Genetic and genomic approaches for managing Fusarium pathogens

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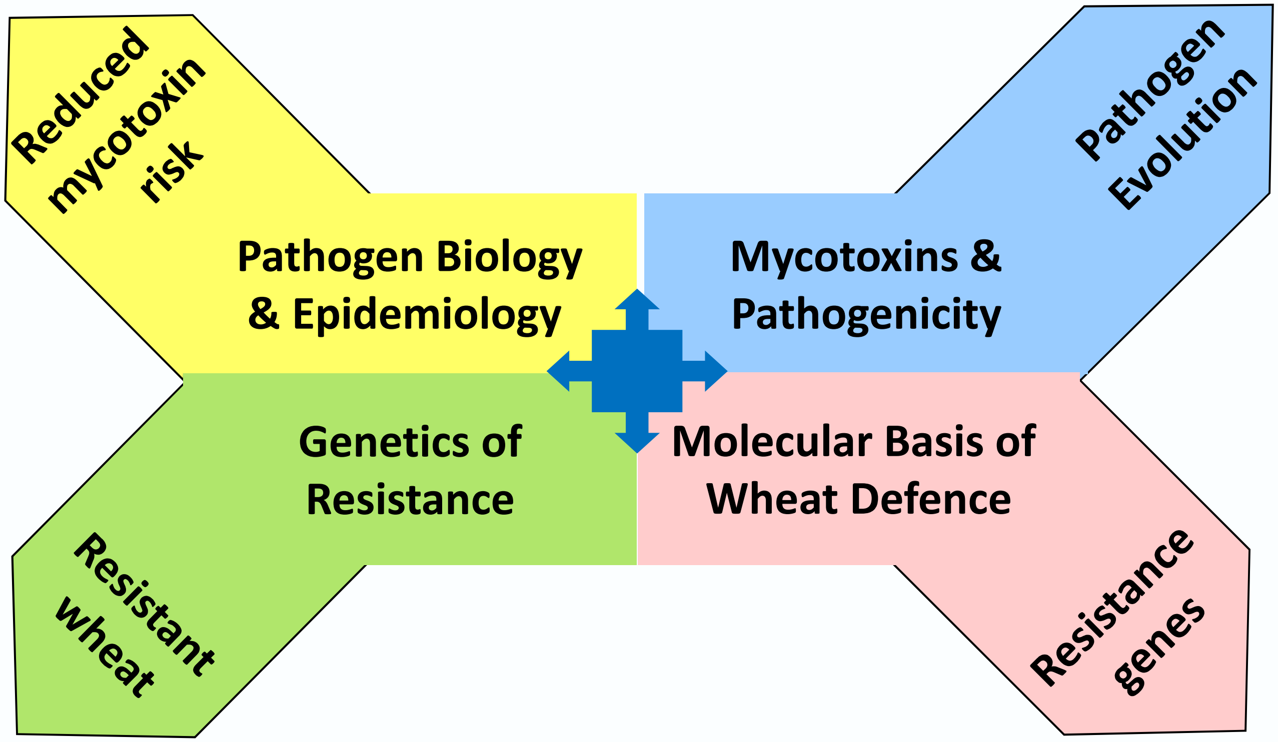
CSIRO PLANT INDUSTRY
www.csiro.au

Wheat - Fusarium Research in CSIRO



Reduced mycotoxin risk

Pathogen Biology & Epidemiology

Pathogen Evolution

Mycotoxins & Pathogenicity

Genetics of Resistance

Resistant wheat

Molecular Basis of Wheat Defence

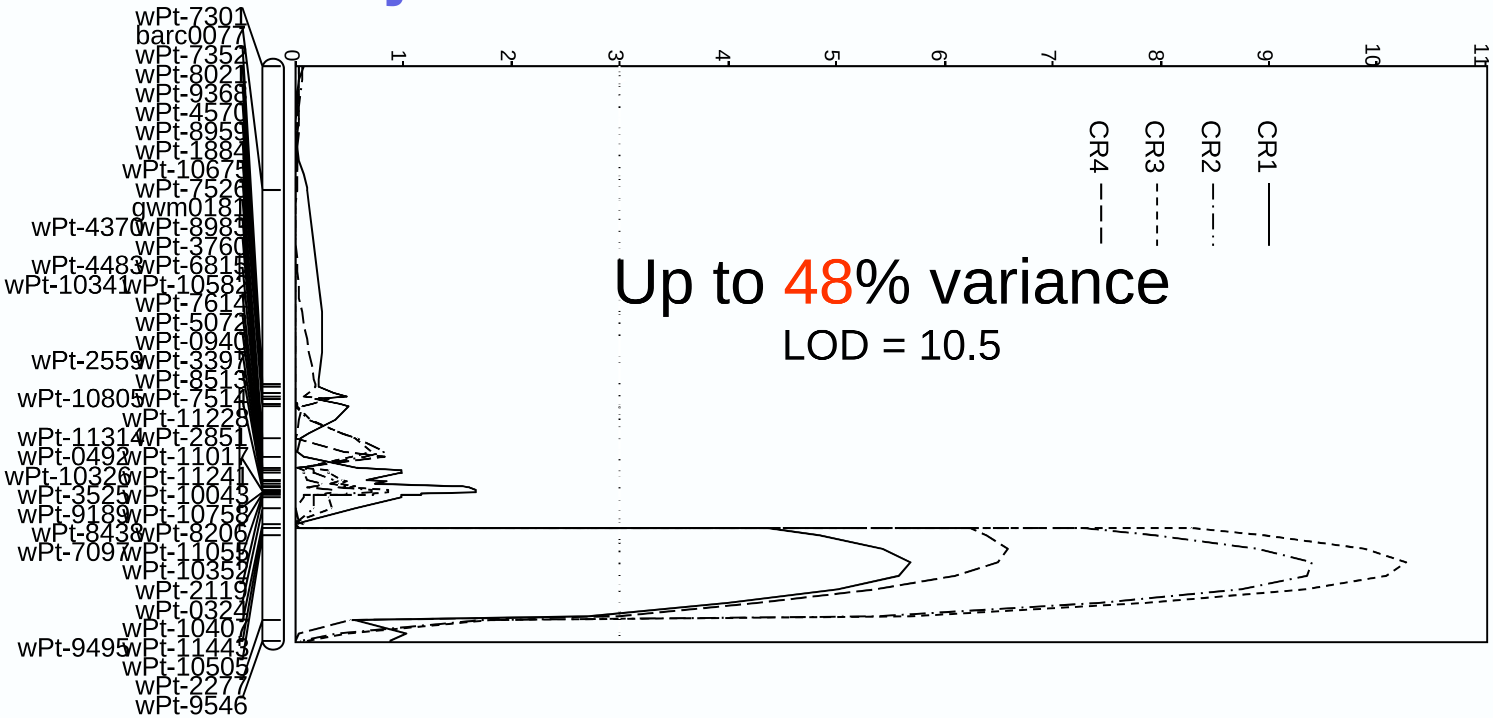
Resistance genes

Four inter-connected strands
to deliver industry and science outputs

Identify novel sources of resistance

Species	No of acc. screened	Species	No of acc. screened
T. aestivum	1301	*T. ovatum*	1
T. bicorne	2	*T. polonicum*	4
T. boeoticum	6	*T. pseudo-boeoticum*	1
T. carthlicum	4	*T. sinskajae*	1
T. comosum	2	*T. spelta*	29
T. compactum	7	*T. speltoides*	2
T. crassum	1	*T. sphaerococcum*	5
T. cylindicum	2	*T. thaoudar*	3
T. dicoccoides	376	*T. timopheevi*	7
T. dicoccum	4	*T. triunciale*	1
T. durum	161	*T. umbellulatum*	3
T. juvenale	1	*T. uniaristatum*	2
T. longissimum	2	*T. urartu*	3
T. macha	2	*T. vavilovii*	4
T. militinae	1	*T. ventricosum*	2
T. monococcum	4	*T. zhukovskyi*	3
unknown	563	*T.tauschii*	3
Total		**2,514**	

A major CR locus on 3BL in CR6



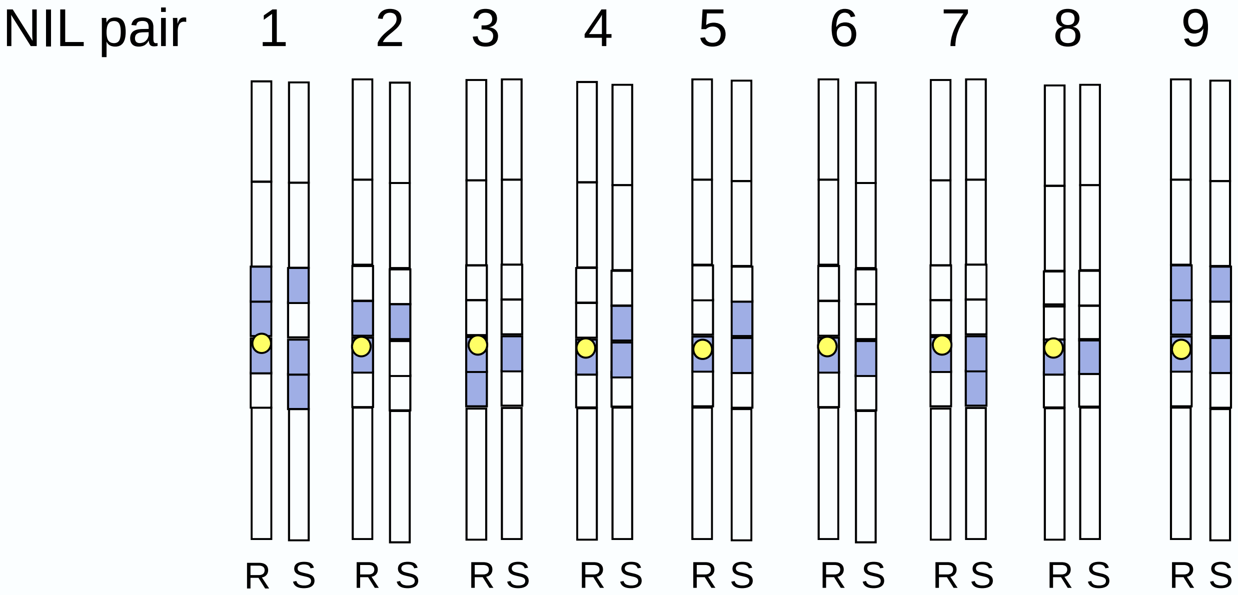
Up to 48% variance

LOD = 10.5

Population	RR	rr	Diff %
Janz/CR6	19.61	27.54	28. 8
Aus13832/CR6	19.77	28.86	31. 5
Janz*2/CR6	25.46	36.71	30. 6
Drysdale//Janz/CR6	21.48	37.11	42. 1

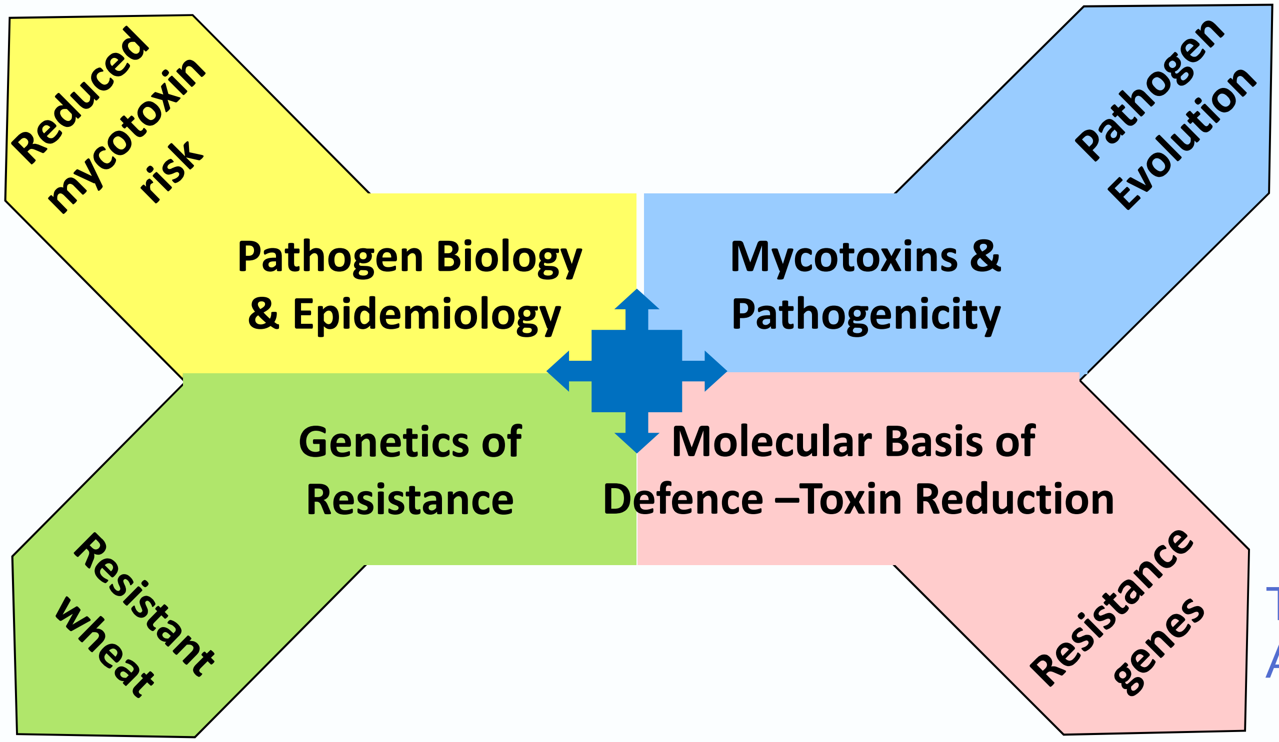
Ma J et al. (2010) Theor Appl Genet 120:1119

Develop markers & identify candidate R gene(s) based on genome sequencing of NILs



CSIRO/CAS project

Wheat - Fusarium Research in CSIRO



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Genetics of Resistance

Molecular Basis of Defence –Toxin Reduction

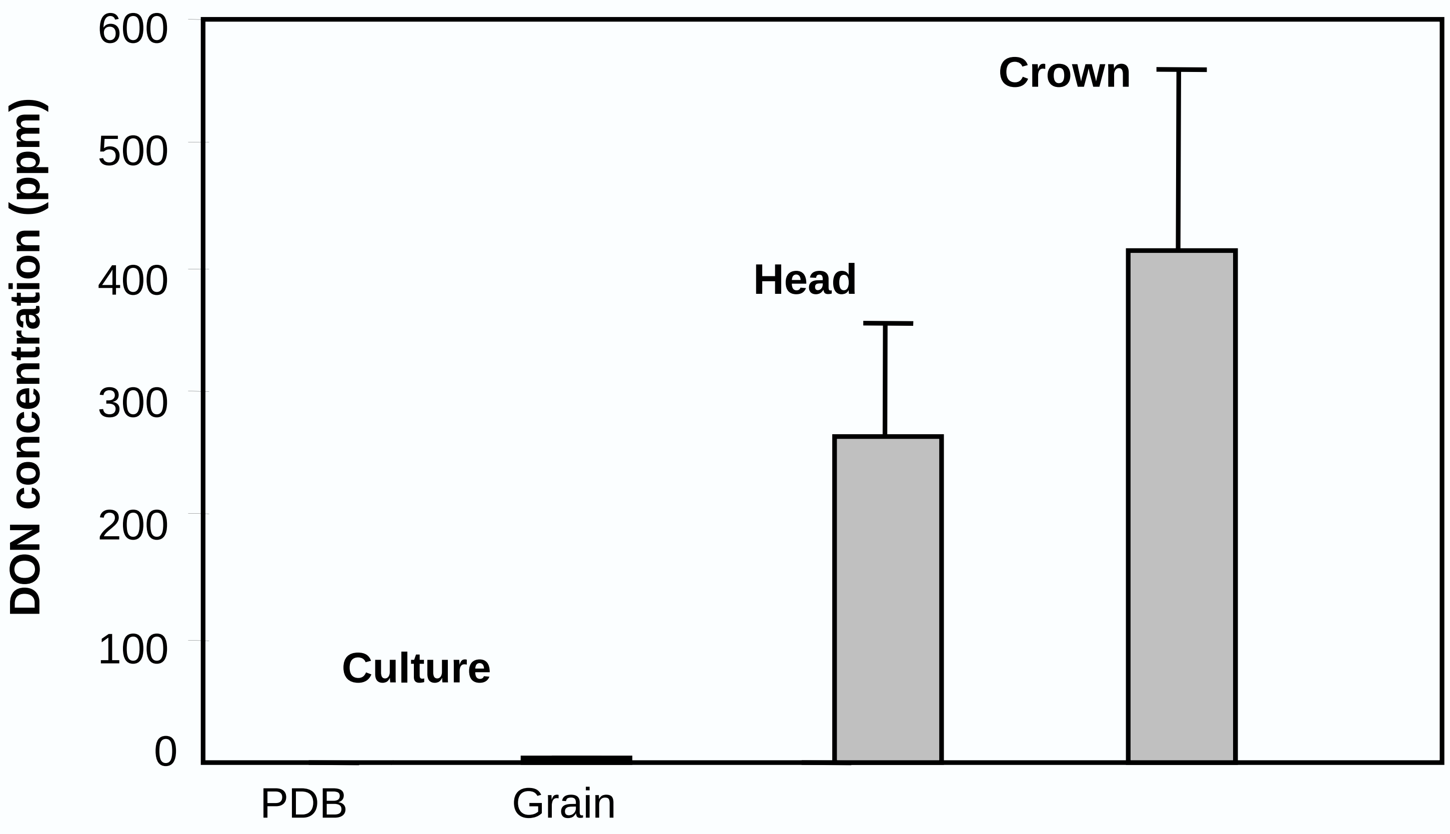
Resistant wheat

Resistance genes

Transgenic Approach

Non-transgenic Approach

What are host signals that activate DON production in *F. graminearum*?

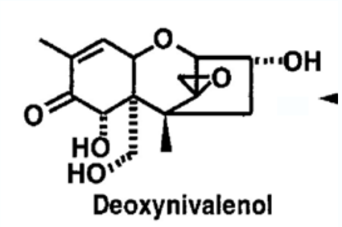


Mudge et al.(2006) Physiol. Molec. Plant Pathol. 69:73-85.

Can we identify host signals triggering DON biosynthesis in *F. graminearum?*

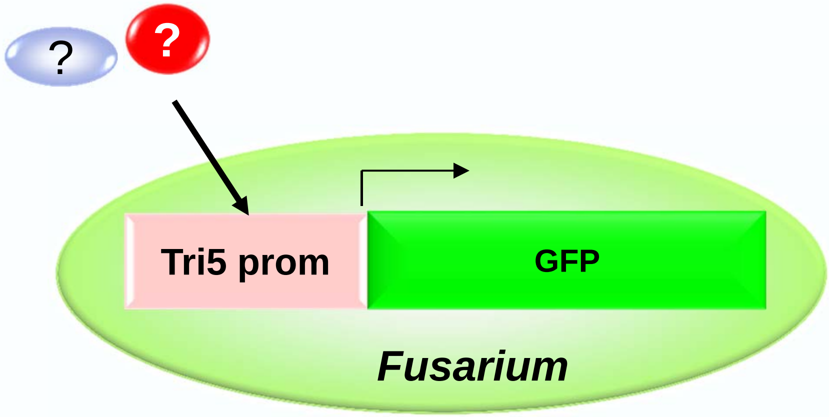
Trichodiene synthase (TRI5)

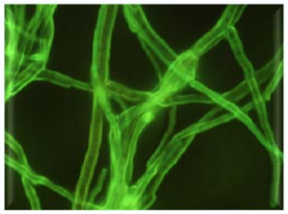
Farnesyl diphosphate → **Trichodiene** → **Trichothecene biosynthetic pathway**



Deoxynivalenol

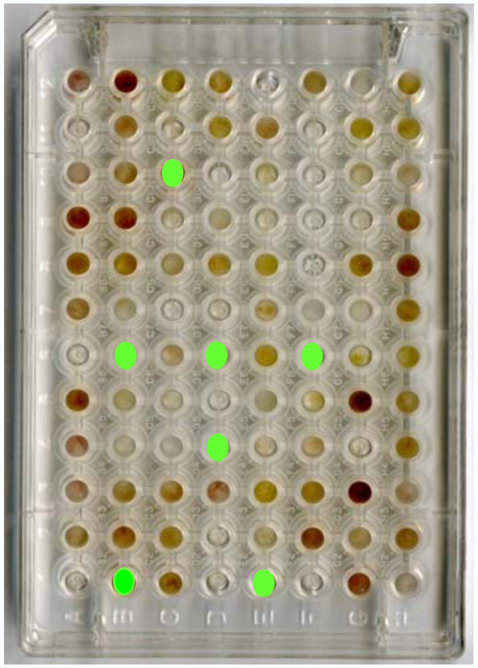
Signals for DON accumulation



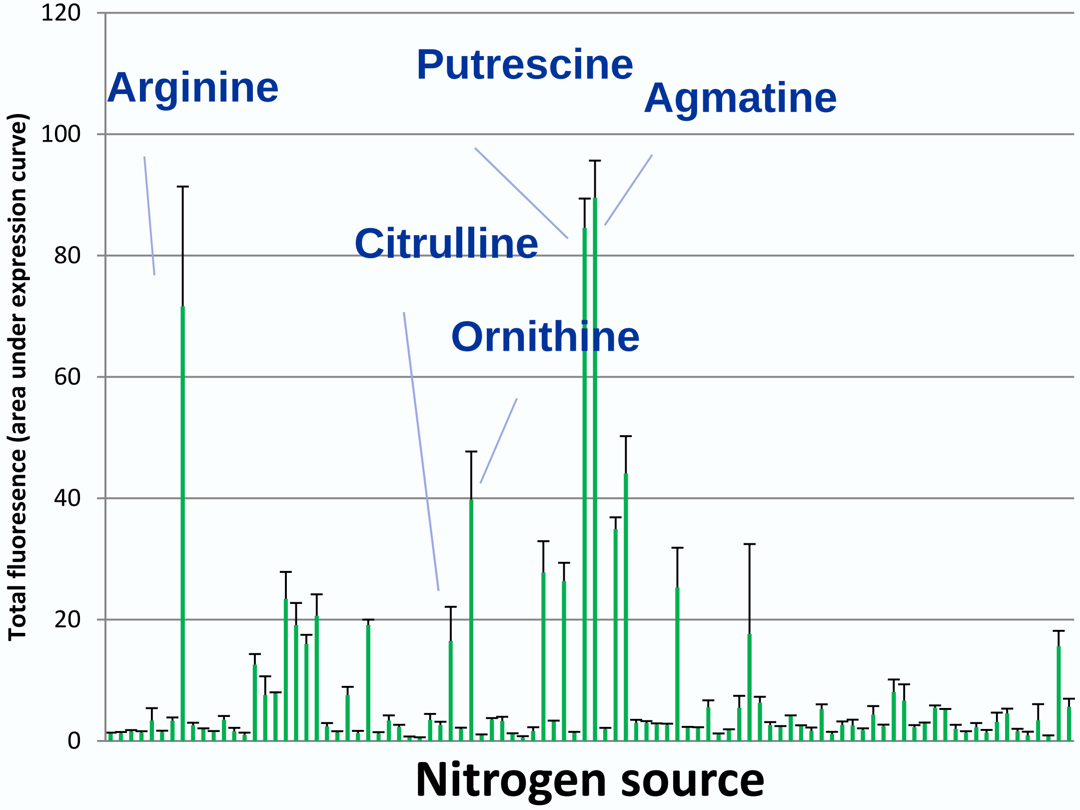


Hyphae express GFP when toxin is made

Polyamines activate DON biosynthesis in *F. graminearum*

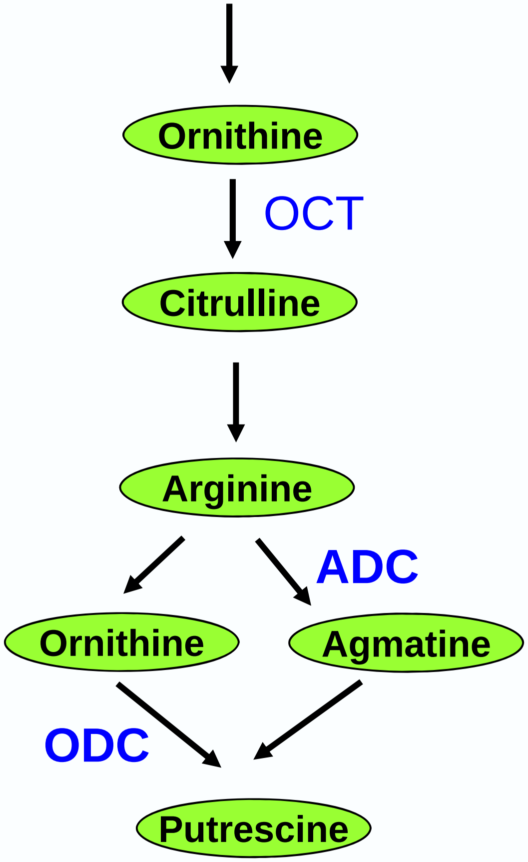


Grow *TRI5-GFP* under different N sources



Gardiner et al 2010; Kazan et al. 2011

The polyamine pathway is up-regulated in wheat during infection


Ornithine

OCT

Citrulline

Arginine

ADC

Ornithine

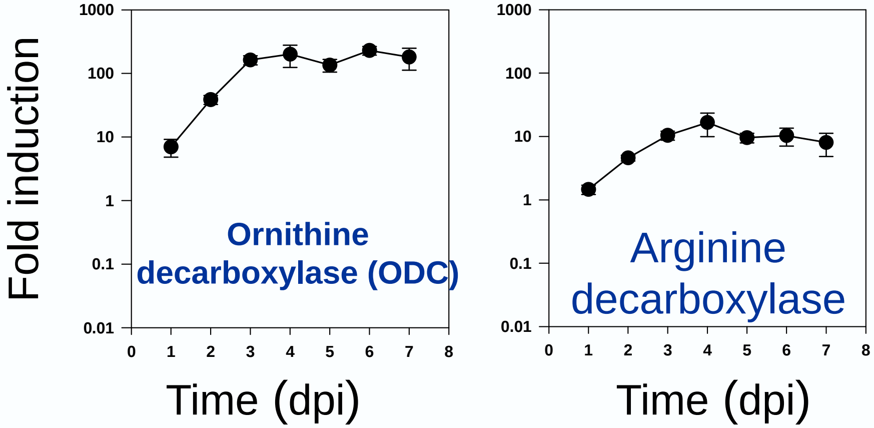
Agmatine

ODC

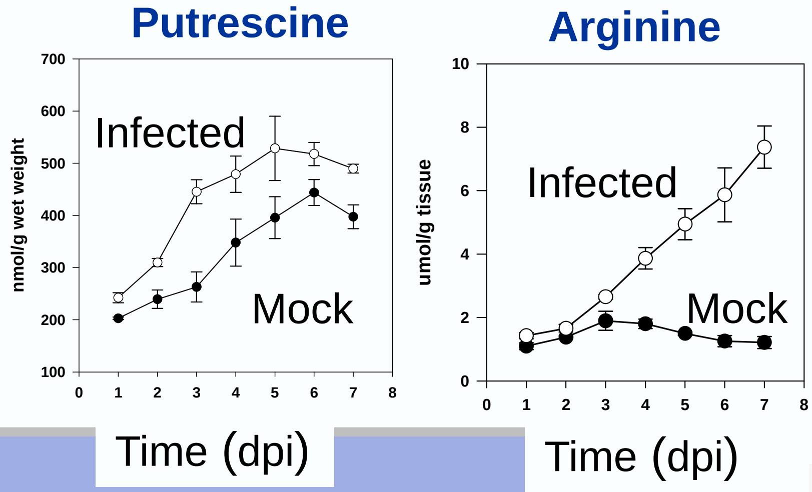
Putrescine

polyamine pathway

Polyamine biosynthesis genes are up-regulated during infection



polyamine levels are increased during infection

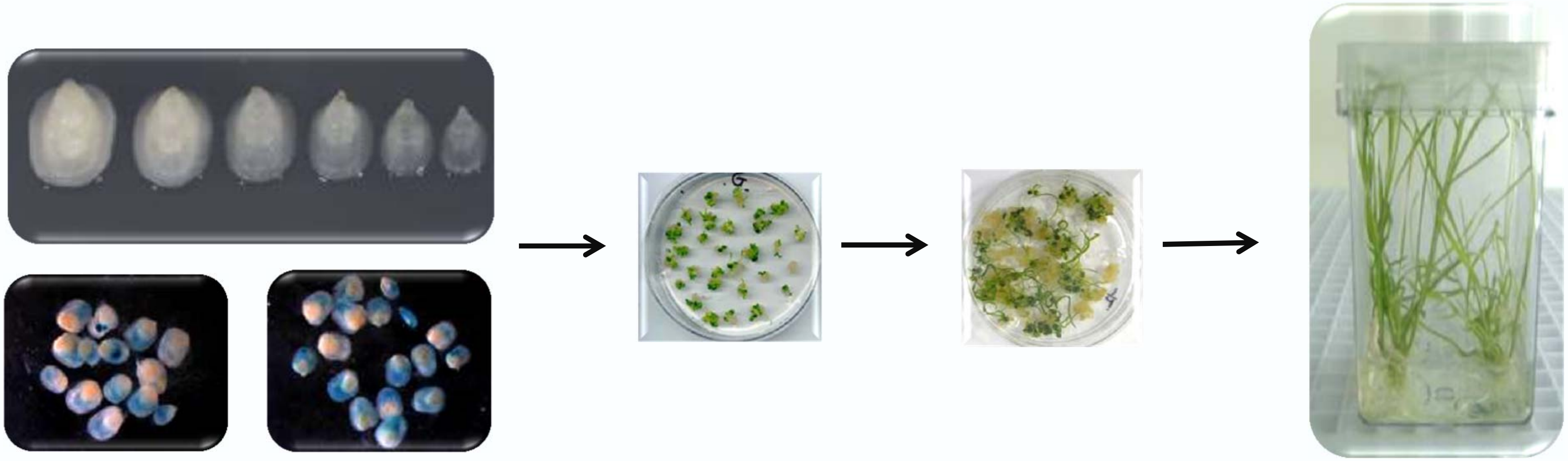


Gardiner et al 2010; Kazan et al. 2011

High-throughput wheat transformation

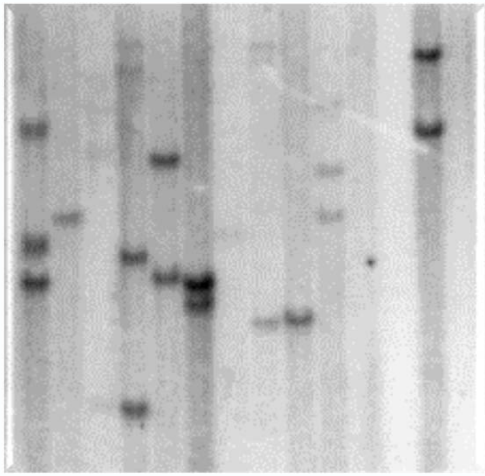
- *Agrobacterium*-based system using scutellum tissue as explant
  (commercially in confidence method licensed from Japan Tobacco).

- Works well with "Fielder" (40% eff. i.e. 40 plants from 100 embryos)
  Australian bread wheat (25% eff.) and durum wheat (25% eff.)



Isolation of embryos —— 3 months ——→ Transfer to glasshouse

High-throughput wheat transformation



Selection using different markers equally effective

BAR (herbicide resistance)
NPTII (antibiotic resistance)
HYG (antibiotic resistance)

Low Transgene Copy Numbers

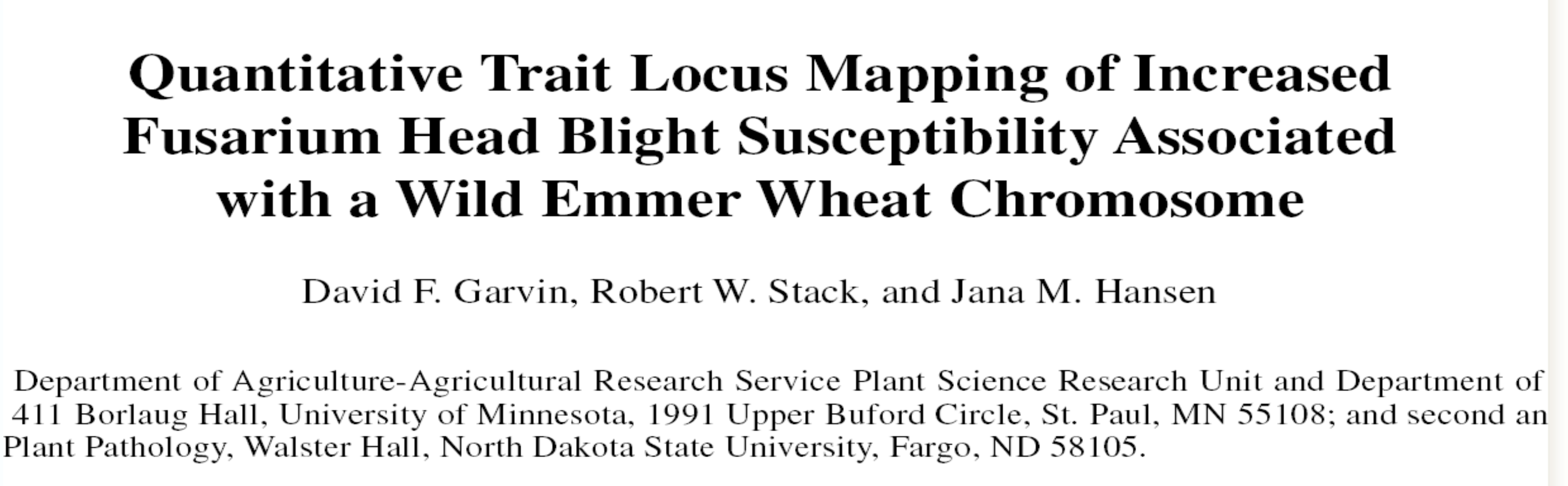
1 copy	14%
2 copies	25%
3 copies	28%

Non-transgenic Approach:

Can we identify wheat mutants deficient for candidate disease susceptibility genes?

"Disease resistance breeding focuses on deploying resistance genes. However, genes that increase disease susceptibility by encouraging pathogen proliferation have been suggested to be relatively common in wheat. Perturbation of these genes increases disease resistance"

Konzak, C. F. 1987. Mutations and mutation breeding. Pages 428-443. in: Wheat and Wheat Breeding.



Quantitative Trait Locus Mapping of Increased Fusarium Head Blight Susceptibility Associated with a Wild Emmer Wheat Chromosome

David F. Garvin, Robert W. Stack, and Jana M. Hansen

Department of Agriculture-Agricultural Research Service Plant Science Research Unit and Department of
411 Borlaug Hall, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108; and second an
Plant Pathology, Walster Hall, North Dakota State University, Fargo, ND 58105.

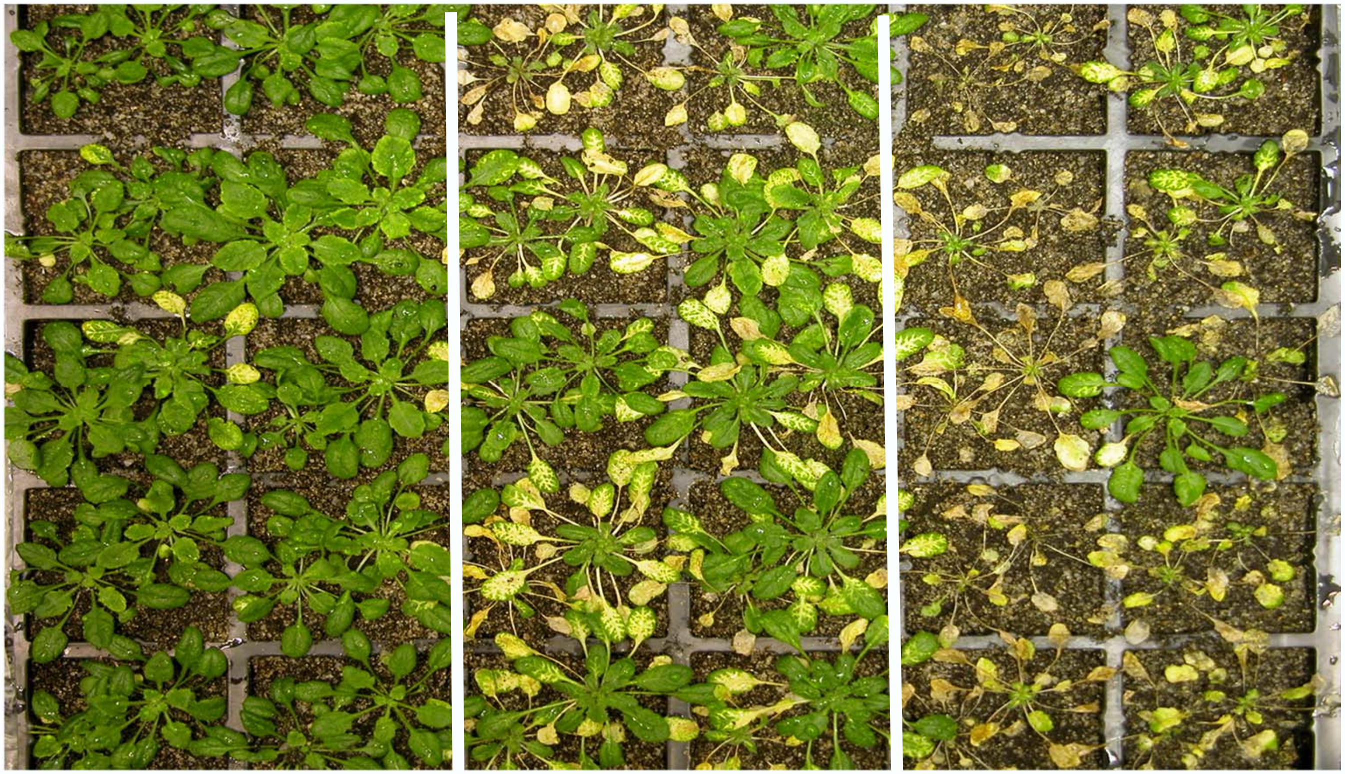
Phytopathology (2009) 99:447-452.

Susceptibility genes: e.g. MEDIATOR subunit 25

No susceptibility (*med25* mutant)

Wild-type (*MED25*)

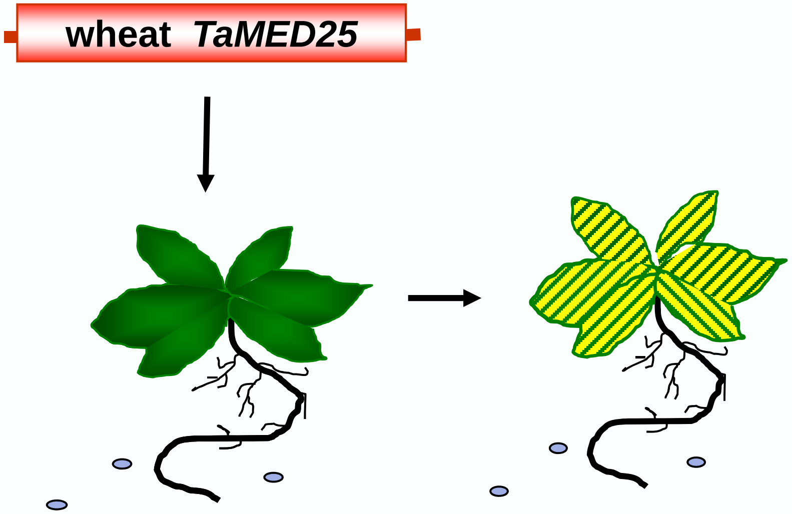
More susceptibility gene product (*35S:MED25*)



***Fusarium* inoculations**

Kidd et al (2009) The Plant Cell

Can wheat MED25 confer susceptibility when introduced into the med25 mutant background?



wheat *TaMED25*

Resistant
(*med25*)

susceptible
(35S:TaMED25/
***med25*)**

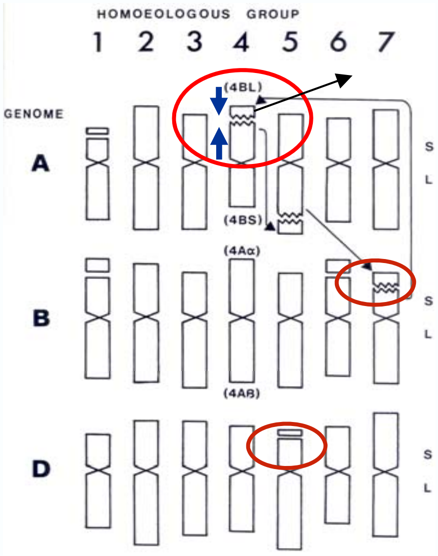
TaMED25/
med25

WT

med25

Deleting susceptibility genes in wheat?

Induce mutations in Wheat (e.g. deletions)



Identify individual mutants via high-throughput Quantitative PCR and combine different homoelog mutations by crossing

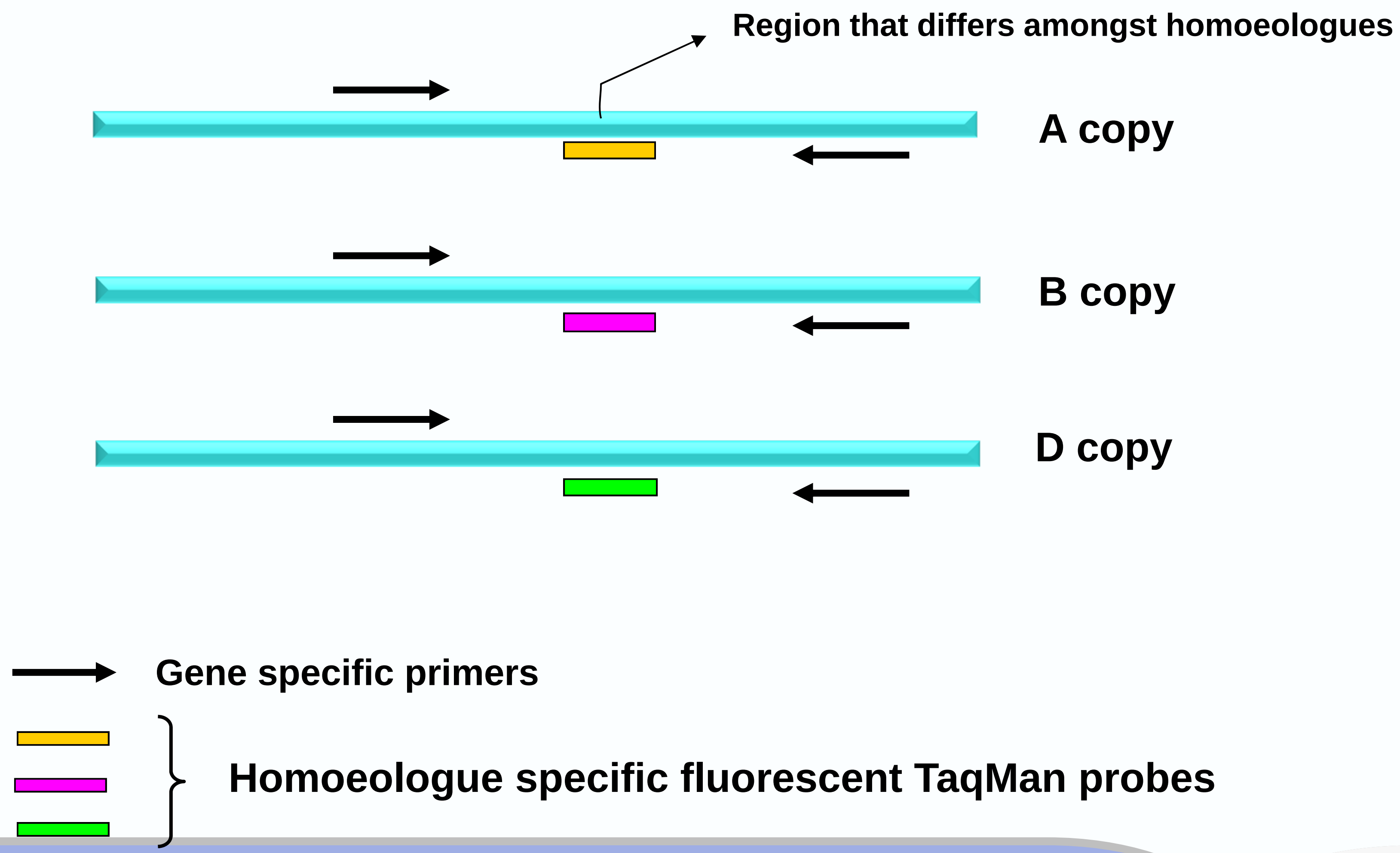
Resistant
(susceptibility gene deleted)

Susceptible
(WT)

Fusarium assays to test gene function

Introduction of resistance to susceptible lines
Via marker assisted selection

High-throughput identification of homoeologous target gene deletions



Region that differs amongst homoeologues

A copy

B copy

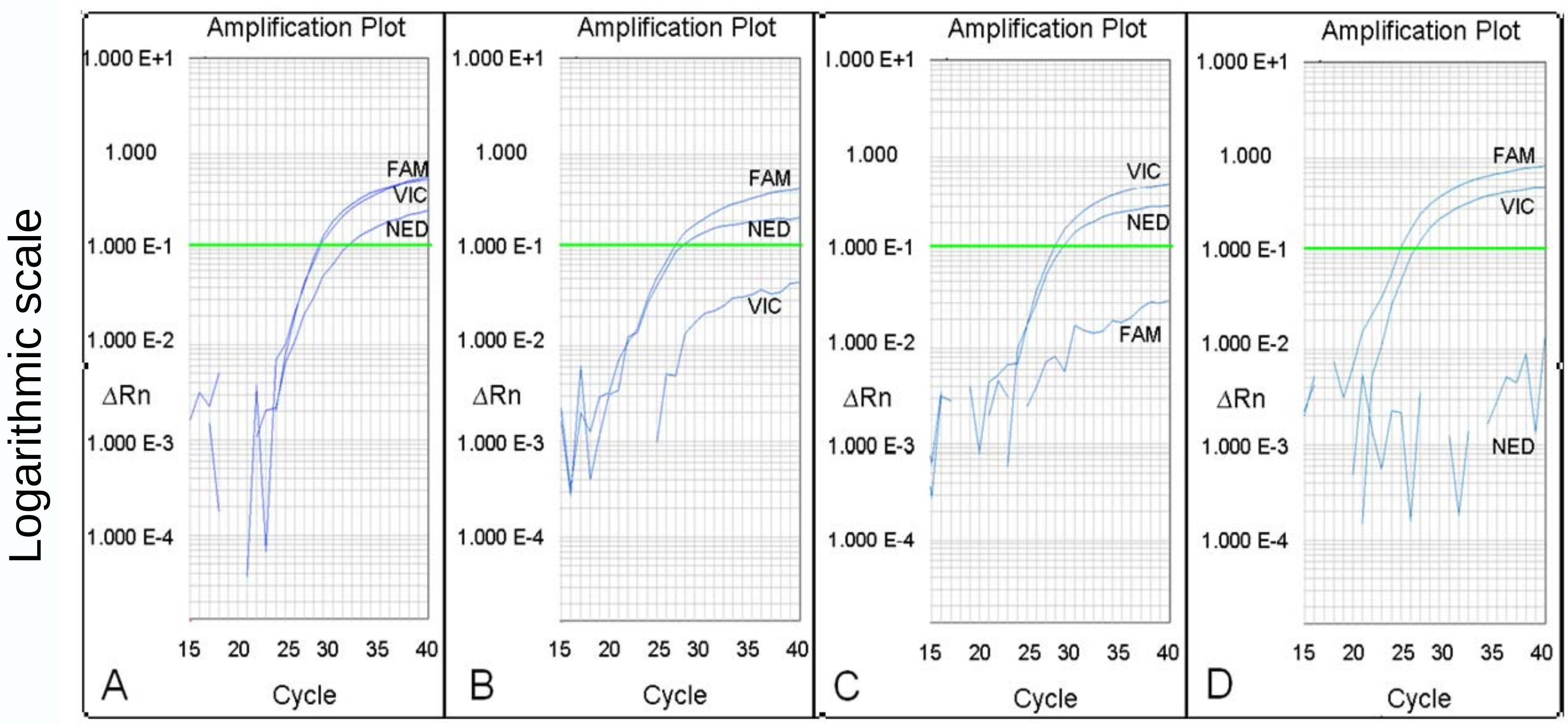
D copy

→ Gene specific primers

🟨 🟪 🟩 } Homoeologue specific fluorescent TaqMan probes

Identification of *med25* wheat mutants

‘VIC’ = A-specific probe; ‘FAM’ = B-specific probe; ‘NED’ = D-specific probe



Chara (WT)

TaMED25-A deleted

TaMED25-B deleted

TaMED25-D deleted

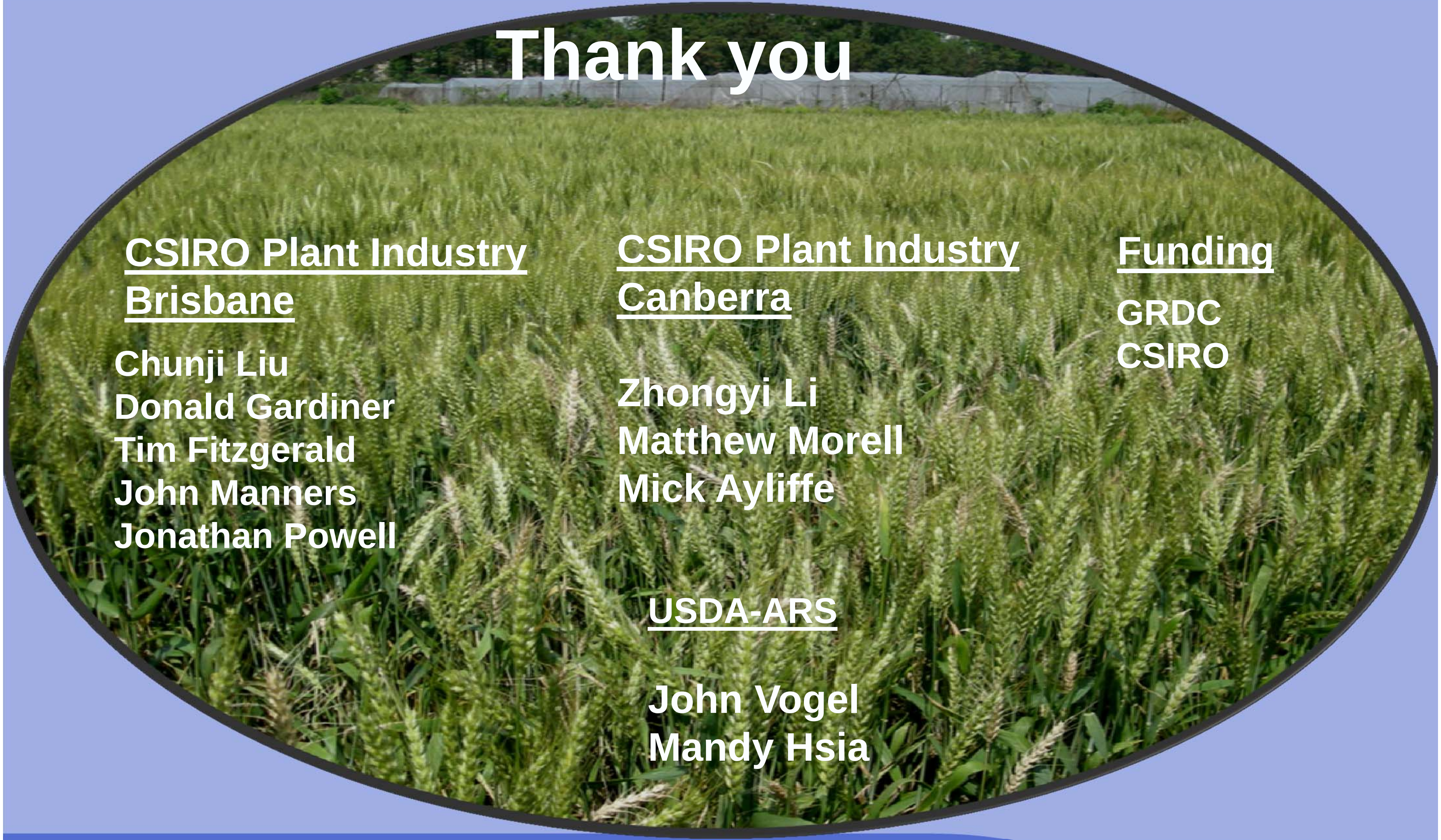
Fitzgerald et al., 2009. BMC Plant Biol

Fusarium CR screening of deletion mutants

Brachypodium as a new model for testing host resistance against *Fusarium* pathogens

Fusarium Inoculated

Mock Inoculated



Thank you

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