

Transgenic resistance to Fusarium head blight in barley



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The disease problem

- FHB prevalent in main US barley growing regions since 1993
- *Fusarium graminearum* produces the mycotoxin deoxynivalenol (DON)
 - Potential effects on human and animal health
 - Malting industry sets a 0.5 $\mu\text{g/g}$ limit on barley purchases
 - Potential for fungal regrowth during malting

FHB resistance*

- Type 2 = resistance to disease spread
 - Point inoculations do not spread in barley
- Type 1 = resistance to initial infection
 - About 30 resistant barley accessions identified
- Type 5 = resistance to toxins

*Mesterházy 1995 Plant Breeding 114:377-386

Resistance in Barley

- Quantitative resistance
 - 19 loci identified
 - Many bi-parental mapping populations
 - Association mapping (part of the Barley CAP)
- Good target for transformation
 - Resistance genes from other sources
 - Over-express or silence native genes

General Protocol

- Particle bombardment
 - Select using bialaphos (bar gene)
- Adapted two-rowed malting cultivar Conlon
 - Model cultivar Golden Promise not adapted to North Dakota environment
 - Problems in both greenhouse and field
 - Direct transformation of adapted malting barley cultivar reduced need for crossing
 - GP also does not meet US malting quality

Field Trial Protocols

- 4 to 6 replicates of hill plots
- CI4196 (resistant check) & Conlon (susceptible check) repeated every 10 hills
- Grain spawn inoculation, half of reps misted
- Measure severity (% infected kernels), incidence (% infected spikes), DON
- Follow APHIS and North Dakota Dept. of Agriculture regulations to prevent transgene escape

Tri101 & PDR5

- Tri101 = 3-OH acetyl transferase from *F. sporotrichioides*
 - Converts DON to 3A-DON, 1/10 as toxic
 - 3A-DON less effective at inhibiting protein synthesis and preventing plant's defense response
- PDR5 = ATP-dependent membrane-bound multidrug efflux transporter from *S. cerevisiae*
 - Shunts trichothecene toxins across the plasma membrane
 - removes DON from cells
- Collaborators: Tom Hohn (previously ARS), Ann Blechl, Nancy Alexander, Susan McCormick (ARS), Muthusamy Manoharan, Richard Horsley, Steven Neate, Paul Schwarz (NDSU).

Tri101 & PDR5

- Tri101
 - Recovered 7 transgenic events, 3 diploid
 - Gene transmission and expression through the T_4
- PDR5
 - Recovered 6 events, 2 diploid
 - Gene transmission and expression through the T_4

Disease Testing – Tri101

	Greenhouse 1		Greenhouse 2		Field 2001		Field 2002	
Genotype	FHB	DON	FHB	DON	FHB	DON	FHB	DON
Conlon	22	10	16	15	13	14	2.7	6
CI4196	nt	nt	3	6	nt	nt	1.9	19
Harrington	nt	nt	19	6	nt	nt	nt	nt
Zhedar 2	nt	nt	3	7	nt	nt	nt	nt
Tri101-1	7	3	5	1	12	12	2	10
Tri101-2	9	4	3	1	12	19.5	1.5	10
Tri101-3	8	5	1	0	8	23	0	0

Disease Testing – PDR5

	Greenhouse 1		Greenhouse 2		Field 2001		Field 2002	
Genotype	FHB	DON	FHB	DON	FHB	DON	FHB	DON
Conlon	19	21	27	10	13	14	2.7	6
CI4196	nt	nt	10	8	nt	nt	1.9	19
I94-403	nt	nt	27	12	nt	nt	3.3	3
PDR5-1	7	14	16	3	8	12	2	6
PDR5-2	4	4	13	2	5	8	1.4	12

New Tri101 Research

- Biochemical properties of FsTri101 compared to FgTri101 by Ivan Rayment's group (UW-Madison)
- FsTri101 high specificity for nivalenol
- FgTri101 high specificity for DON
- Protein engineering to improve FgTri101 activity and stability

Antifungal Genes

- Rice chitinase + thaumatin like protein (tlp)
 - Antifungal effects in wheat
 - 3 events, one expressed both genes, others expressed just the tlp
 - Backcrossed to wild-type Conlon and Lacey (6-rowed) before field testing
- Collaborators - M. Manoharan (UAR), S. Muthukrishnan (KSU), S. Neate, R. Horsley, P. Schwarz, and Dennis Tobias (NDSU), Phil Bregitzer (ARS), Clara Pritsch (Univ. Rep. Uruguay)

Field Trials

Genotype	Average FHB	Average DON
Conlon	6.4	12.0
Lacey	9.4	31.9
CI4196	0.3	10.8
Chi+tlp	12.5	23.6
Chi+tlp Conlon BC	8.5	12.3
Chi+tlp Lacey BC	20.1*	30.7
Tlp	9.9	41.4
Tlp Conlon BC	10.7	13.6
Tlp Lacey BC	11.0	31.7

* One line at 33.5%

Antifungal/antitoxin Genes

- Bombarded with singles and pairs of genes
 - Thaumatin like protein from rice
 - Chitinase from rice
 - Endo and exoChi from *F. venenatum*
 - Glucanase from *F. venenatum*
 - Tri12 from *F. sporotrichioides*
- 140 lines advanced and field tested for 2 years
 - 14 selected for further testing
- Collaborators: Pat Okubara, Phil Bregitzer (ARS), Stephen Neate, Paul Schwarz, Robert Brueggeman (NDSU), Ruth Dill-Macky (UMN)

Field Trials

Genotype	Transgene	FHB severity†	FHB incidence†	DON µg/g
Conlon	Wt	4.3	53.8	10
CI4196	Wt	0.8	16.3	9.7
321	Tri12	3.6	56.7	6.2*
823	Tlp	4.1	51.7	5.9*

† FHB severity=% infected spikelets; FHB incidence=% infected spikes

* Significantly different from wt Conlon (p<0.05)

Values are means from inoculated nurseries conducted in 2005-2007 and 2010-2011

Next Steps

- Backcross transgenes from 321 and 823 into breeding lines/cultivars with partial resistance and acceptable malting quality
 - Quest – University of Minnesota
 - ND20448 – North Dakota State University
- Test for additive effects in replicated field trials over multiple years
 - compare performance of parent genotypes and backcross-derived lines

Collaborations Underway

- Ivan Rayment (Univ. of Wisconsin)
 - FgTri101 and modifications
- Steve Scofield (USDA-ARS)
 - Identifying candidate genes by virus-induced gene silencing (VIGS) in wheat
- Michael Lawton (Rutgers Univ.)
 - Identifying candidate genes by transformation of *Physcomitrella patens*

A photograph of a field of tall, golden-brown grasses, likely a barley or wheat field, with a line of trees in the background. The text is overlaid on the left side of the image.

Funding

- North Dakota Barley Council
- US Wheat & Barley Scab Initiative
- USDA-Agricultural Research Service

Technicians

- William Morgan
 - Luming Brewer
 - Nancy Hillen
 - Mary Wentz
 - Rachel Krein
 - Maggie Sahr
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- Plus numerous undergraduate students

A photograph of a field of tall, golden-brown grass, likely a meadow or pasture. In the background, there is a line of green trees under a clear sky. The text "Thank You" is overlaid in the center of the image.

Thank You

Questions?