Eur J Plant Pathol (2017) 148:617–629
DOI 10.1007/s10658-016-1118-0

Gene expression analysis of host–pathogen interaction between wheat and *Fusarium graminearum*

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Accepted: 29 November 2016 / Published online: 8 February 2017

Abstract Fusarium head blight (FHB) caused by *Fusarium* species is a devastating disease of wheat (*Triticum aestivum*) worldwide. Mycotoxins such as deoxynivalenol (DON) produced by *Fusarium graminearum* negatively affect plant and animal health, and cause significant reductions in grain quantity and quality. Resistant cultivars are the only effective way to control this disease; however, the molecular mechanisms of defense resistance are still unclear. To get a clear insight, transcriptional profiling of some subtracted differentially expressed host genes and some fungal expressed genes were carried out for the Sumai 3 wheat cultivar (resistant) and Caledonia (susceptible) at 6, 12, 24, 36, 48, 72 and 144 h post inoculation (hpi) using quantitative reverse transcription PCR (qRT-PCR). We document in this study the gene patterns of eight up-regulated host clones with significant matches to *T. aestivum* database and seven fungal clones with significant matches to *F. graminearum* sequences.

Keywords *Fusarium graminearum* · Wheat · Suppression subtractive hybridization · Gene expression · qRT-PCR

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Introduction

Fusarium head blight (FHB) is one of the most devastating diseases in wheat, barley and other grains worldwide. It not only severely reduces grain yield, but also diminishes grain quality (Shroeder and Christensen 1963). Kernels infected by *F. graminearum* (the primary causal agent of FHB in Canada) are often shriveled, with significantly lower kernel weight, and consequently can be easily discarded with the chaff during threshing (Bai and Shaner 2004). Additional losses occur by contamination of grain with deoxynivalenol (DON), a mycotoxin produced by *F. graminearum* (Snijers 1990), that is harmful to animal and human health (Desjardins and Hohn 1997). Severe FHB epidemics have been reported throughout United States, Canada, South America, Europe and Asia during the twentieth century (McMullen et al. 1997).

Currently, there are no fungicide treatments that completely control FHB. However, genetic resistance used in combination with fungicides and crop rotation are the most effective and economical strategy to control FHB disease. The Chinese resistant cultivar Sumai 3 and its derivatives have been a major source of FHB resistance in most breeding programs worldwide (Bai and Shaner 2004). In cv. Sumai 3, infection is limited to the inoculated floret, and disease symptoms usually do not spread to uninoculated spikelets (Type II resistance). In contrast, in susceptible cultivars such as cv. Caledonia, disease symptoms start to spread from the inoculated spikelets to non-inoculated spikelets in about five days after inoculation and cover the entire spike in

ten to fifteen days thereafter (Bernardo et al. 2007). The rapid recognition of invading pathogens, as well as the effective induction of the host defense response differentiates resistant plants from those that are susceptible. Resistance is achieved specifically by the recognition of pathogen elicitors with the plant host receptors, resulting in the induction of signalling events that include changes in ion fluxes, phosphorylation and production of proteins and reactive oxygen species (Dixon et al. 1994; Dangl and Jones 2001). The activation of these signalling events induces the transcriptional and translational machinery leading to the expression of plant defense-related genes (Zhu et al. 1996), such as glutathione S-transferases (GST), peroxidases, cell wall proteins, proteinase inhibitors, hydrolytic enzymes, pathogenesis-related (PR) proteins and phytoalexin biosynthetic enzymes (Hammond and Jones 1996).

Plants that have been invaded by fungal pathogens illustrate morphological, structural and biochemical changes, such as deposition of lignin, callose or phenolic compounds, or synthesis of pathogenesis-related (PR) proteins. The increased levels of PR basic chitinase and basic beta-1,3-glucanase in FHB-infected wheat have been extensively studied, and are believed to play an essential role in plant defence reactions (Han et al. 2005). A study done by Kruger et al. (2002) generated 4838 ESTs from a cDNA library prepared from Sumai 3 spikes infected with *F. graminearum*. They identified 16 non-redundant sequences of *F. graminearum* that may be required for pathogeneicity. However, another 326 non-redundant sequences were only found in their *F. graminearum*-Sumai 3 cDNA library. Another study done by Bernardo et al. (2007) identified differentially expressed genes between cv. Ning7840 (FHB resistant) and cv. Clark (FHB susceptible). Gottwald et al. (2012) reported 2169 differentially expressed genes in FHB resistant cv. Dream that were important in the defense mechanism against fungal virulence factors. More research in this area will improve our understanding of the resistance mechanisms in wheat against FHB infection. In our previous study (Al-Taweel et al. 2014), a total of 31 expressed sequence tags (ESTs) were identified in Sumai 3 wheat spikes infected with *Fusarium* using the SSH (suppression subtractive hybridization) technique. The objective of the present study was to analyse the relative transcript accumulation of previously identified genes in both resistant and susceptible wheat cultivars. This will provide a more thorough understanding of the possible role of the genes in different genetic backgrounds of wheat cultivars. We conducted a quantitative reverse transcription PCR time course study to analyse the expression of eight putative defense related genes in wheat and seven fungal genes that were significantly up-regulated during *Fusarium* infection.

Materials and methods

Plant materials

Sumai 3 is resistant to FHB and was selected from the cross Funo × Taiwan Wheat (*Triticum aestivum*) at the Agricultural Institute of Suzhou Prefecture, China. Caledonia is a soft white winter wheat that is well adapted to cooler climates in the Northeastern United States and southern Ontario, Canada. Sumai 3 and Caledonia were used as FHB resistant and susceptible cultivars, respectively, for this study. For both cultivars, two plants were grown in each pot with 3 replications in a controlled-environment cabinet under conditions of 16 h light (18 °C): 8 h dark (15 °C) until mid-anthesis. The experimental design was a completely randomized design. Plant-Prod (20–20-20 NPK) all-purpose fertilizer (Brampton, ON, Canada) was applied at a rate of 6 g/L every second week.

Fungal strains and method of inoculation

A highly virulent strain of *F. graminearum* (*Fg*2, 3-ADON) was used for inoculation. Two florets after the ten basal spikelets of the main spike on each plant were point-inoculated at mid-anthesis with 10 μL of freshly prepared conidial suspension (10×10^4 macroconidia/mL plus 0.1 mL/L Tween 20). A mock-inoculation control procedure using water and tween20 was carried out in parallel. Following inoculation, plants were incubated for 24 h in a humidity chamber set at 21–22 °C with 100% relative humidity. The infected whole spikes were harvested 6, 12, 24, 36, 48, 72 and 144 h post inoculation (hpi), placed in a 50 mL tube, immediately immersed in

liquid nitrogen and then stored at −80 °C until they could be processed.

RNA extraction and cDNA library synthesis

Total RNA was isolated and the mRNA was purified from the infected- and control- spikes for both cultivars; Sumai 3 and Caledonia using the silica membrane spin column provided with the RNeasy Plant Mini Kit (Qiagen, Maryland, USA) according to the manufacturer's instructions. A cDNA library was constructed using mRNA isolated from infected Sumai 3 spikes as described by Al-Taweel et al. (2014).

Suppressive subtractive hybridization (SSH)

cDNA synthesis and subtraction from the pooled poly(A) + RNA isolated from the infected- and control- Sumai 3 spikes were performed using a PCR Select™ cDNA Subtraction Kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions (Al-Taweel et al. 2014).

SSH library construction and differential screening

For the subtracted cDNA library, suppression subtractive hybridization (SSH) was performed between the driver sequences derived from mock-inoculated Sumai 3 wheat spikes and tester sequences from wheat spikes inoculated with *Fg*. The SSH procedure was performed using the PCR-Select cDNA Subtraction kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's directions. The PCR product, enriched in differentially expressed genes from the subtracted library, was cloned into the pGEM-T Easy vector (Promega, USA). The subtracted clones were transferred into *E. coli* (ElectroMAX DH10B Cells, Invitrogen) by electroporation according to the manufacturer's instructions in order to construct subtracted cDNA library. About 1000 randomly selected white-SSH clones were probed by colony-based PCR, out of which, 200 clones that showed significant signals in differential expression using dot blot hybridization were screened and sequenced (Al-Taweel et al. 2014).

EST sequencing, data filtering and BLAST homology search

The positive clones were sequenced using universal primers T7/SP6. A stringent filtering process was carried out. The plant and the fungal sequences of the ESTs were determined based on BLAST searches and annotation applying the BLAST program (BLASTN and BLASTX) in two databases (GenBank non-redundant (nr) and EST databases) of the National Center of Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov).

Quantitative reverse transcription PCR (qRT-PCR) assay

Total RNAs of FHB- and water-inoculated wheat were extracted for both cultivars; Sumai 3 and Caledonia, at different harvested times after inoculation for qRT-PCR. First-strand cDNA synthesis was performed in a 20-μl reaction by combining 5 μg total RNA applying oligo $(dT)_{18}$ and random hexamers using Maxima First Strand cDNA Synthesis Kit for qRT-PCR (Thermo Scientific, Grand Island, NY, USA). The mix was incubated at 25 °C for 10 min followed by 30 min at 55 °C before terminating the reaction by heating at 85 °C for 5 min. The qRT-PCR expression analyses for selected genes were realised using the CFX-96 real-time PCR detection system with its corresponding software (Life Science Research, Bio-Rad Laboratories, Inc.). The forward and reverse primers for qRT-PCR were designed from the differentially expressed clones of the fungal and wheat ESTs using Primer Express version 3.0 software (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). A set of wheat Ubiquitin (UBQ) primers was also designed for use as an endogenous control (reference gene) to normalize the data for differences in input RNA and efficiency of reverse transcription between the various samples. Quantitative PCR reactions were carried out in 96-well UV plates containing SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Hercules, CA), 100 nM for each of forward and reverse gene-specific primers (Table 1), and 30 ng of cDNA template in a final volume of 10 μl. After an initial activation step of the DNA polymerase at 95 °C for 30 s, samples were subjected to 40 cycles of amplification (denature at 95 °C for 5 s, annealing and extension together at 60 °C for

Table 1 Gene-specific primers used for quantitative reverse transcription polymerase chain reaction (qRT-PCR). Plant and fungal genes selected for expression analysis by qRT-PCR with their primer sequences

No.	Species	Description	Accession No	Direction	Sequence (5′ to 3′)
1	Wheat	UDP-glucosyltransferase	JK007707	F	TAGGGCCGCAACTGCAATC
				R	AAGTTGGTCCAGCTGAGCATG
2	Wheat	Putative glucan endo-1,3-beta-D-glucosidase	JK007724	F	CAGCTCTACAGGTCCAAGGG
				R	CGATGTACTTGATGTTCACGG
3	Wheat	Chitinase III	JK007723	F	CCATTATCTCGCAGTCGCT
				R	CTTCTTGACGTCGGTGCTAC
4	Wheat	Thaumatin-like protein	JK007714	F	ACGACATCTCGGTGATCG
				R	TTATTATTGCCACTGCAGGC
5	Wheat	Peroxidase III	JK007721	F	GACTCCGTTGTCGCTCTTG
				R	CGTGTTAAGGTTCTTCTTGAGG
6	Wheat	Blue copper protein	JK007711	F	GAAGATCACCCTCCTTGCC
				R	TCTCATCACCCACGTTGAAC
7	Wheat	Thioredoxin M	JK007715	F	CCTTGGAGACATGCCTCA
				R	CCAGAGCGGACTTGTATGG
8	Wheat	Methyltransferase	JK007719	F	CTACCTGAAGGATGCGGTC
				R	TTCTTGGTGATGATGATGGAGT
1	Fungus	Superoxide dismutase	XM388897	F	GTCGTCTTCGAGCAGGAGTC
				R	AGGGTTGAAGTGAGGGCC
2	Fungus	Glutamine synthetase	XM390440	F	CCAGGTTGGTCCCTGCA
				R	GAATGCAGTCCAGCGCC
3	Fungus	Trichodiene oxygenase (*TRI4*)	EF685280	F	AGACTACTTCAAGGACACTGGCC
				R	GGTAAGGGAGATTCTCTAGGGTAGC
4	Fungus	Trichodiene synthase (*TRI5*)	AY130290	F	TCTATGGCCCAAGGACCTGTTTGA
				R	TGACCCAAACCATCCAGTTCTCCA
5	Fungus	Trichothecene biosynthesis positive transcription factor (*TRI6*)	AB017495	F	TGTCGCTACTCAGAATGCC
				R	CCCTGCTAAAGACCCTCA
6	Fungus	Hypothetical protein (*TRI9*)	AF359361	F	TATCCACTCAAACACTCACCCC
				R	TGGTAGCGCATAAAGCAGC
7	Fungus	Putative trichothecene biosynthesis gene (*TRI14*)	AF359361	F	CTGATAAGCTTGAACCACCTCG
				R	TTGATCACAACGGGAGTTCC
Reference gene		Ubiquitin (*UBQ*)	AY862401	F	AAGACCCTCACCGGCAAGA
				R	GGATACCGGAGACACCGAGA

30 s). The efficiency of each primer pair was determined using 10-fold cDNA dilution series in order to reliably determine the fold changes. Target gene expression was quantified using the comparative $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak 2008). The expression of each target gene was replicated three times and presented as fold change normalised to the reference gene UBQ and relative to the untreated control sample (mock) for wheat genes and treated sample at 6/36 hpi for fungal genes.

Statistical analysis

Analysis of variance (ANOVA) for gene expression data for all genes was performed within cultivar using the PROC Mixed procedure of SAS software (SAS version 9.3, SAS Institute Inc., NC, USA). The statistical significance of least square means of fold change for each gene was compared using the Bonferroni method. The type 3 test of fixed effects was determined and those with $p \leq 0.01$ were considered significant.

Results

Construction of cDNA library

Two cDNA libraries were constructed after extracting RNA from FHB-infected- and control- Sumai 3 spikes. The first library was a cDNA library constructed using *Fusarium*–infected wheat, which provided a useful resource for the functional genomic research of the *Fusarium*-infected wheat (Al-Taweel et al. 2011). The second library was a subtracted cDNA library using suppression subtractive hybridization technique. This library was created to characterize overall plant and fungal gene expression during disease development from 6 to 144 hpi. In this case, the RNA extracted from wheat spikes inoculated with the highly aggressive *F. graminearum* strain (*Fg*2) was used as the tester and RNA from mock-inoculated wheat spikes was used as the driver. One thousand white colonies were isolated after cloning the subtracted cDNA in pGEM-T Easy vector, and an initial 200 clones were sequenced. The mean size for the sequenced ESTs from this library was 0.7 kb. This result indicates that lengths of the ESTs sequences reported in this study are good enough to retrieve significant hits in GenBank database (Al-Taweel et al. 2014).

Sequence analyses and annotation

In total, 1000 EST clones were obtained and analysed by dot blot hybridization. A total of 200 clones with a significantly higher expression level than the control plants, were subsequently sequenced. For annotation, the obtained EST sequences were compared with the National Center for Biotechnology Information (NCBI) non-redundant and databases for expressed sequence tag (dbESTs) using BLASTN and BLASTX to predict the function of the corresponding genes. Dirty raw reads were removed, such as reads with adaptors, the universal and nested primers, low quality reads, and unknown bases. The sequences with no significant matches in GeneBank accounted for 24% of the ESTs.

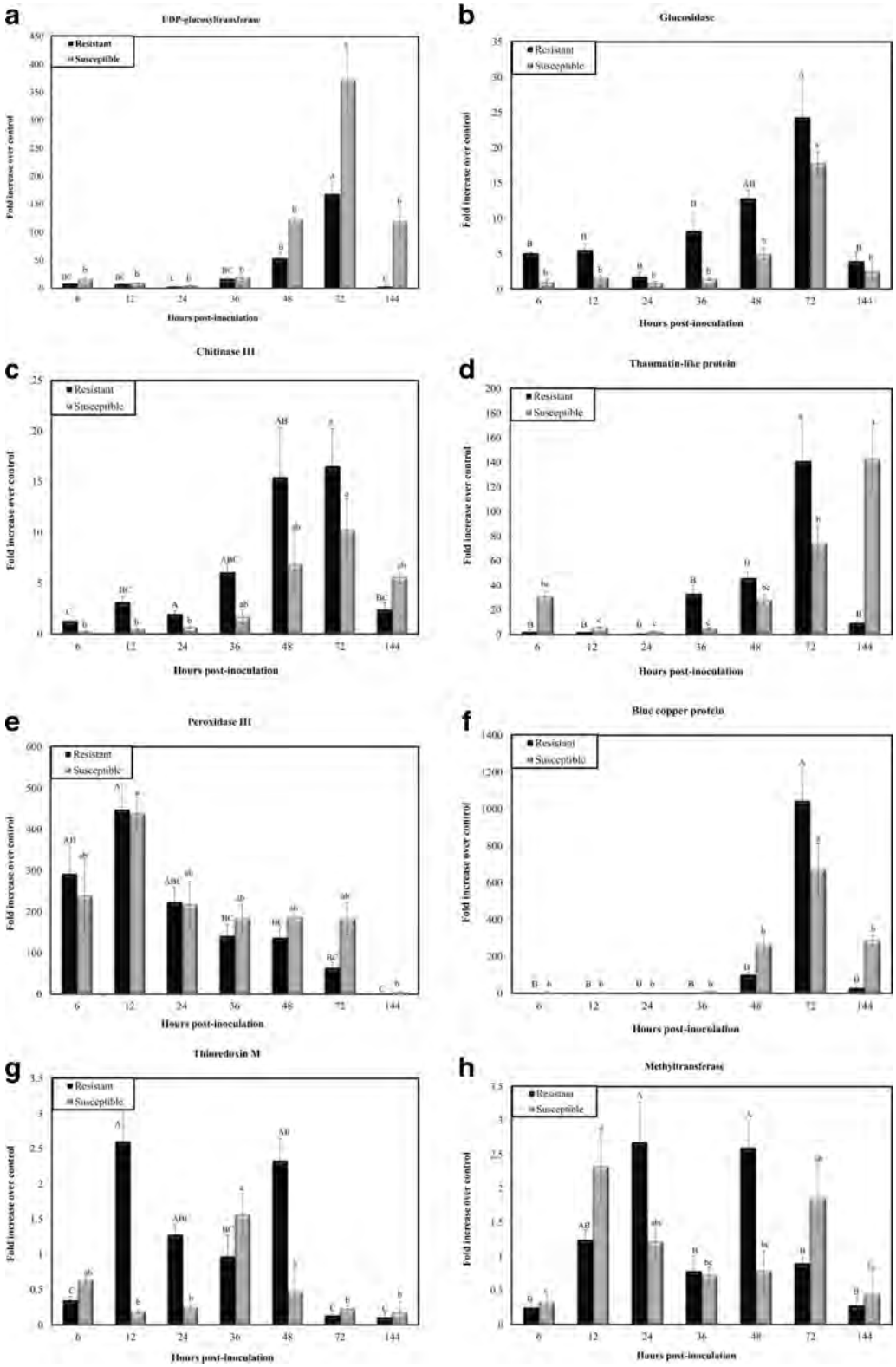
For the plant origin, a total of 31 sequences (25%), out of 126 non-redundant plant ESTs, showed significant similarity to known gene sequences in the database with a stringency level (E value) of 10^{-3} and a score value higher than 80. The identified ESTs were deposited in the NCBI-GeneBank database under accession numbers published in our previous study (Al-Taweel et al. 2014). The sequenced EST clones could be divided into the following different classes: pathogenesis-related proteins (PR protein), DON detoxifying-related genes, oxidative stress-related genes, general plant defence proteins, proteins or enzymes expressed under stress, components of signal transduction and transcription factors, nucleic acid metabolism-related proteins and proteins of unknown function.

For the fungal origin, the comparisons were made by conducting BLASTN searches against EST sequences from *F. graminearum* libraries created using the fungus grown on various culture media. This included EST sequences available in GeneBank for libraries grown on a trichothecene induction medium, complex plant medium, cornmeal and simple substrates, and on nitrogen- and carbon-limited media. Putative functions of genes corresponding to the ESTs were ascertained by BLASTX matches to non-redundant protein sequences in GenBank. In cases where the EST matched an open reading frame (ORF) in the *F. graminearum* genome, the sequence of the entire ORF was used for the searches in GeneBank, in order to better predict function of the gene, and finally only 26% (51 genes) of the non-redundant sequence set was attributable to the fungus.

Quantitative real-time PCR analysis of defense-related genes

Host ESTs analysis

Eight putative defense-related genes were tested in the FHB-resistant cultivar Sumai 3 and the FHB-susceptible cultivar Caledonia at different time points after inoculation with *F. graminearum* using qRT-PCR (Fig. 1). The Y-axis values indicate the relative expression of genes for Sumai 3 and Caledonia compared to their expression in water-inoculated control samples at each sampling time (X-axis) after inoculation. The eight host analysed genes were categorized under three groups; DON detoxifying-related gene, UDP-glucosyltransferase; pathogenesis-related proteins (PR) include glucosidase (*PR-2*), chitinase (*PR-3*), thaumatin-like protein (*PR-5*), peroxidase III (*PR-9*); and oxidative stress-related genes that consist of blue copper protein, thioredoxin M and methyltransferase. The accumulation of UDP-glucosyltransferase transcripts was observed as early as 6 hpi, reached a peak at 72 hpi for both Sumai 3 and Caledonia cultivars and then decreased abruptly at 144 hpi (Fig. 1a). Similarly, the expression of putative



◀ **Fig. 1** Quantitative real-time PCR analyses of eight differentially expressed wheat genes. Fold changes in transcript levels of host-genes; (**a**) UDP-glucosyltransferase (**b**) Glucosidase (**c**) Chitinase III (**d**) Thaumatin-like protein (**e**) Peroxidase III (**f**) Blue copper protein (**g**) Thioredoxin M and (**h**) Methyltransferase. Spikes were sampled for inoculated and mock-inoculated plants in resistant (Sumai 3) and susceptible (Caledonia) wheat at specified hours post inoculation with *F. graminearum*. Relative gene quantification was calculated by comparative ΔΔCT method. The infected samples were quantified relative to the controls (water-inoculated samples) at the same sampling times. All data were normalized to the Ubiquitin expression level. Values are means ± SE of three independent experiments. Statistical analysis was performed within the cultivar. Means with the same letter for fold change in transcript levels are not significantly different

glucan endo-1,3-beta-D-glucosidase was also observed as early as 6 hpi in response to the pathogen and peaked at 72 hpi in both resistant and susceptible cultivars (Fig. 1b). For chitinase III, transcript expression initiated at 6 hpi, increased at 48 hpi, and reached to a peak at 72 hpi in both cultivars (Fig. 1c). Thaumatin-like protein was expressed as early as 6 hpi for Caledonia and 36 hpi for Sumai 3 with highest fold change occurring at 72 hpi for Sumai 3 and 144 hpi for Caledonia (Fig. 1d). Transcript accumulation of Peroxidase class III gene was observed at 6 hpi, reached a peak at 12 hpi and then decreased gradually towards 144 hpi in both cultivars (Fig. 1e). The accumulation of putative blue copper protein transcripts was observed at 6 hpi. Gene expression increased at 48 hpi and reached a peak at 72 hpi, the level of expression then declined by 144 hpi (Fig. 1f). The Thioredoxin M gene was expressed as early as 6 hpi and the transcripts were detected at their highest level at 12 hpi for Sumai 3 and at 36 hpi for Caledonia (Fig. 1g). Acumulation of methyltransferase transcripts was observed at 6 hpi and reached a peak at 24 hpi, then level of gene expression decreased at 36 hpi and again increased at 48 hpi in cv. Sumai 3. In contrast, for cv. Caledonia the highest fold change in gene expression was observed at 12 hpi, gradually decreased from 12 hpi to 48 hpi and then again increased at 72 hpi (Fig. 1h).

Real-time quantitative PCR data demonstrated that the maximal transcript accumulation patterns were at 72 hpi for both cultivars for the genes UDP-glucosyltransferase, glucosidase, chitinase III, thaumatin-like protein and blue copper protein (Fig. 1). A comparison between FHB-resistant cultivar (Sumai 3) and the FHB-susceptible cultivar (Caledonia) showed that pathogen-induced transcripts of glucosidase, chitinase III, thaumatin-like protein, thioredoxin M, and methyltransferase accumulated mostly in the resistant wheat at different time points (24, 36 and 48 hpi) and not in the susceptible one (Fig. 1).

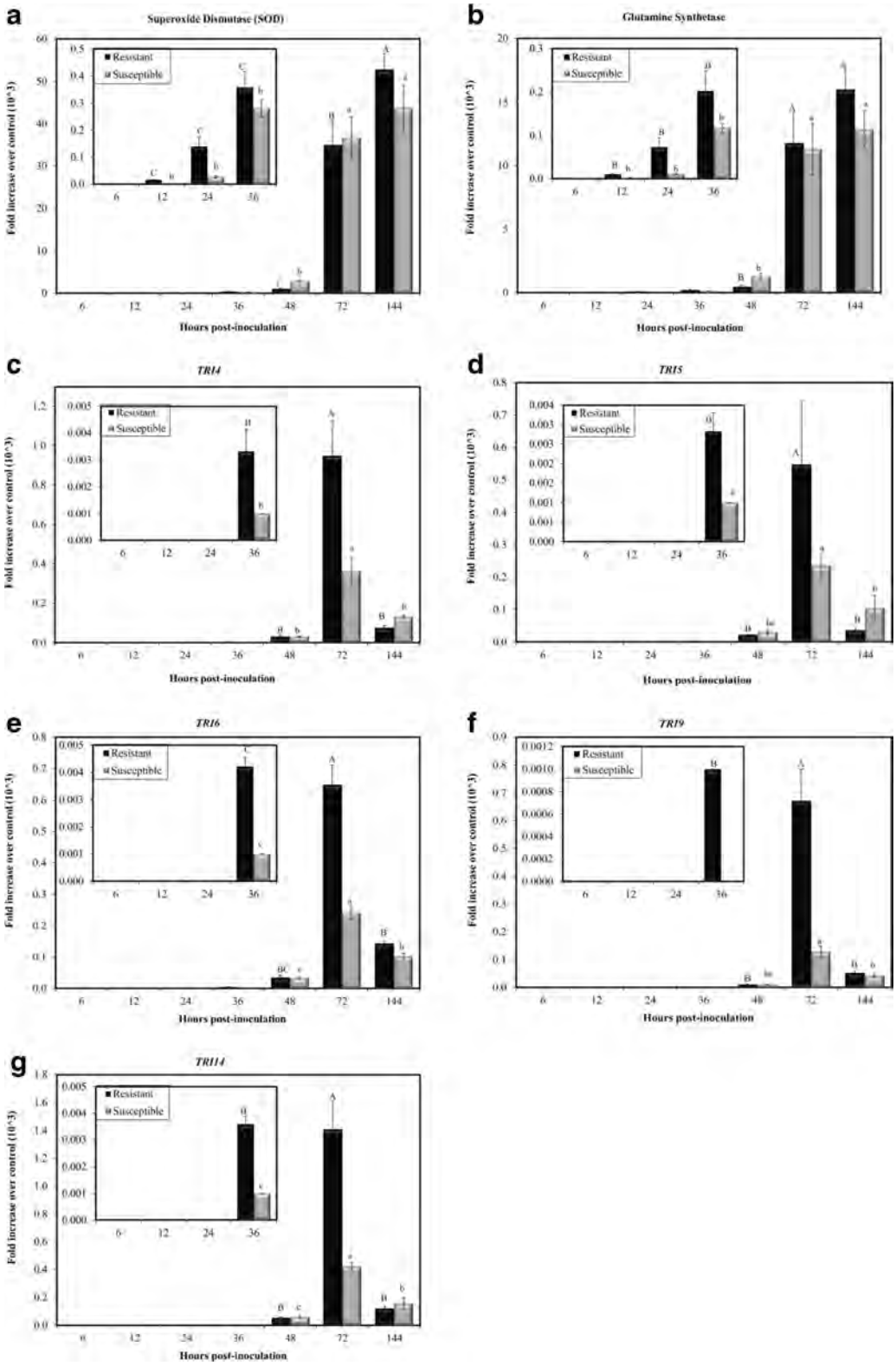
Pathogenic ESTs analysis

ESTs from the subtracted cDNA libraries were examined for genes homologous to known fungal virulence or pathogenicity genes. Out of 51 pathogenic- ESTs identified, we are presenting in this study the transcript accumulations of seven of them categorized into two groups based on their roles in pathogenesis and mycotoxin production; the first one is pathogenicity-related genes which contain superoxide dismutase (*FgSOD*) and glutamine synthetase. The second group of which is DON biosynthesis-related genes which include trichodiene oxygenase (*FgTRI4*), trichodiene synthase (*FgTRI5*), trichothecene biosynthesis positive transcription factor (*TRI6*), hypothetical protein (*FgTRI9*) and putative trichothecene biosynthesis gene (*FgTRI14*).

Figure 2 shows the initial expression of transcript abundance for the pathogenicity-related genes was detected as early as 24 hpi and their transcripts reached the highest level at 144 hpi for *FgSOD* and glutamine synthetase. All *FgTRI* genes were expressed as early as 36 hpi, peaking at 72 hpi and then decreased drastically at 144 hpi for both Sumai 3 and Caledonia cultivars. The relative level of expression of *TRI* genes for Sumai 3 cultivar was considerably higher than in Caledonia at most of the time points. The mock-inoculated wheat spikes were harvested at the same sampling times as negative controls, and no fungal gene transcripts were detected from these, whereas wheat Ubiquitin transcripts were detected (data not shown).

Discussion

This is the first time that both the FHB host and pathogen gene expression profiles have been addressed and simultaneously investigated in a time course study starting from 6 to 144 hpi. Transcriptomics is a powerful approach for comprehensive analysis of plant-pathogen interactions. Using cDNA library and subtracted cDNA library, we identified host genes differentially expressed in response to the pathogen and analyzed their transcriptome patterns at different times post inoculation using qRT-PCR. Resistance to FHB is usually inherited as a quantitative trait. Several chromosomes and QTLs



◀ **Fig. 2** Quantitative real-time PCR analyses of seven *F. graminearum* genes. Fold changes in transcript levels of pathogen genes; (**a**) Superoxide dismutase, (**b**) Glutamine synthetase, (**c**) *TRI4* (**d**) *TRI5* (**e**) *TRI6* (**f**) *TRI9* and (**g**) *TRI14*. Spikes were sampled for inoculated and mock-inoculated plants in resistant (Sumai 3) and susceptible (Caledonia) wheat at specified hours post inoculation with *F. graminearum*. Relative gene quantification was calculated by comparative ΔΔCT method. The infected samples were quantified relative to the controls (water-inoculated samples) at the same sampling times. All data were normalized to the Ubiquitin expression level. Values are means ± SE of three independent experiments. Statistical analysis was performed within the cultivar. Means with the same letter for fold change in transcript levels are not significantly different

are known to influence this resistance reaction (Buerstmayr et al. 2009), indicating that multiple genes affect the resistance.

All the differentially expressed genes in this study were up-regulated during the infection process, indicating their essential roles in defense response against FHB. These genes were identified and analyzed by qRT-PCR. The first gene identified was UDP-glucosyltransferase. Detoxification is one of the main host defense mechanisms to alleviate the effects of trichothecene accumulation. It has been shown that UDP-glucosyltransferase exhibits the ability to detoxify trichothecenes by actively converting DON to DON-3-glucosides (Gardiner et al. 2010). Poppenberger et al. (2003) found an *Arabidopsis* gene encoding a UDP-glucosyltransferase (*DOGT1*) enzyme which is responsible for detoxification of the trichothecene, DON. It has been shown that over expression of the DOGT1 gene in *Arabidopsis* can significantly enhance DON tolerance. Therefore, detoxification of trichothecenes by glycosylation can be considered as an effective natural defense mechanism used by wheat plants to reduce mycotoxin accumulation during *Fusarium* infection. The accumulation of UDP-glucosyltransferase transcripts was significantly less in the resistant cv. Sumai 3 compared to the susceptible cv. Caledonia at all time points in this study. These results contradict reports by Gottwald et al. (2012), in which the level of expression of UDP-glucosyltransferase gene was higher in Sumai 3 at 0, 8, 32 and 96 hpi compared to the susceptible cv. Florense-Aurora. However, at 48, 72 and 120 hpi fold increase over control was higher in cv. Florense-Auror. The sampling time points used in Gottwald et al. (2012) was slightly different from the current study and this may also have an influence on the gene expression patterns. Sumai 3 is highly resistant to FHB, therefore we expect that other defense mechanisms and environmental conditions at the time of inoculation kept the DON concentration at a minimal level so that it required less UDP-glucosyltransferase transcript accumulation to detoxify DON. This may explain the reduced levels of gene expression of UDP- glucosyltransferase in Sumai 3 compared with Caledonia at 72 hpi (Fig. 1a). Moreover, the different genetic background of susceptible cultivars may have an impact on the expression patterns of UDP-glucosyltransferase gene during *Fusarium* infection.

The expression of the glucosidase gene was found to be up-regulated after FHB infection in resistant cultivar as early as 6 hpi. However, in susceptible cultivar, a 5-fold increment was observed at 48 hpi, before that fold change was <2. A study done by Li et al. (2001) also observed an up-regulation of glucosidase gene as early as 24 hpi. In our study glucosidase transcripts were observed to accumulate as early as 6–12 hpi in resistant cultivars. Glucosidases are important components of plant response to pathogen infection and they are classified as PR-2 proteins.

The study indicated high expression of most of the host-tested genes at 72 hpi, including glucosidase and chitinase (Fig. 1b & c). A transgenic wheat line carrying a combination of a wheat β-1,3-glucanase and chitinase genes enhanced resistance against *F. graminearum* in greenhouse and/or field trials (Mackintosh et al. 2007). These two enzymes are important as they are able to degrade β-1,3-glucans and chitin, which are the two main structural components of fungal cell walls, resulting in growth inhibition of fungi (Arlorio et al. 1992). Pritsch et al. (2000) found that the fungus develops infection hyphae, colonizes ovary and floral bract tissue, and sporulates within 72 hpi, which may explain this high expression level at 72 hpi.

Among the other analysed genes, Chitinase III, is a putative gene for plant defense, showed up-regulation in cv. Sumai 3 at early time points of fungal stress which gradually increased and peaked at 72 hpi. A similar expression pattern was observed in the susceptible cv. Caledonia, however the accumulation of transcripts was significantly higher in the resistant cultivar compared to the susceptible cultivar ($p = 0.0096$). Chitinase can degrade chitin in fungal cell walls and releases oligometric products that can activate a cascade of secondary defense responses (Li et al. 2001). Chitinase genes were also found to be differentially expressed in *F. graminearum* infected Sumai 3 spikes (Kruger et al. 2002).

The up-regulation of thaumatin-like protein (Tlp) upon *F. graminearum* infection is interesting. The *TaTLP* gene encodes an important member of (PR-5) proteins and they are involved in plant defense response to many fungi (Van et al. 2006). In our study, *TaTLP* accumulation increased at 36 hpi, with a peak at 72 hpi, followed by a decline in the resistant cv. Sumai 3. In the susceptible cv. Caledonia, the accumulation was highest at 144 hpi. During 36, 48 and 72 hpi the level of expression of *TaTLP* gene in Sumai 3 remained higher than in Caledonia. It has been reported that Tlp inhibits hyphal growth and/or spore germination of various pathogenic fungi through a membrane permeability mechanism (Abad et al. 1996; Mahdavi et al. 2012) or through degradation of fungal cell walls by their β-1, 3-glucan binding and endo- β-1,3-glucanase activity. During infection *Fusarium* hyphae are formed within 72 hpi. The expression of *TaTLP* was observed as early as 6 hpi and peaked at 72 hpi (Fig. 1d) in the resistant cultivar, but in the susceptible cultivar the expression initiated at 6 hpi then declined and gradually increased again at 36 hpi. This confirms the observation of more fungal hyphae in the susceptible cv Caledonia than in the resistant cv. Sumai 3 (Wegulo 2012).

Oxidative stress related genes such as peroxidase III, blue copper protein, thioredoxin M, and methyltransferase were up-regulated in the presence of *Fusarium* infection in both cultivars. All these genes expressed due to oxidative stress. There was no specific pattern of gene expression. As a direct result of pathogenic invasion, plants are exposed to two different toxic stresses; the first one comes from the direct impact of toxins produced by the pathogen, and the second stress arises from the plant's production of ROS as a part of plant defence responses. In order to protect itself against ROS-induced cellular damage, plants also produce antioxidants, including peroxidases (POXs), thioredoxin M, and methyltransferase (Foroud et al. 2012). Also it has been reported that blue copper proteins have roles in reduction and oxidation process during plant infection (Shleev et al. 2005). Therefore, the results shown in (Fig. 1e–h) suggest that although fungal inoculation may lead to an induction of oxidative gene activity in wheat spikes, the variability in their expression depends on the role of the enzyme, the intensity of oxidative stress and available substrates for reactions.

On the other hand, pathogen genes expressed in the plant may produce proteins/products required for pathogenesis processes and the severity of fungal infection relies on obtaining enough nutrients while repressing any host-defense response mechanisms, and control essential adaptations to the nutritional environment encountered by the pathogen. Highly expressed genes in the pathogen during the infection process may have a distinct role in pathogenesis, conferring compatibility in host-pathogen interactions (Hawthorne et al. 1994). Identification of such fungal genes and knowing the regulatory elements that turn genes on/off during successful pathogenesis could reduce the severity of infection and may reveal new information that can be targeted for sustainable disease management.

The pathogen induces several genes to defend itself during fungal-host interaction such as *FgSOD*. *FgSOD*s are metalloproteins present in all aerobic organisms, converting superoxide (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen (Fridovich 1995). *FgSOD*s prevent damage to cellular membranes caused by ROS, acting as a primary defense during oxidative burst to which organisms are exposed (Natvig et al. 1996). Additionally, many pathogens have themselves developed ROS-inactivating systems, where catalases (CAT) and peroxidases (PX), which break down H_2O_2 to H_2O and O_2, are the main enzymatic antioxidants together with SODs. The clear role of ROS and ROS-inactivating systems in microbe pathogenicity and in overcoming host resistance is still an open question (Lanfranco et al. 2005). Glutamine synthetase was found in all organisms and plays a key role in the metabolism of nitrogen by catalyzing the condensation of ammonium ion and glutamate to form glutamine in a reaction which requires ATP. This reaction is essential for the synthesis of glutamine and provides a mechanism for ammonia re-assimilation and detoxification (Stephenson et al. 1997).

In addition to the above-mentioned genes, five genes from the *F. graminearum FgTRI* cluster; *FgTRI4*, *FgTRI5*, *FgTRI6*, *FgTRI9* and *FgTRI14* were selected for gene expression analysis. These genes are involved in pathogenesis and mycotoxin production. The accumulation of each transcript was analysed both in the resistant and the susceptible cultivars using qRT-PCR time course study. It has been reported that the level of toxin biosynthesis in the spike during *Fusarium* colonization is determined by the oxidative conditions encountered by the pathogen. Ponts et al. (2006) reported that H_2O_2 increases DON production by *F. graminearum*. The *FgTRI6* and *FgTRI14* genes play essential roles for mycotoxin production as they

enhance pathway-specific transcription factors and positively regulate the other *FgTRI* genes. Seong et al. (2009) have reported that *FgTRI6* gene is responsible for mediating the pathogenicity and trichothecene accumulation of *F. graminearum* strains. The *FgTRI6* mutants showed localized necrosis and limited spread from the point of infection. Similarly, it is reported that deletion of *FgTRI14* in *F. graminearum* decreased the ability of DON production in plant, resulting in a reduction in pathogenicity (Dyer et al. 2005).

FgTRI5 is the first gene in the DON biosynthesis pathway which encodes the trichodine synthase enzyme that converts farnesyl pyrophosphate to trichodine. The level of expression of the *FgTRI5* gene was higher in the resistant cultivar than the susceptible cultivar; this could be explained as the pathogen has to produce more toxins to withstand the resistance reactions of the plants. Similar results have been observed by (Hallen-Adams et al. 2011), where *Fg*-infected Alsen spikes (moderately resistant) showed fungal expression of *FgTRI5* was higher than the cv. Wheaten (susceptible). In addition, they found the initial expression for *FgTRI* genes started at 24 hpi. In this study, the expression of *FgTRI* genes was initiated at 36 hpi (Fig. 2c–g) showing that the oxidative stress generated during colonization may have roles in activating the DON biosynthesis pathway.

As demonstrated in Fig. 2c–g, the transcript accumulation of the *FgTRI* genes (*FgTRI4*, *FgTRI5*, *FgTRI6*, *FgTRI9*, and *FgTRI14*) was maximal at 72 hpi. Taking into account the host-gene expressed at 72 hpi, we may hypothesize that within 72 hpi, the fungus develops infectious hyphae and *FgTRI* transcripts start accumulating, leading to cascades of defense response genes to be expressed at maximal levels. This hypothesis is supported by one of the mechanisms that explain the reduction of *Fusarium* trichothecene mycotoxin accumulation in grains. This strategy named as type V resistance to *Fusarium* that includes mechanisms by which the trichothecenes are transformed chemically by plants, leading to mycotoxin-degradation or detoxification (Boutigny et al. 2008). Among the detoxification strategies are, glycosylation, acetylation and de-epoxidation of trichothecenes (Boutigny et al. 2008). This is in agreement with what we found in the transcriptome profiling of the differentially expressed genes tested in the host (UDP-glucosyltransferase, glucosidase, Chitinase III, Thaumatin-like protein, Blue copper protein). The expression level of the host genes and pathogen genes maximize at 72 hpi. This shows clearly the strong correlation between host-expressed genes and the pathogenic genes. We hypothesise that in order to resist the mycotoxin accumulation in the host, the expression of PR genes is stimulated to the same level (Fig. 2a & b).

The objective of this study was to analyse the relationship between wheat and *F. graminearum* and understand the mechanism and genes involved in the host-pathogen interaction. In this study, we report the expression pattern of eight differentially expressed host-genes and seven fungal genes in response to *F. graminearum* infection both in resistant and susceptible wheat-cultivars. A better understanding of the natural processes which limits the accumulation of trichothecenes in the plant will open novel ways to breed for wheat varieties with reduced mycotoxin risk. Additionally, identification of a number of host-pathogen genes provides an excellent tool for a future molecular analysis of the correlation between wheat and *F. graminearum.* These genes from the fungus and its resistant host may play an important role in the interaction between the pathogen and the host in the FHB system. More research on this area may enlighten the mechanisms of wheat resistance to FHB infection and complementary studies are required to identify whether the proteins encoded by these genes contribute to the resistance phenotype.

Acknowledgments We thank Dr. Guo Xiaowei for doing all the growth cabinet work of wheat spike inoculations with *Fusarium* spores and the time sensitive harvest of samples. We also thank Dr. Carrie Selin and James Tucker for their critical review of the manuscript. We acknowledge Natural Sciences and Engineering Research Council of Canada (NSERC), Western Grains Research Foundation (WGRF), Agri-Food Research and Development Initiative (ARDI), and the Wheat Breeding Research Cluster (05152) of the Canadian Agri-Science Clusters Initiative for the financial support.

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