Developing Fusarium head blight resistant wheat

Gary J. Muehlbauer
University of Minnesota

FHB resistance

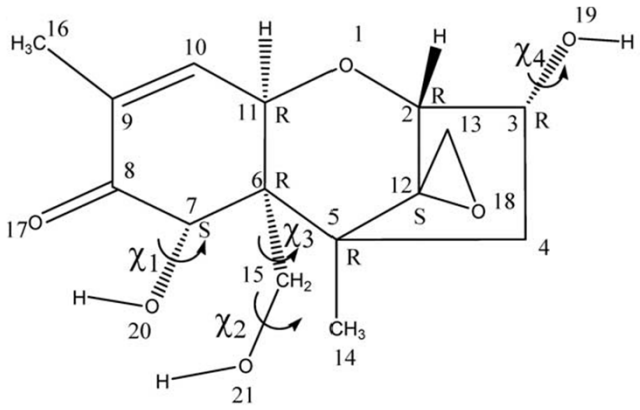
Wheat
Type II R

Wheat
susceptible

Barley

Trichothecenes are virulence factors on wheat

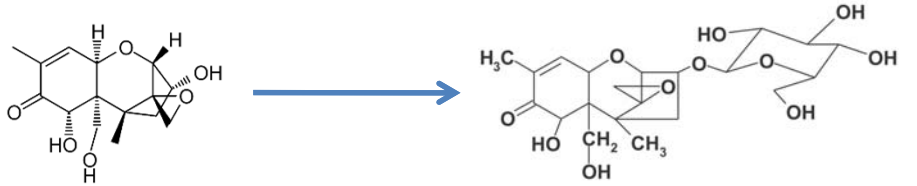
Deoxynivalenol (DON)



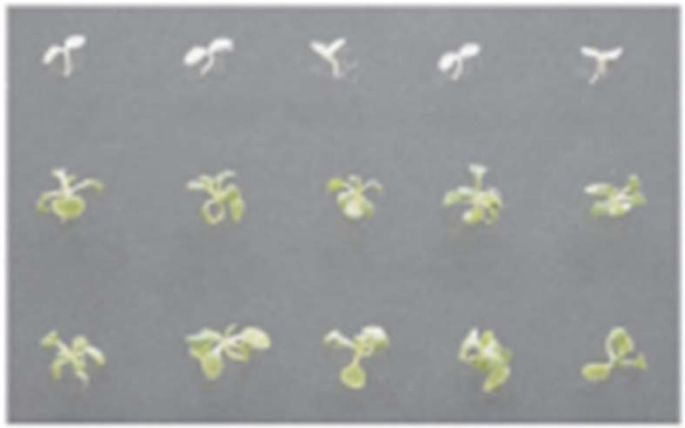
Tri5 mutant – knockout in trichodiene synthase resulting in trichothecene nonproducing Fusarium

Proctor et al., 1995

Arabidopsis UDP-glucosyltransferase provides resistance to DON



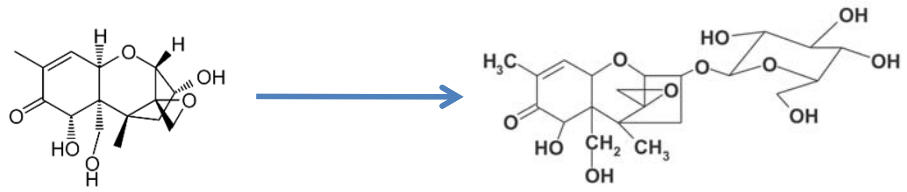
Deoxynivalenol DON-3-O-glucoside



Poppenberger et al., 2003 J. Biol. Chem.

Fhb1 cosegregates with increased DON-glucoside to DON ratio

UDP glucosyltransferase activity converts DON to DON-3-glucoside



Deoxynivalenol DON-3-*O*-glucoside

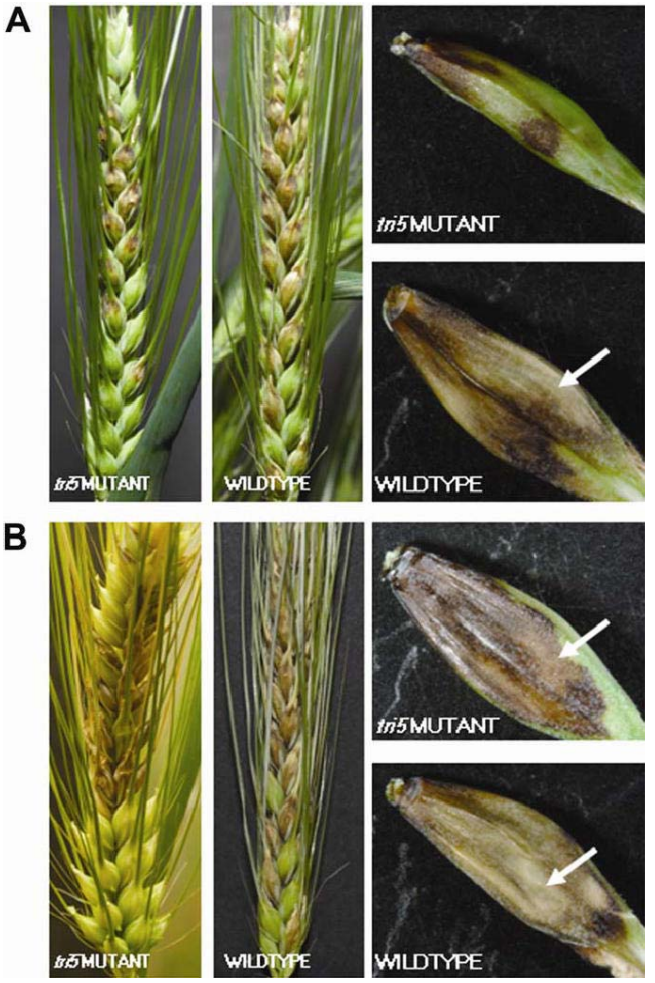
Lemmens et al., 2005 MPMI

Wheat
Type II R

Wheat
susceptible

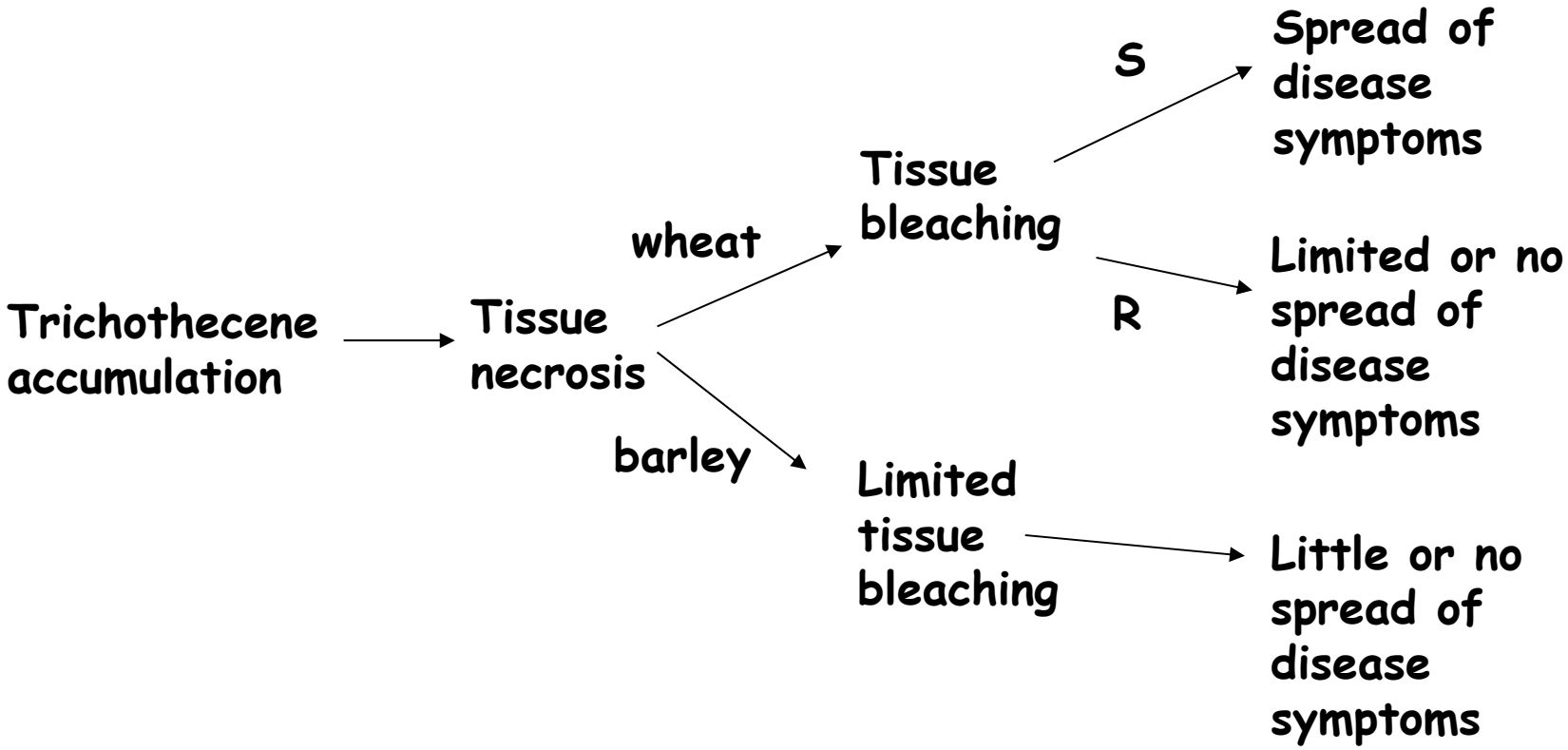
Disease severity is greater in barley inoculated with a wildtype strain of *Fusarium graminearum*

Disease severity:
Wildtype *strain* = 59.5 +/- 2.5
Tri5 mutant *strain* = 46.2 +/- 7.9



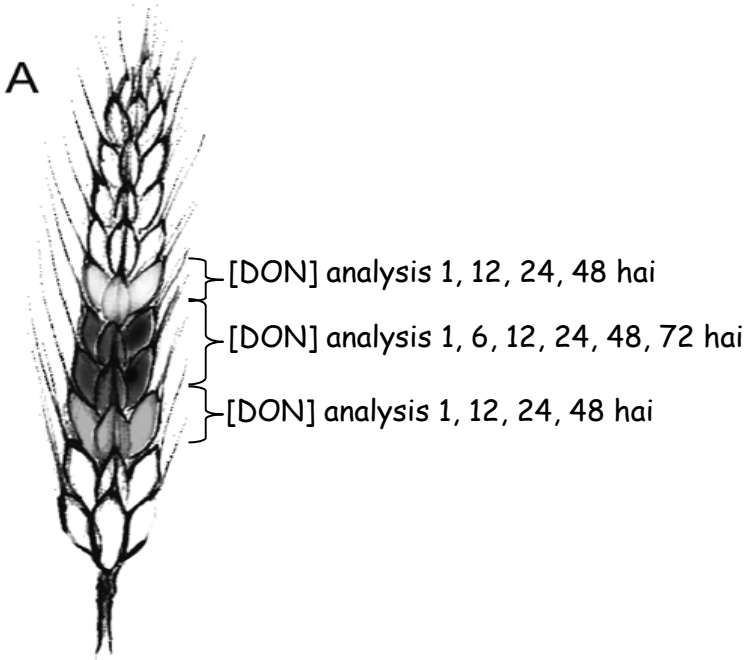
Boddu et al., 2007 MPMI

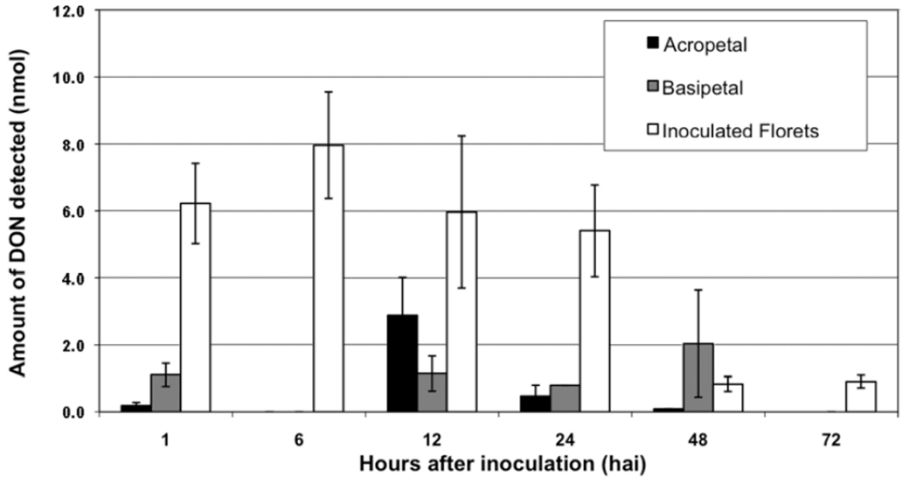
Barley and wheat respond differentially to infection and trichothecene accumulation



Is the different response between barley and wheat due to the ability to detoxify trichothecenes?

DON is transported to acropetal and basipetal florets in barley

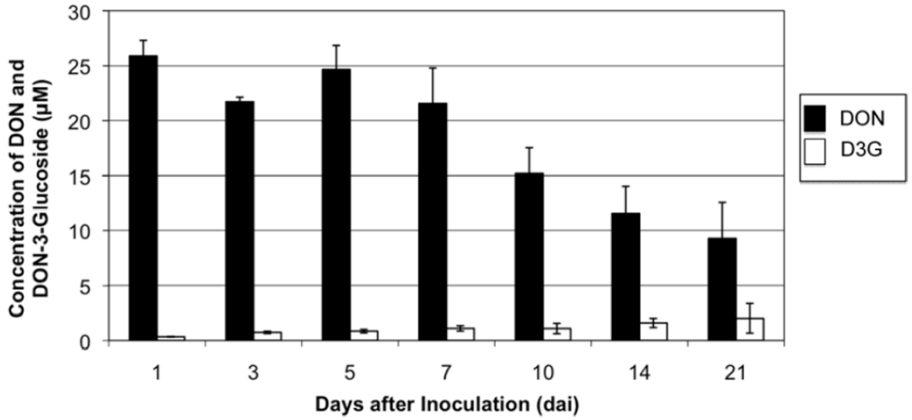




Gardiner et al., 2010 MPMI

DON is converted to DON-3-O-glucoside (D3G) in barley

DON/D3G analysis
1, 3, 5, 7, 10, 14, 21 dai



Gardiner et al., 2010 MPMI

Identify barley genes that respond to *in planta* trichothecene accumulation and DON treatment

Tri5 mutant and wildtype inoculation

DON and water treatment

Identify differentially expressed genes

Identify differentially expressed genes

Trichothecene responsive genes

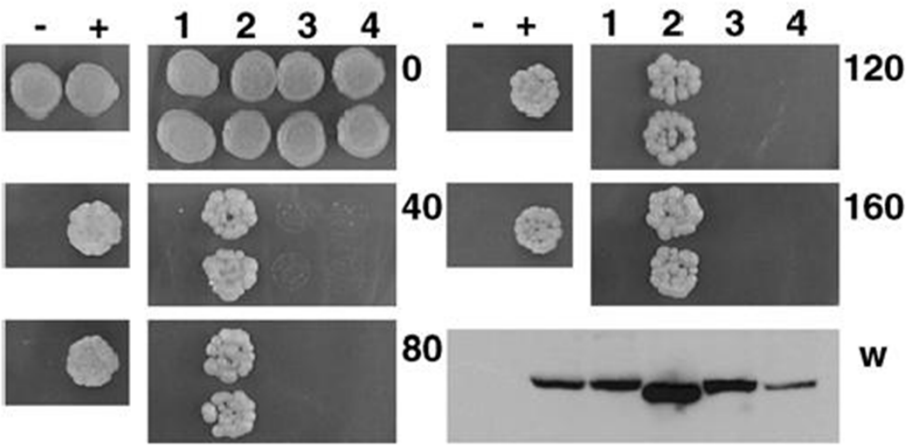
Boddu et al., 2007 MPMI
Gardiner et al., 2010 MPMI

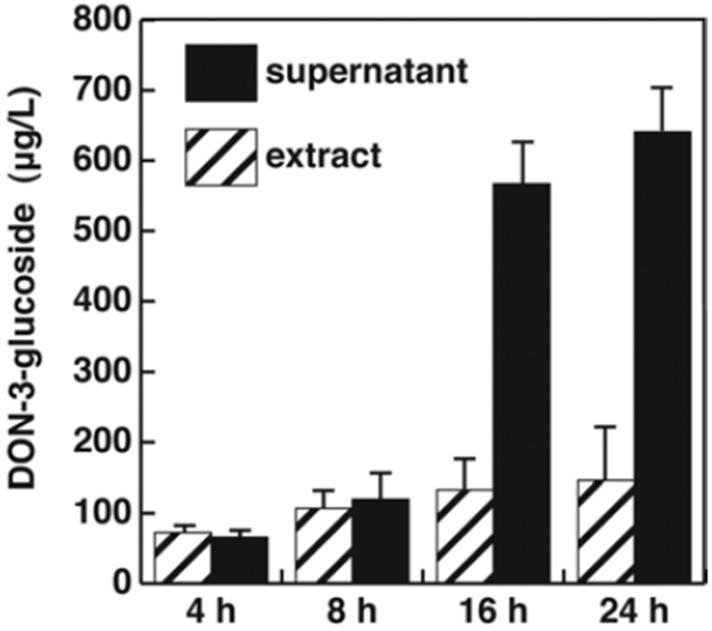
DON application and *in planta* trichothecene accumulation induced barley genes

- Cytochrome P450s
- Cysteine synthase (enzyme for biosynthesis of glutathione)
- Glutathione-S-transferases
- 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 (DON to DON-3-O-glucoside)**
- Transporters (MATE, ABC)
- Transcription factors

Gardiner et al., 2010 MPMI

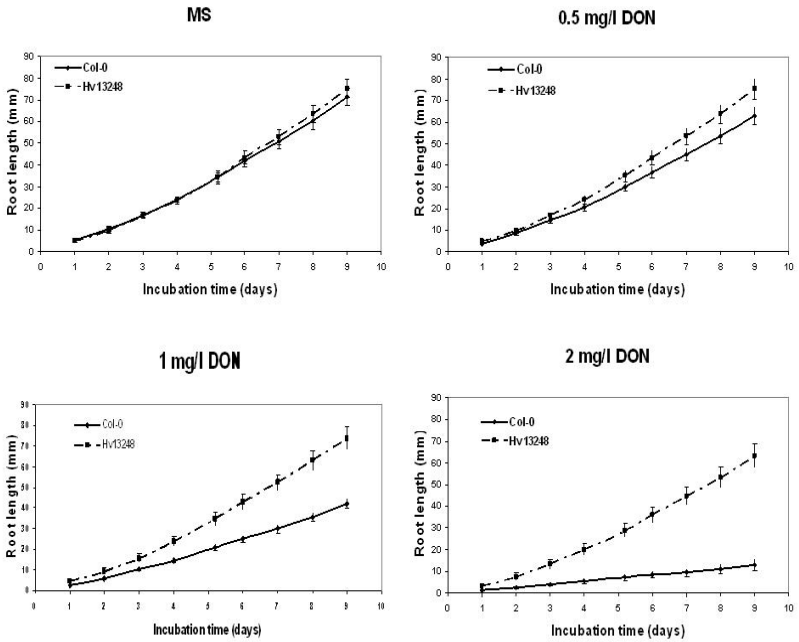
Barley UDP-glucosyltransferase (*HvUGT13248*) converts DON to DON-3-O-glucoside (D3G)

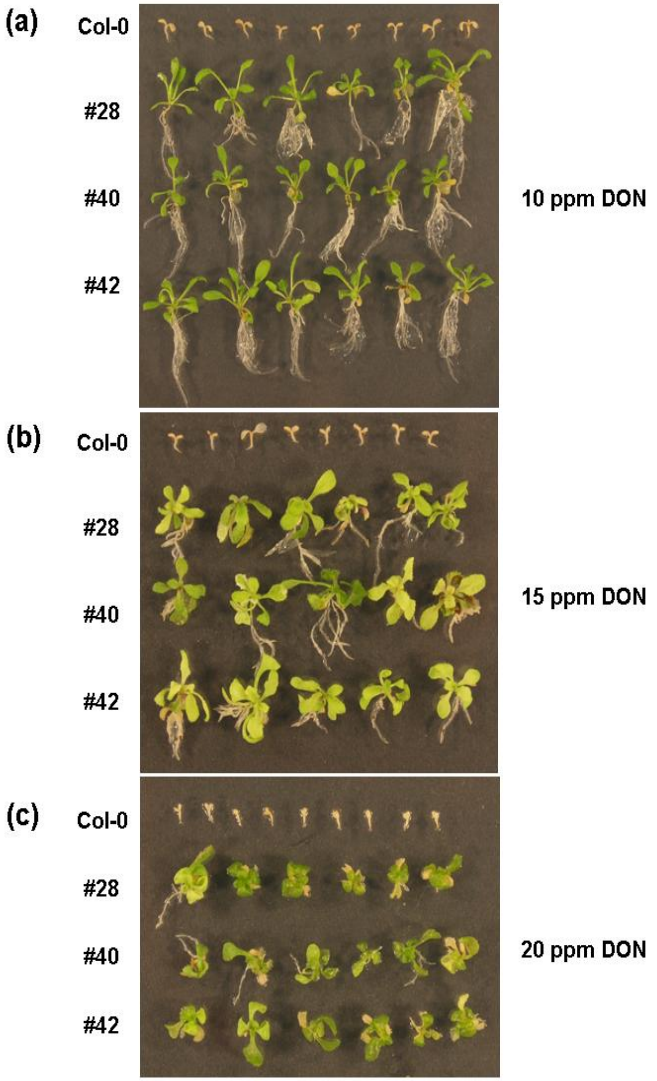




Schweiger et al., 2010 MPMI

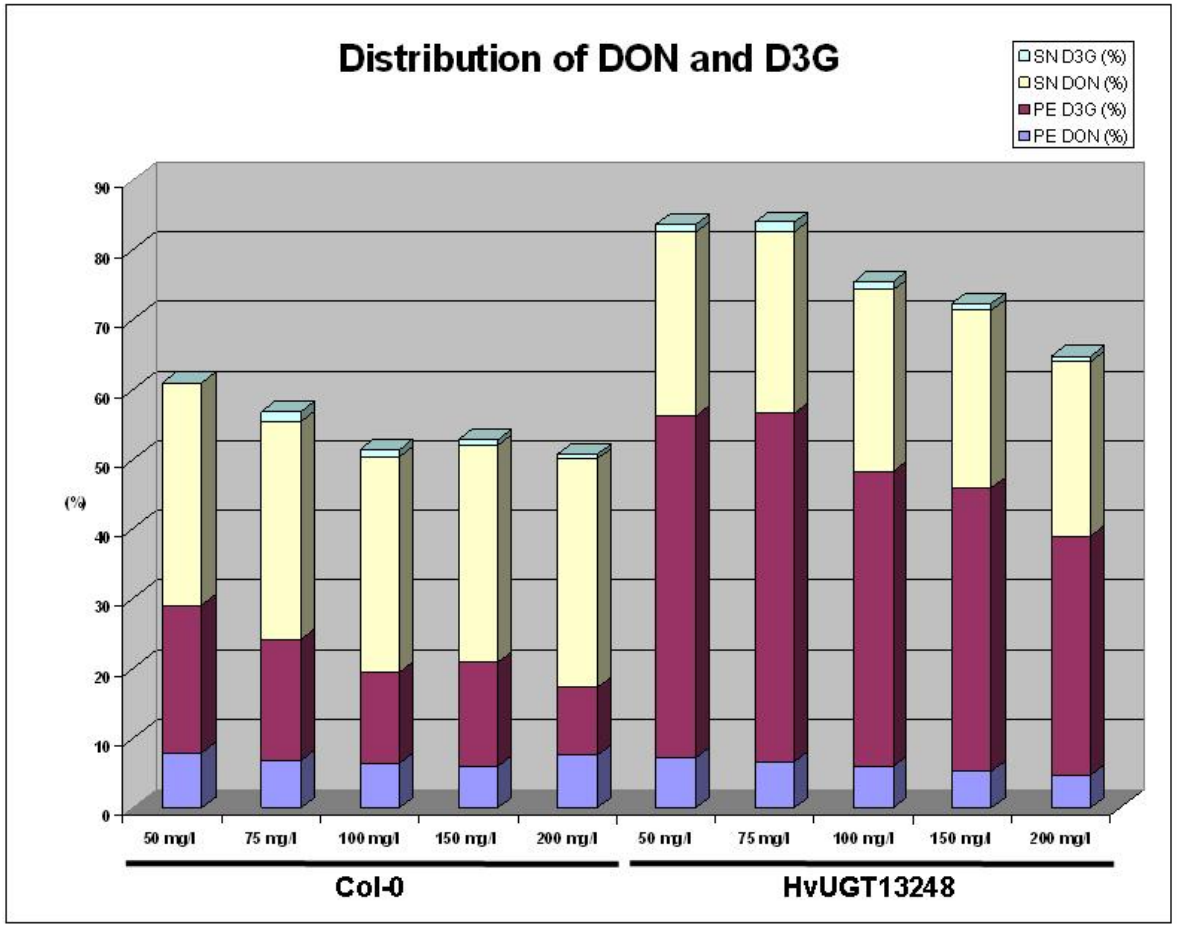
Transgenic Arabidopsis carrying a barley UDP-glucosyltransferase (*HvUGT13248*) exhibits resistance to DON





Shin et al., 2012 J. Exp. Bot.

Transgenic Arabidopsis carrying a barley UDP-glucosyltransferase (*HvUGT13248*) conjugates DON to D3G



Shin et al., 2012 J. Exp. Bot.

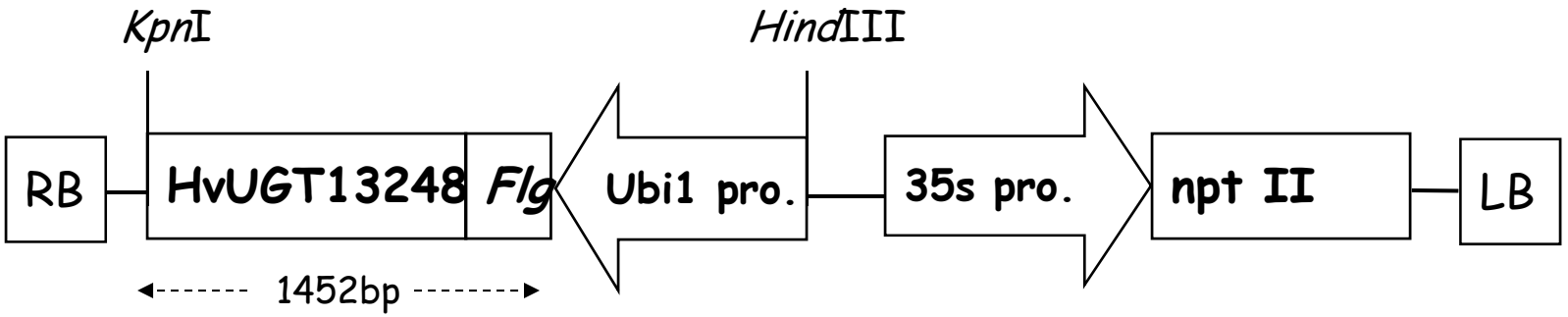
Arabidopsis carrying *HvUGT13248* confers resistance to 3,15-NIV

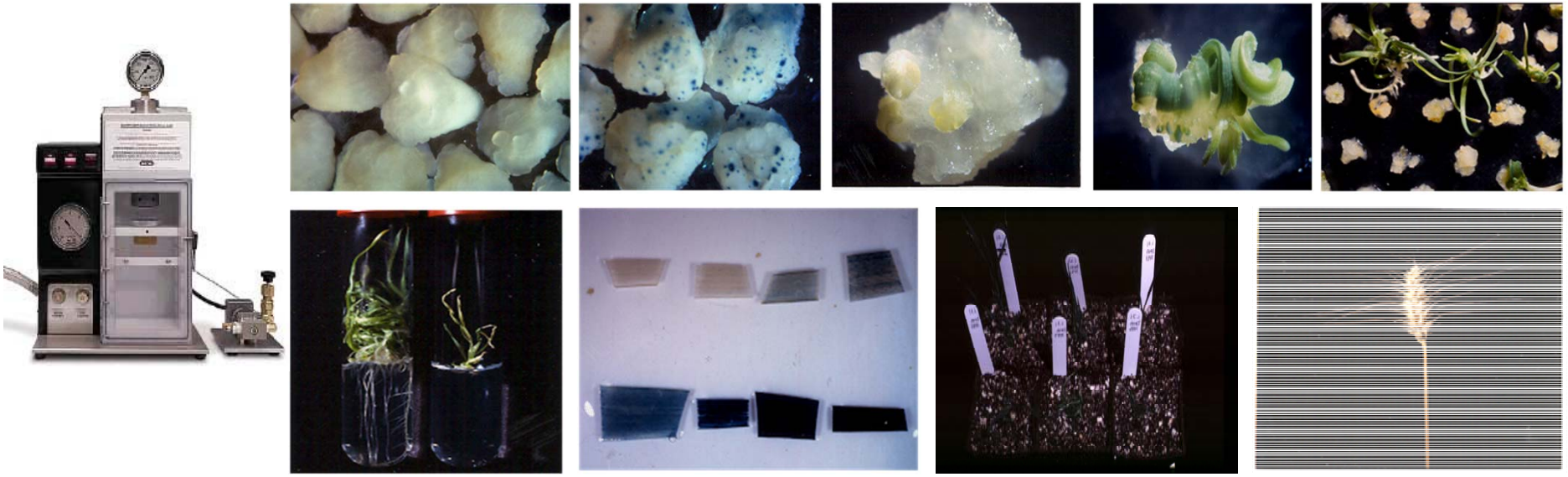
Transgenic Arabidopsis carrying *HvUGT13248* do not exhibit morphological changes

Genotypes	Days to flowering	Number of rosette leaves	Plant height (cm)	Number of shoots
Control (Col-0)	26	8.7 +/-1.04	37.8 +/-3.81	6 +/-1.09
#28	26	8.9 +/-1.14	39.5 +/-3.49	5.3 +/-1.54
#40	26	8.2 +/-1.03	**34.7*** +/-4.74	6 +/-1.05
#42	26	8.4 +/-0.71	35.5 +/-4.18	5.6 +/-0.93

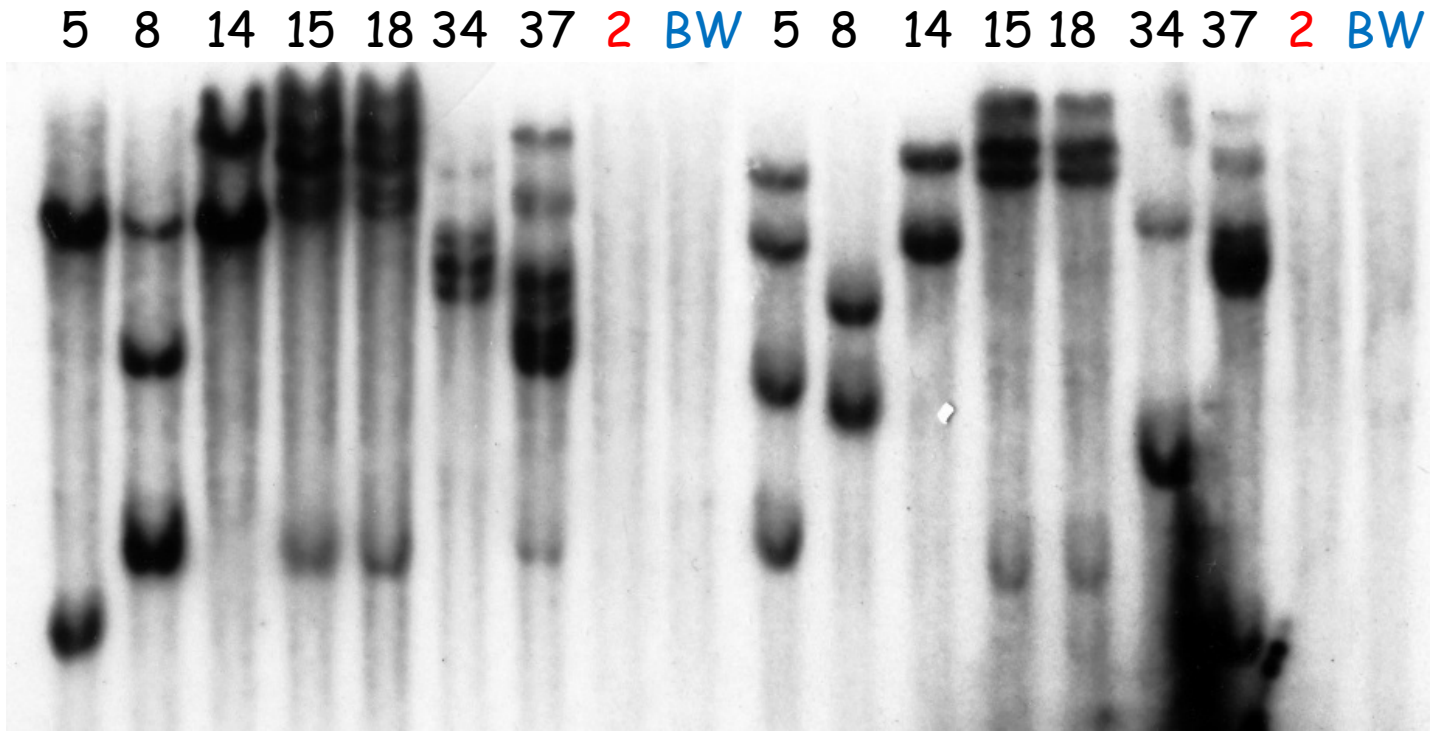
Shin et al., 2012 J. Exp. Bot.

Wheat transformation

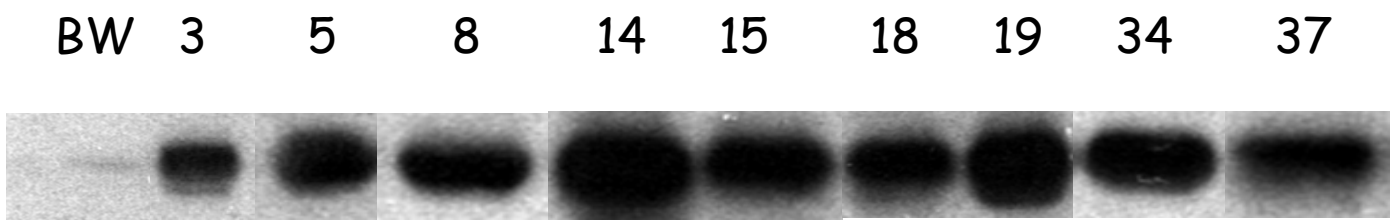




Verification of transgenic plants



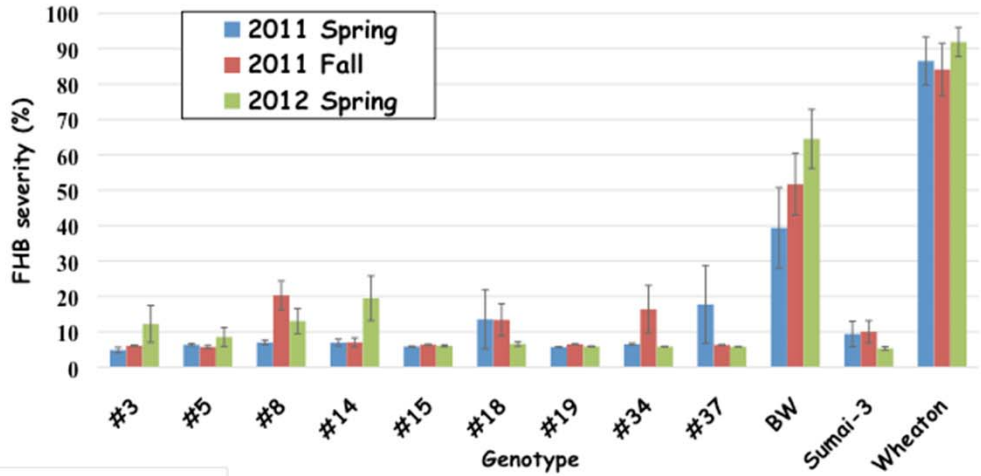
DNA blot

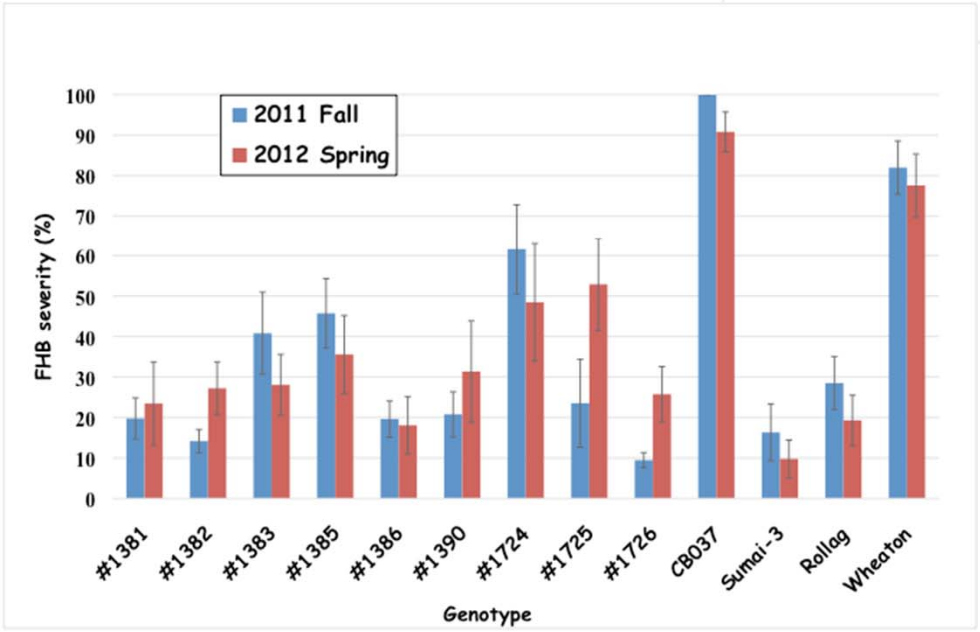


Protein blot

Transgenic wheat expressing *HvUGT13248* confers Type II resistance

Bobwhite background





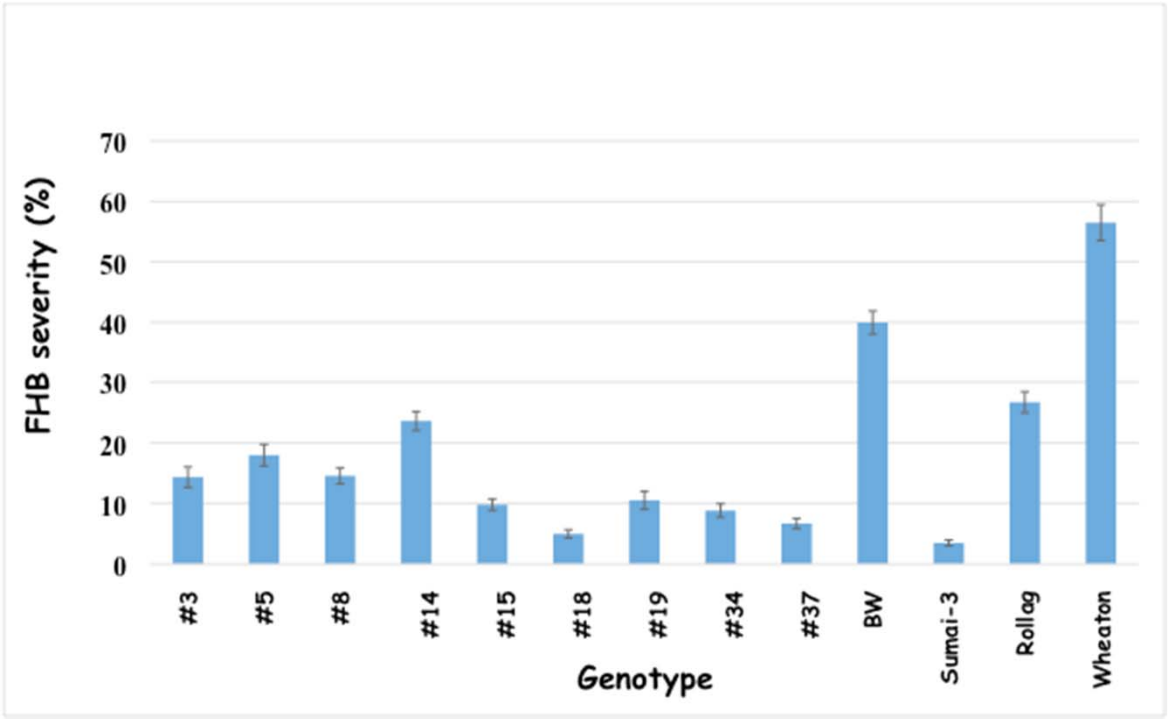
CB037 Background (collaboration with Tom Clemente)

Transgenic wheat expressing *HvUGT13248* confers resistance in the field

Genotype	FHB Inc. (%)	FHB Sev. (%)
Wheaton	96.25	56.48
Roblin	97.5	34.6
Sumai3	47.5	3.50
Rollag	91.25	26.66
Alsen	90	24.93*
Wheaton (non)	33.75	5.17
Bobwhite	96.25	39.96
#3	72.5	14.34**
#5	75	17.97**
#8	76.25*	14.56**
#14	88.75	23.58*
#15	72.5*	9.82***
#18	50***	5.01***
#19	65*	10.53***
#34	63.75	8.37***
#37	56.25**	6.70***

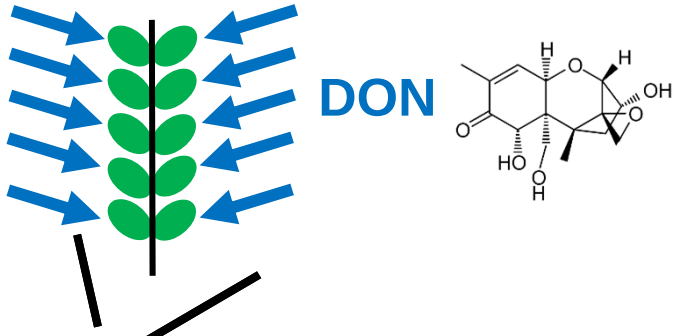
Collaboration with Ruth Dill-Macky

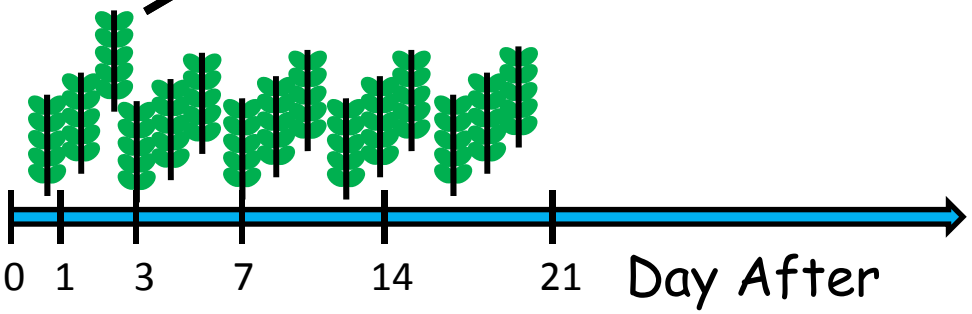
Transgenic wheat expressing *HvUGT13248* confers reduced FHB severity in the field



Collaboration with Ruth Dill-Macky

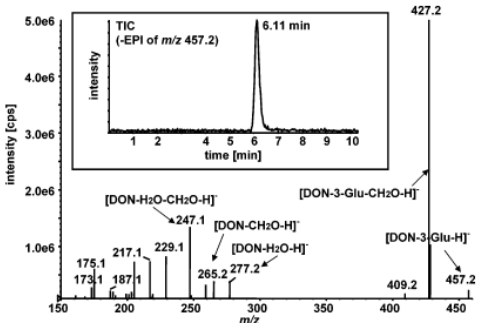
Does HvUGT13248 catalyze DON to D3G conjugation in wheat?





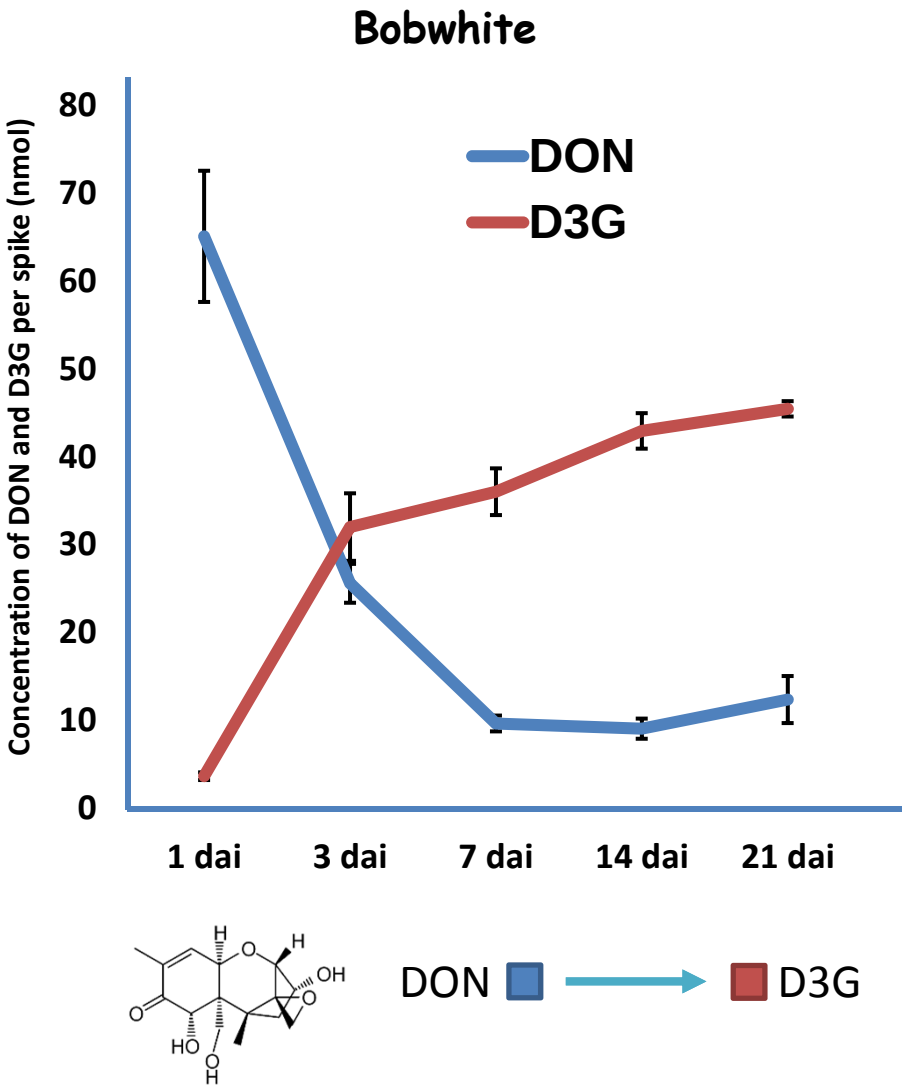
Metabolite extraction

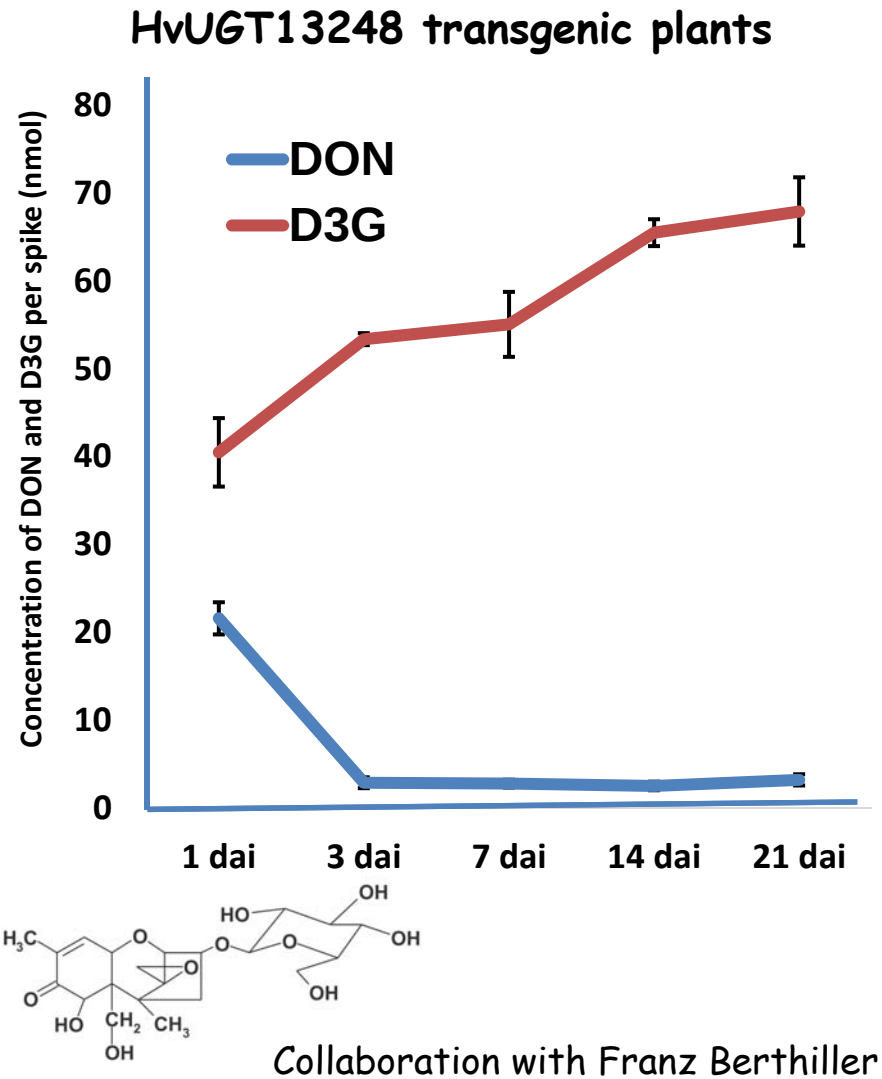
LC-MS/MS



Collaboration with Franz Berthiller

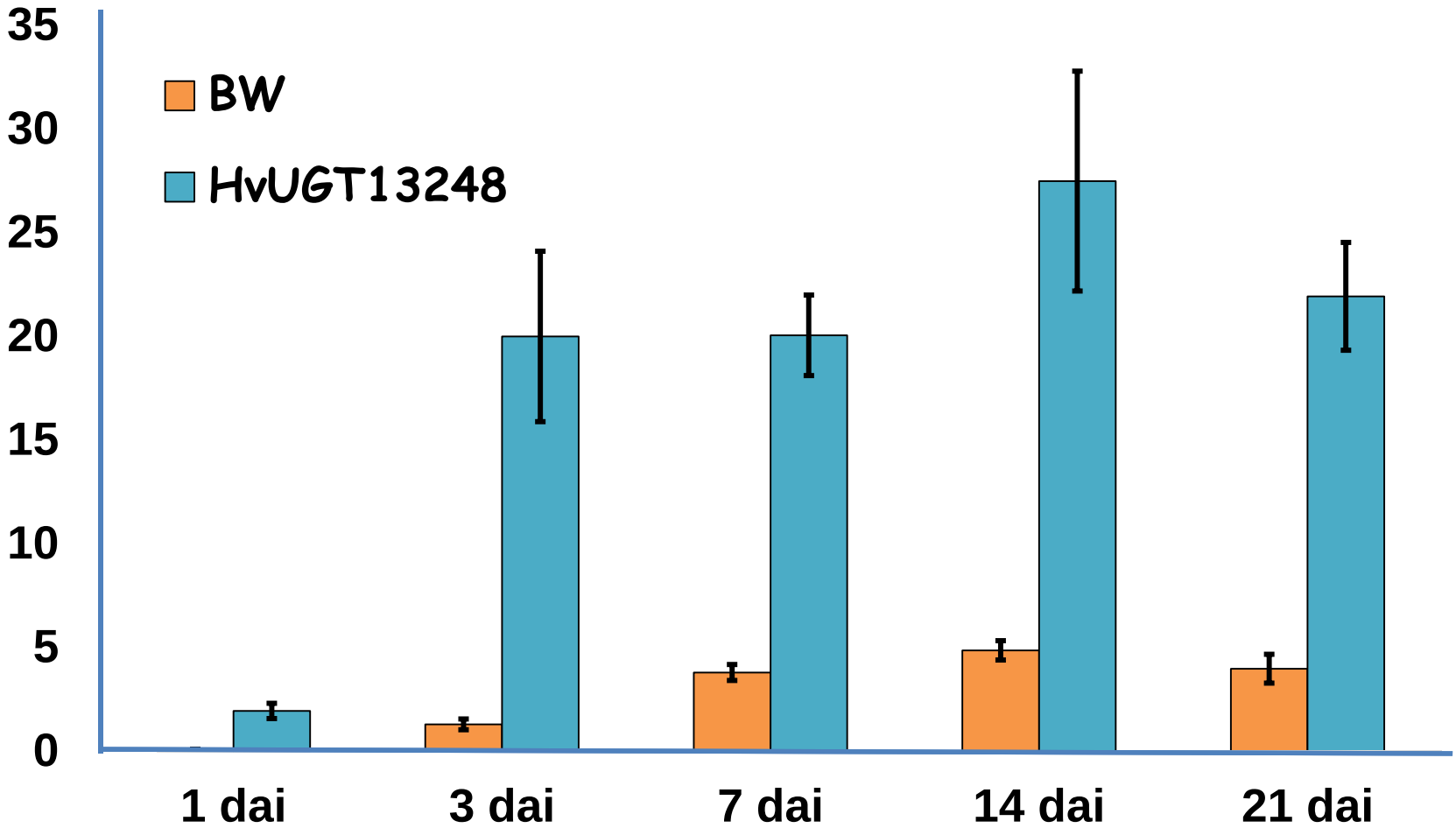
HvUGT13248 catalyzes DON to D3G





Collaboration with Franz Berthiller

Molar ratio of D3G to DON



Collaboration with Franz Berthiller

Summary

- Barley transports DON and converts DON to DON-3-O-glucoside
- *HvUGT13248* confers resistance to DON in yeast
- *HvUGT13248* confers resistance to DON and NIV in Arabidopsis
- HvUGT13248 converts DON to D3G
- Transgenic wheat carrying *HvUGT13248* confers Type II resistance in the greenhouse and resistance in the field

Acknowledgements

Muehlbauer lab
Hatice Bilgic
Jayanand Boddu
Seungho Cho
Stephanie Gardiner
Shane Heinen
Anna Hofstad
Yadong Huang
Haiyan Jia
Warren Kruger
Xin Li
Sanghyun Shin

University of Nebraska
Tom Clemente

U. of Minnesota
Jim Anderson
Ruth Dill-Macky
Yanhong Dong
Kevin Smith
Brian Steffenson

USDA-ARS, Peoria, IL
Susan McCormick

University of Natural Resources and Life Sciences, Austria
Gerhard Adam
Wolfgang Schweiger
Marc Lemmens
Paula Kovalsky Paris
Juan Antonio Torres-Acosta
Franz Berthiller

Vienna U. of Technology
Christian Hametner

USDA ARS
USDA United States Department of Agriculture National Institute of Food and Agriculture