Strength in Numbers: Resistance Gene Cassettes for Durable Disease Control in Cereals

Brian J. Steffenson

Department of Plant Pathology
University of Minnesota
St. Paul
Barley Diseases

- Stem rust
- Fusarium head blight
- Spot blotch
- Leaf rust
- Net blotch
- Powdery mildew
- Stripe rust
- Barley/Cereal Yellow Dwarf Viruses
- Bacterial leaf streak
Deployment of Resistant Cultivars

- **Effective**: yield reductions during epidemics are very low
- **Environmentally benign**: no contamination from pesticides
- **Economical**: breeding has a high return on investment
- **Ease of use**: producers need only plant the seed with no additional inputs
Powdery Mildew Resistance Breeding
Repeated Failure in Europe

Host genes
(sources)

- \( M_{lg} \)
  - (landrace Pflug’s Intensive)

- \( M_{la6} \)
  - (\( H. \) vulgare ssp. spontaneum)

- \( M_{lg} + M_{la6} \)
  - (Pflug’s Intensive \& \( H. \) v. ssp. spontaneum)

Barley cultivar
(Year)

- Union
  - (1961)
  - (1962)

- Maris Badger
  - (1963)
  - (1964)

- Impala
  - (1965)
  - (1966)
  - (1967)

Pathogen genes

- \( VirM_{lg} \)
- \( VirM_{la6} \)
- \( VirM_{lg} + M_{la6} \)

Lucas 1998
Boom and Bust Cycles of Plant Breeding

Single deployed resistance genes are rapidly overcome
Qualitative resistance: due to major effect R-genes that are race-specific, operating at all growth stages

- Many R-genes deployed as singlets were overcome quickly
- Durable single R-genes: $R_{pg1}$ vs. stem rust & $mlo$ vs. powdery mildew
- Gene pyramids: combining three or more R-genes together in one cultivar

Quantitative resistance: due to multiple quantitative trait loci (QTL) that are non-race specific, operating at the adult plant stage
Gene Pyramiding by Conventional Breeding

- Laborious, time-consuming & complex
- Must select for individual resistance loci in the presence of other resistance loci
- Most efficient if resistance loci are tagged by molecular markers

Gene pyramiding schemes

RP=Recurrent Parent; DP=Donor Parent; IRP=Introgression Recurrent Parent; BC=Backcross
Minnesota Barley Breeding Pipeline

Year 1
- Crossing Block
  - Parents (greenhouse)

Years 2-3
- Inbreeding
  - F₁ (greenhouse)
  - F₂ (field)
  - F₃ (greenhouse)
  - Winter Nursery

Years 4-5
- Line Evaluation
  - Prelim. Yield Trials
  - Adv. Yield Trials

Years 6-7
- Industry Testing
  - Pilot Malting
  - Plant-scale Brewing

Year 8
- Release

Kevin Smith, “outstanding” in his field

Malting facility
Brewing facility
Developing Durably Resistant Barley

Each colored circle represents a different R-gene.
Developing Durably Resistant Barley

Each colored circle represents a different R-gene.

Reduces wheat generation time to allow faster crossbreeding to introduce single disease-resistance genes.
A Better Way to Develop Durably Resistant Cultivars?

Ideally, it would be great to:

- Efficiently clone many R-genes from any given germplasm
- Assemble sets of R-genes into different multi-gene cassettes
- Transfer the cassettes efficiently into agronomically superior barley cultivars with high yield, adaptation and end-use quality
- The cassettes would segregate as a single locus, simplifying the breeding process, and provide greater longevity of resistance
Developing Diverse & Durably Resistant Barley

Exploit full R-gene diversity from all genepools
Facilitate rapid development of new multi-disease resistant cultivars
Eliminate linkage drag
Sustain effective resistance beyond normal life of cultivar
Association Genetics-Resistance
Gene Enrichment Sequencing

◆ Combines AG and RenSeq protocols

◆ Efficient means to identify & clone—not just one—but potentially the entire complement of resistance genes

◆ Proof of concept: *Aegilops tauschii*: D-genome donor of wheat, but now being done with wild barley
Predicated on Common Resistant Gene Protein Motifs

- Most R-genes encode proteins that have nucleotide binding and leucine-rich repeat motifs ("NLR genes")

- Plants contain 100s of such R-genes with many residing in complex clusters of linked paralogs

- Baits have been designed from NLR sequences of target species & related species, transcriptome data, & NLR gene models

McHale et al. 2006
Assembly of Germplasm Panel
Wild Barley Diversity Collection

Wild barley in Syria

Diversity of stem rust reactions

Number of accessions & country of origin of Wild Barley Diversity Collection

N=150-200
Disease Phenotyping

Wild Barley Disease Phenotyping

- Stem rust (6 races)
- Leaf rust (12 races)
- Stripe rust (4 races)
- Net blotch (4 races)
- Spot blotch (4 races)
- Powdery mildew (40 races)
- Septoria (1 race)
- Scald (1 race)

N=150-200

Stem rust infection types on barley
~59,000 baits: NLR sequences from target species & related species, transcriptome data, & NLR gene models

DNA libraries enriched & hybridized to baits; bound DNA recovered by magnetic beads

Enriched libraries sequenced with 250 bp paired-end reads

Assembly of NLR repertoire & extraction of NLR k-mers (i.e. all possible subsequences of length k obtained via sequencing
**K-mer Based Association Mapping**

- **A.** Raw rust phenotype: \( k \)-mer (N=8) matrix

- **B.** Pre-filtering, correlated \( k \)-mers of individual accessions/races

- **C.** Regression with PCA covariates to account for structure
K-mers identified by association mapping are plotted in a matrix according to their sequence identity to NLRs (x-axis)

Measure of k-mer association with disease phenotype is given on y-axis
Identification of Stem Rust Resistance Genes
AG-RenSeq on *Ae. tauschii*

**Resistance Gene**

*Sr33*

*Sr45 & Sr46*

*SrTA1662*
Pan-genome variation was exploited by combining association genetics with R-gene enrichment sequencing.

Four stem rust resistance genes cloned from D genome donor of wheat in ~6 months @ ~$10k/gene.

Bonus: stripe rust & Hessian fly R-genes identified from same panel & are in queue for cloning!

Major advance for efficiently isolating many R-genes that can be used to engineer broad-spectrum resistance in cereals.
Constructing R-Gene Cassettes
"The Big 5"

- Sr45 from *Aegilops tauschii*
- Lr67 from *Triticum aestivum*
- Sr50 from *Secale cereale*
- Sr35 from *Aegilops tauschii*
- Sr22 from *Triticum boeoticum*

Ming Luo/Mick Ayliffe
CSIRO
Australia
Efficient Wheat Transformation
Japan Tobacco Method

Agrobacterium tumefaciens

1. Treat foreign DNA and plasmid with restriction enzyme and DNA ligase.
2. Introduce the recombinant plasmid into cultured plant cells.
3. Regenerate new plant from cultured cells.

DNA containing the gene of interest

Ti plasmid

T DNA

Site where restriction enzyme cuts

Recombinant Ti plasmid

Inserted T DNA carrying new gene

Rust Resistant Wheat

Ishida et al. 2015 Methods Mol. Biol.
Field Testing of Transgenic Wheat Lines

- **Planting:** May 22
- **Site:** Rosemount, MN
- **Race:** QTHJC

**Plants at tillering stage**

**Rating scales:**
- severity & infection type

**Inoculating**

**Rust scoring**
Stem Rust Field Results 2018

% Terminal Stem Rust Severity

Fielder WT  | Fielder Null

4.4  | 4-10  | 19-10  | 19-12  | 52-1  | 60-10  | 60-22

C  | C  | C  | C  | C  | C  | C

Controls

Transgenics with 5-gene constructs

Fielder  | Big 5
Genetically Modified Barley: A Possibility for Agriculture?

- Long & expensive deregulation path
- Strong public and industry opposition
- Even with wheat, there is a lack of interest by major biotechnology companies

Wheat—the cereal abandoned by GM

Genetic modification of wheat for disease resistance could help stabilize food production

By Brande B. H. Wulff
and Kanwarpal S. Dhugga

03 Aug 2018
361:451-452 DOI: 10.1126/science.aat5119
The chance of having transgenic barley deployed in agriculture is the same as a:
Summary

- Diseases are a major constraint to barley yield and quality worldwide

- AG-RenSeq will enable the rapid and efficient capture of pan-genome variation for R-genes within a species

- With a suite of cloned R-genes in hand, cassettes of up to 7 genes be constructed

- R-gene cassettes can now be routinely transferred into adapted cultivars, simplifying the breeding process and extending the longevity of the deployed resistance

- Such cultivars will reduce the impact of diseases, making barley a more competitive and economically viable crop
Ongoing and Future Research

- Using AG-RenSeq, we are focusing on cloning the R-gene complement to eight important diseases in a wild barley panel.

- If GM barley will not be commercialized, gene editing protocols will be investigated as an alternative for developing durably resistant cultivars.

- AG-RenSeq can be applied to breeding germplasm, whereby perfect markers can be identified for R-genes and used in marker-assisted and genomic-selection.
Acknowledgements

Funding:
- The Lieberman-Okinow Endowment
  University of Minnesota

Collaborators:
- Steffenson Lab
- 2Blades
- Bill & Melinda Gates Foundation
- USDA United States Department of Agriculture
  Agricultural Research Service
- Cereal Disease Lab: St. Paul, MN
- John Innes Centre
- CSIRO
Thank you!