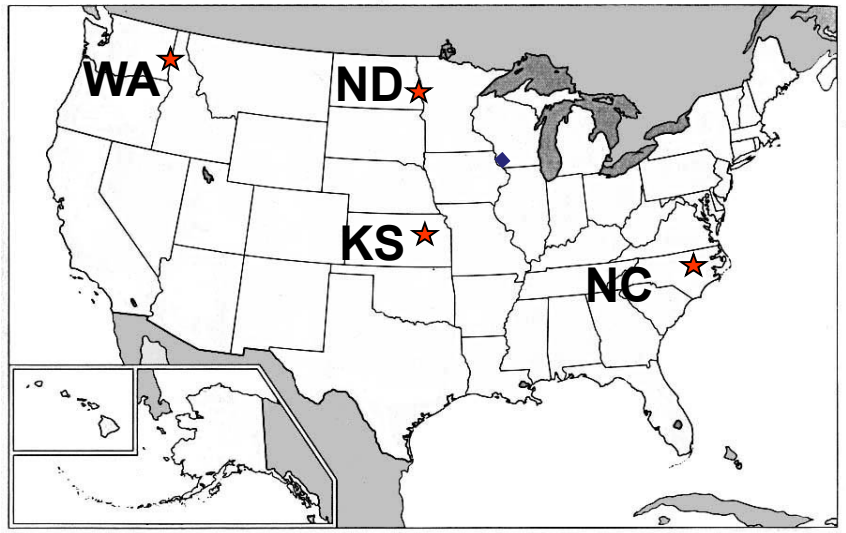
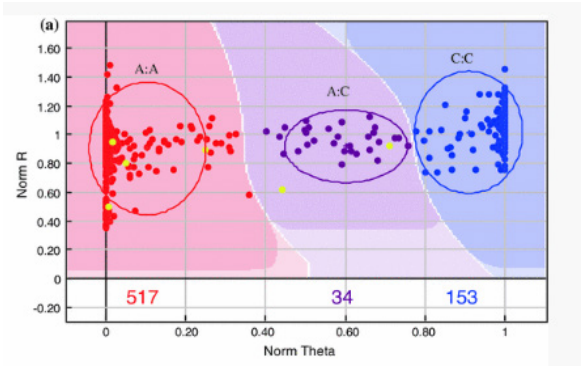
Regional Small Grains Genotyping Labs

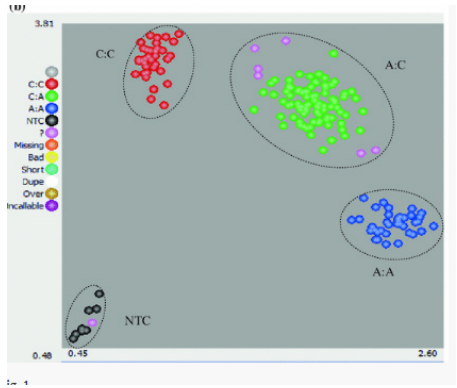
Agricultural Research Service

Provide enabling genotyping services

- Established in 2001 with strong support from stakeholders
- Van Sanford, D. Anderson, J. Campbell, K. Costa, J. Cregan, P. Griffey, C. Hayes, P. Ward, R. 2001. Discovery and Deployment of Molecular Markers Linked to Fusarium Head Blight Resistance. Crop Science. 41. 638-644.
- Facilitate the use of molecular markers to accelerate improvement in small grains
  - Invest in equipment/expertise
  - Develop new technologies
  - Identify new loci that influence traits
  - Permanent fixtures
- Services provided
  - High-throughput DNA extraction
  - Single marker assays – KASP, SSR, STS
  - Genome-wide assays – *Sequencing* and arrays
  - Analysis – MAS, GS modeling





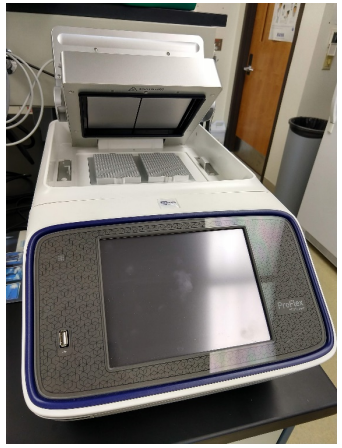
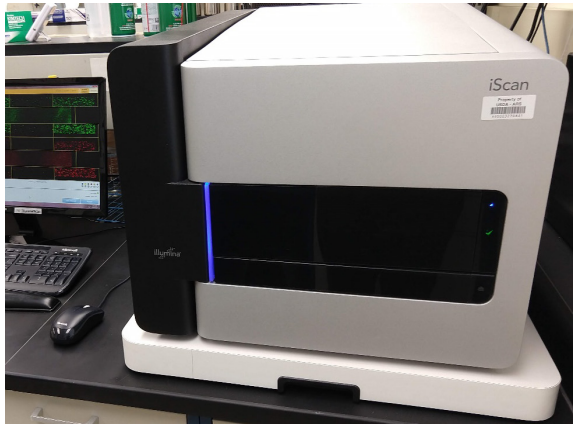
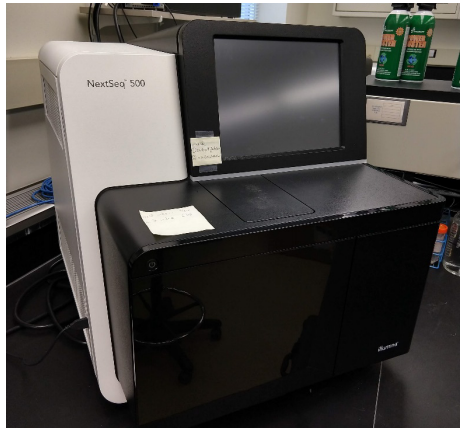
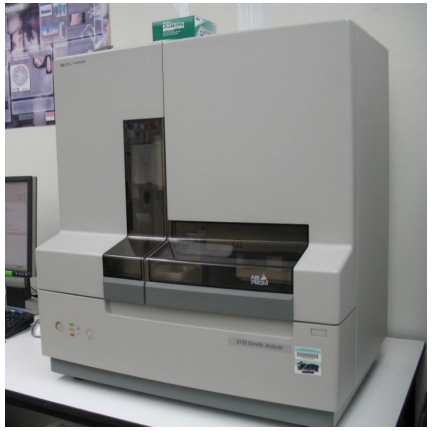


North Central Small Grains Genotyping Lab

- Lead PI - Jason Fiedler
  - 11/11/18
  - Molecular biology, bioinformatics, QTL mapping, GS
- Two full-time technicians
- One post-doc
- Two part-time undergraduates
- Cat4 scientist and another technician in process

Edward T. Schafer Agricultural Research Center

Current capabilities – equipment + expertise

LH Robotics	Proflex x 6	iScan	NextSeq500	ABI 3130
				
DNA extraction & normalization, cherry picking **384-768** lines per day	KASP **4,600** data points per day	90K Wheat, 50K Barley 6K Oat, 3K Pgt **192** lines per day	Sequencing	Fragment analysis **3,000** data points per day

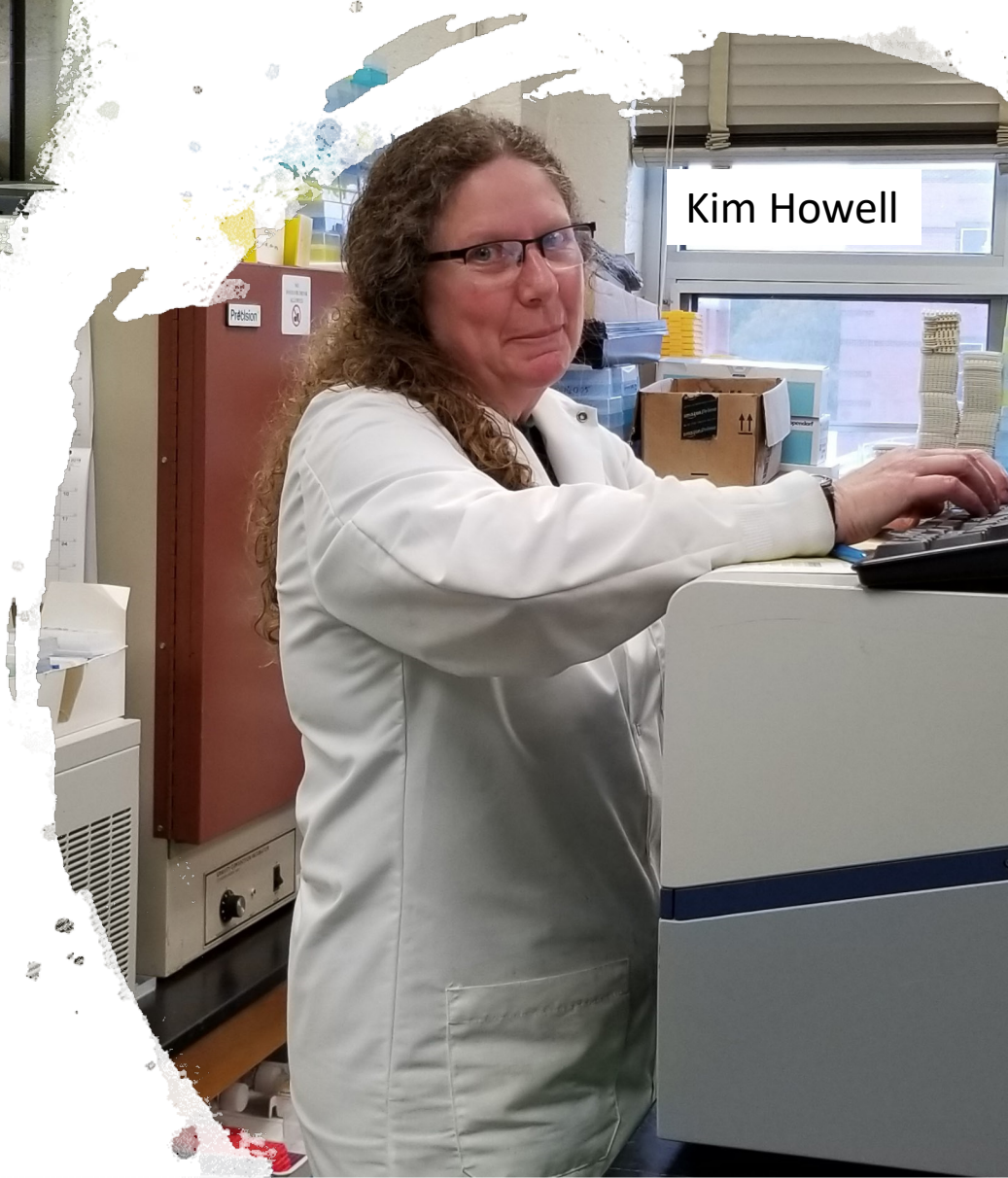
Goal is to increase throughput/speed of every technique and provide additional services

FHB efforts in collaboration with Fargo scientists

- **Shaobin Zhong** - Fine mapping a FHB resistance QTL from PI 277012 & genotyping *Fusarium* isolates from North Dakota.
- **Xuehui Li** – Mapping FHB resistance in HRSW breeding germplasm and domesticated emmer.

- **Steven Xu & Elias Elias** – Dur-CP - Genotyping/sequencing of FHB-resistant durum breeding material

- Always looking for more collaborations



Eastern Regional Small Grains Genotyping Lab

Lead PI: Gina Brown-Guedira

Marker-Assisted Selection for FHB Resistance

Eastern Reginal Genotyping Lab

2-3 breeder projects per year – *Fhb1*, other FHB QTL

- Use *Fhb1* marker developed by G. Bai
- Developed KASP assays flanking SRWW QTL

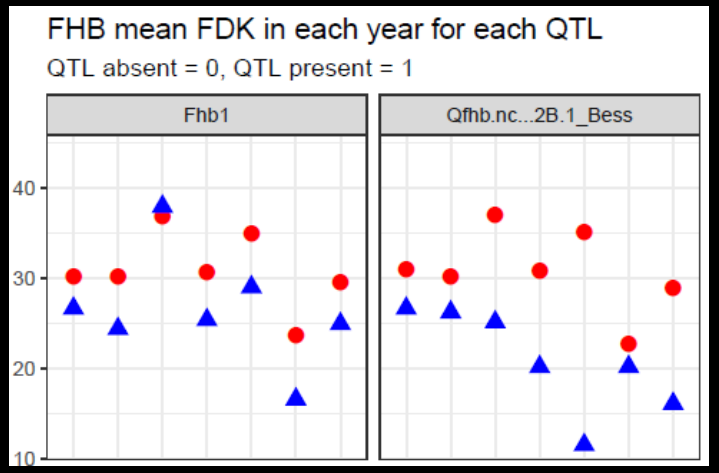
QTL pyramiding for Doubled Haploid production

- 1200 – 2000 F1s each year with ~20 markers – *Fhb1*, FHB QTL, Rht, Ppd, HF, Rust resistance, quality
- Breeder's select DH mother plants – sent to Murphy lab, Heartland Plant Innovations

Introgression into SRWW of PI 277012 resistance

- GP80 line obtained from S. Xu, Fargo, ND
- Resistance QTL on 5A are linked to *Vrn-A1* locus
- BC2F1 with NC-Neuse spring type line
- Crosses to winter lines (ie. Hilliard)
- Selection done in F2s for winter type recombinants
- Murphy crossed recombinant plants to elite Mid-Atlantic and Southern lines; 2500 F2s in GH for SSD
- Additional crosses are being made winter 2020 to broadly adapted elite from KY

Haplotyping Inbred Material



Collaboration with NCSU

Markers on Uniform Scab Screening Nurseries

- Southern, Northern, Preliminary Northern
- 61 loci/ alleles total, 100+ markers
- Diagnostic markers – including *Fhb1* locus
- Markers flanking 10 QTL for FHB resistance
  - QTL_5A_Ning7840, QTL_2DL_Wuhan1
  - Qfhb.nc2B_Bess, Qfhb.nc3B_Bess, QTL_1A_Neuse, QTL_4A_Neuse, QTL_6A_Neuse, QTL_1B_Jamestown, QTL_3BL_Massey, QTL_5A Ernie

FHB marker genotyping of Eastern Winter Wheat nurseries

3-4 Breeder projects per year

Genome Wide Marker Analyses

Uniform Scab Screening Nurseries used as training populations

- ~293 lines in Southern training population, 2011-2018
- ~440 lines in Northern training population, 2014- 2018

Prediction of lines in current year's nursery

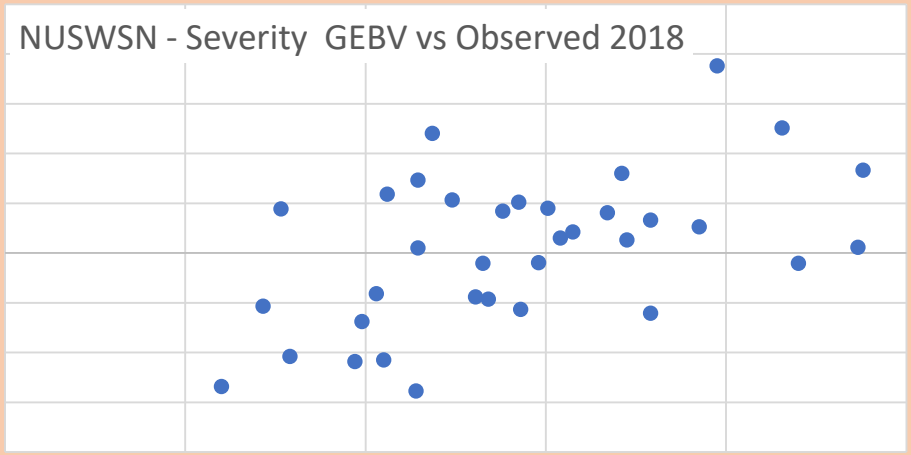
Prediction of untested lines

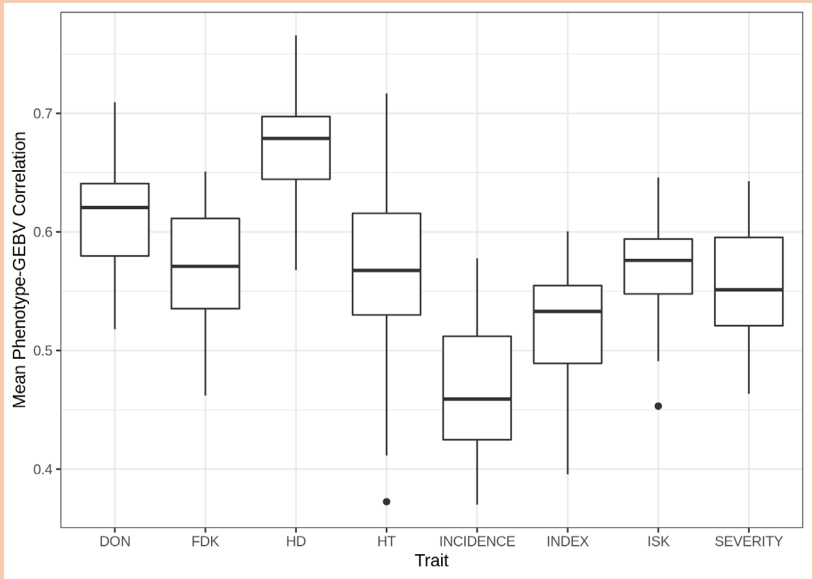
Mapping populations

- NC13-20076 x GA06493-13LE6
- Catawba x NC12-22844

Genome Wide Association Mapping

- Purdue and Arkansas panels





Brian Ward, USDA-ARS

Martin Sarinelli, NCSU

Jeanette Lyerly, NCSU

Roshan Acharya, NCSU

Eastern Reginal Genotyping Lab

Western Regional Small Grains Genotyping Laboratory

Deven R. See

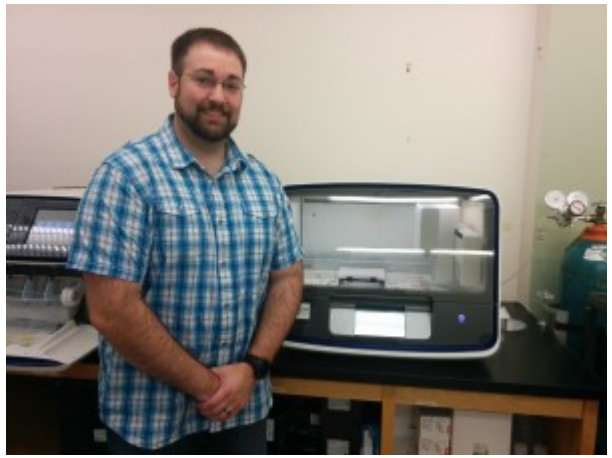
USDA-ARS

Washington State University

Pullman, WA

Western Regional Small Grains Genotyping Laboratory

- Post Doc
  - Marcus Hooker; Big Data
- Technicians
  - Travis Ruff
  - Karol Marlowe
  - Amandeep Dahliwal



FBH Phenotyping activities at WRSGGL

- Spring wheat breeding program
- Varietal Testing Program
- Plant Pathology research farm
  - Mist irrigation system
  - Plant on corn residue
    - Lab-Inoculated corn spread on plot
  - sprayed inoculum daily through anthesis
    - Percent incidence
    - Disease severity
    - FDK percent
    - Don

FBH Genotyping activities at WRSGGL

- Wheat
  - Incorporated Fhb-1 into amplicon sequencing platform
  - Genotype Fhb-1 introgression into winter wheat program
  - Genotype Fhb-1 introgression into spring wheat program
  - Genotype Variety Testing program
  - Exome capture on selected breeding lines and variety testing program
- Barley
  - Collaborate with OSU DH production to genotype and validate gene introgression in DH production system using amplicon sequencing.

CGAHR
Center for Grain & Animal Health Research

USDA Small Grain Genotyping Lab at Kansas

Guihua Bai

1. **Location: Kansas State University, Manhattan KS**
2. **USDA staff (3)**
   - **1 PI (Cat 1)**
   - **1 supporting scientist (GS 11)**
   - **1 Bio Technician (GS 9)**
3. **KSU staff:**
   - **NACA funded Post-doc fellow (2),**
   - **NACA funded graduate student (1-2)**
   - **Self-funded visiting scientists (3)**
   - **Self-funded graduate students (3-4),**
   - **Undergraduate students (1-2)**

Service

- **Hard winter wheat breeding programs in the US Great Plains**
- **Genotype RPN and other elite breeding lines (1500) with >100 markers annually**
- **Conduct 20 plates GBS for breeders/geneticists for QTL mapping/GS**
- **Conversion of other markers to high throughput SNP markers**

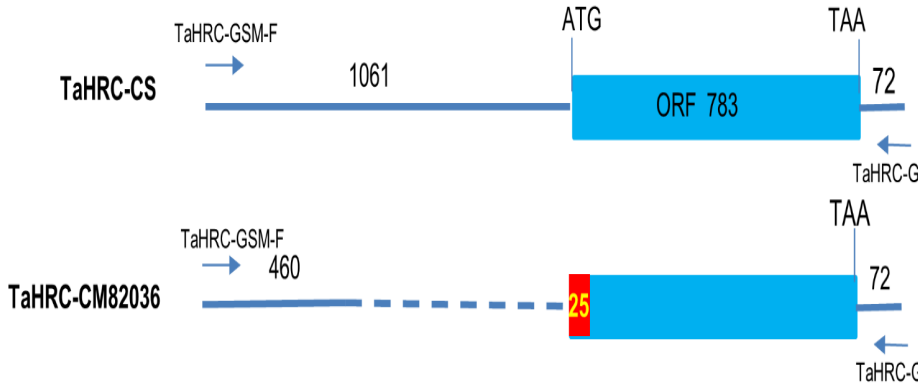
Research

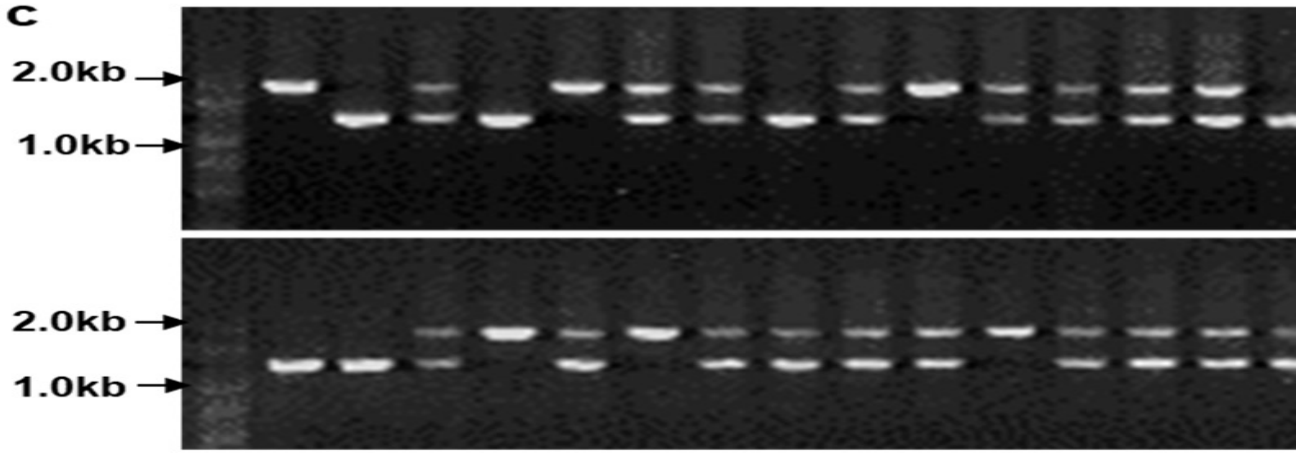
- Identification of QTLs and linked markers for:
  - FHB
  - Preharvest sprouting (PHS)
  - Curl mite (CM)
  - Hessian fly (HF)
  - Three rusts
  - Powdery mildews
  - Yield components
- Fine mapping and cloning genes for:
  - PHS, FHB, HF, CM, yield component
- Marker-assisted transfer of exotic genes to adapted cultivars
- Characterization of gene functions
- Development of high-throughput genotyping technology

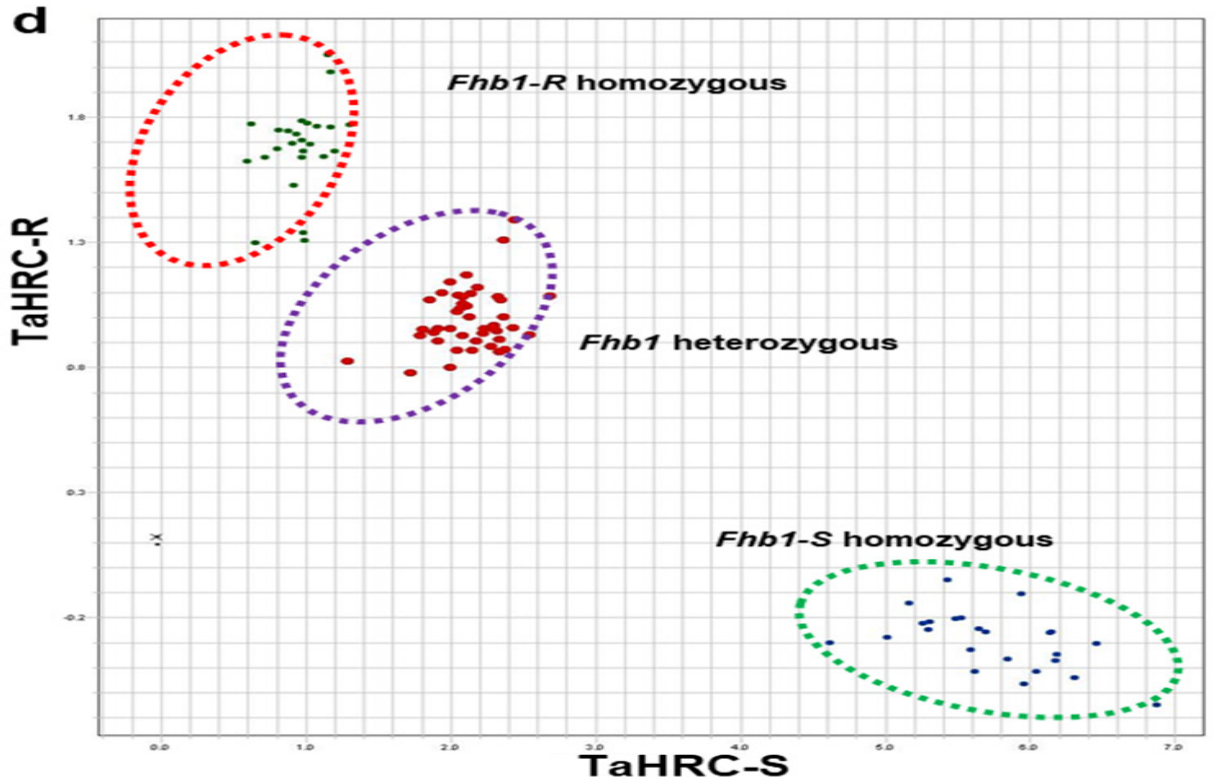
Fusarium Head Blight

1. **Cloned Fhb1 as HRC, demonstrated that loss-of-function mutation results in resistance, and developed functional markers for Fhb1.**
2. **Mapped QTLs from >10 Chinese and US sources.**
3. **Transferred Fhb1 into 16 US hard winter wheat backgrounds and developed germplasm carrying Fhb1 that are used in HWW breeding programs.**
4. **Pyramided Fhb1 with two QTL from chromosome 5A in two HWW.**
5. **Developed MRASeq markers for MABC background screening**
6. **Developed BSMV-mediated CRISPR/Cas9 editing system for candidate gene function validation**

Fhb1







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Nature Genetics 2019 51:1099-1105