

Unraveling the *Triticeae*- *Fusarium graminearum* interaction

Gary J. Muehlbauer

Department of Agronomy and Plant Genetics

University of Minnesota



FHB resistance

Wheat
Type II R



Wheat
susceptible



Barley



- Wheat - type II resistance to spread of infection in the spike
- Trichothecenes implicated in virulence
- Wheat 3BS resistant QTL associated with UDP-glucosyltransferase activity (deoxynivalenol detoxification)
- Barley - endogenous resistance to spread of disease (possible built in resistance (Type II) to trichothecenes)

Barley-*Fusarium* interaction

- Identify major trends in transcript accumulation in barley during *F. graminearum* infection, trichothecene accumulation and DON treatment

Barley GeneChip was used to measure transcript accumulation

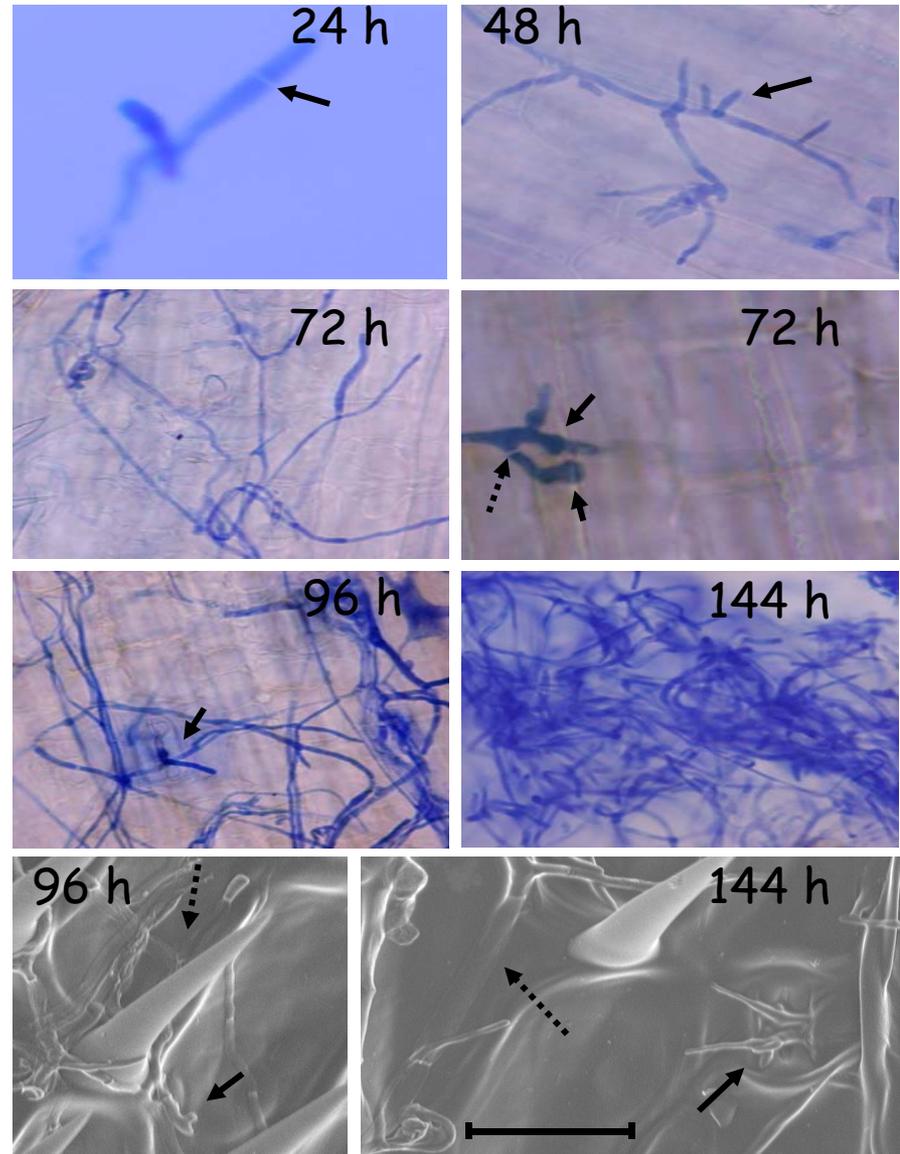
- Examine the fate of DON in barley
- Functionally characterize genes that respond to trichothecenes
- Identify relationship between haplotypes and phenotypes



Fusarium graminearum infection in barley

-Similar infection pathways in wheat

-Events occur earlier in wheat

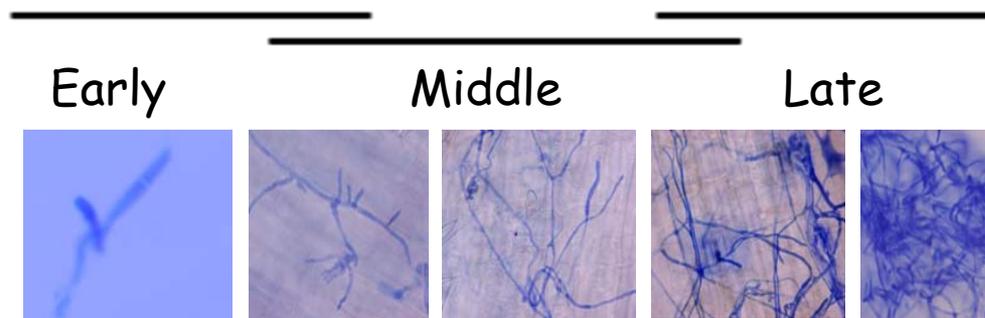
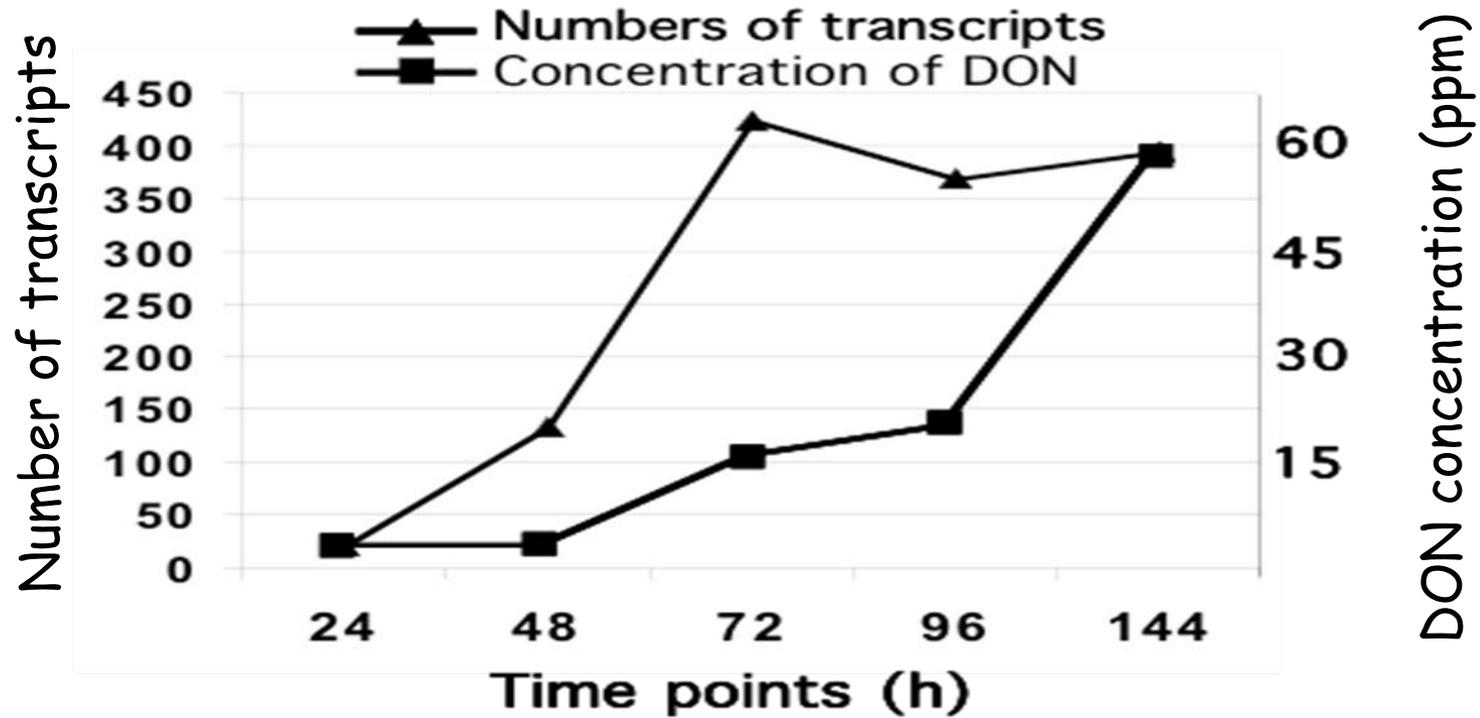


Major classes of genes induced in barley during *F. graminearum* infection (1-6 days after inoculation)

- Defense response related (PR proteins, GSTs, P450s etc.)
- Oxidative burst related
- Phenylpropanoid pathway
- Trichothecene detoxifying (UDP-glucosyltransferases) and transporters (ABC and MATE)
- Tryptophan biosynthetic genes
- Protease inhibitors

- A total of 467 gene transcripts were detected

Three stages of FHB development on barley



Trichothecenes are virulence factors on wheat

- *Tri5* is the first step in the trichothecene biosynthetic pathway
- *Tri5* loss-of-function mutations result in the inability of *F. graminearum* to produce trichothecenes
- Wheat infected with *Tri5* loss-of-function mutations result in reduced virulence - disease spread is reduced, initial infection is similar

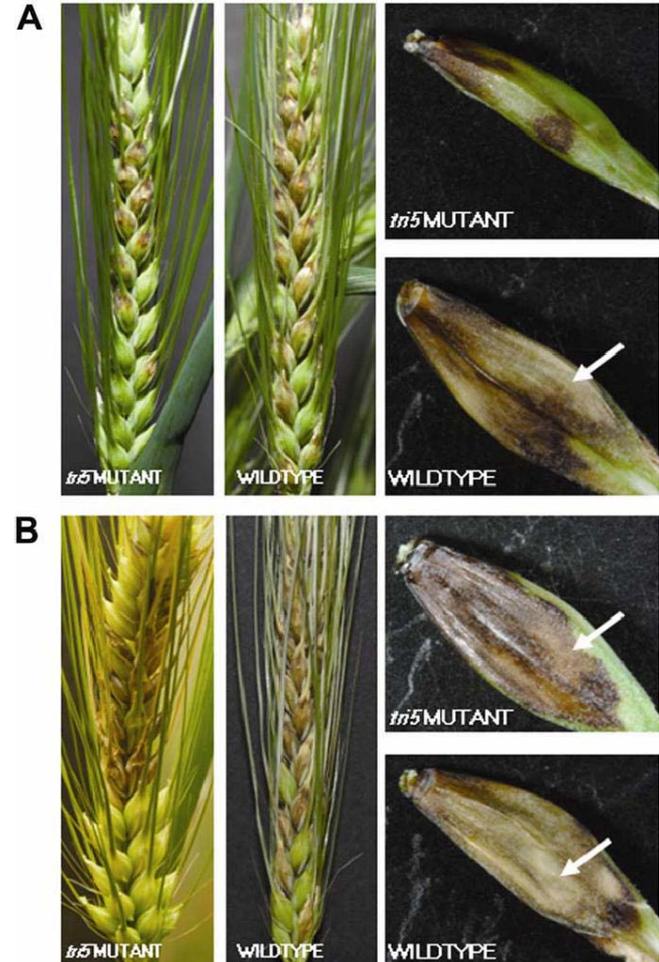


Disease severity is greater in wildtype inoculated barley plants

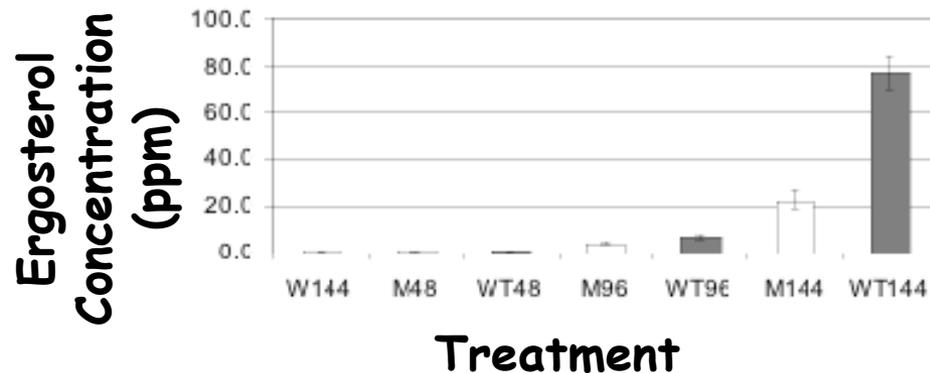
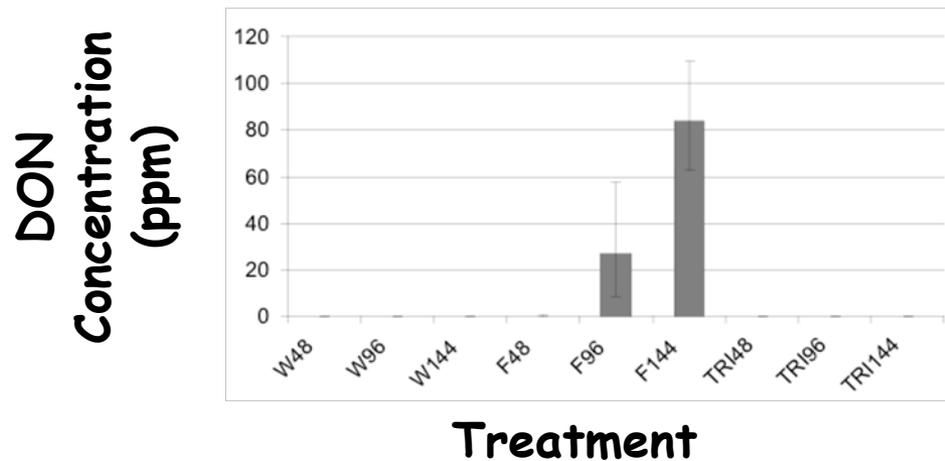
Disease severity:

Wildtype strain = 59.5 +/- 2.5

Tri5 mutant strain = 46.2 +/- 7.9



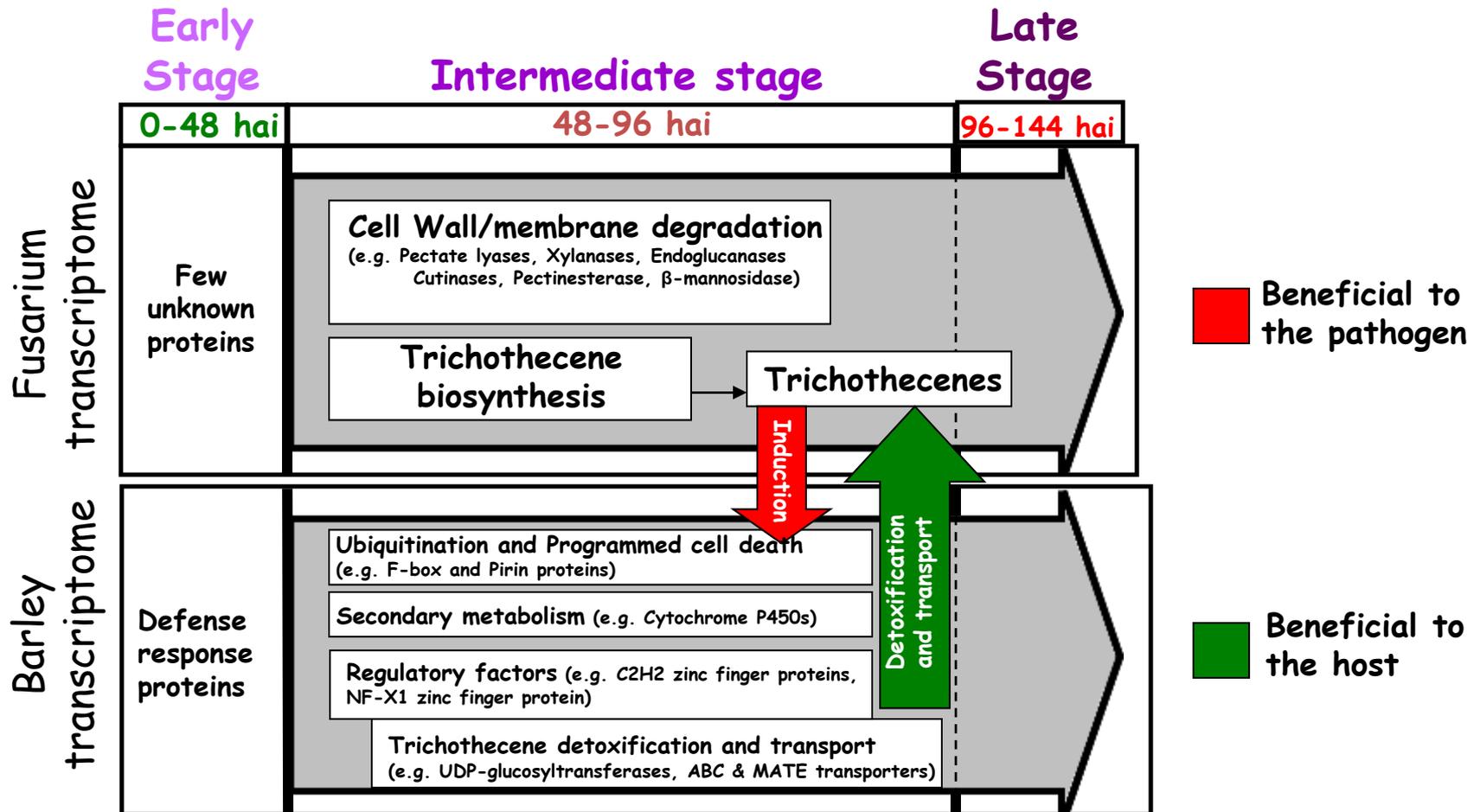
Deoxynivalenol and ergosterol concentration in wildtype, *tri5* mutant and water inoculated barley spikes



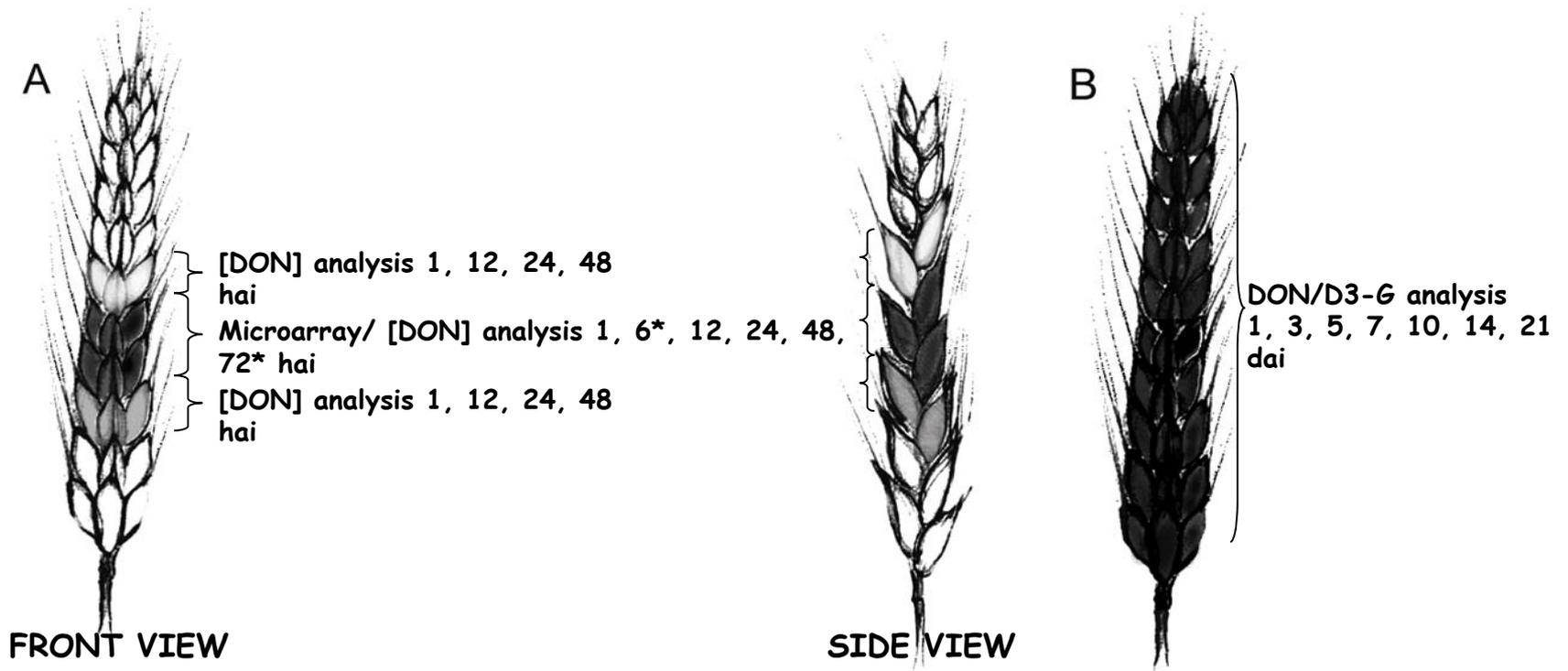
Classes of genes that respond preferentially to trichothecene accumulation

- **Induced (63 transcripts)**
 - Putative trichothecene detoxifying and transporter proteins (e.g., UDP-glucosyltransferases, ABC and MATE transporters)
 - Ubiquitination-related (eg., F-box)
 - Regulatory factors (e.g., zinc finger proteins, NF-X1)
 - Programmed cell death (e.g., pirin)
 - Cytochrome P450s
- **Repressed (none)**

Barley-*Fusarium* interaction

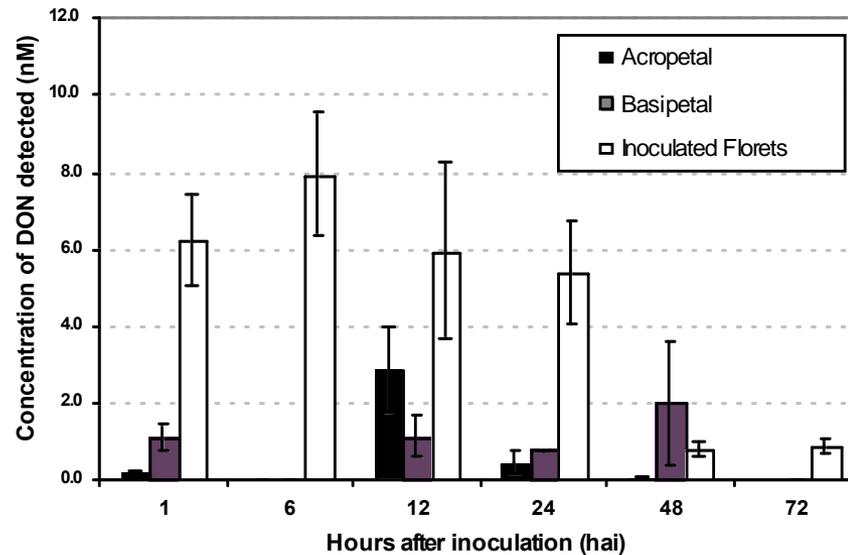


DON inoculation

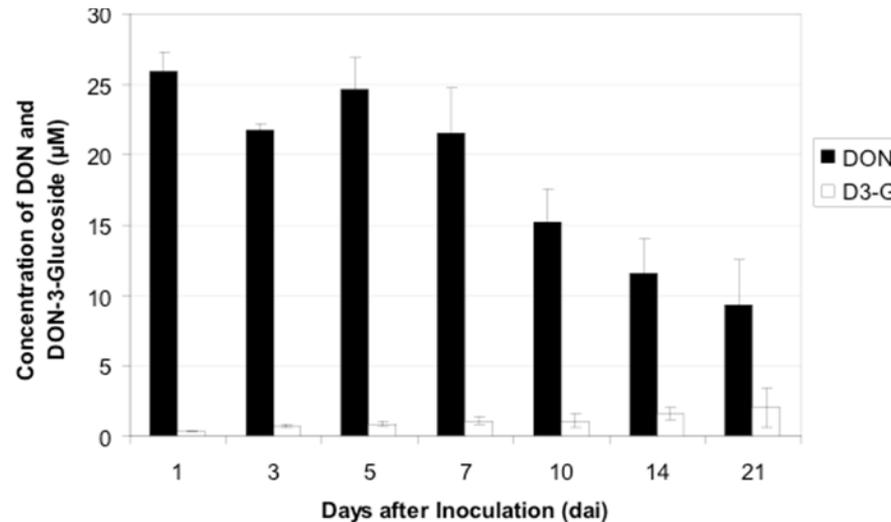


Fate of DON in barley

DON is transported to acropetal and basipetal florets

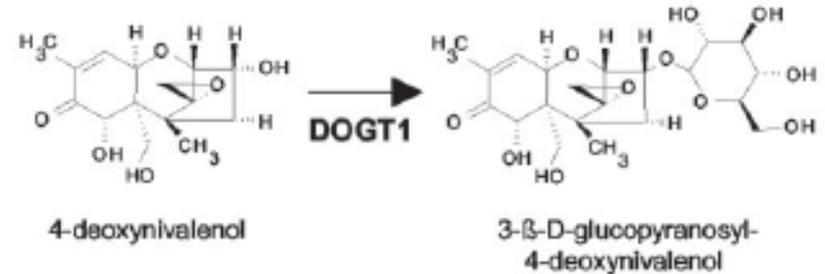


DON is converted to DON-3-O-glucoside

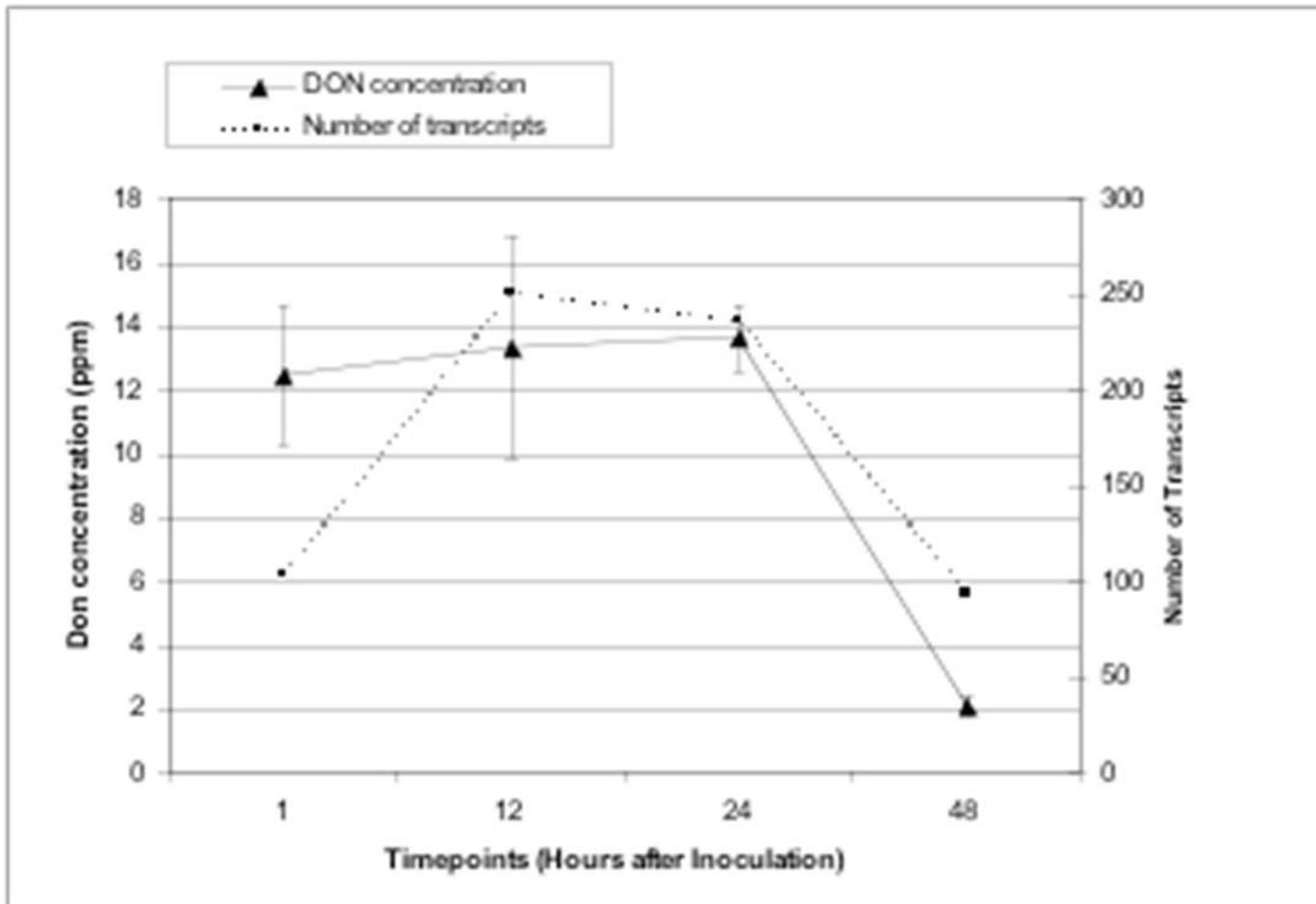


UDP-glucosyltransferase

- *Arabidopsis* DOGT1 encodes a UDP-glucosyltransferase
- Glucose from UDP-glucose is transferred to hydroxy group at carbon 3
- DON-3-O-glucoside is less toxic than DON



Dynamics of gene expression after DON treatment



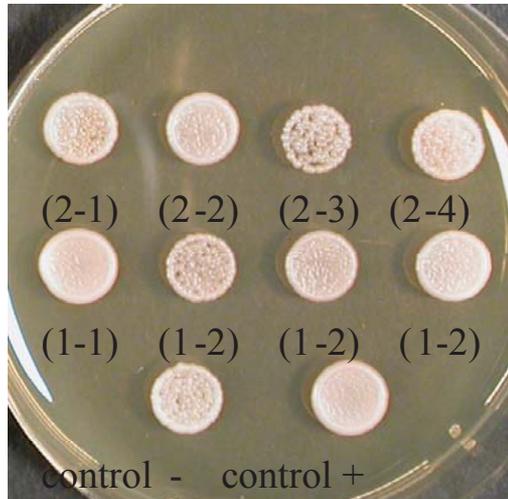
**255 genes
induced**

DON application and *in planta* trichothecene accumulation induced barley genes

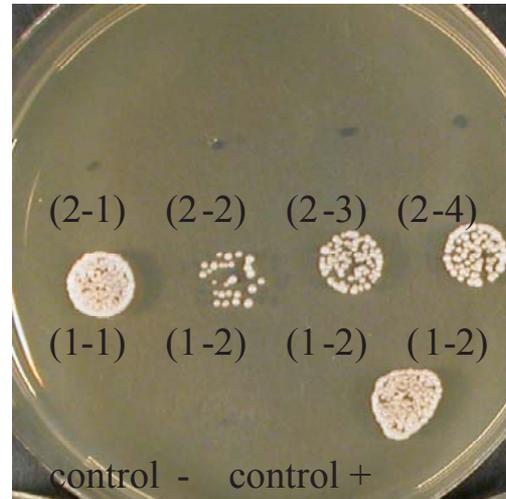
- Cytochrome P450s
- Cysteine synthase (enzyme for biosynthesis of glutathione)
- NF-X1 transcriptional repressor of trichothecene-induced defense responses (Asano et al., 2007)
 - WRKY, NBS-LRR, etc. upregulated in T2 toxin (Type A trichothecene) treated NF-X1 Arabidopsis mutants
- Glucosyltransferases
- Transporters (MATE, ABC)
- Transcription factors

Barley UDP-glucosyltransferase that detoxifies trichothecenes

0 ppm deoxynivalenol

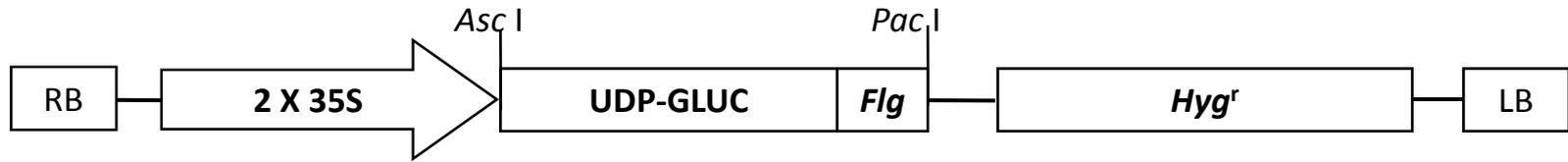


120 ppm deoxynivalenol

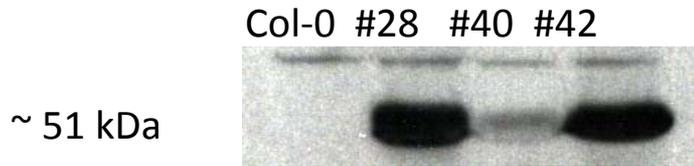


Nine UDP-glucosyltransferases have been identified
Seven have been cloned and four tested
One UDP-glucosyltransferase detoxifies DON

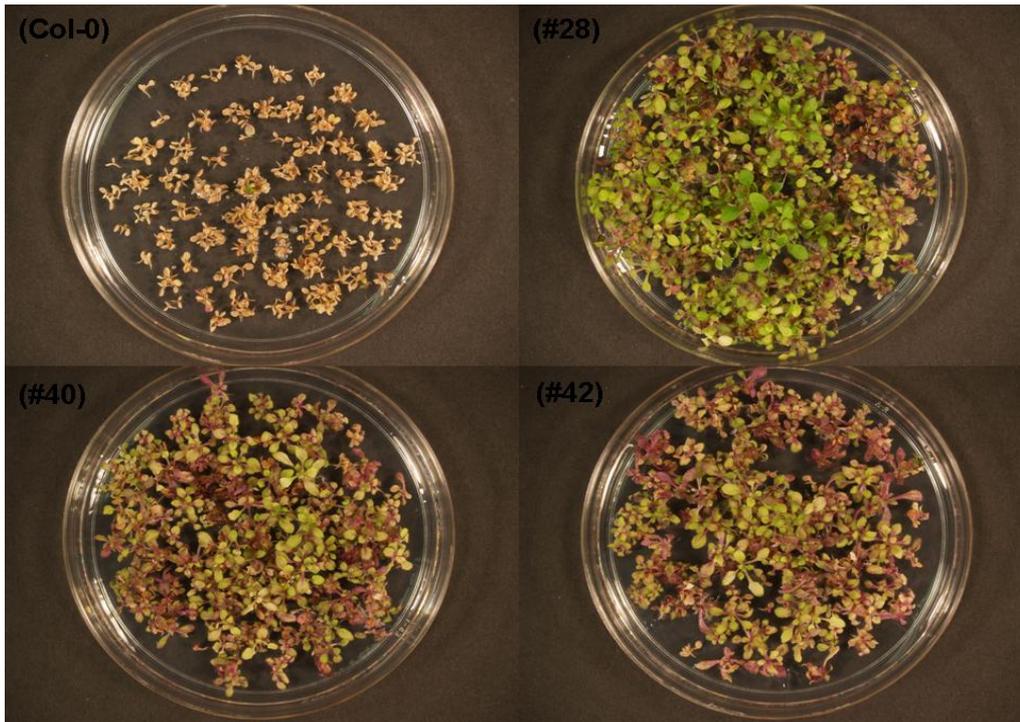
(A) pMDC32-UDP-GLUC



(B)



(C)



UDP-glucosyltransferase overexpressed in Arabidopsis exhibits tolerance to deoxynivalenol (20 ppm)

102 barley genotypes, 80 resistant and 22 susceptible

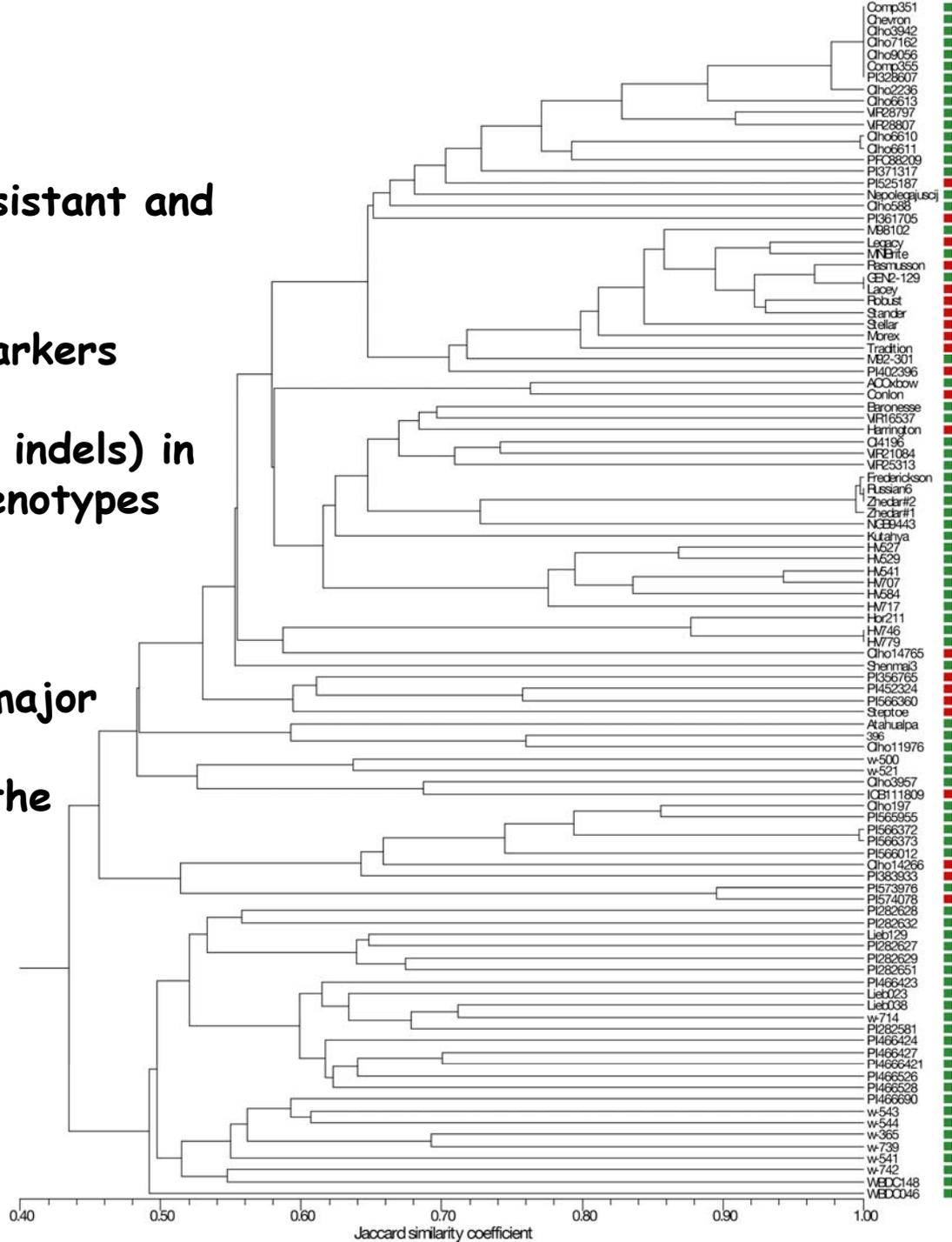
Genotyped with 728 DArT markers

Identified haplotypes (SNPs, indels) in 35 genes from at least 15 genotypes and relate to phenotype

Future goals

- examine haplotypes at major barley FHB QTL
- map SNPs for each of the genes

Green - resistant genotypes
Red - susceptible genotypes



Sequencing barley UDP-glucosyltransferase shows no obvious haplotype (or SNP) that differentiates resistant from susceptible genotypes

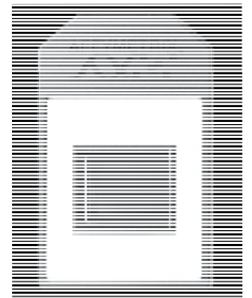
Base pair Genotype	281	311	860	1031	1609	1785	1828	1936	2355	2385	2403	2417	2506	2530	Haplotype
CI4196	G	T	--	A	G	TCC	-	A	G	C	A	T	A	A	A
Frederickson	G	T	--	A	G	TCC	-	A	G	C	A	T	A	A	A
VIR16537	G	T	--	A	G	TCC	-	A	G	C	A	T	A	A	A
Atahualpa	G	T	--	A	G	-CC	-	A	G	C	A	T	A	G	B
OWBR	G	T	--	A	G	TCC	A	A	G	C	A	T	A	G	C
Step toe	G	T	--	A	G	TCC	A	A	G	C	A	T	A	G	C
CIho14765			--	A	G	TC-	T	C	A	T	G	C	G	G	D
OWBD	G	C	--	A	G	TC-	T	C	A	T	G	C	G	G	D
PI282651	G	C	--	G	G	TC-	T	C	G	C	G	C	G	G	E
M69	G	C	AC	G	T	TC-	T	C	G	C	G	C	G	G	F
Morex	G	C	AC	G	T	TC-	T	C	G	C	G	C	G	G	F
PI361705			AC	G	T	TC-	T	C	G	C	G	C	G	G	F
Stander	G	C	AC	G	T	TC-	T	C	G	C	G	C	G	G	F
PI383933	G	C	AC	G	G	TC-	T	C	G	C	G	C	G	G	G
W544			AC	G	G	TC-	T	C	G	C	G	C	G	G	G
Chevron	A	C	AC	G	G	-C-	T	C	G	C	G	C	G	G	H
CIho9056	A	C	AC	G	G	-C-	T	C	G	C	G	C	G	G	H
HV707	A	C	AC	G	G	-C-	T	C	G	C	G	C	G	G	H
PI402396	A	C			G	-C-	T	C	G	C	G	C	G	G	H
PI525187	A	C			G	-C-	T	C	G	C	G	C	G	G	H

Wheat-*Fusarium* interaction

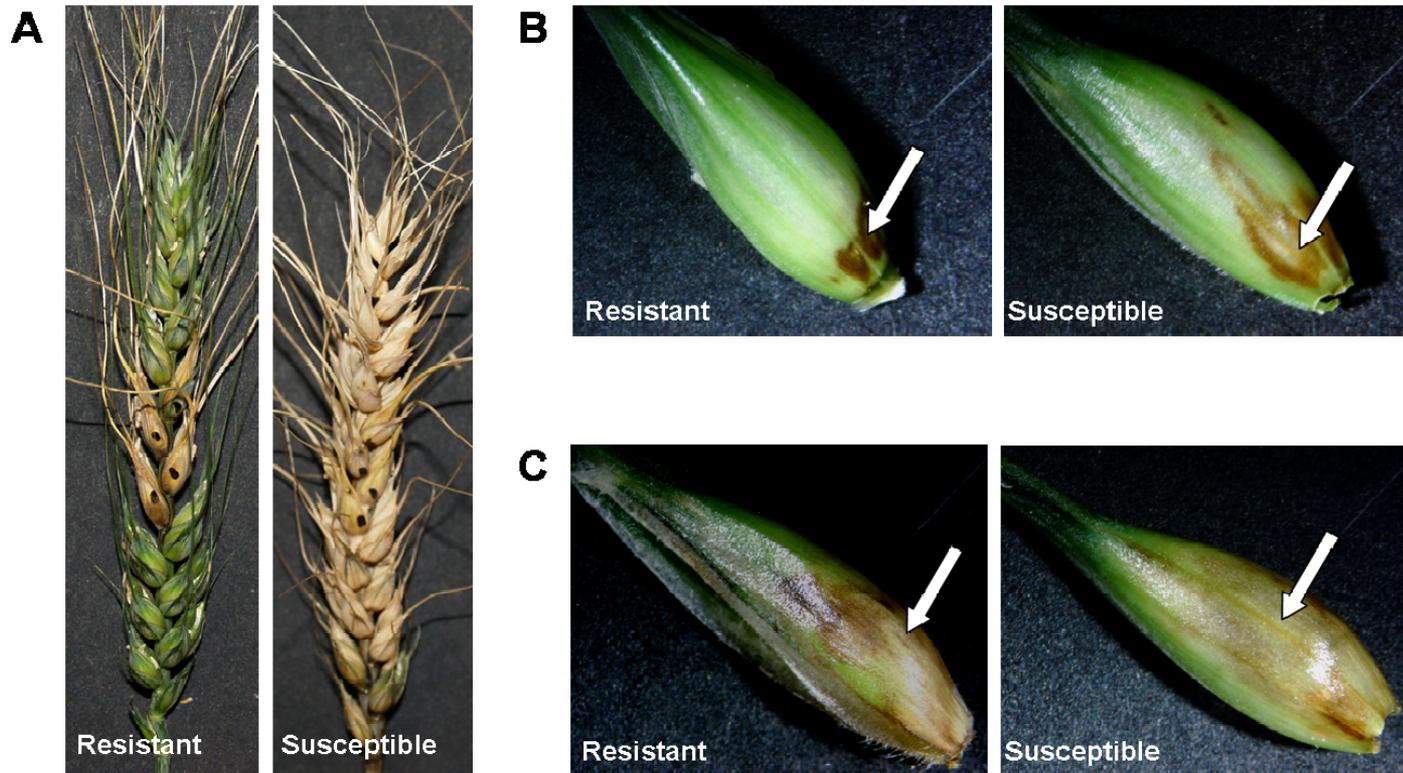
- Examine disease phenotypes between plants carrying a resistant and susceptible allele at the chromosome 3BS FHB resistant locus (Type II resistance)
- Identify wheat genes that respond to *F. graminearum* infection

Wheat Affymetrix GeneChip

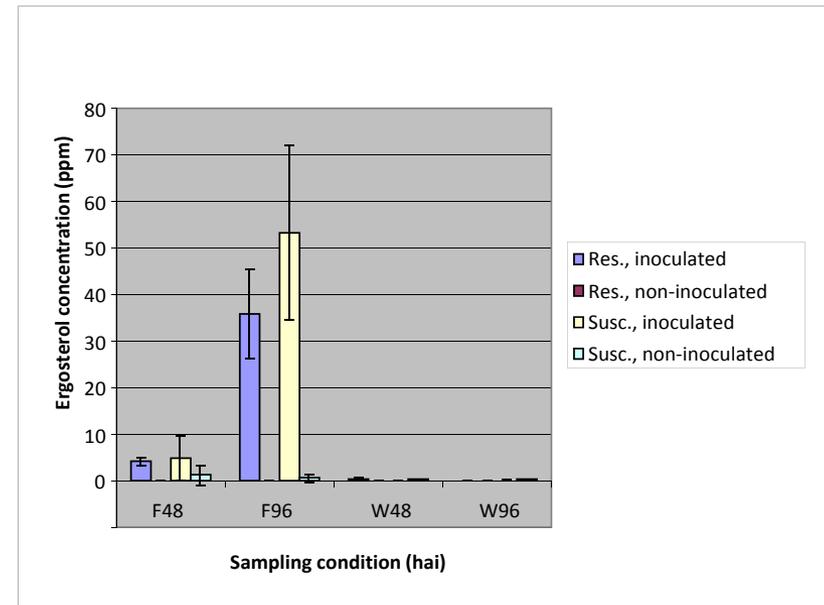
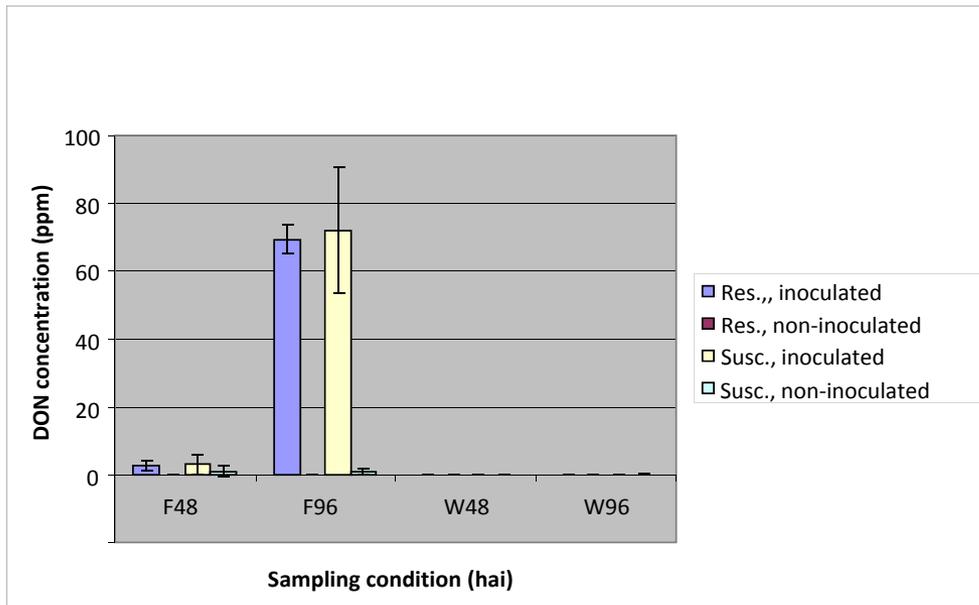
- Identify genes that exhibit differential expression between plants carrying a resistant and susceptible allele at the chromosome 3BS FHB resistant locus (Type II resistance)



Disease phenotypes in type II resistant and susceptible genotypes



Fungal biomass and DON accumulation are similar in inoculated spikelets of resistant and susceptible wheat genotypes



Genes induced and repressed in resistant and susceptible genotypes

Genes induced:

- Defense response genes
- Oxidative burst related genes
- P450s
- Protease inhibitors
- Tryptophan biosynthetic genes
- UDP-glucosyltransferases
- Transporters (ABC, MATE)
- Transcription factors
- Ethylene and jasmonic acid related

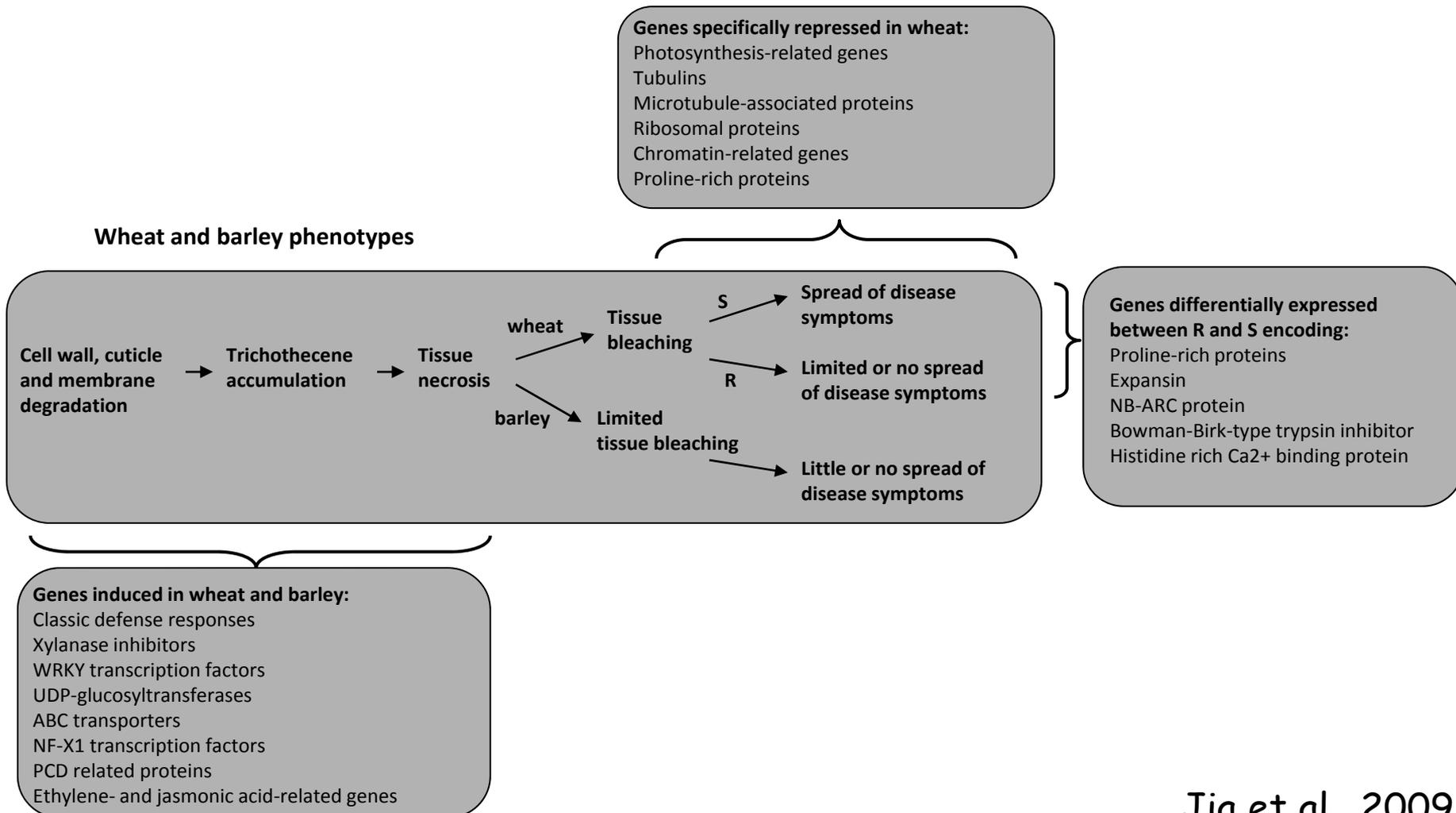
Genes repressed:

- Photosynthesis-related genes
- Tubulins
- Microtubules
- Ribosomal protein genes
- Chromatin related
- Proline rich proteins

Genes differentially expressed between plants carrying the resistant or susceptible allele for the 3BS QTL:

- Expansin
- proline rich protein
- NB-ARC
- Bowman-Birk trypsin inhibitor
- Histidine rich Ca²⁺ binding protein

Triticeae - *F. graminearum* interaction



Acknowledgements

Muehlbauer lab

Jayanand Boddu

Seungho Cho

Stephanie Gardiner

Haiyan Jia

Warren Kruger

Ben Millett

Sanghyun Shin

Collaborators

Jim Anderson

Gerhard Adam

Franz Berthiller

Ruth Dill-Macky

Yanhong Dong

Christian Hametner

Wolfgang Schweiger

Kye-Yong Seong

Kevin Smith

Brian Steffenson

Funding: USDA-CSREES-IFAFS, USDA-ARS U.S. Wheat and Barley Scab Initiative, Minnesota Small Grains Initiative

