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SHORT COMMUNICATION

Components of the gene network associated with genotype-dependent response of wheat to the *Fusarium* mycotoxin deoxynivalenol

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Abstract The *Fusarium* mycotoxin deoxynivalenol (DON) facilitates fungal spread within wheat tissue and the development of Fusarium head blight disease. The ability of wheat spikelets to resist DON-induced bleaching is genotype-dependent. In wheat cultivar (cv.) CM82036 DON resistance is associated with a quantitative trait locus, *Fhb1*, located on the short arm of chromosome 3B. Gene expression profiling (microarray and real-time RT-PCR analyses) of DON-treated spikelets of progeny derived from a cross between cv. CM82036 and the DON-susceptible cv. Remus discriminated ten toxin-responsive transcripts associated with the inheritance of DON resistance and *Fhb1*. These genes do not exclusively map to *Fhb1*. Based on the putative function of the ten *Fhb1*-associated transcripts, we discuss how cascades involving classical metabolite biotransformation and sequestration processes, alleviation of oxidative stress and promotion of cell survival might contribute to the host response and defence against DON.

Keywords *Fhb1* · Trichothecenes · Wheat cDNA microarray

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Introduction

Deoxynivalenol (DON) is a trichothecene mycotoxin commonly produced by *Fusarium graminearum* and *F. culmorum* when they attack wheat heads and cause Fusarium head blight (FHB) disease (Parry et al. 1995). DON acts as a virulence factor for *Fusarium* fungi, aiding in the colonisation of wheat heads (Bai et al. 2001). Like FHB disease, DON induces premature bleaching of wheat spikelets (Lemmens et al. 2005). In the plant cell, DON localises in the cytoplasm, plasmalemma and chloroplasts and is sometimes associated with endoplasmic reticula and ribosomes (Kang and Buchenauer 1999). DON inhibits protein synthesis, alters plant cell plasma membrane permeability (Bushnell and Seeland 2006) and causes chloroplast dissolution (Bushnell et al. 2004). DON treatment induces the accumulation of reactive oxygen species (ROS) and activates plant programmed cell death (PCD) (Masuda et al. 2007; Desmond et al. 2008). At the transcriptional level, plant genes upregulated in response to either DON treatment or DON production by *F. graminearum* included those involved in brassinosteroid inactivation, trichothecene detoxification and transport proteins, cytochrome P450s, ubiquitination-related proteins, programmed cell death-related proteins and transcription factors (Ansari et al. 2007; Boddu et al. 2007; Masuda et al. 2007; Desmond et al. 2008). Some wheat cultivars are resistant to DON-induced bleaching of spikelets and convert DON to the less toxic DON-*3*-glucoside (Lemmens et al. 2005). These traits and the resistance to spread of FHB disease map to a quantitative trait locus (QTL) located on the short arm of chromosome 3B (QTL *Fhb1*). Ansari et al. (2007) found that expression of a transcript encoding a basic leucine zipper protein (bZIP) transcription factor was associated with DON resistance and this QTL, but that this gene was not located within *Fhb1*. This QTL might, in some way, be linked to a glucosyltransferase or other components of a classical detoxification pathway (Coleman et al. 1997) that leads to deposition of glucose-conjugated DON outside of the cytoplasm, i.e. in vacuoles or in the apoplast.

This study set out to determine if the presence of QTL *Fhb1* in wheat results in the accumulation of transcripts associated with classical detoxification or alternative pathways as an early response to DON treatment. While the identity of the genes underpinning the DON tolerance QTL *Fhb1* remained elusive, we relate the observed transcriptomic changes to potential DON tolerance strategies, including metabolite biotransformation and sequestration, and alleviation of oxidative stress. We discuss how such responses could facilitate cell survival and thereby retard fungal colonisation of wheat tissue.

Materials and methods

Plant material

The wheat (*Triticum aestivum*) used in this study included cvs. Frontana, CM82036, Remus and a population of 14 recombinant F_1-derived double haploid (DH) lines originating from a cross between cv. CM82036 and cv. Remus (Buerstmayr et al. 2003). These were kindly supplied by Dr. Hermann Buerstmayr (IFA-Tulln, Austria). The 14 DH lines used in this study had or had not inherited resistance to DON-induced bleaching of spikelets and QTL *Fhb1* from cv. CM82036 (see supplemental "Materials and methods" and supplemental Table S2 for details of the cultivars and DH lines). Callus of DH line E2-24T (that carries *Fhb1*; Buerstmayr et al. 2003) was derived as described in supplemental "Materials and methods". Seeds of wheat cv. Chinese Spring (accession no. Cltr 14108) and its four chromosome 3BS deletion mutant derivatives used in this study were previously described by Ansari et al. (2007).

Adult plant DON tolerance trials

DON tolerance trials were conducted under contained environment conditions [with a 16-h photoperiod, 75% relative humidity and a day/night temperature of 20/12°C]. Plants were grown (two per pot) and central spikelets were treated with DON at mid-anthesis, as previously described (Ansari et al. 2007). Treated spikelets were harvested at 4 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 DON tolerance trial included four heads (one per plant) per treatment per wheat cultivar and was conducted twice.

Microarray analysis

These studies were undertaken prior to the availability of the Affymetrix wheat microarray chip. For this reason, a wheat microarray was constructed such that it included potential DON response-associated transcripts. Some 3066 cDNA clones were isolated from a DON-treated normalised wheat (cv. Frontana) root cDNA library. Suppressive subtractive hybridisation (SSH) was used to isolate 297 clones upregulated in callus of DH line E2-24T (a progeny of cv. CM82036 x cv. Remus carrying *Fhb1* from CM82036) in response to treatment with *F. graminearum* culture filtrate. These clones, in addition to positive and negative controls, were spotted in triplicate on microarray slides. The isolation of clones, sequencing, sequence processing, sequence annotation and phylogenetic analysis, construction of microarrays, target amplification, hybridisation and microarray data analysis are described in the supplemental "Materials and methods". DNA sequences for

ESTs used in these studies and the layout of the arrays are presented in supplemental Table S1.

Microarray analysis compared transcript accumulation in DON-treated wheat spikelets of DH/parent lines inheriting QTL *Fhb1* vs. DH lines not inheriting this QTL. Biological samples comprised equivalent amounts of RNA bulked from four DH/parent lines that carried *Fhb1* (two samples) or that did not carry this QTL (one sample), each representing four different DH lines (see supplemental Table S3 and MIAME in the supplemental "Materials and methods" file). Analyses were conducted using biological samples obtained from two independent experiments and all comparisons were subjected to Cy-3/Cy-5 dye swap, thus resulting in four hybridisations per comparison.

Real-time RT-PCR analysis

Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was used to verify the microarray results for a selected subset of transcripts (using two to four replicate samples per DH/parent line from each of two independent experiments). RT-PCR reaction components, reaction conditions and details regarding the RNA helicase housekeeping gene are given in the supplemental "Materials and methods" and primer sequences are listed in Table S5. Real-time RT-PCR quantification of the accumulation of target transcripts and of the housekeeping RNA helicase gene was performed in separate reactions (duplicate reactions for each sample). The obtained threshold cycle (C_T) values used to calculate the fold change in transcript accumulation with the formula $2^{-(CT\ \text{target transcript}\ -\ CT\ \text{RNA helicase})}$ (Livak and Schmittgen 2001). Non-normally distributed real-time RT-PCR data were analysed using the Kruskal–Wallis test within SPSS (SPSS, Chicago, IL).

Transcript mapping studies

Genomic DNA extracted from leaves of wheat cv. Chinese Spring and its chromosome 3BS deletion mutant lines as previously described (Doyle and Doyle 1987) was subjected to PCR analyses with transcript-specific primers (supplemental Table S5) to determine if selected transcripts were present in gDNA extracts. Details of the PCR reaction components, reaction conditions and of positive control (*bZIP*) PCR analysis are given in the supplemental "Materials and methods". Each PCR was conducted twice.

Supplemental material

- Supplemental Materials and methods (includes MIAME).
- Supplemental Results
- Supplemental Table S1. Wheat 3K microarray feature description.
- Supplemental Table S2. Wheat germplasm, pedigree, FHB-/DON-associated QTL and ploidy status.
- Supplemental Table S3. Experimental design used for microarray analyses.
- Supplemental Table S4. Normalised microarray data.
- Supplemental Table S5. Sequence of transcript-specific primers used for real-time RT-PCR analyses.
- Supplemental Fig. S1. Scatterplot analyses of data from microarray studies.
- Supplemental Fig. S2. Influence of DON treatment on the accumulation of *Fhb1*-associated transcripts in spikelets of the DON tolerant wheat cv. CM82036.
- Supplemental Fig. S3. Accumulation of transcripts in response to deoxynivalenol (DON) treatment in spikelets of double haploid (DH) lines (derived from a cross of wheat cvs. CM82036 and Remus).

Results and discussion

Identification of DON-responsive transcripts associated with, but not exclusively located within, QTL *Fhb1*

Microarray analysis discriminated ESTs representing ten singletons/contigs whose accumulation was significantly higher in spikelets of DH progeny (from a cv. CM82036 × cv. Remus cross) that inherited *Fhb1*, as compared to in those that did not, all samples being DON-treated (Table 1). See supplemental "Materials and methods" and "Results" and supplemental Fig. S1 for further explanation of the microarray and sequence analyses. Real-time RT-PCR analysis confirmed that transcripts were DON-responsive in cv. CM82036 ($P \leq 0.05$) and that their accumulation in DON-treated DH progeny (eight lines containing and eight lacking *Fhb1*) was associated with the inheritance of *Fhb1* from cv. CM82036 (see supplemental Figs. S2 and S3). Fig. 1 depicts the accumulation of transcripts encoding a multidrug resistance protein ABC transporter (*MRP*), two cytochrome P450 enzyme homologs (*CYPs*) and a uridine diphosphate-glucosyltransferase (*UGT*) in DON-treated spikelets of DH lines. Transcripts were classified according to their function (MIPS; http://mips.gsf.de) (Table 1). Most of them are also expressed during plant pathogenesis by mycotoxigenic *Fusaria* (indicated in bold in Table 1), and transcripts encoding similar proteins such as UDP glucosyltransferases (UGTs), multidrug resistance protein ABC transporter (MRP), cytochrome P450 enzymes (CYPs), poly polymerase domain containing protein and AAA$^+$ family ATPase have recently been associated with tricho-

Table 1 Functional classification of expressed sequence tags (ESTs) whose accumulation as an early response to deoxynivalenol treatment (4 h post-treatment) is associated with the presence of the DON tolerance-associated QTL *Fhb1* in wheat[a]

Functional category and accession number[ab]	Number of ESTs[c]	Best hit in the TIGR Plant Transcript Assembly database[d]		Closest protein homolog of the translated transcript/its TA homolog (E value ≤ 8.3e^{-72c})[e]		Subcellular location[f]	Microarray expression ratio (+/− *Fhb1*)[g]
		TA/EST accession	*E* value	Homolog accession	Annotation		
Energy							
FG985280	1 [h]	TA84412_4565	4.3e^{-37}	Q8S914	Wheat alternative oxidase	Mitochondrion	≥ 1.58
Metabolism							
FG985274	1	TA63503_4565	9.7e^{-42}	Q84M02	*Miscanthus* zinc-binding alcohol dehydrogenase 1	Cytoplasm	≥1.67
FG985373	1	TA88294_4565	4.1e^{-68}	Q7XT97	Rice UDP-glucosyltransferase	Not defined	≥4.79
FG985275*	2	TA83881_4565	4.7e^{-186}	Q2V066	Wheat cytochrome P450	Membrane	≥1.71
FG985277*	7	TA87782_4565	8.8e^{-186}	Q2V066	Wheat cytochrome P450	Membrane	≥1.67
Cellular transport, transport facilitation and transport routes							
FG985276	1	TA71286_4565	1.5e^{-125}	Q94E55	Rice MRP3-like ABC transporter	Membrane	≥1.32
Cell rescue, defence and virulence							
FG985272	1	TA83429_4565	2.1e^{-36}	Q0J198	Rice putative mitochondrial phosphate transporter	Mitochondrion	≥3.01
FG985278	1	TA60149_4565	1.8e^{-30}	Q8W3G3	Rice putative CEO poly (ADP-ribose) polymerase (PARP) catalytic domain-containing protein	Nucleus	≥2.17
Unclassified proteins							
FG985281	1	TA109450_4565	5.3e^{-73}	Q2QLK0	Rice cell division protein AAA ATPase family protein	Not defined	≥2.07
FG985279	1	TA62139_4565	3.4e^{-56}	A2XF75	Rice hypothetical protein	Not defined	≥2.13

[a] Transcripts were selected on the basis that microarray analyses indicated that they accumulated to a significantly higher level in spikelets of wheat double haploid lines that inherited QTL *Fhb1* from wheat cv. CM82036, as compared to lines that did not. GenBank accession corresponds to one representative sequence (the longest) of a contig, the sequence of which was used for similarity analyses.
[b] Functional category according to the Munich Information Centre for Protein Sequences (MIPS; http://mips.gsf.de) Functional Catalogue (FunCat) scheme.
[c] Number of EST components in each contig/singleton is listed.
[d] As determined by BLASTn analysis against the TIGR Plant Transcript Assembly database. Those Transcript Assemblies/ESTs in bold had at least one EST match from *Fusarium*-infected or *Fusarium* culture filtrate-treated wheat or barley (>89% identity and $E \leq 6.0e^{-88}$).
[e] Annotation as described for the TIGR Plant Transcript Assembly accessions or for associated Uniprotein [identified by BLASTx analysis of transcripts or transcript homologs using WU BLAST (http://www.ebi.ac.uk/blast2/index.html) (E value≤8.3e^{-72})].
[f] Subcellular location, as determined by GO functional annotation of the Uniproteins.
[g] Microarray expression ratio (+/− *Fhb1*): Wheat spikelets were treated with deoxynivalenol (5 mg ml^{-1} 0.2% Tween20) and RNA was pooled from four double haploids that inherited *Fhb1*, and two pools were prepared from plants that did not inherit this QTL (four double haploids per pool). The expression ratio in the pooled sample that inherited *Fhb1* was compared to expression in each of the pools that did not inherit this QTL (+/−*Fhb1* ratio). The expression ratios represent the inverse of the *M* value (log_2-fold change); values presented are the lowest value observed across both microarray comparisons and, in the case of contigs, the lowest log_2-fold change observed amongst its constituent ESTs.
[h] Two of the DON-induced sequences on the microarray formed a contig representing alternative oxidase, both were upregulated in response to DON, but only one was significant (i.e. $P \leq 0.05$; see supplemental Table S4).
*77% homology in 534nt overlap.

thecene accumulation in spikelets of a FHB-susceptible barley cultivar (Boddu et al. 2007).

Quantitative trait locus *Fhb1* most likely codes for a very early response in the DON and FHB resistance cascade (at least a response initiated earlier than 4 h post-treatment with relatively high amounts of DON) and the *Fhb1*-associated transcripts identified in this study are downstream of this early response. PCR-based mapping (using cv. Chinese Spring and derivative 3BS deletion lines) could not place any of the ten transcripts associated with *Fhb1* exclusively within the genomic region of this QTL and the ten transcripts identified in this study are not homologous to ESTs that previously mapped to the 3BS chromosome bin that includes *Fhb1* (0.78–1.00) (results not shown).

Insights into the pathways associated with defence and resistance to DON

The genes identified in this study map to pathways implicated in classical metabolite biotransformation and

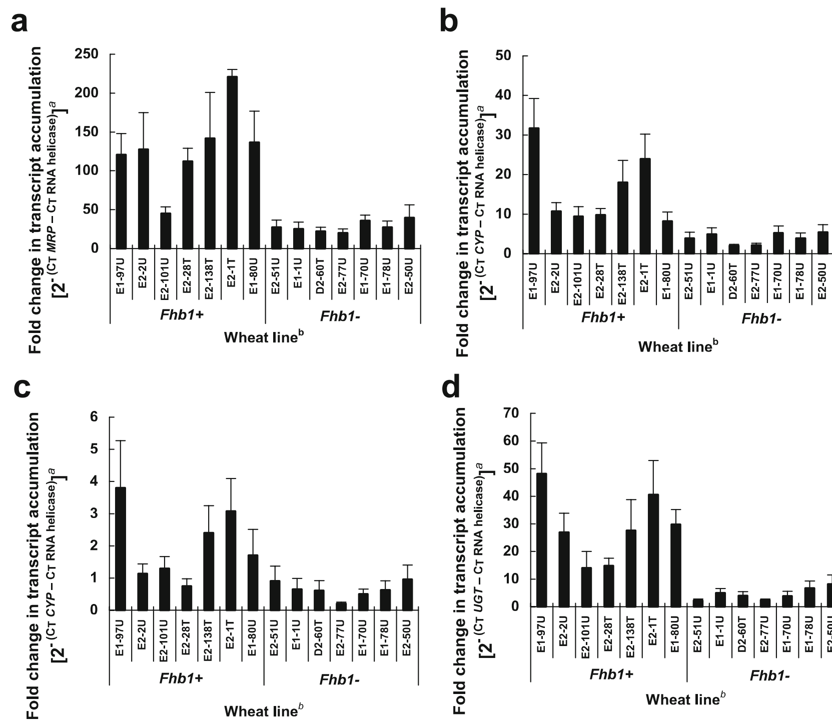

Fig. 1 Accumulation of transcripts homologous to a multidrug resistance protein (*MRP*)-like ABC transporter (**a**), cytochrome P450s (*CYP*) (**b** and **c**) and a UDP glucosyltransferase (*UGT*) (**d**) in response to deoxynivalenol (DON) treatment in spikelets of double haploid (DH) lines (derived from a cross of wheat cvs CM82036 and Remus). [b]Lines are grouped on the basis of whether or not they inherited the DON tolerance-associated QTL *Fhb1* from the short arm of chromosome 3B of cv CM82036. Central spikelets (4) were treated with DON (5 mg ml^{-1} 0.2% Tween20) at mid-anthesis. RNA extracted from treated spikelets 4 h post-treatment was used for real time RT-PCR analysis (2 experiments with each 2–3 replicates per line). [a]Transcript accumulation was quantified as the specific transcript/RNA helicase transcript ratio. *Bars* indicate SEM. GenBank accession numbers=FG985276 (**a**), FG985277 (**b**), FG985275 (**c**) and FG985373 (**d**)

sequestration processes, alleviation of oxidative stress and promotion of cell survival. These genes/pathways are likely to minimise DON-induced cellular damage rather than to play a direct role in toxin tolerance. Manipulation of such mechanisms may provide direction for future breeding strategies aimed at generating FHB-resistant cereal plants.

CYP, UGT and MRP proteins are involved in metabolite biotransformation and the detoxification of xenobiotics (Coleman et al. 1997). Barley orthologs of these DON-responsive *CYP*, *UGT* and *MRP* transcripts were responsive to trichothecene accumulation and Boddu et al. 2007 proposed that the *MRP* transcript encodes a protein for removal of trichothecenes from the cytoplasm. The elucidated characteristics of orthologs of the wheat *CYP*, *UGT* and *MRP* (Table 1) lead us to assume that the identified wheat transcripts may have a role in biotransformation of hormones and other metabolites during the host defence response to DON. However, these proteins are often multifunctional and one cannot rule out the possibility that they play a direct role in the detoxification of DON. The translational products of the DON-responsive *CYP* genes cluster with the CYP72 clan (as determined by phylogenetic analysis; results not shown), members of which are involved in plant hormone homeostasis (Nelson 2006). Although CYPs often activate chemicals prior to glycosylation, DON naturally possesses the hydroxyl group necessary for glycosylation at the C-3 position. The translational product of the *Fhb1*-associated *UGT* transcript is more similar to the *Arabidopsis* indole-*3*-acetate (IAA) beta-glucosyltransferase (UniProt accession Q9SYK9) than to the UGT DOGT1 (UniProt accession Q9ZQ94) that glycosylates DON (Poppenberger et al. 2003) (69% in 134 vs. 34% in 121aa overlaps). Handa et al. (2008) identified a MRP homolog as a potential candidate for FHB resistance and DON accumulation controlling QTLs on wheat chromosome 2DS. Whether MRP proteins can sequester glycosylated DON into plant vacuoles remains to be determined; in yeast DON export from the cytoplasm into the extracellular space is facilitated by a pleiotropic drug resistance (PDR)-like ABC transporter (Poppenberger et al. 2006). Based on the function of its *Arabidopsis* homolog of the DON-responsive MRP (*At*MRP3; GenBank protein ID: NP_187915) and the fact that DON is known to disrupt chloroplast integrity (Bushnell and Seeland 2006), it may be that the DON-responsive MRP sequesters chlorophyll catabolites into the vacuole, thus avoiding any cell

damage that might result from their photodynamic action (Takamiya et al. 2000).

Both DON and DON producers can induce oxidative stress in wheat cells (Zhou et al. 2005; Golkari et al. 2007; Desmond et al. 2008). The higher accumulation of transcripts encoding alternative oxidase (AOX), mitochondrial phosphate transporter (MPT) and a clone eighty one (CEO) protein in DON-tolerant as compared to in susceptible genotypes suggests that the tolerance response might include additional or more efficient mechanisms to modulate the levels of reactive oxygen species (ROS) and regulate oxidative stress responses. AOX acts as an alternative to the cytochrome *c* electron transfer pathway. The overexpression of the wheat homolog of the *Fhb1*-associated AOX (*Waox1a*) in *Arabidopsis* resulted in decreased ROS production following abiotic stress (Sugie et al. 2006). MPTs catalyse the influx of P_i into mitochondria, which is required for the oxidative phosphorylation of ADP to ATP (Takabatake et al. 1999), and ATP generation and export from the mitochondria to the cytoplasm is proposed to help minimise ROS production (Jones 2006; Noctor et al. 2007). The closest characterised protein homolog of the *Fhb1*-associated *CEO* is the *Arabidopsis* radical-induced cell death 1 (RCD1) protein (albeit homology is low: 31.2% in a 573aa overlap; results not shown) which regulates oxidative stress responses (Katiyar-Agarwal et al. 2006).

Maintenance of cell viability and protection against DON-induced PCD (Desmond et al. 2008) may be a secondary effect of the proteins encoded by the wheat DON-inducible *AOX* and *CEO* (resulting from their role in regeneration of redox homeostasis). In tobacco, AOX has been associated with maintenance of cell viability in response to stress (Ordog et al. 2002; Robson and Vanlerberghe 2002). AOX also protected soybean cells against hydrogen peroxide-induced cell death (Amor et al. 2000). *rcd1* (*CEO*) mutant *Arabidopsis* displayed not only an increased sensitivity to apoplastic ROS (Ahlfors et al. 2004), but also typical characteristics of plant PCD following ozone exposure (Overmyer 2002).

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References

Ahlfors R, Lång S, Overmyer K, Jaspers P, Brosché M, Tauriainen A, Kollist H, Tuominen H, Belles-Boix E, Piippo M, Inzé D, Palva ET, Kangasjärvi J (2004) Arabidopsis RADICAL-INDUCED CELL DEATH1 belongs to the WWE protein–protein interaction domain protein family and modulates abscisic acid, ethylene, and methyl jasmonate responses. Plant Cell 16(7):1925–1937

Amor Y, Chevion M, Levine A (2000) Anoxia pretreatment protects soybean cells against H_2O_2-induced cell death: possible involvement of peroxidases and of alternative oxidase. FEBS Lett 477:175–180

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

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-Microb Interact 20:1364–1375

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) Proceedings of the 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 and The American Phytopathological Society, St. Paul, Minnesota, pp 270–281

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): doi:10.1111/J.1364-3703.2008.00475.X

Doyle JJ, Doyle JL (1987) Isolation of DNA from fresh plant tissue. Focus 12:13–15

Golkari S, Gilbert J, Prashar S, Procunier JD (2007) Microarray analysis of *Fusarium graminearum*-induced wheat genes: identification of organ-specific and differentially expressed genes. Plant Biotechnol J 5(1):38–49

Handa H, Namiki N, Xu D, Ban T (2008) Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. Mol Breeding 22:71–84

Jones DP (2006) Disruption of mitochondrial redox circuitry in oxidative stress. Chemico-Biol Interact 163:38–53

Kang Z, Buchenauer H (1999) Immunocytochemical localization of *Fusarium* toxins in infected wheat spikes by *Fusarium culmorum*. Physiol Mol Plant Pathol 55(5):275–288

Katiyar-Agarwal S, Zhu J, Kim K, Agarwal M, Fu X, Huang A, Zhu J-K (2006) The plasma membrane Na+/H+ antiporter SOS1 interacts with RCD1 and functions in oxidative stress tolerance in *Arabidopsis*. Proc Natl Acad Sci USA 103(49):18816–18821

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-Microb Interact 18(12):1318–1324

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

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

Nelson DR (2006) Plant cytochrome P450s from moss to poplar. Phytochem Rev 5:193–204

Noctor G, De Paepe R, Foyer CH (2007) Mitochondrial redox biology and homeostasis in plants. Trends Plant Sci 12(3):125–134

Ordog SH, Higgins VJ, Vanlerberghe GC (2002) Mitochondrial alternative oxidase is not a critical component of plant viral resistance but may play a role in the hypersensitive response. Plant Physiol 129:1858–1865

Overmyer K (2002) Hormonal regulation of radical-induced programmed cell death in ozone-sensitive mutants of *Arabidopsis thaliana*. Academic dissertation, University of Helsinki, Finland

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

Poppenberger B, Berthiller F, Lucyshyn D, Sieberer T, Schuhmacher R, Krska R, Kuchler K, Glössl J, Luschnig C, Adam G (2003) Detoxification of the *Fusarium* mycotoxin deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis thaliana*. J Biol Chem 278(48):47905–47914

Poppenberger B, Adam G, Berthiller F, Krska R, Kuchler K, Luschnig C, Glössl J, Lucyshyn D, Schuhmacher R, Sieberer T (2006) Method for the detoxification of mycotoxins. United States Patent Application Publication No. US2006/0183202 A1

Robson CA, Vanlerberghe GC (2002) Transgenic plant cells lacking mitochondrial alternative oxidase have increased susceptibility to mitochondria-dependent and -independent pathways of programmed cell death. Plant Physiol 129:1908–1920

Sugie A, Naydenov N, Mizuno N, Nakamura C, Takumi S (2006) Overexpression of wheat alternative oxidase gene Waox1a alters respiration capacity and response to reactive oxygen species under low temperature in transgenic Arabidopsis. Genes Genet Syst 81(5):349–354

Takabatake R, Hata S, Taniguchi M, Kouchi H, Sugiyama T, Izui K (1999) Isolation and characterization of cDNAs encoding mitochondrial phosphate transporters in soybean, maize, rice, and Arabidopsis. Plant Mol Biol 40:479–486

Takamiya K-I, Tsuchiya T, Ohta H (2000) Degradation pathway(s) of chlorophyll: what has gene cloning revealed? Trends Plant Sci 5 (10):426–431

Zhou W, Kolb FL, Riechers DE (2005) Identification of proteins induced or upregulated by Fusarium head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). Genome 48 (5):770–780