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ORIGINAL PAPER

Transcript profiling of the phytotoxic response of wheat to the *Fusarium* mycotoxin deoxynivalenol

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Abstract Deoxynivalenol (DON) is a trichothecene mycotoxin commonly produced by *Fusarium graminearum* and *F. culmorum* during infection of cereal plants, such as wheat and barley. This toxin is a fungal virulence factor that facilitates the development of Fusarium head blight (FHB) disease. Wheat cultivar (cv.) Remus is susceptible to DON; the toxin causes premature bleaching of spikelets and inhibits root growth. This study used custom-made wheat cDNA arrays to analyse the effect of DON on the transcriptome of heads of the toxin-sensitive wheat cv. Remus at both 4 and 24 h post-toxin treatment. DON-induced transcripts encoded an array of proteins collectively associated with a range of cellular functions, such as metabolite transformation and detoxification, the ubiquitin-proteasome proteolytic pathway, jasmonate biosynthesis and signalling, carbohydrate metabolism, and phenylpropanoid biosynthesis. This study is the first to demonstrate that the fungal virulence factor DON modulates jasmonate biosynthesis and signalling. It also highlights the fact that the toxin-mediated accumulation of transcripts associated with metabolite transformation and detoxification, proteolysis and phenylpropanoid accumulation is not unique to DON-resistant wheat genotypes. Therefore, the respective encoded proteins are likely part of the general wheat defence against DON. Comparative analysis of the results of this and other studies suggests that it is likely to be the rapidity and magnitude rather than the components of the response that are critical in determining resistance to DON and thus the spread of FHB disease in wheat heads.

Keywords Trichothecene · Detoxification · Fusarium head blight · Microarray · Phenylpropanoid · Ubiquitination

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Introduction

Fusarium graminearum and *F. culmorum* cause Fusarium head blight (FHB) disease epidemics on small-grain cereals (Walter et al. 2010). Although FHB has been the focus of much research, this disease continues to cause serious yield losses (Brown et al. 2010; Goswami and Kistler 2004) and to threaten human and animal health via the contamination of food and feed with *Fusarium* mycotoxins—most commonly, deoxynivalenol (DON) (Parry et al. 1995; Pestka 2007). DON inhibits eukaryotic protein synthesis (Rocha et al. 2005; Pestka 2007) and is toxic to humans, animals and plants (Rocha et al. 2005). In cereal plants, DON acts as a disease virulence factor that aids the spread of the fungus within wheat heads and thus increases the severity of FHB disease symptoms (Bai et al. 2001). Barley and certain wheat genotypes are resistant to the phytotoxic effects of DON and some of the genetic factors associated with this resistance have been identified (Lemmens et al. 2005; Ansari et al. 2007; Walter et al. 2008).

In DON-susceptible wheat, phytotoxicity phenotypically manifests as bleaching of leaves and green spikelets as well as inhibition of seed germination, root generation, and shoot, root and callus growth (Ansari et al. 2007; Lemmens et al. 2005; Rocha et al. 2005). At the cellular level, DON inhibits protein synthesis (Miller and Ewen 1997) and causes degenerative changes in cellular membranes; damage to the plasma membrane can result in electrolyte leakage and cell death (Bushnell et al. 2004; Wojciechowski et al. 1995). Transcriptome studies in wheat and barley indicated that drug efflux, metabolite transformation and detoxification, the oxidative stress response and maintenance of cell survival are critical components of a host response to DON (Ansari et al. 2007; Walter et al. 2008; Doohan et al. 2008; Gardiner et al. 2010). Boddu et al. (2007) investigated the transcriptome response of a FHB-susceptible barley genotype to a DON-producing *F. graminearum* strain in comparison with a DON-minus mutant derivative of this fungus. Their results indicated that an FHB-susceptible barley cultivar (cv.) responds to trichothecene accumulation with at least two, albeit contrasting, responses; namely, (1) activation of trichothecene detoxification and transport, which would reduce the impact of trichothecenes, and (2) activation of protein ubiquitination and cell death, which would promote successful establishment of the disease. The impact of these with regard to the effects of DON probably depends on the timing of induction and the concentration of the toxin.

The objective of this research was to examine the toxin responses in heads of a wheat genotype that is distinct from other wheat genotypes and barley in not exhibiting inherited DON resistance traits. DON was applied to spikelets of the toxin-susceptible cv. Remus and the quantitative and temporal transcriptomic changes within treated tissue were assayed using custom-made wheat cDNA microarrays that were enriched for wheat transcripts differentially expressed in the presence of DON (Walter et al. 2008). The observed gene expression changes in wheat heads confirm previous findings that relate the transcriptional activation of toxin biotransformation and transport pathways, as well as ubiquitin-mediated protein degradation, to the wheat response to DON (Walter et al. 2008; Gardiner et al. 2010). More importantly, it showed that defence responses to the pathogen (as summarised by Walter et al. 2010) can also be transcriptionally activated by the fungal virulence factor, DON.

Materials and methods

Plant material

Seeds of *Triticum aestivum* cv. Remus were kindly supplied by Dr. Hermann Buerstmayr (IFA-Tulln, Austria). The cv. Remus is susceptible to FHB and DON-induced bleaching (Buerstmayr et al. 2003; Lemmens et al. 2005).

Adult plant toxin trials

Plants were grown (two per pot) as described by Doohan et al. (1999) with a 16-h photoperiod, 75% relative humidity and a day/night temperature of 20/12°C. At mid-anthesis, each of the three florets of four central spikelets per wheat head were treated with 15 µl of either 5 mg ml^{-1} 0.2% (v/v) Tween20 DON (Sigma-Aldrich, USA) or 0.2% (v/v) Tween20 (controls). DON was applied between the palea and the lemma, as described by Lemmens et al. (2005) and Ansari et al. (2007). Treated spikelets were harvested at 4 or 24 h post-treatment, flash-frozen in liquid N_2, freeze-dried and stored at −70°C prior to RNA extraction (Ansari et al. 2007). Each experiment included four heads (one per plant) per treatment per wheat cultivar and was conducted twice.

Microarray analysis

Microarray analysis was conducted to compare transcript accumulation in DON-treated versus control (0.2% Tween20-treated) spikelets of cv. Remus at 4 and 24 h post-treatment. The minimum information about a microarray experiment (MIAME) and the experimental design of this study are respectively outlined in the Supplemental Information and Supplemental Table S1. Each microarray comparison comprised two biological samples (each representing a bulked RNA from two independent plants) per treatment, time point and experiment, whereby each biological sample was subjected to dye swap (i.e. labelling of control and treatment target cDNA with Cy-3 and Cy-5 were reversed: total=four hybridisations per treatment, time point and experiment).

A custom-made wheat microarray was used as a platform for the described microarray studies. This spotted glass array was constructed by ARC Seibersdorf (Seibersdorf, Austria) and comprised 3,066 clones with expressed sequence tags (ESTs) inserts from a differential display cDNA library derived after DON-treatment of wheat roots (Ansari et al. 2007), 297 EST clones from a suppression subtractive hybridization cDNA library, which were up-regulated in callus of the wheat double-haploid line E2-24 T (a progeny of cv. CM82036 × cv. Remus carrying *Fhb1* from CM82036) in response to *F. graminearum* culture filtrate (Walter et al. 2008), as well as positive and negative controls, all of which were spotted in triplicate. The construction of microarrays, target amplification, hybridisation and data analysis were as previously described (Walter et al. 2008) and is outlined in the Supplemental Information.

Sequencing, sequence processing and sequence annotation

ESTs of interest were sequenced by either the Austrian Research Centre (Seibersdorf, Austria) or by MWG Biotech (Ebersberg, Germany). Sequences were trimmed, assembled and annotated as described by Walter et al. (2008). Protein functions were ascribed based on those described for the respective wheat homologues in the database and/or based on those described for homologues identified by BLASTX analyses against the UniProt Knowledgebase using the WU BLAST tool (release 2.0MP-WashU) of the European Bioinformatics Institute (http://www.ebi.ac.uk/blast2/index.html). Transcripts were allocated to functional categories according to their predicted protein function by using the Munich Information Centre for Protein Sequences (MIPS) Functional Catalogue (FunCat) scheme (release 2.1) (http://mips.gsf.de).

Real-time RT-PCR analysis

Real-time RT-PCR analysis was used to verify the microarray results for a selected subset of transcripts and was conducted using the oligonucleotide primers and conditions previously described (Walter et al. 2008). Real-time quantification of the expression of target transcripts and of the control RNA helicase gene in wheat heads of cv. Remus was performed in separate reactions. Analysis was conducted twice for each of two replicate samples per treatment from each of two independent experiments. The threshold cycle (C_T) values obtained by real-time RT-PCR were used to calculate the fold change in transcript accumulation with the formula $2^{-(CT\ target\ transcript\ -\ CT\ RNA\ helicase)}$ (Livak and Schmittgen 2001). Data were not normally distributed (as determined using the Kolmogorov-Smirnov normality test within SPSS; SPSS release 15.0.1, SPSS, USA) and were analysed using the Mann-Whitney Rank sum test within SPSS.

Results and discussion

A custom-made wheat microarray was used to determine the temporal effect of DON on the transcriptome of a toxin-susceptible (and FHB-susceptible) wheat genotype (4 and 24 h post-treatment). Although this array contained fewer transcript probes than the wheat Affymetrix genechip, it was specifically designed to represent the DON-responsive wheat transcriptome and was successfully exploited to identify wheat genes associated with the DON resistance innate to the wheat cv. CM82036 (Walter et al. 2008; Doohan et al. 2008). Correlations of data from the two independent experiments were statistically significant ($P \leq 0.01$) (Supplemental Fig. S1 and Table S3). Transcripts discussed hereafter were expressed ≥fourfold higher or≤0.25-fold lower in DON-treated relative to control (Tween20-treated) samples [i.e. the normalised expression (M) values were ≥2 or ≤−2, respectively; $P \leq 0.05$] (Tables 1 and 2). Across both time points analysed, DON treatment resulted in the up-regulated transcription of ESTs representing 66 singletons/contigs and the down-regulation of seven singletons/contigs (Tables 1 and 2). Although differential transcript accumulation was only confirmed for three genes by RT-PCR (see Supplemental Fig. S2) the DON-induced expression of many of the ESTs (indicated in *bold* in Table 1) has previously been confirmed (Walter et al. 2008).

Generally, the effect of DON was more pronounced at 24 h compared with at 4 h post-treatment, indicating that despite the application of a high concentration of toxin (i.e. 5 mg ml^{-1}), the response of wheat cv. Remus was not very rapid and was slower than in heads of the DON-resistant cv. CM82036, in which the greatest effect of DON on the transcriptome occurred at 4 h post-DON treatment (Doohan et al. 2008; Walter et al. 2008). This suggests that the swiftness with which wheat mounts defence against the toxin is critical in determining resistance to DON and thus the spread of FHB disease in wheat heads. In heads of wheat cv. Remus, a 24-h DON treatment resulted in the ≥fourfold up-regulation of ESTs, representing 66 singletons/contigs; ten of which were also up-regulated ≥fourfold at 4 h post DON-treatment (Table 1). Only one transcript was DON-up-regulated ≥fourfold at 4 h but not at 24 h post-treatment (Table 1). DON inhibits protein synthesis (Miller and Ewen 1997), and presumably the genotype-associated temporal differences in transcriptome responses to DON occur because the translational machinery of cv. Remus is more susceptible to DON than that of cv. CM82036.

Results provide evidence that there is commonality between DON-resistant and susceptible wheat genotypes with respect to defence pathways activated in response to toxin treatment

The majority of the DON-responsive transcripts are ‘unclassified proteins’, according to the MIPS functional catalogue (Tables 1 and 2). This indicates that much remains to be learned with respect to the plant cellular cascades that are activated in response to DON. However, the recent release of a shotgun assembly of wheat cv. Chinese Spring genome (http://www.cerealsdb.uk.net/) and the anticipated annotation of this genomic information over the next few years are likely to associate many uncharacterised DON-responsive transcripts with cellular functions. Ten of the 66 transcripts up-regulated >fourfold by DON encode metabolism-related proteins, indicating that signif-

Table 1 Functional classification of expressed sequence tags (ESTs) significantly induced in spikelets of the wheat cultivar Remus in response to deoxynivalenol (4 or 24 h post-treatment)

Functional category and unique clone identifier[ab]	Number of ESTs[c]	Best hit in the TIGR Plant Transcript Assembly database[d]		Closest protein homologue of the translated transcript/its TA homologue (E value ≤$1e^{-15}$)[e]		Expression ratio (T/C)[f]	
		TA/EST accession	E value	Homologue accession	Annotation and abbreviation	4 h micro-array	24 h micro-array
Metabolism							
0023	1	TA63216_4565	$2.1e^{-78}$	A8R7D3	Barley putative phenylalanine ammonia-lyase	5.61	24.1
F141[g]	1	TA63229_4565	$2.8e^{-79}$	Q43664	Wheat phenylalanine ammonia-lyase	—	9.20
F1164[g]	1	TA63195_4565	$2.0e^{-93}$	Q43664	Wheat phenylalanine ammonia-lyase	7.48	15.34
0115	1	TA83881_4565	$1.4e^{-138}$	Q2V065	Wheat cytochrome P450	—	20.79
1344	1	TA70864_4565	$5.4e^{-54}$	Q6T485	Wheat cytochrome P450	4.53	9.17
2365	6	TA87782_4565	$2.9e^{-188}$	A4D8H9	Barley P450 monooxygenase CYP72A39	—	7.63
0265	2	TA69924_4565	$1.7e^{-34}$	Q336U3	Rice proline dehydrogenase family protein	—	6.47
2013	1	TA74360_4565	$9.5e^{-67}$	Q84QK0	Rice 12-oxo-phytodienoic acid reductase	—	7.58
2416	2	TA62139_4565	$1.7e^{-57}$	A2XF75	Rice putative SAM-dependent methyltransferases domain-containing protein	—	8.32
F318	1	TA68946_4565	$1.8e^{-51}$	Q84LA1	Wheat fructan 1-exohydrolase	10.76	41.85
Energy							
F364	1	CJ579467	$1.0e^{-27}$	P00068	Wheat cytochrome C	—	5.18
Cell cycle and DNA processing							
F511	1	TA60573_4565	$6.3e^{-45}$	A8MRL0	*Arabidopsis* histone H3.3	—	5.34
Transcription							
3224	9	AY781358	$1.7e^{-111}$	Q2TN74	Wheat DREB5 transcription factor	6.16	11.70
Protein synthesis							
2498	1	TA68012_4565	$7.7e^{-45}$	Q0IWI9	Rice valyl-tRNA synthetase-like protein	—	7.35
2712	1	TA53038_4565	$3.4e^{-57}$	Q7F807	Rice putative arginine-tRNA ligase	—	7.11
Protein fate (folding, modification, destination)							
1223	1	BJ258948	$2.3e^{-99}$	A2WWE6	Rice ubiquitin carrier protein	—	4.47
Cellular transport, transport facilitation and transport routes							
1446	1	TA89435_4565	$6.3e^{-107}$	Q6YUU5	Rice putative multidrug resistance protein (MDR)	—	4.41
1788	1	TA71286_4565	$2.1e^{-126}$	Q94E55	Rice MRP3-like ABC transporter	—	4.85
2850	2	CK197852	$7.2e^{-110}$	Q6H511	Rice Ligand-effect modulator 3-like	4.54	6.03

Cellular communication/signal transduction mechanism							
0766	1	DR741624	$6.1e^{-97}$	Q75GE8	Rice putative calmodulin-domain/ calcium-dependent protein kinase AK1	—	9.47
Cell rescue, defence and virulence							
1775	1	TA88925_4565	$3.0e^{-107}$	Q9FQB5	Maize glutathione S-transferase GSTU24	—	4.15
1960	4	TA72809_4565	$6.0e^{-162}$	Q8RW00	Wheat glutathione S-transferase GSTU3	—	4.01
Unclassified proteins							
0430	2	TA64686_4565	$6.9e^{-161}$	Q2QXF1	Rice Harpin-induced protein 1-domain containing protein	—	7.50
0453	1	TA69536_4565	$2.3e^{-39}$	Q8RX53	*Deschampsia antarctica* hypothetical protein	—	7.66
1216	1	CK162048	$1.7e^{-55}$	Q6ESF0	Rice putative germin-like protein	—	10.5
1247	1	DR740943	$9.2e^{-47}$	A2XFX5	Rice putative EF-hand domain-containing protein	—	5.58
1395	1	TA92770_4565	$2.9e^{-122}$	Q6ETK8	Rice ATPase domain-containing protein	5.78	—
1444	1	CD920828	$5.2e^{-28}$	Q6L5C0	Rice t-snare domain-containing protein	—	6.02
1980	1	CA613673	$1.5e^{-32}$	Q0J9L1	Rice C2 domain-containing protein	—	18.30
2305	1	—	—	Q9AQU7	Rice putative rhomboid-like protein family protein	—	4.31
Unclassified proteins							
2515	1	TA109040_4565	$6.6e^{-47}$	A2WZ80	Rice putative EF-hand domain-containing protein	—	6.41
2532	1	BJ230821	$4.7e^{-60}$	A2XRZ0	Rice putative oxidoreductase	—	4.33
2673	1	TA76803_4565	$2.9e^{-97}$	A2XDI0	Rice putative Cytb561-domain containing protein	—	5.03
F502	1	TA54696_4565	$1.9e^{-163}$	A1YJE1	Wheat ZIM motif-containing protein	—	8.44
0067	1	—			—	—	5.2
0226	1	—			—	—	7.21
0264	1	—			—	—	5.62
0394	1	—			—	—	9.82
0538	1	CA727871	$1.0e^{-96}$		—	—	4.61
0816	1	TA102420_4565	$2.4e^{-17}$		—	—	10.70
0867	1	—			—	—	6.76
0970	1	TA77074_4565	$3.1e^{-112}$		—	—	11.7
1032	2	—			—	—	11.41
1106	1	—			—	—	4.26
1187	1	BJ233360	$1.8e^{-40}$		—	—	6.58
1217	1	—			—	—	8.11
1310	3	AL816069	$1.4e^{-46}$		—	—	4.38
1335	1	TA91388_4565	$7.2e^{-36}$		—	—	4.25

Table 1 (continued)

Functional category and unique clone identifier[ab]	Number of ESTs[c]	Best hit in the TIGR Plant Transcript Assembly database[d]		Closest protein homologue of the translated transcript/its TA homologue (E value ≤$1e^{-15}$)[e]		Expression ratio (T/C)[f]	
		TA/EST accession	E value	Homologue accession	Annotation and abbreviation	4h micro-array	24h micro-array
1405	2	TA89953_4565	$9.3e^{-92}$		—	—	10.09
1450	1	TA97148_4565	$1.5e^{-99}$		—	—	4.19
1504	1	TA86589_4565	$2.4e^{-87}$		—	—	15.30
1578	2	TA96871_4565	$5.5e^{-118}$		—	31.44	28.76
Unclassified proteins							
1621	5	CK162662	$3.1e^{-73}$		—	18.97	42.31
1770	2	—			—	—	41.00
1920	2	CN010964	$3.3e^{-86}$		—	—	5.76
1934	1	TA111253_4565	$1.2e^{-86}$		—	5.19	47.40
2101	1	CK199259	$1.5e^{-38}$		—	—	7.33
2158	1	TA107757_4565	$1.3e^{-26}$		—	—	4.48
2431	1	—			—	—	15.3
2438	1	—			—	—	5.53
2516	1	—			—	—	9.60
2539	1	—			—	—	4.30
2620	4	TA86588_4565	$4.5e^{-111}$		—	19.32	79.57
2628	3	TA97935_4565	$9.6e^{-83}$		—	—	9.77
3140	1	—			—	—	4.04
F433	1	TA91388_4565	$2.2e^{-94}$		—	—	4.16

[a] Functional category according to the Munich Information Centre for Protein Sequences (MIPS; http://mips.gsf.de) Functional Catalogue (FunCat) scheme

[b] Unique clone identifier corresponds to one representative sequence (the longest) of a contig, the sequence of which was used for similarity analyses. Transcripts in *bold* were identified as being DON-induced not only in this but also in other studies (Doohan et al. 2008; Walter et al. 2008)

[c] Number of EST components in each contig/singleton is listed

[d] As determined by BLASTn analysis against the TIGR Plant Transcript Assembly database

[e] Annotation as described for the TIGR Plant Transcript Assembly accessions or for associated Uniproteins [identified by BLASTX analysis of transcripts or transcript homologues using WU BLAST (http://www.ebi.ac.uk/blast2/index.html) (E value≤$1e^{-15}$)]

[f] Microarray expression ratio [treatment (*T*)/control (*C*)]: wheat spikelets were treated with deoxynivalenol (5 mg ml^{-1} 0.2% Tween20) or 0.2% Tween20 (control). The expression ratios represent the inverse of the *M* value (log_2-fold change). Expression ratios for treatment samples are presented only if they differed significantly ($P \leq 0.05$; $M \geq 2$) from control. In case of contigs values presented are those observed for the longest EST per contig (see first column for respective unique clone identifier), except for Contig2620, Contig2850 and Contig3244 where the longest EST was not common (i.e. >fourfold induced in response to DON) at both time points and thus values are given for the longest EST>fourfold DON-induced at both time points (unique identifiers 1686, 2222 and 2422, respectively)

[g] 88% nucleotide homology in a 167-nt overlap

Table 2 Functional classification of expressed sequence tags (ESTs) significantly repressed in spikelets of the wheat cultivar Remus in response to deoxynivalenol (4 or 24 h post-treatment)

Functional category and unique clone identifier[ab]	Number of ESTs[c]	Best hit he TIGR Plant Transcript Assembly database[d]		Closest protein homologue of the translated transcript/its TA homologue (E value≤1e^{-15})[e]		Expression ratio (T/C)[f]	
		TA/EST accession	E value	Homologue accession	Annotation and abbreviation	4 h micro-array	24 h micro-array
Protein synthesis							
F901	1	CK213178	6.4e^{-104}	Q3S4H9	Wheat eukaryotic translation initiation factor 5A3	—	0.22
Unclassified proteins							
0734	1	TA57045_4565	5.9e^{-85}	Q8LRM8	Wheat translationally-controlled tumor protein homologue	—	0.16
2763	2	TA62247_4565	9.3e^{-156}	Q7XXR0	Rice putative stem-specific protein TSJT1 (Os03g53270)	—	0.22
3110	1	—			—	—	0.20
F115	1	TA60035_4565	2.2e^{-86}	Q6H7P8	Rice zinc finger A20 and AN1 domain-containing stress-associated protein 4 (OsSAP4)	—	0.23
F156	1	CK195422	5.0e^{-74}	Q40019	Barley HvB12D protein	—	0.21
F551	1	CK217833	1.2e^{-50}	Q8LRM8	Wheat translationally controlled tumor protein homologue	—	0.12

[a] Functional category according to the Munich Information Centre for Protein Sequences (MIPS; http://mips.gsf.de) Functional Catalogue (FunCat) scheme

[b] Unique clone identifier corresponds to one representative sequence (the longest) of a contig, the sequence of which was used for similarity analyses

[c] Number of EST components in each contig/singleton is listed

[d] As determined by BLASTN analysis against the TIGR Plant Transcript Assembly database

[e] Annotation as described for the TIGR Plant Transcript Assembly accessions or for associated Uniproteins [identified by BLASTx analysis of transcripts or transcript homologues using WU BLAST (http://www.ebi.ac.uk/blast2/index.html) (E value≤1e^{-15})]

[f] Microarray expression ratio [treatment (*T*)/control (*C*)]: wheat spikelets were treated with deoxynivalenol (5 mg ml^{-1} 0.2% Tween20) or 0.2% Tween20 (control). The expression ratios represent the inverse of the *M* value ($\log_2$-fold change). Expression ratios for treatment samples are presented only if they differed significantly ($P \leq 0.05$; $M \leq -2$) from control. In case of contigs values presented are those observed for the longest EST per contig (see first column for respective unique clone identifier)

icant metabolic re-programming is taking place following DON-toxin treatment (Table 1). Other DON-upregulated transcripts were associated with cellular transport, cell rescue and defence, reaffirming previous findings which showed that DON and DON production by *F. graminearum* induce the up-regulation of cereal transcripts associated with the biotransformation and transport of toxic metabolites (Boddu et al. 2007; Walter et al. 2008). These include a transcript encoding multidrug resistance protein 3 (MRP3), which was among the cellular transport-associated transcripts previously shown to be DON-induced in a toxin-resistant wheat and to be linked to the DON tolerance and FHB resistance quantitative-trait locus *Fhb1* (Walter et al. 2008). The up-regulation of *MRP3* in the DON-susceptible wheat cv. Remus reaffirms the hypothesis of Walter et al. (2008) that this and the DON-induced cytochrome P450 (CYP) enzyme encoding transcripts most likely play a role in cellular defence rather than DON detoxification. DON-induced CYPs (Table 1; Walter et al. 2008) could function in the biosynthesis/transformation of hormones and defence metabolites (Werck-Reichhart et al. 2002) or could activate other DON-induced metabolites for subsequent detoxification via glutathione-*S*-transferases (GSTs) and/or ABC transporters (Coleman et al. 1997). Gardiner et al. (2010) observed DON-induced up-regulation of six barley GSTs and non-enzymatic formation of DON-glutathione conjugates in vitro, but stated that this does not preclude the enzymatic formation of these products in vivo. The up-regulation of tau class GSTs in wheat cv. Remus in response to DON (Table 1) is likely to reflect their involvement in the oxidative stress response that is triggered by DON (Walter et al. 2008). DON is known to damage plant cell membranes (Bushnell and Seeland 2006), which would subsequently increase the level of reactive oxygen species (Desmond et al. 2008). The DON-induced induction of a transcript encoding the integral plasma membrane protein ligand effect modulator 3 (LEM3) might indicate that DON has indeed permeabilised the plasma membrane in DON-susceptible wheat cv. Remus heads. In yeast, LEM3 is required for transport of the membrane phospholipid phosphatidylcholine across the plasma membrane (Graham 2004) and wheat LEM3 might be required to repair the cell membrane following toxin damage.

Ubiquitin conjugation targets proteins for degradation via the proteasome and is an important protein modification that allows rapid adjustment of several phytohormone signalling pathways in response to external and internal stimuli (McSteen and Zhao 2008; Dreher and Callis 2007). DON-induced accumulation of a ubiquitin carrier protein transcript (Table 1) contributes to the increasing amount of evidence that ubiquitin-proteasome components play an important role in the plant response to DON. Accordingly, wheat cDNAs encoding a putative ubiquitin ligase, ubiquitin-specific protease and proteasome subunit enhanced DON-resistance upon transgenic expression in a DON-sensitive yeast strain (Lucyshyn et al. 2008). The *Arabidopsis AtNFXL1* gene, which potentially encodes a ubiquitin ligase, is induced by the trichothecene T2-toxin (Masuda et al. 2007) and its barley homologue is induced by DON (Gardiner et al. 2010). *AtNFXL1* acts as a negative regulator of salicylic acid-associated defence reactions in response to T2-toxin (Asano et al. 2008). While deletion of the *AtNFXL1* gene results in *Arabidopsis* being hypersensitive to T-2 toxin (Asano et al. 2008), deletion of its yeast homologue, *FAP1,* significantly increased yeast resistance to T-2 toxin (Jossé et al. 2011). *FAP1* is a TORC1 (target of rapamycin complex 1) pathway-related gene (Kunz et al. 2000). The TOR pathway is a central regulator of cell growth, cell death, nutrition, starvation, hormone and stress responses in diverse eukaryotes (Smeekens et al. 2010; Stanfel et al. 2009). There is further evidence that the TOR pathway is involved, possibly even differentially regulated, as part of the wheat response to DON. Translationally controlled tumour protein (TCTP) is an upstream regulator of TOR (Brioudes et al. 2010) and this, as well as previous research, showed that DON up-regulated the transcription of a *TCTP* homologue in both DON-resistant and DON-susceptible wheat [see Supplemental Table S1 in Walter et al. (2008)].

Phenylalanine ammonia lyase (PAL) catalyses the first step in the phenylpropanoid pathway, i.e. the conversion of phenylalanine to *trans*-cinnamic acid (Rhodes 1985). A number of transcriptome studies support the idea that PAL and the phenylpropanoid pathway play a role in the resistance of wheat to *F. graminearum*. DON and FHB resistance was associated with enhanced *PAL* transcription in wheat head tissue infected with *F. graminearum* (Steiner et al. 2009). In barley, the production of the phenylpropanoid-derived flavonoids was correlated with resistance to *Fusarium* infection (Skadhauge et al. 1997). Three *PAL* transcripts were up-regulated in heads of cv. Remus in response to 4-h and/or 24-h DON treatment (Table 1). Hence, the role of PAL in FHB resistance might be quantitative or dependent on additional phenylpropanoid pathway components not activated in a DON- and FHB-susceptible wheat genotype.

DON induces the accumulation of wheat transcripts involved in the production of the key plant regulator jasmonic acid and the mobilisation of fructan

We recently reviewed the systemic response of wheat to FHB, including the role of hormones and defence-associated pathways in disease resistance (Walter et al. 2010). Transcriptional responses to both DON treatment and trichothecene accumulation in wheat or barley, respec-

tively, indicate that jasmonic acid (JA) production and signalling are manipulated by DON in susceptible wheat. In both FHB-susceptible barley and wheat, trichothecene accumulation or -treatment induced up-regulation of transcripts encoding 12-oxophytodienoic acid reductase (Boddu et al. 2007; this study). The 12-oxophytodienoic acid reductase enzyme catalyses the transformation of 12-oxophytodienoic acid into JA (Howe and Browse 2007), accumulated in response to *F. graminearum* infection (Zhou et al. 2006) and its expression is enhanced by the *Fusarium* toxin DON; thus indicating that this enzyme might be part of a jasmonic-acid-related wheat defense response to *Fusarium* pathogens and their toxin DON, as previously suggested by Li and Yen (2008) and Walter et al. (2010). However, JA is also a central regulator of plant development and physiology, and therefore it might as well serve functions other than defence to DON. Another protein encoded by a DON-induced transcript contains a ZIM-motif (Table 1), which is characteristic of JAZ transcriptional repressor proteins that repress the JA signalling pathway via interaction with MYC2 (Chini et al. 2007). It is possible that the DON-mediated up-regulation of this transcript leads to suppression of MYC2-induced JA signalling.

Fructans are increasingly recognised as important components of the stress response in plants, due to their action as sugar reserves and protective agents against abiotic stress (Valluru and Van den Ende 2008). DON treatment up-regulated a transcript encoding fructan-1-exohydrolase (FEH) >40-fold. Even as early as 4 h post-DON treatment, it was up-regulated by >tenfold (Table 1). This suggests that toxin treatment causes the hydrolysis of fructans, and according to the hypothesis by Valluru and Van den Ende (2008), FEH hydrolysis of fructan could generate an optimal mixture of higher and lower degree polymerized (DP) fructans, sucrose and hexoses, that would provide superior membrane protection. It would be a logical cellular response to a toxin that permeabilises the cell membrane (Bushnell et al. 2004; Wojciechowski et al. 1995), or it could be that wheat is mobilising its carbohydrate reserves as a response to the depletion of nutrient reserves in toxin-stressed tissue. Another enzyme that leads to increased fructan (sucrose:fructan 6-fructosyltransferase) accumulated in spikes of the FHB resistant wheat cv. Wangshuibai in response to inoculation with *F. graminearum* (Wang et al. 2005).

Conclusions

This research helps us to understand how a susceptible host responds to DON and such studies are a critical requirement for targeted breeding programmes that aim to develop wheat with durable type II resistance to FHB. They tell us that it is not a simplistic story—as in all aspects of life, timing of responses is a critical determinant of the outcome of the wheat–*Fusarium* interaction. It remains to be determined as to how JA biosynthesis and signalling affect FHB resistance. One suspects that it is not a simplistic signalling cascade, but future studies should examine the effect of DON on JA levels in both resistance and susceptible plants. It is certain that sugar signalling and partitioning are worthy of further analysis in FHB resistance research. The fate of mobilised fructans should be determined. Membrane instability may contribute to the deleterious effects of the toxin on wheat and it may be possible to manipulate it to improve FHB resistance.

References

Ansari KI, Walter S, Brennan JM, Lemmens M, Kessans S, McGahern A, Egan D, Doohan FM (2007) Retrotransposon and gene activation in wheat in response to mycotoxigenic and non-mycotoxigenic-associated *Fusarium* stress. Theor Appl Genet 114:927–937

Asano T, Masuda D, Yasuda M, Nakashita H, Kudo T, Kimura M, Yamaguchi K, Nishiuchi T (2008) *AtNFXL1*, an *Arabidopsis* homologue of the human transcription factor NF-X1, functions as a negative regulator of the trichothecene phytotoxin-induced defense response. Plant J 53:450–464

Bai G-H, Desjardins AE, Plattner RD (2001) Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia 153(2):91–98

Boddu J, Cho S, Muehlbauer GJ (2007) Transcriptome analysis of trichothecene-induced gene expression in barley. Mol Plant-Microbe Interact 20:1364–1375

Brioudes F, Thierry A-M, Chambrier P, Mollereau B, Bendahmane M (2010) Translationally controlled tumor protein is a conserved mitotic growth integrator in animals and plants. Proc Natl Acad Sci USA 107(37):16384–16389

Brown NA, Urban M, van de Meene AML, Hammond-Kosack KE (2010) The infection biology of Fusarium graminearum: Defining the pathways of spikelet to spikelet colonisation in wheat ears. Fungal Biol 114(7):555–571

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107(3):503–508

Bushnell WR, Seeland TM (2006) Effects of DON on barley leaf tissues, summary of results. The effects of deoxynivalenol on Barley leaf tissue. In: Canty SM, Clark A, Van Sanford D (eds) National fusarium head blight forum; 2006 Dec 10–12. Research Triangle Park, North Carolina, USA, pp 35–36

Bushnell WR, Seeland TM, Perkins-Veazie P, Krueger DE, Collins J, Russo VM (2004) The effects of deoxynivalenol on Barley leaf tissue. In: Tsuyumu S, Leach JE, Shiraishi T, Wolpert T (eds) Genomic and genetic analysis of plant parasitism and defense. APS Press, St. Paul, pp 270–281

Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R (2007) The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448(7154):666–671

Coleman J, Blake-Kalff M, Davies E (1997) Detoxification of xenobiotics by plants: chemical modification and vacuolar compartmentation. Trends Plant Sci 2(4):144–151

Desmond OJ, Manners JM, Stephens AE, MacLean DJ, Schenk PM, Gardiner DM, Munn AL, Kazan K (2008) The *Fusarium* mycotoxin deoxynivalenol elicits hydrogen peroxide production, programmed cell death and defence responses in wheat. Mol Plant Pathol 9(4):435–445

Doohan F, Arunachalam C, Jiang S, Khan M, Egan D, Erard G, Walter S (2008) The wheat response to deoxynivalenol: does maintenance of hormone homeostasis and alleviation of oxidative stress play an important role in toxin tolerance? Cereal Res Commun 36:233–237

Doohan FM, Weston G, Rezanoor HN, Parry DW, Nicholson P (1999) Development and use of a reverse transcription-PCR assay to study expression of *Tri5* by *Fusarium* species in vitro and in planta. Appl Environ Microbiol 65(9):3850–3854

Dreher K, Callis J (2007) Ubiquitin, hormones and biotic stress in plants. Ann Bot 99:787–822

Gardiner SA, Boddu J, Berthiller F, Hametner C, Stupar RM, Adam G, Muehlbauer GJ (2010) Transcriptome analysis of the barley–deoxynivalenol interaction: evidence for a role of glutathione in deoxynivalenol detoxification. Mol Plant-Microbe Interact 23 (7):962–976

Goswami RS, Kistler HC (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5 (6):515–525

Graham TR (2004) Flippases and vesicle-mediated protein transport. Trends Cell Biol 14(12):670–677

Howe GA, Browse J (2007) Jasmonate synthesis and action in higher plants. In: Encyclopedia of Life Sciences. Wiley, Chichester. doi:10.1002/9780470015902.a9780470020138

Jossé L, Li X, Coker RD, Gourlay CW, Evans IH (2011) Transcriptomic and phenotypic analysis of the effects of T-2 toxin on *Saccharomyces cerevisiae*: evidence of mitochondrial involvement. FEMS Yeast Res 11(1):133–150. doi:10.1111/j.1567-1364.2010.00699.x

Kunz J, Loeschmann A, Deuter-Reinhard M, Hall MN (2000) FAP1, a homologue of human transcription factor NF-X1, competes with rapamycin for binding to FKBP12 in yeast. Mol Microbiol 37 (6):1480–1493

Lemmens M, Scholz U, Berthiller F, Dall'Asta C, Koutnik A, Schuhmacher R, Adam G, Buerstmayr H, Mesterházy Á, Krska R, Ruckenbauer P (2005) The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for *Fusarium* head blight resistance in wheat. Mol Plant-Microbe Interact 18(12):1318–1324

Li G, Yen Y (2008) Jasmonate and ethylene signaling pathway may mediate Fusarium head blight resistance in wheat. Crop Sci 48 (5):1888–1896

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Lucyshyn D, Abolmaali S, Weindorfer H, Shams M, Wiesenberger G, Wilhelm E, Lemmens M, Adam G (2008) Ubiquitin and Fusarium resistance: lessons from wheat cDNAs conferring deoxynivalenol resistance in yeast. Cereal Res Commun 36:437–441

Masuda D, Ishida M, Yamaguchi K, Yamaguchi I, Kimura M, Nishiuchi T (2007) Phytotoxic effects of trichothecenes on the growth and morphology of *Arabidopsis thaliana*. J Exp Bot 58 (7):1617–1626

McSteen P, Zhao Y (2008) Plant hormones and signaling: common themes and new developments. Devel Cell 14(4):467–473

Miller JD, Ewen MA (1997) Toxic effects of deoxynivalenol on ribosomes and tissues of the spring wheat cultivars frontana and casavant. Nat Toxins 5:234–237

Parry DW, Jenkinson P, McLeod L (1995) *Fusarium* ear blight (scab) in small grain cereals—a review. Plant Pathol 44(2):207–238

Pestka JJ (2007) Deoxynivalenol: toxicity, mechanisms and animal health risks. Anim Feed Sci Technol 137:283–298

Rhodes MJC (1985) The phyiological significance of plant phenolic compounds. In: Van Sumere CF, Lea PJ (eds) Annual Proceedingsof the Phytochemical Society of Europe, vol 25: The biochemistryof plant phenolics. Oxford University Press, Oxford, pp 99–117

Rocha O, Ansari K, Doohan FM (2005) Effects of trichothecene mycotoxins on eukaryotic cells: a review. Food Addit Contam 22 (4):369–378

Skadhauge B, Thomsen KK, Wettstein D (1997) The role of the barley testa layer and its flavonoid content in resistance to *Fusarium* infections. Hereditas 126(2):147–160

Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. Curr Op Plant Biol 13(3):273–278

Stanfel MN, Shamieh LS, Kaeberlein M, Kennedy BK (2009) The TOR pathway comes of age. Biochim Biophys Acta 1790 (10):1067–1074

Steiner B, Kurz H, Lemmens M, Buerstmayr H (2009) Differential gene expression of related wheat lines with contrasting levels of head blight resistance after *Fusarium graminearum* inoculation. Theor Appl Genet 118(4):753–764

Valluru R, Van den Ende W (2008) Plant fructans in stress environments: emerging concepts and future prospects. J Exp Bot 59 (11):2905–2916

Walter S, Brennan J, Arunachalam C, Ansari K, Hu X, Khan MR, Trognitz F, Trognitz B, Leonard G, Egan D, Doohan FM (2008) Components of the gene network associated with genotype-dependent response of wheat to the *Fusarium* mycotoxin deoxynivalenol. Funct Integr Genomics 8(4):421–427

Walter S, Nicholson P, Doohan FM (2010) Action and reaction of host and pathogen during *Fusarium* head blight disease. New Phytologist 185(1):54–66

Wang Y, Yang L, Xu H, Li Q, Ma Z, Chu C (2005) Differential proteomic analysis of proteins in wheat spikes induced by *Fusarium graminearum*. Proteomics 5(17):4496–4503

Werck-Reichhart D, Bak S, Paquette S (2002) Cytochrome P450. In: CR Somerville, EM Meyerowitz (eds) The Arabidopsis Book. American Society of Plant Biologists, Rockville, pp 1–28

Wojciechowski S, Chelkowski J, Kostecki M (1995) Influence of deoxynivalenol on electrolyte leakage in cereal seedling leaves. Acta Physiol Plant 17(4):357–360

Zhou W, Eudes F, Laroche A (2006) Identification of differentially regulated proteins in response to a compatible interaction between the pathogen *Fusarium graminearum* and its host, *Triticum aestivum*. Proteomics 6:4599–4609