

Molecular characterization of a gene induced during wheat hypersensitive reaction to stripe rust

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Abstract

A novel gene induced during hypersensitive reaction (HIR) in wheat was identified using *in silico* cloning and designated as *TaHIR2*. The *TaHIR2* gene was deduced to encode a 284-amino acid protein, whose molecular mass and isoelectric point (pI) were 31.05 kD and 5.18, respectively. Amino acid sequence analysis demonstrated the presence of stomatins, prohibitin, flotillins, HflK/C (SPFH) domain and prohibitin homologue for the *TaHIR2* protein. Phylogenetic analysis of 13 HIR genes from different monocots indicated that *TaHIR2* was highly homologous to *HvHIR2*. Transient expression analysis using particle-mediated bombardment showed that the *TaHIR2* fusion protein was located in the onion epidermal cells. Quantitative RT-PCR analyses revealed that *TaHIR2* transcripts were significantly accumulated in adult wheat leaves with maximum induction at 18 h post inoculation with the stripe rust, whereas slightly up-regulation could also be observed in the compatible reaction at the seedling stage. These results suggest that *TaHIR2* may play an active role in wheat defense against stripe rust.

Additional key words: cell death, cloning, defense, expression profile, *Puccinia striiformis*, resistance, *Triticum aestivum*.

Introduction

Plants have evolved sophisticated multifaced mechanisms to prevent the invasions by pathogens. Hypersensitive response (HR) is one of the earliest active defense responses against a wide range of pathogens, involving the rapid cell death surrounding infection sites (Lam *et al.* 2001). Plant cell death occurring during the HR resembles animal programmed cell death (PCD) and may play a vital role in preventing further spread of the pathogen into healthy tissues (Heath 2000). HR is often

conditioned by the presence of an avirulence (*Avr*) gene in the pathogen. The direct or indirect product of an *Avr* gene is recognized by the corresponding resistance (*R*) gene in the host plant in many plant-pathogen interactions (Flor 1971). Exquisite perception of the pathogen by the plant is followed by the interplay of a cascade of signaling transduction pathways, involving reactive oxygen species, ion fluxes and salicylic acid (Heath 2000). Then, a series of complex biochemical changes

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Abbreviations: C_T - threshold value; GS - growth stage; HIR - hypersensitive induced reaction; hpi - hour post inoculation; HR - hypersensitive response; ORF - open reading frame; qRT-PCR - quantitative reverse transcription polymerase chain reaction; SAR - systemic acquired response; RT-PCR - reverse transcription PCR; SPFH - stomatins, prohibitin, flotillins, HflK/C.

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occur near the infection sites including rapid accumulation of phytoalexins, pathogenesis related (PR) proteins and the deposition of callose and lignin in cell walls (Greenberg 1997, Anand *et al.* 2009). As a result of HR, a systemic acquired response (SAR) can be initiated to achieve effective disease resistance (Métraux *et al.* 2002). Hence, HR plays a crucial role for the plant defense against pathogen.

There have been numbers of hypersensitive induced reaction (HIR) genes cloned from different plant species, implicating in HR. The first evidence of HIR gene's involvement in plant HR was from tobacco (Karrer *et al.* 1998). The *NG1* gene in tobacco was transiently assayed to be able to cause formation of HR-like lesions and resulting in induction of an HR-specific PR2 protein, pathogenesis-related acidic β -glucanase in tobacco. In maize, three *NG1* homologs - *ZmHIR1*, *ZmHIR2* and *ZmHIR3* were isolated (Nadimpalli *et al.* 2000). The elevated expression of *ZmHIR3* in maize was partially dominant over lesion mimic mutant *Les9*, and reduced expression of *ZmHIR3* could be detected when the mutant gene *Les9* was crossed into the Mo20W genetic background, suggesting the involvement of the maize HIR genes in HR. In barley, four HIR genes, *HvHIR1*, *HvHIR2*, *HvHIR3* and *HvHIR4* with conserved stomatins, prohibitin, flotillins, HflK/C (SPFH) domain were identified. Barley lesion mimic mutants exhibiting spontaneous HR had up to a 35-fold increase in *HvHIR3* expression implicating the importance of *HvHIR3* in HR (Rostoks *et al.* 2003).

Recently, *TaHIR1* and *TaHIR3* were identified from

wheat (*Triticum aestivum* L.) after infections with the stripe rust pathogen. *TaHIR1* was cloned using the RACE technology (Yu *et al.* 2008). *TaHIR1* were rapidly up-regulated in response to rust infection and was predicted to play an important role in wheat protection against the stripe rust. *TaHIR3* was isolated using *in silico* cloning (Zhang *et al.* 2009). More transcripts of *TaHIR3* were accumulated at the adult-plant stage than that at the seedling stage after infection with the fungus indicating that *TaHIR3* was involved in the HR adult-plant resistance.

Increasing evidences have been documented on functional analyses of plant HIR genes. Jung and Hwang (2007) reported that the transgenic *Arabidopsis* over-expressed with a *Capsicum annuum* HIR gene, *CaHIR1*, conferred resistance to *Pseudomonas syringae*. The *CaHIR1* interacted with a leucine-rich repeat (LRR) CaLRR1 protein, resulting in suppressed cell death. Recently, the *CaHIR1* gene was predicted to positively regulate HR cell death by mediating diverse plant responses to biotic and abiotic stresses (Jung *et al.* 2008).

To better understand the molecular mechanisms of wheat HR and the role of specific HIR genes in the wheat-stripe rust pathogen interaction, it is necessary to characterize more genes associated with HR. Here we identified a wheat HIR gene, namely *TaHIR2*. The structure of the *TaHIR2* gene was analyzed using a series of bioinformatics tools, and the *TaHIR2* protein was located in onion epidermal cells using transient gene expression, and the expression profile after rust fungi infection was analyzed using quantitative RT-PCR.

Materials and methods

Plants and inoculation: Wheat (*Triticum aestivum* L.) genotype Xingzi 9104 and the *Puccinia striiformis* f. sp. *tritici* (*PST*) pathotype CY32 were used in this study. Adult plants of Xingzi 9104 are resistant to CY32, compromising the incompatible reaction. Seedlings of Xingzi 9104 are highly susceptible to CY32, forming the compatible reaction (Zhang *et al.* 2009). Fresh urediniospores were separately applied onto the surface of flag leaves at the boot stage (GS40 - GS43) and primary leaves at the seedlings stage of wheat (GS11 - GS12) (Zadoks *et al.* 1974), with a paintbrush following the procedures described by Kang and Li (1984). Mock inoculation was performed with sterile water, referred to as controls. All plants were kept in a dew chamber with 100 % humidity in dark for 24 h, and transferred to a growth chamber with temperature of 15 ± 2 °C, relative humidity of 85 ± 5 %, a 16-h photoperiod and irradiance of $125 \mu\text{mol m}^{-2} \text{s}^{-1}$. Leaves of the inoculated and control plants were sampled at 0, 12, 18, 24, 48, 72 and 120 h post inoculation (hpi), quickly frozen in liquid nitrogen and stored at -80 °C prior to RNA extraction. Three independent biological replications were performed.

RNA preparation and the 1st strand cDNA synthesis: Total RNA was prepared from sampled wheat leaves using *Trizol*TM reagent (*Invitrogen*, Carlsbad, CA, USA) according to the manufacturer's instructions. Quality and integrity of the total RNA were determined using a formamide denaturing gel along with an RNA ladder (*Invitrogen*) for comparison, and the quantity was tested using a *NanoDrop*TM 1000 spectrophotometer (*Thermo Fisher Scientific*, Waltham, MA, USA). The 1st strand cDNA was synthesized with 2 μg of total RNA using the RT-PCR system (*Promega*, Madison, WI, USA).

Cloning of *TaHIR2* and sequence analyses: The cDNA sequence of barley (*Hordeum vulgare*) *HvHIR2* gene (GenBank accession AY137516) was extracted and used as a query probe to search wheat EST database in GenBank. Homologous wheat ESTs were retrieved and used for *in silico* extension as previously described by Xia *et al.* (2010). To verify the final assembled sequence, primers fp1 (5'-ATGGGTGGCGTTTTGGGT-3') and rp1 (5'-TTAGAGAGTATTGGCCTGGAGGA-3') were used to amplify *TaHIR2* ORF. The resulting PCR products

were cloned into the *pGEM-T Easy vector* (Promega) and sequenced with an *ABI PRISM 3130XL* genetic analyzer (Applied Biosystems, Foster City, USA). Multiple alignments of the deduced amino acid sequences were performed using *MegAlign* running under the *Lasergene software package* from DNASTAR (Madison, WI, USA) (Burland, 2000). Phylogenetic analysis was conducted with *MEGA 4.0* (Tamura *et al.* 2007).

Subcellular localization of the TaHIR2-GFP fusion protein: To generate the *TaHIR2-GFP* fusion construct, the *TaHIR2* ORF was amplified with a forward primer containing a *HindIII* site (5'-CCCAAGCTTATGGGTGGCGTTTTGGG-3') and a reverse primer (5'-CGCGGATCCGAGAGTATTGGCCTGGAGGAG-3') containing a *BamHI* site. PCR products were cloned into *pGEM-T Easy vector* (Promega) and sequenced. The *TaHIR2* fragment was then released with *BamHI* and *HindIII* digestion and introduced into the *pCaMV35S:GFP* vector. The resulting plasmid *pCaMV35S::TaHIR2-GFP* contained an in-frame fusion between *TaHIR2* and *GFP* gene. Onion inner peel (2 × 2 cm²) was cultured inside-out at the central of Murashige and Skoog (MS) medium plate. The fusion construct and control (*pCaMV35S:GFP*) were transformed into onion epidermal cells through particle bombardment at a helium pressure of 7582.1 kPa (1100 psi) using *PDS-1000/He system* (Bio-Rad, Hercules, CA, USA). After bombardment, the transformed onion epidermal cells were cultured in a growth chamber at 24 °C for 16 - 24 h. GFP signals were observed with a *Zeiss LSM 510* confocal laser microscope (Carl Zeiss, Jena, Germany).

Results

Cloning of a TaHIR2 cDNA: To clone *TaHIR2* cDNA, a strategy of a combination of *in silico* cloning and RT-PCR techniques was employed. A cDNA fragment of 1257 bp sequence containing a predicted ORF (121 - 876) was identified. This ORF encodes a protein sharing 99 % similarity with the barley *HvHIR2* protein. The putative initiation codon was in the context of ACCATGG based on the Kozak consensus initiator A/GNNATGG (Kozak 1990). Using the primer pair fp1/rp1, an 855-bp cDNA fragment was amplified and confirmed by sequence analysis to be highly homologous (97 %) to *HvHIR2* (Fig. 1). The corresponding wheat gene was named *TaHIR2* (GenBank accession FJ028662).

Sequence analyses of TaHIR2: The *TaHIR2* gene was predicted to encode a protein with 284 amino acids. Using *Compute pI/MW tool* analysis, molecular mass of the *TaHIR2* protein was predicted to be 31.05 kD with a pI of 5.18. The *TaHIR2* protein was predicted to contain

Expression profiles using qRT-PCR analyses: Leaf samples were harvested at 0, 12, 18, 24, 48, 72 and 120 hpi and three biological replicates were performed independently. Reverse transcription was performed on 2 µg of total RNA using the *MMLV* reverse transcriptase (Promega) with the random hexamer pd(N)₆ primer. Templates were the 10× diluted cDNAs from each time-point leaf sample. The wheat 18S rRNA (GenBank accession AY049040) was used as the internal reference in qRT-PCR analyses (Zhang *et al.* 2009). Primers for qRT-PCR of *TaHIR2* were designed using the *Primer Express* program (Applied Biosystems), and the primer sequences were as follows: fp2 5'-GGTGGCGTTTTGGGTTTAGT-3', rp2 5'-CAAGGGCACGGTATTGGAC-3' (amplifying a 235-bp fragment). qRT-PCR reaction mixtures contained 12.5 mm³ 2× *SYBRGreen PCR MasterMix* (Applied Biosystems), 10 pM of each primer, 2 mm³ template, and sterile distilled water added to a total volume of 25 mm³. Thermal conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All qRT-PCR reactions, including non-template controls, were performed in three times using a *7500* real-time PCR system (Applied Biosystems). Dissociation curves were generated for each reaction to ensure specific amplification. Threshold values (C_T) were generated from the *ABI PRISM 7500* software (Applied Biosystems). The relative expression ratios were calculated by the comparative ΔΔC_T method of relative gene quantification (Pfaffl 2001, Peng *et al.* 2008). A probability (*P*) value ≤ 0.05 was used to determine the significance of difference between time-course points, or when relative quantity of RNA was at least two fold higher or lower than that of leaves from control plants.

the SPFH domain (6 - 193) and prohibitin homologues (5 - 165) without obvious signal peptide sequences or transmembrane region. In addition, *TaHIR2* protein contained four types of motifs, including a tyrosine kinase (82 - 88), two casein kinase II (229 - 232; 245 - 248) phosphorylation sites, four N-myristoylation sites (2 - 7; 200 - 205; 213 - 218; 277 - 282) and four protein kinase C phosphorylation sites (48 - 50; 229 - 231; 245 - 247; 252 - 254).

The predicted amino acid sequence of *TaHIR2* was compared with *HIR2* sequences from barley, maize, and rice using *MegAlign* analyses (Fig. 2). The alignment demonstrated that the *TaHIR2* gene was highly conserved among these four cereal crops, especially for *HvHIR2* and *TaHIR2*, whose encoding proteins only differed by four amino acid residues (Fig. 2A). *TaHIR2* shared highest homology (98 %) with the barley *HvHIR2* protein, followed by the maize *ZmHIR2* (90 %) and the rice *OsHIR2* (90 %), respectively.

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1 tctggtccagcgtgaagaagcggaaaacccaggcgcceaacga
46 ccttccttccggcgaaaccatcctccgactcctccctcatctccctgctcgcgaggctcagctcaggataaac
121 atgggtggcgttttgggttttagtgcaggttgatcagcaactgttgctatcaaagaacttttgggaagttaat
1 M G G V L G L V Q V D Q S T V A I K E T F G K F N
196 gagtcctggagcctggttgcacttcttgccttggccttaggcaacgagattggttacctctcactcgt
26 E V L E P G C H F L P W C I G Q R I V G Y L S L R
271 gtgaacagctagacgtccgatgtgaacaaaacaaggataatgtcttggactgttggcttctgtccaa
51 V K Q L D V R C E T K T K D N V F V T V V A S V Q
346 taccgtgcccttgttgataaggcatctgatgccttctacaactgagcaacacaaagcaacaatccagtcgtac
76 Y R A L V D K A S D A F Y K L S N T K Q Q I Q S Y
421 gtctttgatgttattagagccactgtccaaagctggacgatgcatttgtcagaagatgacattgca
101 V F D V I R A T V P K L E L D D A F V Q K D D I A
496 aaagctgttgaagaggagcttgaaggcaatgtctatgtatgggtatgagattgtcgaactctgatagttgac
126 K A V E E E L E K A M S M Y G Y E I V Q T L I V D
571 attgagcctgatgtgcatgtcaagagggcaatgaatgagatcaatgcagcttctaggatgaggtcggcagccaac
151 I E P D V H V K R A M N E I N A A S R M R S A A N
446 gacaaagcagaggctgtaaagattctccagattaaacgagcagaggagaagcggagtcaggacttggctggt
176 D K A E A V K I L Q I K R A E G E A E S K Y L A G
721 gtggcattgcaaggcagcgtcaggctatcgtggatggctgagagacagcgtcctcgccttctctgagaacgtc
201 V G I A R Q R Q A I V D G L R D S V L A F S E N V
796 cctggcaccactgcaaaggacatcatggacatggttctggttaccagtaacttcgacaccatgaaagagattggg
226 P G T T A K D I M D M V L V T Q Y F D T M K E I G
871 gcctcgccaagtcttcttgggttctcctccctcctggggcctcaaggatgtggcatcgagatcaga
251 A S S K S S S V F I P H G P G A V K D V A S Q I R
946 gatggctccttcaggccaacactctctaaaggaggaccatgaaagggatagcttctggttttgaagagttgt
1187 ggatgggttctgtgaagaaagttagccagattcctgaatgctacttgttctacttgaagtgagcctggat
1246 gctgaccagtct 1257

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Fig. 1. The cDNA sequence of *TaHIR2* and its deduced amino acid sequence. Triple bases in the *box* show the start and stop codons.

Thirteen HIR genes from the above mentioned four cereal species were used to establish a Neighbor-joining phylogenetic tree (Fig. 2B). The analysis showed a high degree of similarity among the HIR1, HIR2, HIR3, and HIR4 groups across the species analyzed. *TaHIR2* was grouped together with the proteins encoded by *HvHIR2*, *OsHIR2*, and *ZmHIR2*. The other HIR members were clustered into three families respectively. These results suggest a high level of conservation among HIR proteins from different plant species.

Subcellular localization of *TaHIR2* in onion cells: To examine subcellular localization of *TaHIR2*, a transient expression system using onion epidermis cells was performed. The fusion *pCaMV35S::TaHIR2-GFP* and the control *pCaMV35S::GFP* were respectively introduced into onion epidermal cells through particle bombardment. The observation with confocal laser microscope illustrated that *TaHIR2-GFP* fusion protein was targeted to the whole transformed cells, which seemed to be in

agreement with the *PSORT* prediction (Fig. 3).

Induction of the *TaHIR2* gene in adult wheat leaves by infections of *PST*: The qRT-PCR was used to determine the expression profiles of *TaHIR2* during the infections of *PST* (Fig. 4). After inoculation with CY32, *TaHIR2* transcripts were accumulated more abundantly at the adult-plant stage compared with the seedling stage. In the incompatible reaction at the adult-plant stage, *TaHIR2* was dramatically up-regulated as early as 12 hpi, and peaked at 18 hpi with 42.4 fold. The expression of *TaHIR2* decreased thereafter, though, still much higher within 24 to 72 hpi. At 120 hpi, the expression level of *TaHIR2* fell down to 1.8 fold. By contrast, in the compatible reaction, *TaHIR2* transcripts showed slightly elevated expression from 12 to 24 hpi. The maximum induction of the *TaHIR2* transcripts occurred at 18 hpi by 3.1 fold. After 48 hpi, *TaHIR2* expression was slightly repressed.

Discussion

Plant HIR genes consist of a few members in each species (Nadimpalli *et al.* 2000, Rostoks *et al.* 2003), namely four barley HIR genes, *HvHIR1*, *HvHIR2*, *HvHIR3* and

HvHIR4 (Rostoks *et al.* 2003); three maize genes *ZmHIR1*, *ZmHIR2* and *ZmHIR3* (Nadimpalli *et al.* 2000); three rice genes, *OsHIR1*, *OsHIR2* and *OsHIR4*

(annotated in GenBank); and two wheat genes, *TaHIR1* and *TaHIR3* (Yu *et al.* 2008, Zhang *et al.* 2009). The *HIR* genes are highly conserved among these four monocots. In the present work, we characterized one of the novel *TaHIR2* genes using the *in silico* cloning combined with RT-PCR.

Basically, various types of domains and motifs of a protein attribute the accuracy of protein function. The SPFH domain identified in *TaHIR2* is characteristic for *HIR* proteins, prohibitins, stomatins, and other proteins associated with membrane functions (Tavernarakis *et al.* 1999). *TaHIR2* also contained the prohibitin homologue, which is involved in cell-cycle control, replicative senescence, and tumor suppression (McClung *et al.*

1995). Furthermore, four types of conserved motifs are observed in *TaHIR2*. N-myristoylation, one of the motifs, is predicted to be anchored to membranes and thus facilitate the functioning of *HIR* proteins (Rostoks *et al.* 2003). All the structural features of *TaHIR2* should better serve future research into the characterization of *TaHIR2* and other SPFH domain-containing proteins.

The small LRR proteins are located in the plant plasma membrane for regulating cell-cell interactions of developmental processes and responses to biotic or abiotic stresses (Hipskind *et al.* 1996). The positive interaction of CaLRR1 with CaHIR1 negatively regulated the CaHIR1-mediated cell death (Jung and Hwang 2007). The finding suggests intracellular components

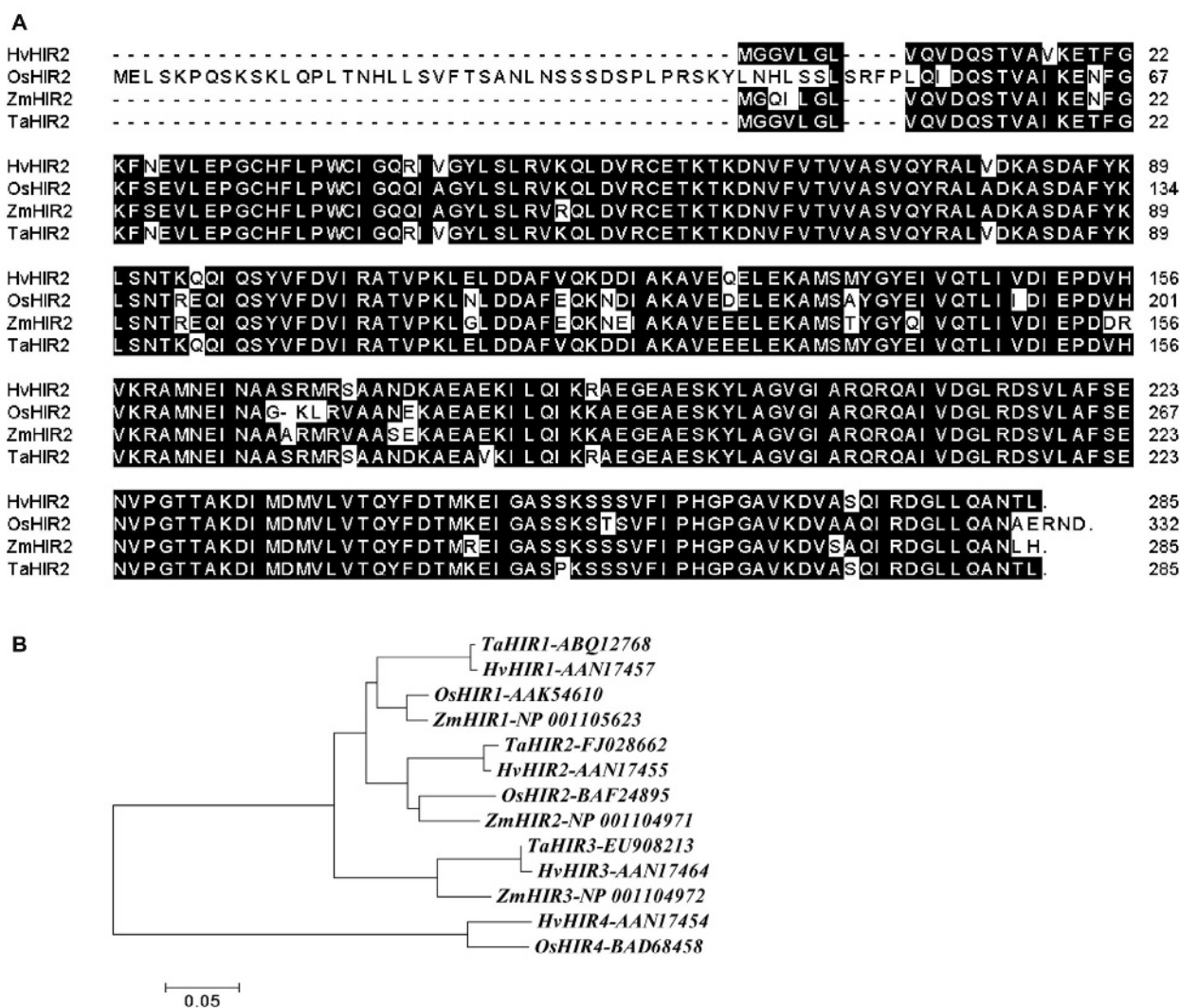


Fig. 2. *A* - Multiple alignments of deduced amino acid sequences of *HIR2* from barley (*Hordeum vulgare*, *Hv*), maize (*Zea mays*, *Zm*), rice (*Oryza sativa*, *Os*), and wheat (*Triticum aestivum*, *Ta*) generated by *MegAlign* from *DNASTAR* software. GenBank accessions for the genes are *HvHIR2*, AAN17455; *OsHIR2*, BAF24895; *ZmHIR2*, NP_001104971; *TaHIR2*, FJ028662; *HvHIR4*, AAN17454 and *OsHIR4*, BAD68458. The identical amino acid residues are indicated with black background. *B* - Phylogenetic tree of *HIR* proteins from four cereal crops. Branches were labeled with plant species name followed by the gene with the GenBank accessions.

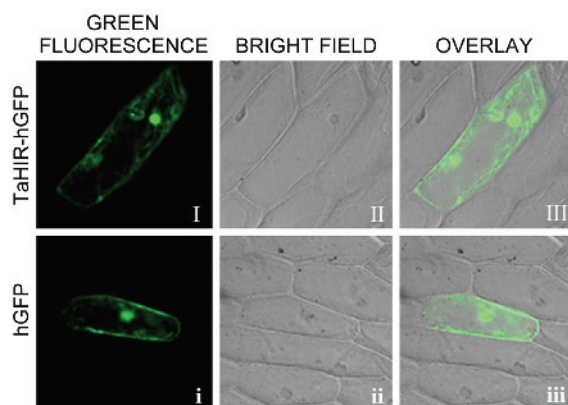


Fig. 3. Subcellular localization of the TaHIR2 fusion protein. The TaHIR2-GFP fusion and GFP control constructs were transformed into onion epidermal cells by particle bombardment. GFP signals in cells expressing the TaHIR2-GFP (*I*) and GFP control (*i*) constructs were observed with a confocal microscope. The images in the middle (*II*, *ii*) and on the right (*III*, *iii*) were the same fields observed under bright and overlay field microscopy, respectively.

involvement in collectively adjusting the function of HIR proteins. Our transient assay revealed intracellular localization of TaHIR2, intimating putative interaction of TaHIR2 with other molecules in cells. Given the expression of TaHIR2 fusion protein in *E. coli* (data not shown), further biochemical characterization of TaHIR2 is needed to be studied.

In previous research, highly inductions of *TaHIR1* and *TaHIR3* transcripts after *PST* infection indicated the two HIR genes' involvement in wheat defense (Yu *et al.* 2008, Zhang *et al.* 2009). In this paper, *TaHIR2* was dramatically up-regulated at the adult-plant stage after stripe rust infection suggesting the involvement of *TaHIR2* in HR associated response of adult-plant resistance to stripe rust. This result is similar to the previous reports of *ZmHIR3*, *TaHIR1*, and *TaHIR3* (Nadimpalli *et al.* 2000, Yu *et al.* 2008, Zhang *et al.* 2009). However, expression profiles of the three wheat HIR genes differed slightly. *TaHIR1* and *TaHIR3* reached maximum induction at 48 hpi (Yu *et al.* 2008, Zhang

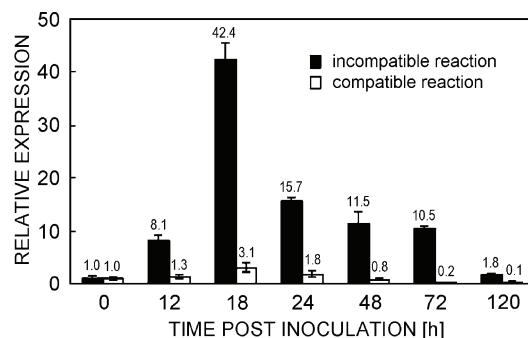


Fig. 4. Real time qRT-PCR analyses of expression profiles of *TaHIR2* in wheat leaves of the adult-plant (incompatible) and seedling stages (compatible) in response to stripe rust infection. Data were normalized to the 18S rRNA expression level. The relative quantification values across different time points are showed above each vertical bar. Error bars represent the standard deviation among three independent replicates.

et al. 2009). *TaHIR2* peaked at earlier 18 hpi. This implies that *TaHIR2* responds to *PST* infection more rapidly, and may play an active role in wheat defense associated with HR formation, since HR cell death occurs at 24 hpi in the incompatible wheat-stripe rust interaction (Wang *et al.* 2007).

So far, a number of different plant HIR genes were isolated, however, functional characterizations have not been fully determined, except *CaHIR1*. Transgenic *Arabidopsis* over-expressed with *CaHIR1* displayed resistance to biotrophic, hemi-biotrophic and necrotrophic pathogens, as well as to osmotic stress (Jung and Hwang 2007, Jung *et al.* 2008). Moreover, a cascade of physiological events required for HR, including the strong expression of PR proteins, the accumulation of salicylic acid, H_2O_2 , and K^+ efflux, were also intimated in the transgenic *Arabidopsis* plants (Jung and Hwang 2007). The evidence demonstrated that HIR genes are well implicated in HR and positively regulate HR cell death to pathogen attacks. Further functional analysis of *TaHIR2* by gene silencing mediated by barley stripe mosaic virus in wheat is in progress (Zhou *et al.* 2007).

References

- Anand, T., Bhaskaran, R., Raguchander, T., Samiyappan R., Prakasam, V., Gopalakrishnan, C.: Defense responses of chilli fruits to *Colletotrichum capsici* and *Alternaria alternata*. - Biol. Plant. **53**: 553-559, 2009.
- Burland, T.G.: DNASTAR's laser gene sequence analysis software. - Methods mol. Biol. **132**: 71-91, 2000.
- Flor, H.H.: Current status of the gene for gene concept. - Annu. Rev. Phytopathol. **9**: 275-296, 1971.
- Greenberg, J.T.: Programmed cell death in plant-pathogen interactions. - Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 525-545, 1997.
- Heath, M.C.: Hypersensitive response-related death. - Plant mol. Biol. **44**: 321-334, 2000.
- Hipskind, J.D., Nicholson, R.L., Goldsbrough, P.B.: Isolation of a cDNA encoding a novel leucine-rich repeat motif from *Sorghum bicolor* inoculated with fungi. - Mol. Plant Microbe Interact. **9**: 819-825, 1996.
- Jung, H., Hwang, B.K.: The leucine-rich repeat (LRR) protein, CaLRR1, interacts with the hypersensitive induced reaction (HIR) protein, CaHIR1, and suppresses cell death induced by the CaHIR1 protein. - Mol. Plant Pathol. **8**: 503-514, 2007.
- Jung, H.W., Lim, C.W., Lee, S.C., Choi, H.W., Hwang, C.H., Hwang, B.K.: Distinct roles of the pepper hypersensitive

- induced reaction protein gene *CaHIR1* in disease and osmotic stress, as determined by comparative transcriptome and proteome analyses. - *Planta* **227**: 409-425, 2008.
- Kang, Z.S., Li, Z.Q.: [Discovery of a normal T. type new pathogenic strain to Lovrin10.] - *Acta Cllegii Septentrionali Occidentali Agr.* **4**: 18-28, 1984. [In Chinese]
- Karrer, E.E., Beachy, R.N., Holt, C.A.: Cloning of tobacco genes that elicit the hypersensitive response. - *Plant mol. Biol.* **36**: 681-690, 1998.
- Kozak, M.: Downstream secondary structure facilitates recognition of initiator codons by eukaryotic ribosomes. - *Proc. nat. Acad. Sci. USA* **87**: 8301-8305, 1990.
- Lam, E., Kato, N., Lawton, M.: Programmed cell death, mitochondria and the plant hypersensitive response. - *Nature* **411**: 848-853, 2001.
- McClung, J.K., Jupe, E.R., Liu, X.T., Dell'Orco, R.T.: Prohibitin: potential role in senescence, development, and tumor suppression. - *Exp. Gerontol.* **30**: 99-124, 1995.
- Métraux, J.P., Nawrath, C., Genoud, T.: Systemic acquired resistance. - *Euphytica* **124**: 237-243, 2002.
- Nadimpalli, R., Yalpani, N., Johal, G.S., Simmons, C.R.: Prohibitins, stomatins, and plant-disease response genes compose a protein superfamily that controls cell proliferation, ion channel regulation, and death. - *J. biol. Chem.* **275**: 29579-29586, 2000.
- Peng, S.M., Luo, T., Zhou, J.Y., Niu, B., Lei, N.F., Tang, L., Chen, F.: Cloning and quantification of expression levels of two MADS-box genes from *Momordica charantia*. - *Biol. Plant.* **52**: 222-230, 2008.
- Pfaffl, M.W.: A new mathematical model for relative quantification in real-time RT-PCR. - *Nucl. Acids Res.* **29** (Suppl.): e45, 2001.
- Rostoks, N., Schmierer, D., Kudrna, D., Kleinhofs, A.: Barley putative hypersensitive induced reaction genes: genetic mapping, sequence analyses and differential expression in disease lesion mimic mutants. - *Theor. appl. Genet.* **107**: 1094-1101, 2003.
- Tavernarakis, N.M., Driscoll, M., Kyrpidis, N.C.: The SPFH domain: implicated in regulating targeted protein turnover in stomatins and other membrane-associated proteins. - *Trends Biochem. Sci.* **24**: 425-427, 1999.
- Tamura, K., Dudley, J., Nei, M., Kumar, S.: MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. - *Mol. Biol. Evol.* **24**: 1596-1599, 2007.
- Wang, C.F., Huang, L.L., Buchenauer, H., Han, Q.M., Zhang, H.C., Kang, Z.S.: Histochemical studies on the accumulation of reactive oxygen species (O₂⁻ and H₂O₂) in the incompatible and compatible interaction of wheat-*Puccinia striiformis* f. sp. *Tritici*. - *Physiol. mol. Plant Pathol.* **71**: 230-239, 2007.
- Xia, N., Zhang, G., Liu, X.Y., Deng, L., Cao, G.L., Zhang, Y., Wang, X.J., Zhao, J., Huang, L.L., Kang, Z.S.: Characterization of a novel wheat NAC transcription factor gene involved in defense response against stripe rust pathogen infection and abiotic stresses. - *Mol. Biol. Rep.* **37**: 3703-3712, 2010.
- Yu, X.M., Yu, X.D., Qu, Z.P., Huang, X.J., Guo, J., Han, Q.M., Zhao, J., Huang, L.L., Kang, Z.S.: Cloning of a putative hypersensitive induced reaction gene from wheat infected by stripe rust fungus. - *Gene.* **407**: 193-198, 2008.
- Zadoks, J.C., Chang, T.T., Konzak, C.F.: A decimal code for the growth stages of cereals. - *Weed Res.* **14**: 415-421, 1974.
- Zhang, G., Dong, Y.L., Zhang, Y., Li, Y.M., Wang, X.J., Han, Q.M., Guo, J., Huang, L.L., Kang, Z.S.: Cloning and characterization of a novel hypersensitive induced-reaction gene from wheat infected by stripe rust pathogen. - *J. Phytopathol.* **157**: 722-728, 2009.
- Zhou, H.B., Li, S.F., Deng, Z.Y., Wang, X.P., Chen, T., Zhang, J.S., Chen, S.Y., Ling, H.Q., Zhang, A.M., Wang, D.W., Zhang, X.Q.: Molecular analysis of three new receptor-like kinase genes from hexaploid wheat and evidence for their participation in the wheat hypersensitive response to stripe rust fungus infection. - *Plant J.* **52**: 420-434, 2007.