

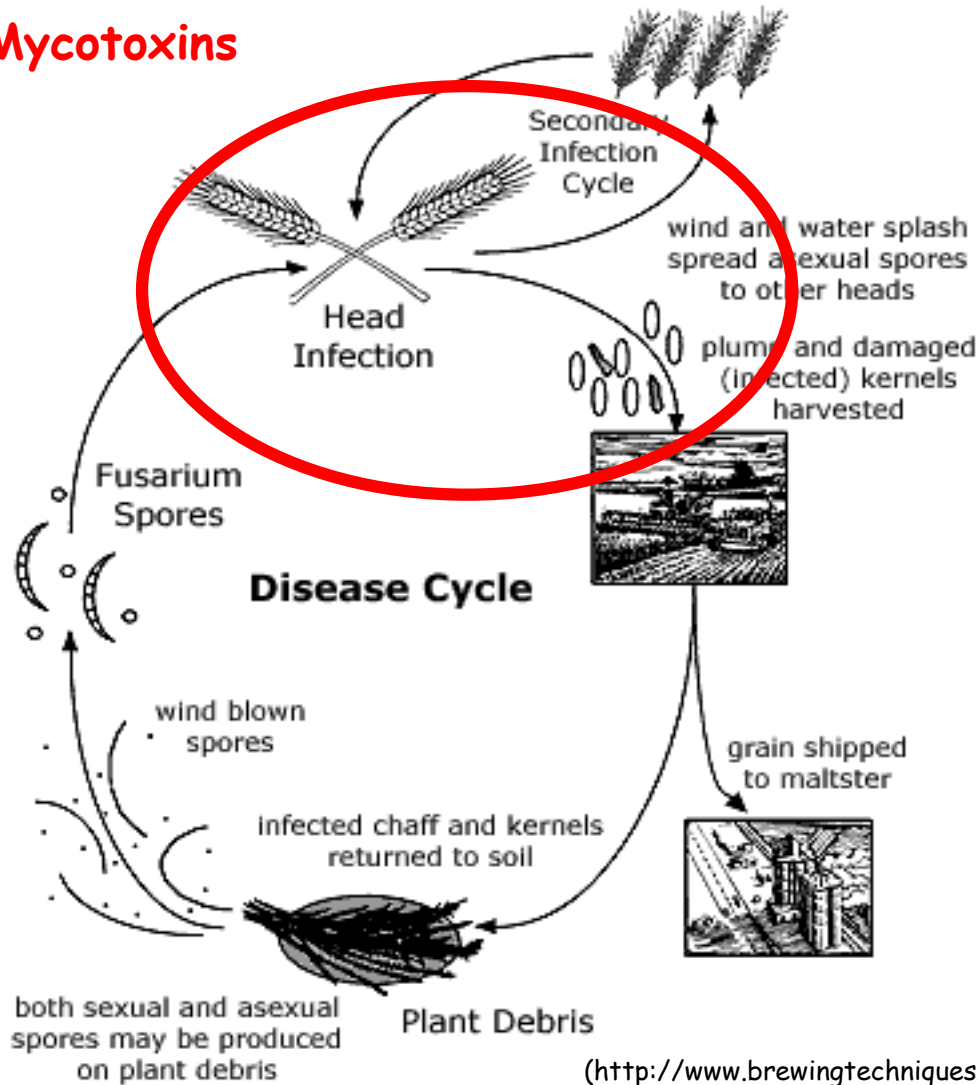
# Detoxification of *Fusarium graminearum* mycotoxins in *Brachypodium distachyon* and resistance

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Université Paris Sud - ORSAY

# Fusarium head blight (FHB)

## Mycotoxins



Infected ears of wheat



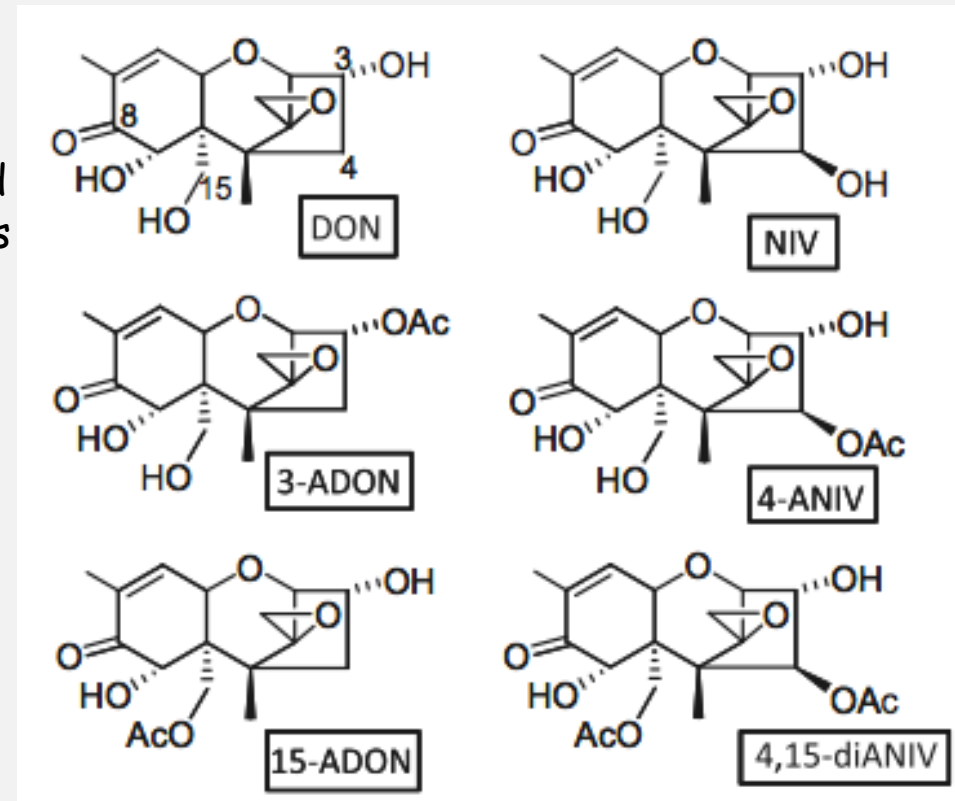
Non-infected and contaminated seeds

Infection cycle of *F. graminearum*

(<http://www.brewingtechniques.com>)

# Type B trichothecenes produced by *Fusarium graminearum*

- Nivalenol (NIV)
  - Deoxynivalenol (DON)
- + acetylated derivatives
- Vomiting
  - Inhibition of protein synthesis
  - Phytotoxicity on wheat and *A. thaliana*

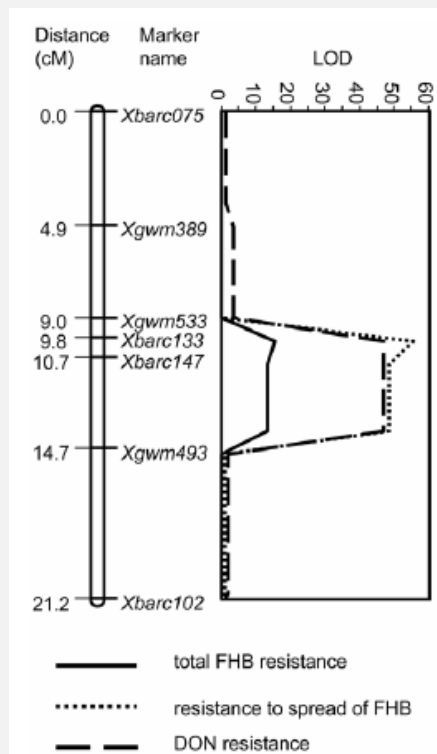


Alexander et al., 2011

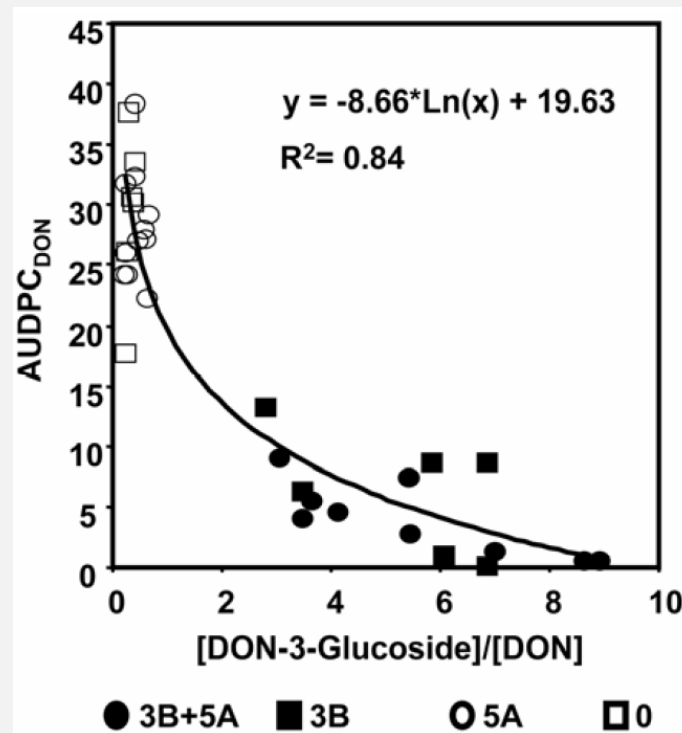
# Resistance to FHB in wheat is quantitative

- Quantitative trait loci (QTL) have been identified on all wheat chromosomes
  - More 100 QTLs: three major QTLs

Buerstmayr et al., 2009



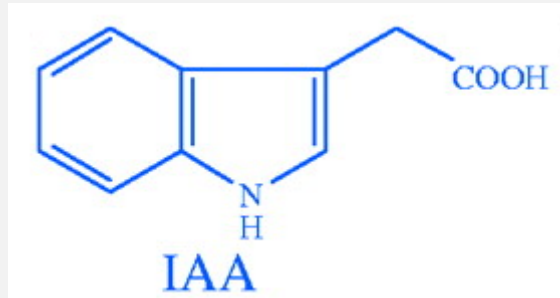
Lemmens et al., 2005



The major QTL *Fhb1* (Qfhs.ndsu-3BS) is correlated with the formation of **DON-3-O-glucose (D3G)**

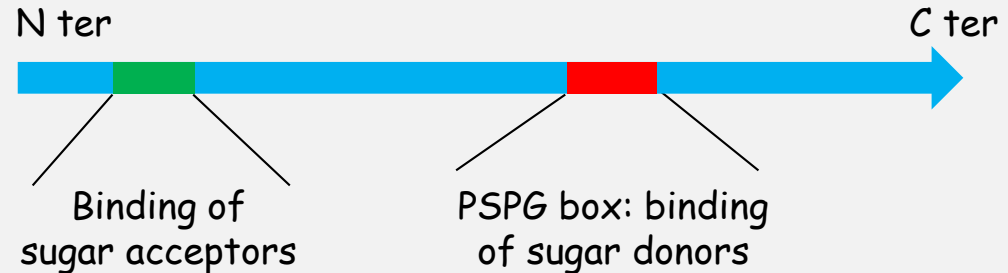
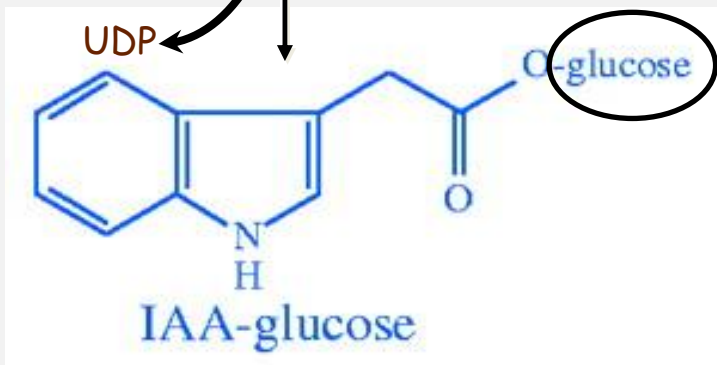


# Plant family 1 UDP-glycosyltransferases (UGTs)



UDP-Glucose  
(rhamnose,  
xylose, galactose)

UGT

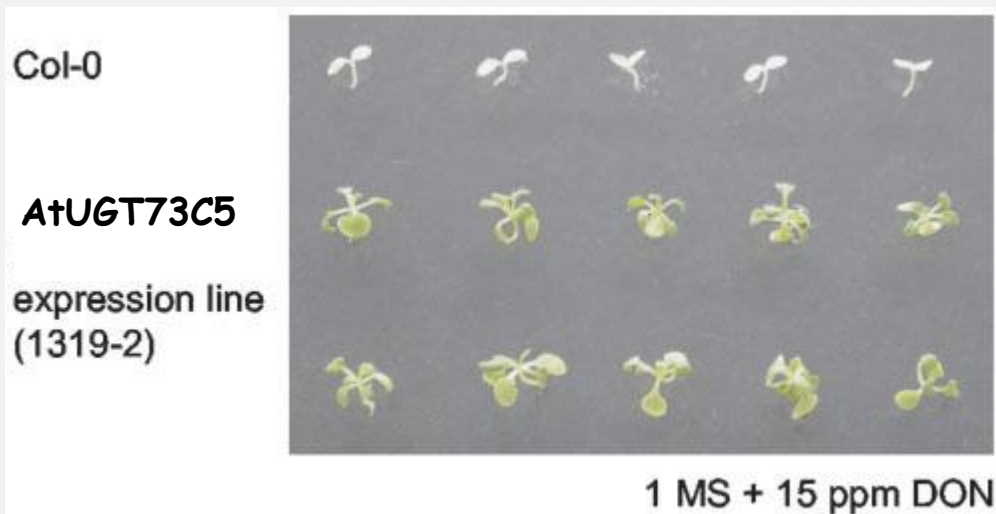
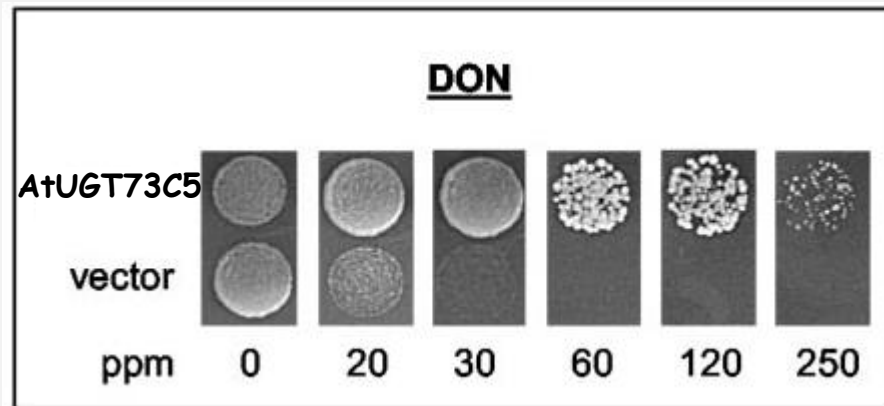


Osmani et al., 2009

UGTs involvement in the plant :

- Biosynthesis
- Stocking
- **Detoxification**

# Involvement of UGTs in DON detoxification



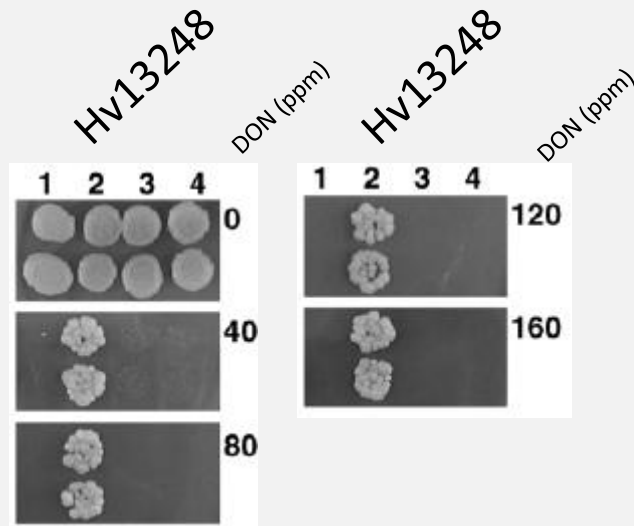
**AtUGT73C5 conjugates  
DON into D3G in yeast  
and in *Arabidopsis***

# Involvement of UGTs in DON detoxification

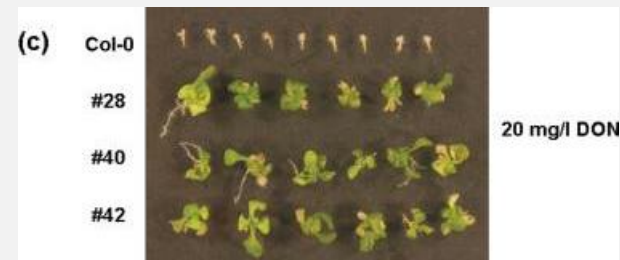
Probe set	Annotation	Range of fold change ratios <sup>a</sup>	
		1 h	12 h
Contig7127_at	NF-X1	17.43 to 42.32***	4.891 to 144.3*
Contig22479_at	Cytochrome P450	10.01 to 99.27*	891.4 to 2568***
Contig13248_at	UDP-glucosyltransferase	31.78 to 1555*	0.824 to 527.6
HD08H17r_at	F-box	15.63 to 266.3*	150.8 to 947.7***
Contig20431_at	Gibberellin receptor	8.674 to 169.3*	2.848 to 27.67*
Contig14507_at	Senescence-associated	0.920 to 6.498	1,915 to 13,216***

Gardiner *et al.*, 2010

- Identification of four genes encoding UGTs in barley by large transcriptional approach



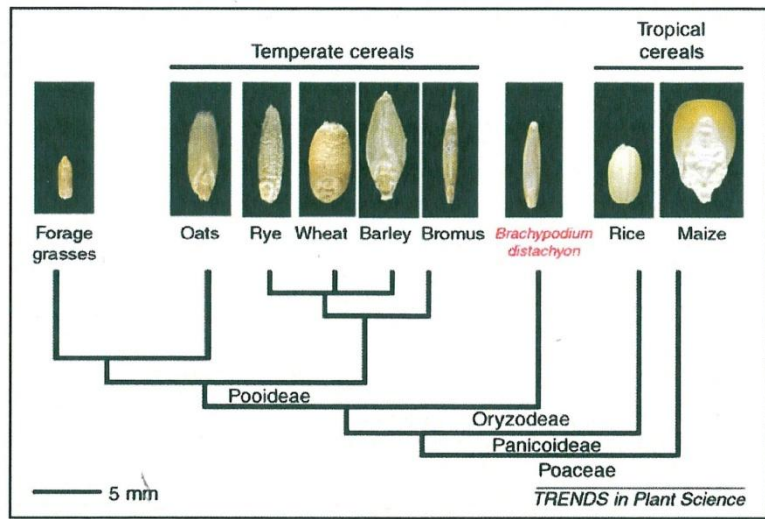
Schweiger *et al.*, 2010



Shin *et al.*, 2012

- Hv13248 confers DON resistance in yeast and in *Arabidopsis* and leads to the production of D3G

# Model cereal species: *Brachypodium distachyon*



Opanovicz et al. 2008



- Numerous genetic and genomic resources (T-DNA, TILLING, genome sequence, etc)

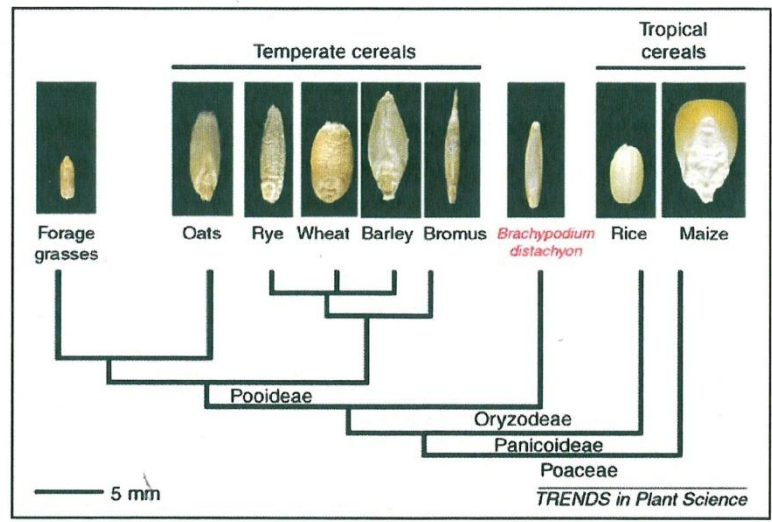


Bd

- Autogamy
- Small size
- Short life cycle (10 to 12 weeks in LD conditions)



# Model cereal species: *Brachypodium distachyon*



Opanovicz et al. 2008

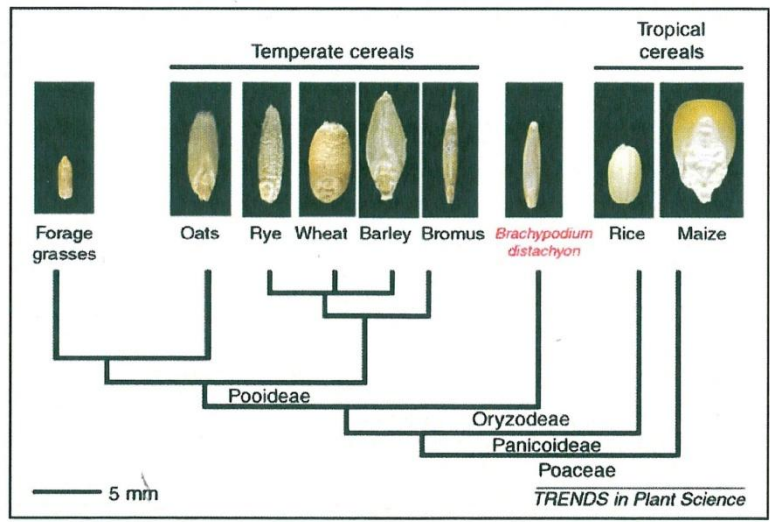


Bd

- Autogamy
- Small size
- Short life cycle (10 to 12 weeks in LD conditions)



# Model cereal species: *Brachypodium distachyon*

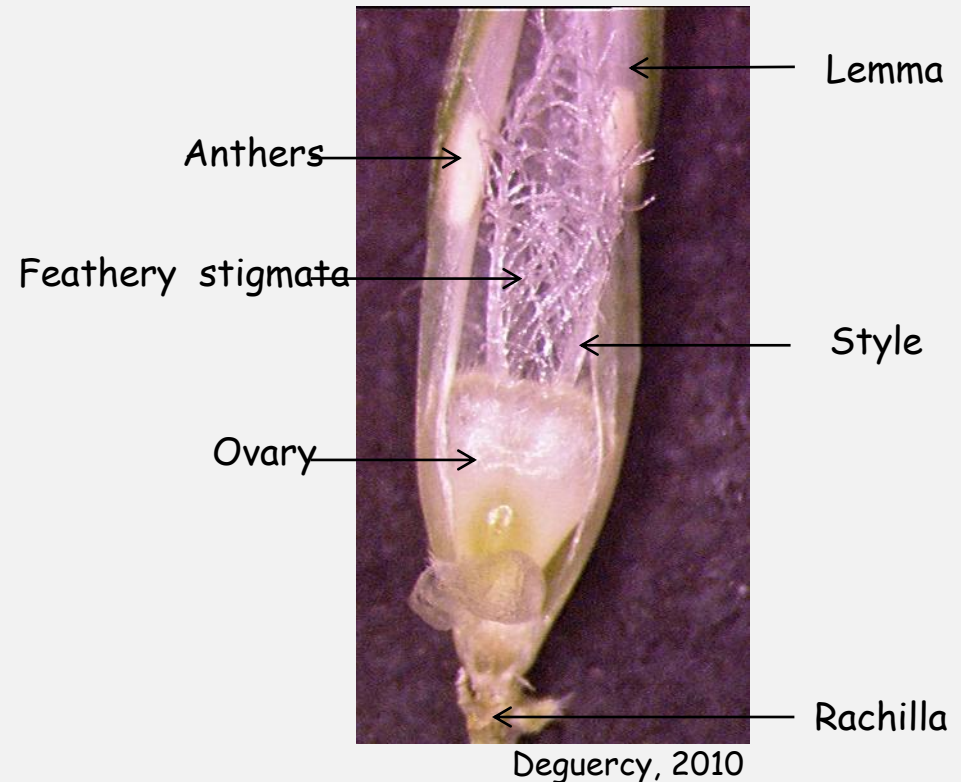


Opanovicz et al. 2008



Bd

- Autogamy
- Small size
- Short life cycle (10 to 12 weeks in LD conditions)



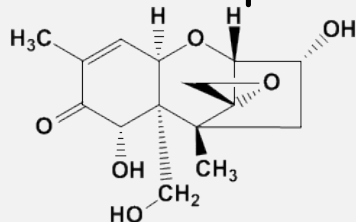
# *Brachypodium distachyon* is a host plant for *Fusarium graminearum*

168 hpi



Mock      *F. graminearum*  
Deguercy, 2010

- Deoxynivalenol is detected in the infected spikes



96 hpi



Point  
inoculation

Peraldi et al., 2011

168 hpi



Spray  
inoculation

Bars: 1 cm



# Aims

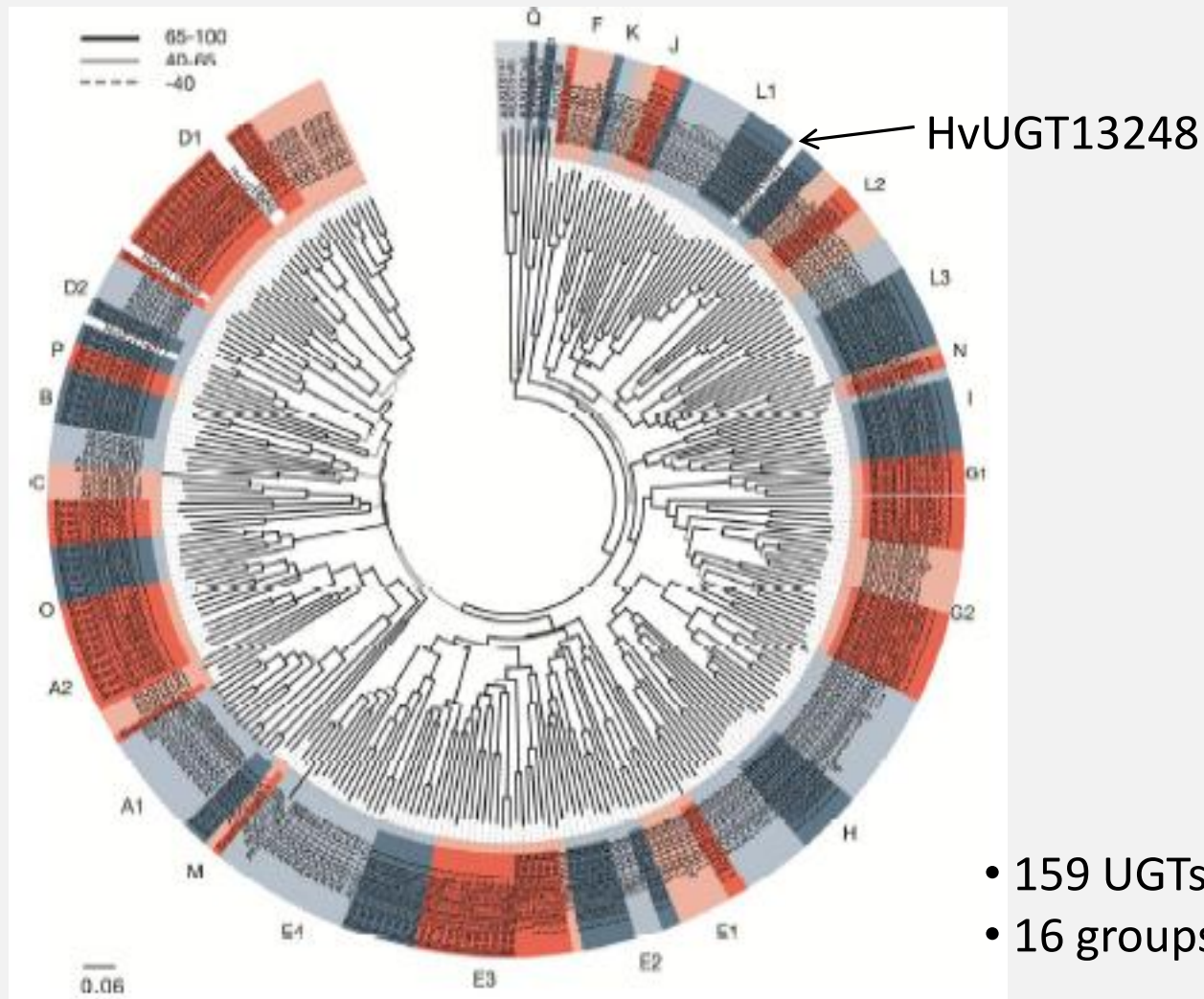
- Identification of UGT(s) able to conjugate DON in D3G
- *In planta* functional analysis of the candidate UGT(s)



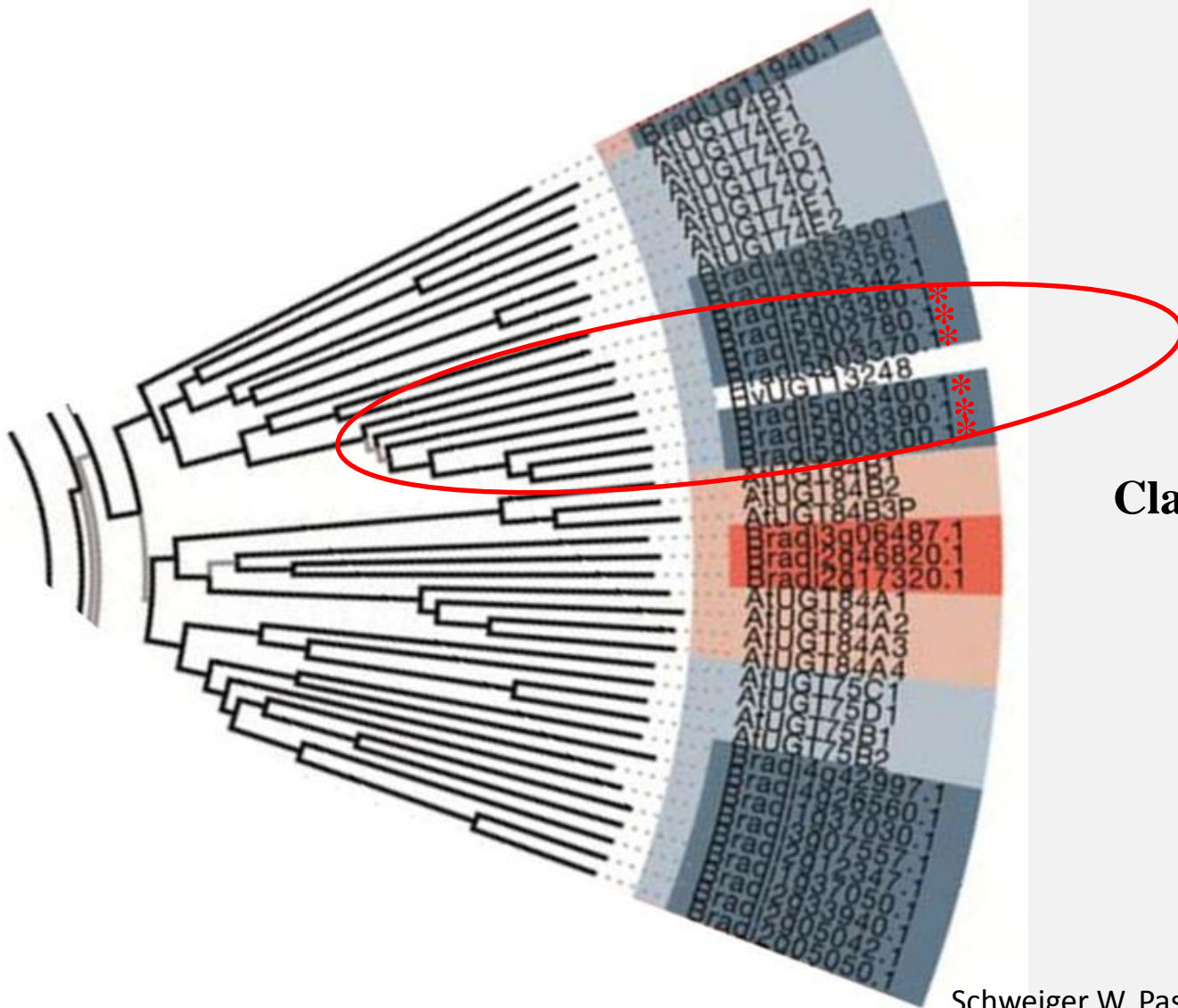


# Identification of UGT(s) able to conjugate DON in D3G

# Identification of a cluster of orthologs of HvUGT13248 in *B. distachyon*



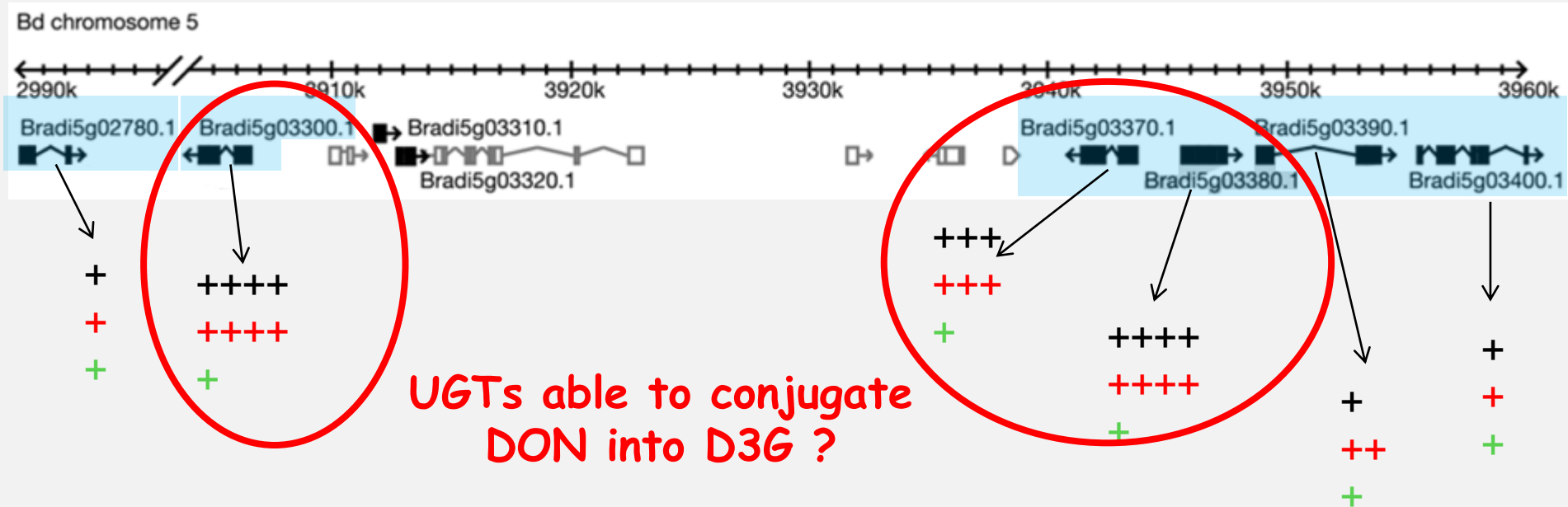
# Identification of a cluster of orthologs of HvUGT13248 in *B. distachyon*



## Clade L1

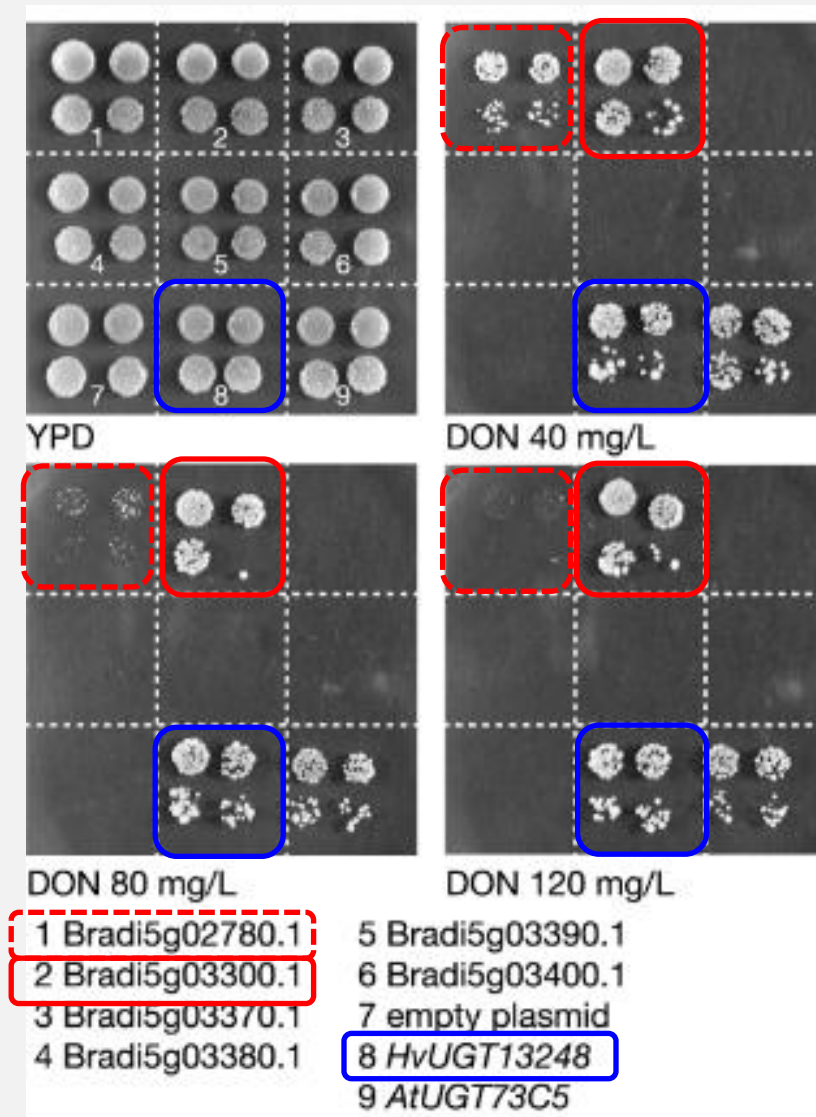
# Expression regulation of a cluster of orthologs of HvUGT13248 in *B. distachyon*

+: DON (12 hpi)  
+: *Fg*DON<sup>+</sup> (96 hpi)  
+: *Fg*DON<sup>-</sup> (96 hpi)





# Involvement of UGTs in DON detoxification

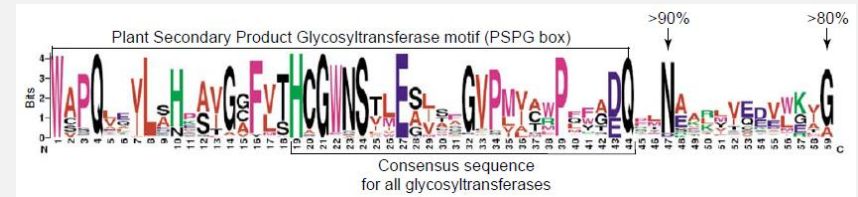
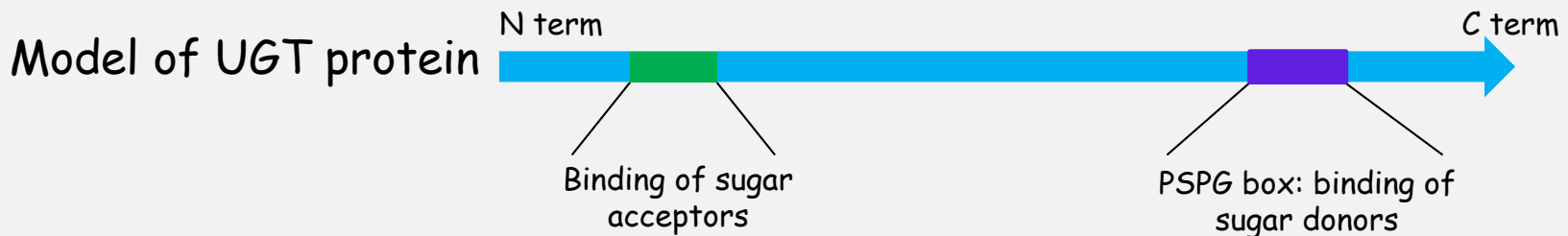




***In planta functional analysis of the  
UGT-encoding Bradi5g03300 gene***

# Identification of *B. distachyon* lines altered in the *Bd5g03300* gene

Screening of the *B. distachyon* TILLING collection for mutants in the 3' end of the *Bd5g03300* gene (URGV, Evry, France)



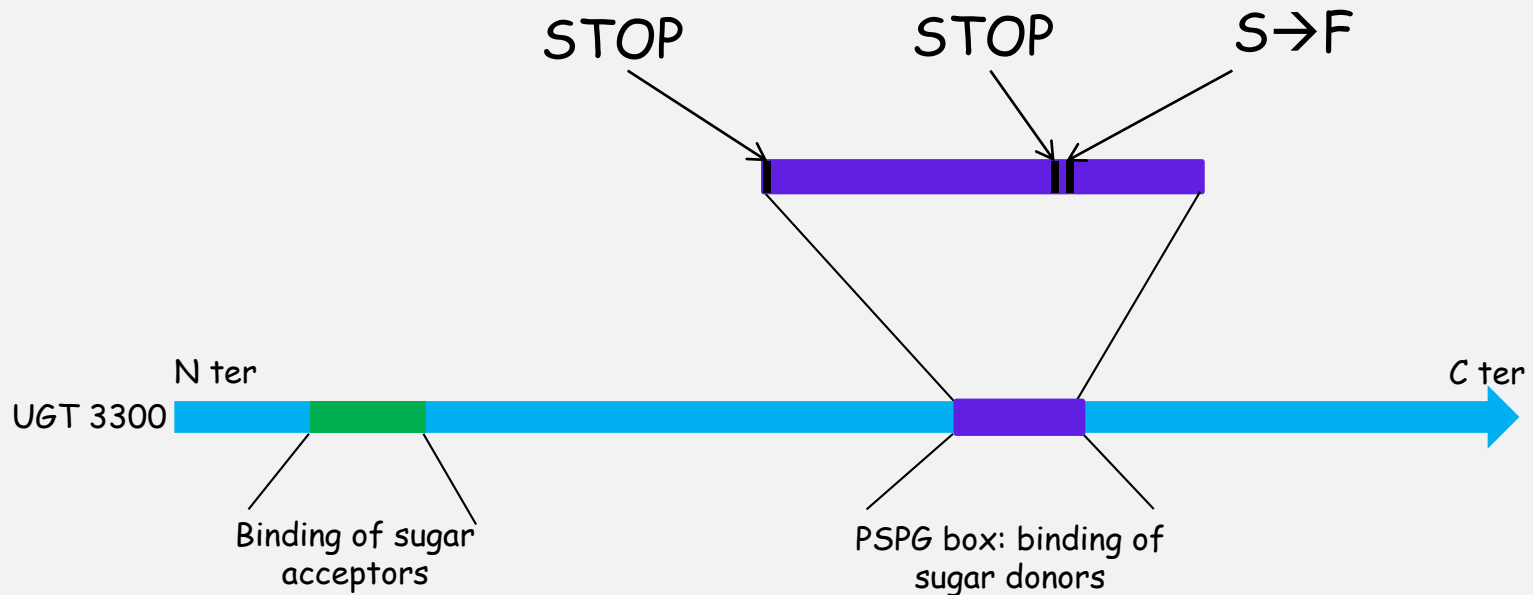
## Model of *Bd5g03300* gene



# Identification of *B. distachyon* lines altered in expression of *Bd5g03300*

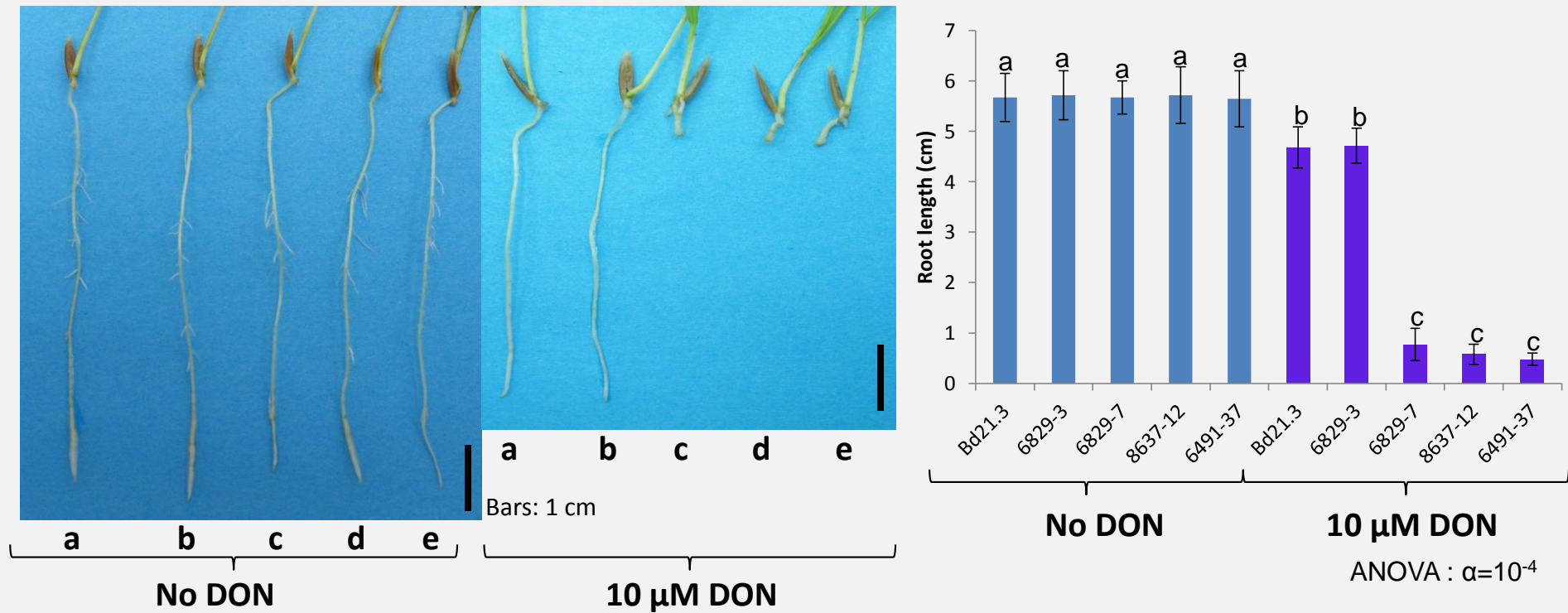
5530 M2 families screened → 16 mutant families identified

Three of interest  
after sequencing





# TILLING mutants are more sensitive to DON



a: Bd21.3 WT

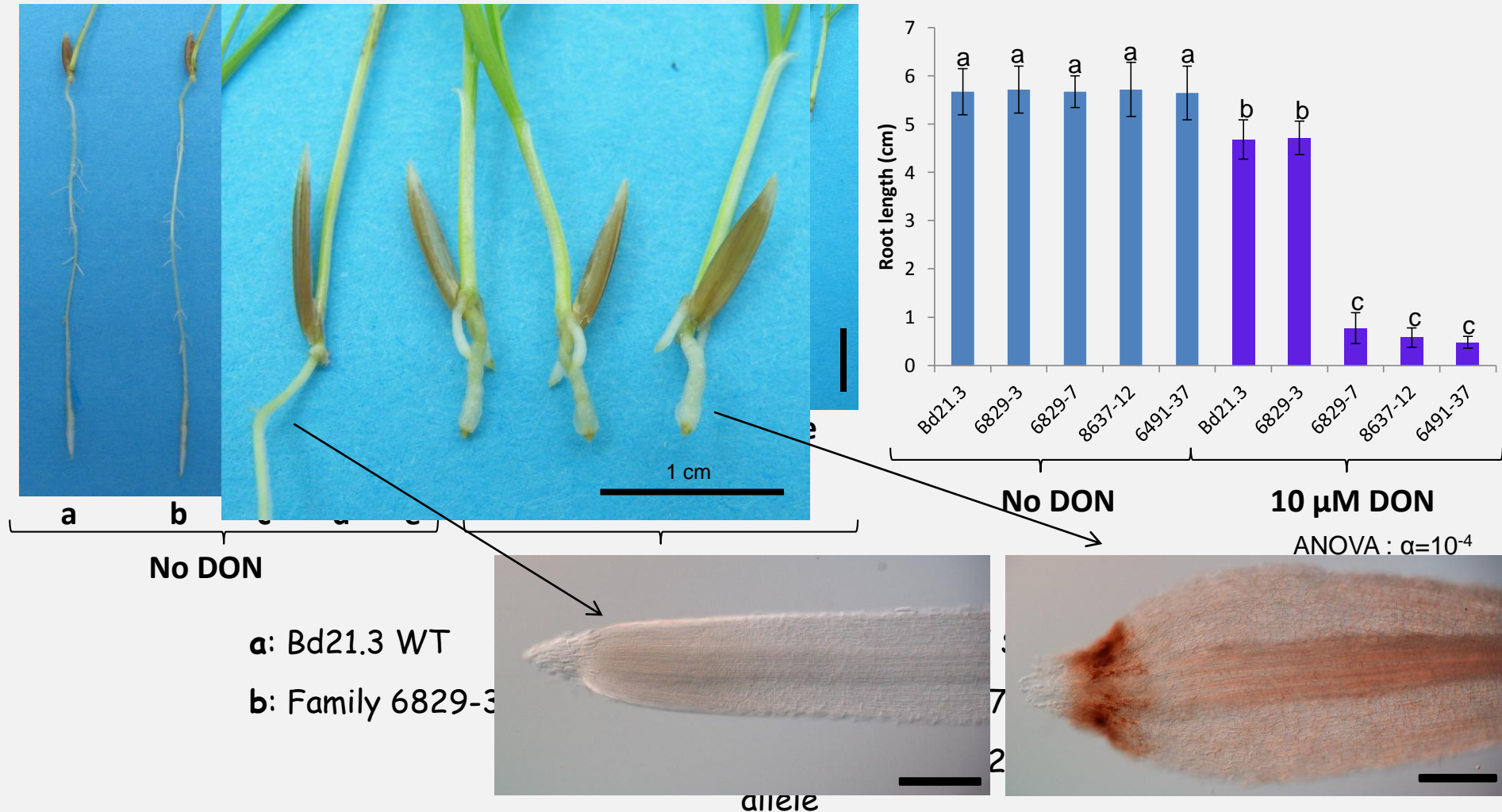
b: Family 6829-3 WT allele

c: Family 6829-7 STOP allele

d: Family 6491-37 STOP allele

e: Family 8637-12 Hydrophobic allele

# TILLING mutants are more sensitive to DON



a: Bd21.3 WT

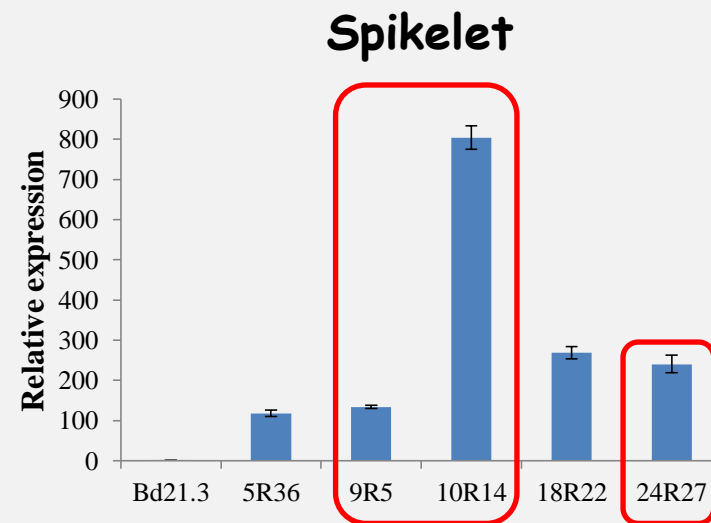
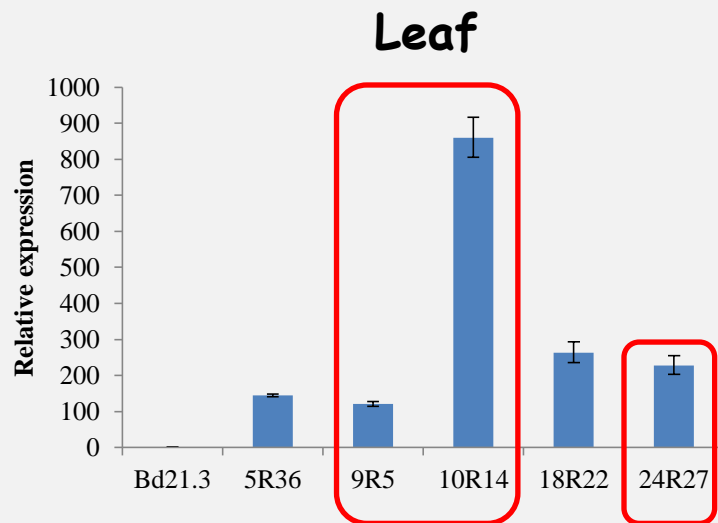
b: Family 6829-3

allele

# Construction of lines over-expressing the *Bd5g03300* gene

(Collaboration with O. Bouchake-Coussa; IJBP)

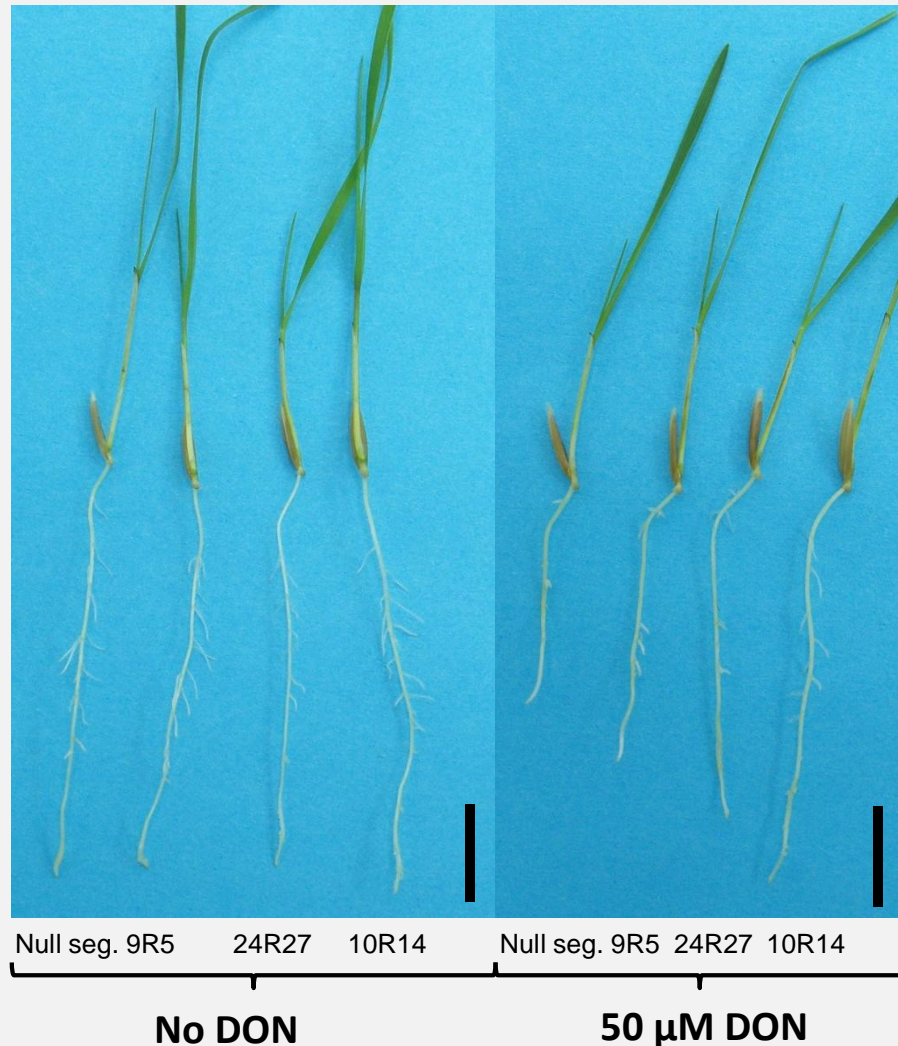
Level of *Bd5g03300* over-expression measured by qRT-PCR



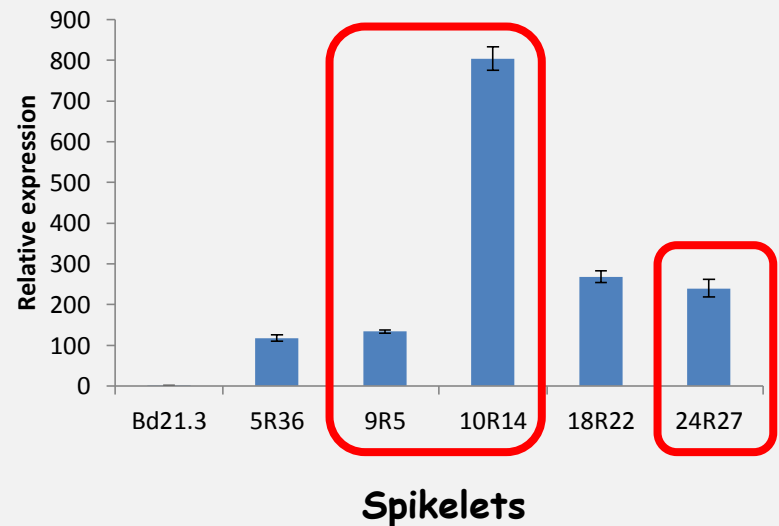
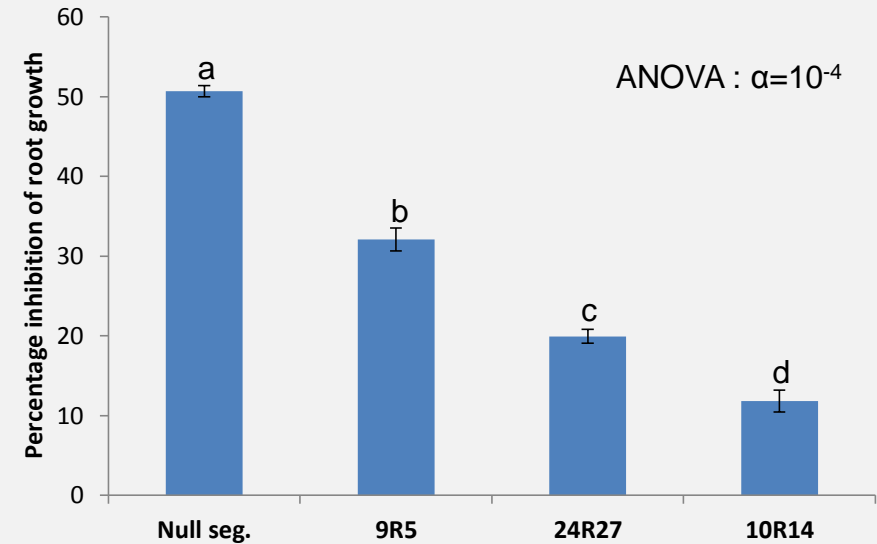
Reference genes: *UBC18* and *ACT7*  
Compared to the endogenous level of Null segregant T0-9



# Increased resistance to DON is correlated with the level of *Bradi5g03300* expression



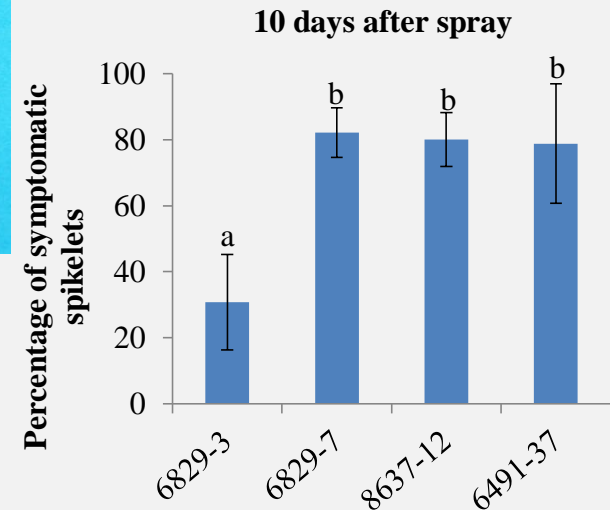
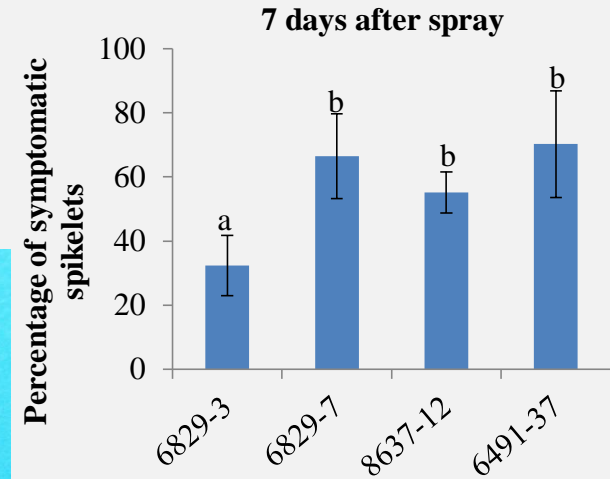
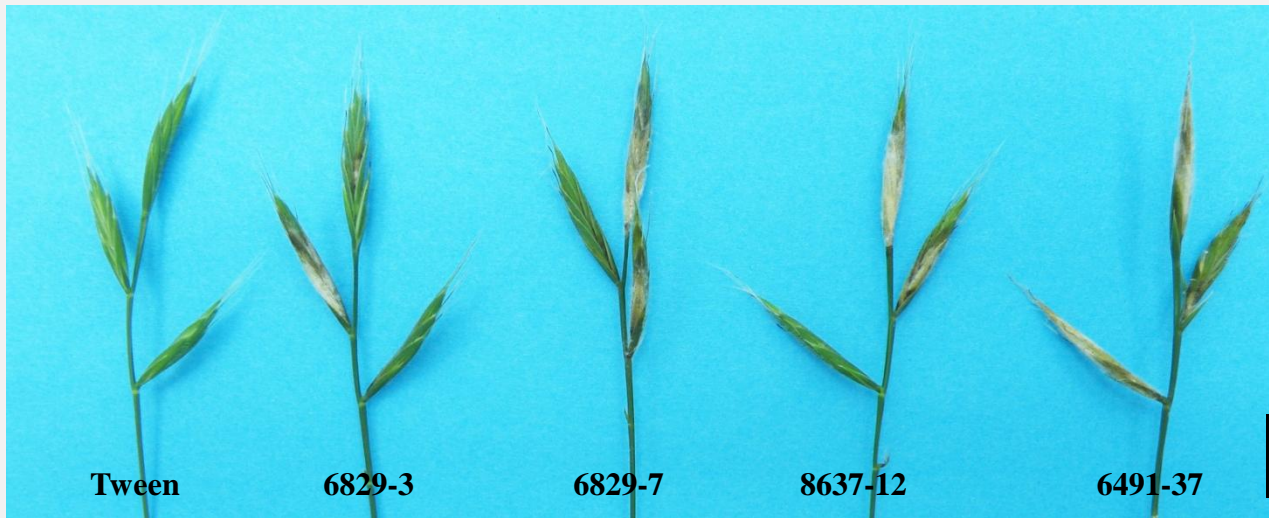
Bars: 1 cm





# Increased susceptibility of TILLING lines to FHB

## Spray inoculation (strain *FgDON+*)

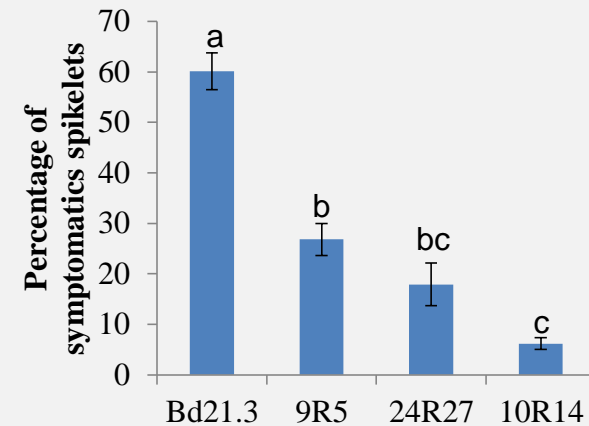
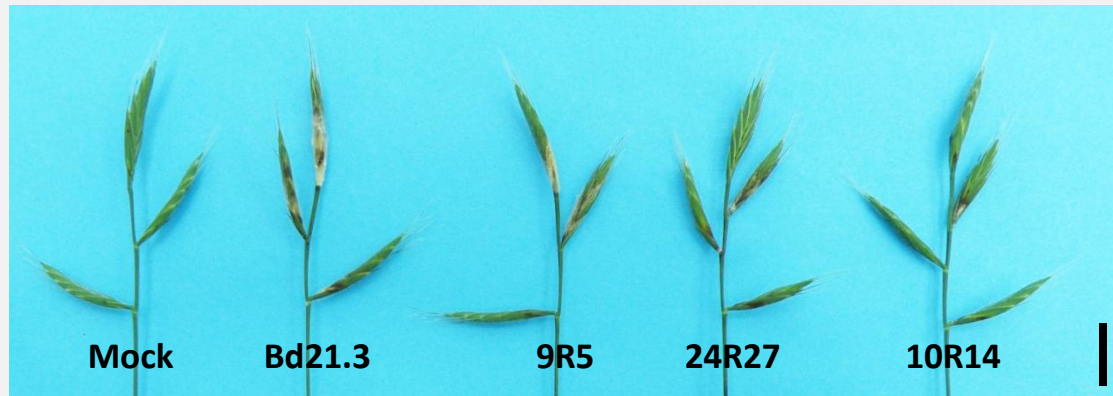


Duncan test ,  $p\text{-value} \leq 0.01$

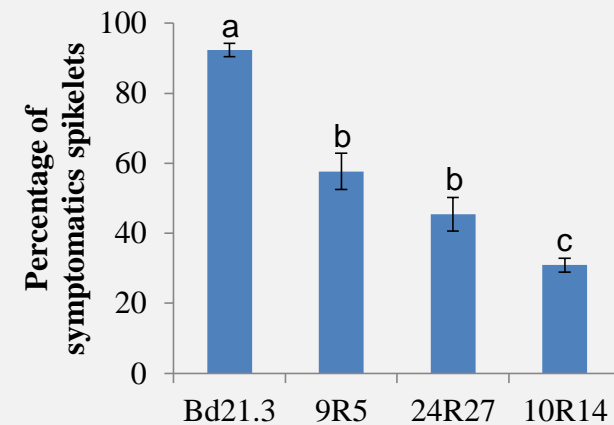
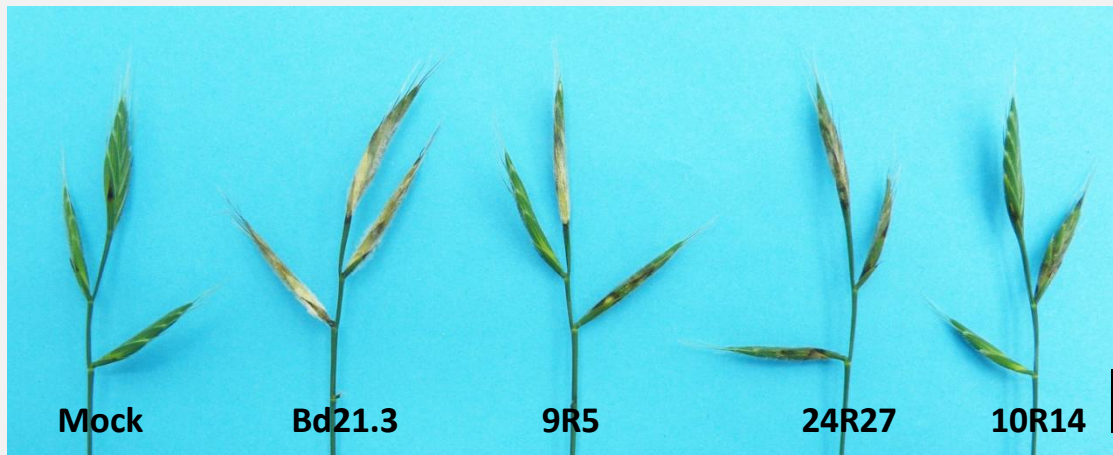
# Increased resistance of OE lines to FHB

## Spray inoculation (strain *FgDON+*)

7 days after spray

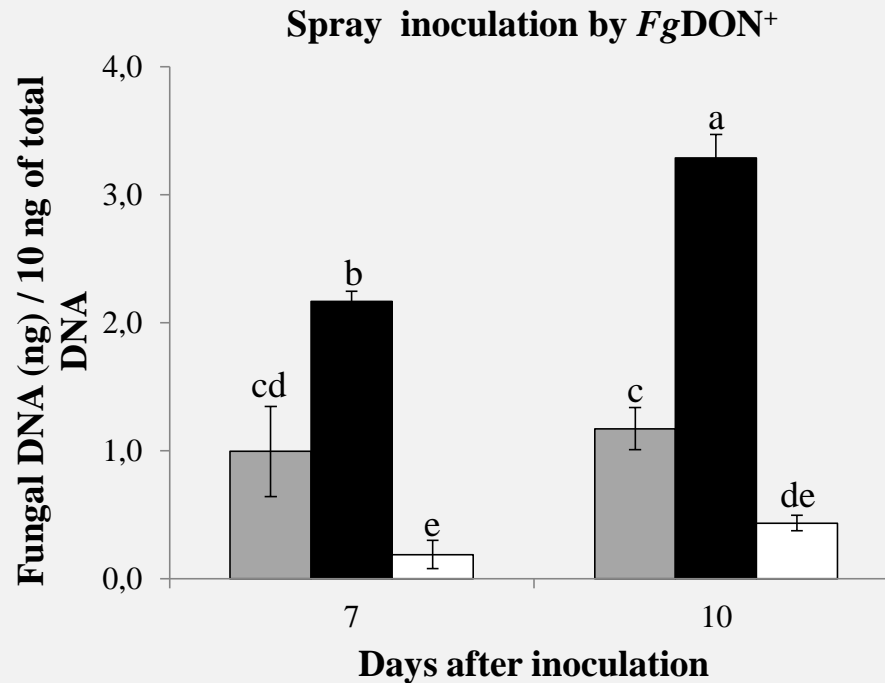


14 days after spray



Duncan test ,  $p\text{-value} \leq 0.01$

# Correlation between the altered response to FHB and fungal biomass



Duncan test:  $p$ -value  $\leq 0.01$

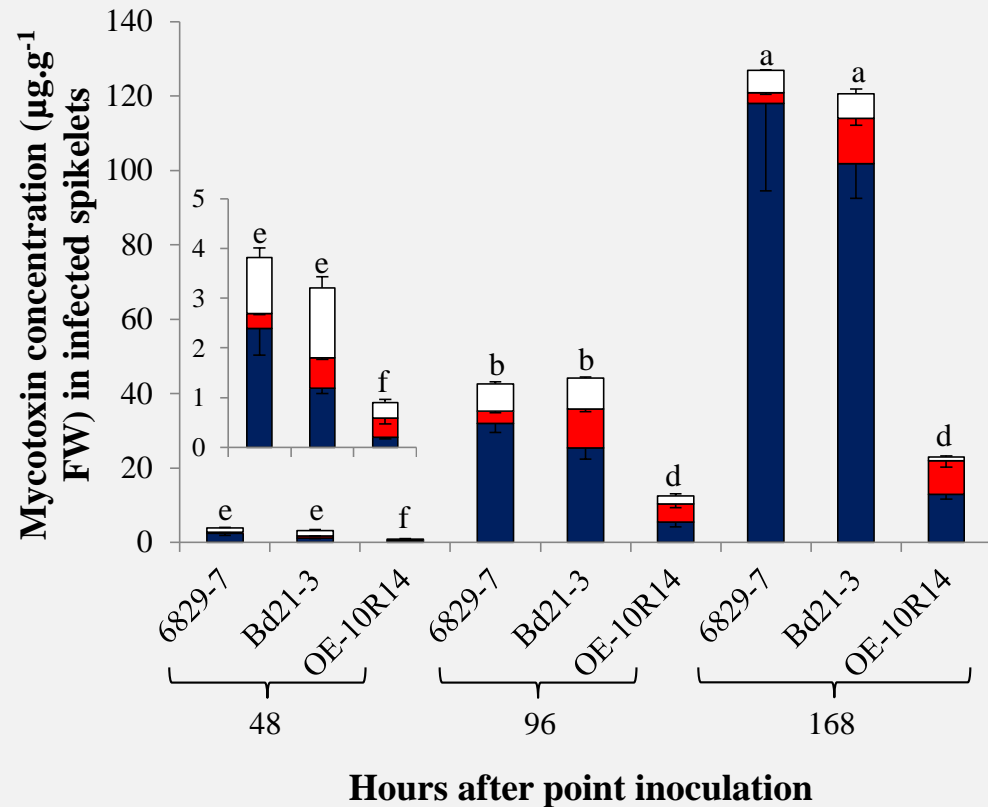
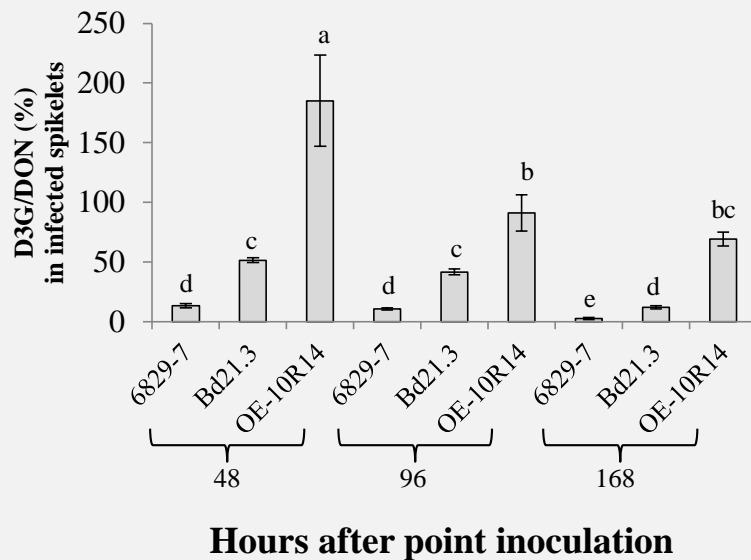
■ Bd21-3  
(WT)

■ 6829-7  
(Mutant)

□ OE-10R14  
(Over-expressor)

# The Bradi5g03300 UGT conjugates DON in planta

(Collaboration with V. Atanasova-Penichon; INRA MycSA)

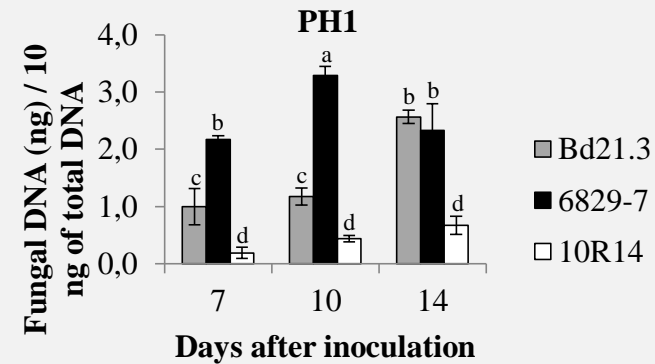
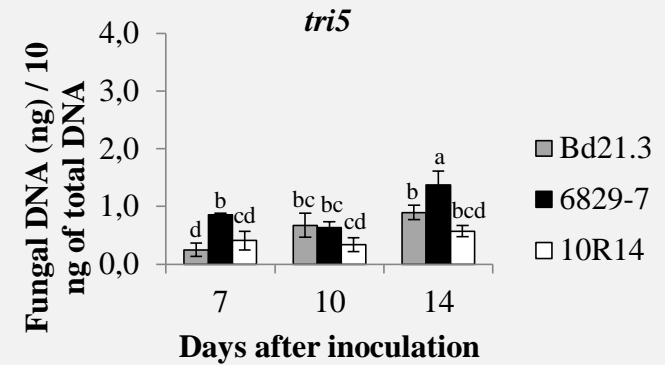
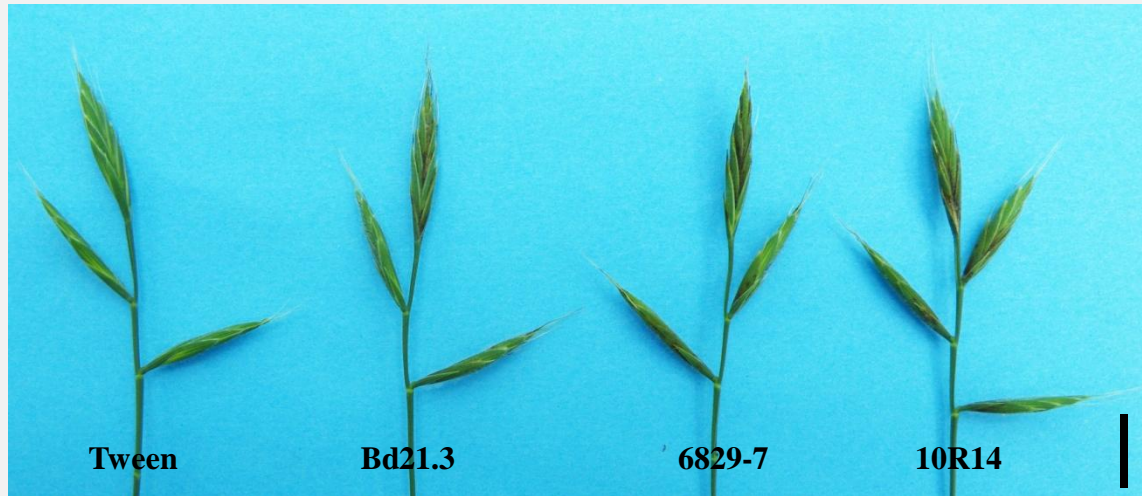


■ DON ■ D3G □ 15-ADON

Student test:  $p$ -value  $\leq 0.01$



# Response to infection by a strain unable to produce DON is unaltered



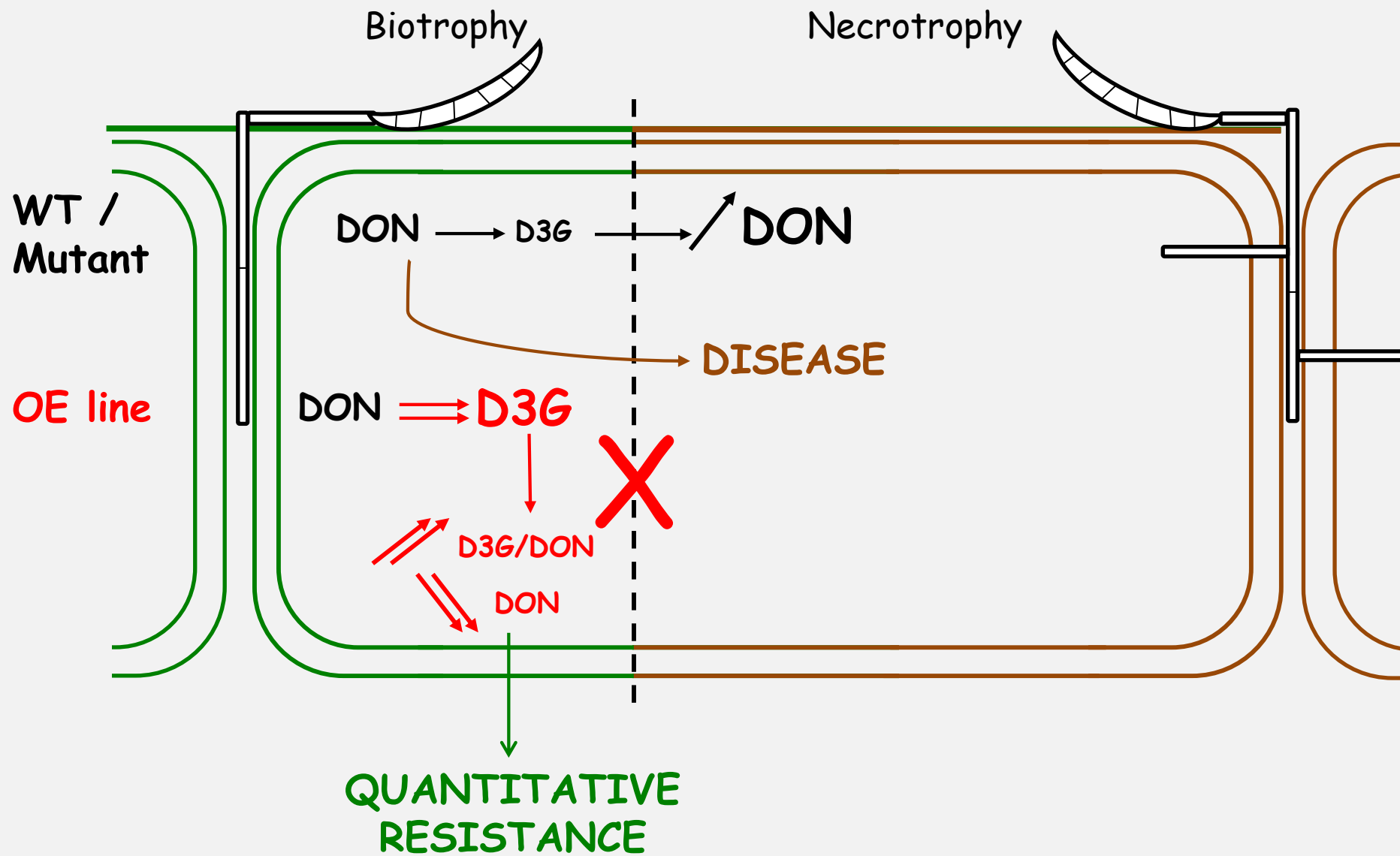


# Conclusion

## Role of DON detoxification in FHB resistance

- Strong correlation between early DON detoxification and resistance to FHB
  - TILLING mutants: increased root sensitivity to DON and susceptibility to *Fusarium graminearum*
  - Over-expressing lines: increased root tolerance to DON and resistance to *Fusarium graminearum*

# Conclusion



# Summary and prospects

## Role of DON detoxification in FHB resistance

- **Strong correlation between early DON detoxification and resistance to FHB**
  - TILLING mutants: increased root sensitivity to DON and susceptibility to *Fusarium graminearum*
  - Over-expressing lines: increased root tolerance to DON and resistance to *Fusarium graminearum*
- **Translation to wheat** (Coll. UMR GDEC, Clermont-Ferrand, France / DIM Astrea PhD project)
  - Expression analysis of wheat orthologs during infection
  - Functional validation of candidate gene(s) in wheat
  - Allele mining & Search for resistance-associated alleles





Jean-Claude Pasquet  
Audrey Chaix-Bryan  
Miriam Gatti  
 Valentin Changenet  
 Catherine Macadré  
 Patrick Saindrenan  
 Jean-Marc Seng



Vincent Thareau

# Collaborations



Richard Sibout  
 Oumaya Bouchabké-Coussa  
 Camille Soulhat  
 Sébastien Antelme



Marion Dalmais



Vessela Atanasova-Pénichon



Gerhard Adam  
 Wolfgang Schweiger  
 Gerlinde Wiesenberger



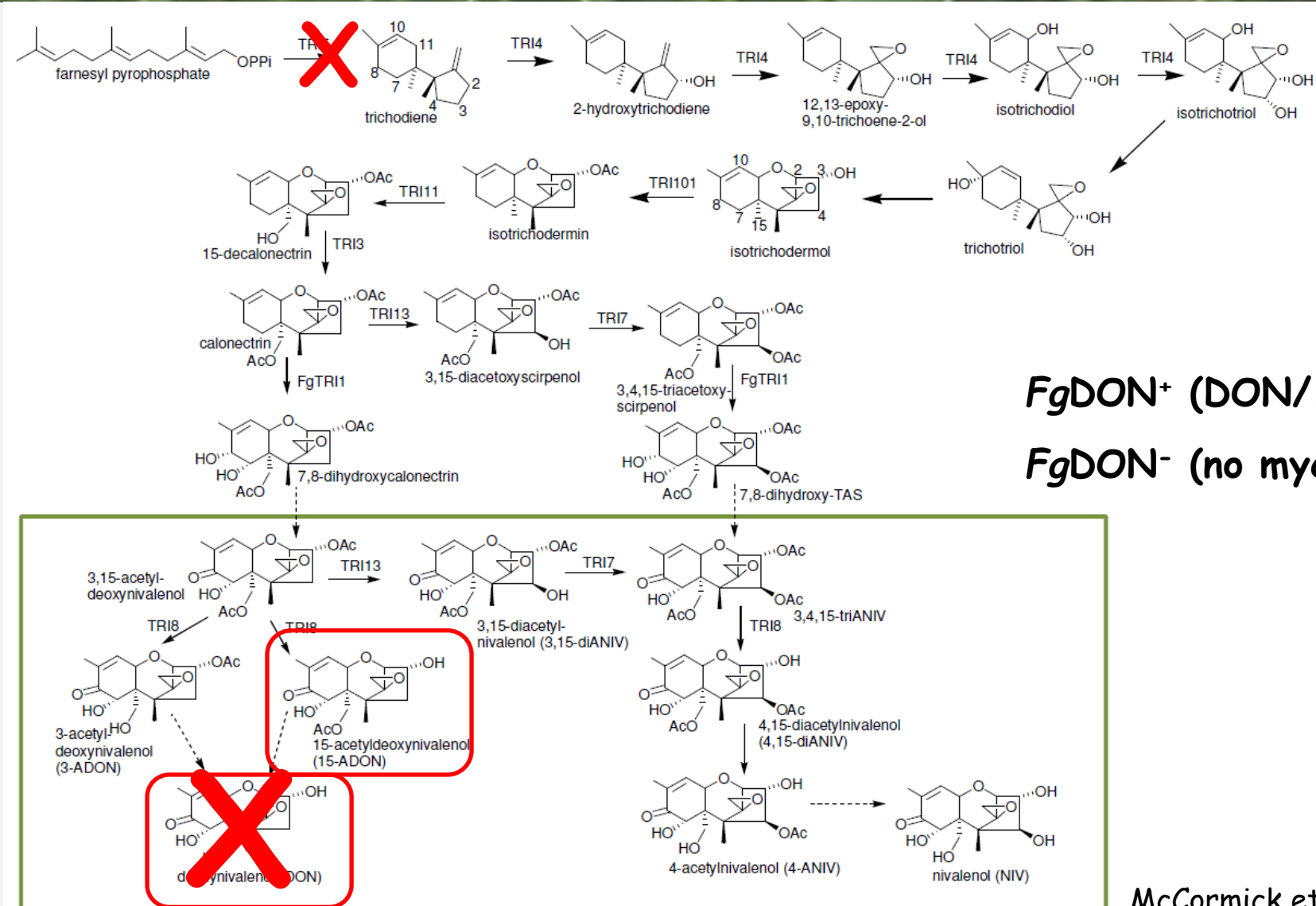
Florence Cambon  
 Caroline Tassy  
 Philippe Leroy  
 Thierry Langin





**Detailed characterization of the  
interaction between  
*B. distachyon* and *F. graminearum***

# Biosynthesis of type B trichothecenes in *Fusarium graminearum*



**FgDON<sup>+</sup> (DON/15-ADON)**

**FgDON<sup>-</sup> (no mycotoxin)**



# DON is involved in fungal aggressiveness on *Bd* spikes



21 dpi *Fg*DON<sup>+</sup>

21 dpi *Fg*DON<sup>-</sup>



WT DON

21 dpi (Maier et al., 2006)

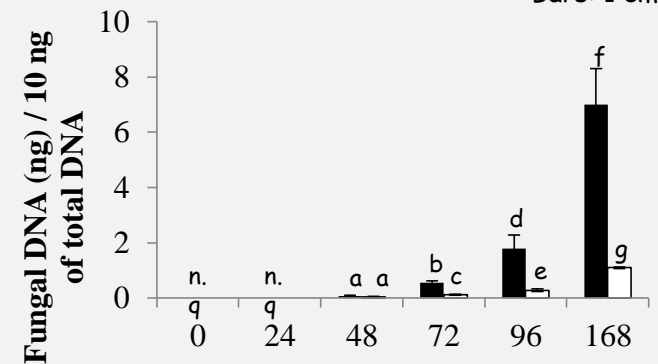


96 hpi *Fg*DON<sup>+</sup>



96 hpi *Fg*DON<sup>-</sup>

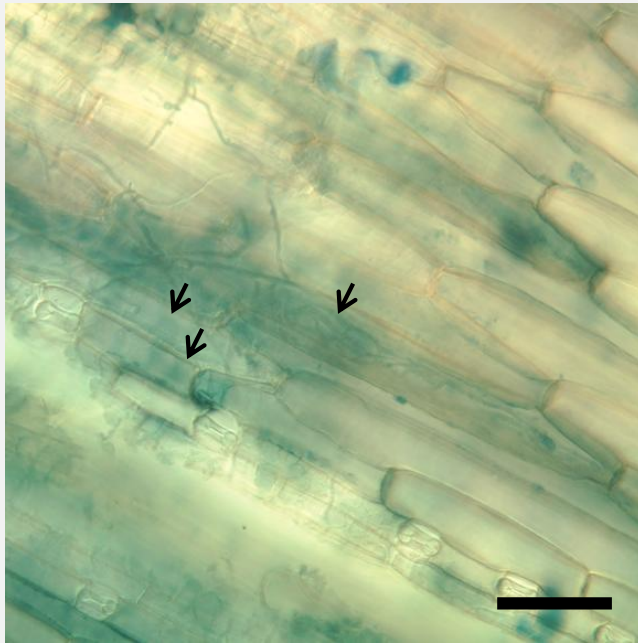
Bars: 1 cm



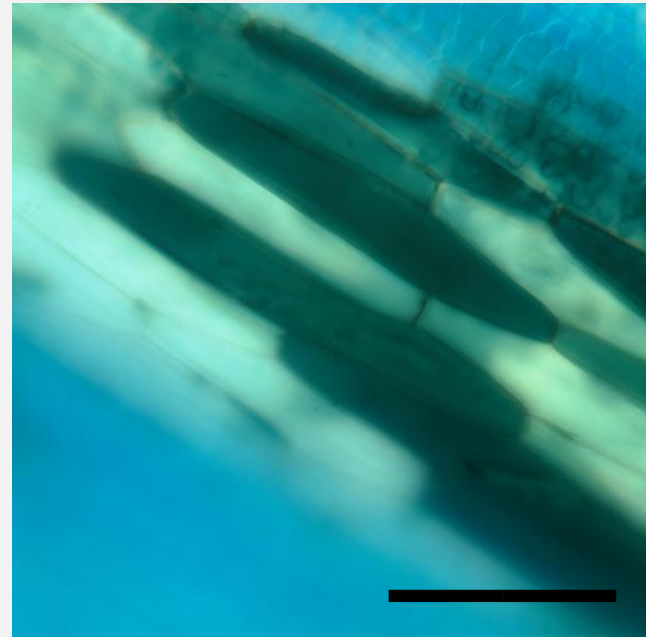
Hours after inoculation

Student test:  $p$ -value  $\leq 0.01$

# A role for DON in preventing early cell death?



72 hpi *Fg*DON<sup>+</sup>

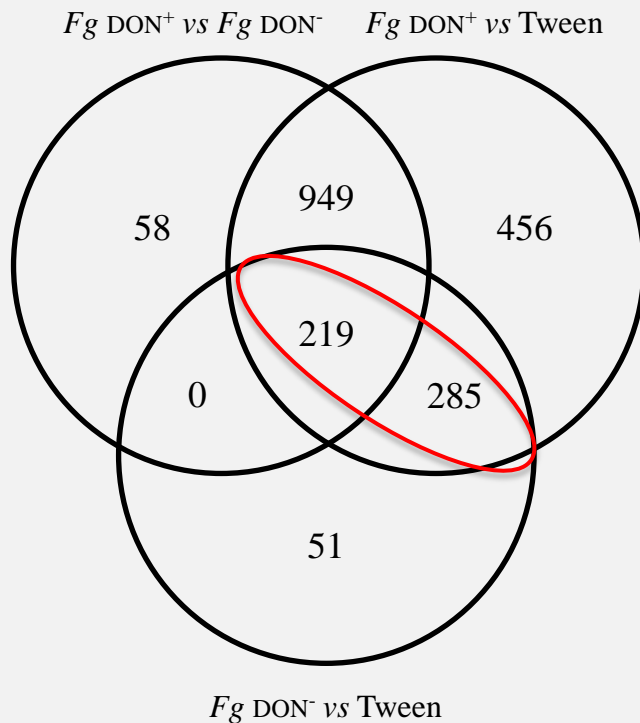


72 hpi *Fg*DON<sup>-</sup>

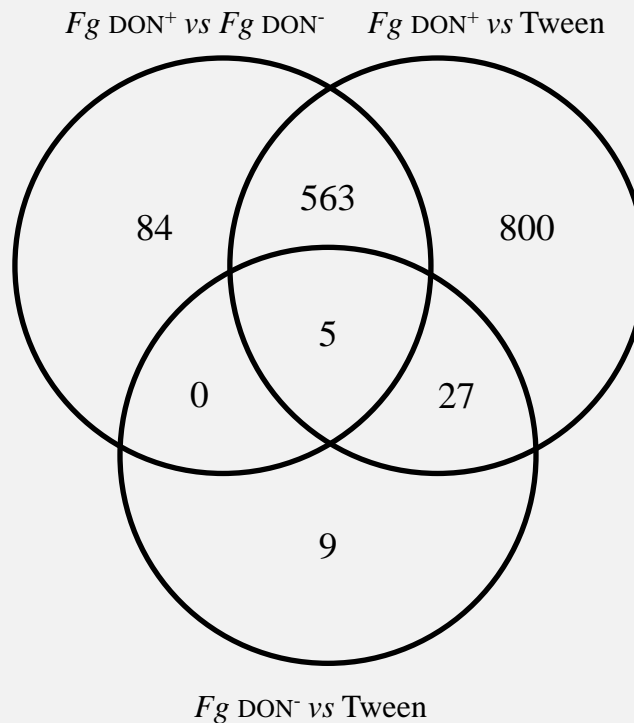
Bars: 100  $\mu$ m

# Transcriptomic analysis of the plant response to *Fusarium graminearum*

## Up-regulated



## Down-regulated



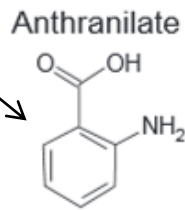
$p\text{-values} \leq 0.05$

# Involvement of the tryptophan pathway in the *Bd* / *Fg* interaction

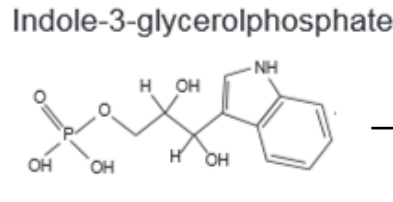
Shikimate



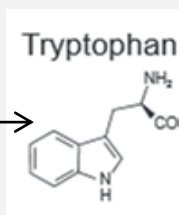
Chorismate



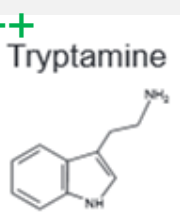
++/++  
IPGS,...



Trp synthase

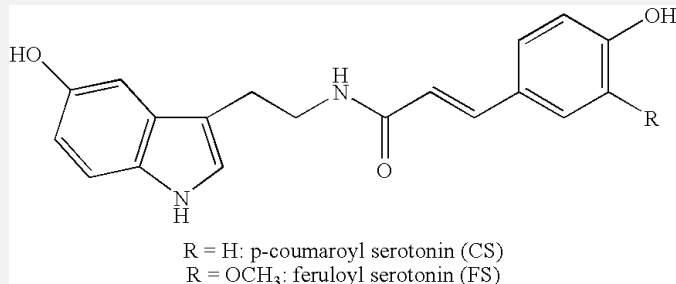


++++/+++  
TDC



++/++

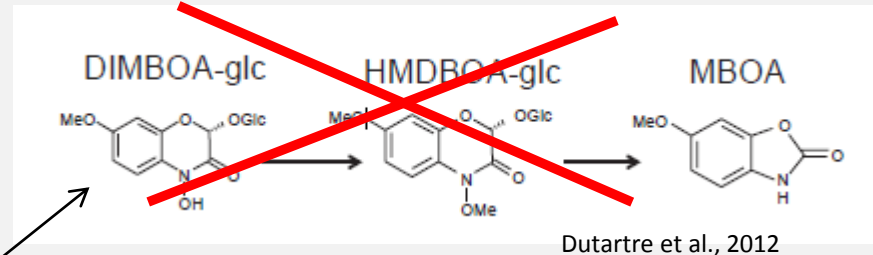
Tryptamine 5-hydroxylase  
(CYP71P1)



+  
Transferase(s)

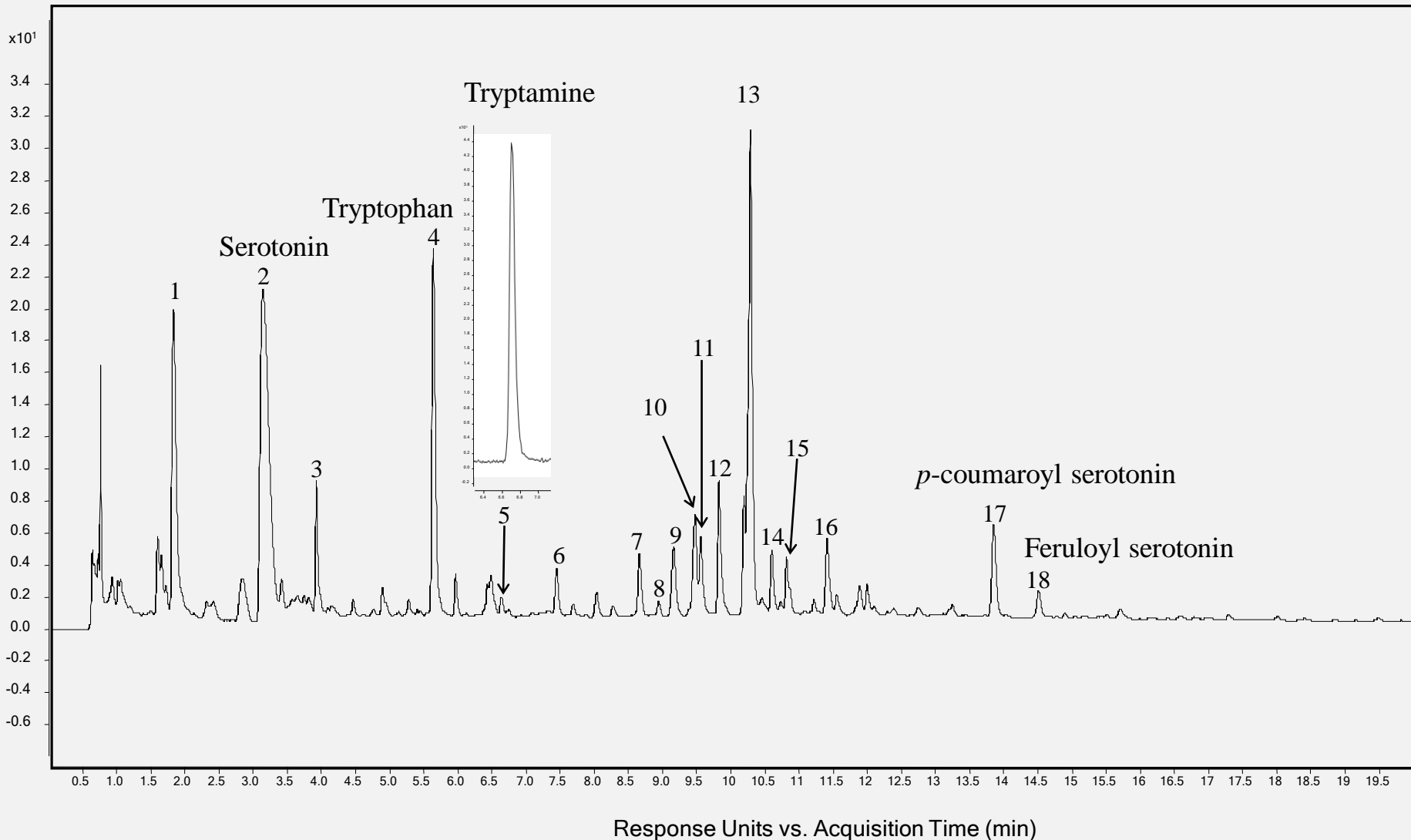


+: *FgDON*<sup>+</sup>  
+: *FgDON*<sup>-</sup>

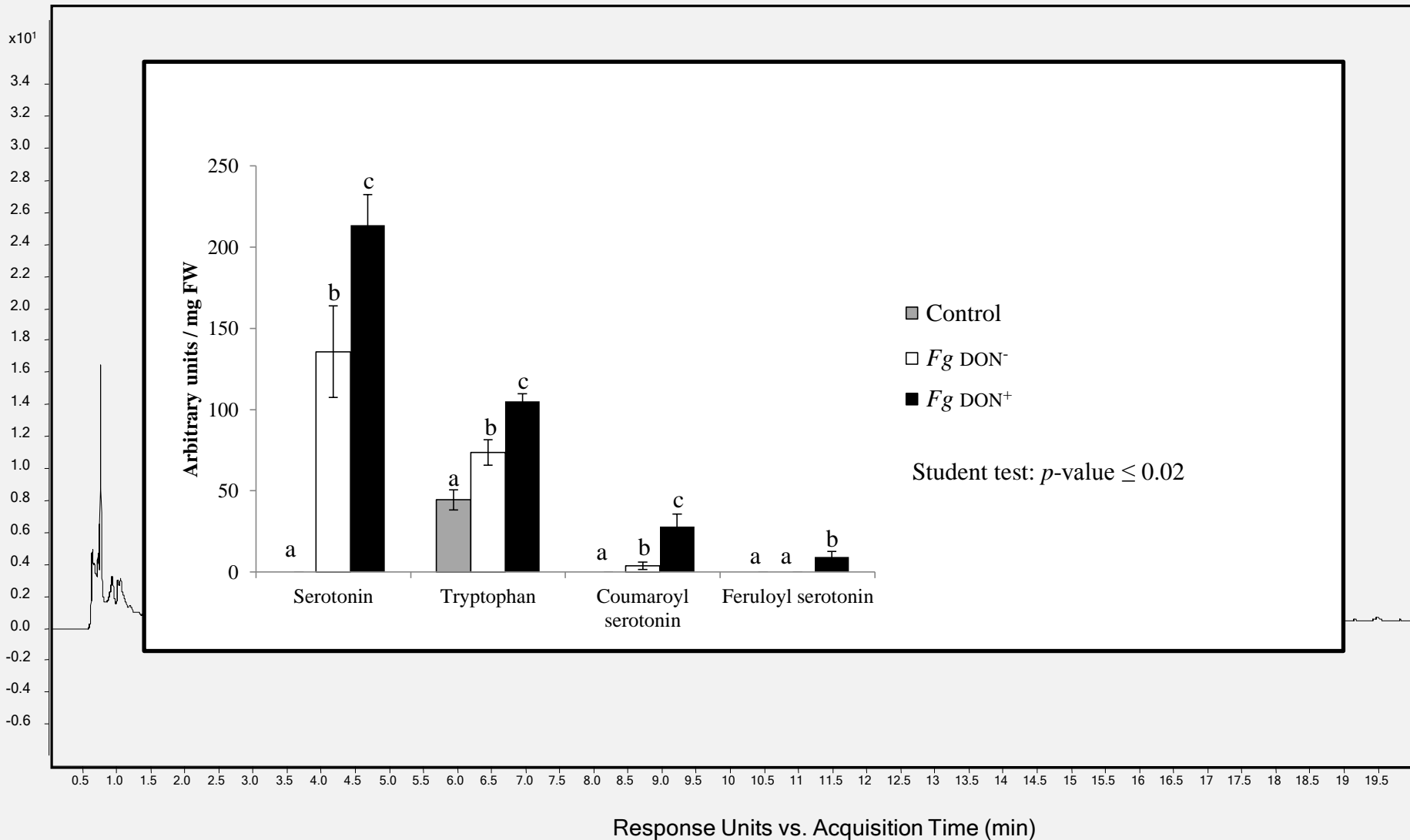




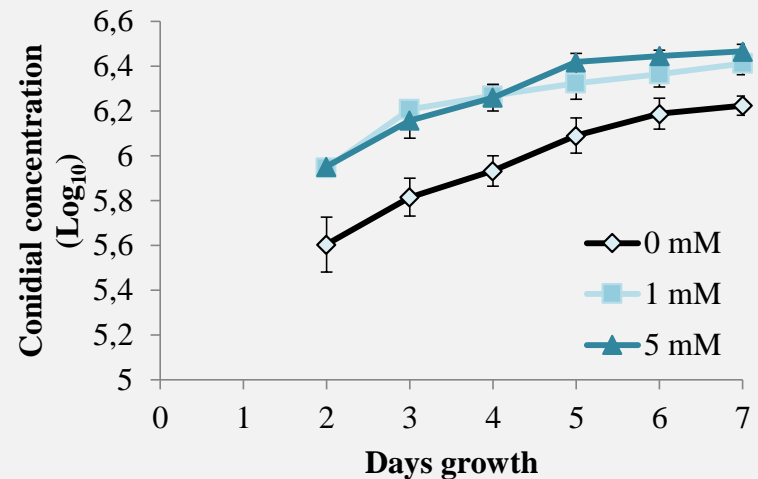
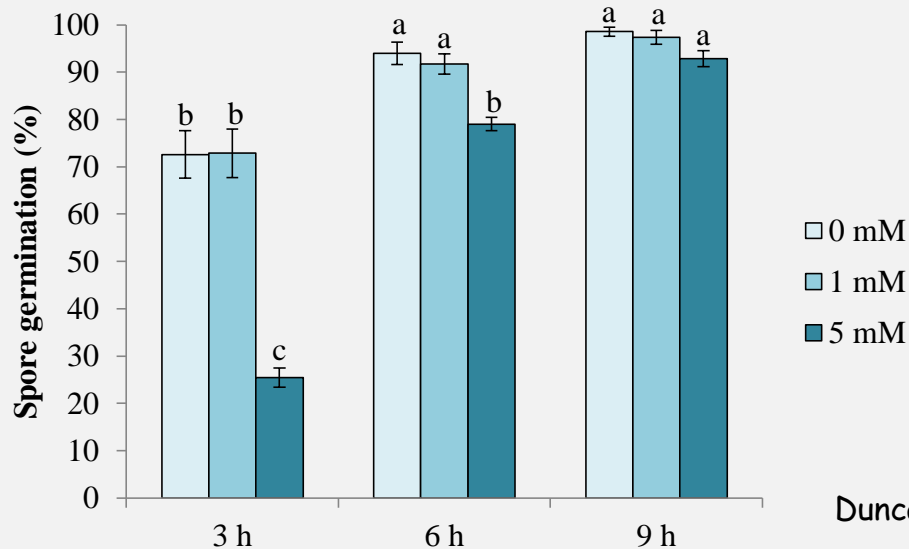
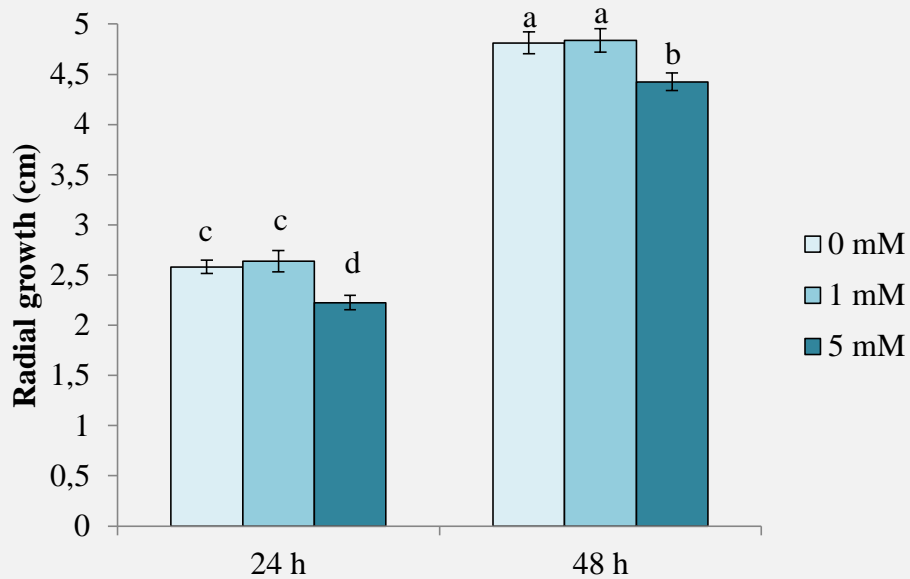
# Involvement of the tryptophan pathway in the *Bd* / *Fg* interaction



# Involvement of the tryptophan pathway in the *Bd* / *Fg* interaction

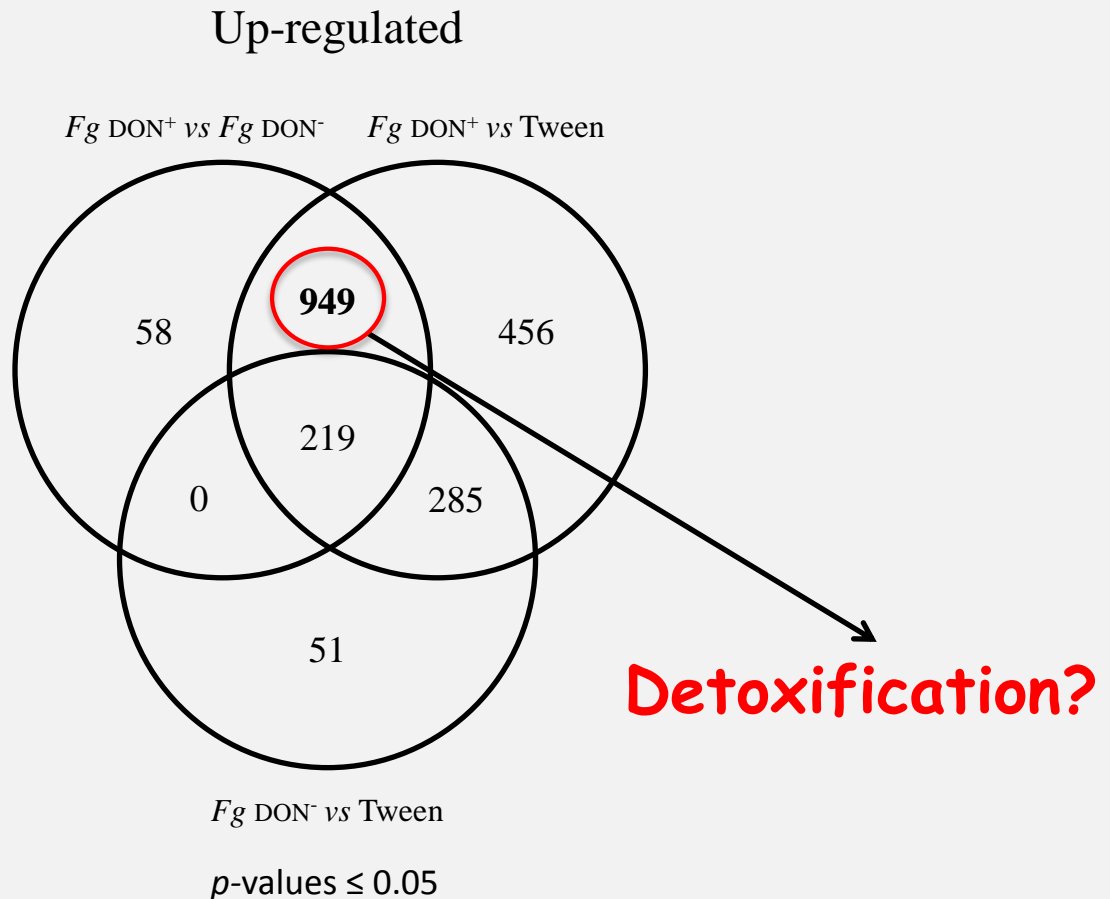


# *In vitro* effect of serotonin on *Fg*



Duncan test:  $p\text{-value} \leq 0.01$

# Transcriptomic analysis of the plant response to *Fusarium graminearum*

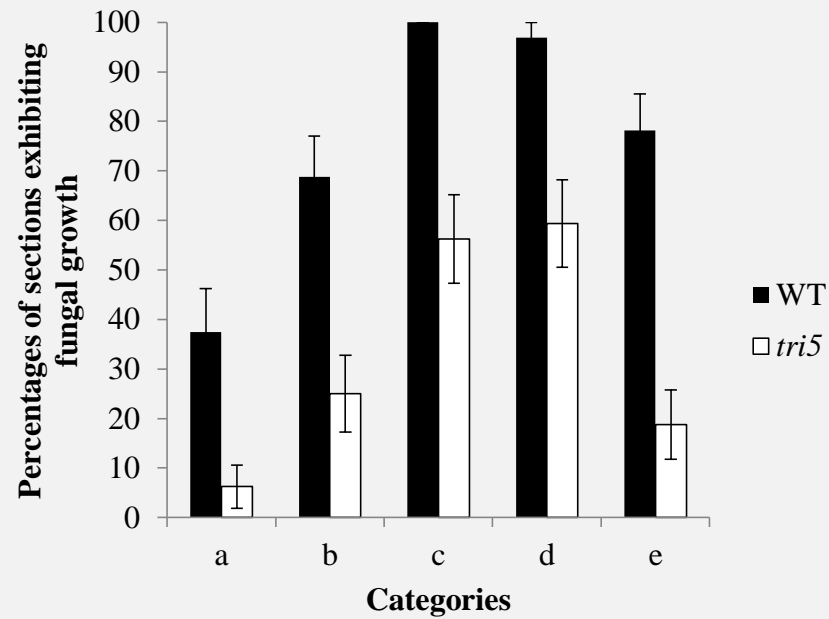




# Genes most highly induced in *FgDON*<sup>+</sup> vs *FgDON*<sup>-</sup>

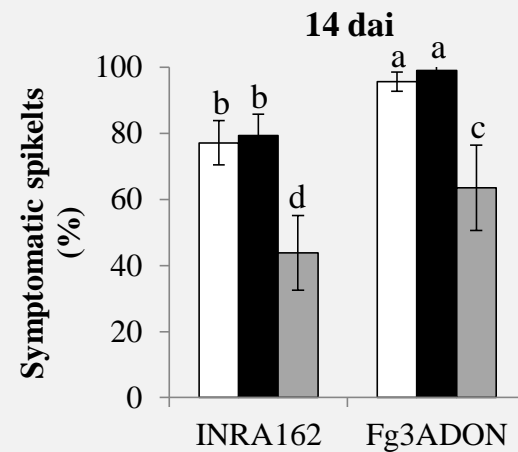
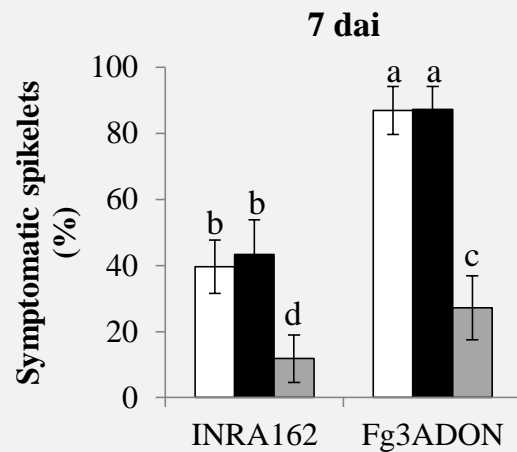
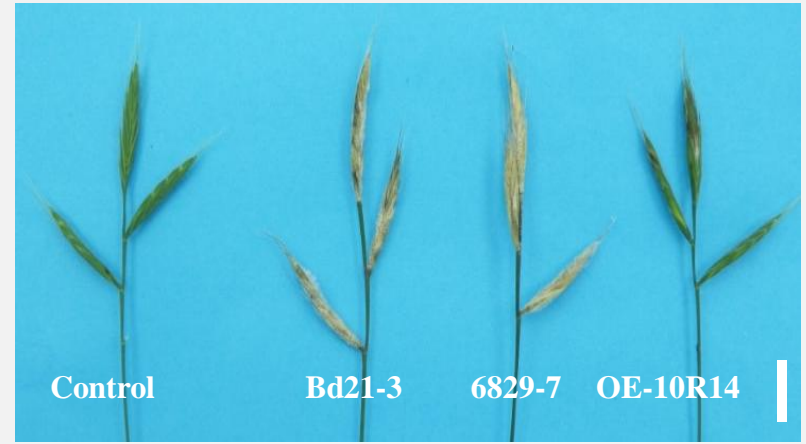
		Induction (Log <sub>2</sub> )	Protein function
Detoxification	Bd3g06800	7,68	unknown
	Bd3g19390	7,67	60S acidic ribosomal protein P0-like
	Bd2g25050	7,20	ethylene-responsive transcription factor ERF019-like
	Bd2g48520	7,19	receptor-like protein kinase
	Bd5g03320	7,12	UGT No Nter domain
	Bd1g10230	7,10	maf-like protein
	Bd2g18560	7,08	unknown
	Bd2g60980	7,02	unknown
	Bd2g41000	6,98	unknown
	Bd2g48530	6,90	unknown
	Bd1g77490	6,82	OsFBX427 - F-box domain containing protein
	Bd3g18330	6,76	Unknown
	Bd2g17800	6,68	mitogen-activated protein kinase kinase kinase 2-like
	Bd5g03300	6,59	UGT
	Bd2g17310	6,49	unknown
	Bd1g17640	6,46	unknown
	Bd1g53240	6,39	60S acidic ribosomal protein P2B-like
	Bd4g35650	6,35	dehydration-responsive element-binding protein 1A-like
	Bd2g26820	6,32	unknown
	Bd2g17820	6,17	mitogen-activated protein kinase kinase kinase A-like
	Bd1g75310	6,16	Cytochrome P450 monooxygenase
	Bd3g49010	6,13	auxin-responsive family protein
	Bd2g47510	6,10	mitogen-activated protein kinase kinase kinase A-like
	Bd5g22610	6,08	protein translation factor SUI1 homolog 2-like
	Bd3g22880	6,08	MATE efflux family protein 5-like

# Characterization of DON<sup>+</sup> and DON<sup>-</sup> strains on *Brachypodium distachyon*



Data representing 30 infected spikes per strain

# The Bd5g03300 UGT is also involved in resistance to *F. graminearum* NIV and 3 ADON strains



□ Bd21-3 ■ 6829-7 ▒ OE-10R14