Transcriptome Analysis of Wheat and Barley Near-Isogenic Line Pairs Carrying Contrasting Alleles for Different Fusarium Head Blight Resistance QTLs

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DEDICATION

I dedicate this dissertation to my parents, Guihua Jia and Ming Fan.

ABSTRACT

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is a major disease problem on wheat and barley around the world. Grain yield and quality are reduced due to infection and the accumulation of trichothecene mycotoxins such as deoxynivalenol (DON). Previously, quantitative trait loci (QTL) conferring FHB resistance and reduced DON accumulation have been identified on wheat and barley chromosomes. A major FHB resistance QTL (*Fhb1*) was identified on wheat chromosome 3BS. Two major QTLs associated with reduced FHB severity have been detected on barley chromosome 2H Bin 8 and 2H Bin 10, which are associated with heading date and spike type, respectively. A QTL associated with reduced deoxynivalenol (DON) accumulation was identified on chromosome 3H Bin 6. Near-isogenic line (NIL) pairs carrying the resistant and susceptible allele for a wheat QTL and three barley QTL were used to examine DON concentration, fungal biomass and transcript accumulation during *F. graminearum* infection. Based on the disease phenotypes, transcript accumulation data, and comparative analysis of the wheat and barley host response to *F. graminearum* infection, we developed an integrated model for the wheat and barley-*F. graminearum* interactions. In addition, gene transcripts that are differentially expressed in the NIL pairs during infection provide a set of genes that may play a role in FHB resistance.

TABLE OF CONTENTS

Acknowledgements ……..i
Dedication ……..iii
Abstract ……..iv
Table of Contents ……v
List of Tables ……..vi
List of Figures ……...……vii

Chapter 1
Literature Review ……1
Fusarium head blight (FHB) on wheat and barley ……...…...2
Infection of FHB on wheat and barley ……...….2
FHB resistance in wheat and barley ……. ….....4
Genetic resistance to FHB in wheat and barley ……....4
Wheat and barley responses to FHB ……. ……....7
RNA profiling using microarrays ……..10

Chapter 2
Transcriptome Analysis of a Wheat Near-Isogenic Line Pair Carrying Fusarium Head Blight Resistant and Susceptible Alleles ……12

Introduction ……...……13
Results ……...17
Discussion ……25
Materials and Methods ……..36
Tables ……....43
Figures ……...47

Chapter 3
Transcriptome Analysis of Three Barley Near-Isogenic Line Pairs Carrying Contrasting Alleles for Different Fusarium Head Blight Resistance QTLs ……..54

Introduction ……...55
Results ……...58
Discussion ……65
Materials and Methods ……..73
Tables ……78
Figures ……...92

Bibliography ……93

LIST OF TABLES

Chapter 2

Table 1. Deoxynivalenol and ergosterol concentration in a wheat near-isogenic line pair carrying either the *Fhb1* resistant or susceptible allele point-inoculated with *F. graminearum* …….43

Table 2. Number of induced or repressed gene transcripts detected in a wheat near-isogenic line pair carrying either the *Fhb1* resistant or susceptible allele at 48 and/or 96 hai after *F. graminearum* inoculation compared to water ……....44

Table 3. Transcripts that exhibited differential accumulation (P≤0.0001; ≥ 2.0 fold) between plants carrying the resistant and susceptible *Fhb1* allele after *F. graminearum* and water inoculation ……45

Chapter 3

Table 1. Deoxynivalenol and ergosterol concentration in inoculated barley spikes …….78

Table 2. Transcripts that exhibited differential accumulation between the resistant and susceptible genotypes in the chromosome 2H Bin 8 NIL pair after *F. graminearum* and water inoculation ……...79

Table 3. Transcripts that exhibited differential accumulation (P≤0.0001; ≥ 2.0 fold) between the resistant and susceptible genotypes in the chromosome 2H Bin 10 NIL pair after *F. graminearum* and water inoculation……..86

Table 4. Transcripts that exhibited differential accumulation (P≤0.0001; ≥ 2.0 fold) between the resistant and susceptible genotypes in the chromosome 3H Bin 6 NIL pair after *F. graminearum* and water inoculation …….90

LIST OF FIGURES

Chapter 2

Figure 1. Disease phenotypes on wheat plants carrying the resistant or susceptible *Fhb1* allele after point-inoculation with *F. graminearum*. A. 21 days after inoculation (dai); B. 48 hours after inoculation (hai); C. 96hai. Arrows point to the necrotic (A) and bleached (B) regions. The light green areas in A were not pathogen-induced bleaching. Resistant indicates plants carrying the *Fhb1* resistant allele. Susceptible indicates plants carrying the *Fhb1* susceptible allele ……47

Figure 2. Venn diagrams showing the number of gene transcripts in the resistant and susceptible genotypes that displayed increased- (A) or decreased (B) transcript accumulation ($P \leq 0.0001$, ≥ 2.0 fold) after *F. graminearum* inoculation compared to water. More information about the differentially expressed transcripts can be found in Supplementary Tables 1, 2, 3, 4, 5, and 6 ……48

Figure 3. Heat maps showing increased (A) or decreased (B) abundance ($P \leq 0.0001$, ≥ 2.0 fold) of gene transcripts in specific classes in the resistant and susceptible genotypes after *F. graminearum* inoculation compared to water. The color contrast used in A was slightly different from B to show those gene transcripts exhibiting low accumulation. R and S = resistant and susceptible genotype, respectively; F and W = *F. graminearum* and water inoculation, respectively; 48 and 96 = hours after inoculation. Lighter color indicates greater transcript accumulation ……....50

Figure 4. Real-time RT-PCR validation of expression ratio between resistant and susceptible genotypes after *F. graminearum* inoculation. F48=48 hours after *F. graminearum* inoculation; F96=96 hours after *F. graminearum* inoculation. Each column was the average expression ratio calculated from three biological replications. Error bar was the standard error ……...52

Figure 5. A model for the wheat/barley-*F. graminearum* interaction. Genes induced in wheat and barley were gene transcripts that exhibited increased accumulation in *F. graminearum* infected barley (Boddu et al., 2006; 2007) and wheat (this study). Only those gene transcripts that were induced in barley and were predominantly induced in wheat were included. Genes specifically repressed in wheat were gene transcripts that were only found to exhibit reduced transcript accumulation in wheat during *F. graminearum* infection. Genes differentially expressed between resistant and susceptible wheat genotypes were gene transcripts that showed accumulation difference between the genotypes after *F. graminearum* and water inoculation. R indicates plants carrying the *Fhb1* resistant allele; S indicates plants carrying the *Fhb1* susceptible allele ……53

Chapter 3

Figure 1. Venn diagram showing no overlap among the differentially expressed transcripts found in the chromosome 2H Bin 8, 2H Bin 10, and 3H Bin 6 NIL pairs. The number in each circle shows the number of differentially expressed transcripts in each NIL pair …….92

CHAPTER 1

LITERATURE REVIEW

Fusarium head blight (FHB) on wheat and barley

Fusarium head blight or scab, caused primarily by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch], is an economically important disease on wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in North America and worldwide (McMullen et al. 1997; Parry 1995). Disease develops in the spike and causes shriveled, lower-weight kernels. During infection, *F. graminearum* produces trichothecene mycotoxins such as deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), and 3-acetyldeoxynivalenol (3-ADON), which reduce grain quality and are harmful to human and animals (Dalcero et al. 1997; Desjardins and Hohn 1997; Desjardins 2006; Miller and Ewen 1997; Mirocha et al. 1998). DON contamination in the grain increases the economic losses to wheat and barley growers. The maximum acceptable DON level for human consumption is 0.5-2 ppm in wheat (McMullen et al. 1997). DON contaminated malting barley grain can result in beer that exhibits "beer gushing", or increased foaming after opening. The brewing industry has a very stringent requirement for DON concentration, which is < 0.5 ppm (McMullen et al. 1997).

Infection of FHB on wheat and barley

Wheat and barley exhibit different disease phenotypes. Wheat spikes emerge from the flag leaf before anthesis and are susceptible to *F. graminearum* until the soft dough stage (Leonard and Bushnell 2003). Barley flowers undergo anthesis before the spikes emerge from the leaf sheath and the newly-emerged flowers are susceptible to infection. In susceptible wheat genotypes, disease symptoms usually spread in the spike from one

floret to another. However, barley genotypes, including susceptible ones, exhibit resistance to disease spread in the spike (Leonard and Bushnell 2003).

The infection pathway on wheat spikes is complex. The fungus invades through multiple pathways including: stomatal pores, subcuticular growth, between crevices in floral bracts (lemma, palea and glumes) or directly through floral bracts. During the infection process, the fungus colonizes ovary and floral bract tissue, and may produce asexual spores within 72 hours after infection (Leonard and Bushnell 2003; Pritsch et al. 2000). As infection progresses, infected tissues become increasingly necrotic (Kang and Buchenauer 2000b; Leonard and Bushnell 2003; Pritsch et al. 2000). During *F. culmorum* (a closely related *Fusarium* sp. that also causes FHB) infection of wheat, cell walls become degraded, indicating that cell wall-degrading enzymes are active in *F. culmorum* (Kang and Buchenauer 2000a; Kang and Buchenauer 2000b; Kang and Buchenauer 2002). In addition, fungal gene transcripts encoding potential plant cell wall degrading enzymes and trichothecene biosynthetic enzymes were detected in barley infected with *F. graminearum* (Guldener et al. 2006). Trichothecene mycotoxins are produced by *F. graminearum* and these mycotoxins are known to inhibit protein synthesis and increase virulence (Desjardins and Hohn 1997; Proctor et al. 1995).

Many of the *F. graminearum* infection pathways in wheat are also present in barley. For example, the fungus can invade through stomatal pores, grow between floral bracts, exhibit subcuticular growth and penetrate the floral bracts directly (Boddu et al. 2006). In contrast to wheat, one pathway of infection on barley is via colonizing brush hairs (ovary epithelial hairs) at the tip of the floret. After colonizing the brush hairs, the developing caryopsis is infected (Skadsen and Hohn 2004). As in wheat, trichothecene

mycotoxins also increase the aggressiveness of *F. graminearum* infection on barley (Boddu et al. 2007). However, Jansen et al. (2005) demonstrated that trichothecenes are not virulence factors during infection through the fruit coat in wheat and barley.

FHB resistance in wheat and barley

Five types of resistance have been described in wheat, of which type I (resistance to initial infection) and type II (resistance to spread within a spike) are the most studied. The other types of resistance reported in the literature include resistance to DON accumulation, resistance to kernel infection, and tolerance (Mesterhazy 1995). Type II resistance has been extensively studied and identified in several wheat genotypes. Most wheat breeding programs screen for type II resistance (Bai and Shaner 2004). However, barley usually exhibits natural type II-like resistance, FHB infection in barley usually does not spread from initially-infected spikelets to adjacent ones. As a result, type I resistance is more important in barley. To evaluate type II resistance in wheat and type I resistance in barley, point inoculation and spray inoculation are used, respectively.

Genetic resistance to FHB in wheat and barley

Wheat. To identify regions of the wheat genome that control FHB resistance, multiple QTL mapping studies have been conducted. The Chinese cultivar Sumai 3 exhibits type II resistance and has been the target of many mapping studies. In these studies, a major QTL has been identified on chromosome 3BS (*Qfhs.ndsu-3BS* or *Fhb1*) (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002; Liu et al. 2006; Waldron et al. 1999; Zhou et al. 2002). A minor QTL on 6BS (Sumai 3) was also detected in a

population of RILs derived from Sumai 3/Stoa (Waldron et al. 1999). *Fhb1* in Sumai 3 explains 25-60% of the variation in FHB resistance and is the largest QTL for type II resistance identified. Sumai 3 and its derivatives have become widely used as a FHB resistance source in wheat breeding programs (Kolb et al. 2001). Poppenberger et al. (2003) showed that an *Arabidopsis* UDP-glucosyltransferase converts DON to DON-3-glucoside. Lemmens et al. (2005) found that plants carrying the *Fhb1* resistant allele convert DON to DON-3-glucoside, a derivative that exhibits reduced toxicity. Thus, they proposed that *Fhb1* either encodes an enzyme such as UDP-glucosyltransferase to detoxifify deoxynivalenol or a regulator of this enzyme (Lemmens et al. 2005). However, the exact function of *Fhb1* is not known and it has not been cloned yet. Identification of QTL from sources other than Sumai 3 has been reported. A QTL at a similar location on chromosome 3BS, which may be allelic to *Fhb1,* was found in another Chinese wheat cultivar, Wangshuibai (Lin et al. 2004; Ma et al. 2006; Mardi et al. 2005). Wangshuibai also has a high level of resistance to DON accumulation. FHB resistance QTL have been found on chromosome 2B 3B, 4BL and 5A in wheat Ernie, whose resistance appears to differ from that in Sumai 3 (Liu et al. 2007). A QTL on chromosome 5A is frequently detected in resistant wheat cultivars and QTL on chromosomes 2A, 2B, 2D, 3A, 4D, 6B and 7A have also been found in different genetic backgrounds (Buerstmayr et al. 2003; Chen et al. 2007; Cuthbert et al. 2007; Draeger et al. 2007; Jiang et al. 2007; Otto et al. 2002; Somers et al. 2006; Zhou et al. 2002).

Barley. Multiple QTLs for FHB resistance have been identified on all barley chromosomes. The three major FHB resistance QTLs are located on barley chromosome 2H Bin 8, chromosome 2H Bin 10 and chromosome 6H Bin 6. However, each of these

QTL is associated with a morphological or agronomic trait such as heading date (HD), spike type, and grain protein content (GPC) (Canci et al. 2003; Mesfin et al. 2003; See et al. 2002). The close association of the FHB resistance QTL with these traits has delayed the incorporation of these QTL in breeding programs, primarily because it is not known if the association is due to linkage or pleiotropy.

The chromosome 2H Bin 8 FHB resistance QTL (designated Qrgz-2H-8) is associated with heading date (Abramson 1996; Dahleen et al. 2003; de la Peña et al. 1999; Horsley et al. 2006; Mesfin et al. 2003). Resistance alleles at this QTL are late heading, whereas susceptible alleles at this QTL are normal heading. Recently, Nduulu et al. (2007) conducted a fine mapping study and identified a recombinant that exhibited resistance to FHB and normal heading date, indicating that the relationship between FHB and HD in the Qrgz-2H-8 region is likely caused by tight linkage instead of pleiotropy.

The two-rowed spike type has been found associated with FHB resistance in several studies (Chen et al. 1991; Steffenson et al. 1996; Takeda and Heta 1989; Zhou et al. 1991). Genetic mapping showed that the two row/six row (*Vrs1*) spike type is associated with the chromosome 2H Bin 10 FHB resistance QTL (Abramson 1996; de la Peña et al. 1999; Mesfin et al. 2003; Takeda 1990; Zhu et al. 1999). Two-rowed spike type (*Vrs1*) exhibits enhanced resistance compared to six-rowed spike type (*vrs1*). The *Vrs1* locus has been isolated and encodes a transcription factor containing a homeodomain and a leucine zipper motif (Komatsuda et al. 2007). It is not known if the *Vrs1* locus is pleiotropic or genetically linked to FHB resistance.

A QTL for GPC accounting for over 55% of the phenotypic variation was identified on chromosome 6(6H) and was coincident with a major QTL for kernel

discoloration (Canci et al. 2003). Kernel discoloration is related to FHB and *F. graminearum* is one of the causal pathogens. High GPC is associated with FHB resistance. It is not known if the high GPC locus is pleiotropic or linked to FHB resistance.

A QTL for reduced DON accumulation was detected on chromosome 3H Bin 6 (Smith et al. 2004). This QTL was detected in the Fredrickson/Stander population at three days after inoculation. Interestingly, the Stander (FHB susceptible parent) allele exhibited low DON accumulation. The chromosome 3H Bin 6 QTL explained 18-35% of the phenotypic variation.

The genetic variation for FHB resistance in wheat and barley germplasm may not be sufficient to develop cultivars by traditional breeding. Therefore, genetic engineering provides an additional approach to improve FHB resistance (Dahleen et al. 2001). Transgenic wheat lines exhibited improved resistance to *F. graminearum* by expressing transgenes encoding β-1,3-glucanase, class II chitinase, ribosome-inactivating protein, thaumautin like protein 1, α-1-purothionin, and Arabidopsis NPR1 (Balconi et al. 2007; Chen et al. 1999; Mackintosh et al. 2007; Makandar et al. 2006; Shin et al. 2008). Transgenic barley expressing *Tri101* did not reduce FHB or DON levels (Manoharan et al. 2006).

Wheat and barley responses to FHB

Wheat responses to *F. graminearum* infection have been studied previously (Bernardo et al. 2007; Golkari et al. 2007; Hill-Ambroz et al. 2006; Jansen et al. 2005; Kong et al. 2005; Kong et al. 2007; Kruger et al. 2002; Pritsch et al. 2001; Steiner et al.

2009; Yu and Muehlbauer 2001; Zhou et al. 2005). Spikes respond to infection by inducing a variety of defense responses. Multiple studies have shown that *F. graminearum* infection induces transcript accumulation of several classes of biotic and abiotic stress-related genes in both resistant and susceptible cultivars (Hill-Ambroz et al. 2006; Kong et al. 2005; Pritsch et al. 2000; Pritsch et al. 2001; Yu and Muehlbauer 2001; Zhou et al. 2005). Hill-Ambroz et al. (2006) conducted cDNA macroarray analysis and detected 75 transcripts that were induced in the first 24h after *F. graminearum* inoculation. Two of the induced genes were mapped to wheat chromosomes 3AS and 6BL, both regions contain major QTL for FHB resistance. Bernardo et al. (2007) used a cDNA array with 4,806 clones to study the *F. graminearum*-induced changes in gene expression between unrelated FHB- resistant and susceptible wheat cultivars. Three genes with unknown function were found to be up-regulated in FHB-resistant cv. Ning 7840 at most time points. Recently, Steiner et al. (2009) reported differential gene expression of related wheat lines with contrasting levels of head blight resistance after *F. graminearum* inoculation. Transcript-derived fragments differentially expressed between the sister lines displayed homology to a UDP-glucosyltransferase, phenylalanine ammonia-lyase, DNA-J like protein, pathogenesis-related family protein and one unknown protein (Steiner et al. 2009).

Although there have been multiple studies investigating the wheat response to *F. graminearum* infection, an understanding of the differences and similarities between the resistant and susceptible interactions is largely unknown. Transcripts encoding a bZIP transcription factor were found upregulated in DON-treated plants segregating for the *Fhb1* resistant allele (Ansari et al. 2007). Additionally, reactive oxygen species and DNA

laddering were produced on DON-inoculated wheat stems, which were characteristic of programmed cell death (PCD) responses (Desmond et al. 2008). Although there have been multiple studies investigating the wheat response to *F. graminearum* infection and DON inoculation, a genome-wide examination of the wheat response to *F. graminearum* infection and comparison between near-isogenic lines carrying contrasting alleles for *Fhb1* have not been studied.

More is known about the overall barley response to *F. graminearum* infection (Boddu et al. 2006). Using the Barley1 Affymetrix GeneChip®, Boddu et al. (2006) examined the host response in the susceptible cultivar Morex to *F. graminearum* infection. Four hundred and sixty-seven differentially accumulating transcripts were detected in *F. graminerum*-treated plants compared with the water control. A variety of genes encoding defense response proteins, oxidative stress response proteins, proteases, predicted trichothecene detoxifying enzymes and transporters, and tryptophan biosynthetic and catabolic enzymes were identified. Based on the histology of infection, DON levels and transcript accumulation data, three stages of *F. graminearum* infection were defined. In the early stage (0 to 48 hai), limited fungal development and low DON concentration were found along with little change in transcript accumulation. The characteristics of the intermediate stage (48 to 96 hai) were increased fungal development and active infection, higher DON concentration, and increased transcript accumulation. The late stage (96 to 144 hai) exhibited hyphal mat development, higher DON accumulation, and a slight decrease in the number of transcripts observed. Finally, the GeneChip® data generated in this study provide a baseline dataset in barley during *F. graminearum* infection. Boddu et al. (2007) also examined the impact of *F.*

graminearum-derived trichothecenes on virulence and transcriptome difference on barley spikes inoculated with wild type (trichothecene producing) and *tri5* mutant (trichothecene nonproducing) *F. graminearum* strains. They found that barley exhibited specific responses to trichothecene accumulation. Two opposing host responses were proposed. One class of induced genes encoded predicted trichothecene-detoxifying enzymes and transporters that may reduce the impact of trichothecenes, and the other class encoded proteins related to cell death and ubiquitination that may promote establishment of the disease. As in the wheat-*F. graminearum* interaction, little is known about the difference between the resistant and susceptible barley-*F. graminearum* interactions. In addition, little is known about the similarities and differences between the host responses in barley and wheat.

RNA profiling using microarrays

RNA profiling using microarray technology allows large-scale examination of temporal and spatial transcript accumulation. Affymetrix GeneChip® Wheat and Barley Genome Arrays can simultaneously analyze 55,052 and 22,439 transcripts, respectively (Close et al. 2004; Close 2005). Affymetrix GeneChip® technology can be used to profile gene expression patterns in resistant and susceptible wheat and barley genotypes during *F. graminearum* infection and to identify genes that are differentially expressed during infection. The wheat and barley arrays are useful to study host-pathogen interactions and to define the host response in FHB-resistant and susceptible genotypes. It offers the most comprehensive and informative platform for wheat and barley gene expression research. The wheat and barley GeneChips have been used to examine transcript accumulation

during pathogen infection (Boddu et al., 2006; Bolton et al., 2008; Caldo et al., 2006; Coram et al., 2007; Coram et al., 2008a, b, c; Zhang et al., 2006), abiotic stress (Svensson et al., 2006) and in wheat-barley addition lines (Bilgic et al., 2007; Cho et al., 2006).

CHAPTER 2

TRANSCRIPTOME ANALYSIS OF A WHEAT NEAR-ISOGENIC LINE PAIR CARRYING FUSARIUM HEAD BLIGHT RESISTANT AND SUSCEPTIBLE ALLELES

INTRODUCTION

Fusarium head blight (FHB) or scab, caused primarily by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch], is an economically damaging disease on wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in North America and worldwide (McMullen et al. 1997; Parry 1995). From 1991 to 1997, the estimated loss due to FHB was about $1.3 billion in the United States (Johnson et al. 2003). Disease develops in the spike resulting in low-weight kernels and reduced yield. FHB not only decreases grain yield but also lowers grain quality by accumulating trichothecene mycotoxins such as deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), and 3-acetyldeoxynivalenol (3-ADON), which are harmful to humans and animals (Desjardins and Hohn 1997; Desjardins 2006).

F. graminearum invades wheat spikes and causes FHB through a series of complex pathways and mechanisms (Bushnell et al. 2003). The fungus can enter through the stomatal pores, or grow between or directly through the floral bracts. *F. culmorum* (a *Fusarium* species that can also cause FHB*)* infection of wheat results in cell wall degradation, and barley infected by *F. graminearum* exhibits the accumulation of fungal gene transcripts that encode cell wall-degrading enzymes (Kang and Buchenauer 2000a); (Guldener et al. 2006). These results indicate that cell wall-degrading enzymes are an important component of infection. Also during infection, fungal gene transcripts encoding trichothecene biosynthetic enzymes are detected (Guldener et al. 2006) and trichothecene mycotoxins are produced that inhibit protein synthesis and increase virulence (Desjardins and Hohn 1997; Proctor et al. 1995). Finally, the fungus colonizes the ovary and floral bracts, and the infected tissue becomes necrotic and bleached

(Leonard and Bushnell 2003; Pritsch et al. 2000).

Resistance to FHB in wheat is primarily defined by type I (resistance to initial infection) and type II (resistance to spread of the disease within a spike) resistance (Mesterhazy 1995). type I and II resistance have been identified in several wheat genotypes and used in breeding programs to increase resistance (Bai and Shaner 2004). Interestingly, barley, including susceptible genotypes, exhibits natural type II resistance. Thus, comparing the responses in wheat and barley to *F. graminearum* infection may reveal the mechanisms of type II resistance and a greater understanding of the wheat-*F. graminearum* interaction.

To identify regions of the wheat genome that control FHB resistance, multiple QTL mapping studies have been conducted. Mapping studies with the type II resistant cultivar Sumai 3 and its derivatives have identified a major QTL on chromosome 3BS referred to as *Fhb1* (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002; Liu et al. 2006; Waldron et al. 1999; Zhou et al. 2002). The Sumai 3-derived *Fhb1* allele explains 20-60% of the variation for FHB resistance (Pumphrey et al. 2007). Lemmens et al. (2005) showed that plants carrying the *Fhb1* resistant allele converts DON to DON-3-glucoside, a derivative that exhibits reduced toxicity. Poppenberger et al. (2003) showed that an *Arabidopsis* UDP-glucosyltransferase converts DON to DON-3-glucoside. Thus, Lemmens et al. (2005) proposed that the wheat *Fhb1* locus encodes a DON-glucosyltransferase or alternatively a gene that regulates a DON-glucosyltransferase.

Wheat responds to *F. graminearum* infection by inducing several classes of biotic and abiotic stress response genes (Ansari et al. 2007; Bernardo et al. 2007; Golkari et al. 2007; Hill-Ambroz et al. 2006; Jansen et al. 2005; Kong et al. 2005; Pritsch et al. 2000;

Pritsch et al. 2001; Zhou et al. 2005). Ansari et al. (2007) showed that transcripts encoding a bZIP transcription factor were upregulated in DON-treated plants segregating for the *Fhb1* resistant allele. Steiner et al. (2009) used genetic stocks carrying resistant and susceptible alleles at the *Fhb1* locus and another QTL for FHB resistance on chromosome 5A and found 14 genes differential expressed between the resistant and susceptible genotypes. In addition, DON inoculation of wheat stems revealed the production of reactive oxygen species and DNA laddering, both characteristic of programmed cell death (PCD) responses (Desmond et al. 2008). Although multiple studies investigating the wheat response to *F. graminearum* infection and DON inoculation have been conducted, a genome-wide assessment of the wheat response to *F. graminearum* infection, and comparisons between near-isogenic lines (NILs) carrying the resistant and susceptible *Fhb1* alleles have not been conducted.

More is known about the overall barley response to *F. graminearum* infection. Using the Barley1 Affymetrix GeneChip probe array, which represents 22,349 barley genes (Close et al. 2004), the host response in barley to *F. graminearum* infection was examined (Boddu et al. 2006). Four hundred and sixty-seven gene transcripts responded to *F. graminerum* infection and the gene expression patterns defined early, intermediate and late stages of infection in barley. The greatest number of host transcripts and initial DON accumulation were observed at the intermediate stage, indicating that trichothecene accumulation plays an important role in inducing the host response. The Barley1 GeneChip was also used to examine transcript accumulation in barley after infection with trichothecene-producing and -nonproducing strains of *F. graminearum* (Boddu et al. 2007). Genes specifically responding to trichothecene accumulation were detected and

two opposing host responses were proposed. One class of induced genes encoded trichothecene-detoxifying enzymes and transporters that may reduce the impact of trichothecenes, and the other class encoded proteins related to cell death and ubiquitination that may promote establishment of the disease.

In this study, we used the Affymetrix Wheat GeneChip probe array to examine the transcript accumulation in a NIL pair carrying resistant and susceptible alleles at the wheat *Fhb1* locus during *F. graminearum* infection. We also studied the disease phenotypes in this NIL pair. The objectives of this study were: (1) to examine the relationship between DON accumulation, fungal biomass and disease severity in a wheat NIL pair carrying the *Fhb1* resistant and susceptible alleles; (2) to describe transcriptome changes during the wheat-*F. graminearum* interaction; (3) to identify genes that define the *Fhb1* resistant and susceptible phenotype; and (4) to develop an integrated model of the wheat and barley host response to *F. graminearum* infection.

RESULTS

Disease severity, DON accumulation, and fungal biomass in a wheat near-isogenic line pair carrying the *Fhb1* resistant and susceptible alleles

Disease severity on a wheat near-isogenic line (NIL) pair carrying either the *Fhb1* resistant or susceptible allele was assessed at 21 days after point inoculation with *F. graminearum*. The difference in percent disease severity was statistically significant ($P \leq 0.0001$) between the two NILs carrying the resistant (21 +/- 4.0) and susceptible (52 +/- 13) alleles (Figure 1A). On the *Fhb1*+ NIL, disease symptoms were restricted to the initially-inoculated spikelets and did not spread to the adjacent spikelets to the same extent as the *Fhb1*- NIL. In contrast, on the *Fhb1*- NIL the disease symptoms spread to the adjacent spikelets. At 48 hours after inoculation (hai), the inoculated spikelets on both the resistant and susceptible NILs showed necrotic tissue (Figure 1B) and by 96 hai showed bleaching (Figure 1C). For both timepoints, the susceptible NIL exhibited more severe disease phenotypes.

The accumulation of DON (Table 1) and ergosterol (an indicator of fungal biomass) (Table 1) in the point-inoculated and non-inoculated wheat spikelets of the NIL pair was quantified at 48 and 96 hours after *F. graminearum* and water inoculation. DON concentration increased in *F. graminearum*-inoculated wheat spikelets at 96 hai compared to 48 hai in both the resistant and susceptible NILs. However, the difference between the resistant and susceptible NILs was not significant at either time point ($P<0.01$). Ergosterol concentration also increased in *F. graminearum*-inoculated wheat spikelets at 96 hai compared to 48 hai. The difference between the resistant and susceptible NILs was not significant ($P<0.01$). We did not detect DON or ergosterol in the water-

inoculated spikelets. Non-inoculated wheat spikelets adjacent to the point-inoculation sites were also sampled at 48 and 96 hai and assayed for DON and ergosterol concentration (Table 1). We did not detect DON or ergosterol in the non-inoculated spikelets adjacent to the *F. graminearum*- or water-inoculated spikelets in the resistant NIL at either time point. However, in the susceptible NIL, we detected low amounts of DON and ergosterol at both time points in the non-inoculated spikelets adjacent to the *F. graminearum*-inoculated spikelets.

Wheat host response to *F. graminearum* infection

The Affymetrix Wheat GeneChip was used to examine transcript accumulation in an NIL pair carrying either the *Fhb1*-resistant or -susceptible allele at 48 and 96 hours after point inoculation with *F. graminearum* or a mock water control. We first examined the number of transcripts exhibiting differential accumulation between *F. graminearum* and mock water inoculated plants ($P \leq 0.0001$, ≥ 2.0 fold). By summing the unique transcripts, we detected 2,141 transcripts exhibiting increased accumulation after *F. graminearum* infection at 48 and/or 96 hai (Figure 2A). Of these 2,141 transcripts, 1,904 and 2,065 were detected in the resistant and susceptible genotypes, respectively (Figure 2A). Three groups were detected: (a) 1,828 transcripts in both the resistant and susceptible genotypes (Supplementary Table 1); (b) 76 transcripts only in the resistant genotype (Supplementary Table 2); and (c) 237 transcripts only in the susceptible genotype (Supplementary Table 3). More transcripts were detected in the resistant genotype (1,618) than the susceptible genotype (1,370) at 48 hai. However, at 96 hai more transcripts were detected in the susceptible genotype (2,018) than the resistant

genotype (1,638).

We also detected 2,847 unique transcripts exhibiting decreased accumulation after *F. graminearum* inoculation at 48 and/or 96 hai. Of these 2,847 transcripts, 1,990 and 2,637 were detected in the resistant and susceptible genotypes, respectively (Figure 2B). Three groups were identified including: (a) 1,780 transcripts in both the resistant and susceptible genotypes (Supplementary Table 4); (b) 210 transcripts only in the resistant genotype (Supplementary Table 5); and (c) 857 transcripts only in the susceptible genotype (Supplementary Table 6). More gene transcripts exhibited decreased accumulation in the resistant genotype (774) than the susceptible genotype (171) at 48 hai. However, at 96 hai more genes exhibited decreased accumulation in the susceptible genotype (2,630) than the resistant genotype (1,684).

Wheat gene annotations

Annotations were assigned to all of the genes that exhibited differential transcript accumulation between *F. graminearum* and mock water inoculation and placed into broad classes including: cell death related, cell wall related, chromatin related, defense response, hormone-related, membrane related, metabolism, miscellaneous, photosystem related, regulatory, ribosomal protein, stress response, transporter, putative trichothecene detoxification and transport, ubiquitination, and unknown (Table 2; Supplemental Tables 1, 2, 3, 4, 5, and 6). There were a variety of genes within the general broad classes that exhibited either increased or decreased transcript accumulation in *F. graminearum* infected plants in both genotypes at 48 and/or 96 hai (Table 2). These genes were not identified by the same probeset but were given a similar annotation. Examples of these

genes encode proteins with the following general functions including: defense response (e.g., pathogenesis-related, phenylpropanoid pathway enzymes, oxidative burst related, cytochrome P450s) and metabolism (e.g., tryptophan biosynthesis). We examined the gene transcripts that exhibited increased or decreased transcript accumulation (Supplemental Tables 1, 2, 3, 4, 5, and 6) with regard to what is known about host responses in the wheat/barley-*F. graminearum* interaction, other plant-pathogen interactions, and the disease symptoms we observed during *F. graminearum* infection of wheat (Figure 3; Supplemental Tables 7 and 8).

Several plant hormones including jasmonic acid (JA) and ethylene (ET) are involved in plant–pathogen interactions (Glazebrook 2005; Spoel et al. 2007). Thus, we examined our data for gene transcripts encoding JA- and ET-related proteins that exhibited differential accumulation in *F. graminearum* inoculated compared to mock water control inoculated plants in both genotypes. We identified 14 transcripts exhibiting increased accumulation that either encode JA biosynthetic enzymes (e.g., 12-oxo-phytodienoic acid reductase) or respond to JA (e.g., jasmonate-induced protein) (Figure 3A). Eight transcripts encoding ethylene-induced esterase exhibited increased accumulation after *F. graminearum* infection (Figure 3A). These results indicate that the JA and ET signaling pathways are functioning during the wheat-*F. graminearum* interaction.

Trichothecenes have been shown to be virulence factors in wheat and barley (Proctor et al. 1995). Two classes of genes have been shown to reduce the impact of trichothecenes including: UDP-glucosyltransferases (Poppenberger et al. 2003) that detoxify trichothecenes, and ABC transporters (Muhitch et al. 2000) that transport

trichothecenes out of the cytoplasm. We detected 12 gene transcripts encoding ABC transporters and 16 gene transcripts encoding UDP-glucosyltransferases that were induced in both genotypes during infection (Figure 3A). We only detected one ABC transporter and four UDP-glucosyltransferases that were repressed in both genotypes. These data indicate that wheat is responding to trichothecene accumulation by expressing transcripts encoding putative trichothecene detoxifying and transport functions.

We also examined our data for gene transcripts encoding transcription factors. The Arabidopsis NF-X1 transciption factor has been shown to be induced upon treatment of T-2 toxin, a trichothecene mycotoxin, and that it functions as a negative regulator of trichothecene-induced defense responses (Masuda et al. 2007; Asano et al. 2007). We identified two gene transcripts that encode NF-X1-like transcription factors that exhibited increased accumulation (Figure 3A). We also identified thirteen transcripts encoding WRKY transcription factors in both genotypes (Figure 3A).

Cell walls are one form of defense against fungal pathogens and xylan is a primary component of grass cell walls (Carpita and Gibeaut 1993). To infect plants, pathogens such as *F. graminearum* appear to use xylanases to degrade xylans. During *F. graminearum* infection of barley, fungal gene transcripts encoding xylanases were detected (Guldener et al. 2006). Thus, we searched our data for xylanase inhibitors and found increased accumulation of 10 gene transcripts encoding xylanase inhibitors in the resistant and susceptible wheat genotypes during infection (Figure 3A). Interestingly, we also found 13 transcripts that were repressed that encode proline-rich proteins that comprise a major component of the plant cell wall (Figure 3B). These results indicate that the cell wall is a primary point of interaction between wheat and *F. graminearum*.

Previous studies have suggested that PCD occurs during *F. graminearum* infection of barley and wheat (Boddu et al. 2007; Desmond et al. 2008). Thus, we examined our data for gene transcripts that encode proteins related to PCD. Nine PCD-related gene transcripts were found up-regulated in both genotypes after *F. graminearum* inoculation (Figure 3A). Three, one and one transcripts encode homologs to the rice *SPOTTED LEAF11* and *SPOTTED LEAF7* genes, and tomato pirin gene, respectively. The other four transcripts encode senescence-associated proteins. These genes have been implicated in PCD responses in various plant-pathogen interactions (Zeng et al. 2004; Yamanouchi et al. 2002; Kojo et al. 2006; Orzaez et al. 2001). PCD is also characterized by chromatin degradation and H_2O_2 production, and both have been found in the wheat stems inoculated with DON (Desmond et al. 2008). We also found 70 chromatin-related genes encoding histones (histone H1, H2A, and H2B, centromeric histone 3, and histone deacetylase 2) that exhibited decreased accumulation after *F. graminearum* inoculation, indicating a reduction in chromatin function (Figure 3B). These results indicate that PCD is functioning during the wheat-*F. graminearum* interaction.

By 96 hai, we observed necrosis and bleaching symptoms on the spikelets of both resistant and susceptible genotypes, indicating a reduction in overall plant biochemical and cellular activity and structure. Corresponding with these phenotypes is the reduced accumulation of 27 gene transcripts encoding photosystem-related functions (e.g., chlorophyll a-b binding protein, photosystem I reaction center subunits, photosystem II reaction center protein, and photosystem II 10 kDa polypeptides) (Figure 3B). We also identified 41 ribosomal gene transcripts that exhibited reduced accumulation in both resistant and susceptible genotypes, only four ribosomal gene transcripts were increased

(Figure 3B). Moreover, we identified 12 and 10 transcripts encoding tubulins and microtubule-associated proteins that were repressed in both genotypes (Figure 3B). The reduction in these transcripts demonstrates the impact *F. graminearum* infection has on basic cellular functions.

Differential response of wheat NILs carrying either the *Fhb1* resistant or susceptible allele to *F. graminearum* infection

We compared the transcript profiles of the resistant and susceptible genotypes during *F. graminearum* infection and mock water inoculation ($P \leq 0.0001$; $\geq$ 2-fold). Twenty-seven transcripts were differentially expressed between the resistant and susceptible genotypes during *F. graminearum* and/or water inoculation (Table 3). Based on the map locations of previously mapped ESTs displayed on the Graingenes website (http://wheat.pw.usda.gov/GG2/index.shtml), the genetic location of 12 of these genes was identified (Table 3). If a transcript contains a single feature polymorphism (SFP), it affects its affinity to the target probe on the GeneChip and may obscure the transcript accumulation value. Of the 27 transcripts, we detected 13 with at least one SFP. It is possible that those transcripts exhibiting SFPs may not be true transcript accumulation differences between the resistant and susceptible genotypes. Subtracting the transcripts with SFPs, ten transcripts exhibited an increase in the resistant genotype compared to the susceptible genotype and four transcripts exhibited a decrease in the resistant genotype compared to the susceptible genotypes (Table 3). The ten transcripts showing increased accumulation in the resistant genotype encode a proline-rich protein, expansin, NB-ARC, putative band 7 protein, Bowman-Birk trypsin inhibitor and five unknown proteins. The

four transcripts showing decreased accumulation in the resistant genotype encode a histidine-rich Ca^{2+} binding protein and three unknown proteins. In total, 10 out of 14 of the transcripts that exhibited a difference between the resistant and susceptible genotypes during *F. graminearum* infection also exhibited a difference in the mock water control, indicating that the expression of these genes was independent of *F. graminearum* infection. The remaining four transcripts (Ta.12536.1.S1_at: putative Band 7 protein, Ta.14246.1.S1_at: unknown, Ta.28760.1.S1_a_at: unknown, and TaAffx.111425.2.S1_at: unknown) were only detected in *F. graminearum*-inoculated plants, indicating that they are dependent upon *F. graminearum* infection. The transcript accumulation differences for these four genes was validated by performing real-time RT-PCR on RNA isolated from the resistant and susceptible genotype infected with *F. graminearum* (Figure 4).

DISCUSSION

We examined transcript accumulation and disease phenotypes in a wheat NIL pair carrying resistant and susceptible alleles at the *Fhb1* locus during *F. graminearum* and water inoculation. The *Fhb1* locus is the largest effect QTL identified in wheat for type II FHB resistance (Waldron et al. 1999). Lemmens et al. (2005) proposed that *Fhb1* encodes a DON-glucosyltransferase that functions to detoxify DON by converting it to DON-3-glucoside, or alternatively *Fhb1* acts as a regulatory gene that controls the expression of a DON-glucosyltransferase. However, the exact function of *Fhb1* is not known and it has not been cloned yet. Using the NIL pair for *Fhb1* allowed us to minimize the genetic background effect and examine disease phenotypes and transcript accumulation patterns mainly caused by resistant and susceptible alleles at the *Fhb1* locus during *F. graminearum* infection. This study represents a comprehensive examination of the wheat transcriptome during *F. graminearum* infection, a comparison of the response to *F. graminearum* infection in plants carrying the resistant and susceptible alleles at the *Fhb1* locus, and an opportunity to develop an integrated model for wheat and barley during *F. graminearum* infection.

Fhb1 restricts spread of disease symptoms but does not provide resistance to initial infection

In our study, the percent FHB disease severity was significantly different between the plants carrying the resistant or susceptible allele at the *Fhb1* locus. In the resistant NIL, disease symptoms were restricted to the initially inoculated spikelets and did not spread to the adjacent spikelets over time, confirming type II resistance conferred by the

resistant *Fhb1* allele. Spread of disease symptoms was observed in the susceptible genotype. These results are consistent with published data on *Fhb1* (Liu et al. 2006). However, we did not observe a significant difference in DON concentration in inoculated spikelets in the resistant genotype compared to the susceptible genotype. In contrast, Lemmens et al. (2005) reported that the wheat *Fhb1* resistant allele reduced the concentration of DON by conjugating it to glucose and forming DON-3-glucoside, a derivative of DON that exhibits a reduction in toxicity. These authors inoculated spikes of plants carrying the resistant and susceptible alleles for *Fhb1* with purified DON and sampled them at 7 to 16 days, providing an opportunity for the plants carrying the *Fhb1* resistance allele to detoxify DON. In our study, we point-inoculated spikes of plants carrying the resistant or susceptible *Fhb1* allele with *F. graminearum* and sampled the inoculated spikelets at 48 and 96 hai, possibly not providing the time necessary for the plants carrying the *Fhb1* resistant allele to convert DON to DON-3-glucoside. We also observed DON accumulation and fungal biomass in the non-inoculated spikelets in the susceptible genotype but not in the resistant genotype. Pritsch et al. (2001) showed that the fungus did not grow to adjacent non-inoculated spikelets in Sumai3 and Wheaton (a highly susceptible genotype) within 48 hai. Our results indicate that between 48 and 96 hai the fungus infects adjacent non-inoculated spikelets in the susceptible genotype. Taken together, our results showed that point-inoculated plants carrying the *Fhb1* resistant allele exhibited reduced disease severity in the whole spike at maturity, but did not result in reduced DON concentration or fungal biomass in the inoculated spikelets during the early stages of infection.

Dynamics of gene expression during the wheat-*F. graminearum* interaction

Overall, our results show that the number of gene transcripts exhibiting higher accumulation was moderately increased between 48 and 96 hai, while there was a large increase in the number of transcripts that showed decreased accumulation between 48 and 96 hai. Thus, our results reveal a general trend that wheat tissues induce a high number of gene transcripts during the early stages of infection and an equivalent number of gene transcripts that are repressed later in disease development. Interestingly, members of many gene classes were induced and repressed (Table 2). Previous studies of the host response in wheat and barley to *F. graminearum* infection did not identify many repressed genes. In two studies, three and 16 transcripts exhibited less accumulation in barley after *F. graminearum* inoculation (Boddu et al. 2006; Boddu et al. 2007), and 16 transcripts exhibited reduced accumulation in wheat after *F. graminearum* infection (Golkari et al. 2007). A potential explanation for the distinct results in the previous studies and the one reported here are the different inoculation and sampling methods. In the barley studies (Boddu et al. 2006; Boddu et al. 2007), spikes were spray- or dip-inoculated and whole spikes were used for the transcriptome analysis, providing a mixture of infected and uninfected tissues. In the current study, only the central four wheat spikelets were inoculated and sampled for gene expression analysis, resulting in samples with little healthy tissue. The decrease in the amount of healthy tissue used for the RNA preparations in the current study likely resulted in the increased number of transcripts exhibiting decreased accumulation. In the Golkari et al. (2007) study, only the 24 hai timepoint was sampled. In our study, the majority of the genes that exhibited reduced transcript accumulation were found at the 96 hai timepoint, indicating that the

timing of reduced transcript accumulation occurs later in disease development.

The wheat/barley-*F. graminearum* interaction

Wheat and barley are members of the Triticeae family, are highly related and are hosts of *F. graminearum.* The initial *F. graminearum* growth and infection pathways that eventually result in tissue necrosis are similar in barley and wheat (Figure 5; Pritsch et al. 2000; Boddu et al. 2006; 2007). However, they exhibit different resistance phenotypes. Barley exhibits limited tissue bleaching and a natural type II resistance (resistance to spread of the disease in the spike), even in susceptible genotypes (Boddu et al. 2007). In contrast, infected wheat tissues become bleached, even in type II resistant genotypes, and susceptible genotypes exhibit spread of the disease symptoms. Thus, a comparison of host response in wheat and barley to *F. graminearum* infection may provide a greater understanding of the wheat/barley-*F. graminearum* interaction.

In our previous studies examining gene expression in barley during *F. graminearum* infection, a model for the barley-*F. graminearum* interaction was proposed (Boddu et al. 2006; Boddu et al. 2007). The basic model states that the interaction is characterized by early (0-48 hai), intermediate (48-96 hai) and late (96-144 hai) stages. We noted that the host response was greatest (largest number of transcripts detected) in the intermediate stage coincident with the initial analytical detection of DON. In addition, we examined fungal gene expression during *F. graminearum* infection of barley and found accumulation of transcripts encoding cell-, cuticle-, and membrane-degrading enzymes, and trichothecene biosynthetic enzymes (Guldener et al. 2006). The majority of these fungal gene products were first observed during the intermediate stage of

infection, indicating that the ability to degrade the cuticle, cell walls and membranes, and synthesize trichothecenes likely aids the fungus in initial infection and colonization of barley spikes. Two general host responses to trichothecene accumulation were proposed (Boddu et al. 2007). One response is to induce genes that encode trichothecene detoxifying and transport functions, providing protection to the toxic effects of trichothecenes. The other general response is to induce genes that are diagnostic for PCD. Thus, PCD likely occurs during *F. graminearum* infection and may result in increased susceptibility due to the necrotrophic phase of *F. graminearum* infection.

We compared the host response in wheat to *F. graminearum* infection (this study) to the host response in barley to *F. graminearum* infection (Boddu et al. 2006; 2007). The differences in inoculation and sampling between wheat and barley will undoubtedly cause differences in the host responses. However, our primary goal was to determine the conserved responses, with a secondary goal of identifying differences. Our approach was to compare the annotations of the gene transcripts detected in wheat and barley during *F. graminearum* infection. The basic model of the barley-*F. graminearum* interaction is supported, with some differences, by the genome-wide gene expression profiles observed in the wheat-*F. graminearum* interaction. In the wheat study presented here, we only examined 48 and 96 hai and precisely placing the events on a timeline of infection was complicated by the lack of multiple timepoints and the integration of the timing of wheat host responses to barley host responses. Nevertheless, we identified a set of host responses that are conserved in barley and wheat during *F. graminearum* infection (Figure 5).

In addition to the standard defense responses (e.g., pathogenesis-related proteins,

oxidative burst, phenyl propanoid pathway genes) observed in most plant-pathogen interactions and in wheat/barley-*F. graminearum* interactions, wheat and barley exhibit several specialized responses to *F. graminearum* infection (Figure 5). Similar to barley, wheat responds to initial infection by inducing the expression of xylanase inhibitors to reduce the impact on cell wall integrity of fungal-derived xylanases and possibly other cell wall, cuticle and membrane degrading enzymes. In addition, wheat and barley respond to infection by inducing the expression of UDP-glucosyltransferases and ABC transporters that may reduce the impact of trichothecene mycotoxins. In Arabidopsis, UDP-glucosyltransferases have been shown to convert DON to DON-3-glucoside, a less toxic derivative (Poppenberger et al. 2003). In tobacco, overexpression of an ABC transporter resulted in enhanced resistance to trichothecenes (Jasinski et al. 2003; Sanchez-Fernandez et al. 2001). Handa et al (2008) proposed that an ABC transporter is a candidate gene for the wheat QTL for FHB resistance located on chromosome 2D. Based on the production of H_2O_2 and DNA laddering in wheat inoculated with DON and the induction of PCD-related genes in barley inoculated with *F. graminearum*, PCD has been proposed (Desmond et al. 2008; Boddu et al. 2007). In our current study, we detected wheat gene transcripts (e.g., pirin) that are diagnostic for PCD related processes. We also detected a set of regulatory genes encoding NF-X1 and WRKY transcription factors in wheat and barley. In Arabidopsis, Asano et al. (2007) showed that NF-X1 is a negative regulator of trichothecene-induced defense responses. Thus, the NF-X1 genes likely play a role in the defense response to trichothecene accumulation during *F. graminearum* infection. WRKY transcription factors were identified in both wheat and barley and are genes known to play a role in biotic and abiotic stress responses (Liu et al.

2007; Mare et al. 2004; Shimono et al. 2007; Wei et al. 2008) and likely regulate the response to *F. graminearum* infection. Furthermore, both wheat and barley induce genes that are involved with ethylene and jasmonic acid biosynthesis and/or responses. Jasmonic acid and ethylene are usually associated with defense against necrotrophic pathogen invasion and cross-talk between the ethylene and jasmonic acid signaling pathways was shown to maximize the host response to the pathogen (Glazebrook 2005; Spoel et al., 2007). Our results indicate that the similar transcript accumulation patterns may relate to the events leading up to necrosis and cell death that are common in both wheat and barley (Figure 5).

Although many of the induced genes are conserved between wheat and barley, in wheat we observed the repression of a large set of genes encoding chromatin functions (e.g., histone H1, H2A, and H2B, centromeric histone 3, and histone deacetylase 2), ribosomal proteins, tubulin proteins, microtubule-associated proteins, proline-rich proteins, and photosynthetic activities (e.g., chlorophyll a-b binding protein, photosystem I reaction center subunits, photosystem II reaction center subunits, and photosystem II 10 kDa polypeptides) (Figure 5). Trichothecenes were shown to interact with the peptidyl transferase site of ribosomal proteins, resulting in inhibition of translation (McLaughlin et al. 1977). Although we do not know if repression of ribosomal transcripts is due to the direct action of trichothecenes, our results show that during the wheat-*F. graminearum* interaction genes encoding ribosomal proteins are repressed. Our results also show that genes involved with photosynthesis function are repressed, indicating that chloroplast function is reduced during infection. Indeed, chloroplast degradation has also been linked to PCD during plant-pathogen interactions (Genoud et al. 2002; Williams and

Dickman 2008). Thus, our results also indicate that PCD and chloroplast function are related in the wheat-*F. graminearum* interaction. The repression of tubulin, microtubule-associated protein and proline-rich protein genes, indicates degradation of plant cellular structures. Compared to barley which exhibits limited tissue bleaching, infected wheat tissues become completely bleached, likely leading to the repression of photosynthesis-related genes and other genes required for cellular structure, protein translation and chromatin integrity. Thus, these gene transcripts are likely repressed during tissue bleaching in wheat and they are not repressed in barley due to the limited amount of bleaching (Figure 5). It is possible that the different inoculation and sampling methods between wheat and barley resulted in the different responses. However, it is interesting that the wheat host responses are consistent with the observed disease phenotypes. Taken together, our study revealed many similarities but some important differences in wheat and barley infected with *F. graminearum* and highlighted the utility of examining the response in two hosts to infection by a single pathogen.

Host responses differing between plants carrying the resistant and susceptible *Fhb1* allele

The use of an NIL pair carrying the resistant and susceptible allele for the *Fhb1* locus in this study provided the opportunity to identify genes that exhibited differential transcript accumulation in the resistant or susceptible genotype and provided the opportunity to identify genetic differences between the genotypes. We identified 14 gene transcripts that exhibited differential accumulation between the plants carrying the resistant and susceptible alleles for *Fhb1* and 13 additional transcripts that contained

SFPs. One of the transcripts that exhibited differential accumulation, Ta.20928.1.S1_at encodes a Bowman-Birk type trypsin inhibitor and was previously mapped as an EST to chromosome 3BS (Table 3; http://wheat.pw.usda.gov/GG2/index.shtml). Of the 13 gene transcripts that exhibited SFPs, five were previously mapped as ESTs to chromosome 3BS. These results demonstrate the utility of GeneChip technology to identify genetic and gene expression differences between genotypes.

DON and ergosterol accumulation in the inoculated wheat spikelets on the resistant and susceptible NILs were not quantitatively different ($P<0.01$) at either 48 or 96 hai. Thus, by analyzing the transcriptome profiles in just the inoculated spikelets, we are likely not able to detect all the genes that were differentially expressed between the genotypes. Nonetheless, our results showed that the resistant genotype exhibited greater numbers of transcripts that exhibited increased and decreased accumulation at 48 hai (Figure 2). We found more gene transcripts at 96 hai in the susceptible genotype. Overall, these results indicate that the resistant genotype responds early after infection, whereas the susceptible genotype exhibits a slower but eventually greater response to infection. This overall response may play a role in the different phenotypes observed in plants carrying the resistant and susceptible *Fhb1* allele (Figure 5).

We also compared our transcript accumulation results to other groups that have studied the wheat-*F. graminearum* interaction. Bernardo et al. (2007) examined resistant and susceptible genotypes during *F. graminearum* infection and identified three genes with unknown function that exhibited transcript accumulation differences; however, the genotypes were not isogenic so a direct comparison was difficult. Ansari et al. (2007) showed that the expression of a bZIP transcription factor exhibited differential

accumulation in plants carrying resistant and susceptible alleles for the *Fhb1* locus and may play a role in *Fhb1*-related defense responses. Steiner et al. (2009) identified 14 gene transcripts that exhibited accumulation differences between resistant and susceptible genotypes. Based on the annotations, there was no overlap between genes that exhibited differential expression between the resistant and susceptible genotypes in the Bernardo et al. (2007), Steiner et al. (2009) and Ansari et al. (2007) studies and our study.

Fourteen gene transcripts exhibited differential accumulation between the resistant and susceptible genotypes. However, only four were found specifically induced during *F. graminearum* infection and the other 10 are likely involved with the basal defense response. Among these 14 gene transcripts, ten exhibited increased accumulation in the resistant compared to susceptible genotypes and four exhibited decreased accumulation in the resistant genotype. The gene transcripts that exhibited increased accumulation in the resistant genotype encoded cell wall biogenesis functions (e.g., proline-rich protein, expansin), a Bowman-Birk type trypsin inhibitor, an NB-ARC protein, a putative band 7 protein and unknown proteins. Expansins are involved in cell expansion and other processes during cell-wall modification events (Sampedro and Cosgrove 2005), while proline-rich proteins are components of the cell wall (Otte and Farz 2000). Interestingly, proline-rich proteins were generally found to be repressed in both genotypes (Figure 3B). The increased accumulation in gene transcripts encoding expansin and proline-rich proteins in the resistant genotype indicates that cell wall functions may play a role in protecting the resistant genotype. Protease inhibitors have been shown to limit germination and growth of fungal pathogens in wheat and other crops (Cordero et al. 1994) and may be participating in the resistant response. The NB-

ARC domain is a signal motif shared by plant resistance genes and regulators of cell death in animals (van der Biezen and Jones 1998), indicating that this gene may play a role in resistance and cell death responses. The other four gene transcripts encoding a putative histidine-rich Ca2+-binding protein and three unknown proteins exhibited decreased accumulation in the resistant genotype compared to the susceptible genotype. The overall small number of gene transcripts exhibiting differential accumulation between the resistant and susceptible genotype is likely due to the lack of distinct differences in the disease phenotypes up to 96 hai. Taken together, these genes are characteristic of the differences between the resistant and susceptible response in wheat (Figure 5).

MATERIALS AND METHODS

Plant materials, fungal strain and growth conditions

A wheat NIL pair 260-1-1-2 *Fhb1*+) and 260-1-1-4 (*Fhb1*-) carrying resistant and susceptible alleles, respectively for *Fhb1* was used in this study. *Fhb1* was originally identified in a wheat recombinant inbred line (RIL) population derived from cv. Sumai 3 × cv. Stoa (Waldron et al. 1999). Sumai 3 carries a type II FHB resistance QTL (*Fhb1* or *QFhs.ndsu-3BS*) on chromosome 3BS (Liu et al. 2006; Waldron et al. 1999). Stoa is a cultivar that is moderately susceptible to FHB. A FHB-resistant RIL derived from the Sumai 3/Stoa population, RI 63 that carries the resistant *Fhb1* allele was crossed with an FHB-susceptible line, MN97448. A NIL pair homozygous for the *Fhb1* region, designated 260-1-1-2 (*Fhb1*+, resistance allele) and 260-1-1-4 (*Fhb1*-, susceptible allele), was derived from a self from an F_7 line that was heterozygous for the *Fhb1* region (Pumphrey et al. 2007). Dr. Jim Anderson (University of Minnesota) provided this NIL pair to our laboratory.

To genetically characterize the NIL pair, 15 SSR markers on chromosome 3BS positioned from 0 – 71 cM (Somers et al., 2004) were used to genotype the two NILs. Only four of the markers, gwm533, gwm493, barc87 and barc133, positioned between approximately 6 – 16 cM, were found polymorphic between the NILs.

The monosporic isolate of *F. graminearum* strain Butte 86 (provided by Dr. R. Dill-Macky, University of Minnesota) was used for fungal inoculations. Butte 86 was deposited at the Fungal Genetics Stock Center (accession number: NRRL38661). It is a DON and 15ADON producer.

The seeds were planted in Scotts MetroMix 200 in 6-inch pots (five seeds per pot)

and grown in a growth chamber at 20°C for 16h (7am-11pm) with light and at 18°C for 8h (11pm-7am) under darkness. The light intensity at pot level was approximately 170 ± 20 μE $m^{-2}s^{-1}$. Five ml of Osmocote 14/14/14 (Marysville, OH) was applied to each pot at one week after planting. Plants were watered everyday until sampling.

Experimental design

Three biological replications of the experiment were conducted with a completely random design. Wheat spikes at anthesis of the NIL pair carrying the resistant or susceptible allele for *Fhb1* were point-inoculated with a freshly prepared spore suspension (10^5 macroconidia/ml in 0.2% (vol/ vol) Tween 20) or with water in Tween 20 (mock control) at anthesis. A pipette was used to inoculate 10μl of inoculum (1,000 spores) to two spikelets on the central two rows of a spike. The wheat spikes were covered in a clear plastic bag until sampling. The inoculated spikelets and the adjacent noninoculated spikelets were sampled at 48 and 96 hai. Inoculations and sampling were conducted at 9 am (2 hours into the light cycle). Inoculated spikelets from ten spikes for each genotype/treatment/timepoint/replication were sampled for RNA isolation. A total of 24 samples (two genotypes, two treatments, two timepoints, and three replications) were obtained for the RNA isolations.

Disease severity on wheat spikes after *F. graminearum* inoculation

Wheat carrying the *Fhb1* resistant or susceptible allele were grown and point inoculated as described above, covered with clear plastic bags for 2 days, and scored at 21 days after inoculation. Three replications with 40 plants per genotype per replication

were examined. Percent disease severity was obtained by counting the number of diseased spikelets out of the total number of spikelets. T-tests were performed between the resistant and susceptible NILs to determine statistical significance.

Determination of DON and ergosterol concentration

For each genotype/treatment/timepoint/replication combination, the same two rows of inoculated wheat spikelets (not including the rachis) used for the RNA isolations were used for DON and ergosterol analysis. DON and ergosterol concentration was also determined on two non-inoculated spikelets that were adjacent (one above and one below, combined for assays) to the inoculated spikelets. DON concentration was measured by gas chromatography and expressed as parts per million (ppm) (Jones and Mirocha 1999; Mirocha et al. 1994; Mirocha et al. 1998). Ergosterol concentration was determined following standard protocols (Dong et al. 2006).

Affymetrix GeneChip® wheat genome array

The wheat GeneChip® (Affymetrix, Santa Clara, CA) consists of 61,127 probe sets representing 55,052 gene transcripts. It was designed from sequence information from the *Triticum aestivum* UniGene Build #38 of publicly available ESTs (build date April 24, 2004), ESTs from the wheat species *T. monococcum*, *T. turgidum*, and *Aegilops tauschii*, and GenBank full-length mRNAs from all species through May 18, 2004 (Affymetrix: http://www.affymetrix.com/index.affx). ESTs from 210 libraries were used to design the Wheat GeneChip. One hundred and four libraries were from abiotic or biotic stress conditions, seven of which were from *F. graminearum*-inoculated spikes.

The number of ESTs from the stress and *F. graminearum* treated libraries were 39% and 2.6% out of the total, respectively.

RNA extraction, labeling, and GeneChip wheat genome array hybridizations

Total RNA was isolated, labeled and hybridized to the Affymetrix Wheat GeneChip following the standard protocol described in Boddu et al. (2006). The Biomedical Image Processing Facility at the University of Minnesota performed the Genechip hybridizations, washing and scanning according to Affymetrix procedures (http://www.bipl.ahc.umn.edu/affymetrix.html/).

GeneChip data analysis

GeneChip data was obtained from wheat spikelets carrying the resistant and susceptible *Fhb1* allele 48 and 96 hours after *F. graminearum* and mock water inoculation. Analysis of the data was conducted using GeneData Expressionist Pro version 4.5 (GeneData, San Francisco, CA). The data was first normalized by robust multichip analysis (RMA; (Irizarry et al. 2003) using the Refiner module in Expressionist. Correlation coefficients were calculated between replications and they ranged from 97 - 99%. N-way Analysis of Variance (ANOVA) test (P value ≤ 0.0001) was conducted using the Analyst module to identify transcripts exhibiting differences in accumulation by genotype effect (resistant or susceptible), treatment effect (*F. graminearum* or water mock), time effect (48 hai or 96 hai), genotype by treatment, genotype by time, treatment by time, and genotype by treatment by time effect. A false discovery rate of 0.04% was calculated based on the Benjamini and Hochberg method (1995). Transcripts showing

greater than two-fold differential accumulation between treatments and genotypes were selected.

A list of 32,578 unreliable probe sets have been identified on the wheat chip (personal communication with Ute Baumann, Australian Center for Plant Functional Genomics). These unreliable probe sets contain 5' probe sets, which often hybridize very poorly as opposed to the usual 3' anchored probe sets. We removed the above unreliable probe sets from our analysis.

Gene annotations were based on the probe set annotations at HarvEST (http://harvest.ucr.edu/) using HarvEST WheatChip Version 1.45. The functional classes included cell death related, cell wall related, chromatin related, defense response, putative trichothecene detoxification and transport, hormone-related, membrane related, metabolism, miscellaneous, photosystem related, regulatory, ribosomal protein, stress response, transporter, ubiquitination, and unknown. Wheat genes were placed into a functional class if the E value is $\leq 10^{-10}$, otherwise they were regarded as unknown. All the probe sets on the wheat array were blasted against the *F. graminearum* genome sequence at the *F. graminearum* database (Broad Institute: http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/). Genes were classified as *F. graminearum* if the percent nucleotide identity to the *F. graminearum* genome sequence was $\geq 95\%$ and the e value was $\leq 10^{-84}$. Sixty-five *F. graminearum* probe sets were removed from further analysis.

All data including CEL, DAT, CHP and EXP files have been uploaded to the Plant Expression database (PLEXdb; http://www.plexdb.org/). The accession number for the experiment is TA20.

Real-time RT-PCR validation

Real-time RT-PCR was used to validate the microarray results for the four gene transcripts that showed differential expression (≥ 2.0 fold) between the resistant and susceptible genotypes specifically after *F. graminearum* inoculation. Primers were designed with Primer Express® Software, according to standard parameters for real-time RT-PCR assays (Bio-Rad Laboratories, Hercules, CA). Primer sequences were: Ta.12536.1.S1_at (F: GCCGCATACCAAGCAAGAGTAG; R: AGTCATAGCTCAAGGCAGCCTG), Ta.14246.1.S1_at (F: TATTCGGACCCCTTTTGCAC; R: TCGGTTAATCCTCTCCCATAGG), Ta.28760.1.S1_a_at (F: TCTTCGACTGGCACTTCAACTG; R: GATAGGACCCCAGACAGCAAAA), TaAffx.111425.2.S1_at (F: AGGAGGCGCTGTCTGAATCTAT; R: GCCCCAGCATACAGTTGAAAC), wheat actin (F: CCGGCATTGTCCACATGAA; R: CCAAAAGGAAAAGCTGAACCG). Bio-Rad iScript^{TM} cDNA Synthesis Kit (170-8891) was used to synthesize cDNA from the same RNA samples used for microarray experiments. Real-time RT-PCR was carried out with the Chromo 4 Real-Time System using 96- well plates. iTaq SYBR Green Supermix with ROX was used (cycling conditions: 95ºC, 3 min; (95ºC, 15 sec; 60ºC, 45 sec;) 40 cycles; 95ºC, 1 min; 58ºC, 1min; melting curve from 58 to 98ºC, read every 0.5ºC, hold 10sec; end). RNA from the resistant and susceptible genotypes inoculated with *F. graminearum* from all three biological replications used in the GeneChip experiment were used for the validation. Transcript accumulation of the four target genes and the positive control gene, wheat actin, were measured for *F. graminearum* treated RNA samples of a single biological replication on each 96-well plate. Each

genotype/treatment/time point combination was technically replicated three times. Negative control wells containing the reaction mixture with water as template were included on each plate. MJ Opticon Monitor Software version 3.1 was used for visualizing and analyzing the real-time RT-PCR data (Bio-Rad Laboratories, Hercules, CA). The obtained threshold cycle (Ct) values were used to calculate the fold change in transcript accumulation = $2^{(\Delta Ct_{target}-\Delta Ct_{actin})}$.

SFP analysis

Single feature polymorphisms (SFPs) were detected between the resistant and susceptible genotypes by applying an algorithm described in Cui et al. (2005) and Rostoks et al. (2005) with modifications.

Table 1. Deoxynivalenol and ergosterol concentration in a wheat near-isogenic line pair carrying either the *Fhb1* resistant or susceptible allele point-inoculated with *F. graminearum*.

Sample	Deoxynivalenol concentration[a] (ppm)				Ergosterol concentration[b] (ppm)			
	F48	F96	W48	W96	F48	F96	W48	W96
Resistant, inoculated	2.83 ±1.36	69.37±4.25	0	0	4.07±0.75	35.7±9.73	0	0
Resistant, non-inoculated	0	0	0	0	0	0	0	0
Susceptible, inoculated	3.04±2.89	71.97±18.52	0	0	4.97±4.84	53.23±18.55	0	0
Susceptible, non-inoculated	0.92±1.54	0.69±0.89	0	0	1.17±2.02	0.5±0.7	0	0

[a,b]Deoxynivalenol and ergosterol concentrations +/- standard deviation were derived from 3 replications.

F and W = *F. graminearum* and water inoculation, respectively; 48 and 96 = hours after inoculation.

Deoxynivalenol and ergosterol concentration between the resistant and susceptible genotypes was not significantly different ($P<0.01$).

Resistant indicates plants carrying the *Fhb1* resistant allele. Susceptible indicates plants carrying the *Fhb1* susceptible allele. Inoculated indicates spikelets that were inoculated and sampled. Non-inoculated indicates spikelets adjacent to the inoculated spikelets that were sampled.

Table 2. Number of induced or repressed gene transcripts detected in a wheat near-isogenic line pair carrying either the *Fhb1* resistant or susceptible allele at 48 and/or 96 hai after *F. graminearum* inoculation compared to water.

Class	Induced	Repressed
Cell death	9	3
Cell wall	39	50
Chromatin	1	70
Defense	314	128
Hormone	41	27
Membrane-related	11	12
Metabolism	255	224
Miscellaneous	138	235
Photosystem	3	27
Regulatory	161	127
Ribosomal protein	4	41
Stress response	18	23
Transporter	33	22
Putative trichothecene detoxification and transport	28	5
Ubiquitination	4	5
Unknown	769	781
Total	1828	1780

Table 3. Transcripts that exhibited differential accumulation (P≤0.0001, ≥2.0 fold) between plants carrying the resistant and susceptible *Fhb1* alleles after *F. graminearum* and water inoculation.

Probe set name	SFP[a]	E-Score	Annotation[b]	Class	Expression ratio between comparisons[c]								Map Location[d]
					R-F48/ S-F48	R-F96/ S-F96	R-W48/ S-W48	R-W96/ S-W96	S-F48/ R-F48	S-F96/ R-F96	S-W48/ R-W48	S-W96/ R-W96	
Ta.10095.1.S1_at		3E-74	Proline-rich protein-like	Cell wall	3.2	2.5	2.5	2.8	0.3	0.4	0.4	0.4	
TaAffx.128740.2.S1_s_at		1E-74	Expansin EXPB11 protein precursor	Cell wall	2.1	1.7	2.1	1.7	0.5	0.6	0.5	0.6	1AS 7AS 7BS
Ta.25981.1.A1_at		3E-47	NB-ARC domain containing protein	Defense	4.9	3.6	3.9	5.1	0.2	0.3	0.3	0.2	4AL 7DS
Ta.20928.1.S1_at		8E-52	Bowman-Birk type trypsin inhibitor	Defense	7.0	7.2	3.8	2.4	0.1	0.1	0.3	0.4	3AS 3BS 3DS
Ta.26119.1.A1_at	6	3E-31	Putative 3-isopropylmalate	Metabolism	3.2	2.2	2.1	3.2	0.3	0.5	0.5	0.3	3AS 3BS 3DS 5AL 5DL
Ta.438.3.S1_at	4,9,11	1E-135	Putative nuclease I	Metabolism	4.1	5.7	4.2	6.7	0.2	0.2	0.2	0.1	3BS
Ta.12536.1.S1_at		4E-40	Putative Band 7 protein	Miscellaneous	1.5	2.1	1.4	1.6	0.7	0.5	0.7	0.6	1BL
Ta.28185.1.S1_at		1E-101	Putative histidine-rich Ca2+-binding protein	Regulatory	0.5	0.3	0.3	0.4	2.1	3.5	2.9	2.5	
Ta.28185.1.S1_x_at	7	1E-101	Putative histidine-rich Ca2+-binding protein	Regulatory	0.3	0.2	0.2	0.3	3.2	5.4	4.3	3.3	
Ta.9489.1.S1_at	3,4	3E-58	F-box domain containing protein	Ubiquitination	2.0	2.2	2.0	2.4	0.5	0.5	0.5	0.4	3AL 3BL 3DL
Ta.12679.1.A1_at				Unknown	0.5	0.5	0.4	0.4	2.2	1.9	2.6	2.8	1AL 1BL 2AL
Ta.14246.1.S1_at				Unknown	2.2	1.7	1.5	1.4	0.5	0.6	0.6	0.7	
Ta.15214.1.S1_at	3,6,8			Unknown	0.1	0.0	0.1	0.1	11.0	20.0	17.5	14.5	
Ta.16149.1.A1_at	6,8	3E-60	Protein At1g22700	Unknown	0.5	0.4	0.5	0.4	2.1	2.2	2.2	2.4	3AS 3BS 3DS
Ta.16149.1.A1_x_at	8,9	3E-60	Protein At1g22700	Unknown	0.4	0.5	0.5	0.5	2.3	1.8	2.2	2.2	3AS 3BS 3DS
Ta.22694.1.A1_at				Unknown	7.3	4.7	8.0	8.3	0.1	0.2	0.1	0.1	
Ta.23324.1.S1_at	1			Unknown	0.4	0.1	0.7	0.5	2.5	10.8	1.5	2.2	

Ta.24841.1.S1_at				Unknown	0.2	0.1	0.2	0.3	6.0	6.9	6.1	3.3	
Ta.24841.2.S1_at	1,4,9			Unknown	0.0	0.0	0.0	0.0	27.6	85.7	49.7	26.7	
Ta.24948.1.S1_at	8,10	5E-62	Os01g0106800 protein	Unknown	0.3	0.3	0.3	0.4	3.8	3.6	3.4	2.6	
Ta.26157.1.A1_at	6			Unknown	0.2	0.2	0.2	0.2	5.3	5.3	5.0	6.3	3AS 3BS
Ta.28760.1.S1_a_at				Unknown	2.0	1.2	1.9	1.5	0.5	0.8	0.5	0.7	
Ta.6066.2.S1_a_at				Unknown	0.1	0.1	0.1	0.1	9.3	8.1	10.3	15.3	
Ta.6873.1.S1_at	10,11			Unknown	0.1	0.2	0.2	0.2	7.1	6.1	4.1	4.8	
Ta.8964.1.A1_at	10			Unknown	0.3	0.3	0.3	0.3	3.4	3.7	3.6	3.7	
TaAffx.111425.2.S1_at				Unknown	2.2	1.6	1.5	1.5	0.5	0.6	0.7	0.7	
TaAffx.78237.1.S1_at				Unknown	3.6	4.6	2.0	3.7	0.3	0.2	0.5	0.3	7AS 7BS 7DS

[a]The thirteen transcripts in bold were identified to have one to three single feature polymorphims (SFPs) in the probe set. The numbers in the SFP column indicate the probe numbers where SFPs were detected.

[b]Wheat gene annotations were obtained from HarvEST WheatChip version 1.54. Annotations were assigned when the BLASTX E-score was $<10^{-10}$.

[c]Expression ratio was calculated using the mean values of transcript expression from three replications derived from RMA normalized data using Genedata Expressionist Pro. R, S, F, and W are resistant genotype, susceptible genotype, *F. graminearum* , and water inoculation, respectively. Number 48 and 96 indicate hours after inoculation.

[d]The map location was obtained by conducting BLASTN searches of the wheat probeset sequences against the mapped wheat ESTs at GrainGenes (http://wheat.pw.usda.gov/GG2/blast.shtml).

Figure 1. Disease phenotypes on wheat plants carrying the resistant or susceptible *Fhb1* allele after point-inoculation with *F. graminearum*. **A**. 21 days after inoculation (dai); **B**. 48 hours after inoculation (hai); **C**. 96hai. Arrows point to the necrotic (A) and bleached (B) regions. The light green areas in A were not pathogen-induced bleaching. Resistant indicates plants carrying the *Fhb1* resistant allele. Susceptible indicates plants carrying the susceptible allele.

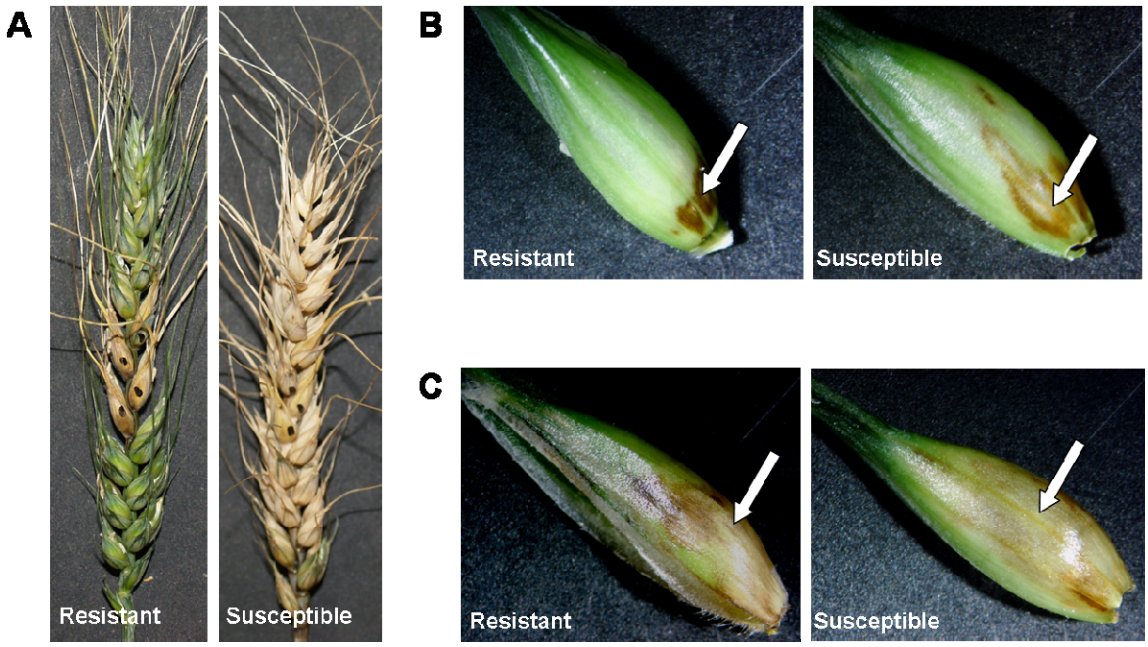


Figure 2. Venn diagrams showing the number of gene transcripts in the resistant and susceptible genotypes that displayed increased- (**A**) or decreased (**B**) transcript accumulation (P≤0.0001, ≥ 2.0 fold) after *F. graminearum* inoculation compared to water. More information about the differentially expressed transcripts can be found in Supplementary Tables 1, 2, 3, 4, 5, and 6.

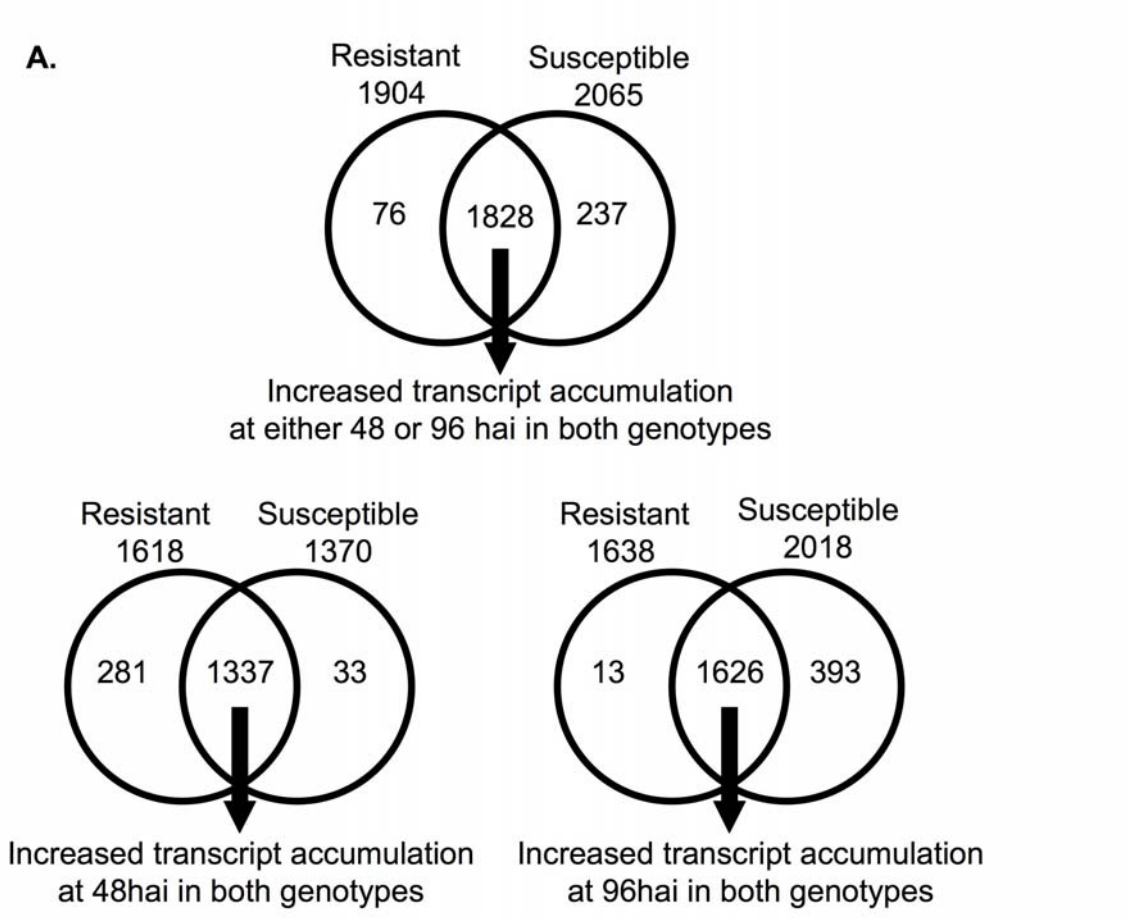


Figure 2.

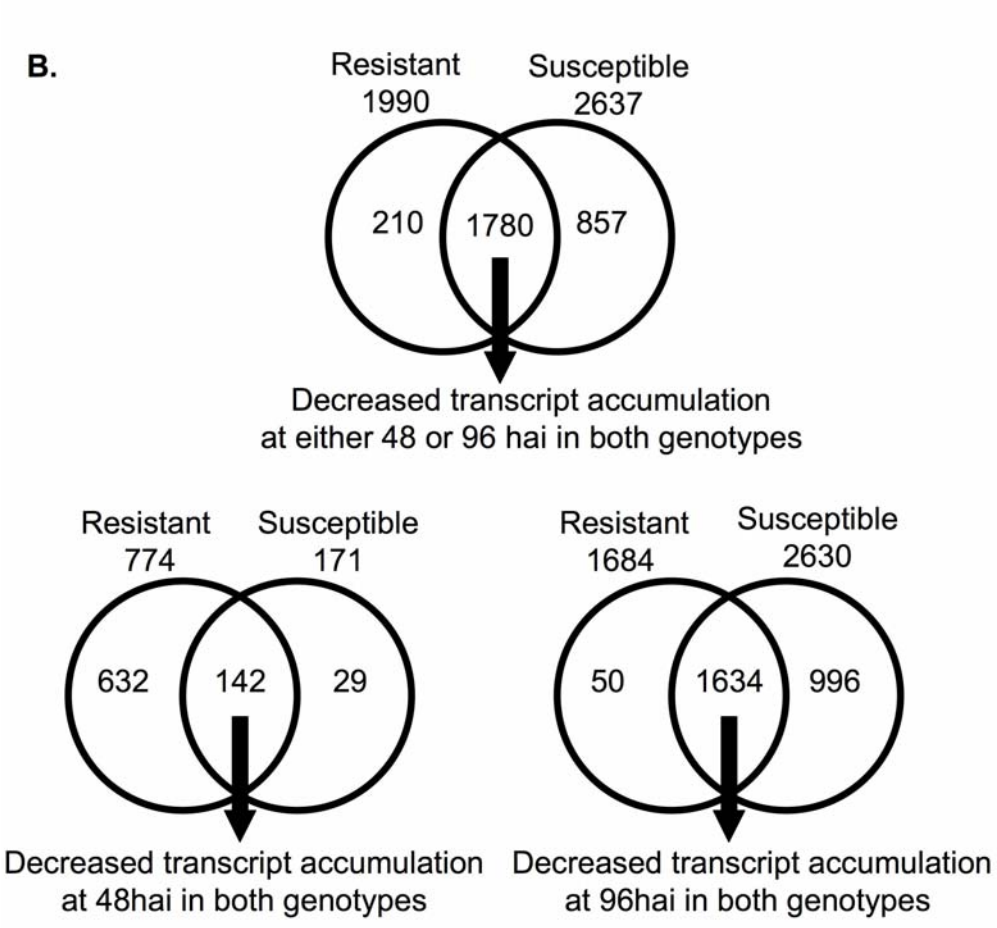

B.
Resistant
1990
Susceptible
2637
210
1780
857
Decreased transcript accumulation
at either 48 or 96 hai in both genotypes
Resistant
774
Susceptible
171
632
142
29
Decreased transcript accumulation
at 48hai in both genotypes
Resistant
1684
Susceptible
2630
50
1634
996
Decreased transcript accumulation
at 96hai in both genotypes

Figure 3. Heat maps showing increased (**A**) or decreased (**B**) abundance (P≤0.0001, ≥ 2.0 fold) of gene transcripts in specific classes in the resistant and susceptible genotypes after *F. graminearum* inoculation compared to water. The color contrast used in A was slightly different from B to show those gene transcripts exhibiting low accumulation. R and S = resistant and susceptible genotype, respectively; F and W = *F. graminearum* and water inoculation, respectively; 48 and 96 = hours after inoculation. Lighter color indicates greater transcript accumulation.

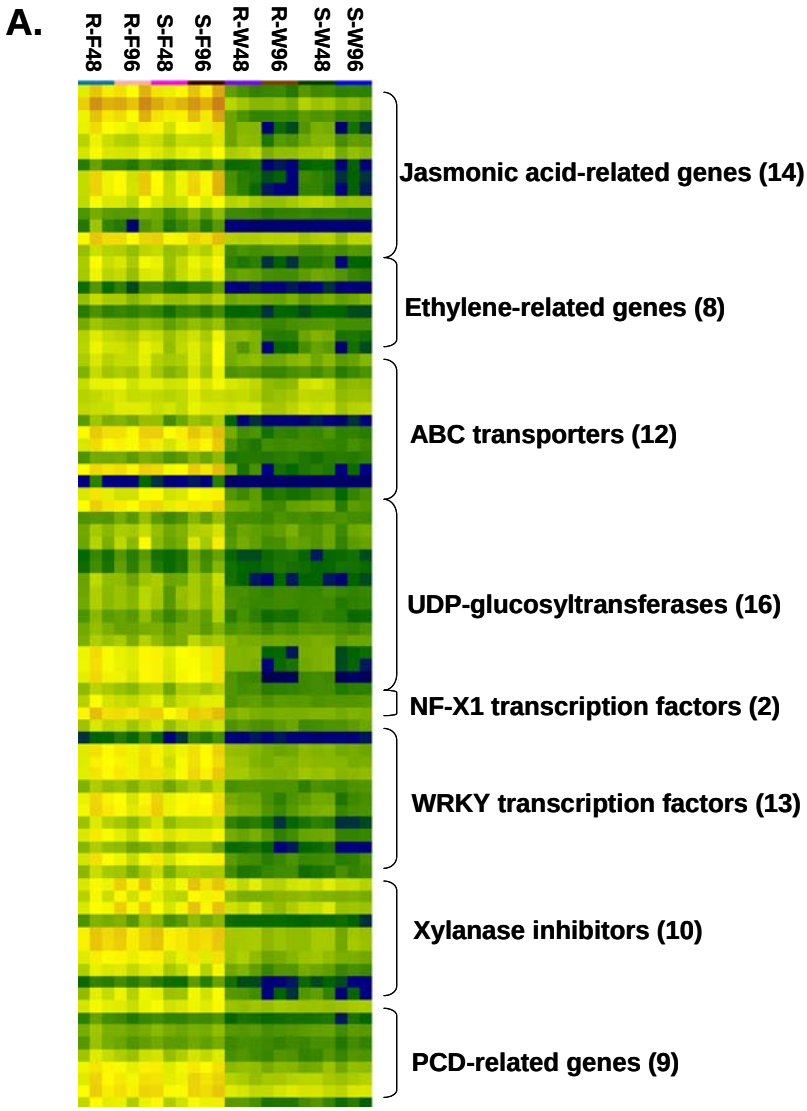


Figure 3.

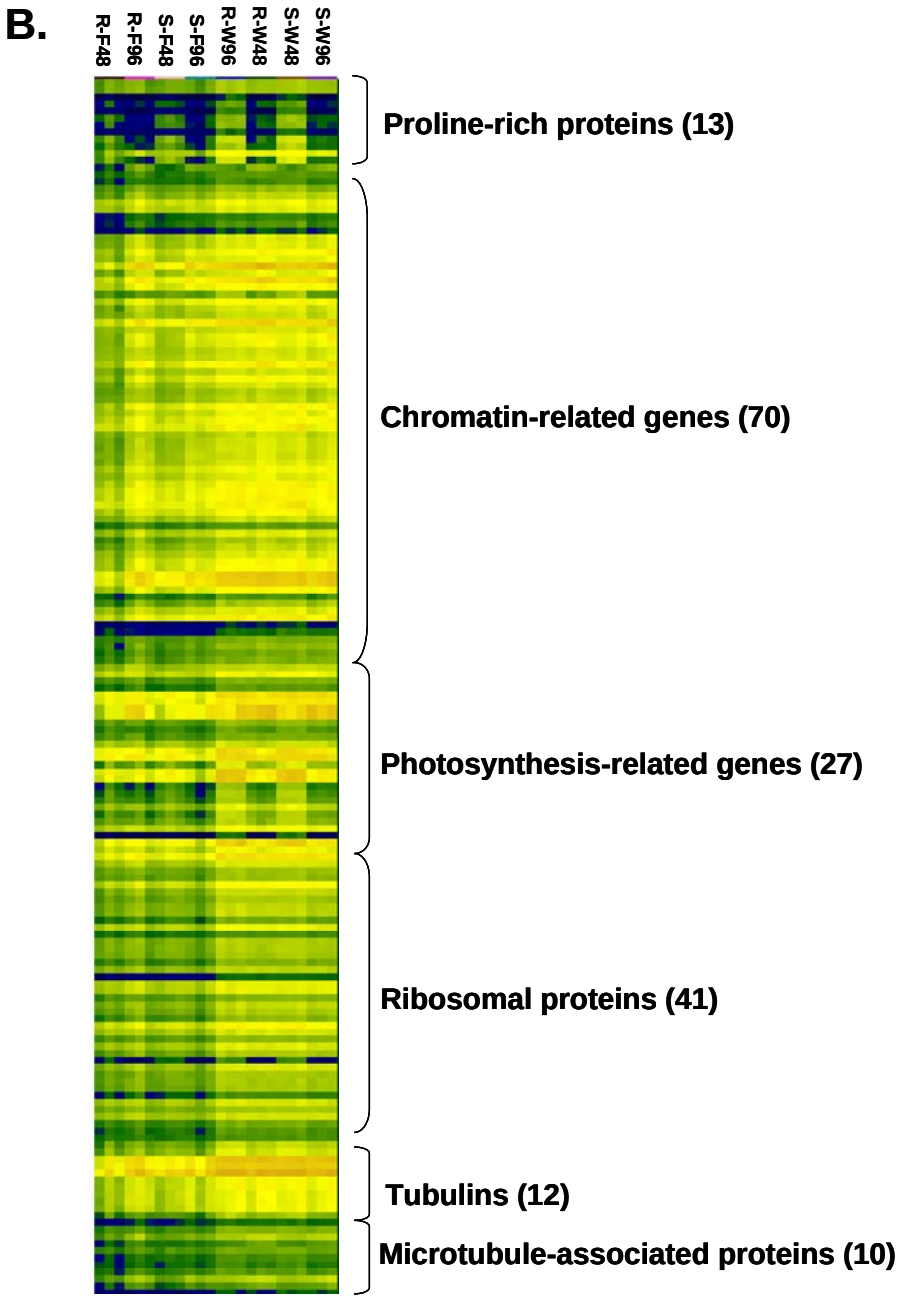

B.
R-F48
R-F96
S-F48
S-F96
R-W96
R-W48
S-W48
S-W96
Proline-rich proteins (13)
Chromatin-related genes (70)
Photosynthesis-related genes (27)
Ribosomal proteins (41)
Tubulins (12)
Microtubule-associated proteins (10)

Figure 4. Real-time RT-PCR validation of expression ratio between resistant and susceptible genotypes after *F. graminearum* inoculation. F48=48 hours after *F. graminearum* inoculation; F96=96 hours after *F. graminearum* inoculation. Each column was the average expression ratio calculated from three biological replications. Error bar was the standard error.

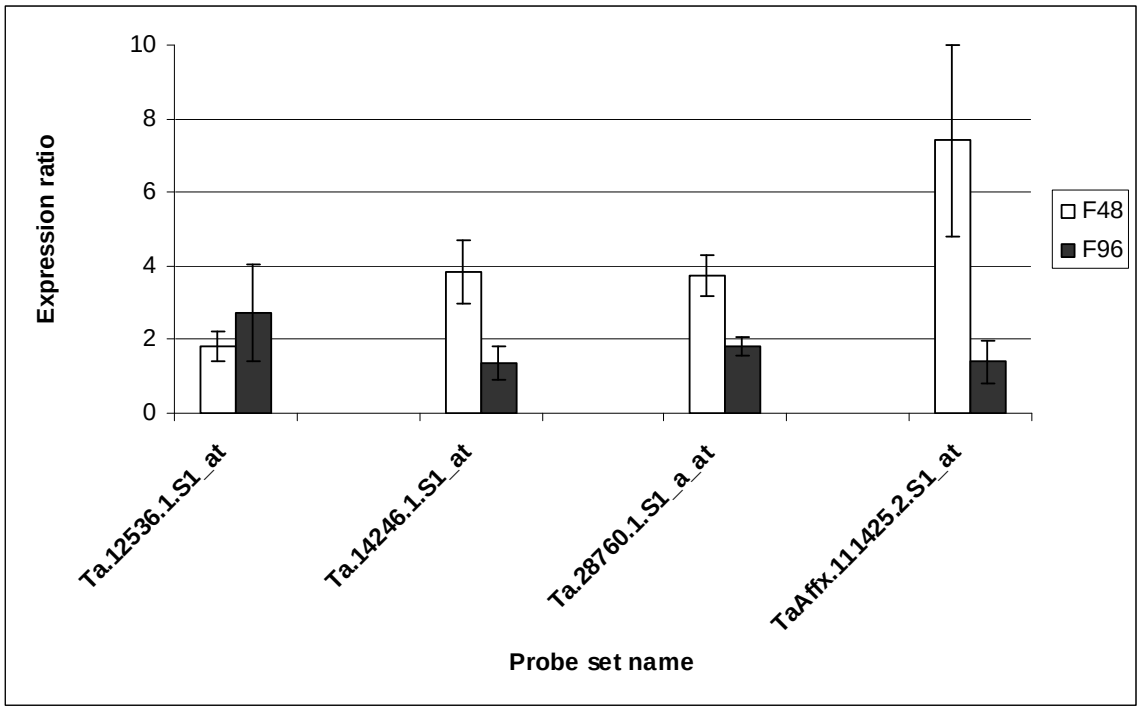
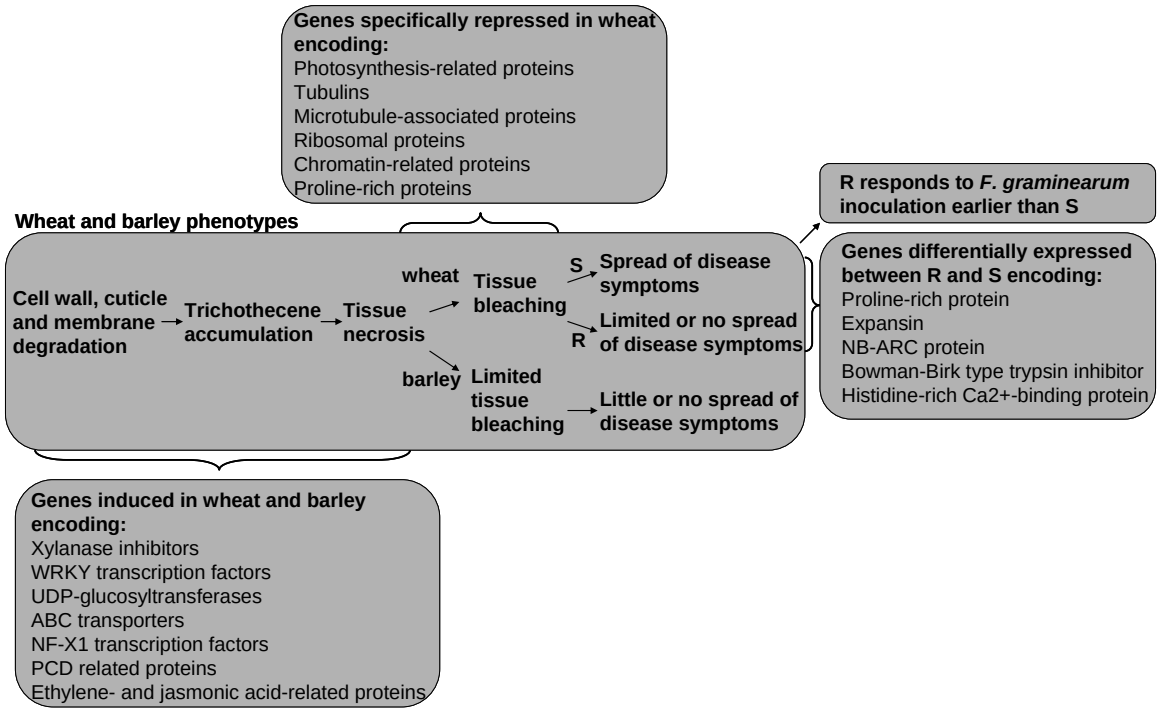


Figure 5. A model for the wheat/barley-*F. graminearum* interaction. Genes induced in wheat and barley were gene transcripts that exhibited increased accumulation in *F. graminearum* infected barley (Boddu et al., 2006; 2007) and wheat (this study). Only those gene transcripts that were induced in barley and were predominantly induced in wheat were included. Genes specifically repressed in wheat were gene transcripts that were only found to exhibit reduced transcript accumulation in wheat during *F. graminearum* infection. Genes differentially expressed between resistant and susceptible wheat genotypes were gene transcripts that showed accumulation difference between the genotypes after *F. graminearum* and water inoculation. R indicates plants carrying the *Fhb1* resistant allele; S indicates plants carrying the *Fhb1* susceptible allele.



CHAPTER 3

TRANSCRIPTOME ANALYSIS OF THREE BARLEY NEAR-ISOGENIC LINE PAIRS CARRYING CONTRASTING ALLELES FOR DIFFERENT FUSARIUM HEAD BLIGHT RESISTANCE ALLELES

INTRODUCTION

Fusarium head blight (FHB) reduces grain yield and quality in wheat and barley (McMullen et al. 1997; Parry 1995). Contamination of grain by trichothecene mycotoxins such as deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON), produced by *Fusarium graminearum* during infection, are the primary causes of reduced grain quality (Dalcero et al. 1997; Desjardins and Hohn 1997; Desjardins 2006; Miller and Ewen 1997; Mirocha et al. 1998). DON contamination in the grain increases the economic losses to barley growers. DON contaminated malting barley grain can result in beer that exhibits "gushing", or increased foaming after opening (Johnson and Nganje 2000; Schwarz et al. 2006). Therefore, the brewing industry has a very stringent requirement for DON level, which is < 0.5 ppm (McMullen et al. 1997).

The most efficient and cost-effective method to control FHB is the deployment of genetic resistance. Resistance to FHB is partial and quantitatively inherited. Multiple quantitative trait loci (QTL) with FHB resistance have been identified on all barley chromosomes. The three major FHB resistance QTLs are located on barley chromosome 2H Bin 8, chromosome 2H Bin 10 and chromosome 6H Bin 6. However, each of these QTL is associated with a morphological or agronomic trait such as heading date (HD), spike type, and grain protein content (GPC) (Canci et al. 2003; Mesfin et al. 2003). The close association of the FHB resistance QTL with these traits has delayed the incorporation of these QTL in breeding programs, primarily because it is not known if the association is due to linkage or pleiotropy. The chromosome 2H Bin 8 FHB resistance QTL (designated Qrgz-2H-8) is associated with heading date (Abramson 1996; Dahleen et al. 2003; de la Peña et al. 1999; Horsley et al. 2006; Mesfin et al. 2003). Resistance

alleles at this QTL are late heading, whereas susceptible alleles at this QTL are normal heading. Recently, Nduulu et al. (2007) conducted a fine mapping study and identified a recombinant that exhibited resistance to FHB and normal heading date, indicating that the relationship between FHB and HD in the Qrgz-2H-8 region is likely caused by tight linkage instead of pleiotropy. Genetic mapping showed that the chromosome 2H Bin 10 FHB resistance QTL is associated with the two row/six row (*Vrs1*) spike type (de la Peña et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Zhu et al. 1999). Two-rowed spike type (*Vrs1*) exhibits enhanced resistance compared to six-rowed spike type (*vrs1*). It is not known if the *Vrs1* locus is pleiotropic or genetically linked to FHB resistance QTL. A QTL for DON accumulation was detected on chromosome 3H Bin 6 (Smith et al. 2004). This QTL was detected in the Fredrickson/Stander population at three days after inoculation. Interestingly, Stander (FHB susceptible parent) carries the low DON accumulation allele.

Multiple transcriptome studies have been conducted to examine barley and wheat host responses to *F. graminearum* infection. The overall response of susceptible barley (cultivar Morex) to *F. graminearum* infection has been studied using the Barley1 Affymetrix GeneChip® (Boddu et al. 2006). Four hundred and sixty seven differentially accumulating gene transcripts were detected defining three stages of the barley-*F. graminearum* interaction. Boddu et al. (2007) also examined the impact of *F. graminearum*-derived trichothecenes on virulence and transcriptomic response on barley spikes. They found that barley exhibited specific responses to trichothecene accumulation. Genes induced were mostly involved in trichothecene detoxification and transport, ubiquitination, programmed cell death, and secondary metabolism. Two types of barley

responses were proposed upon trichothecene accumulation: genes that may promote infection and those that may impede infection. To examine the difference between the resistant and susceptible host-*F. graminearum* interactions, several RNA profiling studies have been conducted in wheat utilizing microarray technology and resistant and susceptible genotypes (Bernardo et al. 2007; Desmond et al. 2008; Hill-Ambroz et al. 2006; Steiner et al. 2009; Walter et al. 2008). Generally, very few genes were found differentially expressed between the resistant and susceptible genotypes. Transcripts of biotic and abiotic-stressed related genes were induced in both resistant and susceptible genotypes after *F. graminearum* infection. Although many microarray studies have been conducted in wheat and barley during *F. graminearum* infection, little is known about the difference between the resistant and susceptible barley-*F. graminearum* interactions.

To begin to develop an understanding of the molecular basis of barley resistance to FHB, we examined three NIL pairs carrying resistant and susceptible alleles for QTL located on chromosome 2H Bin 8, 2H Bin 10, and 3H Bin 6. The primary goals of this study were: (1) to examine the relationship between DON accumulation, fungal biomass and transcript accumulation in three barley NIL pairs carrying different FHB/DON resistance QTLs; and (2) to examine transcript accumulation differences for resistant and susceptible alleles at three major FHB resistance QTLs.

RESULTS

Mycotoxin and fungal biomass accumulation in barley NIL pairs carrying contrasting alleles for different FHB resistance QTLs

The accumulation of DON and ergosterol (indicating the amount of fungal biomass) (Table 1) in the spray-inoculated barley spikes of the NIL pairs carrying resistant and susceptible alleles at the chromosome 2H Bin 8 and 2H Bin 10 FHB resistant QTL, and the 3H Bin 6 DON QTL was assayed at 48 and 96 hours after *F. graminearum* and water inoculation. DON concentration increased in *F. graminearum*-inoculated barley spikes at 96 hai compared to 48 hai in both the resistant and susceptible lines for each pair. However, the difference between the resistant and susceptible NILs in each pair was not significant at either time point ($P<0.05$). Ergosterol concentration also increased in *F. graminearum*-inoculated barley spikes at 96 hai compared to 48 hai. The difference between the resistant and susceptible NILs was not significant ($P<0.05$). None and low amounts of DON or ergosterol was detected in the water-inoculated spikes.

The percent disease severity was different between the two NILs carrying the resistant and susceptible alleles for the chromosome 2H Bin 8 (54% and 89% for resistant and susceptible lines, respectively) and 2H Bin 10 NIL pairs (data not shown) at 14 days after inoculation. However, no clear difference in disease severity was found between the resistant and susceptible genotypes of the chromosome 3H NIL pair (data not shown).

Differential response of barley NILs carrying FHB resistant or susceptible allele to *F. graminearum* infection

We compared the transcript profiles of NIL pairs carrying the resistant and

susceptible alleles at the chromosome 2H Bin 8, 2H Bin 10, and 3H Bin 6 loci during *F. graminearum* infection and mock water inoculation ($P \leq 0.0001$; 2-fold difference). Eighty six, 47, and 8 transcripts were differentially expressed between the resistant and susceptible genotypes during *F. graminearum* and/or water inoculation in the chromosome 2H Bin 8, 2H Bin 10, and 3H Bin 6 NIL pairs, respectively (Tables 2, 3, and 4). Annotations for all these genes were obtained from HarvEST Barley Version 1.68 and grouped into broad classes including: cell wall-related, chromatin-related, defense, hormone, membrane-related, metabolism, miscellaneous, regulatory, ribosomal protein, stress response, transporter, ubiquitination, and unknown protein. A number of the differentially expressed transcripts have been previously mapped on the barley single nucleotide polymorphism (SNP) map (http://harvest.ucr.edu/, Table 2, 3, and 4).

Chromosome 2H Bin 8 NIL pair. Forty-four and 42 transcripts were induced and repressed respectively, in the resistant NIL compared to the susceptible NIL after *F. graminearum* and water inoculation (Table 2). Among the 44 induced transcripts, ten (transposon protein, pectinesterase, two cytochrome P450s, transferase family protein, purple acid phosphatase 1, S-adenosylmethionine decarboxylase proenzyme, mannan endo-1,4-beta-mannosidase 1 precursor, proline-rich protein precursor, and unknown) and two (glutathione S-transferase and catalytic/hydrolase) transcripts were induced specifically after *F. graminearum* and water inoculation, respectively. Among the 42 repressed transcripts, 13 (chitinase IV, uroporphyrinogen III methyltransferase, steroid nuclear receptor, glutathione S-transferase, anthranilate N-benzoyltransferase protein 2, putative cytosolic aldehyde dehydrogenase, cytochrome P450, auxin-regulated dual specificity cytosolic kinase, putative ribonucleotide reductase, putative BLE2 protein, and

three unknowns) and two (expressed protein and unknown) transcripts were repressed specifically after *F. graminearum* and water inoculation, respectively. Transcript HVSMEk0011G13r2_at (transposon protein) was repressed at 48 hai in the resistant genotype compared to the susceptible. Transcript Contig3047_s_at (cytochrome P450) was repressed at both 48 and 96 hai. Interestingly, all ten transcripts that were specifically induced after *F. graminearum* inoculation were detected at 48 hai while almost all the repressed transcripts were found at 96 hai. In addition to the transcripts specifically responding to *F. gramienarum* or water, there were two transcripts (Contig3461_at: glutaredoxin; EBpi01_SQ005_P17_at: putative ripening-related protein) that exhibited at least 15 fold higher expression in the resistant genotype compared to the susceptible after both *F. graminearum* and water inoculation. Among the 86 differentially expressed transcripts between the resistant and susceptible genotypes, 11 of them were previously mapped on the barley SNP map (http://harvest.ucr.edu/). Seven, two, one, and one were mapped to chromosome 2HL, 2HS, 3HS, and 4HL, respectively (Table 2). With available marker densities on barley maps, we could not precisely map six out of the seven transcripts on chromosome 2HL to the 2H Bin 8 QTL region (GrainGenes: http://wheat.pw.usda.gov/GG2/index.shtml). However, contig7815_s_at (anthranilate N-benzoyltransferase protein 2) was mapped close to the chromosme 2H Bin 8 QTL region based on association genetics analysis (personal communication, Jon Massman).

Due to the possibility that single feature polymorphisms (SFPs) may alter the transcript abundance value, we examined the transcripts for SFPs. Eleven of the 86 transcripts contained at least one SFP. SFPs can also be used to detect polymorphisms that can be used to differentiate genotypes. SFPs detected in Contig2403_at and

Contig3173 were previously mapped on the barley SNP map to chromosome 2HL and 2HS, respectively (http://harvest.ucr.edu/; Table 2).

Chromosome 2H Bin 10 NIL pair. Twenty-four and 23 transcripts were induced and repressed respectively, in the resistant NIL compared to the susceptible NIL after *F. graminearum* and water inoculation (Table 3). Among the 24 induced transcripts, six transcripts (26S protease regulatory subunit 6B homolog, cytochrome P561, pyrophosphaste-energized vacuolar membrane proton pump, expressed protein, and two unknowns) were induced specifically after *F. graminearum* inoculation. Among the 23 repressed transcripts, five (Pi-starvation-induced protein, aspartate aminotransferase, protein kinase, hemolysin, and unknown) and two (unknowns) transcripts were repressed specifically after *F. graminearum* and water inoculation, respectively. In contrast to the chromosome 2H Bin 8 NIL pair, all transcripts that were specifically induced or repressed after *F. graminearum* inoculation were detected at 96 hai. Among the 47 differentially expressed transcripts between the resistant and susceptible genotypes, one and three were previously mapped on the barley SNP map to chromosome 2HL and 1HL, respectively (http://harvest.ucr.edu/). The transcript that mapped to 2HL encodes a cis-zeatin O-glucosyltransferase 1 and was found up-regulated in the resistant genotype after *F. graminearum* infection at both time points and after water inoculation at 48 hai.

Chromosome 3H Bin 6 NIL pair. Four transcripts each showed increased and decreased abundance in the resistant genotype compared to the susceptible genotype in the chromosome 3H Bin 6 NIL pair (Table 4). Among the four transcripts exhibiting increased abundance, only one (hypothetical protein) was specifically induced after *F. graminearum* inoculation. Among the four transcripts showing decreased expression, one

(putative ribosomal RNA apurinic site specific lyase) was specifically repressed after *F. graminearum* and one (elongation factor 2) was specifically repressed after water inoculation. The four transcripts showing increased accumulation in the resistant genotype encoded an elongation factor 2, a pectinesterase precursor, a conserved hypothetical protein, and an unknown protein. The four gene transcripts showing decreased accumulation in the resistant genotype encoded a ribosomal RNA apurinic site specific lyase, a transcription factor APFI, a histone H3 and an unknown protein. One of the differentially expressed transcripts (putative ribosomal RNA apurinic site specific lyase) between the resistant and susceptible genotypes was mapped to chromosome 3HL based on the barley SNP map (http://harvest.ucr.edu/).

No overlap among the differentially expressed transcripts between genotypes from the three QTL NIL pairs

When we compared the differentially expressed transcripts between resistant and susceptible genotypes from the three QTL NIL pairs, there was no overlap among them (Figure 1). The differentially expressed transcripts that were detected specifically after *F. graminearum* infection revealed distinct temporal patterns. In the chromosome 2H Bin 8 NIL pair, all transcripts induced in the resistant compared to the susceptible genotype were detected at 48 hai and almost all were repressed at 96 hai. In the chromosome 2H Bin 10 and chromosome 3H Bin 6 NIL pairs, both induction and repression occurred at 96 hai. At each time point, the number of induced or repressed transcripts in the resistant genotype was always higher after *F. graminearum* challenge compared to mock water inoculated in all three of the NIL pairs, indicating the fungus attack triggered greater

responses in the resistant barley host.

Comparative analyses with other Barley GeneChip studies

To further study the host response to *F. graminearum* infection, we performed comparative analyses of all the transcripts showing differential expression between resistant and susceptible NILs with results from three other barley-*F. graminearum*/trichothecene GeneChip studies.

Boddu et al. (2006) examined the host response in the susceptible barley cultivar Morex to *F. graminearum* infection. Four hundred and sixty-seven differentially accumulating transcripts were detected in the *F. graminearum*-treated plants compared with the water control. Seven of these transcripts (HVSMEl0019G11f2_at: unknown, Contig3047_s_at: cytochrome P450, Contig10779_at: uroporphyrin-III C-methyltransferase-like protein, Contig17841_at: unknown, Contig3381_s_at: subtilisin-chymotrypsin inhibitor 2, Contig4326_s_at: chitinase IV, and rbaal11f18_at: protein kinase) showed differential transcript accumulation between the resistant and susceptible genotypes in the chromosome 2H Bin 8 pair.

Boddu et al. (2007) also used the Barley GeneChip to examine the transcriptome pattern in barley after inoculation with trichothecene-producing (wild type) and -nonproducing (*tri5* mutant) strains of *F. graminearum*. Transcripts responding to pathogen-derived trichothecene accumulation were detected**.** One transcript (Contig3047_s_at: cytochrome P450) from this list was found differentially expressed between the resistant and susceptible genotypes in the chromosome 2H Bin 8 NIL pair.

Gardiner et al. (unpublished data) examined the barley host response to

application of purified DON. Microarray analysis was conducted to detect gene transcripts that exhibited a difference between DON inoculation and mock water inoculation. Two hundred and fifty five gene transcripts were found differentially expressed between DON and water inoculation. Three transcripts exhibited decreased accumulation in response to DON application compared to a mock water control. One of them (EBed01_SQ002_G15_at: unknown protein) was found differentially expressed between the resistant and susceptible genotypes in the chromosome 2H Bin 8 NIL pair. One of the transcripts induced by purified DON and in planta *F. graminearum*-derived trichothecenes, Contig3047_s_at (cytochrome P450), was found differentially expressed between the resistant and susceptible genotypes in the chromosome 2H Bin 8 NIL pair.

DISCUSSION

We investigated gene expression profiles and disease phenotypes in three barley NIL pairs carrying contrasting alleles for different FHB resistance QTLs during fungal and water inoculation. The chromosome 2H Bin 8 and 2H Bin 10 QTLs were classified as major QTLs for FHB resistance (de la Peña et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Zhu et al. 1999). The chromosome 3H Bin 6 QTL explained 18-35% of the phenotypic variation in DON accumulation at three days after inoculation (Smith et al. 2004). This is the first report of the barley transcriptome in response to *F. graminearum* infection utilizing NIL pairs carrying resistant and susceptible alleles at key FHB resistant QTLs. This dataset allowed us to examine the host response in resistant and susceptible barley genotypes to *F. graminearum* infection.

Resistance provided by different FHB resistance QTLs

In this study, we examined three pairs of barley NILs carrying the resistant or susceptible allele for three key FHB resistance QTLs. The chromosome 2H Bin 8 FHB resistance QTL was detected in the Chevron/M69 population (de la Peña et al. 1999) and validated in two progeny populations (Canci et al. 2004). This QTL is also associated with reduced DON and late heading date. The Fredrickson/Stander population was used to detect the chromosome 2H Bin 10 FHB resistance QTL (Mesfin et al. 2003) and the chromosome 3H Bin 6 DON accumulation QTL (Smith et al. 2004). The chromosome 2H Bin 10 FHB resistance QTL is coincident with a QTL for low DON accumulation and 2-row spike type. Interestingly, at the chromosome 3H Bin 6 QTL, reduced DON concentration was associated with a Frederickson allele, which provides reduced FHB

severity (Smith et al. 2004). However, the disease severity was similar between the chromosome 3H Bin 6 NIL pair (Smith et al. 2004).

Under the conditions used in our experiments we could not detect a statistical difference in DON accumulation between the resistant and susceptible genotypes at 48 and 96 hai. The DON concentration was 2.4, 1.4 and 2.9 fold higher at 96 hai in the susceptible NIL compared to the resistant in the chromosome 2H Bin 8, 2H Bin 10, and 3H Bin 6 NIL pairs. At 48 hai, no DON was detected in the resistant or the susceptible genotypes of the chromosome 2H Bin 8 and 3H Bin 6 NIL pairs although there was fungal growth already. In contrast, both DON and ergosterol were detected at 48 hai in the chromosome 2H Bin 10 NIL pair.

Our results obtained from the growth chamber are different from those obtained in the field for the chromosome 2H Bin 8 and Bin 10 QTLs, and those in the greenhouse for the chromosome 3H Bin 6 QTL. de la Peña et al. (1999) identified the chromosome 2H Bin 8 FHB QTL as also associated with DON reduction via inoculating the nurseries by natural infection and spreading *F. graminearum*-infected maize and barley seeds. Mesfin et al. (2003) showed that the chromosome 2H BIN 10 FHB QTL also reduced DON concentration through inoculating plants in the field with grain spawn and spray inoculations. In addition, in both the de la Peña et al., (1999) and Mesfin et al., (2003) studies, the determination of DON concentration was based on cleaned grain collected from the nursery at harvest. The chromosome 3H Bin 6 QTL was identified from point inoculating florets and measuring DON in that floret 3 dai (Smith et al. 2004). Our plants were spray-inoculated in the growth chamber and whole spikes were sampled at 48 and 96 hai for DON assay. Thus, it is likely that the differences between the studies are due

to different inoculation and sampling methods used.

Transcriptome differences between resistant and susceptible barley NILs

Chromosome 2H Bin 8 NIL pair. Several interesting transcripts up-regulated in the resistant genotype compared to the susceptible genotype specifically after *F. graminearum* inoculation encode a proline-rich protein, a pectinesterase, a transposon, and cytochrome P450s. The higher accumulation of proline-rich protein transcript suggested that cell wall responses may help protect the resistant line. Pectinesterase is also a cell wall modifying enzyme. In addition to influencing numerous physiological processes, it has also been shown to play a role in plants responding to pathogen and pest attack (Ithal et al. 2007; Jammes et al. 2005; Puthoff et al. 2003). A gene for pectinesterase has been found upregulated in soybean during nematode infection (Ithal et al. 2007). It is possible that pectinesterase induction may provide resistance to FHB severity and DON accumulation in barley. Cytochrome P450 CYP709C1 (Contig3047_s_at) was found capable of hydrolating fatty acids and implicated in detoxificaiton during plant defense (Kandel et al. 2005). Transcripts encoding a glutoredexin (Contig3461_at) and a putative ripening-related protein (EBpi01_SQ005_P17_at) showed much higher expression in the resistant genotype compared to the susceptible after *F. graminearum* and water inoculation. The glutaredoxin gene was shown to be different based on sequencing data between the genotypes. The differential expression is likely due to SFP differences. The resistant genotype is early heading while the susceptible genotype is late heading. The higher expression of the putative ripening-related protein in the resistant genotype may be

related to the heading date trait.

Chromosome 2H Bin 10 NIL pair. Among the transcripts induced or repressed in the resistant genotype after *F. graminearum* and water inoculation compared to the susceptible genotype, Contig13294 (cis-zeatin O-glucosyltransferase) was mapped to chromosome 2HL but was not found within the chromosome 2H Bin10 QTL region. The systematic name for cis-zeatin O-glucosyltransferase is UDP-glucose:cis-zeatin O-β-D-glucosyltransferase. In wheat, Lemmens et al. (2005) suggested that a major FHB resistance QTL (*Fhb1*) on chromosome 3BS encodes a UDP-glucosyltransferase that converts DON to a less toxic derivative or functions as a regulator of a UDP-glucosyltransferase. It is possible that this cis-zeatin O-glucosyltransferase plays a key role associated with the chromosome 2H Bin 10 QTL. It may work coordinately with the QTL and provide resistance to FHB and DON accumulation in the resistant genotype.

Other gene transcripts were also detected that exhibited accumulation differences between the resistant and susceptible alleles at the chromosome 2H Bin 8 FHB QTL. Transcripts encoding a retrotransposon and a transposon were up- and down-regulated in the resistant genotype, respectively. A retrotransposon was found activated in wheat in response to mycotoxigenic and non-mycotoxigenic-associated *F. graminearum* stress (Ansari et al. 2007). We also detected an F-box domain containing protein up-regulated in the resistant genotype after inoculation compared to the susceptible genotype. An F-box domain-containing protein was also found induced in Morex barley responding to pathogen-derived trichothecene accumulation (Boddu et al. 2007). F-box domain-containing proteins have been shown to be related to ubiquitination (Bai et al. 1996). The upregulation of this gene suggested that ubiquitination may be induced by *F.*

graminearum infection and important in disease resistance. A transcript encoding glutathione peroxidase-like protein exhibited the highest expression difference (higher in the resistant) between the resistant and susceptible genotypes. Glutathione peroxidases have been shown to protect plants from oxidative damage under biotic and abiotic stress (Csiszár et al. 2002; Miao et al. 2006). Transgenic tobacco plants with overexpressed glutathione peroxidase displayed enhanced stress tolerance (Yoshimura et al. 2004).

Chromosome 3H Bin 6 NIL pair. There were only eight transcripts differentially expressed in the chromosome 3H Bin 6 NIL pair. This may be due to the minor effect of this resistance QTL. Smith et al. (2004) inoculated a single spikelet of barley plants with *F. graminearum* in the greenhouse and analyzed DON accumulation at 72 hai. The DON concentration was significantly different between the two parents only in one experiment when they mapped the QTL (Smith et al. 2004). They validated this QTL using near-isogenic lines selected from this mapping population. The mean DON accumulation in the homozygous susceptible allele NIL were 2.5 fold higher than the resistant allele lines. This DON QTL was not associated with either FHB severity or DON concentration in field experiments (Mesfin et al. 2003). We did not find distinct differences in FHB severity between the resistant and susceptible NILs, which was consistent with the results in their QTL validation experiment (Smith et al. 2004). The lack of distinct phenotypic difference between the NIL pair is the likely reason for the small number of transcripts showing differential expression between the NILs.

Comparative analyses with other Barley GeneChip studies

A cytochrome P450 (Contig3047_s_at) differentially expressed between the

resistant and susceptible chromosome 2H Bin 8 NILs after *F. graminearum* and water inoculation was found in three other Barley GeneChip studies on susceptible barley Morex (Buddu et al. 2006; 2007; Gardiner et al. unpublished data). This cytochrome P450 was induced in Morex in response to purified DON and to *F. graminearum*-derived trichothecenes. Interestingly, this gene was induced significantly by *F. graminearum* inoculation compared to water in both resistant and susceptible genotypes (data not shown). The change was higher in the susceptible genotype compared to the resistant after both *F. graminearum* and water inoculation. Cytochrome P450s are involved in a wide range of secondary metabolic processes (Schuler and Werck-Reichhart 2003). They are induced by fungal elicitors in plants, i.e., *F. graminearum* infection of wheat (Kong et al. 2005) and barley (Boddu et al. 2006; 2007). Thus, the cytochrome P450 gene identified in these studies may be an important host response that differentiates the resistant and susceoptible genotypes.

Differences in transcript accumulation among the three NIL pairs

The differences in transcript accumulation among the three NIL pairs may be due to: (1) the three QTLs were on different chromosome locations and contained different genetic composition; and (2) the alleles at each QTL exhibit distinct genetic mechanisms. Differential gene expression in different Arabidopsis genotypes has been found in response to salicylic acid (West et al. 2006). Thus, it is possible that the diverse gene expression among the three barley NIL pairs was due to sequence diversity. This work represents a unique set of results in examining the response to a pathogen. Alternatively, it is possible that these QTLs have distinct resistance/susceptibity mechanisms. The

majority of differentially expressed transcripts occurred at either 48 or 96 hai in those three NIL pairs. Thus, our data also suggested that the timing of transcript accumulation changes may play a key role in the resistance mechanisms of these QTLs.

Previously, we examined transcript accumulation in a wheat NIL pair carrying contrasting alleles for the chromosome 3BS FHB resistance QTL (*Fhb1*) in response to *F. graminearum* (Jia et al. accepted by MPMI). An integrated model for the wheat and barley-*F. graminearum* interactions was developed based on the disease symptoms, transcript accumulation data, and comparative analysis of wheat and barley host responses to *F. graminearum* infection (Jia et al. accepted by MPMI). Only data from the susceptible barley cultivar Morex and the wheat NIL pair carrying the resistant and susceptible allele for the chromosome 3BS QTL were used to build the model. We can now incorporate the data from this study into the model to extend the previous model. This study detected transcriptome differences between resistant and susceptible barley genotypes in response to *F. graminearum* infection examined in three NIL pairs. Since both resistant and susceptible barley genotypes exhibit Type II resistance to *F. graminearum*, there was little or no spread of disease symptoms from initial infected spikelets to adjacent spikelets. However, the FHB resistant barley genotypes did have lower percent disease severity compared to the susceptible at 14 dai (de la Peña et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Zhu et al. 1999). Overall, more genes were found differentially expressed during *F. graminearum* infection between the resistant and susceptible genotypes in barley compared to wheat. Only four genes (four unknowns) were found in wheat showing transcript abundance differences between the resistant and susceptible genotypes after *F. graminearum* inoculation. There were total of 130 genes

(not including 11 gene transcripts exhibiting SFPs) found differentially expressed between the resistant and susceptible genotypes in the barley NILs after *F. graminearum* infection but not in the wheat NILs. These gene expression differences may be signatures of resistance or susceptibility and are important to follow up on.

MATERIALS AND METHODS

Plant materials and growth conditions

One resistant and susceptible near isogenic line (NIL) pair differing for the three FHB resistance QTLs: 2H Bin 8 QTL (Qrgz-2H-8), 2H Bin 10 QTL, and 3H Bin 6 QTL and were used in this study. The 2H Bin 8 NIL pair was generated by backcrossing a progeny from the cv. Chevron （FHB resistant, late heading, 6-row）x cv. M69 (FHB susceptible, normal heading, 6-row) population carrying the Chevron allele for the EBmac0521 and Bmag0140 SSR marker interval with M69 as the recurrent parent (Nduulu et al. 2007). Flanking SSR markers EBmac0521a and Bmag0140 were used to verify the genotypes. The 2H Bin 10 NIL pair was developed from a barley RIL population derived from cv. Frederickson x cv. Stander. Frederickson is a 2-row cultivar with a major FHB resistance QTL on chromosome 2H Bin 10. Stander is a 6-row cultivar that is susceptible to FHB. The NIL pair used in this study was developed through self-pollinating an F_7 heterozygote at the chromosome 2H Bin 10 QTL region and selecting homozygotes for the resistant and susceptible allele. Two SSR markers flanking the two row/six row gene *Vrs1*, MBP-52 and MWG865, were used to verify the genotypes. The 3H Bin 6 QTL NIL pair was developed via selfing F_7-derived lines from the Frederickson (FHB resistant) x Stander (FHB susceptible) recombinant inbred line mapping population that were heterozygous for the chromosome 3H QTL region. SSR markers Bmag0122 and Bmac0067 were used to confirm the genotypes.

Five seeds were planted in each 6-inch pot using Scotts MetroMix 200 as the growing medium. Plants were grown in a controlled growth chamber with a 16h

photoperiod and 20/18°C day/night temperature. Fertilizer Osmocote 14/14/14 (Marysville, OH) was applied at 5ml/pot one and three weeks after planting.

Fungal strain, inoculation, sampling and experimental design

Barley spikes were spray-inoculated with a freshly prepared spore suspension (10^5 macroconidia/ml) of *F. graminearum* strain Butte 86 or with water 2 to 3 days after emerging from the boot. The *F. graminearum* isolate used was deposited at the Fungal Genetics Stock Center (accession number: NRRL38661). Butte 86 is a DON and 15ADON producer. Each side of the spike was sprayed two times with the inoculum. The entire inoculated spikes were covered by clear plastic bags and sampled at 48 and 96 hai. Inoculations and sampling were conducted at 9am (which was 2 hours into the light cycle). Three biological replications of the experiment were conducted with a completely random design. Eight to ten entire spikes for each genotype/treatment/timepoint/replication were sampled for RNA isolation. A total of 24 samples were examined for each NIL pair experiment.

Quantification of mycotoxin and ergosterol accumulation

For each sample, the same tissue used for RNA isolation was used for DON and ergosterol quantification. DON concentration was determined by gas chromatography and reported as parts per million (ppm) (Jones and Mirocha 1999; Mirocha et al. 1994; Mirocha et al. 1998). Ergosterol concentration was measured following Dong et al. (2006).

Barley1 Affymetrix GeneChip®

The Barley1 Affymetrix GeneChip® (Affymetrix, Santa Clara, CA) includes 22,792 probe sets representing 22,439 barley genes (Close et al. 2004). Barley ESTs (HarvEST, http://harvest.ucr.edu/) including ESTs derived from *F. graminearum*-infected barley plants were used to design the GeneChip. The number of ESTs from stress (biotic and abiotic) and *F. graminearum* treated libraries were 18% and 1.8% out of the total, respectively.

RNA extraction, labeling, and GeneChip hybridizations

Total RNA was extracted, labeled and hybridized to the Barley1 Affymetrix GeneChip following the standard protocol described in Boddu et al. (2006). The Biomedical Image Processing Facility at the University of Minnesota performed the hybridization, washing and scanning according to Affymetrix procedures (http://www.bipl.ahc.umn.edu/affymetrix.html/).

GeneChip data analysis

GeneChip data was obtained from barley spikes 48 and 96 hours after *F. graminearum* and water inoculation and analyzed using GeneData Expressionist Pro version 4.5 (GeneData, San Francisco, CA). As a first step, the data were normalized by robust multichip analysis (RMA)(Irizarry et al. 2003) in Expressionist. Correlation coefficients were calculated between replications, which ranged from 96-99%, 90-99%, and 85-97% for the 2H Bin 8, 2H Bin 10, and 3H Bin 6 NIL pairs, respectively. N-way Analysis of Variance (ANOVA) test (P value ≤ 0.0001) was conducted to identify

differentially expressed genes by genotype effect (resistant or susceptible). A false discovery rate of 0.7%, 2%, and 16% was calculated for the 2H Bin 8, 2H Bin 10, and 3H Bin 6 NIL pairs, respectively according to Benjamini and Hochberg (1995). Transcripts exhibiting a two or higher -fold differential accumulation between genotypes were selected.

Gene annotations were obtained from HarvEST (http://harvest.ucr.edu/) using HarvEST Barley Version 1.68. Barley genes were placed into a functional class if the E value is $\leq 10^{-10}$, otherwise they were regarded as unknown. The functional classes of the differentially expressed transcripts included: cell wall, chromatin, defense, hormone-related, membrane-related, metabolism, miscellaneous, photosystem-related, regulatory, ribosomal protein, stress response, transporter, ubiquitination, and unknown proteins. All the probe sets on the barley GeneChip were BLASTed against the *F. graminearum* genome sequence at the *F. graminearum* database (Broad Institute: http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/). Genes were annotated as *F. graminearum* if the percent nucleotide identity to the *F. graminearum* genome sequence was $\geq 95\%$ and the e value was $\leq 10^{-84}$.

All GeneChip data (CEL, DAT, CHP and EXP files) have been stored in the Plant Expression database (PLEXdb; http://www.plexdb.org/). The accession numbers for the chromosome 2H Bin 8, 2H Bin 10, and 3H Bin 6 experiments are BB70, BB69, and BB68, respectively.

SFP analysis

Single feature polymorphisms (SFPs) between the resistant and susceptible lines

in each NIL pair was examined using an modified algorithm based on Cui et al. (2005) and Rostoks et al. (2005).

Table 1. Deoxynivalenol and ergosterol concentration in inoculated barley spikes.

Sample[a]	Deoxynivalenol concentration[b] (ppm)				Ergosterol concentration[c] (ppm)			
	F48	F96	W48	W96	F48	F96	W48	W96
2H Bin 8 NIL R (Early HD)	0	8.03±3.55	0	0	0.47±0.55	4.23±3.10	0	0
2H Bin 8 NIL S (Normal HD)	0	18.97±6.57	0	0	0.19±0.03	4.13±1.15	0	0.50±0.87
2H Bin 10 NIL R (2-row)	0.24±0.24	7.61±1.07	0	0	0.39±0.03	4.83±1.63	0.02±0.04	0.33±0.21
2H Bin 10 NIL S (6-row)	0.31±0.07	10.43±4.64	0	0	0.68±0.21	7.69±2.66	0	0.68±0.36
3H Bin 6 NIL R (Stander)	0	2.73±1.71	0	0.33±0.57	0.32	15.4	0.22	0.18
3H Bin 6 NIL S (Frederickson)	0	7.96±3.46	0	0	1.6	19.3	0.32	1.3

[a]R and S = Lines carrying resistant and susceptible alleles of the QTL, respectively

[b,c]Deoxynivalenol and ergosterol concentrations shown were the mean value and standard deviation calculated from 3 replications. The ergosterol data for the 3H NIL pair was only from one replication.

F and W = *F. graminearum* and water inoculation, respectively; 48 and 96 = hours after inoculation.

Table 2. Transcripts that exhibited differential accumulation (P≤0.0001; ≥ 2.0 fold) between the resistant and susceptible genotypes in the chromosome 2H Bin 8 NIL pair after *F. graminearum* and water inoculation.

Probe set name[a]	E-Score	Annotation[b]	Class[c]	Expression ratio[d]				Map location[e]
				R-F48/ S-F48	R-F96/ S-F96	R-W48/ S-W48	R-W96/ S-W96	
Contig10206_s_at	1.00E-47	Proline-rich protein precursor	Cell wall	2.1	1.8	1.5	1.2	
Contig11000_at	2.00E-14	Kinesin motor protein, putative	Chromatin	0.1	0.2	0.1	0.1	
Contig6865_at	1.00E-180	Cytochrome P450 86A2	Defense	2.9	1.3	1.4	1.5	
Contig6865_x_at	1.00E-180	Cytochrome P450 86A3	Defense	2.6	1.3	1.3	1.4	
Contig3047_s_at	0	Cytochrome P450 CYP709C1	Defense	0.4	0.4	0.5	0.6	
Contig16307_at	9.00E-83	Cytochrome P450 family protein	Defense	4.2	2.9	5.3	5.3	
Contig9632_at	2.00E-84	Glutathione S-transferase GST 22	Defense	1.8	0.9	1.8	2.0	
Contig6010_at	7.00E-40	Glutathione S-transferase GST 31	Defense	1.0	0.4	1.0	0.8	
Contig14534_at	9.00E-86	Putative cytochrome P450	Defense	4.2	2.2	3.9	3.3	2HL 66.12
Contig3381_s_at	4.00E-32	Subtilisin-chymotrypsin inhibitor 2	Defense	0.2	0.6	0.5	0.6	
Contig4324_s_at	1.00E-109	Chitinase IV	Defense, PR	0.4	0.4	0.8	0.5	

Contig4326_at	1.00E-137	Chitinase IV	Defense, PR	0.1	0.0	0.4	0.5	2HL 71.12
Contig4326_s_at	1.00E-137	Chitinase IV	Defense, PR	0.4	0.2	1.4	1.0	
HVSMEn0019F17r2_at	9.00E-30	Putative auxin-regulated dual specificity cytosolic kinase	Hormone, Auxin	0.7	0.5	0.9	0.7	
Contig398_s_at	6.00E-91	6,7-dimethyl-8-ribityllumazine synthase	Metabolism	4.8	4.4	4.1	4.8	
Contig7815_s_at	2.00E-91	Anthranilate N-benzoyltransferase protein 2	Metabolism	0.8	0.4	1.1	0.9	2HL 73.75
Contig3371_at	1.00E-120	C13 endopeptidase NP1	Metabolism	8.2	4.7	3.0	6.6	
Contig17756_at	1.00E-102	Carbonic anhydrase precursor	Metabolism	1.6	2.3	1.3	1.3	2HL 63.53
Contig10149_at	1.00E-177	Catalytic/ hydrolase	Metabolism	1.8	0.9	1.9	2.0	
Contig888_at	0	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplast precursor	Metabolism	0.3	0.5	0.2	0.3	
rbah53j14_s_at	0	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplast precursor	Metabolism	0.5	0.5	0.4	0.4	
Contig8703_at	0	Isocitrate lyase	Metabolism	0.4	0.3	0.5	0.2	
HY04F23u_s_at	5.00E-96	Mannan endo-1,4-beta-mannosidase 1 precursor	Metabolism	2.0	1.9	1.0	1.6	
Contig1795_at	0	Phenylalanine ammonia-lyase	Metabolism	1.8	2.6	1.4	1.6	
Contig9787_at	1.00E-106	Protein-tyrosine phosphatase containing protein	Metabolism	0.1	0.1	0.1	0.1	
Contig14459_at	1.00E-126	Purple acid phosphatase 1, putative	Metabolism	2.3	1.9	1.3	1.9	

Contig2403_at	1.00E-172	Putative cysteine proteinase 1	Metabolism	1.3	2.1	1.4	1.4	2HL 62.82
Contig14339_at	1.00E-48	Putative cytosolic aldehyde dehydrogenase RF2D	Metabolism	0.8	0.5	0.9	0.9	
HB08E12r_at	2.00E-18	Putative pectinesterase	Metabolism	2.0	1.8	1.4	1.7	
HVSMEm0003A13r2_at	3.00E-12	Putative ribonucleotide reductase	Metabolism	0.5	0.7	0.6	0.6	
HI05A17u_x_at	2.00E-35	S-adenosylmethionine decarboxylase proenzyme	Metabolism	2.1	1.8	1.8	1.7	
Contig10749_at	2.00E-22	Transferase family protein	Metabolism	2.3	1.2	1.2	1.8	
Contig10779_at	1.00E-167	Uroporphyrinogen III methyltransferase	Metabolism	1.0	0.5	1.0	0.8	
Contig3461_at	8.00E-52	Glutaredoxin	Miscellaneous	23.1	27.0	24.2	24.1	
HT12D12u_s_at	5.00E-48	Glutaredoxin	Miscellaneous	0.0	0.0	0.0	0.0	
HVSMEf0020F06r2_at	4.00E-30	Nuclear movement protein-like	Miscellaneous	0.0	0.0	0.0	0.0	
Contig2804_x_at	2.00E-15	Nup133 nucleoporin family protein	Miscellaneous	0.1	0.3	0.2	0.2	
rbah41a21_s_at	6.00E-16	Nup133 nucleoporin family protein	Miscellaneous	0.5	0.8	0.6	0.4	
Contig9507_at	1.00E-136	Outer envelope protein, putative	Miscellaneous	0.2	0.2	0.2	0.2	
EBpi01_SQ005_P17_at	3.00E-12	Putative ripening-related protein	Miscellaneous	25.3	26.8	32.5	16.3	
Contig927_at	1.00E-59	Retrotransposon protein	Miscellaneous	3.8	2.4	2.2	2.2	

Contig9075_s_at	9.00E-66	Retrotransposon protein, putative, Ty1-copia subclass	Miscellaneous	1.7	3.0	1.0	1.2	2HL 96.82
AF064561_at	0	Starch branching enzyme IIb	Miscellaneous	0.4	0.3	0.3	0.3	
Contig3762_s_at	0	Starch branching enzyme IIb	Miscellaneous	0.6	0.5	0.4	0.4	
HVSMEk0011G13r2_at	2.00E-12	Transposon protein, putative, CACTA, En/Spm sub-class	Miscellaneous	2.1	1.3	1.4	1.8	
Contig19484_at	1.00E-100	Tubby protein, putative	Miscellaneous	0.4	0.4	0.3	0.4	
rbaal9h21_s_at	7.00E-56	Putative Photosystem II 10 kDa polypeptide, chloroplast	Photosystem	3.3	3.8	2.6	2.4	
Contig18339_at	4.00E-78	Calcium-dependent protein kinase	Regulatory	7.5	7.2	6.2	6.0	
Contig14348_at	4.00E-60	MADS-box transcription factor 26	Regulatory	0.3	0.4	0.4	0.5	
Contig1983_at	9.00E-17	PCI domain containing protein	Regulatory	3.8	4.0	3.4	3.0	
Contig7367_at	1.00E-104	Receptor kinase LRK10 - Avena sativa (Oat)	Regulatory	6.0	14.0	1.6	3.4	3HS 6.70
HV12L19u_at	2.00E-49	Serine/threonine kinase receptor-like protein	Regulatory	3.6	2.6	3.1	4.5	
rbaal11f18_at	1.00E-72	Serine/threonine kinase receptor-like protein	Regulatory	2.7	1.8	5.3	3.7	
Contig17841_at	3.00E-60	Steroid nuclear receptor, ligand-binding	Regulatory	0.7	0.5	0.9	0.8	2HL 66.12
Contig3173_at	2.00E-23	Low temperature and salt responsive protein	Stress response	0.0	0.0	0.0	0.0	2HS 51.75
rbags19n19_s_at	3.00E-59	Salt tolerant protein	Stress response	2.3	3.2	2.1	1.7	

Contig15794_at	2.00E-70	Erg28 like protein	Unknown	0.3	0.4	0.4	0.3	
Contig383_at	4.00E-84	Expressed protein	Unknown	0.7	1.0	0.7	0.4	
Contig24230_at	1.00E-33	GRAB2 protein	Unknown	0.8	0.5	0.7	0.7	
Contig2236_at	5.00E-25	Hypothetical protein OJ1126B12.18	Unknown	0.3	0.6	0.3	0.3	
Contig8346_at	1.00E-118	Hypothetical protein OJ1200_C08.123	Unknown	11.6	6.7	10.3	10.5	2HS 32.33
Contig17632_at	5.00E-54	Hypothetical protein P0408G07.18	Unknown	4.2	3.9	4.2	4.3	
Contig1246_at	1.00E-87	Os07g0683600 protein	Unknown	0.8	0.3	0.8	0.7	
Contig1247_at	0	Osr40c1 protein	Unknown	1.3	2.0	1.1	1.0	4HL 51.30
HV_CEa0015C01r2_at	1.00E-40	Putative BLE2 protein	Unknown	0.5	0.2	0.8	0.9	
Contig11524_at			Unknown	0.0	0.0	0.0	0.0	
Contig13656_at			Unknown	1.5	2.0	1.0	1.4	
Contig14789_at			Unknown	1.6	2.1	1.7	1.7	
Contig15743_s_at			Unknown	0.4	0.8	0.5	0.4	
Contig16903_at			Unknown	0.3	0.5	0.3	0.4	
Contig18705_at			Unknown	2.2	1.4	2.6	2.8	

Contig21239_at	Unknown	2.0	1.6	1.7	2.0
Contig2304_at	Unknown	0.4	0.5	0.5	0.6
Contig25428_at	Unknown	4.7	2.7	5.4	6.1
Contig25428_x_at	Unknown	5.4	2.9	5.1	4.9
Contig9258_at	Unknown	0.1	0.2	0.1	0.1
Contig9772_at	Unknown	2.5	2.3	2.1	2.1
EBed01_SQ002_E13_at	Unknown	0.5	0.7	0.3	0.4
EBed01_SQ002_G15_at	Unknown	1.9	2.6	1.6	2.5
EBem09_SQ006_L23_at	Unknown	2.8	2.1	3.1	2.1
EBpi01_SQ004_E15_at	Unknown	0.7	0.8	0.8	0.5
HM02L01u_at	Unknown	2.1	1.6	2.2	2.2
HVSMEl0019G11f2_at	Unknown	1.1	0.4	0.9	1.0
HVSMEl0023H09r2_at	Unknown	6.7	5.4	6.2	4.2
HVSMEn0005L15f_s_at	Unknown	0.3	0.4	0.3	0.3
HY07K02u_at	Unknown	2.6	1.4	1.6	1.7

[a]Probe sets in bold were specifically induced or repressed by *F. graminearum* inoculation in the resistant genotype compared to the susceptible genotype. Probe sets in italics were detected with single feature polymorphisms.

[b]Barley gene annotations were obtained from HarvEST Barley version 1.68. Annotations were assigned when the BLASTX E-score was < 10^{-10}.

[c]PR=pathogenesis-related.

[d]Mean values of transcript expression were calculated from 3 replications. R, S, F, and W are resistant genotype, susceptible genotype, *F. graminearum*, and water inoculation, respectively. Ther numbers 48 and 96 indicate hours after inoculation.

[e]Map locations are based on 2943 SNP markers (http://harvest.ucr.edu/).

Table 3. Transcripts that exhibited differential accumulation (P≤0.0001; ≥ 2.0 fold) between the resistant and susceptible genotypes in the chromosome 2H Bin 10 NIL pair after *F. graminearum a* nd water inoculation.

Probe set name[a]	E-Score	Annotation[b]	Class[c]	Expression ratio[d]				Map location[e]
				R-F48/ S-F48	R-F96/ S-F96	R-W48/ S-W48	R-W96/ S-W96	
Contig174_x_at	5.00E-53	Histone H4	Chromatin	0.1	0.1	0.1	0.1	
Contig59_x_at	3.00E-53	Histone H4	Chromatin	3.4	3.6	3.5	3.7	
EBem06_SQ004_E23_s_at	4.00E-52	Histone H4	Chromatin	0.1	0.2	0.1	0.2	
Contig5347_at	0	Histone-lysine N-methyltransferase	Chromatin	3.6	3.5	3.8	3.5	
Contig82_at	2.00E-68	PREDICTED similar to histone 1, H2ai	Chromatin	2.7	2.7	2.7	3.1	
Contig1871_at	1.00E-136	Class III peroxidase 135 precursor	Defense, PR	0.5	0.4	0.6	0.4	
Contig17991_at	4.00E-22	Putative cytochrome b561	Membrane-related	1.5	2.0	1.3	1.2	
HVSMEf0019M19r2_s_at	2.00E-72	Pyrophosphate-energized vacuolar membrane proton pump	Membrane-related	1.6	2.0	1.1	1.2	
Contig5727_at	1.00E-142	Epoxide hydrolase 2	Metabolism	3.4	4.8	5.7	4.3	
Contig7382_s_at	1.00E-173	Fructose-1,6-bisphosphatase, cytosolic	Metabolism	0.6	0.4	0.5	0.5	
Contig6058_at	0	Putative aspartate aminotransferase	Metabolism	0.6	0.4	1.0	0.5	1HL 92.80

Contig10829_at	1.00E-107	Putative gamma-glutamyl hydrolase	Metabolism	0.2	0.2	0.1	0.2
HVSMEi0003A10r2_s_at	0	Putative linalool synthase	Metabolism	1.3	2.6	1.3	2.4
HB01M17r_at	6.00E-11	Putative NADH-ubiquinone oxidoreductase	Metabolism	7.7	7.6	5.7	6.9
EBma03_SQ003_P14_s_at	1.00E-112	Aberrant root formation protein 4	Miscellaneous	0.4	0.3	0.4	0.4
Contig11534_at	1.00E-123	Agglutinin isolectin 2 precursor	Miscellaneous	7.4	4.5	6.8	5.9
HV04J01r_at	4.00E-24	Glutathione peroxidase-like protein	Defense	8.5	4.9	12.1	11.0
Contig11796_at	0	Hemolysin	Miscellaneous	0.8	0.4	0.9	0.6
Contig2622_at	0	Multicopper oxidase family protein	Miscellaneous	0.4	0.6	0.5	0.9
Contig9573_at	0	Retrotransposon protein	Miscellaneous	2.2	2.2	2.1	2.3
Contig17168_at	6.00E-18	Transposon protein, putative, Pong sub-class	Miscellaneous	0.3	0.4	0.3	0.3
Contig22323_at	8.00E-36	26S protease regulatory subunit 6B homolog	Regulatory	1.4	2.1	1.3	1.4
Contig7505_at	6.00E-61	Protein kinase	Regulatory	0.6	0.4	0.8	0.7
Contig4100_at	3.00E-74	Signal recognition particle 54 kDa protein 1	Regulatory	2.4	2.8	2.5	3.1
Contig4099_at	0	Signal recognition particle 54 kDa protein 3	Regulatory	0.1	0.2	0.1	0.2
HV05A09u_s_at	0	Signal recognition particle 54 kDa protein 3	Regulatory	0.1	0.1	0.1	0.1

Contig11290_at	1.00E-30	Ribosomal protein S19	Ribosomal protein	5.7	6.7	6.2	5.6	1HL 97.68
Contig11291_at	8.00E-31	Ribosomal protein S19	Ribosomal protein	0.1	0.2	0.1	0.1	
Contig4508_at	7.00E-44	Pi starvation-induced protein	Stress response	0.6	0.4	0.8	0.6	1HL 93.95
Contig14226_s_at	2.00E-85	Putative iron deficiency protein Ids3	Stress response	0.4	0.4	0.3	0.2	
Contig13294_at	4.00E-50	Cis-zeatin O-glucosyltransferase 1	Toxin detoxification	2.9	3.8	2.0	1.7	2HL 96.82
Contig7898_at	1.00E-112	Peptide transporter PTR2-B	Transporter	2.6	1.1	4.3	2.4	
Contig14041_at	1.00E-45	F-box domain containing protein	Ubiquitination	3.2	2.1	3.8	3.5	
Contig15522_at	1.00E-13	Ubiquitin carboxyl-terminal hydrolase isozyme L3	Ubiquitination	0.5	0.5	0.5	0.5	
Contig16487_at	4.00E-35	Expressed protein	Unknown	1.4	2.1	1.2	1.4	
Contig5092_s_at	2.00E-42	Expressed protein	Unknown	0.8	0.5	0.9	0.6	
Contig10878_at	0	Hypothetical protein OJ1005_B11.9	Unknown	0.5	0.7	0.4	0.5	
Contig12243_at			Unknown	0.6	0.7	0.5	0.6	
Contig13137_at			Unknown	4.0	3.7	4.5	4.0	
Contig23762_at			Unknown	0.5	0.6	0.4	0.3	
Contig24822_at			Unknown	2.0	2.1	2.0	1.9	

Contig26439_at	Unknown	1.5	2.1	1.3	1.2
Contig4102_at	Unknown	4.7	3.8	4.6	3.9
HA27E07r_at	Unknown	0.5	0.9	0.4	0.3
HVSMEb0004N24r2_at	Unknown	1.6	2.1	1.6	1.6
HVSMEm0021M24r2_at	Unknown	0.2	0.4	0.2	0.2
HZ49G19r_at	Unknown	3.2	3.4	4.1	3.9

[a]Probe sets in bold were specifically induced or repressed by *F. graminearum* inoculation in the resistant genotype compared to the susceptible genotype.

[b]Barley gene annotations were obtained from HarvEST Barley version 1.68. Annotations were assigned when the BLASTX E-score was < 10^{-10}.

[c]PR=pathogenesis-related.

[d]Mean values of transcript expression were calculated from 3 replications. R, S, F, and W are resistant genotype, susceptible genotype, *F. graminearum*, and water inoculation, respectively. Ther numbers 48 and 96 indicate hours after inoculation.

[e]Map locations are based on 2943 SNP markers (http://harvest.ucr.edu/).

Table 4. Transcripts that exhibited differential accumulation (P≤0.0001; ≥ 2.0 fold) between the resistant and susceptible genotypes in the chromosome 3H Bin 6 NIL pair after *F. graminearum* and water inoculation.

Probe set name[a]	E-Score	Annotation[b]	Class	Expression ratio[c]				Map location[d]
				R-F48/ S-F48	R-F96/ S-F96	R-W48/ S-W48	R-W96/ S-W96	
Contig124_at	1.00E-67	PREDICTED: similar to H3 histone, family 2 isoform 2	Chromatin	0.2	0.5	0.2	0.1	
Contig13335_at	2.00E-80	Pectinesterase family protein	Cell wall	3.6	1.7	3.1	2.2	
Contig12254_at	1.00E-145	Putative ribosomal RNA apurinic site specific lyase	Metabolism	0.7	0.6	0.6	0.5	3HL 68.32
Contig536_at	1.00E-179	Elongation factor 2	Regulatory	0.6	0.4	0.6	0.7	
Contig3102_s_at	1.00E-124	transcription factor APFI	Regulatory	0.5	0.7	0.6	0.5	
Contig13039_s_at	5.00E-42	Hypothetical protein P0445D12.15	Unknown	1.6	2.0	1.9	1.9	
Contig21450_at			Unknown	2.0	1.5	2.8	2.5	
Contig3859_s_at			Unknown	2.8	2.9	3.4	23.9	

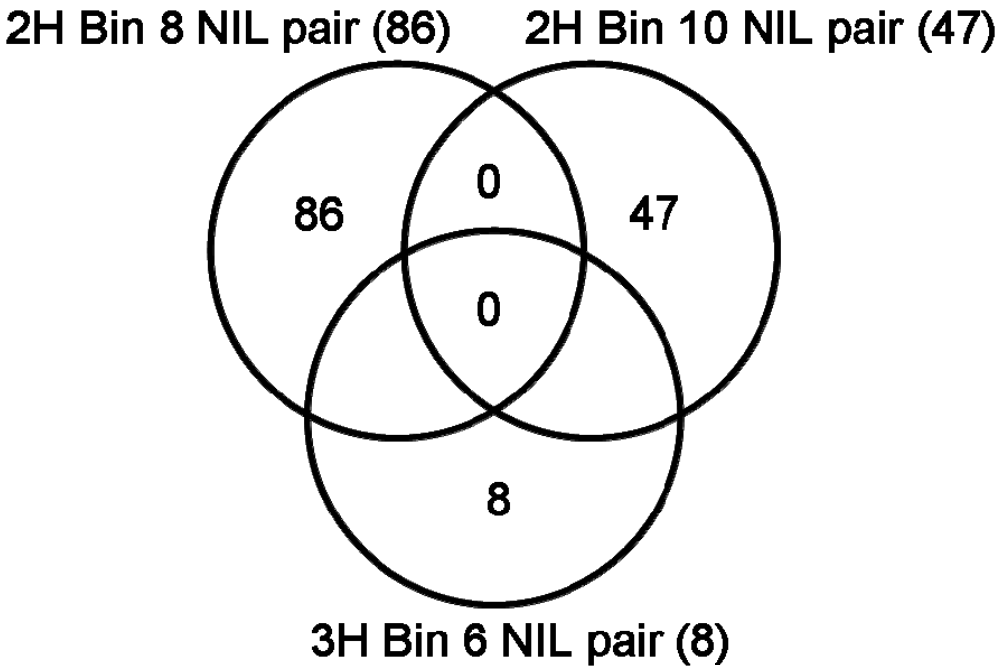
[a]Probe sets in bold were specifically induced or repressed by *F. graminearum* inoculation in the resistant genotype compared to the susceptible genotype.

[b]Barley gene annotations were obtained from HarvEST Barley version 1.68. Annotations were assigned when the BLASTX E-score was < 10^{-10}.

[c]Mean values of transcript expression were calculated from 3 replications. R, S, F, and W are resistant genotype, susceptible genotype, *F. graminearum*, and water inoculation, respectively. Ther numbers 48 and 96 indicate hours after inoculation.

[d]Map locations are based on 2943 SNP markers (http://harvest.ucr.edu/).

Figure 1. Venn diagram showing no overlap among the differentially expressed transcripts found in the chromosome 2H Bin 8, 2H Bin 10, and 3H Bin 6 NIL pairs. The number in each circle shows the number of differentially expressed transcripts in each NIL pair.



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