accession No. for the *Rim2* cDNA and amino acid (Fig. 5) sequences is AF121139

TNPD and Tgm-ORF shows that RIM2 is truncated – with a deletion of 95–373 amino acids in the N-terminal region, compared with the other proteins (Fig. 4B). Thus, these results indicate that *Rim2* also encodes a TNP2-like protein.

Rim2 sequence shares 82% identity with DNA sequences flanking the *Xa21* gene family

DNA database searches revealed that the *Rim2* sequence from −631 to +427 shares 82% identity with the

A

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1   MMWYFPIIPR LRRLFRNKGN ARMLRWHAEE RQQDGMLRHP ADGSQWQNID
51  RKFKEFGMDA RNIRFGLSTD GMNPFGEMSS GHSTWPVTMC IYNLPLAMHE
101 EEVHNDADYY SRPKQPGNDI DVYLRHWLKI LNSCGRRKVS PCGTRTNRRR
151 FKLRALLFVT INDWPALNNI SGQSNKGYKT CTHYMDETES TYLKHCRKVV
201 KMGHRRFLAA NHPVRKKGKH FEHKADHRTE PKHRSGKTVF AMVKDLKVVF
251 GKGPGSQPIE SEDGHRRCGK RNPIFWELPY WEFLDVRHAI DVMHLTKNLC
301 VNLLGFLSVY GKSKDTLEAR NDLKHMEQCS DLHPEPKEKG SHYLSPASYT
351 LSKAEKESMF ECLESIKVPS GYFTNIKRII STKEKKFTNL KSHDCHVLMT
401 QLLPVVIRDI FPDNVRATIT KLCVFMNVIS QKVIDPDRLE ALQNDVVQCL
451 VSFELIFPPS FFNIMTHLLC HLMKVISILG PVYLHNTFPF ERYMGVQKKY
501 VRNHARPEAS IAKGYGTEEV IEFCVEFIED LRPIGVPESR HEGRLREKGT
551 LGRKAIMTVD NDLFRKAHFT ILQHTSLVAP YIEEHLALVR ARNIGKSDAW
601 IARHHIDTFP TWLRQHLMGT PEINQQLAFL ARGPSGSIAT FQGYEDQWVH
651 ILH
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B

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 RIM2   1 MMWYFPIIPRLRRLFRNKGNARMLRWHAEERQQ...DGMLRHPADG
 TNP2 238 VLRYFPLKPRLQRLFTSSKTASFMRWHKEERTK...DGQLRHPSDS
TNP2L 374 VMWYAPIIPRLKRLFRNKDNAKLLRWHKEDRKI...DNMLRHPADG
 TNPD 258 QLRYMPITPRLKRLFLNQETAKQMRWHKEGDRQGQDPDVMVHPSDG
 Tgm-O 96 VLWYLPIIPRFKRLFANEDDAKDLTWHANGRKS...DGMVRHPADC

 RIM2 SQWQNIDRKFKEFGMDARNIRFGLSTDGMNPFGEMSSGHSTWPVTMCIYN
 TNP2 PLWHAFDHQHPDFAEDPRNIRLGLAADGFNPFRTMSVAHSTWPVILTPYN
TNP2L SQWRAIDKEFPDFANDARNLRFALSTDGMNPFGEQSSSHSTWPVTLCIYN
 TNPD EAWQALDRFDPEFARDPRSVRLGLSTDGFTPYSNNSTSYSCWPVFMMPYN
Tgm-O SQWKKIDSLYPNFGKEARNLRLGLASDGMNPYGNLSTQHSSWPVLLVIYN

 RIM2 LPLAM.HEEEVHNDADYYSRPKQPGNDIDVYLRHWLKILNSCGRRKVSPC
 TNP2 IPPWMCMKEPFFFLTLLIPGPSPPGNNIDVYMQPLIEELQELWG..GVNT
TNP2L LPPWLCMKRKFIMMPVLIQGPKQPGNDIDVYLRPLVEELQLLWSKPGVRV
 TNPD LPPNKCMKEEVMFLALIVPGPKDPVTKINVFMEPLIEELKMLW..QGVEA
Tgm-O FPPWLCMKRKYMMLSMMISGPRQPGNDIDVYLSPLIEDLRKLWDE.GVLV

 RIM2 GTRTNRRRFKLRALLFVTINDWPALNNISGQSNKGYKTCTHYMDETESTY
 TNP2 YDASAKENFNVRAALLWTINDYPAYANLSGWSTKGELACPSCHKDTCSKY
TNP2L WDEYKQEHFDLRALLFVTINDWPALSNLSGQSNKGYNACTHCYEDLDCVF
 TNPD YDSHLKCCFTLRAAYLWSIHDLLAYGIFSGWCVHGILRCPICMGDSQAYR
Tgm-O FDGFRKETFQMRAMLFCTINDFPAYGNLSGYSVKGHLACPICEEDTSYIQ

 RIM2 LKHCRKVVKMG.HRRFLAANHPVRKKGKHF.EHKADHRTEPKHRSGKTVF
 TNP2 LQKSHKYCYMG.HRRFLNRYHPYRKDTKSF.DGNEEYRRAPIALTGDMVS
TNP2L LKKCRKVVYLG.HRRFLPVNHPVRKKGKHF.KGNADHRTKPLNRTGDDVL
 TNPD LEHGKKETFFDVHRRLLPYNHPFRKDTKSFRKGKRVRDGPPKRQTGENIM
Tgm-O LKHGRKTVYTR.HRVFLKAHHPYRRLKKAF.NGSQEHEIRRTPLTGEQV
                                                    334*
 RIM2 AMVKDLKVVFGKGPGSQPIESEDGHRRCGKRNPIFWELPYWEFLDVRHAI
 TNP2 EEITGFNIKFGKKVDDNPTLPLN.....WKKRSIFFDLPYWKDSLLRHNF
TNP2L EMVKDIKVVFGKGRGSEPIPKDA.....KGHVPMWKKKSIFWELPYWNVL
 TNPD RQHRDLKPGVGGRFQGY......GKEHNWTHISFIWELPYTKALLLPHNI

 RIM2 DVMHLTKNLCVNLLG.FLSVYGKSKDTLEARNDLKHMEQCSDLHPEPKEK
 TNP2 DVMHIEKNVCESIIGTLLNLEGRTKDHENSRLDLKDMGIRSELHPISLES
TNP2L ETDDGRHYLSPASYTLSKEEKESMFECLSSIKVPSGFSSNIKGIINVPEK
 TNPD DLMHQERNVAESIISMCFDFTGQTKDNMNARRDLAELCDRPHLELRKNPS

 RIM2 GSHYLSPASYTLSKAEKESMFECLESIKVPSGYFTNIKRIISTKEKKFTN
 TNP2 GKHYLPAACYSMSKKEKEIVFEILKTVKVPDGYASNISRRVQLKPNKISG
TNP2L KFLNLKSHDYHVLMTQLLPVVLRGILPPHPEGSIAKGYGTEEVIEFCVDF
 TNPD GSESRPQAPYCLKRQEREEIFQWLKKLRFPDRYAANIKRAVNLDTGKLVG

 RIM2 LKSHDCHVLMTQLLPVVIRDIFPDNVRATITKLCVFMNVISQKVIDPDRL
 TNP2 LKSHDHHILMQQLLPIALRKVLPKHVRTPLIKLCTFFRELCSKVLNPQDL
TNP2L IPDLDPIGIPESRHEGRLSGKGTLGKKTYIGTGNDYFNKAHYTVLQNSSL
 TNPD LKSHDYHILIERLVPVMFRGYFSPDVWKIFAELSYFYKQICAKEISKKLM

 RIM2 EALQNDVVQCLVSFELIFPPSFFNIMTHLL 469...
 TNP2 VRMGKDIAKTLCDLEKIFPPSFFDIMMHLP 701...
TNP2L VEPYVEVHKDYLRSQWPGKNEAWIMRQHME 839...
 TNPD LRFEKEIVVLVCKMEKVFPPGFFNCMQHLL 725...
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5′-flanking region of member *A1* (*A1RIM-5*, positions 1971–3021 according to Genbank Accession No. U72725), and 5′ and 3′-flanking regions of member *C* (*CRIM-5*, positions 2413–3462, and *CRIM-3*, from

◀

Fig. 4A, B Amino acid sequence of the predicted RIM2 protein (**A**) and multiple alignment of regions of RIM2, TNP2 (*Antirrhinum majus*), TNP2-like (TNP2L) (*Sorghum bicolor*), TNPD (*Zea mays*) and Tgm-ORF (Tgm-O) (*Glycine max*) that show sequence similarity (**B**). Residues that are conserved with respect to RIM2 are marked in *bold*. Note that the Tgm-ORF sequence is full length, with 334 amino acid residues

12320 to 13367 according to Genbank Accession No. U72723) of the rice *Xa21* disease resistance gene family (Song et al. 1995, 1997; Fig. 5). The 3′-flanking region sequence of member *A1* is not available for analysis. These similar sequences contain at least five stop codons that disrupt the predicted *RIM2* ORF. In addition, the ORF of *Rim2* from nucleotides 859 to 1479 shares 43% identity with the ORF encoded by the *Xa21*-linked marker *pTA818* (Table 1). The product of the *pTA818* ORF has previously been shown to be 57.4% identical to TNP2, encoded by *Tam1*, and 38.3% identical to TNPD encoded by *En*/*Spm* (Song et al. 1998). These results support and extend the observation that the *Xa21* gene family is a transposon-rich locus (Song et al. 1998).

Rim2 copy number in the rice genome

To estimate the *Rim2* copy number in the genomes of different rice species and subspecies, Southern analysis was conducted using genomic DNA from eight rice lines. These lines include *O. sativa* ssp. *indica* and *japonica*, *O. nivara* and *O. glumaepatula*. As shown in Fig. 6, Southern hybridization revealed four major fragments in rice genomic DNA digested with *Eco*RI, indicating that there exists a family of *Rim2* elements with 3–4 copies in the rice genomes tested. A DNA restriction fragment length polymorphism (RFLP) was found between wild rice (*O. nivara*) and other lines; *O. nivara* lacked the 2-kb band. All six modern *O. sativa* lines exhibit the same *Rim2* hybridization pattern. We did not detect *Rim2* polymorphism in digests prepared using other restriction enzymes, such as *Hin*dIII or *Bam*HI (data not shown).

Discussion

Transposition and transcription of plant TEs can be activated by a number of environmental stresses (Arnault and Dufournel 1994; Wessler et al. 1995; Wessler 1996). In this paper, we have shown that the rice *Rim2* transcript accumulates in plants inoculated with *M. grisea*, the rice blast pathogen, and in cell suspension cultures treated with a fungal elicitor. Because the transcript of *Rim2* accumulates during both incompatible and compatible interactions, it appears that *Rim2* is induced through a non-specific mechanism in the course of pathogen attack. Similar results have been found for other TEs. For instance, the tobacco retrotransposon *Tnt1* is transcriptionally activated by pathogen infection, microbial elicitors, as well as abiotic factors (Pouteau

Table 1 Sequence comparison of *CACTA* transposon proteins with RIM2

Protein	Transposon	Plant species	Degree of identity to *RIM2* (%)	Reference
TNP2	*Tam1*	*Antirrhinum majus*	42	Nacken et al. (1991)
TNP2-like	Putative transposon	*Sorghum bicolor*	55	Llaca et al. (1998)
TNPD	*En/Spm*	*Zea mays*	32	Pereira et al. (1986)
Tgm-ORF	*Tgm*	*Glycine max*	44	Rhodes and Vodkin (1988)
Tdc1-ORF	*Tdc1*	*Daucus carota*	35	Ozeki et al. (1997)
T26I20-ORF	*En/Spm*	*Arabidopsis*	34	Rounsley and Lin (1999)
T10J7-ORF	Putative transposon	*Arabidopsis*	35	Rounsley and Lin (1998)
pTA818-ORF	*Xa21-CACTA*	*Oryza sativa*	43	Song et al. (1998)

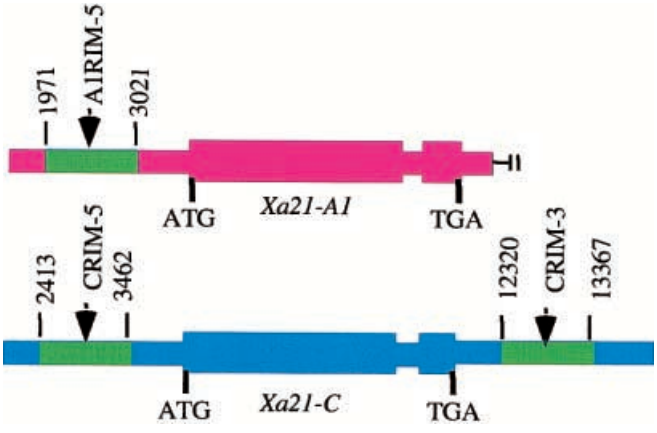

Fig. 5 *Rim2* sequences are present in the 5′ and 3′-flanking regions of the *A1* and *C* members of the rice *Xa21* disease resistance gene family. These *Rim2* homology regions (from −631 to 427 in *Rim2*, see Fig. 4) are named *A1RIM-5*, *CRIM-5* and *CRIM-3* as indicated

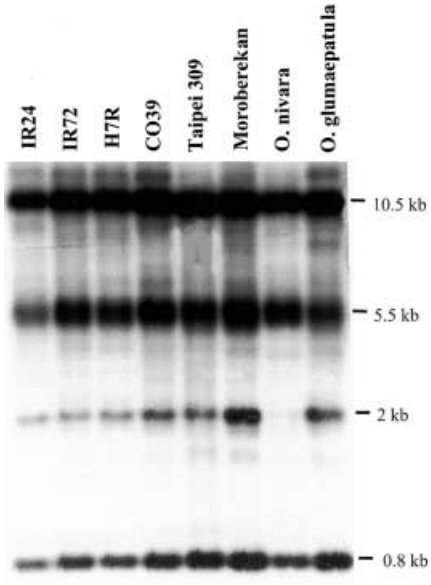

Fig. 6 *Rim2* is present in multiple copies in the rice genome. Eight genomic DNA samples were digested with *Eco*RI, fractionated on agarose gels, blotted onto a nylon membrane, and hybridized with the 786-bp DD-PCR probe *Rim19-8*. The sizes of the four major hybridizing bands are indicated

et al. 1994; Mhiri et al. 1997; Vernhettes et al. 1997). Transcriptional regulatory elements of *Tnt1* share similarities with *cis*-acting elements involved in the activation of plant defense genes, providing a plausible explanation for the molecular basis of retrotransposon activation by pathogen attack (Vernhettes et al. 1997; Grandbastien 1998). Such a defense-related activation mechanism may also be shared by *Rim2* since putative *cis*-acting elements similar to the BI and BII elements of *Tnt1* and the elicitor-responsive elements of *PR-1* genes of parsley are found in the *Rim2* 5′-upstream region (Fig. 3).

The predicted protein RIM2 encoded by the *Rim2* ORF is structurally similar to the TNP2-like proteins encoded by the *En/Spm* family or *CACTA* elements found in many plant species (Table 1 and Fig. 4; see Gierl et al. 1989; Nacken et al. 1991; Llaca et al. 1998; Rounsley and Lin 1999). *CACTA* elements are usually large (up to 15.2 kb long), have conserved TIRs with the consensus sequence CACTA, and encode at least two proteins such as TNP1 and TNP2 of *Tam1* (Nacken et al. 1991), and TNPA and TNPD of *En/Spm* (Pereira et al. 1986). TNP1 and TNPA are thought to be DNA-binding proteins whose targets could be the 9-bp and 12-bp motifs found in the 5′ and 3′ termini of *Tam1* and *En/Spm*, respectively (Gierl et al. 1988; Nacken et al. 1991). TNP2 and TNPD have been proposed to provide transposase activity by interacting with the conserved 13-bp CACTA TIRs for excision. The high degree of protein sequence similarity between RIM2 and other TNP2-like proteins suggests that *Rim2* may also encode a protein with transposase activity. Thus, *Rim2* may be one component of a larger element carrying TNP1-like and TNP2-like proteins and CACTA motifs in its 5′ and 3′ ends. In support of this hypothesis, CACTA TIRs have been found in the rice transposable element *Tnr3* (Motohashi et al. 1996). *Tnr3* is a 1536-bp insertion found in one member of the rice retrotransposon *p-SINE1* family and contains no ORF. In addition, we identified imperfect tandem repeats of a 106-bp sequence in the 3′ noncoding region of the *Rim2* cDNA. Tandem repeats are also found in subterminal regions of *Tnr3* and *En/Spm* family members (Motohashi et al. 1996). The significance of these repeats, however, remains

unknown. We also observed another 4-kb transcript that simultaneously accumulated in response to inoculation with *M. grisea*, and treatment of cells with a cell wall fraction from *M. grisea* as a transcript of the cloned *Rim2* (Figs. 1 and 2). This transcript may originate from another *Rim2* copy or ORF.

Transposons in regions flanking plant genes can affect gene structure and expression (White et al. 1994), and have been proposed to be important in creating allelic diversity in disease resistance genes (Michelmore 1995; Song et al. 1998). For example, 17 elements have been found within or closely linked to the *Xa21* locus (Song et al. 1997, 1998). Furthermore, the *Xa21*-linked marker *pTA818* encodes part of a TNP2-like protein; this sequence may have transposed from a different chromosome to the *Xa21* locus on chromosome 11 in the wild species *O. longistaminata*, resulting in the sequence duplication in IRBB21, the *Xa21* gene donor (Jiang et al. 1995). Interestingly, *Rim2*-like sequences are found in DNA regions flanking members *A1* and *C* of the *Xa21* gene family (Fig. 5). These results further support the observation that TEs are clustered at the *Xa21* gene locus and contribute to its polymorphic nature (Song et al. 1998).

The *En/Spm* family of TEs is mobile and present in numerous copies (Gierl et al. 1989). For example, 25 *Tam1* insertions have been isolated from the T53 line of *Antirrhinum majus* (Nacken et al. 1991). In contrast, *Rim2* is present in only four copies. A polymorphism is found only between *O. nivara* and the other lines, and all six modern *O. sativa* lines share the same *Rim2* hybridization pattern (Fig. 6). These results suggest that the *Rim2* ancestor might have been mobile at one time during the evolution of rice but may since have lost the capacity to transpose.

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