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Transcript Abundance Responses of Resistance Pathways of *Arabidopsis thaliana* to Deoxynivalenol

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Abstract

Mycotoxin deoxynivalenol (DON), produced by *Gibberella zeae* (Schwein.) Petch (teleomorph of *Fusarium graminearum* Schwabe) was known to be both a virulence factor in the pathogenesis of *Triticum aestivum* L. (wheat) and an inhibitor of *Arabidopsis thaliana* L. seed germination. *Fusarium graminearum* causes both Gibberella ear rot in maize (*Zea mays* L.) and Fusarium head blight (FHB) in wheat and barley. *Arabidopsis thaliana* was also a host for the related root rot pathogen *F. virguliforme* Aoki. *A. thaliana* seedling growth was reduced by the pathogen in a proportional response to increasing spore concentrations. Here, the changes in transcript abundances corresponding to 10,560 *A. thaliana* expressed sequence tags (ESTs) was compared with changes in 192 known plant defense and biotic/abiotic stress related genes in soybean roots after infestation with *F. virguliforme*. A parallel comparison with a set of resistance pathways involved in response to the DON toxicity in *A. thaliana* was performed. *A. thaliana* data was obtained from the AFGC depository. The variations of transcript abundances in *Arabidopsis* and soybean treated with pathogen suggest that both plants respond to the pathogen mainly by common, possibly global responses with some specific secondary metabolic pathways involved in defense. In contrast, DON toxin appeared to impact central metabolisms in *Arabidopsis* plants with significant alterations ranging from the protein metabolism to redox production. Several new putative resistance pathways involved in responding to both pathogen and DON infestation in soybean and *A. thaliana* were identified.

Keywords: *Glycine max*, *Arabidopsis thaliana*, *Fusarium*, deoxynivalenol, DON, pathway, interaction.

Introduction

In the field, many fungal species cause plant diseases, several of which produce specific mycotoxins (Bai et al., 2002). Mycotoxins are diverse secondary metabolites formed by fungi through terpenoid, polyketide, and other biosynthetic pathways. One of the important groups of Fusarial mycotoxins, named the trichothecenes, that include deoxynivalenol (DON) and T-2 toxin, are commonly found in cereal grains as a result of fungal infection. Trichothecenes inhibit protein synthesis. DON inhibits a wide set of steps in gene expression but has been shown to reduce the activity of eukaryotic ribosomes (60S subunit) and impairs the initiation or elongation and termination steps of protein synthesis (Ehrlich and Daigle, 1987; Feinberg and Mclaughlin, 1989).

Sudden death syndrome (SDS) of soybean (*Glycine max* L. Merr.) caused by fungal pathogen *Fusarium virguliforme* (Aoki; ex. *F. solani* Mart. Sacc., ex f.sp. *glycines* Roy) produces root cell infection as well as leaf scorch, which mycotxins produced in roots translocated into soybean leaves and resulted in disease symptom. Both root rot and leaf scorch can cause significant yield losses. Iqbal et al. (2005) measured changes in transcript abundance (TA) of 192 known plant defense and biotic/abiotic stress related genes from a soybean root cDNA library within a time course study. They found at least two-fold TA increase among 36 of these resistance genes from day 3 to day 10 after the pathogen was infested in the soil surrounding the roots of a resistance genotype.

Arabidopsis thaliana (L). is compatible with many types of phytopathogens (Bai et al., 2002). *Arabidopsis* is a host for *F. virguliforme* and the growth responses to the pathogen were correlated with the spore concentration (Yuan et al., 2008). It is unclear whether *F. virguliforme* releases just DON or also other trichothecenes (Baker and Nenec, 1994). Similar responses to

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the concentration of DON were seen for *Arabidopsis* root elongation (Shin et al., 2012). Transgenic expression of a barley UDP-glucosyltransferase provided resistance to DON.

Masuda et al. (2007) demonstrated that seedlings showed dwarfism with morphological changes such as petiole shortening, curled dark-green leaves, and reduced cell size under T-2 toxin treatment. When T-2 toxin was applied into the leaves of *Arabidopsis*, it showed an elicitor-like activity in plant cells (Nishiuchi et al., 2006). DON tends to be harmful to the translational machinery in plants but may also inhibit transcription and elicitor-like signaling pathways in *Arabidopsis* cells (Masuda et al., 2007; Shin et al., 2012). Studies showed that pathogens with toxicity (Tox) gene mutations were often nonvirulent (Graniti, 1991), or caused less symptoms than the wild-type counterparts, suggesting that trichothecenes were pathogenesis factors of *Fusarium graminearum* (Desjardins and Hohn, 1997; Awad et al., 2008). Genetic engineered defense response genes provide an alternative strategy to reduce *F. graminearum* infection. Overexpression of a barley class II chitinase (EC 3.2.1.14) gene increased the resistance against *F. graminearum* in wheat (Mackintosh et al., 2007; Shin et al., 2008) and thereby reduced head blight and DON toxicity. DON and glutathione formed a glucose-conjugate to reduce DON toxicity in vivo and wheat transgenic plants carrying barley UDP-glucosyltransferase converted DON into a DON-glucoside and displayed an enhanced tolerance to DON (Shin et al., 2012). To date, there is no a single model of plant vs. toxin interaction and no simple or common resistance mechanism to explain the mechanism of plant resistance to tricothecene toxins like DON and T2. Whether there is a close correlation between DON toxicity and *Fusarium* infection is still not fully understood.

Microarray experiments allow the dissection genome wide patterns of mRNA abundances and improve understanding of the molecular basis of the plant defense responses. These global and simultaneous analyses of TA profiles enable variations in mRNA abundances under specific treatments to be compared. In order to compare the plant resistance mechanism to DON toxicity and infection of Fusarial pathogens, microarray data from *Arabidopsis thaliana* cells challenged with the mycotoxin DON was compared to the data from the plants responding to *F. virguliforme* infestations herein. The cDNA microarray chips used contained over 10,000 different ESTs (AFGC set 2001) was employed in both analyses. The first objective of this approach was to identify genes that were transcriptionally regulated when plants were treated with the toxin. The second objective using the microarray data was to identify resistance pathways where these co-regulated genes were positioned. The parallel comparison on transcriptional activities among *Arabidopsis* and soybean after fungal pathogen *F. virguliforme* pathogenesis and *Arabidopsis* with DON treatment was also performed.

Materials and Methods

Arabidopsis thaliana Seed Germination, RNA Isolation and Microarray Procedure

A. thaliana ecotype 'Columbia' seeds were germinated and RNA was extracted from young seedlings treated with DON (for 6 hours or 24 hours) or water (ftp://smd-ftp.stanford.edu /smd/organi sms/AT), respectively. Microarray (21124.xls and 22172.xls at ftp://smd-ftp.stanford.edu/smd/ organisms/AT/) platform for the gene expression analysis was performed in this experiment. The mRNA samples corresponding to DON treatment and non-treated control were labeled using Cy3- or Cy5-labeled dUTP. One technical replicate was applied by using reversed dye labels compared to the first hybridization (ftp:// smd-ftp.stanford.edu/smd/). Dr. Patrick Hart at Michigan State University carried out the microarray experiment.

Microarray Gene Expression Data Analysis

The cDNA microarray data were normalized by local (local background value was subtracted from the intensity value of each spot) metrics. Stringent quality control measures were applied to all stages of data analysis. The normalization procedure for the microarray data followed the method described by Pevsner (2003) to adjust for differences in the intensity of the two labels. Coefficients of means, variances of the signal intensities in each channel, and ratios of signals from two replicates were calculated by a C++ program written for the purpose. The C++ program was also applied to handle the missing and extra data values.

MapMan™ allows the visualization of transcriptomic and metabolomic data simultaneously (http://gabi.rzpd.de/projects/ MapMan/; Thimm et al., 2004). Classification of Image Annotator in the software was also used to diagram the data display. After the *Arabidopsis* EST-based microarray data were converted into Affymetrix 22K array annotation, all the genes that corresponded from both reverse labeled arrays were subjected to pathway construction using the MapMan platform (http://gabi. rzpd. de/projects/MapMan). Transcripts that were increased in abundance were denoted as blue, and transcripts that were decreased in abundance were denoted as red. The identified metabolic clusters and their relationships in metabolism by the MapMan platform were displayed as bins (1-100) and each bin represented a set of related pathways. In the scale used for the visualized data, a 2-fold (1 on Log2) change was required to produce a visible coloration. A computer platform, *Arabidopsis* Interactions Viewer (renamed Interactome 2.0, http://bar.utoronto.ca/interactions/cgi-bin/arabidop sis_interactions_viewer. cgi) was employed to predict protein-protein interactions (interologs, Geisler-Lee et al., 2007). Output of interlogs was plugged into the Cytoscape software environment (Shannon et al., 2003) for network visualization and modeling to against each other in order to catalog all of their conserved pathways and gene interaction networks. The program was equipped with a plug-in architecture for customizing applications. Visual data were displayed on Log 2 scale and saved as a Cytoscape graph. In the Cytoscape diagram of a transcriptional network, nodes represented structural genes and their regulatory elements and links represented protein-protein (transcription factor DNA binding) interactions. Regulatory genes appeared as 'hubs' in a network and possessed many interactions.

Results

Overview of TA Alterations After DON Treatment

The microarray analysis clearly indicated that deoxynivalenol (DON) treatment resulted in major responses among transcript abundances (TAs; Figure 1; Table 1). TAs in cell cultures derived from *Arabidopsis* seedlings were altered differently by DON over time. Six hours (6h) after DON treatment, 391 transcripts were increased in abundance by more than 2 fold (displayed as 1 fold on Log 2 scale) but 169 transcripts were decreased in abundance by more than 2 fold (displayed as -1 fold on Log 2 scale) and 119 of them were reduced by more than 3 fold. However, only 120 transcripts appeared to be increased in abundance by more than 2 fold 24 hours (24h) after DON was applied (Figure 1; Table 2). Conversely, 181 transcripts were decreased in abundance by more than 2 fold at this time point. Moreover, the transcripts that could be detected by the microarray experiment were significantly reduced by nearly 40% suggesting that the toxicity to transcription and subsequent metabolic perturbations might occur in the process of the time course experiment (data not shown).

Description of Significantly Regulated Pathways in the MapMan Platform

The *Arabidopsis* transcript abundances that were significantly increased (log 2 ≥ 1) and decreased (log2 ≥ -1) were visualized within the MapMan platform to identify metabolic clusters and their relationships in metabolism. The preliminary analysis displayed by the MapMan platform indicated that the transcript abundances following the DON treatment had been dramatically altered in many metabolic clusters. By 6 hours after DON treatment, the 560 genes that had significantly altered TA (≥1 or ≤-1 on Log2 scale) in the two reversed labeled slides were assembled into 12 major bins and each bin represented a set of related pathways (Table 1). Within these 12 bins, more than 41 sub-bins were differentially regulated by DON. The toxicity of DON had a major effect on the pathways of the protein synthesis and degradation. There were 12 sub-bins in bin29 (protein metabolism) and 4 sub-bins in bin27 (RNA synthesis) being strongly affected by DON, which suggested that DON toxicity also targeted to the gene transcription (Table 1A, B). Moreover, TA changes in signaling and hormone metabolism bins were also altered.

At 24h after DON treatment, 301 genes that had TAs significantly altered were assembled into 16 major bins, within which were a total 1,945 genes (Table 2). There were more than 35 sub-bins that contained TAs that were significantly affected by the DON toxin ($P<0.05$). TAs in bin27 (RNA transcription) and bin29 (protein metabolism) were reduced significantly at this stage. In total 1,045 genes encompassed by the bin27 were affected by DON 6h after treatment but only 776 genes remained in the bin 24h after the treatment. The bin29 also showed clearly an inhibition in protein metabolism indicating a suppressed responsiveness by the toxin 24h post DON treatment (Figure 1B). Only 3 of the 12 sub-bins altered at 6h were still preserved at 24h after DON treatment. The number of genes encompassed by these affected sub-bins were reduced from 410 to 79. Therefore, a large proportion of genes involved in protein metabolism that were poisoned by the mycotoxin at 24h were normally abundant at 6h after treatment. In parallel, several other bins such as transporter genes and the genes related with lipid metabolism and redox reactions were altered at 24h, whereas they were not at 6h. Furthermore, about half of the transcripts altered at 24h had unknown functions (713 genes) and can now be annotated as invilvoed in responses to DON. Whether the plants attempt to reconfigure and bypass the inhibited enzymes and damaged pathways still need to be determined.

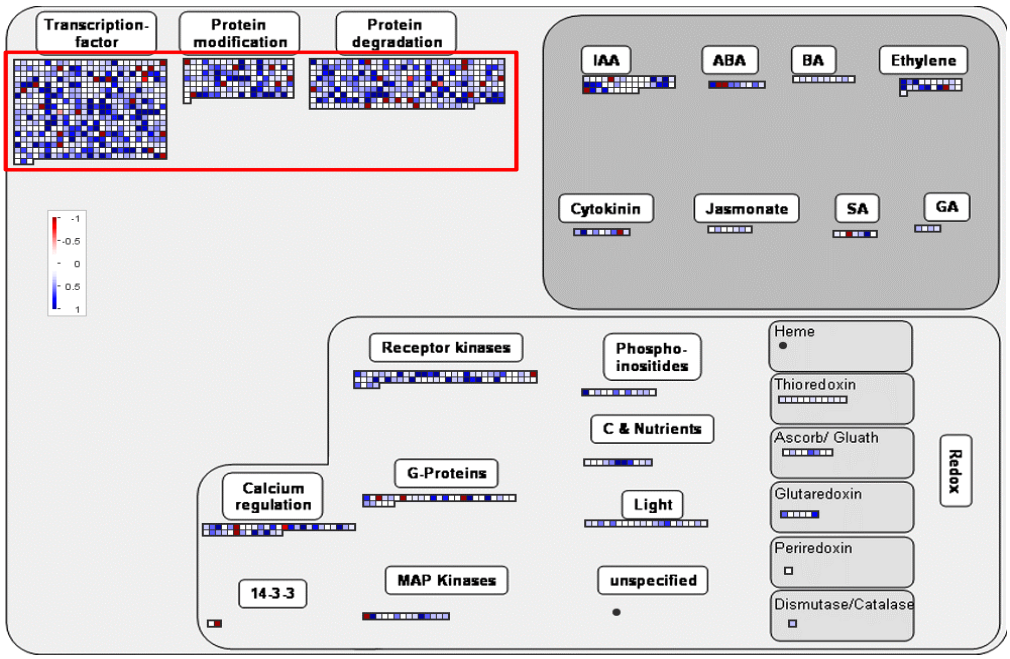
Unlike *F. virguliforme* infestation of soybean and *Arabidopsis*, none of the crucial gene transcripts that potentially involved in secondary metabolism (bin 16) had been increased in abundance after DON toxin was applied. No pathway leading to isoprenoid, phenylpropanoid, and lignin biosynthesis was shown increased in TA at either 6 or 24 h after treatment. Therefore, DON inhibition does not appear to trigger all of the same biotic and abiotic stress response pathways as *F. virguliforme* infestation.

Networks of Protein-protein Interaction

Bin29 contained many genes involved in plant defense schema (Wang et al., 2006) and transcripts in this bin were greatly affected by DON treatment. The 417 genes encompassed in this bin (from the stage of 6 hours after DON treatment) were visualized using the *Arabidopsis* Interactions Viewer (Geisler-Lee et al., 2007) to investigate the potential molecular protein-protein interactions among genes with TA changes. The output of the interlogs from the *Arabidopsis* Interaction Viewer (Geisler-Lee et al., 2007) was plugged into the Cytoscape software environment (Shannon et al., 2003) for network visualization and modeling. In these diagrams of protein –protein interaction networks, nodes represented structural genes and their regulatory elements while links denoted protein-protein interactions. Regulatory genes like transcription factors appeared to be 'hubs' in a network and had many interactions. Four hubs, At2g38560 (TFIIF (-3.258/Log2)), At3g55620 (EMB1624 (1.310/Log2)), At1g36730 (ETIF5 (1.025/Log2)), and At2g47020 (PCRF (1.079/Log2)) were identified 6h after DON was applied (Figure 3). The multiple network characteristics among several hubs (highlighted in red) inferred resistance related protein-protein interactions and displayed a correlation between the complexity of a network and the property of resistance.

Four major hubs, At5g35980 (Protein kinase (-1.269/Log2)), At5g57020 (NMT1 (-3.727/Log2)), At3g59950 (APG4a (-1.716/Log2)), At5g45900 (APG7 (1.051/Log2)), At3g11830 (chaperonin (-5.442/Log2)) were observed 24h after DON treatment. The metabolic networks in bin29 were represented as a linear relationship among the hubs and nodes (Figure 3). However, plants may attempt to avoid DON toxicity by reconfiguration of the metabolic networks. The great number of genes with unknown functions that were identified 24h post DON treatment might be involved in that response. The increased response

A. 6 hours after DON applied



B. 24 hours after DON applied

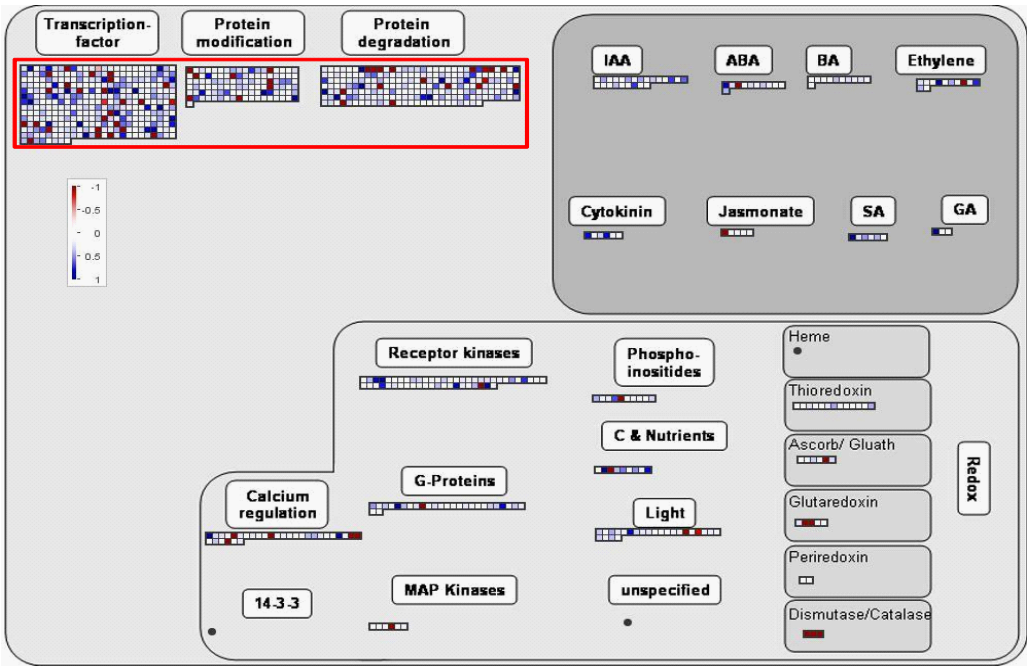


Figure 1. Transcript abundance changes in responses of Arabidopsis cells to DON treatments. The crucial metabolic pathways in the MapMan program were marked in red rectangles . The small blue and red squares denoted increased (positive) and decreased (negative) transcript abundances (TAs) of individual genes. The bar was shown on log 2 scale (1=two fold change). A. 6 hours after DON was applied. B. 24 hours after DON was applied.

among genes of unknown function was in contrast to the reduction in the number of TAs that were involved in other metabolisms in *Arabidopsis* plants at this stage. Whether or how the increased TA of these genes of unknown function provides a bypass for damaged enzymes and compartments to escape the toxin selection is still not known.

Table 1. Description of the Mapman bins (metabolic clusters) with significant changes in TAs by 6 hours after DON treatment. The bin numbers were denoted from 1-100. Sub-bins were listed as decimals. Only bins encompassing genes with significant changes in transcript abundance ($P<0.05$) were listed. The number of elements in each bin present on the array was listed. The five bins highlighted in light blue and two bins in brown contained many TAs highly affected by the mycotoxin.

bin	name	elements	p-value
1	PS	26	0.015
1.2	PS.photorespiration	4	0.046
2.1	major CHO metabolism.synthesis	4	0.019
2.1.2	major CHO metabolism.synthesis.starch	3	0.041
3.1	minor CHO metabolism.raffinose family	4	0.013
3.5	minor CHO metabolism.others	5	0.048
3.6	minor CHO metabolism.callose	3	0.02
3.1.2	minor CHO metabolism.raffinose synthases	2	0.023
3.1.2.2	minor CHO metabolism.raffinose synthases.putative	2	0.023
8	TCA / org. transformation	10	0.035
8.2.4	TCA / org. transformation.IDH	2	0.035
10	cell wall	79	0.027
10.3	cell wall.hemicellulose synthesis	4	0.039
10.8.1	cell wall.pectin*esterases.PME	10	0.032
11	lipid metabolism	67	0.014
11.8	lipid metabolism.'exotics' (steroids, squalene etc)	18	0.037
13	amino acid metabolism	61	0.026
13.1	amino acid metabolism.synthesis	43	0.045
13.1.4	amino acid metabolism.branched chain group	8	0.038
13.1.6.1	amino acid metabolism.aromatic aa.chorismate	6	0.016
19.99	tetrapyrrole synthesis.unspecified	2	0.039
20.1	stress.biotic	60	0.035
27	RNA	544	0.004
27.3	RNA.regulation of transcription	453	0.009
27.3.27	RNA.regulation of transcription.NAC domainTF	16	0.007
27.3.32	RNA.regulation of transcription.WRKY domain TF	12	0.022
27.3.35	RNA.regulation of transcription.bZIP TF	20	0.009
29.2.1	chloroplast/mito - plastid ribosomal protein	9	0.033
29.2.1.1	chloroplast/mito - plastid ribosomal protein.plastid	5	0.005
29.2.2.50	misc ribososomal protein.BRIX	4	0.023
29.2.5	protein.synthesis.release	2	0.033
29.3	protein.targeting	37	0.04
29.3.4	protein.targeting.secretory pathway	17	0.009
29.3.4.99	protein.targeting.secretory pathway.unspecified	7	0.036
29.4.1.57	postranslational modification.RLCK VII	11	0.028
29.5.11.4	protein.degradation.ubiquitin.E3	166	0.014
29.5.11.4.3	protein.degradation.ubiquitin.E3.SCF	74	0.004
29.5.11.4.3.2	protein.degradation.ubiquitin.E3.SCF.FBOX	69	0.002
29.5.11.5	protein.degradation.ubiquitin.ubiquitin protease	9	0.045
30.2.6	signalling.receptor kinases.leucine rich repeat VI	4	0.021
30.7	signalling.14-3-3 proteins	2	0.038

Discussion

A number of fusarial species cause infections that both reduce yield and grain quality on major cereal crops such as wheat, barley, and maize. Mycotoxins produced by these pathogens lead to feed-born intoxications in farm animals (Awad et al., 2008). Mycotoxins have also been considered as aggressiveness factors in pathogens in both wheat and maize. Cumagun et al. (2008) identified loci associated with pathogenicity and aggressiveness in a *F. graminearum* population derived from a cross between a NIV producer lineage 6 from Japan and a DON producer lineage 7 from Kansas. Lineage 7 was the dominant race that caused FHB and Gibberella ear rot in crops (O'Donnell et al., 2000; Xu and Nicholson, 2009). Mycotoxin trichothecenes (that include DON and T2 toxin) appeared to possess multiple inhibitory effects ranging from protein, DNA, and RNA synthesis to signaling and membrane functions in eukaryotes (Rocha et al., 2005). The toxins enhance the spread of *F. graminearum* fungal pathogen (Lori et al., 1997). A close relationship between the concentration of DON and FHB severity and 1,000 kernel weight was observed suggesting that the trait was affected by the production of the toxin (Lori et al., 1997; Hestbjerg et al., 2002). DON also affected root elongation and the roots were less organized compared with the control in A. thaliana (Hart, unpublished data, AAFC). Evidences demonstrated that reduction of toxin production by the pathogen or removal/degradation of the toxin by the host reduced aggressiveness of some pathogens (Foroud and Eudes, 2009). Pathogens with toxicity (Tox) gene mutation appeared to be non-virulent (Graniti, 1991) or caused lesser disease symptoms than the wild-type (Desjardins and

Table 2. Description of the Mapman bins (metabolic clusters) with significant changes in TAs by 24 hours after DON treatment. The bin numbers were denoted from 1-100. Sub-bins were listed as decimals. Only bins encompassing genes with significant changes in transcript abundance ($P<0.05$) were listed. The number of elements in each bin present on the array was listed. The bins highlighted by light blue and brown colors contained many TAs highly affected by the mycotoxin. ($P<0.05$).

bin	name	elements	p-value
1.1.30	PS.lightreaction.state transition	2	0.024
1.3	PS.calvin cyle	5	0.042
3.1.2	minor CHO metabolism.raffinose synthases	2	0.04
3.6	minor CHO metabolism.callose	2	0.033
8	TCA / org. transformation	15	0.01
8.1.1	TCA / org. transformation.TCA.pyruvate DH	2	0.017
8.2.10	TCA / org. transformation	2	0.026
11.2	lipid metabolism.FA desaturation	5	0.036
13.2	amino acid metabolism.degradation	14	0.008
16.7	secondary metabolism.wax	3	0.022
17.8	hormone metabolism.salicylic acid	6	0.049
17.8.1	hormone metabolism.SA.synthesis-degradation	6	0.049
20.2.99	stress.abiotic.unspecified	17	0.001
21	redox.regulation	29	7.00E-04
21.6	redox.dismutases and catalases	3	0.005
23.1	nucleotide metabolism.synthesis	9	0.016
23.1.2	nucleotide metabolism.synthesis.purine	6	0.03
26.19	misc.plastocyanin-like	2	0.048
27	*RNA*	*394*	*0.015*
27.3	*RNA.regulation of transcription*	*333*	*0.008*
27.3.11	RNA.regulation of transcription.C2H2 zinc finger family	18	0.029
27.3.15	RNA.regulation of transcription. HAP3	2	0.033
27.3.21	RNA.regulation of transcription.GRAS TA	5	0.029
27.3.25	RNA.regulation of transcription.MYB domain TA	19	0.01
27.3.69	RNA.regulation of transcription.SET-domain TA	5	0.024
29.2.3	protein.synthesis.initiation	23	0.016
29.4.1.57	protein.postranslational modification.kinase.RLCK VII	7	0.042
29.5.11.4.3	protein.degradation.ubiquitin.E3.SCF	49	0.042
33	development	81	8.00E-04
33.99	development.unspecified	70	0.0002
34.12	transport.metal	13	0.048
34.21	transport.calcium	4	0.018
35.1.41	not assigned.no ontology.hydroxyproline rich proteins	14	0.014
35.1.5	not assigned.no ontology.pentatricopeptide (PPR) P.	65	0.0005
35.2	not assigned.unknown	713	0.011

Hohn, 1997) also suggesting that there was a biological role for the trichothecenes in the virulence of *F. graminearum*.

Here DON toxin was shown to have a profound and pathogen-like impact on the transcript abundance of genes encoding several crucial metabolic pathways in *Arabidopsis* with a scope from the protein metabolism to redox control. In contrast, some of the responses might not be related to pathogenicity. For example, many of the 1,045 genes distributed among bin27 of the RNA transcription related bins were altered in TA by DON. This result contrasted to that of *F. virguliforme* infestation where there was no bin27 pathway effect was observed (Yuan et al 2008). Similarly, during *F. virguliforme* infection, a large proportion of altered TAs that were in protein metabolism related genes assigned to bin29 (protein metabolism) were not concentrated in the same sub-bins of protein synthesis and posttranslational modification altered by DON (Yuan et al., 2008). Only a few of transcripts altered were involved in protein activation, post-translational modification, degradation, and folding. . This was consistent with the known activities of DON as an inhibitor of translation and a pathogenesis factor (Ueno et al., 1969; Feinberg and Mclaughlin, 1989; Ehrlich and Daigle, 1987; Harris et al., 1999; Cumagus et al., 2008; Shin et al., 2012).

The toxic function of DON is an inhibitor for the protein synthesis on the 60S subunit of eukaryotic ribosomes and inhibits the initiation or elongation and termination steps of protein synthesis (Feinberg and Mclaughlin, 1989; Ehrlich and Daigle, 1987). From 6 to 24h after DON treatment, the number of affected transcripts in bin27 and bin29 was reduced significantly. There were only 3 sub-bins left in bin29 24h after DON treatment. This suggested that a large proportion of genes involved in protein metabolism were affected by the mycotoxin initially but metabolism was restored later. However, several other new bins emerged, such as transporter genes and the genes related with lipid metabolism and redox reactions at this stage. About half of the transcripts altered were among bins encompassing genes with unknown functions (713 genes). The results were in

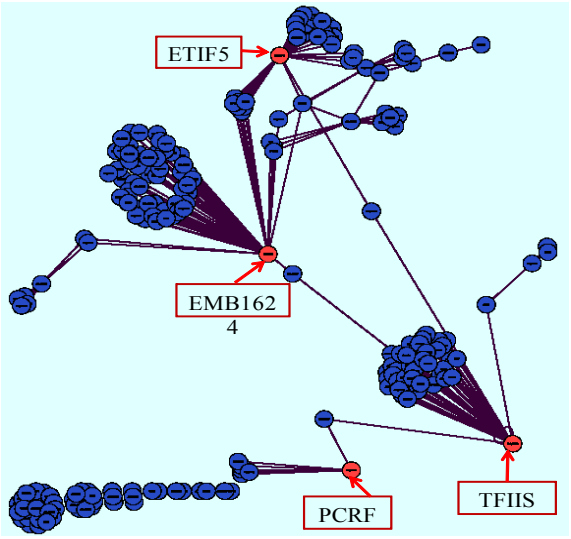


Figure 2. Network of predicted protein-protein interactions among genes from bin 29 in *A. thaliana* 6 hours post DON treatment. The networks were generated by Cytoscape (Shannon et al. 2003) and visual displays were saved as Cytoscape graphs. Major interaction hubs were marked by red.

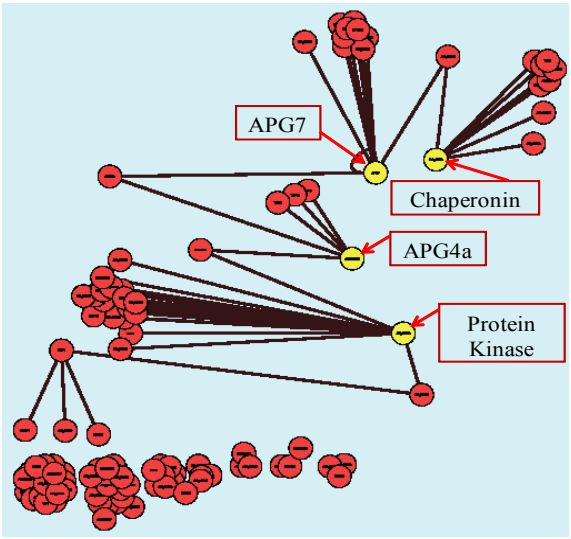


Figure 3. Network predicted protein-protein interactions from bin29 in A. thaliana treated with DON after 24 hours. The networks were generated by Cytoscape (Shannon et al. 2003) and visual displays were saved as Cytoscape graphs. Major interaction hubs were marked by yellow.

concordance with earlier studies of *Fusarium* infested in *Arabidopsis thaliana* (Yuan et al., 2008). If plants rather attempt to reconfigure those inhibited enzymes and bypass the damaged pathways via a backup system is still unknown and functions for these genes can be inferred. Unlike in soybean *F. virguliforme* infestation (Yuan et al., 2008), none of the crucial gene transcripts that were classified as potentially being involved in secondary metabolism (bin 16) had been increased in abundance when DON toxin was applied. No pathway leading to isoprenoid, phenylpropanoid, and lignin biosynthesis was shown increased in the TAs. *F. virguliforme* infection of *A. thaliana* only increase PAL and C3H suggesting phenolics rather than flavonoids were being produced. Therefore, it appears that flavonoids (anthocyanins, proanthocyanidins, flavones, and flavonols) in *Arabidopsis* may indeed not play a significant role in defense.

Dissection of gene transcript abundance regulation may be one of the main strategies used to decipher resistance gene function because many transcript changes may be involved in plant defense processes (Journot-Catalino et al., 2006). By comparison of species, organ, and time specific transcript abundances of thousands of genes simultaneously, investigations of alterations in these transcripts have provided unique opportunities to delve into gene function. To analyze the dynamics of TA change during DON mycotoxin treatments, *Arabidopsis* seedlings were treated with DON toxin and transcript abundances were evaluated in a time course study. Moreover, in specific metabolic pathways, the transcripts that could be detected by the microarray experiment were significantly reduced and a large number of genes of unknown function were emerged 24 hours after DON treatment suggesting that the toxicity to transcription and subsequent metabolic perturbations might have largely occurred in the later stage of the time course experiment. The reasons for the anti-parallel reduction in one pathway and induction of the unknowns still need to be investigated. If a transcription factor or factors underlay the inhibition of groups of genes, each individual metabolic pathway should be interrogated for a common cis-acting element. In contrast, if toxicity occurred in the transcriptome, investigations of adaptive changes in the metabolic transcriptome should be performed.

The similarities and differences inferred among the metabolic responses of *Arabidopsis thaliana* to DON and *Fusarium virguliforme* might be attributed to nature of the pathogenesis factors. The resistance regulation and signaling pathways in Arabidopsis plants to these pathogenesis factors may share similar components. Based on results here, DON toxin appeared to be a remarkable pathogenesis factor with effects on the crucial metabolic pathways ranging from the protein metabolism to redox reactions with significant metabolic alterations in *Arabidopsis* plants. Therefore, the results presented here may provide basic knowledge and an alternative strategy for developing more cultivars with resistance/tolerance to the toxin. Overexpression of defense response genes such as a barley class II chitinase (EC 3.2.1.14) gene that broke chitin, a key component of the cell wall in Fungi enhanced the resistance against *Fusarium graminearum* (Mackintosh et al., 2007; Shin et al., 2008) and thereby reduced FHB (Fusarium head blight) and DON toxicity. Shin et al. (2012) showed that DON conjugated with glutathione into a glucose-conjugate in vivo was no longer toxic. Barley UDP-glucosyltransferase converted DON into a DON-glucoside and therefore, transgenic *Arabidopsis* carrying the barley gene displayed an enhanced tolerance to DON. Therefore, the effective neutralization of DON and other trichothecene toxins can be used to provide resistance/tolerance to the *Fusarium* pathogens and this strategy should be also explored in genetic engineering soybean plants.

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