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PROFILING GENE EXPRESSION PATTERNS OF STRIPE RUST (*PUCCINIA STRIIFORMIS* F.SP.*TRITICI*) RESISTANCE GENE IN NEW WHEAT GERMPLASM

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Abstract

Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating diseases in common wheat (*Triticum aestivum* L.) worldwide. Total RNA for SSH analyses was extracted from wheat germplasm NR1121 infected by Chinese *Pst* race CYR32. To identify differentially expressed genes in response to *Pst* infection in wheat, all 550 Positive clones from the library were subjected to sequencing, while 162 expressed sequence tags (ESTs) were to clustering, BLAST alignment, functional annotation and classification into different categories. The expression of eight genes was detected by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time polymerase chain reaction (real time-PCR). Expression of six genes (acyl-coenzyme A synthetase, glutathione S transferase, Lipid transfer protein, ubiquitin protein ligase 2, cytochrome P450, and serine/threonine protein kinase SNT7) transcripts were induced and up-regulated to their highest levels at 24 hours after *Pst* infection, while that of two genes(serine Hydroxy-methyl transferase and S-adenosylmethionine decarboxylase) were significantly expressed at 48 hours (hpi). These genes were highly induced at an early stage of infection, suggesting they are transcriptionally activated for the host defense response.

Introduction

Stripe rust (yellow rust), caused by the obligate biographic fungus *Puccinia striiformis* f. sp. *tritici* Eriks. (*Pst*), is one of the major diseases in common wheat worldwide (Chen, 2005). It is the most destructive disease in autumn-sown wheat in northwest and southwest China, where Stripe rust resistance is a major breeding objective. Fifteen countrywide Stripe rust epidemics have been recorded, and losses of 6.0, 3.2, 1.8 and 1.3 million metric tons of wheat occurred during 1950, 1964, 1990, and 2002, respectively. Since the appearance of *Pst* race CYR32 in China, wheat cultivars with resistance gene *Yr9* and the derivatives of Fan 6 have become susceptible, resulting in the 2002 epidemic (Wan *et al*., 2004). Therefore, It is essential to identify new Stripe rust resistance genes (Spielmeyer *et al*., 2003; Liao *et al*., 2007; Fu *et al*., 2009), and to detect gene expression, profiling and pattern through dissecting regulatory mechanism and transcriptional network to the phenotypic responses. Recent advances in molecular biology have improved our understanding of plant-pathogen interactions through the isolation of a number of R genes, and analysis of signaling pathways leading to the hypersensitive response (HR) and systemic acquired resistance (SAR) (Ryals *et al*., 1995; Dempsey *et al*., 1999). The completion of *Arabidopsis* and rice genome sequences and the current sequencing of crop plant genomes, together with improved knowledge of plant defense response mechanisms through functional analyses, will pave the way for the development of transgenic crops with increased disease resistance, or the development of novel pesticides capable of activating plant defense responses.

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Suppression subtractive hybridization (SSH) , semi-quantitative RT-PCR and real-time PCR are basic tools used to profile expression, function and characterization of a particular gene at specific stress condition (Higuchi *et al*., 1992; 1993; Silke *et al*., 2003; Wei *et al*., 2007; Zhang *et al*., 2009).

The cDNA libraries were constructed from wheat seedling leaves with compatible and incompatible interactions after *Pst* attack using SMART (switching mechanism at the 5' end of the RNA transcript) (Ma *et al.,* 2007; Wang *et al.*, 2008), respectively. SSH-cDNA libraries were constructed from wheat seedling leaves (Liu *et al.*, 2006; Yu *et al.*, 2007) and adult-plant leaves (Huang *et al.*, 2007) with an incompatible interaction after *Pst* infection. The expression pattern of resistance genes at seedling stage and in adult plants of wheat cultivar Xingzi 9104 has been observed against CYR23 and CYR32 using semi-quantitative RT-PCR (Huang *et al.*, 2007; Yu *et al.*, 2007). Consequently, no studies have reported the expression pattern of disease-resistant genes from SSH-cDNA libraries in at wheat seedling stage against CYR32 using semi-quantitative RT-PCR or either real-time PCR.

In this study, we isolated suppression subtractive hybridization (SSH) cDNA library from incompatible wheat - *Secale cereale* (Austrian rye) line NR1121infected by CYR 32 at seedling stage to explore expression patterns of eight distinct genes using semi-quantitative RT-PCR and real-time PCR to discover regulatory mechanisms of key genes related to stripe rust resistance in wheat.

Materials and Methods

Wheat (*Triticum aestivum* L.) - *Secale cereale* alien disomic subsititution line NR1121 was provided by the College of Agronomy, Northwest A&F University. Chinese *Puccinia striiformis* f.sp. *tritici* (*Pst*) race CYR32 was provided by the College of Plant Protection, Northwest A&F University. NR1121 seedlings showed incompatible reactions (infection type is 0) after CYR32 infection.

Stripe rust inoculations: Seeds of NR1121 were sown in pots with 15 plants each and then grown in the greenhouse for 21 days. The seedlings were divided into two groups. The seedling of one group was inoculated with CYR32. After inoculation, the seedling were placed in a dew chamber at 9^{o}C and 100% of relative humidity for 24 h then transferred to a greenhouse maintained with a cycle 14 h light and 10 h dark at 12-17^{o}C. NR1121 leaves infected with *Pst* at 0 h, 24 h, 48 h, 72 h, 96 h, 168 h, 240 h, and 336 h were collected into 2 ml centrifuge tubes, immediately frozen in liquid nitrogen and then stored at -80^{o}C. Non-inoculated NR1121 leaves smeared with sterile water were used as controls. Three plants of the cultivar Mingxian169 susceptible to CYR32 was used as check in each pot.

SSH-cDNA library construction: Total RNA was extracted from the leaves using the BIOZOL Reagent Kit (BioFlux, Hangzhou City , China). Trace DNA was removed with DNase I. The total RNA was used as a template for cDNA synthesis by AMV reverse transcriptase (Promega, USA) using oligo d (T) 18 as primer. Using the inoculated leaves cDNA as the tester and the control leaves cDNA as the driver, SSH was performed as described by Diatchenko *et al*., (1996).

The products from two rounds of SSH were used for two nested PCR amplifications, and the amplified products were purified and ligated into the pGEM-T easy vector (Promega, USA). The ligation products were transformed into *E.coli* DH5α competent cells. The transformed cells were selected by ampicillin (100 ug/mL) on X-gal/IPTG plates. The white colonies were selected for PCR using Sp6 and T7 promoter sequences as primers. The positive clones were screened and tested for the size of inserts.

Sequencing and bioinformatics analysis: The plasmid DNA was extracted and purified using the U-gene plasmid miniprep kit (Youjing, Anhui Province , China) and sequenced by Beijing Jierui Company (China). After the removal of vector, primer and ligation junction sequences, DNAstar and CAP3 software were used to eliminate redundant and repeat sequences as well as sequences less than 100 bp. The resultant sequences were then submitted to GenBank. The obtained EST sequences were compared to GenBank's dbEST database and non-redundant protein database for a homology search and analysis using NCBI BLAST. The method by Li *et al.,* (2004) was used as the reference for significant sequence homology in the Blast alignment. The functions of ESTs were classified according to the method published by Bevans *et al*., (1998).

Semi-quantitative RT-PCR and Real-Time PCR: The primers for the positive control genes encoding Canaliculus protein, β-Actin, and β-Tubulin as well as the candidate genes (Table 1) were designed according to the specific DNA sequence of these genes and synthesized by TaKaRa Biotechnology Co. (Dalian City, China). Semi-quantitative RT-PCR was performed as described by Okubara *et al*., (2002) with some modifications. Firstly, to ensure that equal amounts of cDNA were used, the positive control encoding Canaliculus protein gene was normalized using primers. Then the specific primers of candidate genes were used to amplify the normalized cDNA. PCR amplifications were performed using *Taq* polymerase(TaKaRa Biotechnology Co., Dalian City, China) under the following conditions: 94°C for 3 min., 94°C for 40 s, 52-60°C for 40 s, 72°C for 60 s for 34 cycles, then 72°C for 5 min., and 4°C for storage. The PCR products were subjected to electrophoresis in 1.0% agarose gel and visualized using the Gel Doc 2000^{TM} gel imaging system (Bio-Rad).

Real-time PCR was performed in the ABI PRISM 7300 real-time quantitative PCR system according to the instruction of the SYBR Premix Ex *Taq*^{TM} kit. The cDNA at different time points were used as the templates and diluted 1 to 5^5 folds. PCR for each of the samples was repeated for forth. Real-time PCR data were processed according to formulas described by Zhang *et al*., (2005).

Results

SSH-cDNA library construction and sequencing: Total RNA for SSH analyses was extracted from NR1121 leaves at 48 hpi, while RNA for gene expression analysis was obtained from all sampling points. DNase I treatment was applied to remove contaminating genomic DNA (Fig. 1).

Analysis of SSH library: The purification of second PCR amplification products link to pGEM-T Easy vector, then transferred into DH5α by heat-shocked. Inserts from white colonies were selected using PCR and a total of 550 clone bands were picked (Fig. 2). The size of the fragment bands ranged between 80 and 750 bp. To identify differentially expressed genes after *Pst* infection in wheat, all 550 positive clones from the library were subjected to sequencing. According to the result of sequencing, remove vector segment and the adaptors at first, then reject the redundant array and inlay clones, while some sequences whose length smaller than 100bp were eliminated. 162 high-quality EST arrays were obtained. These sequences were submitted into NCBI and the accession number of Genbank is from GO254150 to GO254231, GR884577 to GR884652, and GT270714 to GT270718, while these ESTs were to clustering, BLAST alignment, functional annotation and classification into different categories.

Table 1. List of primers used for semi-quantitative reverse transcriptase polymerase chain reaction and Real Time PCR.

Amplified gene	Primer sequence (5'–3') (RT-PCR)	Primer sequence(5'–3') (Real Time PCR)
Canaliculus protein	F-ACATCTGCCGCCGCTCCCTT	
	R-ACATCTGCCGCCGCTCCCTT	
β-Actin		F-CTGGAATGGTCAAGGCTGGT
		R-CTGGAATGGTCAAGGCTGGT
β-Tublin		F-CCAAGTTCTGGGAGGTGATCTG
		R-TTGTAGTAGACGTTGATGCGCTC
Glutathione-S-transferase (GST)	F- ACTACTCCCTCCATGCCTAT	F-TCGGAGAAAACAGTGGAA
	R- CATCCCGTTCACCTTCTACT	R-AGGGATGATGAAGGGAAT
Acetyl-coenzyme A synthetase (AcsA)	F-AGCGAAAAGGTTATGCAGCTA	F-TGTGGACCGGCTTCTTAACTCT
	R-GGAACACGCACGTTATCAAA	R-CATCCCCACGCCAAAATC
Serine/threonine protein kinase SNT7 (S/TPKSNT7)	F-GATCAAGCCGAAGAAGGACA	F-TGTGGACCGGCTTCTTAACTCT
	R-CTTGCACAGCATGGAAGAAG	R-CATCCCCACGCCAAAATC
Ubiquitin proten ligase2 (UPL2)	F-TGATAGCAGCCACATGGACTTT	F-TGATAGCAGCCACATGGACTTT
	R-AGGACCAGGAGCAACTTTTGAG	R-AGGACCAGGAGCAACTTTTGAG
Cytochrome P450 (CP450)	F-ATGCGTTCTGGAAGGTGTC	F-CCTGATGCGGTTATGAGTACGA
	R-TGGTCGATGTAGAGCGTGA	R-CGGTTCATTCGGAGGTAGGTT
Lipid transfer protein2 (LTP2)	F- GCTCGGCAGGAGAATGT	F-CAACCTGAATGGGGAATGG
	R- AAAGGACCGGGCGTTAG	R-TGCCGAAAAGGAAGGAAAA
Serine hydroxy methyl transferase (SHMT)	F-GGCCAAAGTTGATTGTTGCT	F-CGGCACTTTTCTCCAACTGGTC
	R-TTTCAGCTTGGATGTTGCTG	R-ATGGCTCTGGATCTTCCTCACG
S-adenosylmethioninedecarboxylase (SAMDC)	F-AATTCGGAGTGAGGTTGTG	F-CTTGTGTTGCGTCAGTTTGTTCT
	R-CTGGAGCAGTTTGAGTAGGC	R-GCATTTCCATACATTCGCTCAC

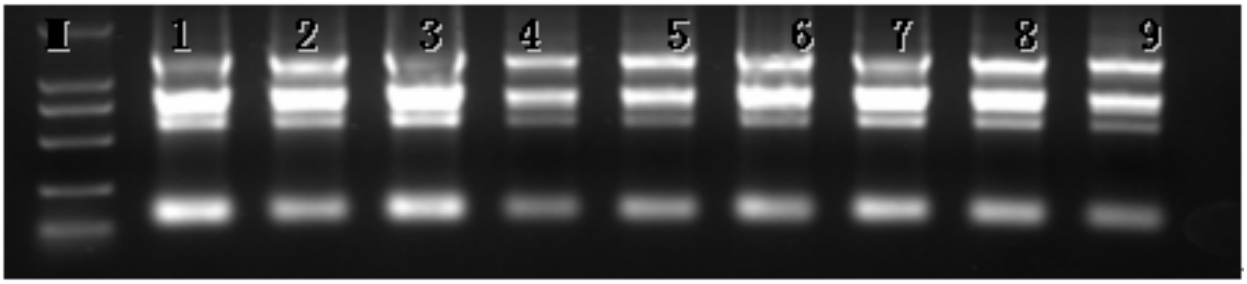

Fig. 1. Detection of total RNA at different time points.
Lane M represented DNA Ladder 2000 (100, 250, 500, 750, 1,000 and 2,000 bp). Lane 1-9 represented the RNA at different time points (0 h, 24 h, 48 h, 48h, 72 h, 96 h, 168 h, 240 h, and 336 h respectively).

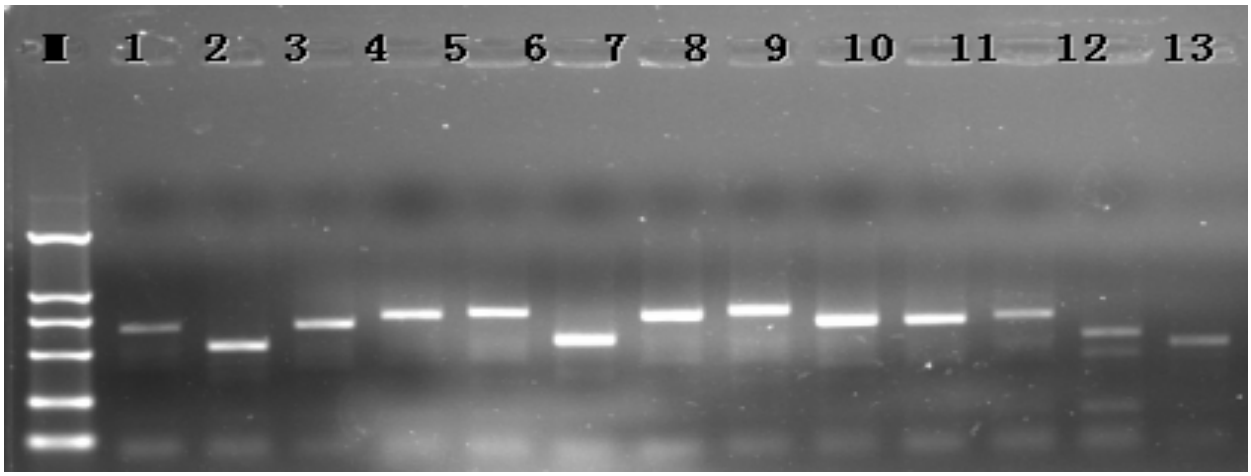

Fig. 2. PCR product of the inserted fragments in the positive clones.
Lane M represented DNA Ladder 2000 (100, 250, 500, 750, 1,000 and 2,000 bp). Lane 1-13 represented randomly selected positive clones.

ESTs sequence alignment: BlastX was used to perform a homology search against the non-redundant protein database in GenBank. 106 out of 162 ESTs showed more than 90% similarity to proteins, Most of which are from the cDNA library derived from *Oryza sativa*, *Arabidopsis thaliana*, *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, and other crops under biotic or abiotic stresses (Fig. 3 and Table 2).

The ESTs were functionally expressed as ESTs related to energy and basic metabolism make up the largest proportion (29.2%), like photosynthesis chloroplast structural proteins and acetyl coenzyme while the Ribulose-1,5-bisphosphate carboxylase/oxygenase showed the highest occurrence frequency. The second category consists of proteins encoding resistance/defense-associated genes, including pathogenesis-related proteins and abiotic stress-related proteins, such as pathogenesis-related protein β-1,3-glucanase, lipid transfer protein, cytochrome P450, and reactive oxygen species (ROS)-reducing GST. The third category comprises proteins encoding signal-processing genes, including SAMDC, YKT61 (similar to yeast SNARE YKT61) and SHMT and other transport-related genes expressed at high frequency.

In the SSH-cDNA library of disease-resistant wheat seedling leaves having an incompatible interaction with *Pst*, EST sequences with a high similarity to sequences of senescence-related proteins, ubiquitin-protein ligase 2, maturase K, membrane transporter YKT61, and SAMDC were discovered for the first time.

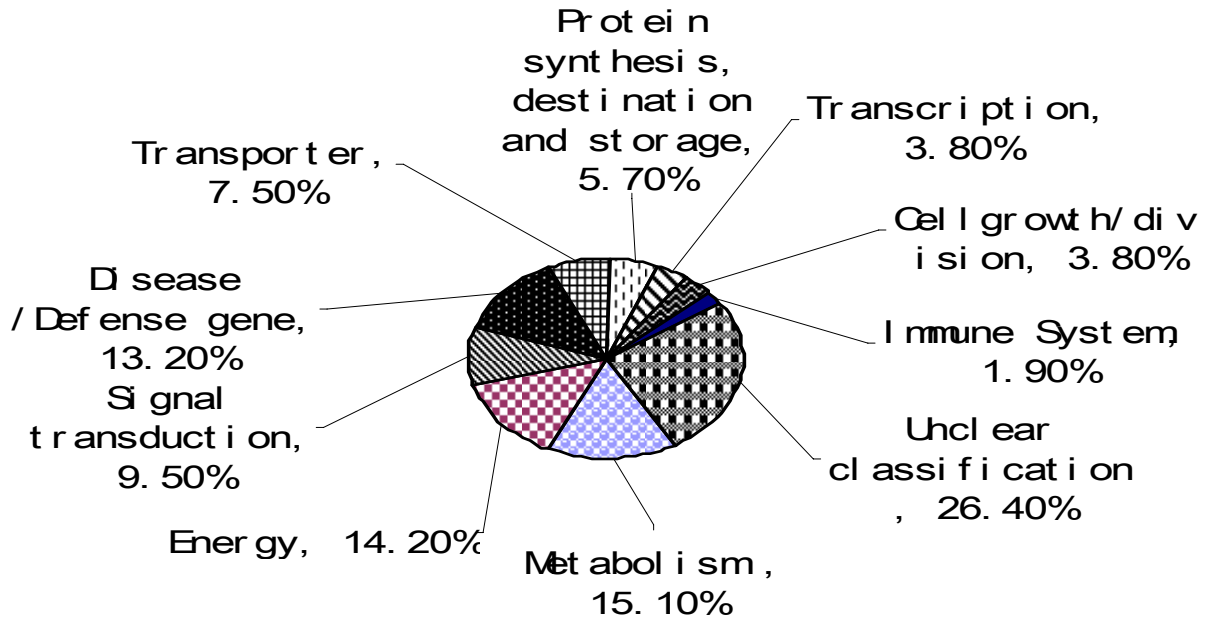

Fig. 3. Pie chart showing the distribution of predicted genes with assigned functions for each category described in Table 2.

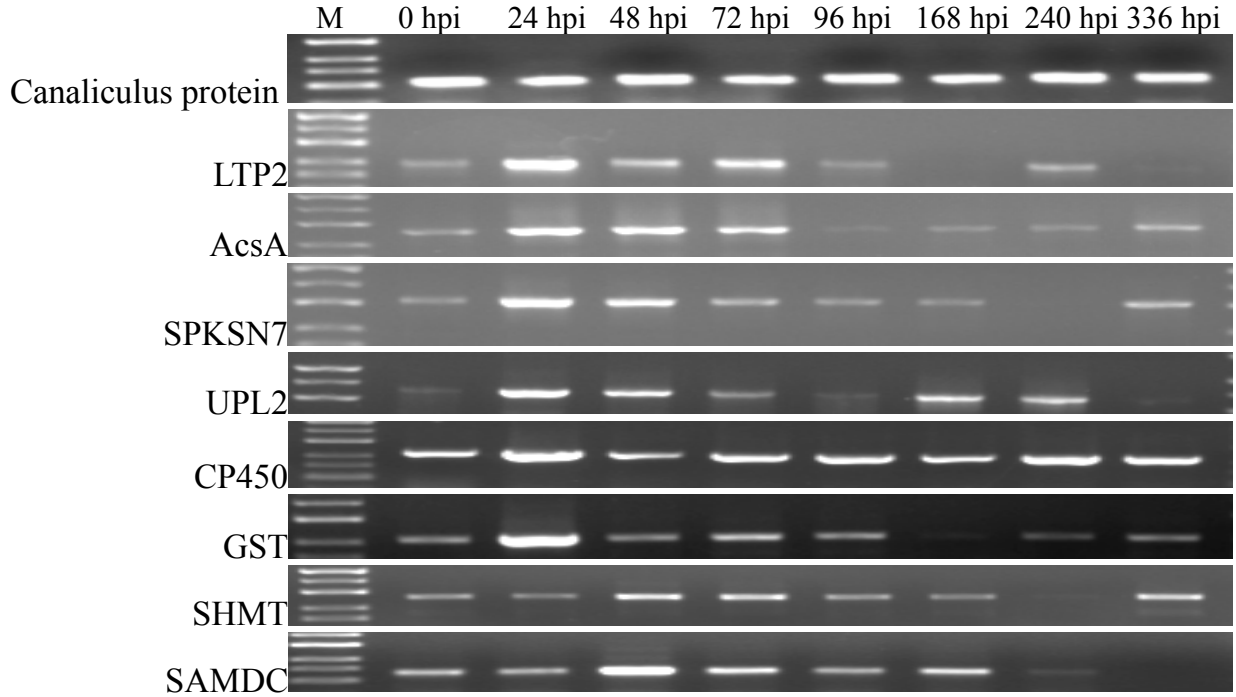

Fig. 4. Semi-quantitative RT-PCR analysis of selected genes at different time points after *Pst* CYR32 infection.

Semi-quantitative RT-PCR and real-time PCR of resistance-related gene expression: The real time PCR was performed to confirm the reliability and reproducibility of semi-quantitative RT-PCR results and to quantify accurately the transcription of eight different genes in different samples.

Semi-quantitative RT-PCR results (Figs. 4, 5) showed that eight candidate genes were expressed at basal levels before *Pst* infection. AcsA and GST were up-regulated to the highest level at 24 hpi after *Pst* infection, and began to drop regularly from 48 hpi until it was below the basal level at 0 hpi. However, the expression level at 336 hpi was

higher than that at 0 hpi. LTP2 and CP450 were up-regulated to the highest expression level at 24 hpi, followed by a fluctuation in the expression with levels higher than that at 0 hpi. It showed that the expression level of these genes was fluctuated significantly after reaching at the peak stage level.

It was also observed that all the eight candidate genes were expressed at basal level before *Pst* inoculation, whereas six (AcsA, GST, SPKSNT7, LTP2, UPL 2, and CP450) out of eight genes up regulated at the maximum level at 24 hpi. Hence SHMD and SAMDC genes were unregulated at the highest level at 48 hpi.

These results demonstrated that the transcription of eight genes were up-regulated at the early stage of stripe rust inoculation, whereas the expression level of LTP2 was higher than that of the other candidate genes, suggesting that this gene may participate in host defense response through different regulatory mechanisms. It was also observed that the results produced by both semi-quantitative RT-PCR and real-time PCR (Fig. 6) regarding to eight different genes were consisted to each other even at pre and post inoculation.

Discussion

The SSH-cDNA library generated from specific conditions is a valuable tool for analyzing the involvement of metabolic genes at a genomic level. The SSH-cDNA library used in this study was established from wheat NR1121 leaves 48 h after Chinese *Pst* race CYR32 inoculation. The results revealed that the genes of known protein functions are mainly involved in energy and basic metabolism, disease resistance and defense, membrane transportation, protein synthesis and metabolism, signal transduction, and transcription regulation. The gene expression profiles is consistent with that in other disease-resistant wheat stocks after *Pst* infection (Liu *et al*., 2006; Huang *et al*., 2007; Yu *et al*., 2007; Wang *et al*., 2008), indicating a similar regulatory mechanism in the Stripe rust-resistant wheat under *Pst* attack.

Disease resistance-related genes have been discovered in the libraries constructed previously and include zinc finger proteins, peroxidase, wound-induced proteins, stress-induced proteins, iron deficiency-induced proteins, GTP binding proteins, serine/threonine protein kinase and so on (Luo *et al*., 2002; Liu *et al*., 2006; Huang *et al*., 2007; Yu *et al*., 2007; Wang *et al*., 2008). In this SSH-cDNA, EST sequences were discovered that display high similarity to the genes encoding SAMDC, membrane transport protein YKT61, maturase K, UPL2, and aging-associated protein. These genes have never been identified in relevant libraries generated previously. SAMDC is a key regulatory enzyme in the polyamine biosynthetic pathway (Hu *et al*., 2005), participating in the resistance of wheat to *Erysiphe grami*nis f. sp. *tritici*(*Egt*) and to abiotic stress (Bhuiyan *et al*., 2007). Maturase K is involved in the splicing of type II introns in RNA transcripts, regulating gene expression through its effect on intron splicing. YKT61 is a membrane transport protein, an organism-specific protein embedded in the phospholipids bilayer of the membrane. It is responsible for soluble substance exchange and small molecule transportation. ESTs highly similarity to this gene were found in the library constructed from disease-resistant wheat samples induced by *Egt* (Wu *et al*., 2008). UPL2 belongs to the group of immune system genes. This enzyme can provide sufficient substrates for the degradation reaction, accelerate the rate of the transportation of target proteins to the proteasome, reduce the lifetime of abnormal proteins, and enhance the efficiency of the ubiquitin degradation pathway. The ubiquitin pathway plays an important role in the nitrogen cycle of degraded non-essential proteins and the utilization of amino acids as alternative energy, which maintains the critical functions of senescent cells (Courtney *et al*., 1994).

Table 2. ESTs and functions identified by BlastX sequence alignment.

Gene Bank Accession No.	Putative gene	Functional catalogue
GO254151, GR884644	Phosphoenol pyruvate carboxylase	Metabolism (16)
GO254157	Acetyl-CoA carboxylase	
GO254171, GR884645	Acyl-CoA dehydrogenase	
GO254185	Magnesium-dependent phosphatase 1	
GO254199, GR884607	Phosphatidyl inositol glycan	
GO254202	phosphatidylserine decarboxylase	
GO254216,GO254226 GR884615	Betaine aldehyde dehydrogenase	
GO254224	Cystathionine beta-synthase	
GR884581	Acyl-CoA synthetase	
GR884649	MYO-Inositol oxygenase	
GR884589	Glycerate dehydrogenase	
GO254155,GO254227,GO254174,GO254179,GO254187,GR884635,GR884636,GR884587,GR884602,GR884616	Ribulose-1,5-bisphosphate carboxylase/oxygenase	Energy (15)
GO254178	Photosystem I 20 kDa subunit/PSI-D	
GO254230, GR884629	Photosystem II protein D1	
GR884626	Thiamine pyrophosphate enzyme	
GR884586	Photosystem I P700 chlorophyll a apoprotein A2	
GO254186	Serine protein kinase SNT7	Signal transduction (9)
GO254162	Thioredoxin M1	
GO254205	Calmodulin-binding family protein	
GO254211, GR884585	Thiamin pyrophosphokinase 1	
GR884609	Protein kinase CK2	
GR884601	Histidine kinase A	
GR884611,GO254194	Thioredoxin F	

Table 2. (Cont'd.).

Gene Bank Accession No.	Putative gene	Functional catalogue
GR884608	Catalase/peroxidase	Disease /Defense gene (14)
GO254166	Zinc knuckle family protein	
GO254167	Glutathione transferase	
GT270714	Pathogenesis-related protein	
GT270715	Hydroxyproline-rich glycoprotein family protein	
GT270717	hydroxypyruvate reductase	
GT270716	Glyoxalase	
GO254188	LTP2	
GO254196,GR884582	Cytochrome P450	
GO254206	Dehydration-responsive protein RD22	
GO254215	Superoxide dismutase	
GO254217	Selenium-binding protein	
GO254225	Beta-1,3-glucanase 1	
GO254193,GR884623	SHMT	Transporter (8)
GT270718	SAMDC	
GO254190	Signal peptidase complex subunit 3	
GO254214,GR884590	YKT61 (similar to yeast SNARE YKT61)	
GR884588	Glyceraldehyde-3-phosphate dehydrogenase	
GO254150	60S ribosomal protein L18a	Protein synthesis, destination and storage (6)
GO254189,GR884595	Esterase/lipase/thioesterase-like protein	
GO254204	Peptide methionine sulfoxide reductase	
GO254210	50S ribosomal protein L15, putative	
GR884597	60S ribosomal protein L11	
GO254182	MYB transcription factor	Transcription (4)
GO254192,GR884579,GR884580	Senescence-associated protein	
GO254201	DNA mismatch repair protein mlh1	Cell growth/ Division (4)
GR884578	Structural constituent of cell wall	
GR884638	MUTL	
GR884610	Extensin-like protein	
GR884577	Ubiquitin-Protein Ligase2	Immune System (2)
GR884619	U-box domain-containing protein	
Unknown (46)	Unclear classification(28)	

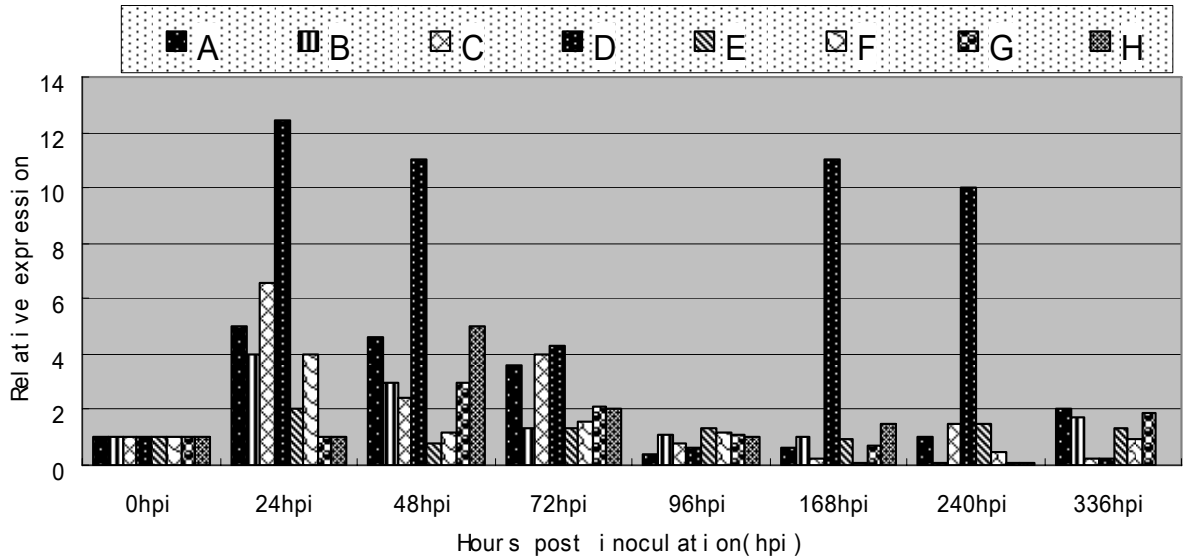

Fig. 5. Semi-quantitative RT-PCR analysis of the expression patterns of candidate genes at different time points.

Note: A, LTP2; B, AcsA; C, SPKSNT7; D, UPL2; E, CP450; F, GST; G, SHMT; H, SAMDC

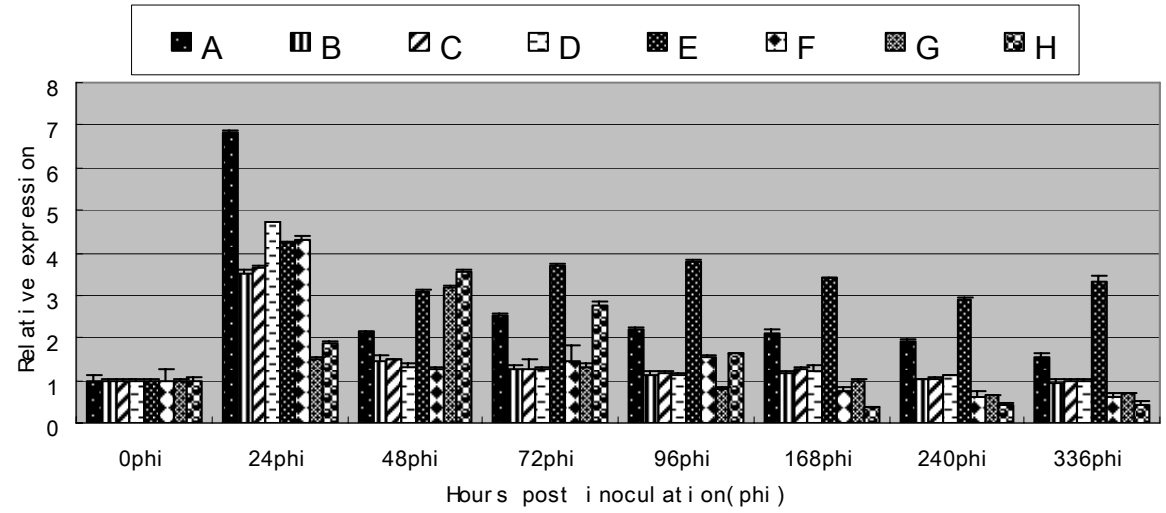

Fig. 6. Real-time PCR analysis of the expression patterns of candidate genes at different time points.

Note: A, LTP2; B, AcsA; C, SPKSNT7; D, UPL2; E, CP450; F, GST; G, SHMT; H, SAMDC

Among the genes of known protein functions derived from the cDNA library of disease-resistant wheat seedling leaves or adult-plant leaves infected by *Pst*, the largest number were involved in energy and basic metabolism as well as disease resistance defense response. In this study, the frequency of two classes of genes was 29.2% and 13.2%, respectively (Li *et al*., 2004). In the relevant libraries in previous studies, the percentage of these two classes of genes were 32.5% and 11.0%, 23.1% and 11.5%, and 40% and 17.1%, respectively (Huang *et al*., 2007; Yu *et al*., 2007; Wang *et al*., 2008). The high-level expression of genes involved in energy and basic metabolism reflects the complex photoautotrophic metabolism of plants (Bevans *et al*., 1998). This finding indicates that plants increase their substance metabolism related to disease resistance when they are induced by pathogen infection or other inducing factors (Ingram *et al*., 1996; Wang *et al*., 2008). This increased metabolism provides the energy needed for resistance to injuries resulting from pathogen infection. The genes that participate in

photosynthesis as well as those encoding chloroplast structural proteins and acetyl coenzyme A constitute the largest proportion of genes related to disease resistance. Ribulose-1,5-bisphosphate carboxylase/oxygenase, showed the highest occurrence frequency. It is a dual-function enzyme and catalyzes a pair of competitive reactions in the chloroplast stroma, viz., carbon dioxide fixation and photorespiratory carbon oxidation (Yoshizawa *et al*., 2004).

The present study revealed that the expression levels of genes involved in disease defense and signal transduction were higher than those of other types of genes. However, genes related to energy and basic metabolism and also signal processing were found to participate in the process associated with the Stripe rust-resistance response. This finding indicates that the host initiates defense mechanisms against the damage caused by a pathogen infection *via* different pathways. Therefore, the process of Stripe rust resistance in wheat involves not only an identification process during pathogenesis and the activation of disease-resistance genes, but also the expression of disease resistance-related genes and disease resistance-related signal transduction. The process of resistance involves many aspects of physiological and biochemical pathways and reflects the systemic coordination of biological activities.

In the incompatible interactions between wheat and *Pst* or *Egt*, expression of hypersensitive-induced reaction protein 1, ASAH1, pathogenesis-related protein 10(*TaPR10*),GST , PR1(systemic acquired resistance hallmark protein), and SAMDC transcripts were highly induced at an early stage of infection (Cao *et al*., 2006; Bhuiyan *et al*., 2007; Yu *et al*., 2007; Wu *et al*., 2008; Zhang *et al*., 2009). In this study, we have shown that transcription of genes ACSA, GST, SAMDC, SHMT, LTP2, SPKSNT7, UPL2, and CP450 is up-regulated after *Pst* CYR32 infection. We hypothesize that these genes up-regulation is linked to an enhanced defense response.

LTP2 plays important roles in lipid transport, cuticle formation and plant defense responses (Cheng *et al*., 2004). The level of LTP2 is up-regulated after *Pst*-induction to inhibit the growth of pathogenic bacteria and to induce systemic plant resistance (Van Loon *et al*., 1999). Cytochrome P450 is a class of multi-functional heme oxidoreductase that plays an important role in the resistance mechanism in adverse conditions. Signal transduction genes, such as serine/threonine kinase, play important roles in plant resistance against pathogens, participating in phosphorylation reactions and regulating the expression of disease-resistance genes and the activation of transcription factors (Seo *et al*., 1995; Dröge-Laser *et al*., 1997; Frye *et al*., 2001). These genes belong to the group of Stripe rust-induced genes and are involved in disease-resistance responses.

The expression patterns of SAMDC and SHMT in wheat after *Pst* infection differ from those after *Egt* infection. SAMDC is a key regulatory enzyme in the biosynthesis of polyamine and exerts its function as an osmotic regulatory protein when plants are under environmental stress (Wang, 1990). The SAMDC gene also functions in wheat resistance to fungal diseases. It may contribute to an increased synthesis of ethylene, which transduces disease resistance signals through its cooperation with jasmonic acid methyl ester and initiates defense responses. In this study, we showed that SAMDC was slightly up-regulated at 24 hpi and reached its highest level at 48 hpi after *Pst* infection. Therefore, SAMDC is a *Pst*-inducible gene. Previous studies have shown that SAMDC can be up-regulated after *Egt* infection (Bhuiyan *et al*., 2007). Meanwhile, SHMT was slightly up-regulated at 24 h after *Pst* inoculation and peaked at 48 hpi. SHMT is a *Pst*-inducible genes. However, its expression did not significantly change during *Egt* infection (Bhuiyan *et al*., 2007).

In this study, the result reflected that one immunized wheat trigger corresponding defense mechanisms against pathogen to strengthen their resistance to disease and related genes expression patterns. We have clearly demonstrated in this report that these gene are associated with resistance to stripe rust in wheat, But the regulation of the expression of these genes may be complex. With the development of technology of functional genomics, comparative genomics and genetic transformation, scientists have been absorbed in genetic engineering technology and RNA technology to analyze resistance mechanisms and the role of gene function (Song *et al*., 1996). The study should further strengthen the understanding pathogenic mechanism of the interaction between the plant and pathogen, signal transduction rules between the various signals, and their metabolism of plant and pathogens respectively, to reveal the molecular mechanism of plant disease resistance mystery and to provide a theoretical basis for enhancing the capacity of plant resistance.

Conclusion

A SSH-cDNA library was constructed from wheat germplasm NR1121 infected by Chinese *Pst* race CYR32. All 550 Positive clones from the library were subjected to sequencing, while 162 ESTs were to clustering, BLAST alignment, functional annotation and classification into ten categories. Expression of six genes (AcsA, GST, LTP2, UPL2, CP450, and SPKSNT7) transcripts were induced and up-regulated to their highest levels at 24 hours after *Pst* infection, while that of two genes(SHMT and SAMDC) were significantly expressed at 48 hours. These genes were highly induced at an early stage of infection, suggesting they are transcriptionally activated for the host defense response.

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