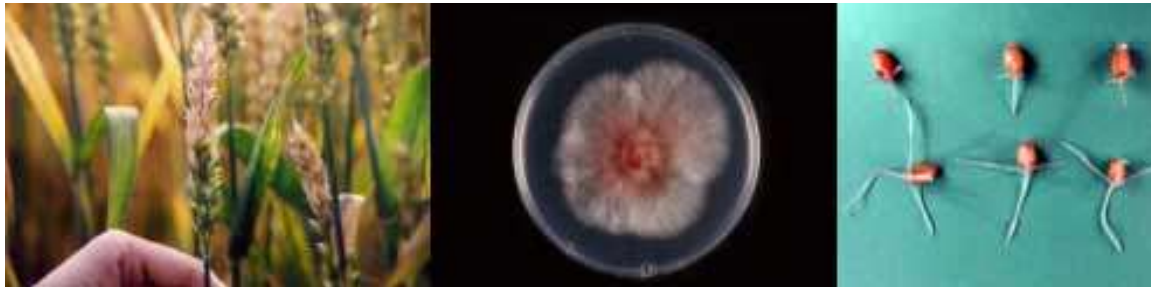# Candidate genes for wheat resistance to head blight and deoxynivalenol

Fiona Doohan

Outline

- Aproaches taken
  - Biocontrol
  - Toxin resistance

  } Candidate genes for FHB/DON resistance

- Conclusions & future work

A series of questions….

BIOCONTROL

- Could we identify agents that control DON and disease?

- Can we determine how they work?

Could we identify biocontrol agents that control DON and disease?

- YES:

  - E.g. *Pseudomonas fluorescens strain MKB158* – controls both seedling blight and FHB
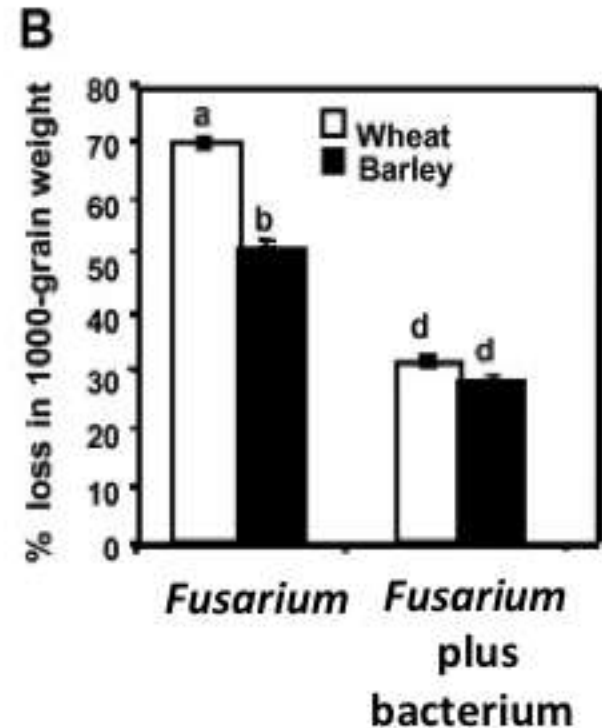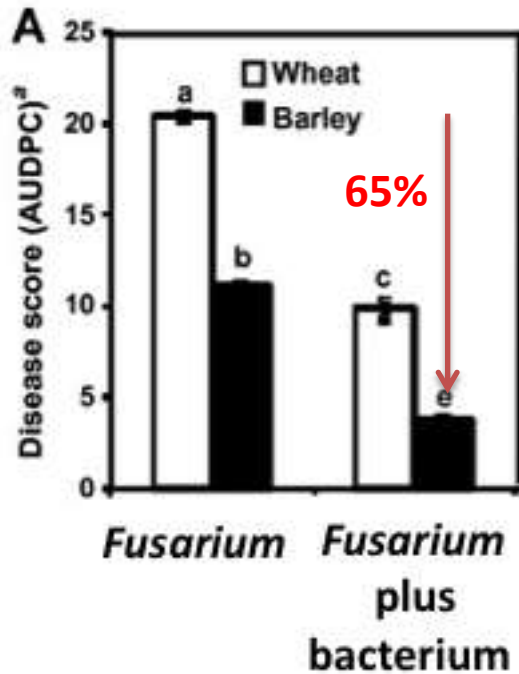

Fusarium
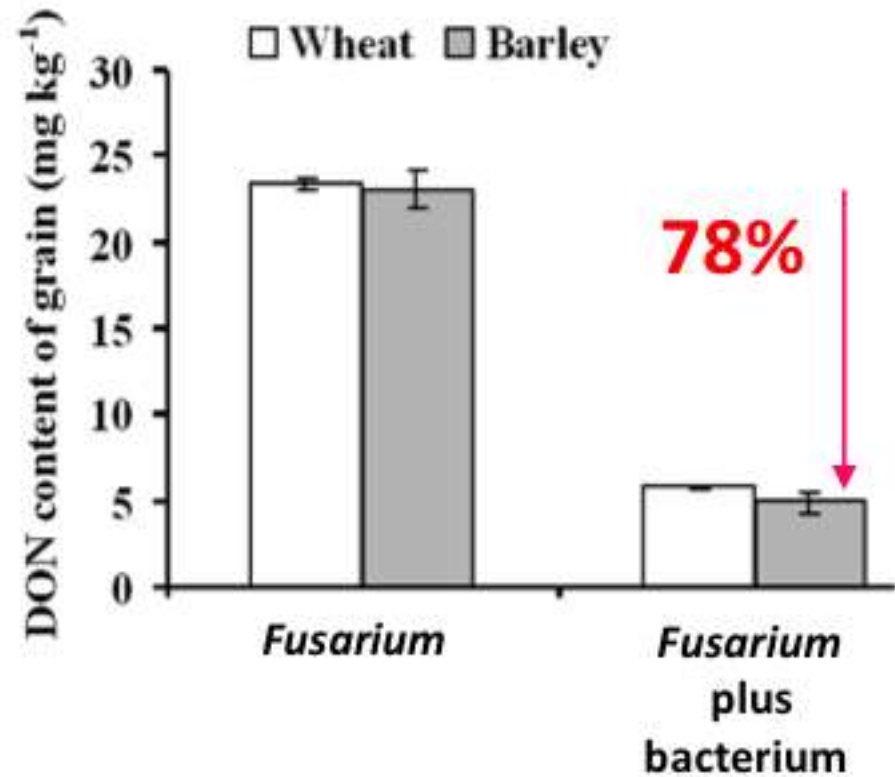

Fusarium **plus bacterium**

Bacterium reduces disease

FIELD

- *P. fluorescens* strain MKB158 applied to heads at flowering, 2 days pre-inoculation with *Fusarium culmorum*
- Disease assessed periodically - AUDPC

Bacterium reduces DON levels in grain

How does it work?

Barley

- Stem base studies- induces systemic resistance *Fusarium*

- Microarray analysis – to identify genes involved in the
  - systemic responses (seedling studies)
  - local responses (head blight studies)

Discriminated primed genes e.g. Serpin gene is primed..

Lipid transfer proteins and protease inhibitors as key factors in the priming of barley responses to Fusarium head blight disease by a biocontrol strain of *Pseudomonas fluorescens*

Carloalberto Petti · Mojibur Khan · Fiona Doohan

Abstract Strains of non-pathogenic pseudomonad bacteria, can elicit host defence responses against pathogenic microorganisms. *Pseudomonas fluorescens* strain MKB158 can protect cereals from pathogenesis by *Fusarium* fungi, including Fusarium head blight which is an economically important disease due to its association with both yield loss and mycotoxin contamination of grain. Using the 22 K barley Affymetrix chip, transcriptome studies were undertaken. jasmonic acid plays a role in pseudomonad priming of host responses. Additionally, the transcriptome studies described herein also reveal new insights into bacterium-mediated priming of host defences against necrotrophs, including the positive effects on grain filling, lignin deposition, oxidative stress responses, and the inhibition of protease inhibitors and proteins that play a key role in programmed cell death.

Postulated that brassinosteroids play a role in FHB resistance
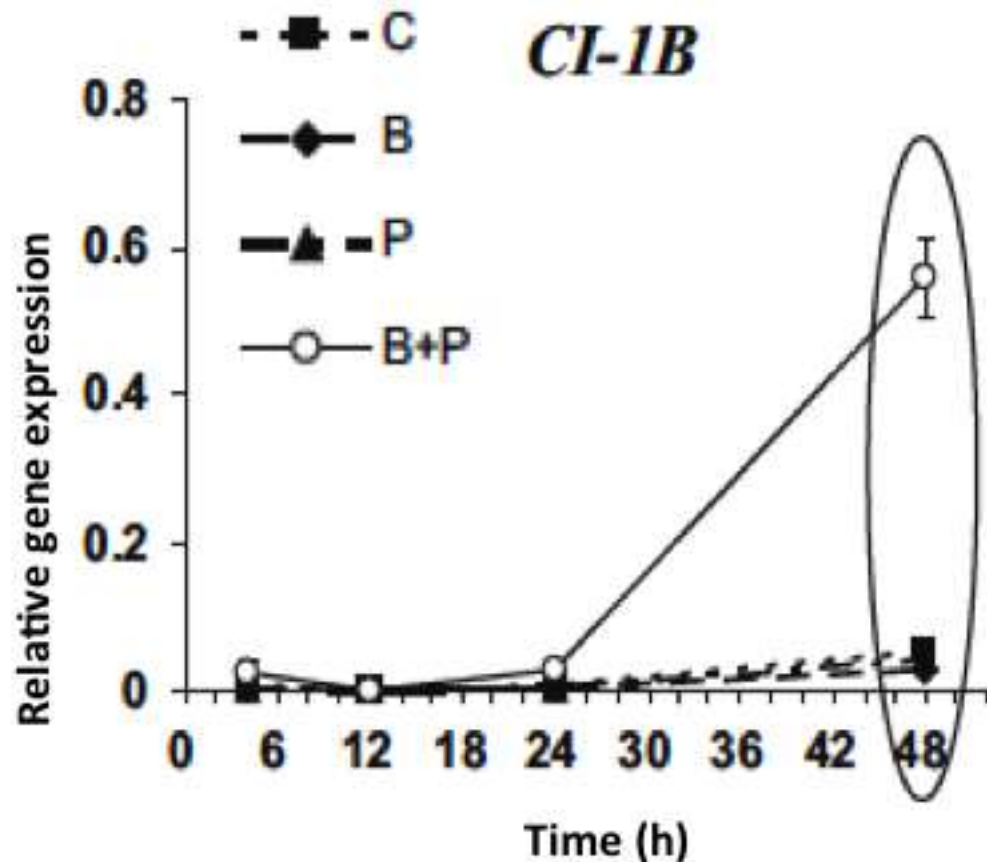
Mutation of a brassinosteroid receptor in barley enhanced FHB resistance....
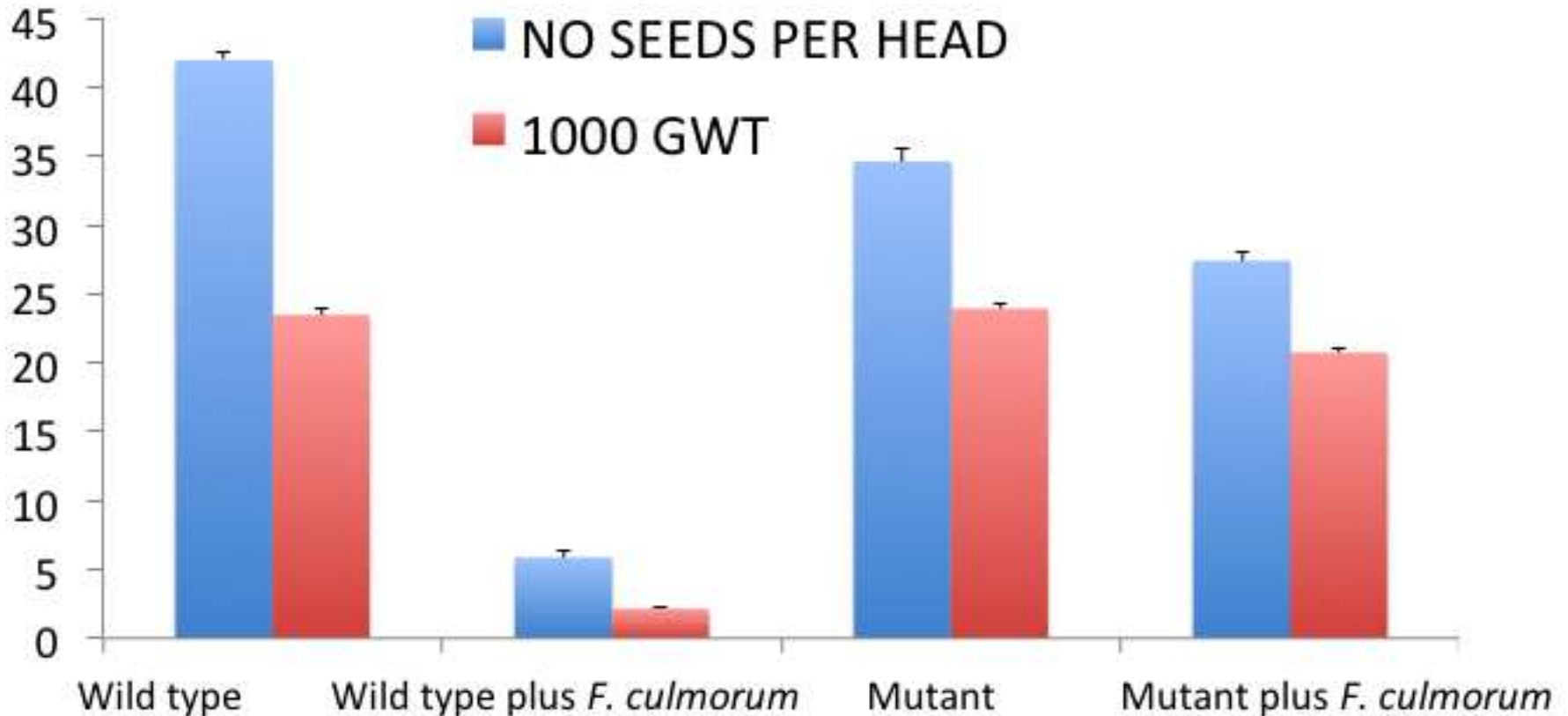
Conclusions from biocontrol work

- Identified bacteria that control disease

- Showed that they activate systemic resistance

- Identified genes activated by the bacterium

- Priming – lower penalty

- Important early players in priming
  - Membrane stability
  - Cell death responses
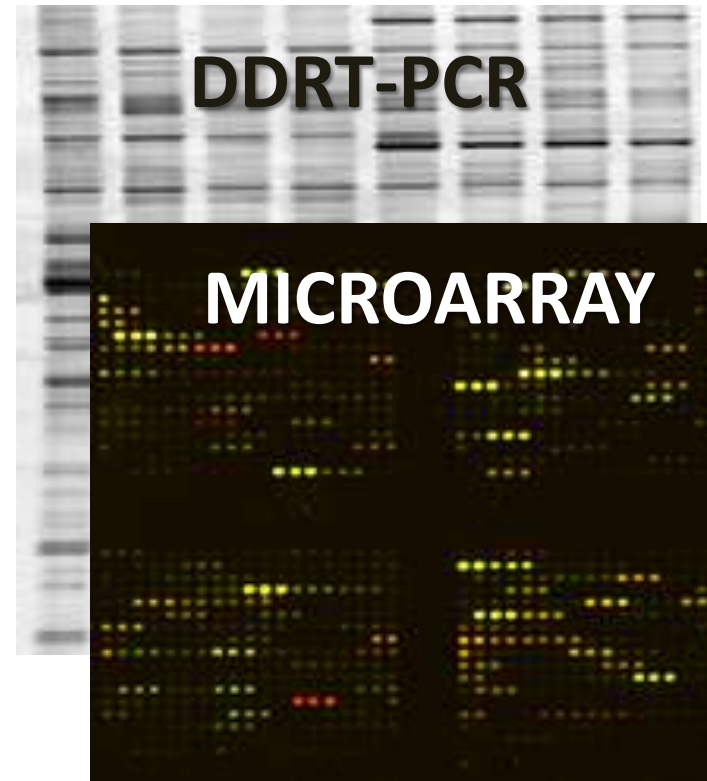
A series of questions….

HOST RESISTANCE

- Could we identify DON-responsive wheat genes?

- Could we discriminate those ASSOCIATED with DON resistance?

Identified genes based on:

Responsiveness to DON
- DDRT-PCR

Association with DON resistance

DDRT-PCR

MICROARRAY

Used a population derived from a CM82036 X Remus cross

CM82036
Resistant to DON and FHB

X

Remus
Susceptible to DON and FHB

→ **Parents**

+

double haploid progeny
(+ or − *Fhb1*)

AUDPC$_{DON}$

☐ Above treated spikelets
■ Below treated spikelets

16
14
12
10
8
6
4
2
0

CM 82036 | E2-101U | E1-97U | E2-2U | Remus | E1-78U | E2-50T | E1-70U

+ *Fhb1*   **− *Fhb1***

S — **QTL *Fhb1***
genome B chromosome 3
L

S — **genome B chromosome 3**
L

(Ansari et al., 2007 ; Buerstmayer et al., 2002 ; Lemmens et al., 2005)
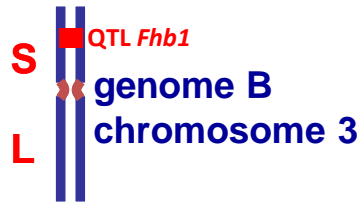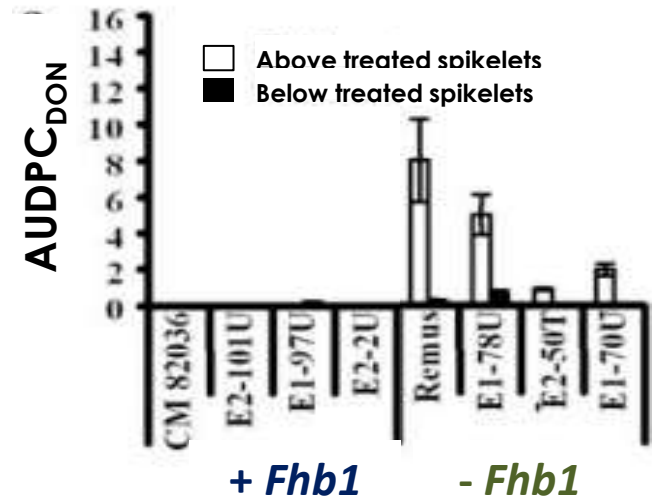
Genes of known Function

- Cytochrome P450s
- Glucosyl transferase
- Elongation factor 1-alpha (*EF-1a*)
- Glutathione S-transferase (*GST*)
- Histone
- Zinc-binding alcohol dehydrogenase (*ADH*)
- Alternative oxidase (*AOX*)
- UDP-glycosyltransferase (*UGT*)
- MRP-like ABC transporter (MRP)

Genes of unKnown function

QTL 3BS-related genes

Example II: Multidrug resistance-related protein (MRP) ABC transporter gene

Realtime RT-PCR from 4h DON (5mg/ml)-treated wheat heads:
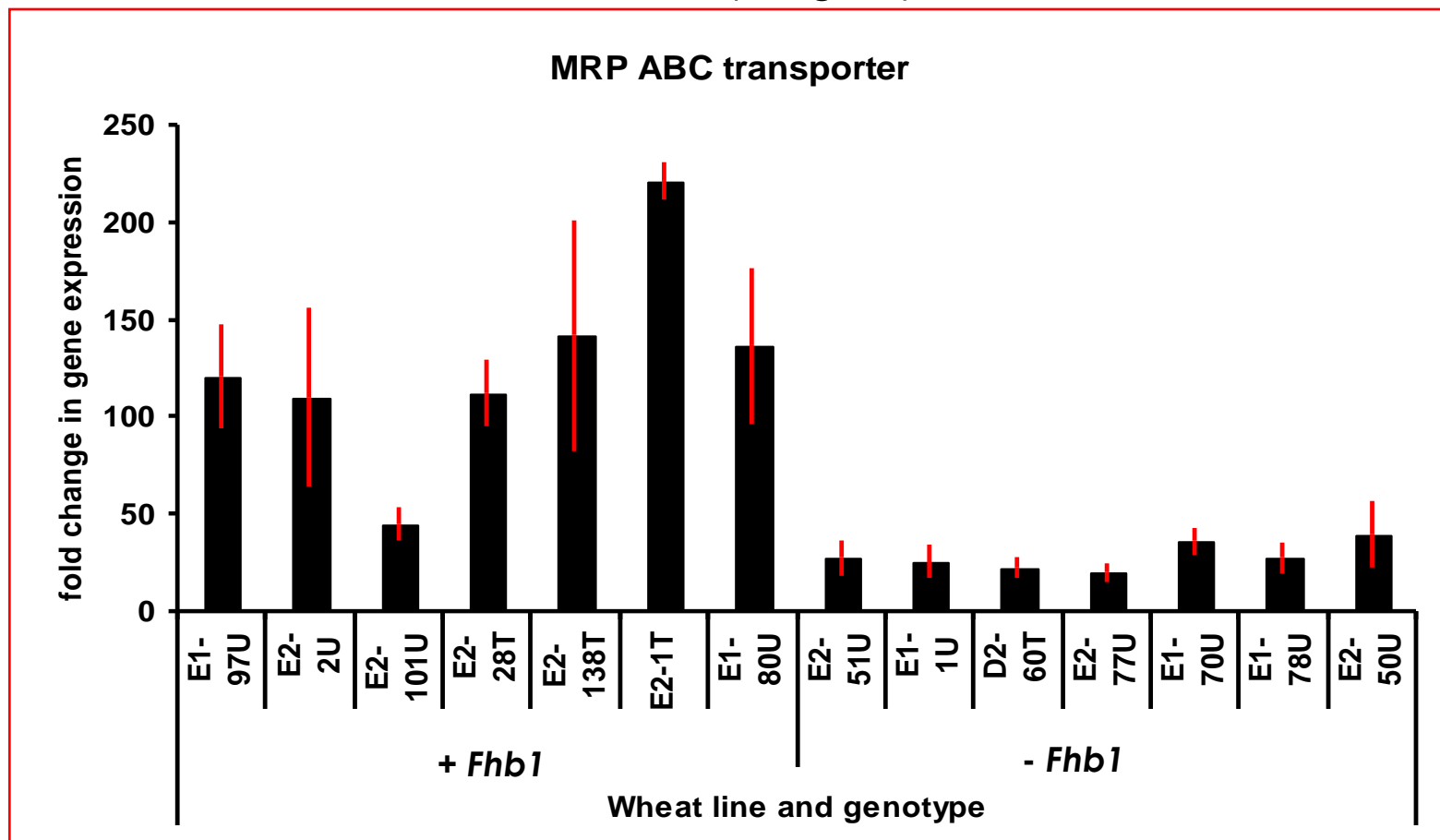
* Error bars indicate standard error of the mean, n=4

A series of questions….

BIOCONTROL

- Could we identify agents that control DON and disease? **YES**

- Can we identify the associated host genes – ISR **- YES**

HOST RESISTANCE

- Could we identify DON-responsive wheat genes?

- Could we discriminate those ASSOCIATED with DON resistance?

CANDIDATE GENES

- Where are we now?

Agenda 2011 - 2015

- Focus on specific genes

- Role in DON/FHB/stress resistance

- 6 targets, including novel genes
  - VIGS – confirm role in DON or FHB resistance
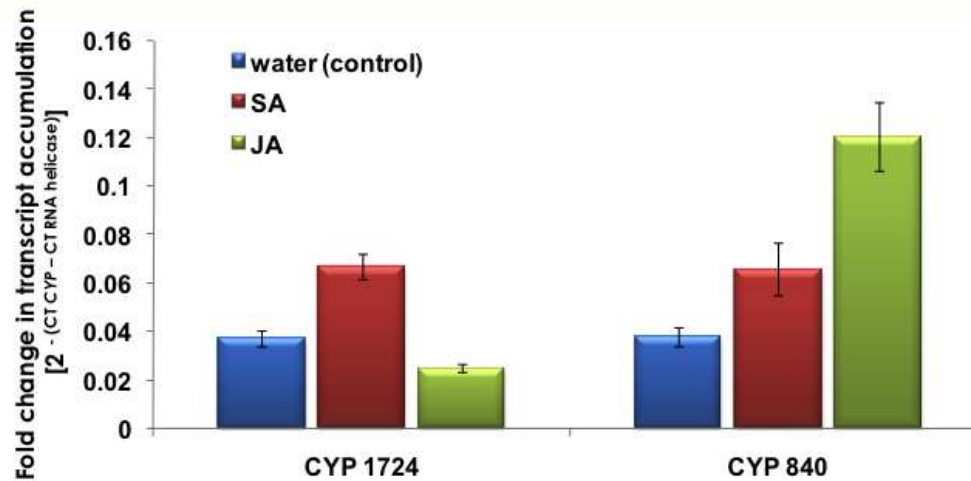 - Overexpression studies
  - Biochemical function

Silencing genes – Virus induced gene silencing
Phenotypic effect of specific genes

- Three of five genes tested enhanced phenotypic DON tolerance, as determined by VIGS
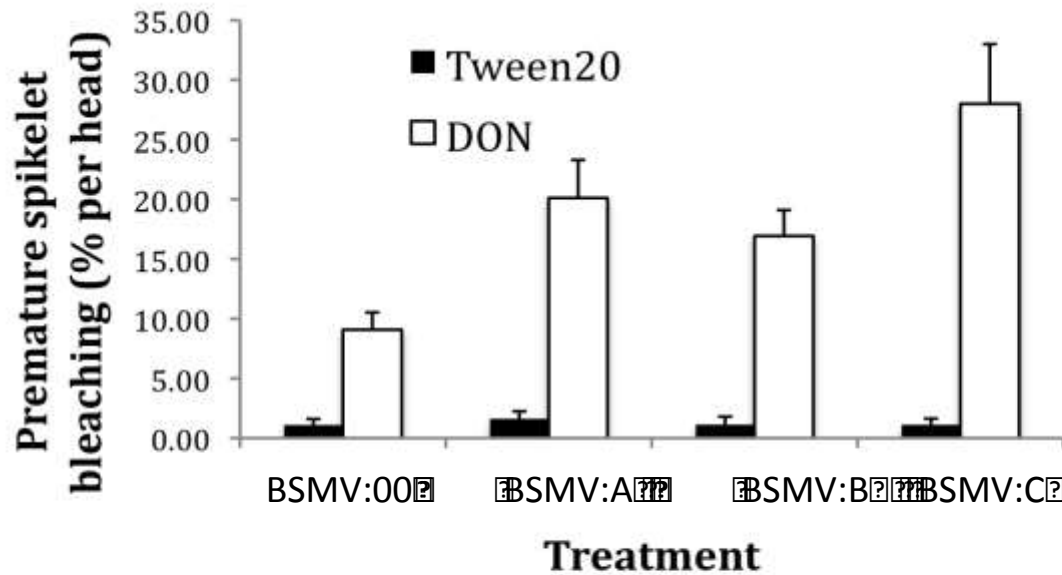
Several are JA-responsive

Fig. 2. Effect of hormones on the accumulation of *CYP* transcripts in roots of wheat cv. CM82036. Seeds of cv. CM82036 were germinated in dark for 48h at 24 ^{0}C and the seedlings were treated with 100 µM salicylic acid (SA) or 200 µM jasmonic acid (JA) or sterile water (control) for 4h in dark at 24 ^{0}C. Roots of treated seedlings were harvested at 4h post treatment, RNA extracted and used for real time RT-PCR analysis. Gene expression was quantified as the *CYP* transcript/RNA helicase transcript ratio. Bars indicate SEM.

Some are specific to DON-production...
# e.g. novel gene
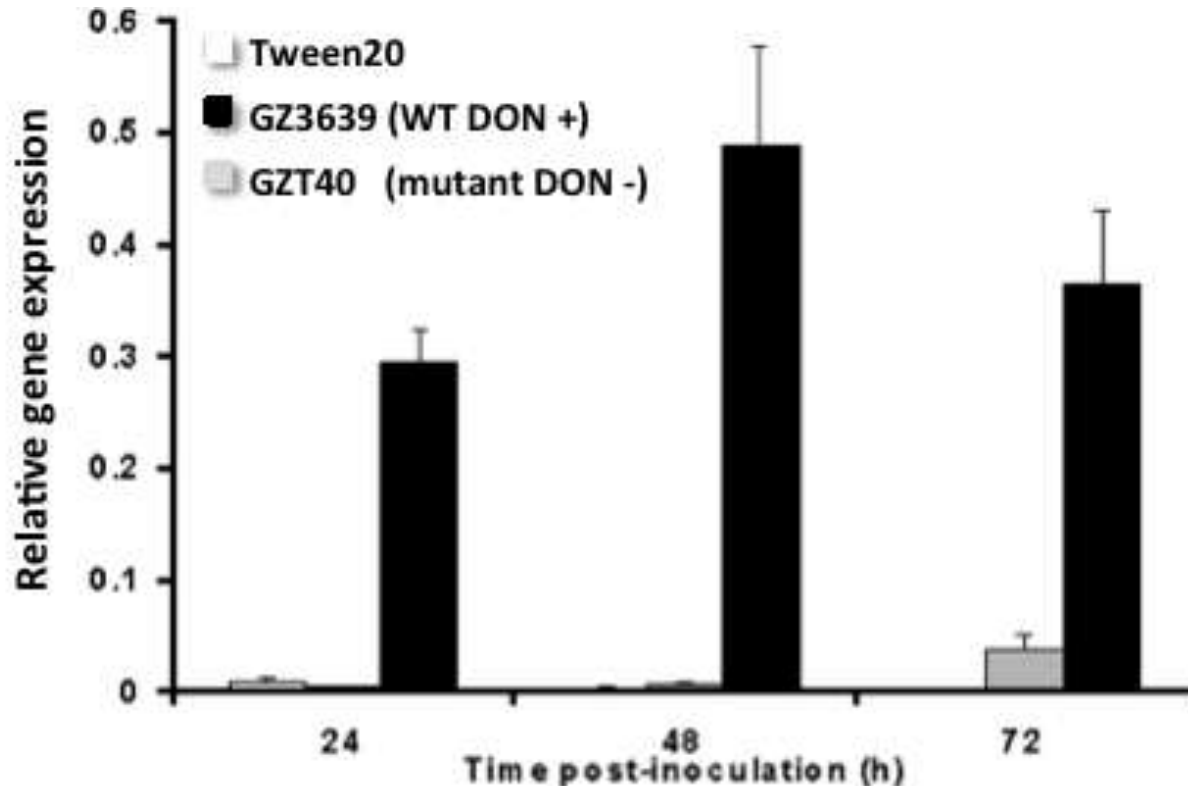
Transcript accumulation in wheat leaves in response to *F. graminearum* strain able or not to produce DON

The gene induction is associated with the DON-producing capacity of *F. graminearum*

One gene of interest is an orphan – evolutionary divergent

- **No significant homology to any characterised gene or protein**

- **Homologs (≥ 46 % identity) found only in the Pooideae subfamily : (Festuca, Triticum, Hordeum, Brachypodium), not found in other plants (Zea, Oryza)**
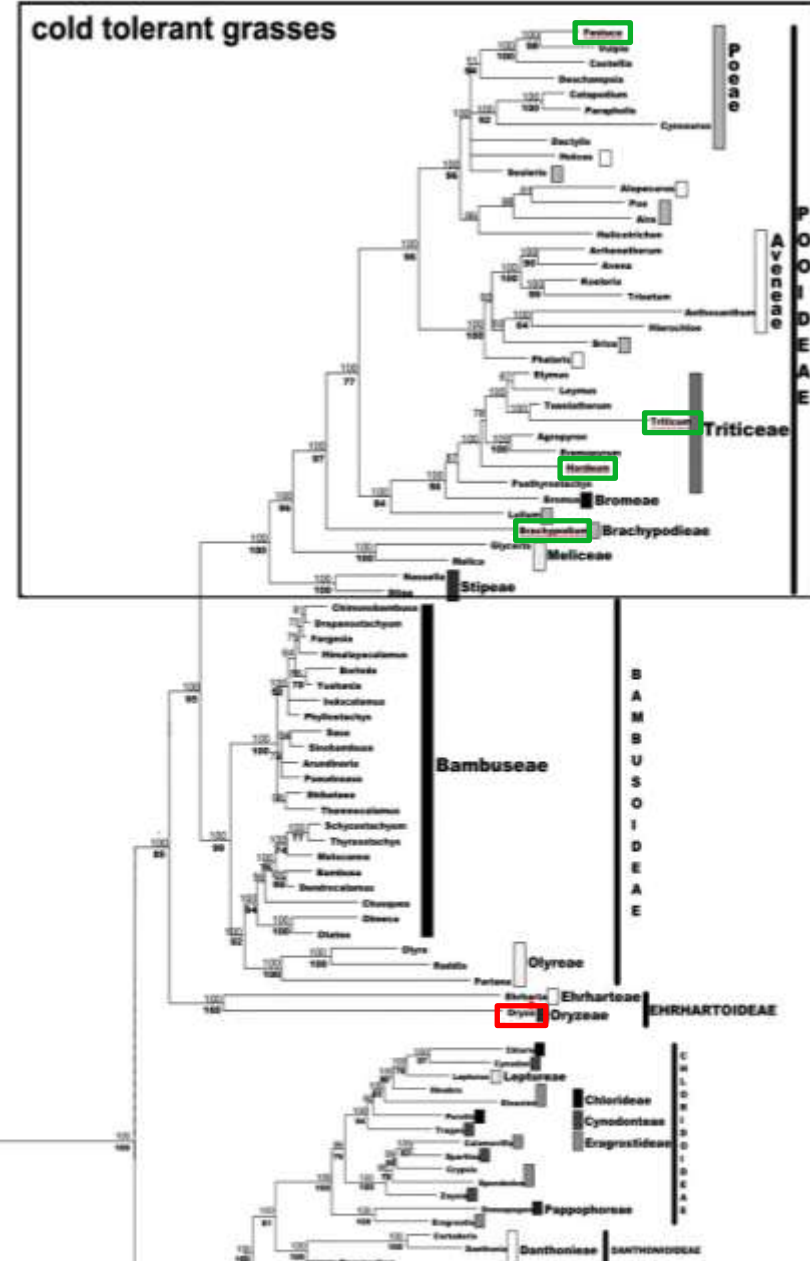
Conclusions..

- Found genes that are involved in host resistance to DON
  - Bacterium-primed
  - Resistance to DON
- Targets for breeding programmes?
  - Several affect DON/FHB resistance
- Mode of action
  - Insights, particularly via Y2H

Acknowledgements