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Microarray analysis of *Fusarium graminearum*-induced wheat genes: identification of organ-specific and differentially expressed genes

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Summary

A wheat cDNA microarray consisting of 5739 expressed sequence tags (ESTs) was used to investigate the transcriptome patterns of the glume, lemma, palea, anther, ovary and rachis dissected from infected wheat spikes after inoculation with the fungus *Fusarium graminearum*, the causal agent of fusarium head blight (FHB) disease. Stringent conditions were employed to reduce the false discovery rate. The significance analysis of microarrays (SAM) was used to identify transcripts that showed a differential response between fungal-challenged vs. control plants. To verify the microarray data, Northern blot analysis was carried out on randomly selected up-regulated clones. We observed 185 (3.2%) up-regulated and 16 (0.28%) down-regulated ESTs in the six organs constituting the wheat spike. Many up-regulated ESTs (46.67%) showed no homology with sequences of known functions, whereas others showed homology with genes involved in defence and stress responses, the oxidative burst of H_2O_2, regulatory functions, protein synthesis and the phenylpropanoid pathway. The monitoring of genes in specific organs avoided the averaging of expression values over multiple organs that occurs when using data from the whole spike. Our data allowed us to uncover new up-regulated genes expressed in specific organs. The study revealed that each organ had a defined and distinctive transcriptome pattern in response to *F. graminearum* infection.

Introduction

cDNA microarrays are powerful tools that can be used to profile plant gene expression in different tissues or organs under different stimuli. Microarray techniques can probe the expression level of thousands of genes simultaneously, thus providing a global gene expression analysis of specific tissues at a particular time or condition (Schena *et al*., 1995). Micro-genomics, the analysis of gene expression at the tissue or organ level, may uncover genes that are differentially expressed in specific tissues. Particular physiological processes are allocated to specific cell types. Most plant microarray experiments described in the literature have used 'bulk material' with numerous contaminating cells, which results in averaged information rather than reflecting the individuality of specific tissues present in the plant. Tissue-specific gene expression data are lost or diluted when assaying such bulk material (Brandt, 2005).

Recently, a genome expression atlas of 24 733 genes for 18 organ or tissue types has been generated in *Arabidopsis* (Ma *et al*., 2005). Each tissue type has a defining genome expression pattern revealed by a subset of genes showing at least a twofold higher expression than in any other non-homologous tissues. This defined transcriptome is especially true for organs biologically distant from each other. In addition, only about 16% of the total number of genes are commonly expressed in all organs, thus showing little 'cross-talk' between different cell types. These include the housekeeping genes for fundamental cellular processes, such as protein synthesis, energy metabolism, DNA synthesis and cell division.

Fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch] is a

devastating disease of wheat (*Triticum aestivum* L.) and other small grains (Gilbert and Tekauz, 2000). Wheat infection by FHB results in significant yield losses and severe grade penalties (www.agriline.it). The fungus produces trichothecene mycotoxins, such as deoxynivalenol (DON), which contaminates infected grains and has been linked to livestock feed refusal, and depression of the immune system, nausea and vomiting in humans (Hall and Van Sanford, 2003). The development of resistant cultivars has long been recognized as the most cost-effective way to combat the disease. Resistance to FHB is very complex and is considered to be quantitatively governed by numerous resistance genes located on different chromosomes (Jahoor *et al*., 2004). Indeed, at least 18 of the 21 wheat chromosomes have been reported to be associated with resistance (Fedak *et al*., 1998; Buerstmayr *et al*., 1999).

Of the many recent plant microarray-based studies, most have addressed transcriptome changes during the development of the plant or under abiotic stress conditions. These include, amongst others, *Arabidopsis* organ development (Ma *et al*., 2005), maize embryo maturation (Lee *et al*., 2002), potato tuber formation (Kloosterman *et al*., 2005), salt stress in rice (Kawasaki *et al*., 2001) and drought, cold and salt stress in barley (Atienza *et al*., 2004). There are fewer reports on the application of microarrays to study plant–pathogen interactions. Studies have been published on *Arabidopsis* (Narusaka *et al*., 2003), rice (Shim *et al*., 2004), maize (Baldwin *et al*., 1999) and barley (Caldo *et al*., 2004). Although public microarray databases are available online for *Arabidopsis* (SIGnAL, http://signal.salk.edu/), maize (http://www.maizegdb.org/) and barley (http://www.affymetrix.com), presently there is no publicly available information for wheat. These databases have expanded our knowledge on the molecular mechanisms involved in plant–pathogen interactions.

In this study, we dissected wheat spikes into six major organs, including glume, lemma, palea, anther, ovary and rachis. Gene expression was monitored by the two-sample design (Yang and Speed, 2003). For each of the six organs, transcripts that were differentially expressed between the fungal-challenged and water control wheat spikes were analysed. Co-hybridization of different colour-labelled cDNA probes to a single microarray slide allowed for the direct comparison between challenged vs. control. By analysing individual organs, new genes that were differentially expressed on fungal attack were identified. Microarrays representing 5739 wheat expressed sequence tags (ESTs), derived from a suppression subtraction hybridization (SSH) library of wheat–*F. graminearum* interactions, were used for organ-specific analysis of the wheat response to infection. The ESTs described here were deposited in the public databases of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) with GenBank accession numbers. The microarray expression data were deposited in the ArrayExpress Gene Expression Database (http://www.ebi.ac.uk/arrayexpress) under accession number E-TABM-97.

Results and discussion

SSH library

The SSH library was constructed by subtracting the control cDNAs from the fungal-challenged cDNAs. This population of subtracted cDNAs was enriched for low-abundance transcripts and thus reduced the number of redundant ESTs spotted on the arrays. Redundant ESTs were grouped into contigs using the CAP3 assembly program (Huang and Madan, 1999) with a minimum match of 40 and a minimum score of 90. Each EST in a contig is a transcript from either the same gene (overlapping sequences) or from multiple paralogous members of a multigene family. For the SSH library, 593 (41%) singletons having only one EST were found. The majority of the remaining ESTs were present as two to four ESTs in a contig. These results demonstrated that the constructed SSH library greatly reduced the large number of redundant transcripts found in a typical cell.

Identification of ESTs differentially expressed in response to *F. graminearum* infection

Significance analysis of microarrays (SAM) and gene filtering resulted in the identification of 201 ESTs whose transcript level was significantly altered in *F. graminearum*-infected tissues. From the 5739 arrayed elements, 185 ESTs (3.2%) were identified as being significantly up-regulated (Table 1) and 16 (0.28%) as down-regulated (Table 2). The CAP3 assembly program was used to group redundant ESTs into contigs with a minimum match of 40 and a minimum score of 90. Of the 185 up-regulated ESTs, 51 (27%) were found as singletons with only one EST. Of the remaining ESTs, 52 (28%) were found in contigs with four or fewer ESTs. Contigs with 5–15 ESTs (21%) were classified as abundant gene transcripts. A single contig (DV799653) from the ovary contained an abundant number of transcripts of unknown function, having 43 (23%) of the total number of ESTs. From all six organs, there were 75 up-regulated and 16 down-regulated unigenes.

Functional annotation and gene classes

Putative functions were assigned to the ESTs, responsive to *F. graminearum*, on the basis of homology searches against

Table 1 Functional classes of expressed sequence tags (ESTs) that were significantly up-regulated in organs of wheat spikes challenged with *Fusarium graminearum*

Accession number*	Organ†	Annotation‡	Number of ESTs§	Expression ratio (T/C)¶
Metabolism				
DV799695	R	Phosphatidylinositol synthase	1	3.97
DV799649	P, R	Methionine synthase	2	1.82
DV799705	G, P, R	Glyceraldehyde-3-phosphate dehydrogenase	4	1.77
DV799631	G, L, P, R	Cytosolic glyceraldehyde-3-phosphate dehydrogenase	1	2.05
DV799735	R	Dihydroneopterin aldolase, putative	2	2.05
DV799729	R	Cinnamate-4-hydroxylase	1	1.83
DV799679	G	CER 1, putative	1	1.49
Energy				
DV799600	L	Ubiquinol-cytochrome *c* reductase	1	1.47
BQ901689	G, P	Chlorophyll *a*/*b*-binding protein 3C-like	2	1.66
DV799694	R	Photosystem II 10K protein	1	2.28
DV799701	R	Photosystem II polypeptide	1	1.7
Transcription				
NP199318	A	Transcription factor (WRKY DNA-binding domain)	1	4.5
Protein synthesis				
DV799739	R	40S ribosomal protein S15A	1	1.63
DV799744	R	40S ribosomal protein S15A	1	1.55
DV799626	G, R	40S ribosomal protein 25S	2	1.61
DV799723	G, P, R	60S ribosomal protein L10	6	1.69
DV799663	G, P	Ribosomal protein L13a	2	1.63
DV799691	G	Ribosomal protein S10	1	1.5
DV799746	P, R	Elongation factor 1α-subunit (TEF1)	3	1.76
DV799748	G, P, R	Elongation factor 1α-subunit (TEF1)	3	1.86
Protein fate (folding, modification, destination)				
DV799611	P, R	Heat shock protein 70	4	1.57
DV799719	R	Vacuolar sorting receptor protein	1	1.65
DV799603	G	Der1-like protein	1	1.63
DV799750	A, R	α-Mannosyltransferase-like	1	3.54
Cellular transport, transport facilitation and transport routes				
NP195236	A	Plasma membrane intrinsic protein (water channel)	1	3.47
DV799737	R	ADP-ribosylation factor	1	2.28
DV799693	G, A	Syntaxin of plants 52	1	1.74
Cellular communication/signal transduction mechanism				
DV799683	R	Leucine-rich repeat protein	2	1.98
DV799732	G	Receptor-like kinase Xa21-binding protein 3	1	1.45
Cell rescue, defence and virulence				
DV799703	G, L, P, R	β-1,3-glucanase II	15	1.89
DV799682	G, L, P	Class I chitinase	11	2.19
DV799658	P	Class I chitinase	1	2.69
DV799607	G, A	Thaumatin-like protein TLP4	2	1.51
DV799670	G	Ascorbate peroxidase	1	1.65
DV799602	G	Monodehydroascorbate reductase	1	1.51
DV799727	R	Metallothionein	4	3.15
BQ901288	G	Polyphenol oxidase	1	1.5
NP174620	A	Disease resistance protein (CC–NBS–LRR class)	1	3.92
NP195056	A	Disease resistance protein (CC–NBS–LRR class)	1	4.56
NP199463	A	Disease resistance protein (TIR–NBS–LRR class)	1	3.33
Unclassified proteins				
BQ901310	G	Hypothetical protein	1	1.57
DV799599	R	Hypothetical protein	1	1.86

Table 1 *Continued*

Accession number*	Organ†	Annotation‡	Number of ESTs§	Expression ratio (T/C)¶
DV799648	G	Expressed protein	1	1.49
DV799668	R	Unknown protein	1	1.77
DV799684	R	Unknown protein	1	1.75
DV799643	R	Unknown protein	1	1.63
DV799690	R	Unknown protein	2	1.77
DV799661	G	Unknown protein	2	1.45
DV799655	G, L, P	Unknown protein	8	2.1
DV799672	G, L, P	Unknown protein	6	2.35
DV799675	R	Unknown protein	2	4.09
DV799712	R	Unknown protein	2	1.95
DV799640	R	Unknown protein	2	2.19
DV799728	O	Unknown protein	3	3.71
DV799653	O	Unknown protein	43	3.73
DV799646	A	Unknown protein	1	1.56
DV799687	R	Unknown protein	1	1.64
DV799757	G, P	Unknown protein	1	2.39
DV799698	O	Unknown protein	1	3.72
DV799696	G	Unknown protein	1	1.59
DV799680	G	Unknown protein	1	1.46
DV799689	G	Unknown protein	1	1.51
DV799704	G	Unknown protein	1	1.52
DV799706	G, A	Unknown protein	1	1.52
DV799758	G	Unknown protein	1	1.48
DV799604	G	Unknown protein	1	1.5
DV799613	R	Unknown protein	1	1.54
DV799615	G	Unknown protein	1	1.42
DV799618	O	Unknown protein	1	3.5
DV799650	G	Unknown protein	1	1.53
DV799665	A	Unknown protein	1	1.48
DV799666	G	Unknown protein	1	1.43
DV799721	G	Unknown protein	1	1.88
DV799734	G	Unknown protein	1	1.44
DV799738	G	Unknown protein	1	2.11

*GenBank accession numbers for unigenes. The GenBank accession number corresponds to one of the ESTs representing the group.

†Organs in which significant up-regulation was detected: A, anther; G, glume; L, lemma; O, ovary; P, palea; R, rachis.

‡The 185 ESTs for which the transcript level was significantly up-regulated at 24 h after fungal inoculation are listed by functional class according to the Munich Information Centre for Protein Sequences (MIPS; http://mips.gsf.de) Functional Catalogue (FunCat) scheme.

§Number of EST components in each contig is listed.

¶Expression ratio (T/C): signal intensity ratio of treated (fungus-challenged) over the water control.

the GenBank non-redundant database using the blastx algorithm with the default parameters. The maximum probability threshold for a sequence match was set at 10^{-5}. Sequences that produced hits with *E* values of greater than 10^{-5} were assigned as unknown protein. The differentially expressed sequences were classified to different functional classes following the Munich Information Centre for Protein Sequences (MIPS; http://mips.gsf.de) Functional Catalogue (FunCat) scheme (Tables 1 and 2).

Unigenes with the most ESTs probably represent differentially expressed genes that are abundantly expressed during *F. graminearum* infection. Genes encoding the pathogenesis-related (PR) proteins, including the β-1-3-glucanases (PR-2), chitinases (PR-3) and thaumatin-like proteins (PR-5), were induced (Table 1; Cell rescue), in agreement with a previous study by Pritsch *et al*. (2000). The induction of chitinase is often associated with the induction of β-1-3-glucanases and other PR proteins (Collinge *et al*., 1993). Chitinase and β-1-3-glucanase inhibit fungal growth by degrading chitin and (1,3)-β-glucan, both found in the cell wall of growing hyphae. The thaumatin-like proteins have been shown to have antifungal activity (Vigers *et al*., 1992). These antifungal proteins are considered

Table 2 Functional classes of expressed sequence tags (ESTs) that were significantly down-regulated in organs of wheat spikes challenged with *Fusarium graminearum*

Accession number*	Organ†	Annotation‡	Number of ESTs§	Expression ratio (T/C)¶
Metabolism				
DW986531	A, R	Succinyl-CoA ligase	1	0.72
Energy				
DW986529	A, R	Light-harvesting complex IIa protein	1	0.71
Cellular transport, transport facilitation and transport routes				
DW986528	R	PIP aquaporin isoform	1	0.73
DW986530	A, R	Mitochondrial phosphate translocator	1	0.68
Unclassified proteins				
DW986532	A, R	Unknown protein	1	0.74
DW986533	A	Unknown protein	1	0.76
DW986534	R	Unknown protein	1	0.74
DW986535	P, A, R	Unknown protein	1	0.75
DW986536	A, R	Unknown protein	1	0.68
DW986537	R	Unknown protein	1	0.77
DW986538	P, A, R	Unknown protein	1	0.71
DW986539	A, R	Unknown protein	1	0.78
DW986540	R	Unknown protein	1	0.72
DW986541	R	Unknown protein	1	0.75
DW986542	R	Unknown protein	1	0.74
DW986543	R	Unknown protein	1	0.76

*GenBank accession numbers for unigenes. The GenBank accession number corresponds to one of the ESTs representing the group.

†Organs in which significant down-regulation was detected: A, anther; G, glume; L, lemma; O, ovary; P, palea; R, rachis.

‡The 16 ESTs for which the transcript level was significantly down-regulated at 24 h after fungal inoculation are listed by functional class according to the Munich Information Centre for Protein Sequences (MIPS; http://mips.gsf.de) Functional Catalogue (FunCat) scheme.

§Number of EST components in each contig is listed.

¶Expression ratio (T/C): signal intensity ratio of treated (fungus-challenged) over the water control.

to be part of the general defence response as they are induced in many different plant–pathogen systems.

The significant increase in the level of transcripts encoding proteins related to active oxygen species (AOSs), such as ascorbate peroxidase (AXP), monodehydroascorbate reductase and metallothionein (MT) genes, indicates the induction of an oxidative stress in response to *F. graminearum* infection (Table 1; Cell rescue). In agreement with our observation, Zhou *et al*. (2005), using two-dimensional displays of proteins, recently identified several up-regulated proteins with antioxidant function, indicating the induction of an oxidative burst of H_2O_2 in *F. graminearum*-infected wheat tissues. Plant cells often produce AOSs, such as superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), as a first reaction to contact with pathogens (Kauss *et al*., 1999). The induced level of AOSs can exert detrimental effects on both host and pathogen tissues by damaging membranes, proteins, chlorophyll and nucleic acids (Pryor, 1997). AXP catalyses the scavenging of AOSs and is part of the plant's defence system against toxic oxygen intermediates (Mittler, 2002).

The level of transcripts encoding heat shock protein 70 (Hsp70) was significantly increased in *F. graminearum*-challenged organs (Table 1; Protein fate). The synthesis of such proteins has been reported to increase after various forms of abiotic and biotic stress (Lu *et al*., 2003; Wang *et al*., 2004). These molecular chaperones function by helping in the folding of nascent polypeptide chains, the refolding of denatured proteins and the prevention of irreversible protein aggregation and insolubilization (van Montfort *et al*., 2002). They increase the rate of folding and thus increase the resistance of cells under stress conditions.

The data (Table 1) suggest that pathogen-induced signal transduction pathways play a role in the wheat–*F. graminearum* interaction. These include sequences that show homology to the receptors of disease resistance genes, proteins such as phosphatidylinositol synthase that are involved in major signalling pathways, putative receptor-like kinase protein and sequences with the conserved domain of the WRKY transcription factor superfamily. These signal transduction pathways have been shown to be highly specific for particular pathogen

races. This result was surprising as the wheat–*F. graminearum* interaction is generally considered to be race non-specific (van Eeuwijk *et al*., 1995). The majority of cloned disease resistance genes encode for proteins with a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region, the latter conferring a high degree of specificity for pathogen strains. The NBS–LRR class of disease resistance gene is abundant in plants, with at least 200 different genes in *Arabidopsis* (Meyers *et al*., 1999). This class of gene has been shown to be inducible on fungal stress in rice (Shim *et al*., 2004), but members of this class of gene were only induced in wheat anthers in our studies. Hart *et al*. (2005) reported several resistance genes encoding proteins with NBS and LRR domains that were significantly up-regulated in *Arabidopsis* cell cultures exposed to DON, a toxin produced by *F. graminearum*. Their findings suggest that DON triggers a pathway which plays a primary role in the initiation of a specific defence response. The WRKY proteins are a superfamily of transcription factors involved in the signal transduction pathway, that are induced early on in pathogen attack, and have up to 100 different members in *Arabidopsis* (Eulgem *et al*., 2000). This class of DNA-binding protein recognizes the W-box of promoters of a large number of defence-related genes and, to date, is unique to plants. These genes were significantly up-regulated exclusively in the anthers of *F. graminearum*-challenged wheat.

The plant's responses to pathogen infection included changes in the translation machinery in the host tissues (Table 1; Protein synthesis). Trichothecene toxins, such as DON, produced by the fungus are able to block eukaryote peptidyl transferase activity at the initiation of elongation, thereby inhibiting protein synthesis (Poppenberger *et al*., 2003). Kruger *et al*. (2002) reported several ribosomal proteins (RPs) and translation elongation factors (TEFs) that were induced in wheat tissues infected by *F. graminearum*. Results from a later study showed that RPs were a target for DON. Di and Tumer (2004) reported a wheat transgenic line over-expressing RPs which resulted in increased resistance to DON. In agreement with previous findings, we identified several RPs and TEFs (TEF-1) that were significantly induced in the fungus-challenged organs of the glume, palea and rachis.

Phenylpropanoid metabolism has been implicated in plant defences against both biotic and abiotic stress (Dixon and Paiva, 1995). The enzymes cinnamate-4-hydroxylase (C4H) and polyphenol oxidase were significantly induced in specific *F. graminearum*-challenged tissues (Table 1; Metabolism, Cell rescue). The C4H enzyme catalyses the second reaction of the phenylpropanoid pathway, which gives rise to a wide array of important metabolites, including lignin (Ro and Douglas, 2004). Lignin is a complex of polyphenolic structures responsible for the thickening and strengthening of plant cell walls. In response to *F. culmorum* infection, the accumulation of lignin was greater in resistant wheat cultivars than in susceptible ones (Kang and Buchenauer, 2000). A rapid deposition of lignin into plant cell walls as an early reaction to fungal attack provides an effective barrier to the penetration and growth of many pathogenic fungi (Dushnicky *et al*., 1998). Another indication of changes in the content of phenolic compounds in tissues infected by *F. graminearum* comes from the increased level of transcripts encoding polyphenol oxidase. These proteins catalyse the oxidation of phenols to quinines (Mayer and Harel, 1979).

A total of 73 (40%) sequences represented novel transcripts of unknown function for which BLAST searches yielded no homology in the databases (Table 1; Unclassified proteins). This result was very similar to that of Kruger *et al*. (2002), who found that 49% of the total FHB-induced ESTs were of unknown function. The BLAST search against the *F. graminearum* genome and COGEME fungal databases showed no differentially expressed transcripts originating from the fungal genomes. These fungal sequences were most probably of low abundance as the RNA was isolated from plant organs just 24 h after inoculation with the fungus.

cDNA microarray controls and RNA blot validation

The universal control system (Stratagene, LaJolla, CA, USA) was used to validate the microarray data. Hybridization across the array was considered to be uniform when consistent signals from replicated spots were observed. The hybridization specificity was evaluated by the lack of signal from the negative controls of alien sequences, 3 × saline-sodium citrate (SSC), human Cot-1 DNA and salmon sperm DNA that were spotted on the arrays. The positive spiked labelled cDNAs were used to validate the normalization of the data for differences in cyanine dye (Cy5/Cy3) incorporation and hybridization efficiencies. The observed yellow spots on the array for the spiked elements showed the normalization of the data.

Five up-regulated genes were randomly selected and subjected to validation by Northern analysis. Representative RNA gel blots are shown in Figure S1 ('Supplementary material'). The relative intensity signals were depicted visually. For the loading control, rRNA band intensities on gels stained with ethidium bromide verified that equimolar amounts of total RNA for both *F. graminearum*-inoculated and water control samples had been loaded on the gel. The overall abundance of transcripts for each of the genes analysed was greater in the

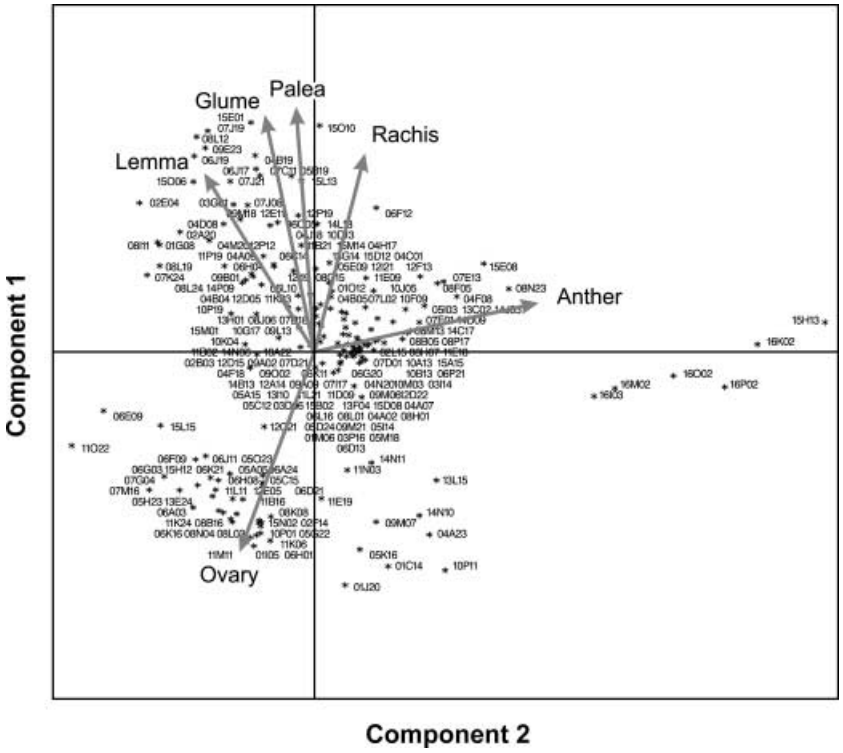

Figure 1 Principal components analysis (PCA) projection of expression data for expressed sequence tags (ESTs) up-regulated in organs infected by *Fusarium graminearum*. Data are spanned according to principal components 1 and 2 that together accounted for 72% of the variation in the dataset. The angles between arrows pointing to different tissues represent the linear correlations between tissue types. Genes with a similar expression pattern profile are plotted close to each other and to the defined component that matches their expression.

samples inoculated with *F. graminearum* than in the controls. These results were in agreement with the microarray data.

Organ-specific transcriptome analysis

Analysis of variance (ANOVA) on ESTs that significantly responded to FHB infection revealed that the expression patterns for 36% of these ESTs differed significantly ($\alpha = 0.01$) between organs (Table S1, 'Supplementary material'). Principal components analysis (PCA) was used to visualize the transcriptome profile of all 185 up-regulated ESTs in the six dissected organs (Figure 1). The analysis was designed to define three components that accounted for most of the variance of the expression data. Figure 1 represents the PCA projection of expression data using components 1 and 2 that together accounted for 72% of the variance in the dataset. The distribution of the 185 EST patterns in relation to the PCA components strongly supported the existence of significant variation between organs and generated a visual proof. The bract tissues of lemma, palea and glume were visually grouped together with the rachis showing a close relationship. The anther and ovary each showed unique transcriptome expression patterns. Studying the organ-specific expression of the *Arabidopsis* genome, Ma *et al*. (2005) showed that each organ or tissue type had a defining transcriptome expression pattern, and that the degree to which organs shared expression profiles was highly correlated with the biological relationship

Table 3 Correlations of gene expression patterns between organs of wheat spikes challenged with *Fusarium graminearum*

Organ	Glume	Lemma	Palea	Anther	Ovary
Lemma	0.67†				
Palea	0.81†	0.65†			
Anther	0.01 (NS)	–0.01 (NS)	0.13 (NS)		
Ovary	–0.60†	–0.27†	–0.57†	–0.16*	
Rachis	0.47†	0.35†	0.62†	0.15*	–0.55†

NS, not significant.
*Significant at $\alpha = 0.05$.
†Significant at $\alpha = 0.01$.

of the organ types. In our study, significantly different expression profiles were observed between the reproductive organs (anther and ovary) and vegetative organs (glume, lemma, palea and rachis).

The organ-specific gene expression patterns of ESTs responding to fungus infection were further reflected in the values of linear correlations between tissues (Table 3). Expression patterns in the three bract tissues, glume, lemma and palea, were found to be positively correlated, with the highest correlation between the glume and palea ($r = 0.80$, $P < 0.0001$). To a lesser degree, EST patterns in the rachis were also positively correlated with those in the bract tissues. The anther EST pattern, in general, showed no significant correlation with

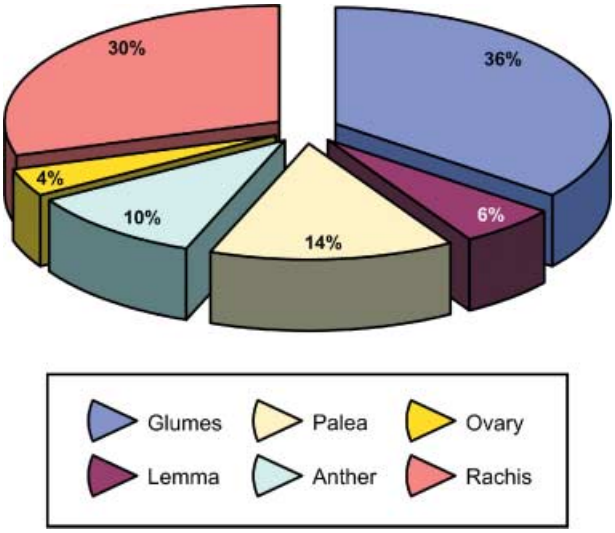

Figure 2 Percentage of 75 unigenes induced in *Fusarium graminearum*-infected organs.

other organs, but the ovary showed a significant negative correlation with all other organs, confirming the organ-specific structure observed in PCA.

Differences between organs also included the number, as well as the nature, of unigenes that were identified as differentially expressed during *F. graminearum* infection. Considering the number of unigenes as a measure, we found that the glume, with 37 of the total 75 unigenes, was the most responsive organ to *Fusarium* infection (Figure 2). The ovary, with only four unigenes, was the least responsive. Glumes provide the first layer of protection to the floral parts and are directly exposed to the macroconidia of *F. graminearum* during spray inoculation. The rachis is also directly exposed to the fungus and, with 31 unigenes detected, was the second most responsive organ. On the other hand, the ovary is deeply embedded within the spike, and the spray inoculation method does not result in immediate contact with the fungus. As revealed by several histopathological studies monitoring the early events during FHB infection, the direct contact of the fungus with the inner surfaces of the lemma, glume and palea, and with the ovary, occurs much later during the progress of infection (Miller *et al*., 2004). Therefore, the higher number of *F. graminearum*-induced unigenes detected in organs such as the glume and rachis, compared with the rest of the organs, may in part be a result of the fact that, at 24 h following infection, fungus penetration and contact with the inner surfaces were probably limited.

The numbers of commonly up-regulated ESTs in the six organs are shown in Table 4. The glume, lemma, palea, rachis, anther and ovary up-regulated exclusively 22, 1, 1, 21, 7 and 4 unigenes, respectively. The number of shared unigenes was highest between the glume and palea (11), two tissues of similar biological function. The bract tissues also shared a considerable number of up-regulated unigenes with the rachis. The biologically distant anther shared only three unigenes with the glume, one with the rachis and none with the lemma and palea. The ovary had no shared unigenes with any of the other five organs. The number of shared unigenes between three and four organs was low, and no unigenes were shared between all organs.

Table 4 Number of up-regulated unigenes exclusive to specific organs (a) and common to two of the six organs (b) following inoculation with *Fusarium graminearum*

Organ	Glume	Lemma	Palea	Anther	Ovary	Rachis
Glume	22a					
Lemma	5b	1a				
Palea	11b	5b	1a			
Anther	3b	0b	0b	7a		
Ovary	0b	0b	0b	0b	4a	
Rachis	6b	2b	8b	1b	0b	21a

Many of the *F. graminearum* up-regulated genes reported in previous studies were also found in this study. PR proteins, a heat shock protein, resistant gene analogues and proteins involved in the H_2O_2 oxidative burst, protein synthesis and phenylpropanoid pathway were significantly up-regulated in FHB-infected wheat organs. Several genes showed a significant increase only in specific organs. For instance, the induction of MT and the WRKY superfamily of transcription factors was exclusively up-regulated in the rachis and anther, respectively. These results demonstrate that precise profiling of molecular changes during the wheat–*F. graminearum* interaction requires the examination of individual organs. Many of these organ-specific genes, responding to FHB infection, may not be detectable when the entire wheat spike is used as the biological sample.

Experimental procedures

Plant material and inoculation with *F. graminearum*

Wheat line 93FHB37, resistant to FHB, derived from the cross Ning 8331(R)/HY611(S), was used in this study. The line 93FHB37 carries three QTLs (Quantitative Trait Loci) mapped to chromosomes 3BS, 6BS and 5AL that have been associated with resistance to FHB (Procunier *et al*., 2000). Plants were grown in a controlled environment in a growth cabinet with a 16-h photoperiod and 18/15 °C day/night temperatures. Mineral fertilization was applied at a rate of 6 g/L every second week with Plant-Prod (20-20-20) all-purpose fertilizer

(Brampton, ON, Canada). The main spike on each plant was spray inoculated at mid-anthesis with a freshly prepared conidial suspension (5×10^4 macroconidia/mL plus 0.1 mL/L Tween 20) of *F. graminearum* (monosporic isolate DAOM 192132, Canadian Collection of Fungal Cultures, Ottawa, ON, Canada). Following inoculation, plants were incubated for 24 h in a humidity chamber set at 21–22 °C with 100% relative humidity. Control plants of the same line were sprayed with water containing 0.2% Tween 20 and incubated under the same conditions. Twenty-four hours after inoculation, the sprayed spikes were harvested and immediately placed in a 50-mL tube containing RNA stabilizer reagent (RNAlater, Ambion, Austin, TX), and stored at –80 °C until processed. Later, the RNA was isolated from inoculated spikes for the synthesis of fungus-challenged and control probes. The same two RNA populations were used for the construction of the SSH library.

Construction of EST microarrays

The PCR-Select cDNA Subtraction Kit (Clontech, Palo Alto, CA, USA) was used to construct the SSH library according to the manufacturer's instructions. This library contained enriched low-abundance transcripts which minimized the number of redundant ESTs spotted on the microarrays. Briefly, water control cDNAs were subtracted against fungal-challenged cDNAs and the subtracted cDNAs were ligated into pGEM-T Easy T/A cloning vector (Promega, Madison, WI, USA). After transformation, amplification and purification of polymerase chain reaction (PCR) inserts using standard procedures, the cDNA products were printed (double spotted) on Corning GAPS II glass slides (Corning Inc. Life Sciences, Acton, MA, USA) with high-speed robotics (Microarray Centre, UHN, Max Bell Research Centre, Toronto, ON, Canada). The printed microarray consisted of 5739 elements representing wheat ESTs derived from the wheat–*F. graminearum* interaction SSH library. In addition, 65 *Arabidopsis* clones carrying ESTs putatively related to plant disease resistance genes were obtained from the Arabidopsis Biological Resource Center (Columbus, OH, USA) and spotted on the arrays.

RNA isolation and cDNA labelling

RNAlater-stabilized inoculated spikes were hand dissected into organs corresponding to the glume, lemma, palea, ovary, anther and rachis (Figure 3). Total RNA was extracted from each organ for each of the fungus-challenged and water control plants using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Prior to the preparation of the probes, the total RNAs isolated from the tissues of three different plants were pooled to reduce the level of biological variation between the samples. Five independent pools of control and fungus-challenged RNAs from each organ were separately prepared to provide probes for the five biological replications. Thus, the signal intensity ratios (T/C, treated over control) corresponding to an arrayed element represent the average level of transcripts from 15 plants (Table 1). The quality and quantification of total RNA was assessed using the RNA 6000 Nano LabChip with the Agilent 2100 Bioanalyser (Agilent Technologies Canada, Mississauga, ON, Canada).

Cyanine (Cy) dyes (Amersham Pharmacia Biotech, UK) were directly incorporated into first-strand cDNA synthesized from 5 μg of total RNA. The pooled RNA was labelled using the fluorescent dyes

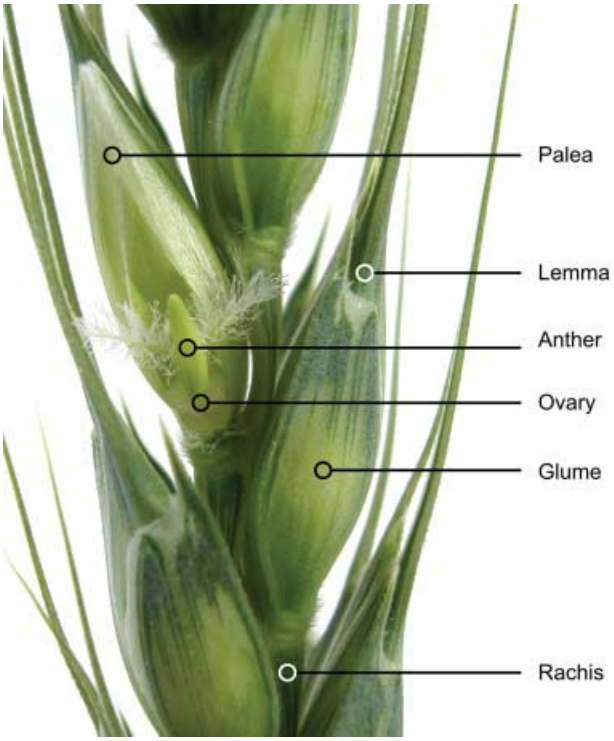

Figure 3 Wheat spike showing organs.

cyanine-3 (Cy3-dCTP for control samples) and cyanine-5 (Cy5-dCTP for fungus-challenged samples). The pooled RNA was mixed with a reaction volume containing the final concentration of 1 × first-strand PCR buffer (Invitrogen, Carlsbad, CA, USA), 10 mM dithiothreitol (DTT), 150 pM 5′-dT20 VN primer (where V is A, C or G, and N is any base), 500 μM each of dATP, dGTP and dTTP, 50 μM dCTP, 25 μM Cy3- or Cy5-dCTP, 0.5 ng spike control alien mRNA (Stratagene, La Jolla, CA) and DEPC (diethyl procarbonate)-treated water up to a volume of 40 μL. The mixture was denatured at 65 °C for 10 min. Superscript II reverse transcriptase (400 U) was added and cDNA synthesis was carried out for 2 h at 42 °C. Reactions were stopped by the addition of 5 μL of 50 mM ethylenediaminetetraacetic acid (EDTA). RNA was hydrolysed by adding 2.0 μL of 10 M NaOH for 20 min at 65 °C, and the reaction was neutralized by the addition of 4 μL of 5 M acetic acid. The fungal-challenged and water control probes were then combined and precipitated at –20 °C by the addition of an equal volume of isopropanol. The pellet was washed with cold 70% ethanol, briefly air dried and dissolved in 5.0 μL of nuclease-free water.

Microarray hybridizations

To prevent non-specific binding, the slides were incubated in pre-hybridization buffer containing 1% bovine serum albumin (BSA) for 45 min at 42 °C. For hybridization, the combined labelled cDNAs (5 μL) were mixed with hybridization solution (100 μL) composed of a 20 : 1 : 1 volume of DIG EasyHyb (Roche Diagnostics, Laval, QC, Canada), yeast tRNA (10 mg/mL) and salmon sperm DNA (10 mg/mL). The combined cDNAs were denatured at 65 °C for 2 min, centrifuged and cooled to 42 °C. The hybridization mixture was directly applied to the slide and the slide was immediately covered with a

coverslip (Hybrislip, Sigma, St Louis, MO). The array was then carefully placed in a CMT-GAPS (Corning Microarray Technology – Gamma Auino Propyl Silane) hybridization chamber (2551; Corning, NY) and incubated in a 42 °C water bath for 18 h. Coverslips were removed by submerging the slides in foil-covered Coplin jars containing 1 × SSC. The slides were then washed three times for 10 min each in plastic containers containing fresh 1 × SSC, 0.1% sodium dodecyl sulphate (SDS) in a 50 °C water bath. After washing, the slides were rinsed by repeatedly plunging in 1 × SSC at room temperature. Liquid was quickly removed from the slide surface by spinning at 500 r.p.m. for 5 min. All reactions and hybridizations were carried out in light-protected conditions and washing was performed in minimum light.

Controls for cDNA labelling and hybridization

An array of 10 artificial synthetic sequences (70-mers), with no homology to any known plant or animal gene (Universal Control Alien Array, Stratagene), was spotted on the slides to serve as negative or positive control for labelling and hybridization efficiency. Additional negative hybridization controls spotted on the array were salmon sperm DNA, human Cot-1 DNA and 3 × SSC. Up to 10 exogenous alien mRNAs were used as spiked RNAs to provide positive hybridization controls. An equimolar amount of a particular alien mRNA was individually added to the Cy5 fungal-challenged and Cy3 water control cDNA labelling reactions. The corresponding spotted alien sequence was co-hybridized with both of these labelled cDNAs. The expected 'yellow spots' on the array indicated the dye incorporation and hybridization efficiency of the differently labelled probes and normalization of the data.

Image acquisition, data collection and analysis

Using the GenePix 4000B AXON scanner and software (GenePix Pro 5, Axon Instruments, Union City, CA, USA), hybridized slides were scanned with two wavelengths corresponding to the dyes used. Signal intensity ratios (T/C, Cy5/Cy3) for arrayed elements were then imported into the software Acuity-3.1 (Axon Instruments). The data were normalized using the linear ratio-based normalization method implemented in the software, so that the centre of the distribution of the ratios shifted to unity. Using the data filtering wizard in Acuity-3.1, stringent conditions were applied to filter out spots with low quality and to remove the unreliable data from the dataset.

The SAM program (http://www-stat.stanford.edu/~tibs/SAM/; Tusher *et al*., 2001) was applied to log base 2-transformed, normalized data to identify elements in which the expression levels were significantly altered in response to *F. graminearum* infection. On the basis of the data from the five biological replications, SAM identified significant differences in mean expression levels. This was based on the change in gene expression relative to the standard deviation of repeated measurements for each arrayed element, and through the evaluation of a percentage of elements that would be identified by chance, called the false discovery rate (FDR). Transcripts identified by SAM were further subjected to a gene filtering process. Those showing a minimum 1.5-fold change in signal intensity between fungus-challenged and control plants in at least three of five replications were considered to be significantly responsive to *F. graminearum* infection.

Only ESTs showing a significant response to *F. graminearum* infection were further analysed to identify any organ specificity in response to the fungal infection. ANOVA was performed on log base 2-transformed expression data for each EST at a threshold *P* value of 0.01. The organ-specific variation of the element's expression patterns between organs was further evaluated by calculating the linear correlation. PCA of expression data was used to visualize the organ-specific expression pattern of ESTs identified as having significantly responded to *F. graminearum*.

Northern blot analysis

Northern blot analysis was used to verify the microarray results for a selected subset of up-regulated genes. From each tissue, 10 μg of total RNA was isolated, transferred to a positively charged nylon membrane (Roche Diagnostics Canada, Laval, QC, Canada) and UV cross-linked. DIG-labelled DNA probes were prepared with the PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH, Indianapolis, IN, USA) using the DNA inserts as template. Pre-hybridization and hybridization were performed according to the manufacturer's instructions. DIG-labelled DNA/RNA hybrids were detected with an enzyme-linked immunoassay using an alkaline phosphatase-labelled anti-DIG antibody with a chemiluminescent reaction, using CDP-Star to detect the phosphatase activity.

Sequence analysis and annotation

The nucleotide sequences of the differentially expressed ESTs were determined on an ABI PRISM 3100 Genetic Analyser (Perkin Elmer Applied Biosystems, Foster City, CA, USA) using the BigDye Termination Cycle Sequencing Kit. To assign putative functions, homology searches were conducted using the BLASTX program against the GENBANK non-redundant (nr) database (NCBI, Bethesda, MD) with default parameters. To determine whether any of the identified ESTs originated from the fungal genome, DNA sequences were also BLAST queried against the *F. graminearum* genome database (http://www.broad.mit.edu/annotation/fungi/fusarium) and the COGEME fungal database (http://cogeme.ex.ac.uk/blast.html). The cut-off value of 10^{-5} was used as a threshold for the expectation scores (*E* value), and only homologies with an *E* value of less than the threshold were regarded as a significant match. The EST annotated proteins were assigned to different functional categories following the MIPS (http://mips.gsf.de) FunCat scheme (Ruepp *et al*., 2004).

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References

Atienza, S.G., Faccioli, P., Perrotta, G., Dalfino, G., Zschiesche, W., Humbeck, K., Stanca, A.M. and Cattivelli, L. (2004) Large scale analysis of transcript abundance in barley subjected to several

single and combined abiotic stress conditions. *Plant Sci*. **167**, 1359–1365.

Baldwin, D., Crane, V. and Rice, D. (1999) A comparison of gel-based, nylon filter and microarray techniques to detect differential RNA expression in plants. *Curr. Opin. Plant Biol*. **2**, 96–103.

Brandt, S.P. (2005) Microgenomics: gene expression analysis at the tissue-specific and single-cell levels. *J. Exp. Bot*. **56**, 495–505.

Buerstmayr, H., Lemmens, M., Fedak, G., Ruckenbauer, P. and Gill, C.B.S. (1999) Back-cross reciprocal monosomic analysis of Fusarium head blight resistance in wheat (*Triticum aestivum* L.). *Theor. Appl. Genet*. **98**, 76–85.

Caldo, R.A., Nettleton, D. and Wise, R.P. (2004) Interaction-dependent gene expression in *mla*-specified response to barley powdery mildew. *Plant Cell*, **16**, 2514–2528.

Collinge, D.B., Kragh, K.M., Mikkelsen, J.D., Nielsen, K.K., Rasmussen, U. and Vad, K. (1993) Plant chitinases. *Plant J*. **3**, 31–40.

Di, R. and Tumer. N.E. (2004) A truncated form of ribosomal protein l3 eliminates ribosome depurination and cell death caused by pokeweed antiviral protein and confers resistance to trichothecene mycotoxins. In: *Proceedings of the 2nd International Symposium on Fusarium Head Blight Incorporating the 8th European Fusarium Seminar, Wyndham Orlando Resort, Orlando, FL, USA, 11–15 December*, Vol. 1, p. 242. East Lansing: Michigan State University.

Dixon, R.A. and Paiva, N.L. (1995) Stress-induced phenylpropanoid metabolism. *Plant Cell*, **7**, 1085–1097.

Dushnicky, L.G., Ballance, G.M., Sumner, M.J. and MacGregor, A.W. (1998) The role of lignification as a resistance mechanism in wheat to a toxin-producing isolate of *Pyrenophora tritici-repentis*. *Can. J. Plant Pathol*. **20**, 35–47.

van Eeuwijk, E.A., Mesterházy, A., Kling, C.I., Ruckenbauer, P., Saur, L., Bürstmayr, H., Lemmens, M., Keizer, L.C.P., Maurin, N. and Snijders, C.H.A. (1995) Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum* and *F. nivale* using a multiplicative model for interaction. *Theor. Appl. Genet*. **90**, 221–228.

Eulgem, T., Rushton, P.J., Robatzek, S. and Somssich, I.E. (2000) The WRKY superfamily of plant transcription factors. *Trends Plant Sci*. **5**, 199–206.

Fedak, G., Gilbert, J., Armstrong, K.C., Procunier, J.D., Tinker, N., Butler, G. and Pandeya, R. (1998) Studies of inheritance of *Fusarium* head blight in wheat. In: *Proceedings of the National Fusarium Head Blight Forum, Michigan State University, East Lansing, MI, USA, 26–27 October*, pp. 25–27. East Lansing: Michigan State University.

Gilbert, J. and Tekauz, A. (2000) Review: Recent developments in research on FHB of wheat in Canada. *Can. J. Plant Pathol*. **22**, 1–8.

Hall, M.D. and Van Sanford, D.A. (2003) Diallele analysis of Fusarium head blight resistance in soft red winter wheat. *Crop Sci*. **43**, 1663–1670.

Hart, L.P., Catal, M. and Wang, Z. (2005) Microarray analysis of Fusarium head blight toxin deoxynivalenol (DON) regulated genes of *Arabidopsis thaliana*. In: *Proceedings of the National Fusarium Head Blight Forum, Hilton Milwaukee City Center, Milwaukee, WI, USA, 11–13 December*, pp. 36–40. East Lansing: Michigan State University.

Huang, X. and Madan, A. (1999) CAP3: a DNA sequence assembly program. *Genome Res*. **9**, 868–877.

Jahoor, A., Eriksen, L. and Backes, G. (2004) QTLs and genes for disease resistance in barley and wheat. In: *Cereal Genomics* (Gupta, P.K. and Varshney, R.K., eds), pp. 199–251. Dordrecht: Kluwer Academic.

Kang, Z. and Buchenauer, H. (2000) Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. *Physiol. Mol. Plant Pathol*. **57**, 255–268.

Kauss, H., Fauth, M., Merten, A. and Jeblick, W. (1999) Cucumber hypocotyls respond to cutin monomers via both an inducible and a constitutive H_2O_2-generating system. *Plant Physiol*. **120**, 1175–1182.

Kawasaki, S., Borchert, C., Deyholos, M., Wang, H., Brazille, S., Kawai, K., Galbraith, D. and Bohnert, H.J. (2001) Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell*, **13**, 889–906.

Kloosterman, B., Vorst, O., Hall, R.D., Visser, R.G.F. and Bachem., C.W. (2005) Tuber on a chip: differential gene expression during potato tuber development. *Plant Biotechnol. J*. **3**, 505–519.

Kruger, W.M., Pritsch, C., Chao, S. and Muehlbauer, G.J. (2002) Functional and comparative bioinformatic analysis of expressed genes from wheat spikes infected with *Fusarium graminearum*. *Mol. Plant–Microbe Interact*. **5**, 445–455.

Lee, J.-M., Williams, M., Tingey, S. and Rafalski, A. (2002) DNA array profiling of gene expression changes during maize embryo development. *Func. Integrative Genomics*, **2**, 13–27.

Lu, R., Malcuit, I., Moffett, P., Ruiz, M.T., Peart, J., Wu, A.J., Rathjen, J.P., Bendahmane, A., Day, L. and Baulcombe, D.C. (2003) High throughput virus-induced gene silencing implicates heat shock protein 90 in plant disease resistance. *EMBO J*. **22**, 5690–5699.

Ma, L., Sun, N., Liu, X., Jiao, Y., Zhao, H. and Deng, X.W. (2005) Organ-specific expression of *Arabidopsis* genome during development. *Plant Physiol*. **138**, 80–91.

Mayer, A.M. and Harel, E. (1979) Polyphenol oxidase in plants. *Phytochemistry*, **18**, 193–215.

Meyers, B.C., Dickerman, A.W., Michelmore, R.W., Sivaramakrishnan, S., Sobral, B.W. and Young, N.D. (1999) Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. *Plant J*. **20**, 317–332.

Miller, S.S., Chabot, D.M.P., Ouellet, T., Harris, L.J. and Fedak, G. (2004) Use of a *Fusarium graminearum* strain transformed with green fluorescent protein to study infection in wheat (*Triticum aestivum*). *Can. J. Plant Pathol*. **26**, 453–463.

Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*. **7**, 405–410.

van Montfort, R.L.M., Slingsby, C. and Vierling, E. (2002) Structure and function of the small heat shock protein/α-crystallin family of molecular chaperones. In *Protein Folding in the Cell* (Horwich, A.L., ed.), pp. 105–156. New York: Academic Press.

Narusaka, Y., Narusaka, M., Seki, M., Ishida, J., Nakashima, M., Kamiya, A., Enju, A., Sakurai, T., Satoh, M., Kobayashi, M., Tosa, Y., Park, P. and Shinozaki, K. (2003) The cDNA microarray analysis using an *Arabidopsis* pad3 mutant reveals the expression profiles and classification of genes induced by *Alternaria brassicicola* attack. *Plant Cell Physiol*. **44**, 377–387.

Poppenberger, B., Berthiller, F., Lucyshyn, D., Sieberer, T., Schuhmacher, R., Krska, R., Kuchler, K., Glossl, J., Luschnig, C. and Adam, G. (2003) Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis thaliana*. *J. Biol. Chem*. **278**, 47 905–47 914.

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A. and Vance, C.P. (2000) Fungal development and induction of defense

response genes during early infection of wheat spikes by *Fusarium graminearum*. *Mol. Plant–Microbe Interact*. **13**, 159–169.
Procunier, J.D., Gilbert, J., Townley-Smith, F., Armstrong, K. and Fedak, G. (2000) Markers for Fusarium head blight disease resistance. U.S. Patent No. 6197518, filed June, 1999.
Pryor, W.A. (1997) Oxidative stress and defense mechanisms in plants: general introduction. *Free Radic. Biol. Med*. **23**, 470.
Ro, D.K. and Douglas, C.J. (2004) Reconstitution of the entry point of plant phenylpropanoid metabolism in yeast (*Saccharomyces cerevisiae*): implications for control of metabolic flux into the phenylpropanoid pathway. *J. Biol. Chem*. **279**, 2600–2607.
Ruepp, A., Zollner, A., Maier, D., Albermann, K., Hani, J., Mokrejs, M., Tetko, I., Guldener, U., Mannhaupt, G., Munsterkotter, M. and Mewes, H.W. (2004) The FunCat, a functional annotation scheme for systematic classification of proteins from whole genomes. *Nucleic Acids Res*. **32**, 5539–5545.
Schena, M., Shalon, D., Davis, R.W. and Brown, P.O. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, **270**, 467–470.
Shim, K.S., Cho, S.K., Jeung, J.U., Jung, K.W., You, M.K., Ok, S.H., Chung, Y.S., Kang, K.H., Hwang, H.G., Choi, H.C., Moon, H.P. and Shin, J.S. (2004) Identification of fungal (*Magnaporthe grisea*) stress-induced genes in wild rice (*Oryza minuta*). *Plant Cell Rep*. **22**, 599–607.
Tusher, V.G., Tibshirani, R. and Chu, G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. USA*, **98**, 5116–5121.
Vigers, A.J., Wiedemann, S., Roberts, W.K., Legrand, M., Selitrennikoff, C.P. and Fritig, B. (1992) Thaumatin-like pathogenesis related proteins are antifungal. *Plant Sci*. **83**, 155–161.
Wang, W., Vinocur, B., Shoseyov, O. and Altman, A. (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci*. **9**, 244–252.
Yang, Y.H. and Speed, T. (2003) Design and analysis of comparative microarray experiments. In: *Statistical Analysis of Gene Expression Microarray Data* (Speed, T., ed.), pp. 35–91. Washington, DC: Chapman & Hall/CRC.
Zhou, W., Kolb, F.L. and Riechers, D.E. (2005) Identification of proteins induced or upregulated by Fusarium head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). *Genome*, **48**, 770–780.

Supplementary material

The authors have provided the following supplementary material, which can be accessed alongside the article at http://www.blackwell-synergy.com.

Figure S1 Northern blot validation of microarray data for a selected number of expressed sequence tags (ESTs) up-regulated in response to *Fusarium graminearum* infection. The first five lanes contained total RNA (10 μg) blotted from the water controls: glumes (G), lemma (L), palea (P), ovary (O) and rachis (R). The following five lanes contained RNA from *Fusarium graminearum*-challenged organs. The selected clones were as follows: (A) clone BQ901699, β-1-3-glucanase; (B) clone DV799683, leucine-rich repeat (LRR) protein; (C) clone DV799622, metallothionein (MT); (D) clone DV799628, unknown; (E) clone DV799757, unknown. Loading controls (F) show the rRNA band intensities of RNA samples loaded on to gels and visualized on the Agilent 2100 Bioanalyser.

Table S1 Complete list of expressed sequence tags (ESTs) that were identified as differentially regulated in response to *Fusarium graminearum* infection, together with the average expression ratio derived from five replications for each organ.