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ORIGINAL PAPER

Quantitative trait loci conferring resistance to Fusarium head blight in barley respond differentially to *Fusarium graminearum* infection

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Abstract Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, reduces grain yield and quality in barley. Resistance to FHB is partial and quantitatively inherited. Previously, major FHB resistant QTL were detected on barley chromosome 2H Bin 8 and 2H Bin 10, and another QTL for reduced deoxynivalenol (DON) accumulation was identified on chromosome 3H Bin 6. To develop an understanding of the molecular responses controlled by these loci, we examined DON and fungal biomass levels and the transcriptome differences in near-isogenic line (NIL) pairs carrying contrasting resistant and susceptible alleles at these QTL during *F. graminearum* infection. No overlap was found among the differentially accumulated transcripts of the three NIL pairs, indicating that the response to infection controlled by the resistance alleles at each QTL may be distinct. Transcripts showing differential accumulation between resistant and susceptible NILs were compared to results from previous wheat/barley–*F. graminearum* studies and integrated into a wheat/barley–*F. graminearum* interaction model.

Keywords Barley · *Hordeum vulgare* · Fusarium head blight · Near-isogenic line · RNA profiling

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Introduction

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch], reduces grain yield and quality in wheat and barley (McMullen et al. 1997). Contamination of grain by trichothecene mycotoxins produced by *F. graminearum* during infection, such as deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON), are the primary causes of reduced grain quality (Desjardins 2006). DON-contaminated grain increases the economic losses to barley growers as the brewing industry has a stringent requirement for DON levels of less than 0.5 ppm in harvested grain used for malting (McMullen et al. 1997).

In barley, two major FHB resistance QTL are located on barley chromosome 2H Bin 8 and chromosome 2H Bin 10 (henceforth referred to as 2Hb8 and 2Hb10, respectively) and a minor QTL for reduced DON accumulation is located

on chromosome 3H Bin 6 (referred to as 3Hb6). The resistant allele at the 2Hb8 QTL is associated with heading date (e.g., de la Peña et al. 1999). Nduulu et al. (2007) identified a recombinant in a fine mapping study that exhibited normal heading date and resistance to FHB, suggesting that the relationship between FHB and HD in the 2Hb8 region is caused by tight linkage instead of pleiotropy. The 2Hb10 FHB resistance QTL is associated with the two-row (*Vrs1*)/six-row (*vrs1*) spike type (e.g., Mesfin et al. 2003). Plants with a two-rowed spike type exhibit enhanced resistance compared to plants with a six-rowed spike type. Recent evidence suggests that resistance associated with this QTL is the pleiotropic effect of the *Vrs1* locus (Sato et al. 2008), though fine mapping of the region is necessary for verification. The 3Hb6 reduced DON accumulation QTL was detected in the Fredrickson × Stander population at 3 days after *F. graminearum* inoculation (Smith et al. 2004). Interestingly, Stander (FHB susceptible) carries the low DON accumulation allele. Although identifying these QTL has been useful for understanding the genetic architecture of FHB resistance, little is known regarding the molecular response to *F. graminearum* infection controlled by each locus.

Multiple transcriptome studies have been conducted to examine the barley and wheat host responses to *F. graminearum* infection. The transcriptomic response of susceptible barley (cultivar Morex) to *F. graminearum* infection and *F. graminearum*-derived trichothecenes has been studied (Boddu et al. 2006, 2007). Several RNA profiling studies in wheat utilizing resistant and susceptible genotypes showed very few transcripts that exhibited differential accumulation between the resistant and susceptible genotypes (Bernardo et al. 2007; Jia et al. 2009; Steiner et al. 2009; Walter et al. 2008). The results of Jia et al. (2009) were incorporated with data on the host response in barley (Boddu et al. 2006, 2007) to propose a barley/wheat–*F. graminearum* interaction model. While a useful model to describe similarities and differences in disease responses between wheat and barley and differences between resistant and susceptible wheat genotypes, there was insufficient data to similarly describe transcripts that are differentially accumulated in resistant and susceptible barley genotypes.

To gain an understanding of the molecular basis of resistance to FHB in barley, three near-isogenic line (NIL) pairs carrying resistant and susceptible alleles for QTL located on 2Hb8, 2Hb10, and 3Hb6 were examined for DON accumulation, fungal biomass, and transcript accumulation during *F. graminearum* infection. The primary objectives of this study were (1) to examine the transcript accumulation differences among these NIL pairs and (2) to compare with other wheat/barley–*F. graminearum* transcriptome studies and integrate the transcript accumulation data into a wheat/barley–*F. graminearum* interaction model.

Materials and methods

Plant materials

NIL pairs differing for the resistant and susceptible alleles for each of three FHB resistance QTL [2Hb8 QTL (Qrgz-2H-8), 2Hb10 QTL, and 3Hb6 QTL] were evaluated in this study. The 2Hb8 NIL pair was generated by Nduulu et al. (2007) through five backcrosses of a progeny from the Chevron (FHB resistant) × M69 (FHB susceptible) population carrying the Chevron allele for the EBmac0521 and Bmag0140 SSR marker interval with M69 as the recurrent parent. Multiple field trials showed that the NIL carrying the Chevron allele at the 2Hb8 region (64–72.7 cM) exhibited reduced FHB severity compared to M69 (Nduulu et al. 2007). Flanking SSR markers EBmac0521a and Bmag0140 were used to verify the genotypes.

The 2Hb10 NIL pair was developed for this study from a barley RIL population derived from the cross between Frederickson (FHB resistant) and Stander (FHB susceptible). The NIL pair used in this study was developed through self-pollinating a single F_6 plant heterozygous for the 2Hb10 region (78.6–89.9) identified from line FEG8-12 of the Frederickson × Stander mapping population (Mesfin et al. 2003) and selecting plants in the next generation homozygous for the Fredrickson and Stander alleles at this region. Growth chamber screening of the 2Hb10 NIL pair showed that the two-row line (54%) statistically ($P<0.05$) reduced the percentage of infected kernels compared to the six-row line (89%). Genotypes of the NIL pair were verified by SSR marker MBP-52 and an STS marker derived from RFLP marker MWG865.

Smith et al. (2004) developed the 3Hb6 QTL NIL pair via selfing a single F_7 plant from the line FEG8-14 of the Frederickson × Stander RIL mapping population that was heterozygous for the chromosome 3H QTL region (52.7–66.6 cM) and selecting progeny that were homozygous for contrasting alleles at the QTL. Greenhouse screens of the 3Hb6 QTL NIL pair showed that the NIL carrying the Stander allele exhibited statistically reduced levels of DON compared to the Frederickson-derived NIL (Beaubien et al. 2008). Genotypes were confirmed with SSR markers Bmag0122 and Bmac0067.

Experimental design

Seeds for the NIL pairs were planted in 6-in. pots (five seeds per pot) containing MetroMix 200 (ScottsMiracle-Gro, Marysville, OH). Plants were grown in a controlled growth

chamber with a 16-h photoperiod and 20°C/18°C day/night temperature and treated with Osmocote (14/14/14, 5 mL per pot; ScottsMiracle-Gro) at 1 and 3 weeks after planting.

Barley spikes were spray-inoculated with a freshly prepared spore suspension (10^5 macroconidia/mL) of *F. graminearum* strain Butte 86 (Fungal Genetics Stock accession number: NRRL38661) or with water 2 to 3 days after emerging from the boot. Each side of the spike was sprayed two times with inoculum. Inoculated spikes were covered entirely by clear plastic bags and sampled at 48 and 96 hai. Inoculations and sampling were conducted at 9:00 a.m., 2 h into the light cycle. Three biological replications of the experiment were conducted with a completely randomized design. Eight to ten spikes for each genotype/treatment/time point/replication combination were sampled for RNA isolation. A total of 24 samples (two genotypes, two treatments, two time points, and three replications) were examined for each NIL pair.

To ensure that the plants were inoculated and infected with *F. graminearum*, we examined disease severity and DON and ergosterol concentration. We examined disease severity on several plants from each genotype at 10–14 days after inoculation and observed disease progression (data not shown). 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; Mirocha et al. 1998). Ergosterol (a measure of fungal biomass) concentration was measured following Dong et al. (2006). *T* tests were performed in Microsoft® Excel® for Mac 2008 (v12.2.4, Microsoft Corporation, Redmond, WA) to determine significance of differences in mycotoxin and ergosterol levels.

GeneChip data analysis

The Affymetrix Barley1 GeneChip (Affymetrix, Santa Clara, CA) represents 22,439 barley genes (Close et al. 2004) and was used in this study. Total RNA was extracted and labeled, and the Barley1 GeneChips were processed following the standard protocols described in Boddu et al. (2006).

GeneChip data was obtained from barley spikes 48 and 96 h 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 (Irizarry et al. 2003) in Expressionist. Correlation coefficients were calculated between replications, which ranged from 96–99%, 90–99%, and 85–97% for the 2Hb8, 2Hb10, and 3Hb6 NIL pairs, respectively. Analysis of variance ($P \leq 0.0001$) was conducted to identify transcripts exhibiting differential accumulation by genotype effect (resistant or susceptible) for each NIL pair. A false discovery rate of 0.7%, 2%, and 16% was calculated for the 2Hb8, 2Hb10, and 3Hb6 NIL pairs, respectively (Benjamini and Hochberg 1995). Transcripts that exhibited a twofold or higher differential accumulation between genotypes for at least one time point were selected for further examination.

Gene annotations were obtained using HarvEST Barley (v 1.68; http://harvest.ucr.edu/). Barley genes were placed into a functional class if the *E* value was less than or equal to 10^{-10}; otherwise, they were regarded as unknown. The functional classes of the differentially expressed transcripts included: cell wall-related, chromatin-related, defense, hormone-related, membrane-related, metabolism, miscellaneous, mycotoxin detoxification, photosystem-related, regulatory, ribosomal protein, stress response, transporter, ubiquitination, and unknown proteins. All probe sets exhibiting a greater than or equal to 95% and an *E* value less than or equal to 10^{-84} to the *F. graminearum* genome sequence were annotated as *F. graminearum* and removed from the data set.

Single feature polymorphisms (SFPs) between the resistant and susceptible lines in each NIL pair were examined as described in Xu et al. (2009). Map locations of SFP-containing probes and differentially expressed genes were identified from the integrated SNP genetic map (Close et al. 2009).

All GeneChip data (CEL, DAT, CHP, and EXP files) are available in the Plant Expression database (http://www.plexdb.org/). The accession numbers for the 2Hb8, 2Hb10, and 3Hb6 experiments are BB70, BB69, and BB68, respectively.

Results and discussion

Location of contrasting alleles in NIL pairs

NIL pairs carrying the FHB resistant and susceptible alleles at the chromosome 2Hb8 (Nduulu et al. 2007), 2Hb10 (developed as part of this study), and 3Hb6 (Smith et al. 2004) QTL regions were used to investigate the genetic difference between the resistant and susceptible alleles at the 2Hb8, 2Hb10, and 3Hb6 QTL regions. To verify the location of contrasting alleles for each NIL pair, SFPs were identified from GeneChip data for each of the NIL pairs and located on barley chromosomes based on the barley SNP map (Close et al. 2009). Five hundred fifty-eight, 584, and 44 SFPs were identified from the 2Hb8, 2Hb10, and 3Hb6 NIL pairs, respectively, though map locations were identified for only 82, 60, and 5 of those SFPs (Supplementary Fig. 1). The 2Hb8 and 3Hb6 QTL NIL pairs were largely defined by the expected introgressed regions. However, the

2Hb10 QTL NIL pair exhibited several unexpected regions of introgression, likely due to the fact that one, eight, and three markers mapping to chromosomes 1H, 2H, and 5H, respectively, were heterozygous in the Frederickson × Stander mapping population line (FEG8-12) used to develop the NIL pair (unpublished results).

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

The accumulation of fungal biomass (as measured by ergosterol accumulation) and DON in spray-inoculated barley spikes of the NIL pairs at the 2Hb8 and 2Hb10 FHB resistant QTL and the 3Hb6 DON QTL were assayed at 48 and 96 h after *F. graminearum* and water inoculation (Table 1). Fungal biomass was significantly different between only the resistant and susceptible genotypes in the 2Hb10 pair at 48 hai; however, the values were within the range detected in the water controls, indicating that the difference is likely not biologically significant. The lack of significant difference at 96 hai indicates that the three resistant QTL do not restrict initial infection. 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. The difference between the resistant and susceptible NILs of the 2Hb8 and 3Hb6 pairs was significant ($P>0.05$) at 96 hai consistent with previous research (Beaubien et al. 2008, de la Peña et al. 1999). We observed a lack of significant differences in the 2Hb10 NIL pair, likely due to differences in the inoculation and sampling strategies used in this study compared to previous studies (cf. Mesfin et al. 2003).

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

We compared the gene transcript profiles of NIL pairs for the 2Hb8, 2Hb10, and 3Hb6 QTL during *F. graminearum* infection and mock water inoculation. Eighty-five, 47, and 8 transcripts exhibited accumulation differences between the resistant and susceptible genotypes during *F. graminearum* and/or water inoculation in the 2Hb8, 2Hb10, and 3Hb6 NIL pairs, respectively (Supplemental Tables 1, 2, and 3). Due to the possibility that SFPs may alter the transcript abundance value, significant transcripts were examined for the presence of SFPs. While SFPs do not necessarily alter detected transcript levels, discussion of transcripts identified in this study that appear to be of importance in the resistance response to FHB is limited to those which have been mapped to the general QTL region or have no SFPs.

2Hb8 NIL pair Forty-four and 41 total transcripts exhibited increased and decreased accumulation, respectively, in the resistant NIL compared to the susceptible NIL after *F. graminearum* and water inoculation (Supplemental Table 1). These numbers were reduced to 28 and 21, respectively, after identifying transcripts that mapped to the expected QTL region or did not have an SFP. Several transcripts exhibiting increased transcript accumulation include four cytochrome P450s (Contig6865_at, Contig6865_x_at, Contig16307_at, Contig14534_at), two retrotransposons (Contig927_at, Contig9075_at), a pectinesterase (HB08E12r_at) and a proline-rich protein precursor (Contig10206_s_at). The proline-rich protein and pectinesterase are involved in cell wall functions. In addition to influencing numerous physiological processes, pectines-

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
2Hb8 NIL R (Late HD)	0	**8.03±3.55**	0	0	0.47±0.55	4.23±3.10	0	0
2Hb8 NIL S (Normal HD)	0	**18.97±6.57**	0	0	0.19±0.03	4.13±1.15	0	0.5±0.87
2Hb10 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
2Hb10 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
3Hb6 NIL R (Low DON/Stander)	0	**2.73±1.71**	0	0.33±0.57	0.32	15.4	0.22	0.18
3Hb6 NIL S (High DON/Frederickson)	0	**7.96±3.46**	0	0	1.6	19.3	0.32	1.3

F48 F. graminearum 48 h after inoculation, *F96 F. graminearum* 96 h after inoculation, *W48* water inoculation 48 h after inoculation, *W96* water inoculation 96 h after inoculation

[a] Lines carrying resistant (R) and susceptible (S) alleles of the QTL, respectively. Bold values indicate significant ($P<0.05$) differences within each NIL

[b] Deoxynivalenol concentration shown was the mean value and standard deviation calculated from three replications

[c] Ergosterol concentration shown was the mean value and standard deviation calculated from three replications. The ergosterol data for the 3Hb6 NIL pair was only from one replication

terases have been shown to play a role in plant responses to pathogen and pest attack (Ithal et al. 2007; Jammes et al. 2005; Puthoff et al. 2003). Retrotransposon sequences have been shown to respond to stress. A retrotransposon was found activated in wheat in response to mycotoxigenic and non-mycotoxigenic-associated *F. graminearum* stress (Ansari et al. 2007). Cytochrome P450s are involved in a wide range of secondary metabolic processes (Schuler and Werck-Reichhart 2003), including the hydroxylation of fatty acids, and have been implicated in detoxification during plant defense (Kandel et al. 2005). They are induced in plants by *F. graminearum* infection of wheat (Kong et al. 2005) and barley (Boddu et al. 2006, 2007), and DON inoculation of barley (Gardiner et al. 2010), and accordingly warrant investigation of their importance in resistance.

Among the 21 transcripts exhibiting decreased accumulation, eight were repressed in the resistant NIL compared to the susceptible NIL specifically after *F. graminearum* inoculation. Several transcripts of interest encode a subtilisin–chymotrypsin inhibitor (Contig3381_at), a nuclear movement protein (HVSMEf0020F06r2_at), and a kinesin motor protein (Contig11000_at). Subtilisin–chymotrypsin inhibitors were found to play an important role in defense against herbivorous pests (Di Gennaro et al. 2005). Additionally, a maize proteinase inhibitor was induced in response to fungal infection as a result of wounding during penetration (Cordero et al. 1994). Nuclear movement proteins were previously shown to be important in maintaining proper number of nuclei per cell (Aumais et al. 2003). A kinesin motor protein exhibited similar transcript level patterns during *F. graminearum* and water treatment. It is unclear how lower levels of these genes might contribute to greater disease resistance, if the differences in transcript accumulation between the NILs are indeed important for the defense response.

2Hb10 NIL pair Twenty-four transcripts exhibited increased accumulation in the resistant NIL compared to the susceptible after *F. graminearum* and water inoculation (Supplemental Table 2). Ten of these gene transcripts either mapped to the expected QTL region or had no SFPs. Two transcripts encoding a cis-zeatin O-glucosyltransferase (Contig13294_at) and a glutathione peroxidase (HV04J01r_at) are related to previous identified responses to *F. graminearum* infection. UDP-glucosyltransferase activity is associated with the wheat Fusarium head blight resistance locus *Fhb1* (Lemmens et al. 2005), and *Arabidopsis* and barley UDP-glucosyltransferases have been identified that exhibit resistance to DON (Poppenberger et al. 2003; Schweiger et al. 2010). Glutathione peroxidases have been shown to protect plants from oxidative damage under biotic and abiotic stress (Csiszár et al. 2002; Miao et al. 2006). Overexpression of a glutathione peroxidase in transgenic tobacco plants enhanced stress tolerance (Yoshimura et al. 2004). That particular glutathione peroxidase was also shown to use glutathione as an electron donor to reduce hydroperoxides (Takeda et al. 2003). Accordingly, the availability of glutathione could be important for stress resistance. Indeed, increased glutathione levels appear to be important in DON resistance (Gardiner et al. 2010).

Among 23 transcripts that exhibited decreased accumulation in the resistant NIL, eight warrant further attention. Two transcripts, a hemolysin (Contig11796_at) and an expressed protein (Contig5092_s_at), were detected specifically 96 h after *F. graminearum* inoculation. A number of chromatin-related transcripts were found to be either up- or downregulated (two and one, respectively) in the resistant line after either *F. graminearum* or water treatment.

3Hb6 NIL pair Of the eight significant transcripts, seven met the additional SFP and mapping requirements for further investigation. Three exhibited increased abundance in the resistant genotype compared to the susceptible genotype (Supplemental Table 3). Among the three transcripts exhibiting increased abundance, one encoded a pectinesterase (Contig13335_at). The functions of the four downregulated transcripts are connected to nucleic acids. Elongation factor 2 (Contig536_at) and rRNA apurinic site-specific lyase (Contig12254_at) were specifically downregulated at levels greater than twofold at 96 h after treatment with *F. graminearum* or water, respectively. The downregulation of the other transcripts, transcription factor APFI (contig3102_at) and a histone (Contig124_at), was not specific to any one time point or treatment.

Comparison of gene transcripts among genotypes and between gene expression studies

There was no overlap between the specific transcripts that exhibited differential accumulation between the resistant and susceptible genotypes among the NIL pairs, though three gene types were found in common between the NIL pairs: histones (2Hb10 and 3Hb6), pectinesterases (2Hb8 and 3Hb6), and transposons (2Hb8 and 2Hb10). The lack of common transcript accumulation among the three NIL pairs may be due to: (1) the three QTL regions contained different regions of contrasting alleles; (2) the FHB resistance allele at each QTL exhibited distinct genetic mechanisms; and/or (3) the genetic backgrounds of each NIL pair is different. Different genetic composition in each of the NIL pairs may result in distinct transcript accumulation patterns. Differential gene expression in different *Arabidopsis* genotypes has been found in response to

salicylic acid (Kliebenstein et al. 2006). Thus, it is possible that the diverse transcript accumulation among the three barley NIL pairs are due to genotype differences. Alternatively, it is possible that these QTL have distinct resistance/susceptibility mechanisms that result in distinct host responses to *F. graminearum* infection.

To further study the host response to *F. graminearum* infection, comparative analyses were performed on all the transcripts identified in this study and the transcripts identified in two other barley–*F. graminearum* GeneChip studies (Boddu et al. 2006, 2007) and a barley–DON study (Gardiner et al. 2010). In each of these studies, the susceptible variety Morex was used. Five transcripts (Contig3047_s_at, cytochrome P450; Contig3381_s_at, subtilisin–chymotrypsin inhibitor 2; Contig4326_s_at, chitinase IV; Contig10779_at, uroporphyrin-III C-methyltransferase-like protein; and Contig17841_at, ligand-binding steroid nuclear receptor) from the Boddu et al. (2006) study showed differential transcript accumulation between the resistant and susceptible genotypes in the 2Hb8 pair. Contig3047_s_at was also identified in the Boddu et al. (2007) and Gardiner et al. (2010) studies. Contig4326_s_at was found in the Gardiner et al. (2010) study. All five common genes exhibited increased transcript accumulation in plants carrying the susceptible allele for the 2Hb8 QTL, indicating that these genes may be involved in the susceptible response or downregulated as part of the resistant response. We did not identify any common genes with the 2Hb10 and 3Hb6 QTL.

The gene expression data coupled with the comparisons to previous studies provides some insight into the function of these QTL. For the 2Hb8 QTL, we identified a set of genes that may be signatures for the resistant and susceptible responses. These genes will require additional study to determine their role in the resistance/susceptible interaction. For the 2Hb10 QTL, a primary question is whether resistance is due to an active mechanism or to the two-row spike morphology that results in a microclimate that is unfavorable to the pathogen. Our results do not resolve this issue but do provide tools in the form of differentially accumulating transcripts to test the idea of an active defense response. The 3Hb6 QTL exhibits reduced DON early in the progression of disease. However, at harvest the DON concentration in the grain is the same as the susceptible allele. Thus, the phenotype derived from this QTL is subtle and it is likely that only a few genes are

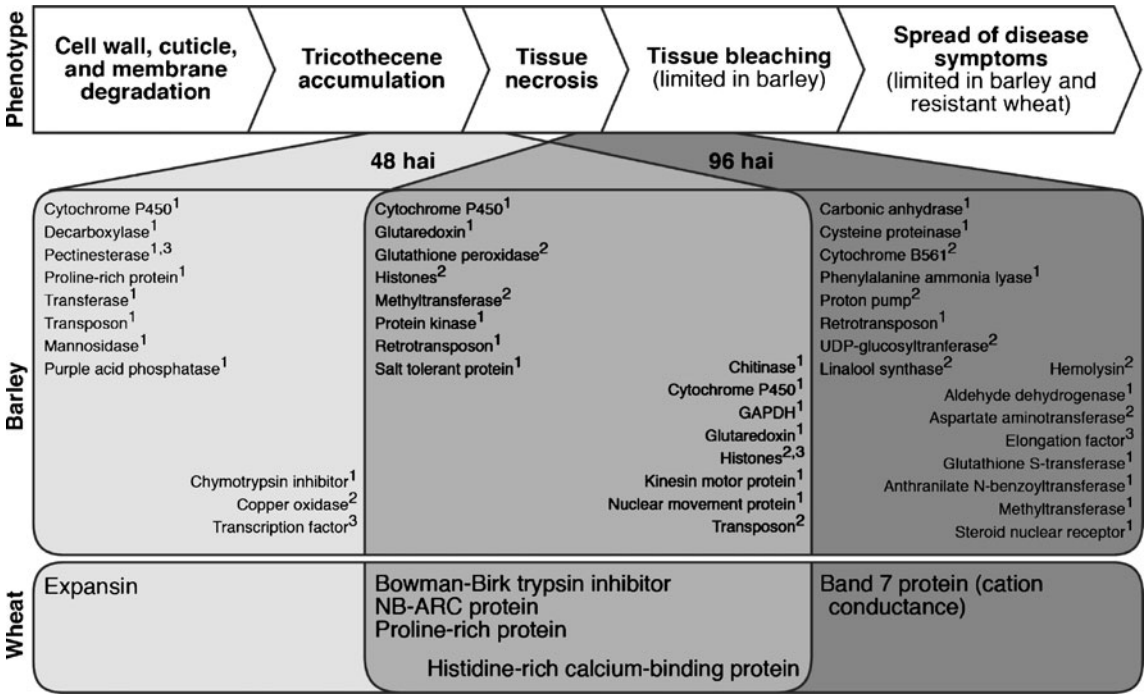

Fig. 1 Model for the barley– and wheat–*Fusarium graminearum* interaction. Barley genes (identified from 2Hb8, 2Hb10, or 3Hb6 NIL pairs, this study) and wheat genes (from *Fhb1* NIL pair; Jia et al. 2009) differentially expressed between resistant and susceptible members of each NIL pair in response to *F. graminearum* treatment were grouped by time point of detection and species. The observed phenotype corresponds to each sampled time point is indicated. Genes were included in this model only if transcripts were mapped to the expected QTL location or contained no SFPs. Gene types with multiple members are listed in multiple groups depending on when individual transcripts were detected (either time point or both time points). Left-justified gene types were detected at higher levels in the resistant NIL, while those that are right-justified are detected at higher levels in the susceptible NIL. *Superscripts* indicate which NIL pair from which the gene type was identified (*1* 2Hb8, *2* 2Hb10, *3* 3Hb6)

impacted. Our results are consistent with the idea that there is only a single gene (or very few) that confer the 3Hb6 phenotype.

Barley and wheat responses to *F. graminearum* infection

Previously, disease symptoms and transcript accumulation were examined in a wheat *Fhb1* NIL pair (Jia et al., 2009) and in the susceptible barley variety Morex (Boddu et al. 2006, 2007) in response to *F. graminearum* infection. Based on these studies, an integrated model for the wheat/barley–*F. graminearum* interactions was developed (Jia et al. 2009). While both barley and wheat are hosts of *F. graminearum*, the phenotypic response of each species is different. Type II resistant wheat limits the spread of disease, but both resistant and susceptible genotypes exhibit bleached tissue (Jia et al. 2009). Barley, even susceptible genotypes, limits both tissue bleaching and disease symptom spread (Boddu et al. 2007), indicating that barley exhibits a basal type II resistance. The previous model illustrated that there are a number of gene types induced or repressed in wheat and barley in response to *F. graminearum*. However, barley genes with differential transcript accumulation patterns between resistant and susceptible genotypes were not included. This study provides that missing data and enables a comparison with those genes detected in wheat and further development of the wheat/barley–*F. graminearum* interaction model (Fig. 1). Overall, more genes were found differentially expressed during *F. graminearum* infection between the resistant and susceptible genotypes in barley (74 transcripts) compared to wheat (14 transcripts). The difference in quantity can be attributed in part to the number of NIL pairs assessed in each study.

Interestingly, the gene transcripts exhibiting differential accumulation in the three barley QTL and wheat *Fhb1* (Jia et al. 2009) were largely different (Fig. 1). The one similarity is the gene transcripts encoding proline-rich proteins that were found in the barley chromosome 2Hb8 and wheat *Fhb1* NIL pairs. However, the available sequence data for these proline-rich protein genes indicates that they are unrelated. This lack of common genes may be highlighting the differences in the resistance mechanisms in these two plant species. The type II resistance mechanism proposed for *Fhb1* is DON detoxification through UDP-glucosyltransferase activity or a regulatory gene that controls an UDP-glucosyltransferase gene (Lemmens et al. 2005). Alternatively, barley exhibits a basal type II resistance (restricted spread of the disease), which could be the result of UDP-glucosyltransferase activity. Consistent with this idea is the identification of a barley UDP-glucosyltransferase gene from the susceptible variety Morex that exhibits resistance to DON when expressed in yeast (Schweiger et al. 2010). Thus, resistance derived from the three barley QTL may supplement basal type II resistance and may not be related to UDP-glucosyltransferase activity. Taken together, our working hypothesis is that the three barley QTL exhibit distinct resistance mechanisms from wheat. The genes we identified provide the tools to begin to address the differences between wheat and barley.

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