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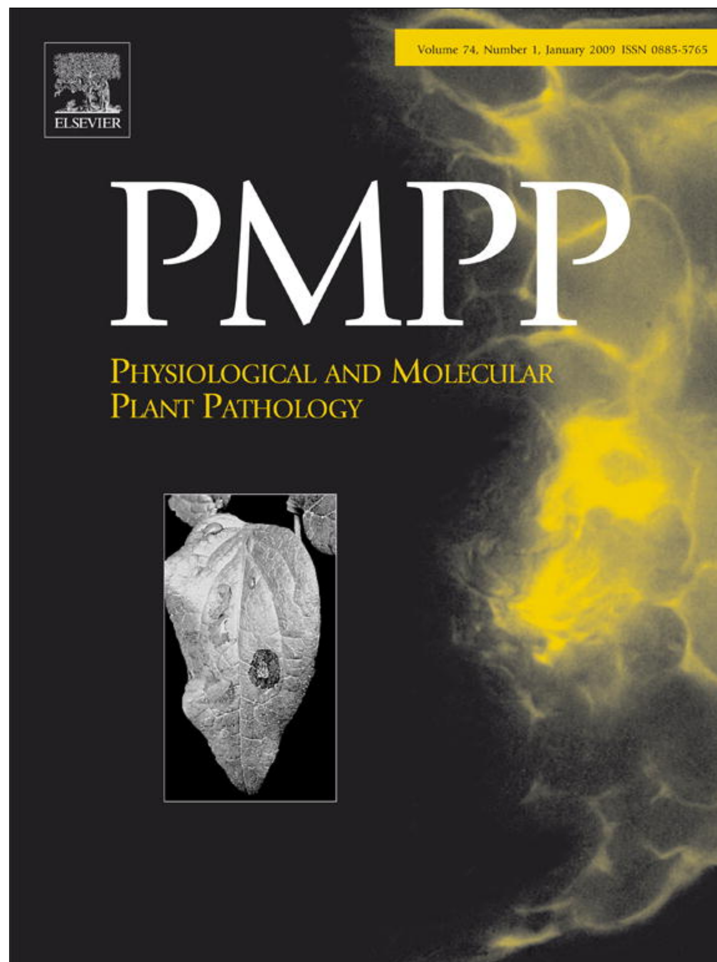

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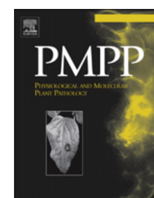

Wild barley accumulates distinct sets of transcripts in response to pathogens of different trophic lifestyles

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ABSTRACT

Wild barley (*Hordeum vulgare* subsp. *spontaneum*) accession ‘Shechem 12–32’ is resistant to multiple pathogens, including *Puccinia hordei* and *Cochliobolus sativus*, causal agents of leaf rust and spot blotch, respectively. Both pathogens begin the infection process as biotrophs, but *C. sativus* switches to necrotrophism after colonization. It is generally considered that plants require distinct arsenals of genes to successfully respond to either biotrophic or necrotrophic pathogens. To characterize the defense responses initiated by resistant wild barley accession ‘Shechem’, a temporal survey of transcript abundance after inoculation with *P. hordei* and *C. sativus* was conducted using the Barley1 GeneChip. A total of 95 and 299 gene transcripts exhibited significant ($p < 0.0001$) differential accumulation in response to *P. hordei* and *C. sativus*, respectively, compared to mock-inoculated controls, with 21 transcripts (6 defense-related) in common. Quantities of differentially accumulated gene transcripts in response to *P. hordei* were evenly distributed across examined time points, while over one-half (183) of the differentially accumulating gene transcripts in response to *C. sativus* were identified at 24 h after infection, the approximate time when the pathogen changes trophic lifestyles. Our results indicate that resistant wild barley exhibits a different host response to biotrophic and hemibiotrophic pathogens, with genes related to oxidative stress having a particularly important role in defense against hemibiotrophs.

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1. Introduction

Fungi are the most important group of plant pathogens and are responsible for the greatest percentage of economic loss due to biotic causal agents. Phytopathogenic fungi can be separated by their trophic lifestyle. Biotrophs depend on living host tissue, use early infection structures to recognize surface features of the host, and attempt to delay host cell death [1,2]. Conversely, necrotrophs feed on dead host tissue, generally achieved by killing host cells through the production and secretion of cell wall-degrading enzymes and toxins [1,3]. There also exists a hemibiotrophic group of pathogens that initially requires living tissue before transitioning to necrotrophism to complete its life cycle. As a matter of survival, plants have therefore developed generally distinct pathways to protect themselves from pathogens of different trophic lifestyles [4,5]. For example, the correct implementation of reactive oxygen species (ROS) production or removal can determine whether the host will successfully resist the pathogen or not [6,7]. Therefore, research of plant–pathogen interactions should not be solely focused on resistance to a single pathogen because the possibility of simultaneously promoting the susceptibility to other pathogens exists.

Two fungal diseases of barley (*Hordeum vulgare* L.) that are of importance include leaf rust and spot blotch, caused by *Puccinia hordei* Otth. and *Cochiliobolus sativus* (S. Ito & Kurib.) Drechs. ex Dastur [anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.], respectively. *P. hordei* is a biotroph that forms rust-colored pustules on leaf sheaths and blades. Yield losses result due to reductions in leaf size, stem strength, kernel plumpness, and number of kernels [8]. Since the 1990s, the pathogen has become increasingly important in North America when pathogen virulence arose for previously resistant genotypes carrying the resistance gene *Rph7* [8,9]. Spot blotch is a serious foliar disease that is particularly important in areas that are warm and moist during the barley-growing season [10], especially the eastern Prairie Provinces of Canada and the Upper Midwest of the United States [8,11]. The disease is characterized by distinct necrotrophic spots that extend beyond vascular bundles. Similar to the leaf rust disease phenotype, reduced photosynthetic capabilities of diseased leaves decreases

Abbreviations: Cs, *Cochliobolus sativus*; hai, Hours after infection; *Ph*, Puccinia hordei; ROS, reactive oxygen species; GST, glutathione *S*-transferase; FDR, false discovery rate; RMA, robust multi-array averaging; HR, hypersensitive reaction.

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yield by reducing kernel size and weight [8]. The pathogen is considered a model hemibiotrophic organism [10].

Recent developments of large-scale microarray platforms have provided additional approaches to investigate host–pathogen interactions [12]. For example, the Affymetrix Barley1 GeneChip, which contains 22,792 probe sets [13], has been used to evaluate the transcript profiles in response to *Blumeria graminis* f. sp. *hordei* [13–15], *Fusarium graminearum* [16,17], and *Puccinia graminis* f. sp. *tritici* [18]. The Barley1 GeneChip has not yet been used to evaluate the response of wild barley to *P. hordei* and *C. sativus*, which can provide an opportunity to explore barley responses to pathogens of differing trophic lifestyles.

A number of studies have utilized microarray-based technology to evaluate transcript accumulation in response to a variety of pathogens, though most have utilized different isolates of the same species [14,15,17–20]. The few notable studies that used distinct species evaluated transcript accumulation in tomato, *Arabidopsis thaliana*, rice, and barley [21–26]. While Güimil et al. [24] inoculated rice with pathogens of differing trophic lifestyles (*Magnaporthe grisea* and *Fusarium monoliforme*), the response to mycorrhizal *Glomus intraradices* was the primary research interest. Accordingly, there is no direct comparison of transcript accumulation between the hemibiotrophic and necrotrophic pathogens beyond the commonalities of each with the mycorrhizal response. Desmond et al. [26] recently compared data of wheat transcript accumulation in response to necrotrophic *Fusarium pseudograminearum* to previously collected data of wheat transcript accumulation in response to biotrophic *Puccinia triticina* [27] and found substantial overlap (60%) of significant transcripts between pathogen treatments. However, this overlap is more likely to be attributed to compatibility differences between the pathogens than to different trophic lifestyles.

As the genetic diversity of barley in breeding populations has become limited [28], novel traits, including disease resistance, have been sought among wild barley populations. *H. vulgare* subsp. *spontaneum*, the wild progenitor of cultivated barley, has been evaluated in a number of studies to identify and characterize resistance to a variety of foliar fungal pathogens [29–33]. Wild barley accession Shechem 12–32 exhibits resistance to six fungal diseases (leaf rust, stem rust, spot blotch, net blotch, Septoria speckled leaf blotch, and powdery mildew; [31]). In this study, we utilized the Barley1 GeneChip to analyze the transcript profiles of wild barley accession Shechem in response to two different incompatible pathogen treatments during the first 48 h of infection. Significant differentially accumulated transcripts unique to and common between treatments of the biotroph *P. hordei* and the hemibiotroph *C. sativus* suggest distinct defense responses based on the trophic lifestyle of the attacking pathogen.

2. Materials and methods

2.1. Plant material

Wild barley *H. vulgare* ssp. *spontaneum* accession Shechem exhibits resistance to a number of important barley diseases including leaf rust and spot blotch [31]. Plants were grown from seeds derived from a single 'Shechem' plant in individual plastic cones (2.8 cm × 16.5 cm deep) containing a mixture of equal parts steamed soil and Metro-Mix 200 (vermiculite, peat moss, perlite, and sand mixture; Sun Gro Horticulture, Elma, Manitoba). Barley lines susceptible to leaf rust (cultivar Harrington) and spot blotch (line ND5883) were grown under similar conditions. A controlled-release fertilizer Osmocote (14-14-14, N-P-K; ScottsMiracle-Gro, Marysville, OH) was added at a rate of 0.6 g per cone. Seeded cones were initially watered with water-soluble fertilizer Peter's Dark Weather (15-0-15, 40 g/L) and stored at 4 °C for one week before transfer to a growth chamber (20–22 °C, 14 h photoperiod, 160 W VHO fluorescent and 60 W incandescent lamps). This cold period treatment was done to break possible dormancy in the wild barley seeds and increase uniformity of germination. Plants were watered as needed. Seedlings were grown to the two-leaf stage for inoculation, about 14 days after removal from the cold room.

2.2. Inoculations and material collection

A single pustule isolate of *P. hordei* (isolate ND8702, race 8) and a single spore isolate of *Cochliobolus sativus* (isolate ND85F, race 1) were used to generate inoculum [31]. Inoculation of seedlings was performed using standard methods [31]. Briefly, *P. hordei* urediniospores (28.5 mg urediniospores per mL) mineral oil (Soltrol 170, Phillips Petroleum, Bartlesville, Oklahoma) were dispensed from gelatin capsules using rust inoculators pressurized by an air pump (27.5 kPa), with each plant receiving about 0.5 mg. *Cochliobolus sativus* inoculum (20,000 *B. sorokiniana* conidia per mL H_2O) was applied at a rate of 0.3 mL per plant using an artist's airbrush pressurized by an air pump (137.9 kPa). Control treatments of mineral oil and water were applied to plants in a similar manner as for leaf rust and spot blotch treatments, respectively. After inoculation, all plants were incubated for 16 h in the dark at 22 °C and 95–100% RH in mist chambers. Plants were initially premisted for 30 min with the humidifiers running continuously, after which the humidifiers ran for 2 min every hour. Plants remained in the mist chambers at the end of the incubation period for an additional 5 h for slow drying before being moved back to the growth chamber. Nine days after inoculation, the infection phenotypes of plants were assessed. Leaf rust infection types were determined by uredinial size using a 0–4 scale [34]. Uredinia of sizes intermediate to two numerical scores were indicated with the inclusion of a plus or minus sign. As leaf rust ratings are not always absolute, the two most predominant reaction types are reported together. Spot blotch disease ratings were determined on a 1–9 scale based on amount of necrosis and chlorosis and relative size of lesions [35].

A split-plot design was used for each of three replications. In a single replication, 320 'Shechem' plants were arranged in two racks, with each treatment plot (80 plants per inoculation of *P. hordei*, *C. sativus*, water, or oil) randomly assigned to one of the four halves of the two racks. Cardboard was used as a physical barrier to prevent cross-contamination between each treatment half-rack. Each treatment plot was further subdivided into sampling subplots. Two consecutive rows (20 plants) of each treatment plot were randomly assigned one of four harvest times (12, 24, 36, or 48 hai). The second leaves of 12–14 plants per treatment were harvested at their assigned times and frozen immediately in liquid nitrogen. The remaining unsampled plants (6–8 plants per treatment) were moved to the greenhouse for disease assessment. Control plants of 'Harrington' and 'ND5883' were assessed only for disease phenotypes.

2.3. RNA extraction and probe preparation

Leaf tissue from each replication, treatment, and time point were pooled. Labeled RNA was prepared for hybridization according to Boddu et al. [16]. Samples were submitted for hybridization to the Affymetrix Barley1 GeneChip (Affymetrix, Santa Clara, CA) and GeneChip processing to the Biomedical Image Processing Facility at the University of Minnesota. Data files are available online from PlexDB's BarleyBase (experiment BB61, http://www.plexdb.org/modules/PD_browse/experiment_browser.php?expNo=BB61).

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2.4. Data analysis

Genedata Expressionist Pro (version 5.0.25; San Francisco, CA) was used to analyze data. Data were condensed and normalized with robust multi-array averaging (RMA) using Refiner Array [36]. Using Expressionist Analyst, a maximum quality *p*-value of 1.0 was set to include all probe sets in the analysis. Data from each pathogen treatment and its control at each time point (three replications of each treatment) with transcript levels above background were analyzed by ANOVA with time and treatment effects. Transcripts with a significance of $p < 0.0001$ and false discovery rate less than 6.4% [37] were selected for further examination. Transcripts were organized into ten groups based on their transcript accumulation profiles by a K-Means clustering analysis. Fold differences were determined using in Microsoft® Excel® for Mac 2004 (v11.3.7, Microsoft Corporation, Redmond, WA). Gene classification and categorization were performed by supplementing previous classifications of probe sets [16,17] and information provided by the top BLASTX hit (top hits with an *e* value greater than 10^{-10} were considered to have an unknown function) by submitting corresponding Arabidopsis accession numbers obtained from HarvEST: Barley (v. 1.68, http://www.harvest-web.org/) to the Munich Information Center for Protein Sequences (MIPS) Functional Catalogue Database (http://mips.gsf.de/projects/funcat). GO annotations of functional category and subcellular localization were condensed to assign each transcript to one of six general categories of defense, metabolism, regulatory, transport, miscellaneous, and unknown function.

3. Results

3.1. Disease phenotypes

Wild barley accession Shechem was screened for foliar disease reactions against *P. hordei* and *C. sativus* (Fig. 1). Cultivar Harrington and line ND5883 were similarly inoculated and observed. Based on the 0 to 4 rating scale for leaf rust [34], 'Shechem' exhibited an infection type mode of 21 and a range of 12–23–. Leaf rust disease ratings are indicated by the two predominant reaction types (see Section 2.1 for more details). Some of the infection sites on 'Shechem' were flanked by necrotic tissue where the pathogen failed to successfully establish itself. In contrast, the leaf rust control 'Harrington' reliably exhibited a susceptible infection type mode of 3–4. The large and erumpent uredinia were indicative of a successful infection. Based on the 0–9 rating scale for spot blotch [35], 'Shechem' exhibited an infection type mode of 3 with a range of 2–4. The susceptible control 'ND5883' gave a susceptible infection type mode of 8 as expected. Minor damage from the mineral oil carrier was observed on some plants inoculated with leaf rust and the oil control. No cross-contamination was observed among treatments.

3.2. GeneChip analysis

To determine what genes are important in the incompatible response to *P. hordei* and *C. sativus* infection, we measured transcript accumulation in 'Shechem' at four time points after pathogen and mock treatment (12, 24, 36, and 48 h) using the Affymetrix Barley1 GeneChip. An analysis of variance (ANOVA, $p < 0.0001$) was performed at each time point for each pathogen treatment compared to its mock-inoculated control. At the $p < 0.0001$ level, transcripts of *P. hordei*-inoculated barley ranged in false discovery rate (FDR) *q*-values [37] of 4.1% (12 hai) to 6.4% (48 hai), while all q-values of *C. sativus*-inoculated barley transcripts were below 2.7% (12 hai; the lowest was 1.2% at 24 hai). Transcripts were also assessed for increased or decreased accumulation at levels greater than two-fold and clustered according to their transcript accumulation profiles (Supplemental Fig. 1). We conducted semiquantitative RT-PCR with three transcripts that exhibited greater than two-fold accumulation for at least one time point in response to both pathogens (Contig2550_x_at, Contig3635_s_at, and Contig4402_s_at) and the results were consistent with the GeneChip data (data not shown).

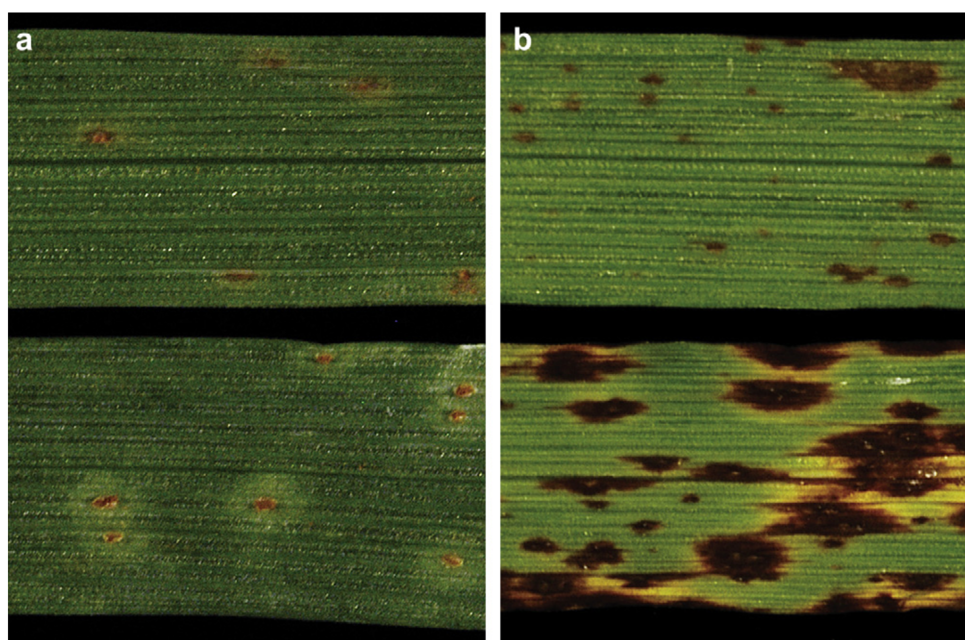

Fig. 1. Disease reactions of wild barley accession 'Shechem' to *P. hordei* and *C. sativus*. a. Inoculation of *P. hordei* on 'Shechem' (top) and control 'Harrington' results in incompatible and compatible responses, respectively. 'Shechem' exhibits small uredinia generally surrounded by necrotic areas where the pathogen ultimately fails to successfully establish. In contrast, 'Harrington' exhibits large and erumpent uredinia indicative of successful infection. b. 'Shechem' (top) and control 'ND5883' exhibit incompatible and compatible reactions, respectively, when inoculated with *C. sativus*.

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Table 1
Totals of significant differentially accumulated transcripts by pathogen and hours after inoculation, detected at $p < 0.0001$, FDR q-value $< 6.4\%$, and at greater than one- and two-fold levels.

		12 hai			24 hai			36 hai			48 hai			Total		
		T	I	D	T	I	D	T	I	D	T	I	D	T	I	D
P. hordei	all	35	30	5	23	16	7	21	17	4	19	11	8	95	72	23
	>2×	17	17	0	7	7	0	9	9	0	8	8	0	38	38	0
C. sativus	all	39	19	20	183	144	39	40	19	21	52	36	16	299	204	95
	>2×	13	11	2	114	109	5	13	13	0	32	32	0	159	152	7

Total (T), increased (I), or decreased (D) levels of transcript accumulation compared to the mock-inoculated control.

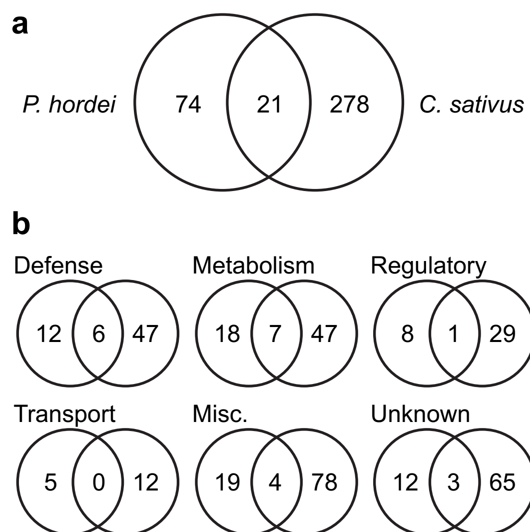

Fig. 2. Venn diagrams of significant differentially accumulated transcripts in barley inoculated with *P. hordei* and *C. sativus*. *P. hordei* and *C. sativus* components of the diagrams are indicated by the left and right circles, respectively, of each Venn diagram. a. The number of transcripts exhibiting increased accumulation within and between pathogen treatments during at least one examined time point after inoculation is indicated. b. Significant differentially accumulated transcripts were separated within each pathogen type by gene classification. The number of unique and common transcripts within each classification is indicated.

A total of 95 unique transcripts were detected in response to *P. hordei* (Table 1, Supplementary Table 1). Of these 95, 72 exhibited increased accumulation. Of the transcripts found to have increased accumulation, 38 exhibited a greater than two-fold increase compared to the mock-infected controls. There were no transcripts that exhibited decreased accumulation greater than two-fold. The number of identified transcripts slowly decreased across time points. The 12 hai time point had more than double the number of transcripts with increased accumulation at greater than two-fold levels than any of the other time points.

The *C. sativus* treatment yielded a greater number of unique transcripts with significant differential accumulation (299 total; Table 1, Supplementary Table 1). Of the 204 transcripts that increased in response to *C. sativus* infection, 152 exhibited a greater than two-fold increase compared to the mock-water control. However, unlike the *P. hordei* treatment, seven transcripts had decreased accumulation at levels greater than two-fold. These seven were identified in the first two time points, 12 and 24 hai. Interestingly, the 24 hai time point had 3.5–4.7 times more transcripts than the other time points, though the percentage of transcripts with a greater than two-fold expression difference at 24 hai is similar to the 48 hai time point (62.2% and 61.5%, respectively) and double that of 12 and 36 hai time points (33.3% and 32.5%, respectively).

Of particular interest were transcripts common between the incompatible responses of the two pathogen treatments. The majority of significant differentially accumulated transcripts (352 of 373, 94.4%), even at levels greater than two-fold (175 of 186, 94.1%), were unique to one of the pathogen treatments (Fig. 2a, Supplementary Table 1). As might be expected, the number of common transcripts was higher with a less stringent p-value. The majority of transcripts at the less stringent p-value of 0.001 (1849 of 2148, 86.1%) were unique to a particular pathogen treatment, even at accumulation levels greater than two-fold (577 of 765, 75.4%), suggesting that the trends we observed in analyses using the more stringent p-value of 0.0001 are not a product of statistical bias. At the p-value of 0.0001, there were 21 transcripts identified for at least one time point in response to both pathogens (Fig. 2a). When transcripts were divided into one of six general categories (defense, metabolism, regulatory, transport, miscellaneous, and unknown function), defense and metabolism had the greatest number of common transcripts (6 and 7, respectively; Fig. 2b). There were no common transcripts found in the transport category (Fig. 2b). Eleven of the 21 common transcripts were found at greater than two-fold levels in response to both pathogens, including all six common defense genes (EBem05_SQ002_D05_s_at, Contig2550_x_at, Contig2788_x_at, Contig3635_s_at, Contig3636_at, Contig4402_s_at; Table 2). Some transcripts were found in common at the same time point, such as Contig3636_at, a receptor kinase, and Contig4402_s_at, *PR10*, in response to both pathogens at 48 hai, while the majority accumulated at different time points between the pathogens. An additional six of the 21 common transcripts were found at levels greater than two-fold in response to *C. sativus*, but less than two-fold in response to *P. hordei*. These six accumulated in response to *C. sativus* at 24 hai only and in response to *P. hordei* at the other three time points only. The remaining four of 21 common transcripts accumulated at levels less than two-fold in response to both pathogens and at only the first three examined time points.

In addition to identifying accumulated transcripts common between the pathogen treatments, we identified transcripts that specifically responded to each pathogen in the resistance responses of 'Shechem.' Due to their well-known function in response to pathogens, we focused on the transcripts categorized as having some role in the defense response. The defense-related classifications of transcripts with increased accumulation at levels greater than two-fold could be sorted into 10 general subclasses (Table 3). Not surprisingly, the subclass of PR proteins had the highest number of total transcripts (28). Four of the common defense-related transcripts were in this PR protein category. The other two common defense-related transcripts were receptor kinases. These six common defense probe sets show different patterns of expression. For example, EBem05_SQ002_D05 is detected at one time point in response to each pathogen (*P. hordei* 36 hai and *C. sativus* 24 hai) and Contig2550_x_at was identified twice in *P. hordei* (12 and 24 hai) and once in *C. sativus* (48 hai; Table 2). There were no common transcripts that were classified as having a function related to oxidative stress (Table 3). Interestingly, the oxidative stress transcripts that were identified in response to each pathogen occurred at different time points – 12 hai for the biotroph *P. hordei* and 24, 36, and 48 hai for the hemibiotroph *C. sativus* (Table 3).

Previous efforts have identified QTL important in the defense reactions to these pathogens [29,33,38–45]. Despite the limited number of mapped Barley1 GeneChip probes [3030; Tim Close, HarvEST (http://www.harvest-web.org/)], we examined the map locations of the significantly accumulated transcripts in relationship to known QTL and R genes that confer resistance to leaf rust (4HS, 4HL, 6HL/*Rph11*, and 7HS [38–44]) and to spot blotch (3HS, 5HL and 7HS/*Rcs5* [29,44,45]). It was hoped that the combination of

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Table 2
Significant differentially accumulated transcripts common between pathogen treatments, detected at $p < 0.0001$, FDR q-value $< 6.4\%$, and indicated fold changes.

	Gene category[a] and annotation		Hours after inoculation							
			12		24			36	48	
***Ph* > 2× [b], *Cs* > 2x**										
Contig10985_at	2	Cytochrome P450	***Ph***[c]		***Cs***					
Contig15533_at	2	Subtilisin-like serine protease	***Ph***		***Cs***					
Contig3635_s_at	1	Receptor kinase	***Ph***						***Cs***	
Contig2550_x_at	1	PR4b	***Ph***		***Ph***				***Cs***	
Contig5469_at	6	Unknown function			***Ph***	***Cs***				
EBem05_SQ002_D05_s_at	1	Endochitinase precursor			***Cs***			***Ph***		
Contig2788_x_at	1	Thaumatin-like			***Cs***			***Ph***	***Cs***	
Contig11773_at	6	Unknown						***Ph***	***Cs***	
HV_CEb0001D02r2_at	3	Regulatory protein-like			***Cs***				***Ph***	
Contig3636_at	1	Receptor kinase			***Cs***			***Cs***	***Ph***	***Cs***
Contig4402_s_at	1	PR10							***Ph***	***Cs***
***Ph* 1-2×, *Cs* >2×**										
Contig5648_s_at	2	Carbohydrate metabolism-related	*Ph*		***Cs***					
Contig632_s_at	5	J-domain protein	*Ph*		***Cs***					
Contig2888_at	2	NADP malic enzyme			***Cs***		*Ph*			
Contig8402_at	2	Fatty acid-derived signaling			***Cs***		*Ph*			
Contig21140_at	2	Serine/threonine kinase			***Cs***		*Ph*			
Contig3284_x_at	5	Heat shock protein			***Cs***				*Ph*	
***Ph* 1-2×, *Cs* 1-2×**										
Contig15480_at	2	Receptor kinase-like	*Ph*	*Cs*						
HA13O21r_s_at	5	Notch protein homolog precursor	*Ph*				*Cs*			
Contig22595_at	5	Ca-binding EF hand	*Cs*		*Ph*					
Contig22948_at	6	Unknown			*Ph*		*Cs*			

[a] Gene categories: 1- Defense; 2- Metabolism; 3- Transport; 4- Regulatory; 5- Miscellaneous; 6- Unknown.
[b] Fold change values in response to the indicated pathogen [*P. hordei* (*Ph*) or *C. sativus* (*Cs*)].
[c] Bold *Ph* or *Cs* indicates greater than 2 fold change in response to indicated pathogen at indicated hour after inoculation.

Table 3
Defense-related gene classes containing significant differentially accumulated transcripts, detected at $p < 0.0001$, FDR q-value $< 6.4\%$, and at levels greater than two-fold, in response to *P. hordei* and *C. sativus*.

	Unique	Common	By pathogen		By hour after inoculation			
					12	24	36	48
All classes[a]	57	6	*Ph*	13	6	2	3	3
			Cs	50	7	25	10	21
Common classes								
Oxidative burst-related								
General	2	0	*Ph*	0	0	0	0	0
			Cs	2	0	1	0	1
Germin-like/Oxalate oxidase	4	0	*Ph*	1	1	0	0	0
			Cs	3	0	2	1	0
Glutathione-s-transferase	5	0	*Ph*	0	0	0	0	0
			Cs	5	0	4	1	0
Pathogen induced	4	0	*Ph*	1	0	0	0	1
			Cs	3	0	3	0	1
PR proteins								
General	16	2	*Ph*	2	1 (a)[b]	1 (a)	0	1 (b)
			Cs	16	3	3 (b)	4	11 (a,b)
Chitinase	6	1	*Ph*	4	2	0	2 (c)	0
			Cs	3	0	3 (c)	0	0
Peroxidase	3	0	*Ph*	0	0	0	0	0
			Cs	3	0	1	2	1
Thaumatin-like	3	1	*Ph*	1	0	0	1 (d)	0
			Cs	3	1	1	0	3 (d)
Receptor kinase-like	3	2	*Ph*	2	1	0	0	1 (e,f)
			Cs	3	1 (e)	2 (f)	2 (f)	2 (f)
Pathogen-unique classes								
Glucosyl transferase	1		*Ph*	1	1	0	0	0
MAPK-related	1		*Ph*	1	0	1	0	0
PAL-related	1		*Cs*	1	1	0	0	0
Protease inhibitor	2		*Cs*	2	1	0	0	1
R genes	2		*Cs*	2	0	2	0	0
Defense-related unknown	4		*Cs*	4	0	3	0	1

[a] Information about specific contigs available in Supplemental Table 1.
[b] Letters a–f indicate individual transcripts detected in response to both *P. hordei* (Ph) and *C. sativus* (Cs) at indicated hours after inoculation. a – Contig2550_x_at [Wheatwin-2 precursor (*PR4b*)]; b – Contig4402_s_at (*PR10*); c – EBem05_SQ002_D05_s_at (Endochitinase precursor); d – Contig2788_x_at (Thaumatin-like protein 5); e – Contig3635_s_at (Brassinosteroid Insensitive 1-associated receptor kinase 1); f – Contig3636_at (Brassinosteroid Insensitive 1-associated receptor kinase 1).

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the mapping, transcript accumulation, and QTL data could be used to elucidate the genes important in the resistance response. However, we found that 89 of our significantly accumulated transcripts have known map locations, with only 26 of those transcripts mapping to the same chromosome arm as previously identified *P. hordei* or *C. sativus* disease resistance QTL (Supplementary Tables 1 and 2; [33]). Only one of the mapped transcripts that share a chromosome arm with a QTL (Contig6013_at, a DNA binding protein) exhibits accumulation at levels greater than two-fold (2.1829 at 36 hai) in response to *P. hordei,* suggesting that the genes of interest, such as leaf rust resistance gene *Rph11*, are among those transcripts not yet mapped. Similarly, the lack of a clear transcript responsible for *C. sativus* resistance in seedlings among the mapped probes suggests additional mapping is required to successfully identify important genes such as *Rcs5* within the QTL region.

4. Discussion

Disease resistance in barley is an important component of the effort to improve yields and reduce production costs in the feed and beverage industries. *P. hordei* and *C. sativus* are particularly important pathogens in the barley-growing regions of the United States and around the world. These pathogens also represent two different trophic lifestyles of fungal pathogens. Research efforts with a number of plant species and pathogens aimed at responses to biotrophic or hemibiotrophic pathogens suggest that some defense mechanisms favor resistance to one trophic lifestyle over another [1,2,4,5]. The aim of this study was to identify transcriptional differences in wild barley resistance responses to biotrophic *P. hordei* and hemibiotrophic *C. sativus* using the Affymetrix Barley1 GeneChip.

Penetration of host cells by biotrophic pathogens is typically prevented through host responses of papilla formation, hypersensitive cell death, and accumulation of hydrogen peroxide [10,46–48]. As both pathogens examined begin the infection process as biotrophs, common significant differentially accumulated transcripts were expected to be detected at the initial examined time points, even though at 12 hai *P. hordei* penetrates stomata and *C. sativus* is only just starting to directly penetrate epidermal cells [49–54]. However, it appears that the penetration method and time frame of penetration are different enough between pathogens to induce distinct genes as there were few transcripts in common at 12 hai. Indeed, across all time points, there were only 21 common, significant differentially accumulated transcripts in response to both pathogens. This lack of commonality between transcript profiles contrasts with recent transcript evaluations of wheat in response to biotrophic *P. triticina* and necrotrophic *F. pseudograminearum* [26,27]. Desmond et al. [26] found 60% overlap of transcripts induced in response to *F. pseudograminearum* with the transcripts Hulbert et al. [27] identified in response to *P. triticina*. Our p- and fold-change values were more stringent (*p*-value of 0.0001 and fold-change of 2 to their 0.05 and 1.5), but even with a less stringent *p*-value of 0.001, the common transcripts only accounted for 13.92% of the total. One factor contributing to this difference is the resistance classification of the inoculated plants. While the barley accession used in our study is resistant to both pathogens, the wheat data of Desmond et al. [26] was collected from *F. pseudograminearum*-susceptible 'Kennedy' and compared to data of *P. triticina*-resistant 'Thatcher' [27]. Resistant and susceptible genotypes have been shown to exhibit similar defense responses, but at slower rates in the susceptible genotypes [20,55–57], which could explain the high percentage of common transcripts between the transcript profiles collected by Desmond et al. and Hulbert et al. [26,27]. This phenomenon could be explored more fully by evaluating the transcript profiles of a barley genotype susceptible to both *P. hordei* and *C. sativus*. We would expect those transcript profiles to exhibit a high level of overlap with the transcript profiles of 'Shechem' evaluated in the current study.

The response to *C. sativus* resulted in greater numbers of transcripts exhibiting increased accumulation at all time points than in response to *P. hordei*, suggesting that the method with which *C. sativus* penetrates cells throughout its biotrophic phase induces genes in addition to those required for resistance against *P. hordei*. Moreover, while the number of detected genes is essentially consistent across all time points for both pathogens, the dramatic increase at 24 hai in response to *C. sativus* corresponds to a shift from biotrophism to necrotrophism within the pathogen [10,49,58]. Further investigation with additional biotrophic and hemibiotrophic pathogens would be necessary to determine whether these phenomena are specific to these pathogens or are common to pathogens of these trophic lifestyles in general. Additionally, sampling at a finer range of hours after infection in future work would allow for more accurate correlations of corresponding points of disease progression.

Similar to other studies investigating gene expression in pathogen-inoculated cereals [16,18,26,59,60], irrespective of pathogen trophic lifestyle, we identified a number of transcripts that encode PR proteins, including chitinases and thaumatin-like proteins. Receptor kinases also appear to be less specific to trophic lifestyle. Our results are in agreement with other spot blotch studies, specifically the accumulation of *PR1* transcripts in barley [61] and the identification of transcripts of *PR* genes by 12 hai in rice using barley cDNA probes [60]. The screening of resistant and susceptible barley isolines by Zhang et al. [18] was useful to determine genes important in defense response against biotroph *P. graminis* f. sp. *tritici*. They identified Contig4402_at (*PR10*) in the incompatible interaction, as did we in response to both pathogens, suggesting an important basal defense role against fungi of this particular gene product. Transcript analysis of a barley genotype susceptible to both *P. hordei* and *C. sativus* should also prove useful to further refine the set of *PR* genes important for resistance to these pathogens, both specifically and relating to trophic lifestyle.

Genes related to oxidative stress were also identified in response to both pathogens. The detection of greater numbers of significant differentially accumulated transcripts in response to *C. sativus* initially suggested that this class of gene products has a more important role in response to hemibiotrophs at the time points assessed in this study. However, this group of detected genes can be divided between those that produce and those that remove ROSs. Transcripts of only one oxidative stress-related gene, Contig2788_x_at, an oxalate oxidase/germin-like gene, were detected in response to *P. hordei*. Transcripts from oxalate oxidase and germin-like genes also were found in response to *C. sativus*, though at later time points. These genes encode hydrogen peroxide-producing products that have been identified in a number of plants, such as barley, grape, pepper, rice, and wheat, in response to a number of pathogens, including bacteria, viruses, or biotrophic or necrotrophic fungi [62–67]. Interestingly, while these genes were identified in response to both pathogens, genes that are important in removing ROIs, such as glutathione *S*-transferases (GST) [68], were found only in response to the hemibiotrophic *C. sativus*. The observed pattern of ROS producers at the earlier stages of infection and reducers towards the later stages is supported by previous research that hypersensitive reaction (HR) as a resistance mechanism functions well against biotrophs and poorly against necrotrophs [6,46,47]. The pattern suggests that 'Shechem' targets biotrophic fungi with ROSs to stop pathogen infection, while further targeting the necrotrophic phase of *C. sativus* by removing ROSs. Indeed, this supports previous research of barley inoculated with *P. hordei* [69] and *C. sativus* [58].

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Transcripts of *Mlo3* (Contig13968_at) were identified only in response to *C. sativus*. The *Mlo* gene family is well-known for its involvement in plant response to *B. graminis* f. sp. *hordei*, specifically that the recessive alleles confer race-nonspecific resistance against the pathogen via cell death related mechanisms. Recent studies have found that barley carrying the wild-type dominant *Mlo* allele is more resistant to necrotrophic fungi (*C. sativus* and *M. grisea*) than *mlo* barley genotypes [58,70]. This resistance appears to result from increased papillae formation due to elevated H_2O_2 levels in epidermal cells, thereby reducing fungal penetration, and decreased H_2O_2 levels in mesophyll cells, resulting in lower levels of cell death. Additional research with *B. graminis* f. sp. *hordei* by Piffanelli et al. [71] supports the concept that *Mlo* negatively regulates cell death in the mesophyll cells near the penetrated epidermal cells. Piffanelli et al. [71] also found that transcript accumulation of GSTs showed similar trends to *Mlo* transcript accumulation. The detection of significant differentially accumulated *Mlo3* transcripts as well as those of GSTs at the approximate time point when *C. sativus* transitions to necrotrophism is therefore interesting. However, whether the product of *Mlo3* simply reduces susceptibility or is indeed responsible for resistance of 'Shechem' to *C. sativus* warrants further investigation. Analyses of transcription within single inoculated cells, as described by Gjetting et al. [64,72], might be a useful approach to further resolve where and when the transcripts of ROS producers and reducers are differentially accumulated.

This study has demonstrated the use of distinct sets of genes by 'Shechem' in its response to those pathogens. In particular, the type of oxidative stress-related transcripts and the timing of their accumulation appear to be important components distinguishing successful defense against pathogens of different trophic lifestyles. However, the targeting of particular oxidative stress-related proteins or those proteins of other classes unique to a trophic lifestyle for use in breeding programs should be carefully evaluated to ensure broad usage. The few defense classes with common genes, namely PR proteins and receptor kinases, might be of most interest for enhancing resistance to a wider range of pathogens.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pmpp.2009.09.006.

References

[1] Mendgen K, Hahn M. Plant infection and the establishment of fungal biotrophy. Trends Plant Sci 2002;7:352–6.
[2] Panstruga R. Establishing compatibility between plants and obligate biotrophic pathogens. Curr Opin Plant Biol 2003;6:320–6.
[3] Howlett B. Secondary metabolite toxins and nutrition of plant pathogenic fungi. Curr Opin Plant Biol 2006;9:371–5.
[4] Glazebrook J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 2005;43:205–27.
[5] Kunkel BN, Brooks DM. Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 2002;5:325–31.
[6] Govrin EM, Levine A. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Curr Biol 2000;10:751–7.
[7] Piffanelli P, Devoto A, Schulze-Lefert P. Defence signalling pathways in cereals. Curr Opin Plant Biol 1999;2:295–300.
[8] Mathre DE. Compendium of barley diseases. 2nd ed. St. Paul: American Phytophathological Society; 1997.
[9] Steffenson BJ, Jin Y, Griffey CA. Pathotypes of *Puccinia hordei* with virulence for the barley leaf rust resistance gene *Rph7* in the United States. Plant Dis 1993;77:867–9.
[10] Kumar J, Schafer P, Huckelhoven R, Langen G. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Mol Plant Pathol 2002;3:185–95.
[11] Bailey KL, Gossen BD, Gugel RK, Morrall RAA. Diseases of field crops in Canada. 3rd ed. Ontario: The Canadian Phytopathology Society; 2003.
[12] Wise RP, Moscou MJ, Bogdanove AJ, Whitham SA. Transcript profiling in host-pathogen interactions. Annu Rev Phytopathol 2007;45:329–69.
[13] Close TJ, Wanamaker SI, Caldo RA, Turner SM, Ashlock DA, Dickerson JA, et al. A new resource for cereal genomics: 22K barley GeneChip comes of age. Plant Physiol 2004;134:960–8.
[14] Caldo RA, Nettleton D, Wise RP. Interaction-dependent gene expression in *Mla*-specified response to barley powdery mildew. Plant Cell 2004;16:2514–28.
[15] Caldo RA, Nettleton D, Peng J, Wise RP. Stage-specific suppression of basal defense discriminates barley plants containing fast- and delayed-acting *Mla* powdery mildew resistance alleles. Mol Plant-Microbe Interact 2006;19:939–47.
[16] Boddu J, Cho S, Kruger WM, Muehlbauer GJ. Transcriptome analysis of the barley- *Fusarium graminearum* interaction. Mol Plant-Microbe Interact 2006;19:407–17.
[17] Boddu J, Cho S, Muehlbauer GJ. Transcriptome analysis of trichothecene-induced gene expression in barley. Mol Plant-Microbe Interact 2007;20:1364–75.
[18] Zhang L, Castell-Miller C, Dahl S, Steffenson B, Kleinhofs A. Parallel expression profiling of barley–stem rust interactions. Funct Integr Genomics 2008;8:187–98.
[19] Eulgem T, Weigman VJ, Chang H-S, McDowell JM, Holub EB, Glazebrook J, et al. Gene expression signatures from three genetically separable resistance gene signaling pathways for downy mildew resistance. Plant Physiol 2004;135:1129–44.
[20] Tao Y, Xie Z, Chen W, Glazebrook J, Chang H-S, Han B, et al. Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 2003;15:317–30.
[21] van Esse HP, Fradin EF, de Groot PJ, de Wit PJGM, Thomma BPHJ. Tomato transcriptional responses to a foliar and a vascular fungal pathogen are distinct. Mol Plant-Microbe Interact 2009;22:245–58.
[22] Thilmony R, Underwood W, He SY. Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and the human pathogen *Escherichia coli* O157:H7. Plant J 2006;46:34–53.
[23] Whitham SA, Quan S, Chang H-S, Cooper B, Estes B, Zhu T, et al. Diverse RNA viruses elicit the expression of common sets of genes in susceptible *Arabidopsis thaliana* plants. Plant J 2003;33:271–83.
[24] Güimil S, Chang H-S, Zhu T, Sesma A, Osbourn A, Roux C, et al. Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. Proc Natl Acad Sci USA 2005;102:8066–70.
[25] McGrann GRD, Townsend BJ, Antoniw JF, Asher MJC, Mutasa-Göttgens ES. Barley elicits a similar early basal defence response during host and non-host interactions with *Polymyxa* root parasites. Eur J Plant Pathol 2009;123:5–15.
[26] Desmond O, Manners J, Schenk P, Maclean D, Kazan K. Gene expression analysis of the wheat response to infection by *Fusarium pseudograminearum*. Physiol Mol Plant Path 2009. doi:10.1016/j.pmpp.2008.12.001.
[27] Hulbert SH, Bai J, Fellers JP, Pacheco MG, Bowden RL. Gene expression patterns in near isogenic lines for wheat rust resistance gene *Lr34/Yr18*. Phytopathology 2007;97:1083–93.
[28] Matus IA, Hayes PM. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 2002;45:1095–106.
[29] Yun SJ, Gyenis L, Hayes PM, Matus I, Smith KP, Steffenson B, et al. Quantitative trait loci for multiple disease resistance in wild barley. Crop Sci 2005;45:2563.
[30] Yun SJ, Gyenis L, Bossolini E, Hayes PM, Matus I, Smith KP, et al. Validation of quantitative trait loci for multiple disease resistance in barley using advanced backcross lines developed with a wild barley. Crop Sci 2006;46:1179.
[31] Fetch Jr TG, Steffenson BJ, Nevo E. Diversity and sources of multiple disease resistance in *Hordeum spontaneum*. Plant Dis 2003;87:1439–48.
[32] Steffenson BJ, Olivera P, Roy JK, Jin Y, Smith KP, Muehlbauer GJ. A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. Aust J Agric Res 2007;58:532–44.
[33] Alsop B. Utilization of *Hordeum vulgare* subsp. *spontaneum* for improvement of disease resistance in cultivated barley [thesis]. St. Paul: University of Minnesota; 2009.
[34] Levine MN, Cherewick WJ. Studies on dwarf leaf rust of barley. US Dep Agric Tech Bull 1952:1057.
[35] Fetch Jr TG, Steffenson BJ. Rating scales for assessing infection responses of barley infected with *Cochliobolus sativus*. Plant Dis 1999;83:213–7.
[36] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4:249–64.
[37] Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci USA 2003;100:9440–5.
[38] Qi X, Jiang G, Chen W, Niks R, Stam P, Lindhout P. Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. Theor Appl Genet 1999;99:877–84.

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[39] Qi X, Fufa F, Sijtsma D, Niks R, Lindhout P, Stam P. The evidence for abundance of QTLs for partial resistance to *Puccinia hordei* on the barley genome. Mol Breeding 2000;6:1–9.
[40] Borovkova IG, Jin Y, Steffenson BJ. Chromosomal location and genetic relationship of leaf rust resistance genes *Rph9* and *Rph12* in barley. Phytopathology 1998;88:76–80.
[41] Borovkova IG, Steffenson BJ, Jin Y, Kilian A, Kleinhofs A, Blake TK. Identification and mapping of a leaf rust resistance gene in barley line Q21861. Genome 1997;40:236–41.
[42] Collins N, Park R, Spielmeyer W, Ellis J, Pryor A. Resistance gene analogs in barley and their relationship to rust resistance genes. Genome 2001;44: 375–81.
[43] Backes G, Madsen L, Jaiser H, Stougaard J, Herz M, Mohler V, et al. Localisation of genes for resistance against *Blumeria graminis* f. sp. *hordei* and *Puccinia hordei* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor Appl Genet 2003;106:353–62.
[44] Steffenson B, Hayes P, Kleinhofs A. Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theor Appl Genet 1996;92:552–8.
[45] Bilgic H, Steffenson BJ, Hayes PM. Comprehensive genetic analyses reveal differential expression of spot blotch resistance in four populations of barley. Theor Appl Genet 2005;111:1238–50.
[46] Gorlach J, Volrath S, Knauf-Beiter G, Hengy G. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 1996;8:629–43.
[47] Hückelhoven R, Fodor J, Preis C, Kogel K-H. Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. Plant Physiol 1999;119:1251–60.
[48] Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB. Subcellular localization of H_2O_2 accumulation in papillae and hypersensitive response during the barley–powdery mildew interaction. Plant J 1997;11:1187–94.
[49] Bisen PS, Channy B. Some observations on the surface of wheat leaves during the early stages of infection by *Helminthosporium sativum* (P.K. & B.). J Indian Bot Soc 1983;62:285–7.
[50] Dehne HW, Oerke EC. Investigations on the occurrence of *Cochliobolus sativus* on barley and wheat. II. Infection, colonization and damage of stems and leaves. Z Pflanzenk Pflanzen 1985;92:606–17.
[51] Yadav BS. Behaviour of *Cochliobolus sativus* during its infection of barley and wheat leaves. Aust J Bot 1981.
[52] Lin KC, Bushnell WR, Smith AG, Szabo LJ. Temporal accumulation patterns of defence response gene transcripts in relation to resistant reactions in oat inoculated with *Puccinia graminis*. Physiol Mol Plant Path 1998;52: 95–114.
[53] Sellam MA, Wilcoxson RD. Development of *Puccinia graminis* f. sp. *tritici* on resistant and susceptible barley cultivars. Phytopathology 1976;66:667–8.
[54] Hu G, Rijkenberg FHJ. Scanning electron microscopy of early infection structure formation by *Puccinia recondita* f. sp. *tritici* on and in suseptible and resistant wheat lines. Mycol Res 1998;102:391–9.
[55] Lamb CJ, Ryals JA, Ward ER, Dixon RA. Emerging strategies for enhancing crop resistance to microbial pathogens. Biotechnology 1992;10:1436–45.
[56] Maleck K, Levine A, Eulgem T, Morgan A, Schmid J, Lawton KA, et al. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nat Genet 2000;26:403–10.
[57] Xiong L, Yang Y. Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. Plant Cell 2003;15:745–59.
[58] Kumar J, Hückelhoven R, Beckhove U, Nagarajan S, Kogel KH. A compromised *Mlo* pathway affects the response of barley to the necrotrophic fungus *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) and its toxins. Phytopathology 2001;91:127–33.
[59] Király L, Kumar J, Hückelhoven R, Kogel KH. *mlo5*, a resistance gene effective against a biotrophic pathogen (*Blumeria graminis* fsp. *hordei*) confers enhanced susceptibility of barley to the necrotrophic fungus *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*). Acta Biol Szeged 2002;46:135–6.
[60] Manandhar HK, Mathur SB, Smedegaard-Petersen V, Thordal-Christensen H. Accumulation of transcripts for pathogenesis–related proteins and peroxidase in rice plants triggered by *Pyricularia oryzae, Bipolaris sorokiniana* and u.v. light. Physiol Mol Plant Path 1999;55:289–95.
[61] Santén K, Marttila S, Liljeroth E, Bryngelsson T. Immunocytochemical localization of the pathogenesis-related PR-1 protein in barley leaves after infection by *Bipolaris sorokiniana*. Physiol Mol Plant Path 2005;66:45–54.
[62] Godfrey D, Able AJ, Dry IB. Induction of a grapevine germin-like protein (*VvGLP3*) gene is closely linked to the site of *Erysiphe necator* infection: a possible role in defense? Mol Plant-Microbe Interact 2007;20:1112–25.
[63] Membré N, Bernier F. The rice genome expresses at least six different genes for oxalate oxidase/germin-like proteins (accession nos. AF032971, AF032972, AF032973, AF032974, AF032975, and AF032976). Plant Physiol 1998;116:868.
[64] Gjetting T, Carver TL, Skøt L, Lyngkjaer MF. Differential gene expression in individual papilla-resistant and powdery mildew-infected barley epidermal cells. Mol Plant-Microbe Interact 2004;17:729–38.
[65] Christensen AB, Thordal-Christensen H, Zimmermann G, Gjetting T, Lyngkjaer MF, Dudler R, et al. The germinlike protein GLP4 exhibits superoxide dismutase activity and is an important component of quantitative resistance in wheat and barley. Mol Plant-Microbe Interact 2004;17:109–17.
[66] Park CJ, An JM, Shin YC, Kim KJ, Lee BJ, Paek KH. Molecular characterization of pepper germin-like protein as the novel PR-16 family of pathogenesis-related proteins isolated during the resistance response to viral and bacterial infection. Planta 2004;219:797–806.
[67] Schweizer P, Christoffel A, Dudler R. Transient expression of members of the germin-like gene family in epidermal cells of wheat confers disease resistance. Plant J 1999;20:541–52.
[68] Wagner U, Edwards R, Dixon DP, Mauch F. Probing the diversity of the Arabidopsis glutathione *S*-transferase gene family. Plant Mol Biol 2002;49:515–32.
[69] Marcel T, Varshney R, Barbieri M, Jafary H, Kock M, Graner A, et al. A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theor Appl Genet 2007;114:487–500.
[70] Jarosch B, Kogel KH, Schaffrath U. The ambivalence of the barley Mlo locus: mutations conferring resistance against powdery mildew (*Blumeria graminis* f. sp. *hordei*) enhance susceptibility to the rice blast fungus *Magnaporthe grisea*. Mol Plant-Microbe Interact 1999;12:508–14.
[71] Piffanelli P, Zhou F, Casais C, Orme J, Jarosch B, Schaffrath U, et al. The barley MLO modulator of defense and cell death is responsive to biotic and abiotic stress stimuli. Plant Physiol 2002;129:1076–85.
[72] Gjetting T, Hagedorn PH, Schweizer P, Thordal-Christensen H, Carver TL, Lyngkjaer MF. Single-cell transcript profiling of barley attacked by the powdery mildew fungus. Mol Plant-Microbe Interact 2007;20:235–46.