Science brief: Transcriptional responses to trichothecene mycotoxins – a systematic review

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Summary

Mycotoxins is the collective description of a wide variety of chemicals which are secreted by a multitude of fungi. A small number of these toxins have been identified as harmful for human and animal health and are therefore under surveillance in many jurisdictions. The mechanisms of toxicity elicited by mycotoxins are pleiotropic and, so far, not fully defined. Comprehensive datasets describing the transcriptome changes in response to mycotoxin treatment are available in publicly accessible databases and may shed light on common signalling pathways affected by mycotoxin exposure.
This report summarizes these available datasets in a systematic way with a focus on the trichothecene toxin deoxynivalenol and its derivatives. It addresses the question whether common signalling pathways stimulated by exposure to mycotoxins, or their metabolites can be identified. Such pathways may serve as targets for the development of sensitive bioassays, including cell-based assays, for mycotoxins.

Overall, the available datasets suggest that:

1. Many cell lines are highly sensitive to mycotoxin exposure.
2. Transcriptome changes precede overt toxicity and may represent a responsive sensor for toxin exposure.
3. Cellular responses to mycotoxins and their metabolites are highly consistent, enabling the detection of co-toxicities and uncharacterised toxins.
4. Genes which are highly sensitive to toxin exposure and the pathways associated with their regulation can be identified.
5. Transcriptome pathway analysis has the potential to suggest mitigation interventions against mycotoxin exposure.

Therefore, cell based assays derived from transcriptome analysis will support the sensitive detection of mycotoxins including their metabolites which may escape immunological or chemical detection.

Regulated mycotoxins

Mycotoxins is collective description of a wide variety of chemicals which are secreted by a multitude of fungi. A small number of these have been identified as harmful for human and animal health and are therefore under surveillance in many jurisdictions. These include the substances shown in **table 1** in with the permitted limits in the EU as mandated by EFSA. Similar limits are imposed by the Food and Drug Administration in the USA (https://www.fda.gov/food/natural-toxins-food/mycotoxins) and the State Administration of Market Regulation in China (https://www.fas.usda.gov/data/china-china-releases-standard-maximum-levels-mycotoxins-foods). The permitted levels of toxins vary depending on the food product in which they are found. Generally, the permitted limits are lower in products of which large quantities are typically consumed (e.g., flour) and higher in products of which only small quantities are consumed (e.g., spices or cooking oils).

mycotoxin	limits EFSA
Aflatoxins	0.1 – 15 µg/kg
Ochratoxin A	0.5 – 20 µg/kg
Patulin	10 – 50 µg/kg
Deoxynivalenol	500 – 1750 µg/kg
Zearalenone	20 – 400 µg/kg
Fumonisins	200 – 4000 µg/kg
T-2 and HT-2 toxin	15 – 1000 µg/kg
Citrinin	100 µg/kg

Table 1: *Mycotoxins under food surveillance (food for human consumption) and permitted limits (https://www.legislation.gov.uk/eur/2006/1881). Around 10 fold higher levels apply to feed products (for farm animal consumption).*

Detection methods for mycotoxins

Mycotoxins are typically detected using chemical or immunological techniques with defined limits of detection (Nolan et al., 2019). The methods have varying degrees of technical and instrumental complexity ranging from lateral flow tests to liquid chromatography-mass spectrometry. The chemical analysis relies on targeted approaches using commercially available standards and are offered by commercial providers. Immunological techniques (including lateral flow tests and ELISA) can be carried out at the site of food and feed producers with limited instrumental requirements.
One drawback of the targeted testing for specific toxins is that toxin metabolites (masked mycotoxins) may escape detection (Gratz, 2017). Cell based assay methods are currently being developed for a variety of environmental and food toxins (Cheli et al., 2014; Legler et al., 2020). Cell based assays have the potential to detect mycotoxins based on their toxic effects rather than the physicochemical properties. Therefore, they may be able to also detect toxin congeners which may escape chemical or immunological detection.

Mechanisms of mycotoxin activity

Mycotoxins elicit a variety of toxic effects. Their main purpose is to provide the fungus with a growth advantage. Therefore, most mechanisms will reduce the growth of the host cell or competing organism. Mycotoxins elicit pleiotropic effects whose mechanistic basis is often not well defined and may affect several molecular targets (Awuchi et al., 2022; Janik et al., 2020). Two mycotoxins whose effects are well defined are Aflatoxin B1 and zearalenone. Aflatoxin B1 (produced by Aspergillus flavus

and A. parasiticus), generates DNA (guanine) adducts after activation to aflatoxin B1-8,9-exo-epoxide by liver enzymes (Smela et al., 2001). Zearalenone (produced by some Fusarium species) acts as potent estrogenic metabolite signalling through the classic estrogen receptors alpha and beta (Ayed-Boussema et al., 2011; Molina-Molina et al., 2014). Trichothecene mycotoxins (like deoxynivalenol and T2/HT2 toxin) are presumed to act primarily by inhibiting translation (Pestka, 2010). This ribotoxic effect is consistent with binding of the trichothecene toxins to the peptidyl-transferase centre of eukaryotic ribosomes (Garreau De Loubresse et al., 2014; Pierron et al., 2016; Shifrin and Anderson, 1999). However other modes of toxicity have also been suggested, including anti-inflammatory effects (Sugiyama et al., 2016), food refusal effects (Zhou and Pestka, 2015), ER stress (Shi et al., 2009), and activation of cell stress response pathways through the p38-fos signalling pathway (Bae and Pestka, 2008). However, all or most of these effects may be interdependent and linked at a molecular level.

Transcriptomic responses to mycotoxin treatment

Search strategy and data analysis

The objective of this report was to identify common signalling and gene expression pattern elicited by trichothecene mycotoxins which provide a rational basis for cell-based assay development.
The GEO (gene expression omnibus) database (Barrett et al., 2009) was searched for available transcriptomic datasets of the regulated mycotoxins. Using the search terms indicated in **supplementary table 1**, relevant GEO records were identified. These records include both, complete experiments and the individual datasets included in these experiments. Experiments can include up to several hundred individual datasets but typically consist of 10 to 20 datasets. Older experiments tend include a lower number of datasets. Most transcriptomic experiments (82 experiments, **supplementary table 1**) are available for aflatoxins, mainly aflatoxin B1.
The focus of this study was on trichothecene mycotoxins. There are only 2 datasets describing experiments with T-2/HT-2 (GSE25491 and GSE10103) which both do not show any significant effect of the toxin treatment. For deoxynivalenol, 40 experiments are recorded in the GEO database. These include experiments in plants, bacteria, whole animals, primary cells, and cell lines (**supplementary figure 1**). Experiments in plants (14 experiments) were excluded from further analysis (**figure 1**) as plant cells would not provide a suitable basis for rapid cell based assays. Three experiment records provided insufficient information about the experimental conditions. Five experiments were derived from whole animals or primary cells unsuitable for the development of cell based assays. Nine experiments were excluded due to poor replication of the data (n<3), the absence of significant results, or the absence of gene expression data.
The remaining 9 experiments (**supplementary table 2**) were analysed by the web-based R pipeline GEO2R (Barrett et al., 2007) or the statistical packages available for Microsoft Excel. Most experiments (5) were generated using Agilent microarrays, 3 experiments were generated using Affymetrix arrays, one experiment was carried out using the Illumina platform of RNA sequencing. The gene lists obtained were analysed using the pathway analysis modules G-Profiler (Raudvere et al., 2019) and Enrichr (Chen et al., 2013). The gene lists were also compared with a Venn diagram comparison module (https://bioinformatics.psb.ugent.be/webtools/Venn/).

Data quality and research gaps

Most of the available datasets have poor levels of replication (n=3) (**supplementary table 2**). Moreover, only 3 experiments utilised more than one concentration of toxin. And where more than one concentration was used, the lower concentration generated few significant responses (Alassane-Kpembi et al., 2017; Kugler et al., 2016; Nossol et al., 2018). Only one of the experiments used more than one time point of analysis (Kugler et al., 2016). And in that case the OD of yeast, rather than a

defined time period, is reported (**supplementary table 2**). The available data are also derived from a very low number of cell types. In fact, only two commercially available cell lines (Caco2 and IPEC-J2) were used in the experiments which could be included in the systematic review. This indicates a clear research gap requiring new analyses in commercially available cell lines, using better replication, a wider range of toxin concentrations and several time-periods of toxin exposure.

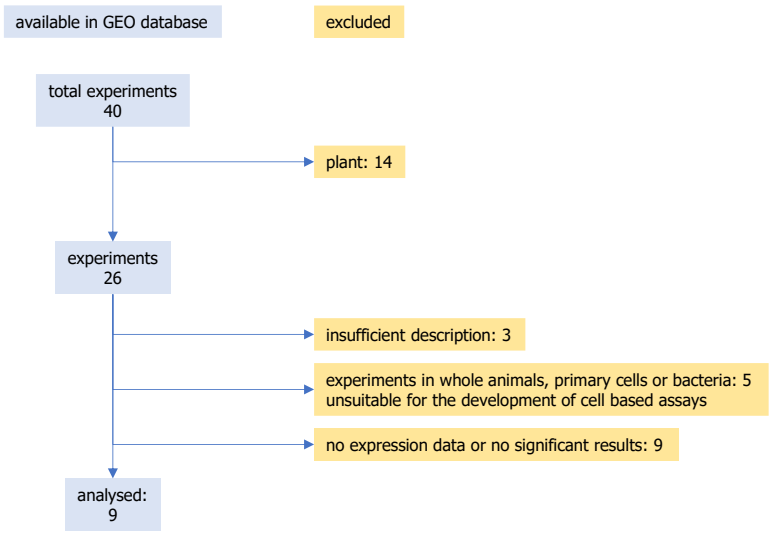

***Figure 1**: Selection of datasets for the systematic review. The 40 experiments found with the search terms "mycotoxin" AND "deoxynivalenol" were filtered according to the indicated criteria.*

Sensitivity of organisms to toxin exposure

Literature data (Cheli et al., 2014) demonstrate that cell lines display a wide spectrum of sensitivity to different mycotoxins (exemplified for sensitivity to deoxynivalenol in **supplementary figure 2**). Collectively, the gene transcription data confirm that cell lines are highly reactive to deoxynivalenol exposure and the expression of a significant number of genes is changed, often with a high amplitude (**figure 2a and 2b**). Therefore, the transcriptome of (or the expression of individual genes in) cell lines offer great potential as a sensitive indicator of mycotoxin exposure.
In contrast, yeast is far less sensitive (by 2 orders of magnitude). In addition, the yeast strains used for the transcriptome experiments in the GEO database carry a mutation in one of the ABC efflux transporter genes (Pdr5) to increase sensitivity (Harris et al., 2021; Kugler et al., 2016). Wildtype yeast strains would show an even higher resistance to mycotoxin exposure. This suggests that yeast, despite their excellent molecular characterisation and the availability of numerous different strains, would not be a good candidate for the generation of cell based assays.

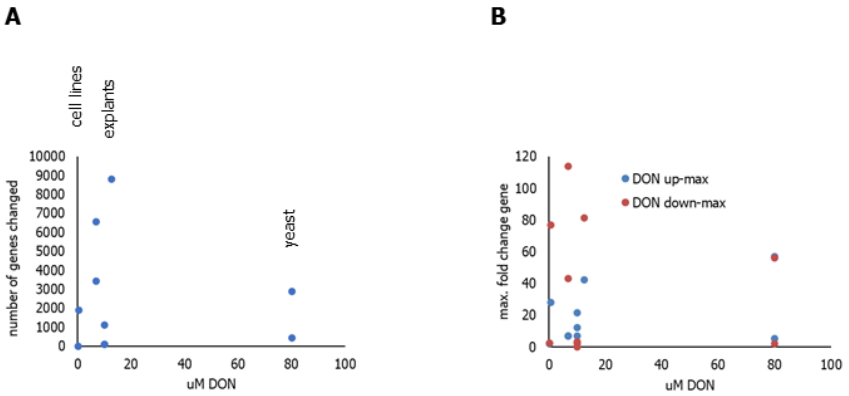

***Figure 2**: Gene expression changes in 9 transcriptomic experiments in the GEO database. **Panel A**: The number of genes changed more than 2-fold with an adjusted p-value of <0.05 was correlated with the concentration of deoxynivalenol (µM DON) used in the respective experiment. **Panel B**: The maximum amplitude of the gene expression changes observed in the 9 transcriptomic experiments is plotted in correlation with the concentration of deoxynivalenol used in the respective experiment. The maximum fold upregulation (relative to control values) in response to DON is shown as a blue circle (DON up-max); the maximum fold downregulation is shown as a red circle (DON down-max).*

Data consistency between and within experiments

Four of the 9 experiments analysed include a comparison of different trichothecene mycotoxins. These include deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3ADON) and 15-acetyl-dexynivalenol (15ADON) (in 3 cases: GSE164334, GSE97821, GSE36954), plus nivalenol (NIV) and fusarenone X (FX) in the latter two of these experiments (Alassane-Kpembi et al., 2017; He et al., 2021; Suzuki and Iwahashi, 2012). One additional experiment compared deoxynivalenol to deoxynivalenol-3-β-d-glucoside, 3-epi-deoxynivalenol, and de-epoxy-deoxynivalenol (GSE 66918). The GSE66918 experiment, however, did not show any significant gene expression changes for these deoxynivalenol metabolites (Pierron et al., 2016) (**supplementary table 2**).

Gene expression patterns in the experiments where deoxynivalenol was compared to its derivatives were very consistent. The fold increase in expression in response to toxin treatment is shown in **figure 3a**. 59 of the top 100 genes upregulated by exposure of human Caco2 gut epithelial cells to DON, 3ADON and 15ADON were identical (**figure 3b**). An analysis of all genes changed by more than 2 fold and an adjusted p-value of < 0.05 shows that between 50% and 70% of the genes upregulated and downregulated by the 3 toxins are identical (**figure 3c**). This is remarkable in view of the fact that 3ADON is far less toxic than DON or 15ADON (He et al., 2021). This indicates that transcriptome changes precede overt toxicity and may represent a more sensitive sensor for toxin exposure than cell viability.
There is a limited overlap in the number of identical genes being changed by DON exposure in a comparison between Caco2 cells (GSE164334) and porcine gut epithelial cells IPEC-J2 (GSE111185). Around 5% of the genes activated by DON exposure and around 10% of the genes repressed in response to DON exposure are identical between the two different gut cell lines (**figure 3d**). This may reflect the difference in species, specific cell line, or analysis platform used.

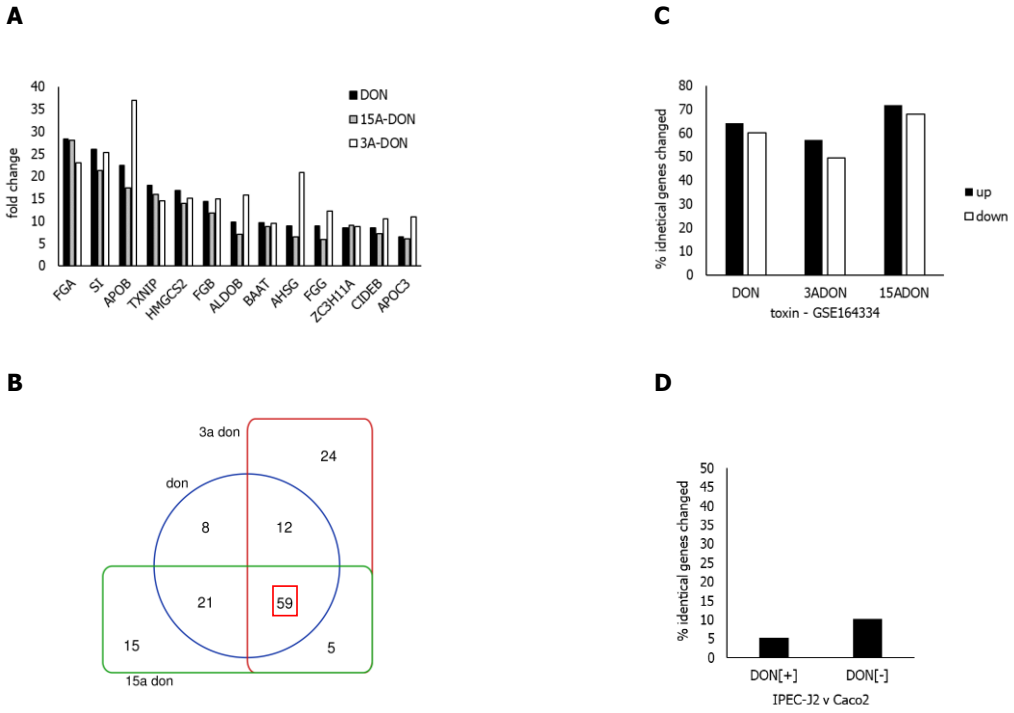

Figure 3*: Similarity of gene expression changes elicited by related mycotoxins in the GEO experiments GSE164334 (human Caco2 cells) and GSE111185 (porcine IPEC-J2 cells).* ***Panel A****: Gene expression changes (in GSE164334) of the top 10 genes regulated by exposure to deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3A-DON) and 15-acetyl-deoxynivalenol (15A-DON).* ***Panel B****: Venn diagram of the top 100 genes (in GSE164334) whose expression is changed by exposure to DON, 3A-DON or 15A-DON. 59 genes are identical between the 3 gene lists.* ***Panel C****: Similarity of all genes (in GSE164334) changed with a significance of p<0.05 and a fold change of more than 2. The number of genes collectively regulated by DON, 3ADON and 15ADON (557 genes upregulated; 615 genes downregulated) were compared to the number genes regulated for each of the individual toxins. Gene lists were compared using a Venn diagram web-application hosted by the University of Gent, Belgium (https://bioinformatics.psb.ugent.be/webtools/Venn/). The percentage of the co-regulated genes is shown.* ***Panel D****: Comparison of regulated genes in Caco2 (GSE164334) and IPEC-J2 cells (GSE111185) in response to DON treatment. Gene names for the experiment GSE11185 using IPEC-J2 cells were generated using the probe name conversion tool in G-Profiler. Gene names were compared using the University of Gent Venn diagram tool.*

Analysis of the data available in GSE97821 (IPEC-J2 cells) and GSE36954 (yeast) demonstrates that nivalenol (NIV) and 3-acetyl-deoxynivalenol (3ADON) treatment leads to a smaller number of genes being changed than exposure with deoxynivalenol (DON), 15-acetyl-deoxynivaleon (15ADON) and fusarenone-X (FX) (structures are given in **supplementary figure 3c**). This reflects the reduced toxicity of NIV and 3ADON. Most gene expression changes in response to DON are also found in response to 15ADON and FX. In the case of IPEC-J2 cells (GSE97821) 97% of genes changed in response to DON are also changed in response to either 15ADON, FX or both. In the case of yeast 79% of DON activated genes are also activated by 15ADON and/or FX (**supplementary figure 3a and 3b**). These data demonstrate that transcriptional responses to mycotoxins are consistent between different trichothecene mycotoxin derivatives/congeners.

The exact mechanism of trichothecene mycotoxins is ambiguous. Different toxicity mechanisms have been suggested which may or may not be interdependent. Pathway analysis of genes activated by DON exposure in Caco2 cells (0.5µM, 24, GSE164334) and human peripheral blood lymphocytes

suggests that the prevailing toxicity mechanism may cell context dependent (**table 2**). In Caco2 cells the predominant pathways identified using the list of DON-activated genes affect cell growth and ribosome function. E.g., the genes activated by DON are similar to those activated in cell carrying a deletion of ribosomal proteins RPL8 or RPL11 (adjusted p-values of 5.08E-12 and 5.08E-10, respectively). In contrast, the genes activated by DON (12.5µM, 20h, GSE19078) are similar to those involved in mitochondrial function. This suggests that mycotoxins mainly elicit ribo-toxicity in epithelial cells and oxidative stress in lymphocytes. This finding confirms a recent study of the proteome of DON treated skin cells in which ribosomal proteins and mitochondrial respiratory chain proteins were found to be the main targets of toxin action (Del Favero et al., 2021).

Caco2 cells [GSE164334]	**p-value**
Lylamine	2.65E-24
Torin-2	8.22E-15
Rhodomyrtoxin-B	1.24E-13
H-89	1.45E-13
PIK-90	1.15E-12
RPL8	5.08E-12
GSTP1	5.78E-12
FOXA1 27270436 ChIP-Seq VCaP Human Prostate Carcinoma	6.43E-11
HNF1A 26855178 ChIP-Seq Caco2 Human Colon Adenocarcinoma	4.84E-10
RPL11	5.08E-10
PRDM5 23873026 ChIP-Seq MEFs Mouse	1.12E-09

human peripheral blood lymphocytes [GSE19078]	**p-value**
mitochondrial electron transport, NADH to ubiquinone (GO:0006120)	0.000322
RNA biosynthetic process (GO:0032774)	0.00207
mitochondrial respiratory chain complex I assembly (GO:0032981)	0.006013
NADH dehydrogenase complex assembly (GO:0010257)	0.006013
RNA polymerase II preinitiation complex assembly (GO:0051123)	0.01733
Eukaryotic Transcription Initiation WP405	0.03706
Mitochondrial complex I assembly model OXPHOS system WP4324	0.04287

***Table 2**: Pathway analysis of deoxynivalenol induced genes in Caco2 cells (GSE164334)* (He et al., 2021) *and human peripheral blood lymphocytes, PBL (GSE19078)* (Hochstenbach et al., 2010). *The lists of genes upregulated by DON exposure were assessed using the Enrichr-KG software (https://maayanlab.cloud/enrichr-kg) limited to GO Biological Process, LINCS Chemical Perturbation signatures, LINCS CRISPR KO signatures, ChEA3 transcription factor enrichment analysis and Wikipathway 2021. The analysis demonstrates that in Caco2 cells the gene list signature induced by deoxynivalenol overlaps with signatures induced by growth inhibitors (e.g., Torin-2, PIK-90) and genetic deletions of ribosomal proteins (RPL8 and RPL11; highlighted in green). This suggests that ribotoxicity is the main insult of DON in Caco2 cells. In human PBLs the gene signatures induced by deoxynivalenol overlaps with gene signatures relevant for mitochondrial electron transport (highlighted in yellow). This suggests that oxidative stress is the predominant toxic effect induced by DON in PBLs. Note that the p-value with which pathways are detected is much lower in PBLs, although the replication is better (n=5 vs n=3 for Caco2 cells). This may reflect the fact that PBLs represent a mixture different cell types.*

Pathway analysis through the Enrichr software suite provides a wide range of outputs. These also include analyses of gene or drug perturbations. Analysis of the GSE164334 gene list of deoxynivalenol induced and repressed genes via L1000CDS2 (https://maayanlab.cloud/L1000CDS2/#/index) reveals

similarities to gene expression changes induced by cancer drugs (e.g., PubChem compounds 265237, 2449454, or 10275789). The drug perturbation module can also identify chemical treatments of cells which reflect a reciprocal transcriptome response (Drug perturbation from GEO down). The gene list derived from the transcriptomic experiment GSE164334 identifies quercetin as a highly significant drug perturbation which may counteract the effects of deoxynivalenol (**supplementary table 3**). Intriguingly a recent paper identified quercetin as a mitigator of DON activity on cell viability in a cell culture model (Kalagatur et al., 2021). This suggests that pathway analysis of transcriptome changes induced by mycotoxins may identify potential mitigation strategies.

Application of data to the development of cell based assays

Cell based assays are usually generated in one of two ways. A reporter gene construct in which a readily measurable reporter protein (fluorescent or luminescent) is linked to a promoter responsive to the stimulus applied (e.g., a toxin). Alternatively, a signalling interaction which is activated by a stimulus is tagged with a fluorescent marker unit such that the stimulus can be readily quantified. Both approaches rely on detailed knowledge of the transcriptomic changes which are elicited by the stimulus and the signalling events which lead to the gene expression changes. Therefore, transcriptomic studies are essential for the design of sensitive and specific cell based assay systems. The secondary data analysis carried out in this study identified several potential gene targets and their associated signalling pathways as suitable starting points for the development of cell based assays.
E.g., the CMSS1 gene (encoding the homologue of a ribosomal protein) is activated 42-fold in response to DON treatment in lymphocytes. Analysis of CMSS1 gene regulation using the Signaling Pathways Project database (Ochsner et al., 2019) identifies cAMP response element binding protein CREB1 as the transcription factor with the highest binding score. Literature data demonstrate an increase in GLP-1 secretion in entero-endocrine cells as a cellular response to DON (Zhou and Pestka, 2015). GLP-1 secretion is associated with cAMP signalling (Parker et al., 2012).

Conclusions

The systematic review of the transcriptome data for trichothecene mycotoxins in the GEO database demonstrates that:

1. Transcriptome analysis in response to mycotoxin exposure is an area with substantial research gaps.
2. Cell lines are highly sensitive to mycotoxin exposure, but sensitivity varies from cell type to cell type.
3. Cell lines respond to the mycotoxin deoxynivalenol and its metabolites with consistent transcriptome changes.
4. Transcriptome changes occur faster and with higher sensitivity than changes in cell viability.
5. The prevalent toxicity mechanism of a mycotoxin is strongly influenced by cell context.
6. Transcriptome analysis can provide information on potential mycotoxin mitigation strategies.

For the development of cell based assays these finding suggest that:

1. Finding cell lines with high sensitivity to mycotoxin exposure is an important prerequisite for assay development.
2. Cell based assays can be used to detect mycotoxins and their metabolites.
3. Cell based assays need to be tailored to the dominant toxin response in a specific cell context.
4. Cell based assay may be a tool to explore toxin mitigation strategies in a high throughput format.

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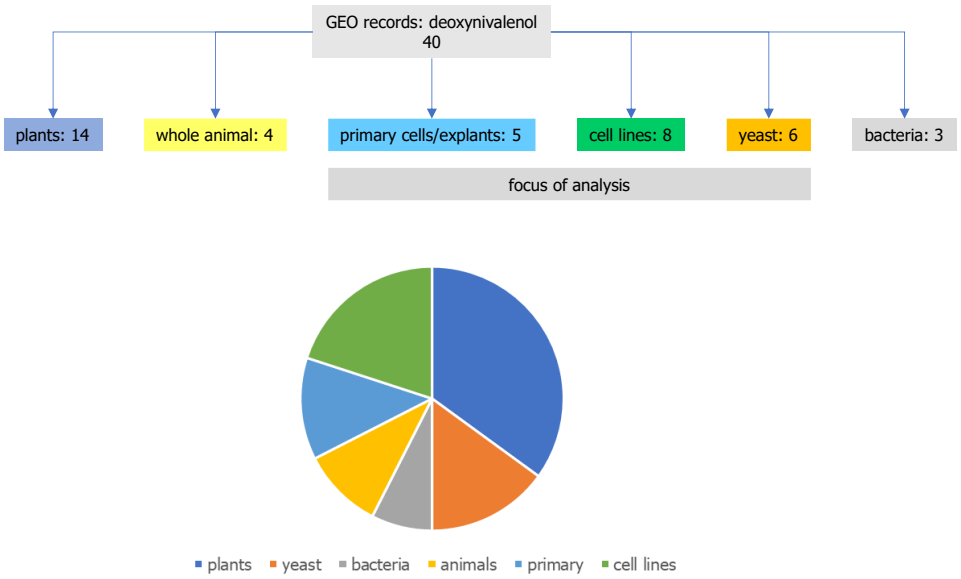
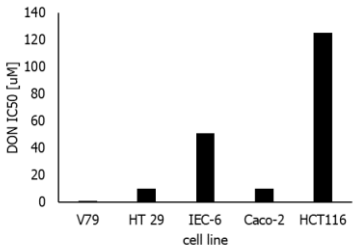
search-terms	rec.	exp.
mycotoxin AND (microarray OR RNAseq OR transcriptome)	1396	288
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND aflatoxin	338	82
aflatoxin	1254	115
aflatoxin AND mycotoxin	1254	115
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND ochratoxin	15	15
ochratoxin	118	20
ochratoxin AND mycotoxin	118	20
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND patulin	8	8
patulin	57	8
patulin AND mycotoxin	57	8
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND deoxynivalenol	19	17
deoxynivalenol	301	40
deoxynivalenol AND mycotoxin	39	25
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND zearalenone	5	5
zearalenone	90	11
zearalenone AND mycotoxin	88	10
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND fumonisin	28	23
fumonisin	535	27
fumonisin AND mycotoxin	535	27
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND (T-2 OR HT-2)	2	2
T-2 OR HT-2	1935	233
HT-2	47	24
T-2 AND mycotoxin	20	2
mycotoxin AND (microarray OR RNAseq OR transcriptome) AND citrinin	9	9
citrinin	73	10
citrinin AND mycotoxin	73	10

***Supplementary Table 1**: Number of records (rec.) and experiments (exp.) retrieved with the indicated search terms from the GEO database.*



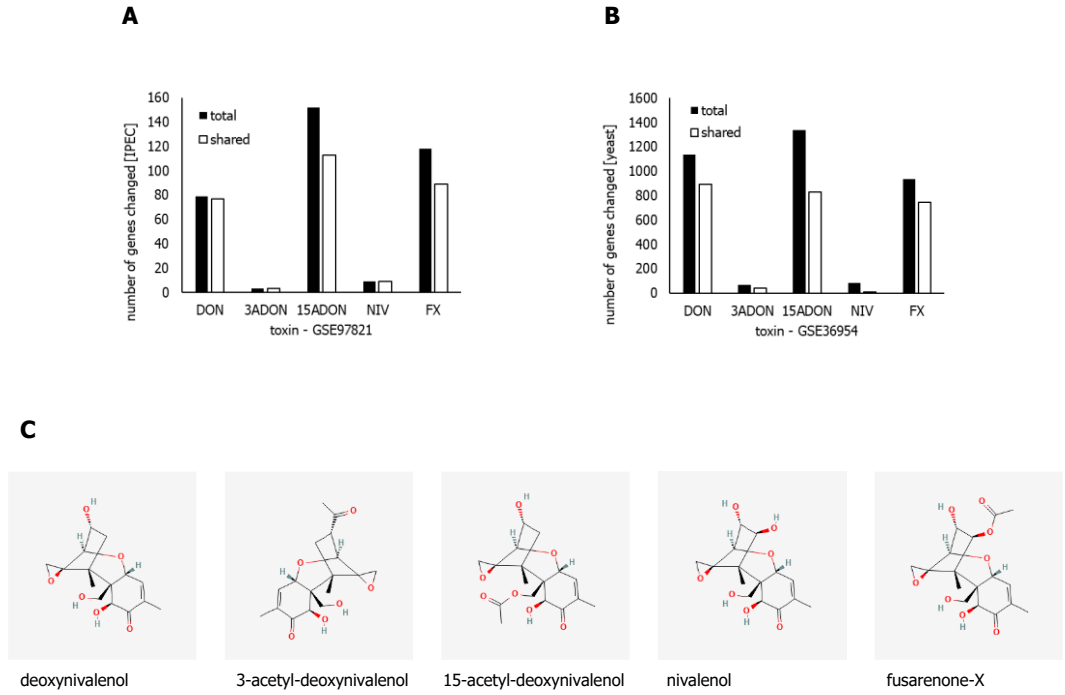
***Supplementary Figure 1**: Distribution of GEO datasets selected with the search terms "mycotoxin" AND "deoxynivalenol" by categories.*



***Supplementary Figure 2**: Sensitivity of different cell lines to deoxynivalenol exposure. The IC50 values were derived from Cheli et al. 2014*

accession	platform	species	cells	replicates	conc. MT	inc. time	probes changed: p<0.05	changed >2 fold	publication	analysis
GSE19078	GPL6480 Agilent	human	PBL primary cells	n=5	12.5uM DON 1.25mM AFB1	20h 20h	8812 146	(1331↓, 1572↑)	PMID: 20702593 Hochstenbach et al. 10	GEO2R
GSE164334	GPL24676 Illumina	human	Caco2 cell line	n=3	500nM DON 500nM 3A-DON 500nM 15A-DON	24h 24h 24h	1901 2218 1681	(869↓, 1032↑) (876↓, 1342↑) (777↓, 904↑)	PMID: 33671637 He et al. 21	Excel
GSE97821	GPL19893 Agilent	pig	jej. explant primary cells	n=4	2uM or 10uM DON 2uM or 10uM 3ADON 2uM or 10uM 15ADON 2uM or 10uM NIV 2uM or 10uM FX	4h 4h 4h 4h 4h	0 for 2uM; 79 for 10uM 0 for 2uM; 3 for 10uM 1 for 2uM; 152 for 10uM 0 for 2uM; 9 for 10uM 128 for 2uM; 118 for 10uM	(0↓, 79↑) (0↓, 3↑) (0↓, 135↑) (0↓, 6↑) (0↓, 110↑)	PMID: 28790326 Alassane-Kpembi et al. 17	GEO2R
GSE66918	GPL19893 Agilent	pig	jej. explant primary cells	n=4	10uM DON 10uM 3G-DON 10uM epi-3-DON 10uM de-epoxy-DON	4h 4h 4h 4h	98 0 0 0	(1↓, 93↑)	PMID: 27381510 Pierron et al. 16	GEO2R
GSE165968	GPL19893 Agilent	pig	jej. explant primary cells	n=8	10uM DON	4h	1103	(5↓, 249↑)	PMID: 34205708 Tremblay-Franco et al. 12	GEO2R
GSE111184 GSE111185	GPL3533 GPL3533 Affymetrix	pig	IPEC-J2 cell line	n=3	680nM DON (apical) 6.8uM DON (apical) 680nM DON (basolateral) 6.8uM DON (basolateral)	72h 72h 72h 72h	2 3417 12 6569	(324↓, 271↑)	PMID: 30423940 Nossol et al. 18	GEO2R
GSE75462	GPL11488 Agilent	yeast	S.cerevisiae defined strain	n=3	81uM DON 40uM DON 40uM DON 81uM DON 81uM DON	o/n + OD1/1.5 OD1 OD1.5 OD1 OD1.5	418 0 0 40 1	(26↓, 1↑) (1↓, 10↑)	PMID: 27245696 Kugler et al. 16	GEO2R
GSE36954	GPL2529 Affymetrix	yeast	S.cerevisiae defined strain	n=3	84uM DON 84uM 3ADON 84uM 15ADON 84uM NIV 84uM FX	2h 2h 2h 2h 2h	2876 222 3215 223 2438	(695↓, 1143↑) (117↓, 70↑) (685↓, 1344↑) (91↓, 85↑) (624↓, 944↑)	PMID: 22897823 Suzuki & Iwahashi 12	GEO2R
GSE63663	GPL2529 Affymetrix	yeast	S.cerevisiae defined strain	n=3	80uM 3GDON	36h	14	(7↓, 7↑)	PMID: 25609182 Suzuki & Iwahashi 15	GEO2R

***Supplementary Table 2**: List of the transcriptomic experiments included in the systematic review. The number of probes which were changed with an adjusted significance value of p<0.05 are shown. Some genes may be represented by more than one probe. Therefore, the number of genes changed will be lower than the number of probes. The number of probes changed more than 2 fold is shown with the arrow indicating whether the genes are upregulated or downregulated in response to toxin exposure.*



***Supplementary Figure 3**: Gene expression changes reported in IPEC-J2 porcine gut epithelial cells (GSE97821) and yeast (GSE36954) in response to exposure to trichothecene mycotoxins deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3ADON), 15-acetyl-deoxynivalenol (15ADON), nivalenol (NIV) or fusarenone-X (FX). **Panel A**: Number of genes changed (IEPC-J2 cells – 10µM toxin, 4h exposure) in response to toxin exposure (solid bars) and number of gene changes shared with at least one other toxin (open bars). **Panel B**: Number of genes changed (yeast cells – 84µM toxin, 2h exposure) in response to toxin exposure (solid bars) and number of gene changes shared with at least one other toxin (open bars). **Panel C**: 2D structural formulas for the toxins used (adapted from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).*

rank	Term - DON	adj. P-value
#1	quercetin 5280343 human GSE7259 sample 3416	2.31E-18
#2	quercetin DB04216 mouse GSE38136 sample 3437	4.21E-13
#3	quercetin 5280343 human GSE7259 sample 3415	1.56E-12
rank	**Term - 3ADON**	**adj. P-value**
#1	quercetin 5280343 human GSE7259 sample 3416	1.61E-16
#2	quercetin 5280343 human GSE7259 sample 3415	8.52E-16
#3	quercetin DB04216 mouse GSE38136 sample 3437	5.64E-13
rank	**Term - 15ADON**	**adj. P-value**
#1	quercetin 5280343 human GSE7259 sample 3416	1.12E-12
#2	quercetin 5280343 human GSE7259 sample 3415	1.29E-09
#3	quercetin DB04216 mouse GSE38136 sample 3437	7.95E-09

***Supplementary Table 3**: Analysis of the gene lists of the top 100 genes induced by deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3ADON) and 15-acetyl-deoxynivalenol (15ADON) treatment in using the Enrichr module Drug perturbation from GEO (down) (https://maayanlab.cloud/Enrichr/). The most closely related gene signatures for all 3 toxin treatments in the GEO database are induced by quercetin.*