# Gene expression profiling of human T cells upon exposure to the mycotoxin Deoxynivalenol

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## Introduction

Deoxynivalenol (DON) is one of the mycotoxins produced by soil fungi of the Fusarium genus and is prevalent worldwide in cereal-based foods. Human consumption of DON-contaminated foods, particularly cereals, imposes a health hazard and exerts adverse effects on several organs including the immune system.

## Aim

***Characterization of effects of DON on the T-lymphocyte cell line Jurkat***

## Materials and Methods

Cell type: Human T-lymphocyte cell line (Jurkat)

Concentration of DON: 0, 0.25, 0.5 and 1 µM

Exposure times: 3, 6, 12 and 24h

Determination of cell viability: WST-1 assay

Gene expression profiling: Agilent whole human genome microarrays (4x44k)

Selection of significant genes: Fold Ratio ≥ |1.5| and P < 0.01 (T-test).

Biological interpretation: Gene set enrichment analysis (GSEA).

## Results

Exposure to DON resulted in a time- and concentration-dependent decrease in viability (Fig 1).

The number of genes affected by DON increased as a function of time and dose (Table 1).

After 3h of exposure, DON induced expression of endoplasmic reticulum (ER) stress response genes and repressed expression of major chaperones which are involved in the unfolded protein response (Fig. 2).

GSEA on the microarray data showed that DON activates MAPK pathway, T cell activation, NFKB pathway and p53-dependent apoptosis after 3h of exposure (Fig. 3).

The effects of DON on these pathways were less pronounced at later time points, from 6h onwards.

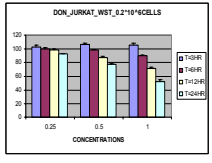


*Figure 1. Cell viability of Jurkat cells exposed to various doses of DON. Viability of the cells was measured by the WST-1 assay. The percentage of viable cells was determined by comparing the optical density of treatment groups to those of the appropriate controls.*

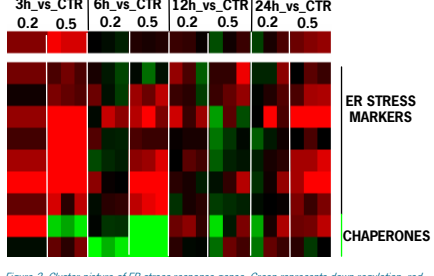


*Figure 2. Cluster picture of ER stress response genes. Green represents down regulation, red upregulation and black means no regulation. ER stress-related genes are mainly upregulated by 0.5 µM DON with highest induction rates within 3 h. Chaperone gene expression was down regulated by 0.5 µM DON at 3 h and 6 h.*

| 1.5 x P<0.0 | 0.2 µM | 0.5 µM |
|---|---|---|
| 3 h | 33 | 529 |
| 6 h | 7 | 578 |
| 12 h | 24 | 476 |
| 24 h | 0 | 318 |

*Table 1. The number of genes significantly regulated by DON at various doses and time periods.*

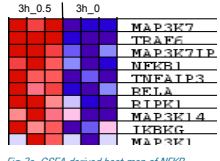


*Fig 3a. GSEA derived heat map of NFKB pathway. Blue represents low expression and red high expression. NFKB pathway related genes are mainly upregulated by 0.5 µM DON within 3 h compared to 3h vehicle controls.*

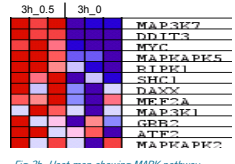


*Fig 3b. Heat map showing MAPK pathway associated genes are mainly upregulated by 0.5 µM DON within 3 h compared to 3h controls.*

## Conclusions

Based on the GSEA results, we hypothesize that DON induces ER stress that leads to disturbance of the calcium homeostasis, which in turn leads to a T-cell activation response and induction of apoptosis.

DON has been reported to bind to 28S ribosomal RNA and inducing ribotoxic stress (Petska et al., 2005, Tox Sci 85: 916-926). Since ribosomes are attached to ER, the ER stress found in our study might be a consequence of ribotoxic stress. Alternatively, the induction of ER stress might be a separate effect of DON, independent from ribotoxic stress.

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