The Gene Discovery and Engineering Resistance (GDER) RA is urgently working to develop strategies that can be used to significantly improve FHB resistance in wheat and barley.

Although the FHB1 locus is the best tool currently available for scab resistance in wheat, it has several limitations.

1. FHB1-mediated resistance can be breakdown under heavy pathogen pressure.

2. Recently, it has been shown that FHB1 has low genetic penetrance in some genotypes.

For these reasons we firmly believe that novel strategies must be found for engineering scab resistance.

My lab’s approach is to identify the wheat genes that are essential for FHB resistance in wheat. These genes will then be used to engineer improved FHB resistance.
The isolation of wheat genes with particular functions has been very difficult

Wheat’s hexaploid genome often prevents conventional mutational analyses

The wheat genome is enormous - 16,000 Mbp genome (128X Arabidopsis)

Wheat is very difficult to transform. This prevents the use of many modern approaches to gene isolation, T-DNA libraries and transposon gene tagging
Genetic analysis of recessive mutations in diploids and hexaploids

The yy phenotype is always masked by Y’s on 1B and 1D.
Capabilities that would be very useful for wheat molecular genetics:

The ability to specifically turn off genes (knockout) so that their functions can be inferred from the knockout phenotype.

The ability to efficiently generate gene knockouts despite the hexaploidy of wheat.

The ability to create knockouts without needing to transform wheat.

An efficient virus-induced gene silencing (VIGS) system for wheat should satisfy these requirements.
OUTLINE FOR THE REMAINDER OF THIS PRESENTATION:

1. What is VIGS?

2. Development of the BSMV-VIGS system for wheat

3. Using BSMV-VIGS to functionally identify genes in FHB and other resistance pathways of wheat.
Virus-Induced Gene Silencing (VIGS)

VIGS is a form of RNA-mediated gene silencing.

Replication of RNA viruses causes large amounts of dsRNA to accumulate which activates the RNA silencing mechanism.

The silencing mechanism targets the sequences represented in the dsRNA for homology-dependent degradation.

If the virus carries sequences homologous to plant genes, transcripts of the plant gene are also degraded.

Because the mechanism is homology-dependent, VIGS can silence homeologous genes present in polyploids.

Viral infection is easy, doesn’t require transformation.
General mechanism of RNA-induced gene silencing

- dsRNA
- RDRP
- siRNAs
- miRNAs
- AGO
- RNA Interference Silencing Complex (RISC)
- DNA/Histone Methylation
- TGS
- mRNA Degradation
- VIGS, PTGS, RNAi
- Translation Block
- Developmental Processes
Barley Stripe Mosaic Virus

BSMV is a member of Hordeivirus family

Positive-sense single strand RNA virus

Tripartite genome: $\alpha$, $\beta$, $\gamma$ RNAs

Hosts: barley, wheat, oat, rice and tobacco


Barley Stripe Mosaic Virus

BSMV Genomic Organization & Protein Function:

α
replicase

β
coat protein
movement proteins

γ
replicase
suppressor of silencing

VIGS targeting sequence

Sub-genomic promoter
BSMV-VIGS Procedure

Clone 200 - 500bp cDNA fragment from target gene into BSMV γ plasmid in antisense orientation.

Linearize α, β and γ DNA plasmids.

*In vitro* transcribe 5’ capped α, β and γ RNAs.

Mix RNAs 1:1:1 in 25μl inoculation buffer (~0.5 μg of each RNA/inoculation).

Rub inoculate RNAs onto wheat leaves.

Observe VIGS phenotype; confirm silencing of intended target gene.
VIGS in Hexaploid Wheat: Silencing the PDS gene results in the appearance of photobleached tissue
The first test using BSMV-VIGS in the dissection of a resistance pathway

A cloned R-gene would provide a very useful positive control.

Silencing the R-gene should cause a resistant plant to become susceptible.

*Lr21* was cloned by Li Huang in Bikram Gill’s group at Kansas State University.

*(Huang et. al., 2003 Genetics 164: 655-664).*

*Lr21* provides hypersensitive resistance to leaf rust caused by *Puccinia triticina.*
Design for the Leaf Rust VIGS Experiment

Two wheat genotypes tested: WI = Susceptible; WGRC7 = Resistant

10 plants inoculated for each treatment; 2 experimental blocks.

Treatments: WI and WGRC7 infected with:
- BSMV:00
- BSMV:PDS4
- BSMV:Lr21
- BSMV:RAR1
- BSMV:SGT1
- BSMV:HSP90

Plants inoculated with BSMV transcripts 7 days after germination.

Plants sprayed with avirulent *P. triticina* (PRTUS6) 8 days later.

Plants scored 10 days later.
Control experiment:
Infection with BSMV:00 does not alter resistance or susceptibility to *Puccinia triticina*
Silencing *Lr21, RAR1, SGT1* or *HSP90* converts incompatible interactions to compatible, indicating that each is essential for *Lr21*-mediated resistance.
Resistance to FHB

The most useful known sources of FHB resistance are QTL-based.

In wheat, the strongest QTL is FHB1 from chromosome 3B of Sumai-3.

FHB1 conditions type II resistance that limits the spread of the fungus.

Type II resistance is assayed by single floret inoculation.

FHB1 resistance is inadequate during FHB epidemics.

We are working to identify the genes involved in resistance so that we can improve resistance.

Our current approach is to use VIGS to test genes that are differentially expressed during FHB interactions, or have annotations suggesting function in resistance.
Demonstration of BSMV:VIGS in adult wheat plants
BSMV:VIGS of PDS in the awns of wheat

qRTPCR analysis of PDS expression in wheat awns

Relative PDS expression

qRTPCR normalized with GAPD
Infection with BSMV:00 does not interfere with type II resistance of Ning 7840

Single floret *Fusarium graminearum* inoculation of BSMV:00 infected Ning 7840 wheat
Expression of chitinase genes after infection with Fusarium graminearum

VIGS assays using three different fragments of chitinase to target gene silencing result in Ning 7840 becoming susceptible to FHB.
Expression of wheat chitinase after silencing by BSMV:097

Chitinase

097 VIGS fragment
qRTPCR product

Relative Chitinase Expression

Pooled Controls

097-7

097-9

097-10

097-12

097-13

097-14

097-15

* Plants scored as susceptible to FHB
Recently, the Muehlbauer lab demonstrated that over-expression of chitinase increases FHB resistance in transgenic wheat.

My group is testing whether a wide range of genes, which are differentially expressed during challenge by *F. graminearum*, make significant contributions to FHB resistance.
VIGS analysis has shown that genes that act to promote ethylene biosynthesis and ethylene-dependent signaling play essential roles in FHB resistance.
Conclusions

We have obtained strong evidence that BSMV-VIGS is an effective tool for the rapid identification of genes functioning in wide range of wheat disease resistance pathways.

We have proven this system’s utility for probing the FHB resistance pathways, and are actively testing a range of new candidate genes.

We are very interested in testing whether genes from the Ethylene signaling pathway can be used to engineer FHB resistance.

BSMV-VIGS can be used to test candidate genes identified on the basis of differential expression, candidates identified in genome-walking experiments, or candidates identified from other functional screens performed in systems (ie., yeast or Physcomitrella).
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