

# DEVELOPING FUNCTIONAL MARKERS FOR VERTICILLIUM WILT RESISTANCE IN ALFALFA

---

Long-Xi Yu

USDA-ARS, PGITRU, Prosser, WA

# Alfalfa Verticillium Wilt

- Verticillium wilt (VW) of alfalfa is a soil-borne disease caused by the fungal pathogen *Verticillium alfalfae* (Inderbitzen et al. 2011).
- VW causes sever loss in alfalfa production in the northern United States and Canada.
- The best method for controlling the disease is through the development and use of resistant varieties.
- Conventional selection for VW resistance in alfalfa requires evaluating host response to the pathogen and is time consuming.
- Developing molecular marker associated with VW resistance and use of MAS would greatly accelerate the breeding program

**Identify genetic loci for VW resistance**



**Resequencing parents and segregated lines**



**Comparative and haplotype analysis**



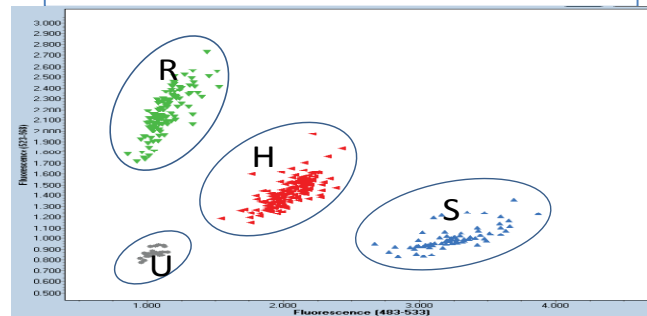
**Developing markers and strategies**



**Validating markers' diagnosticity**



**MAS in breeding programs**



**Improved resistant alfalfa to diseases**

GBS raw sequence reads

Genotyping by sequencing

FreeBayes

TASSEL-Reference

TASSEL-UNEAK

**FastQC (v0.11.2)**  
Initial quality control

**FastqToTagCountPlugin**

**Stacks (v 1.23)** process\_radtags  
De-multiplexing and cleaning reads

**MergeMultipleTagCountPlugin**

**BWA (v 0.5.9)**  
Alignment of reads to reference genome sequence

**TagCountToFastqPlugin**

**UtagCountToTagPairPlugin**

**SAMtools (v 0.1.19)/Picard (v 1.94)**  
Mark duplicate reads

**FastqToTBTPPlugin**

**UExportTagPairPlugin**

**GATK (v 3.1)**  
Extraction of read depth information

**MergeTagsByTaxaFilesPlugin**

Perl scripts and **BEDTools (v2.17.0)**  
Processing of read depth and coverage information

**TagsToSNPByAlignmentPlugin**

**MergeDuplicateSNPsPlugin**

**FreeBayes (v 0.9.15)**  
Sequence variant calling and genotyping

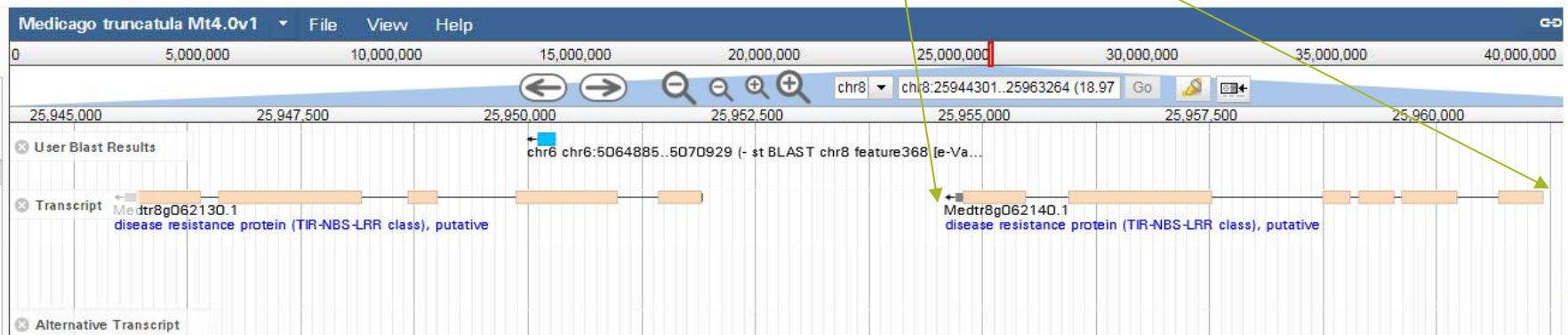
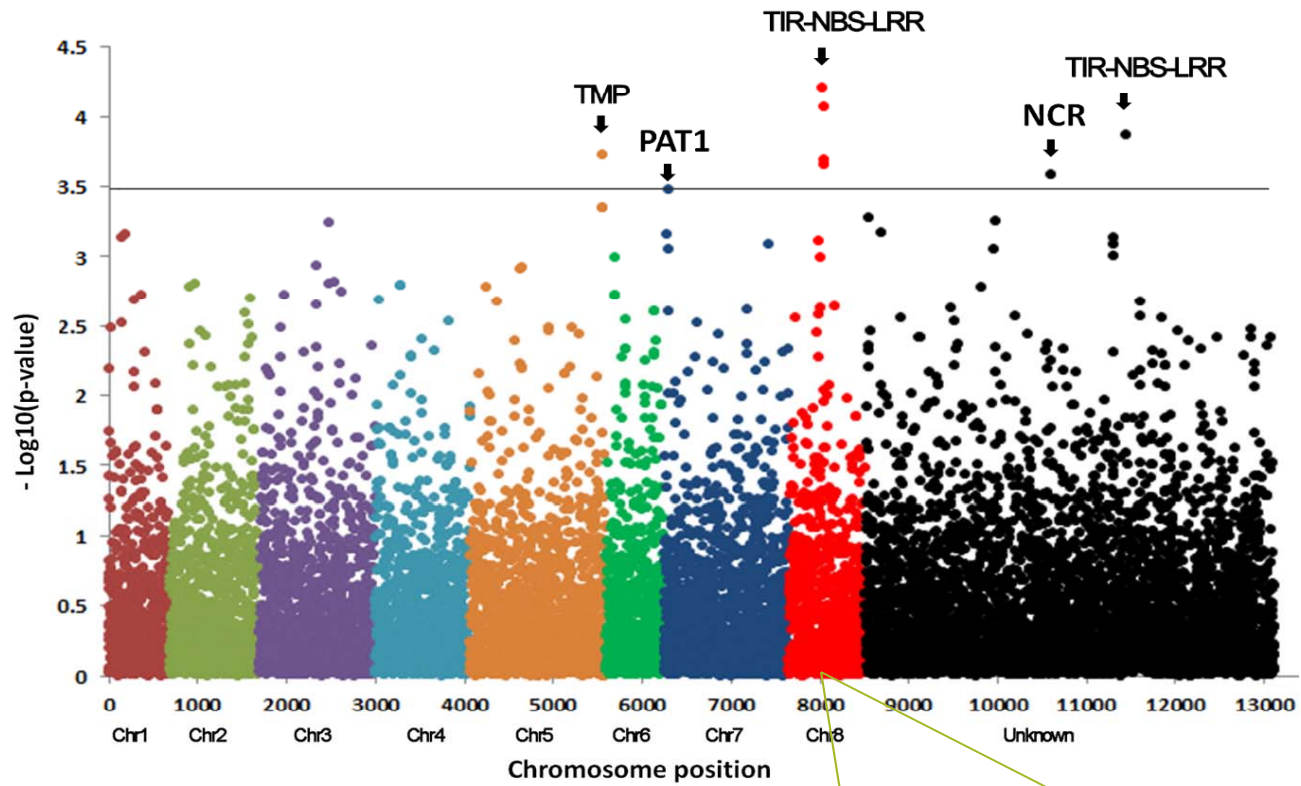
**tbt2vcfPlugin**

**MergeDuplicateSNP\_vcf\_Plugin**

Perl scripts and **VCFTools**  
Filtering genotype calls

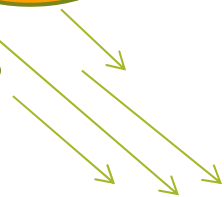
**GBSHapMapFiltersPlugin**

SNPs  
(Variants)





Avirulence



ARTICLE

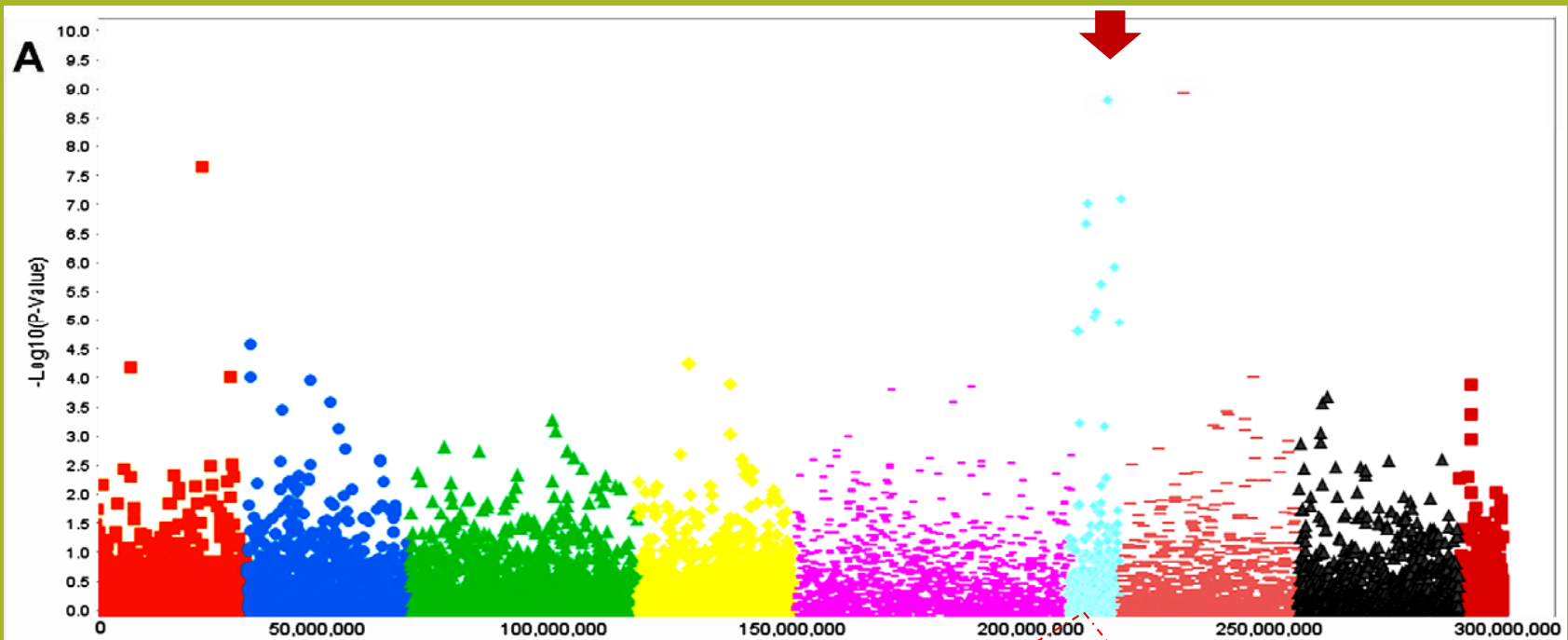
Received 4 Sep 2014 | Accepted 20 Jan 2015 | Published 6 Mar 2015

DOI: 10.1038/ncomms7338

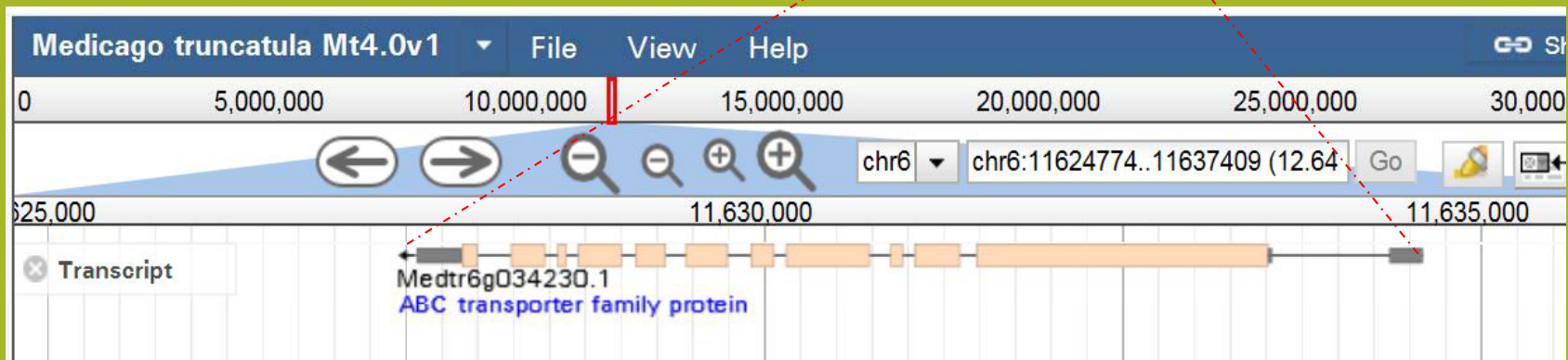
## Two linked pairs of *Arabidopsis* TNL resistance genes independently confer recognition of bacterial effector AvrRps4

Simon B. Saucet<sup>1,\*</sup>, Yan Ma<sup>1,\*</sup>, Panagiotis F. Sarris<sup>1</sup>, Oliver J. Furzer<sup>1</sup>, Kee Hoon Sohn<sup>2</sup> & Jonathan D.G. Jones<sup>1</sup>

Plant immunity requires recognition of pathogen effectors by intracellular NB-LRR immune receptors encoded by *Resistance* (*R*) genes. Most *R* proteins recognize a specific effector, but some function in pairs that recognize multiple effectors. *Arabidopsis thaliana* TIR-NB-LRR proteins RRS1-R and RPS4 together recognize two bacterial effectors, AvrRps4 from *Pseudomonas syringae* and PopP2 from *Ralstonia solanacearum*. However, AvrRps4, but not PopP2, is recognized in *rrs1/rps4* mutants. We reveal an *R* gene pair that resembles and is linked to RRS1/RPS4, designated as RRS1B/RPS4B, which confers recognition of AvrRps4 but not PopP2. Like RRS1/RPS4, RRS1B/RPS4B proteins associate and activate defence genes upon AvrRps4 recognition. Inappropriate combinations (RRS1/RPS4B or RRS1B/RPS4) are non-functional and this specificity is not TIR domain dependent. Distinct putative orthologues of both pairs are maintained in the genomes of *Arabidopsis thaliana* relatives and are likely derived from a common ancestor pair. Our results provide novel insights into paired *R* gene function and evolution.



**B**



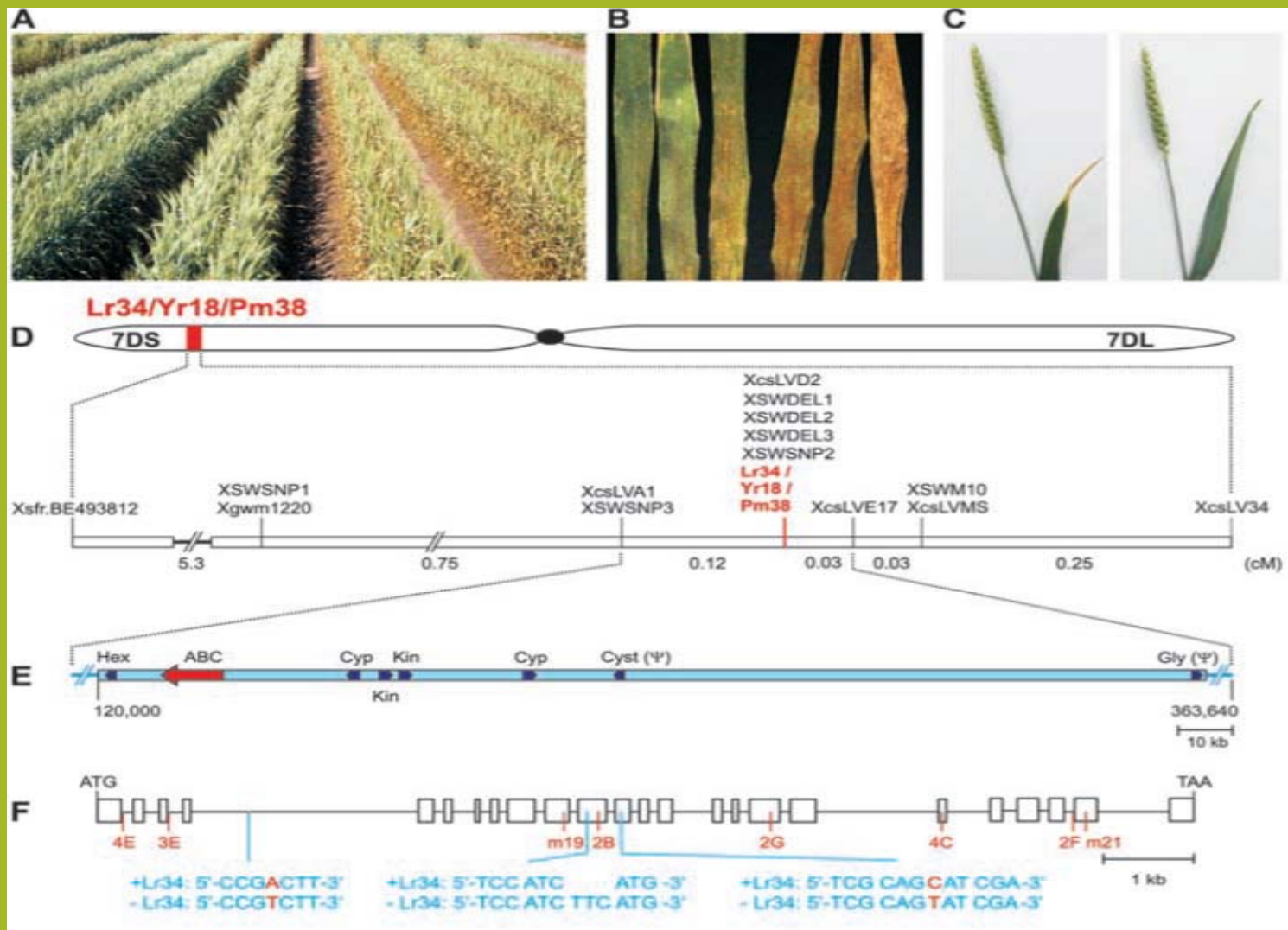


**A Putative ABC Transporter Confers Durable Resistance to Multiple Fungal Pathogens in Wheat**

Simon G. Krattinger, *et al.*

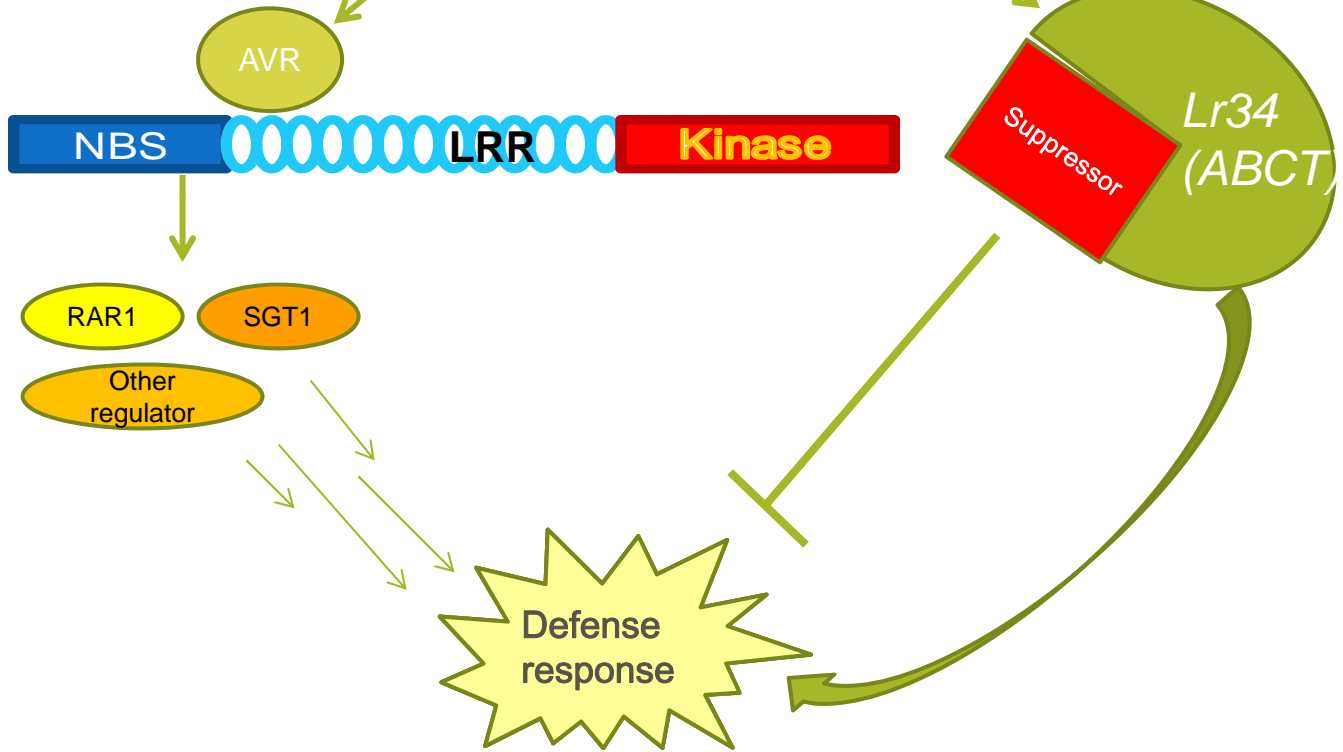
*Science* **323**, 1360 (2009);

DOI: 10.1126/science.1166453





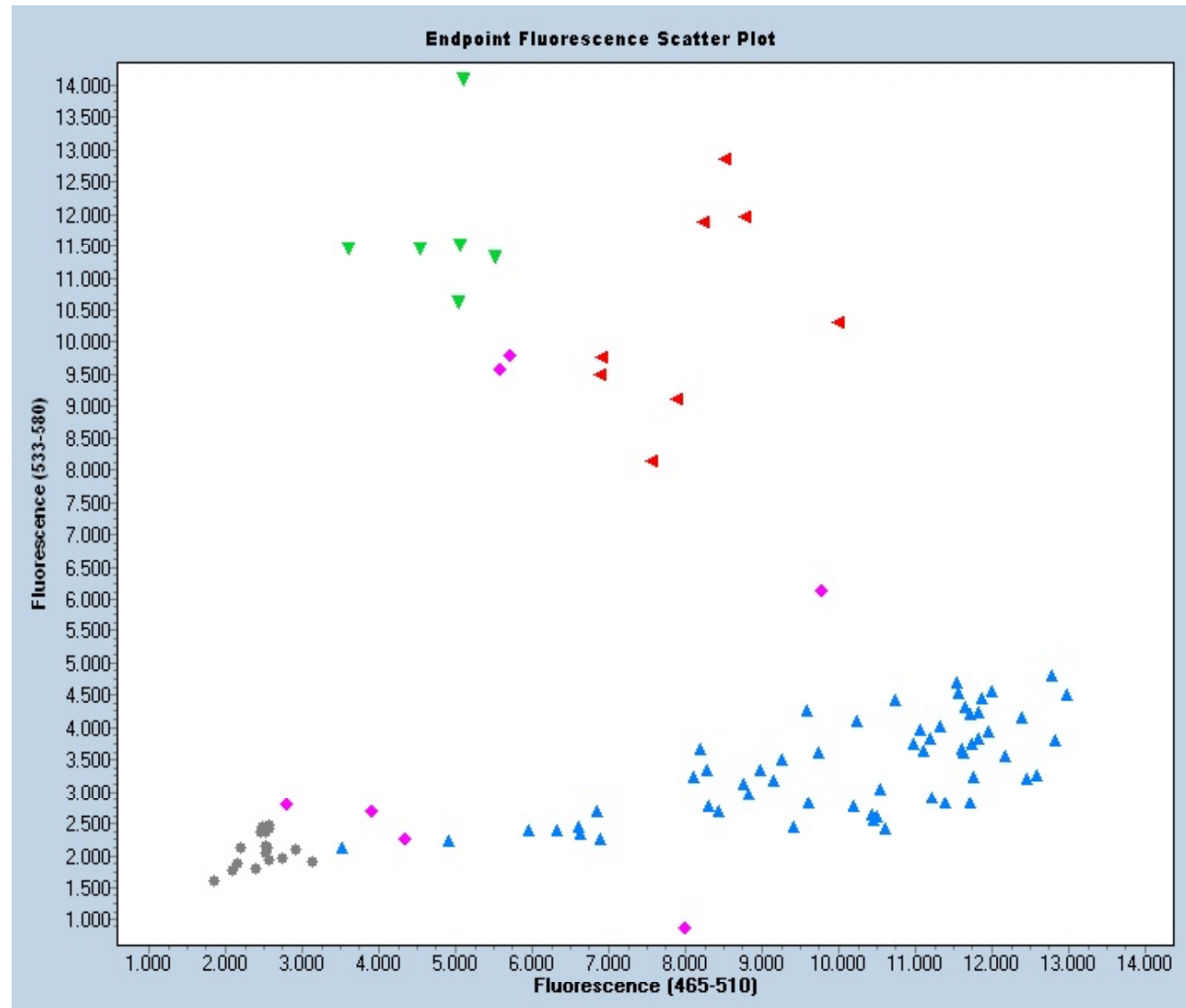
Pathogen



# KASP assay

Marker:  
S6\_330143983

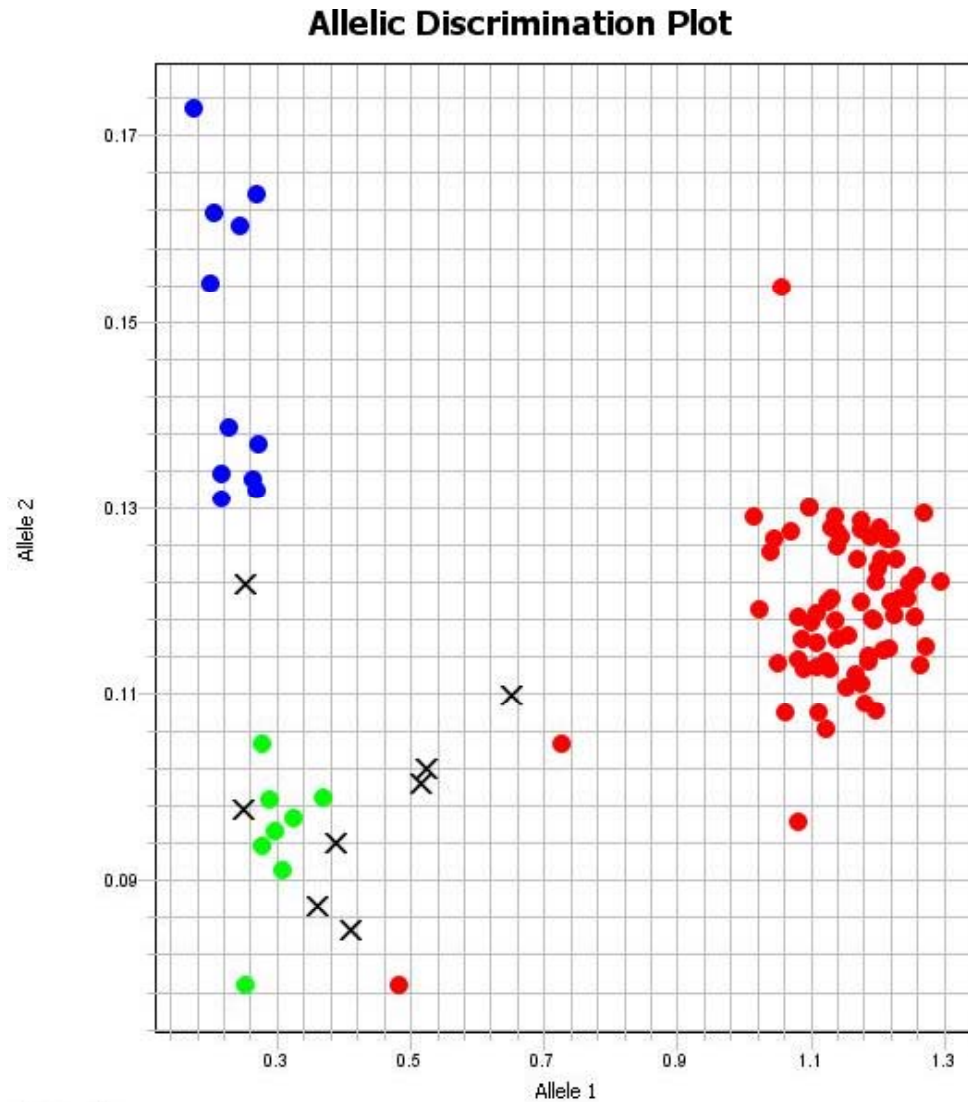
Sample: S&W



# Taqman assay

Marker: AH21CTH  
linked to NBS-LRR

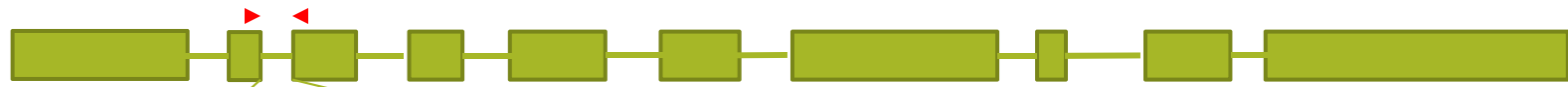
Sample: PGI



#### Legend

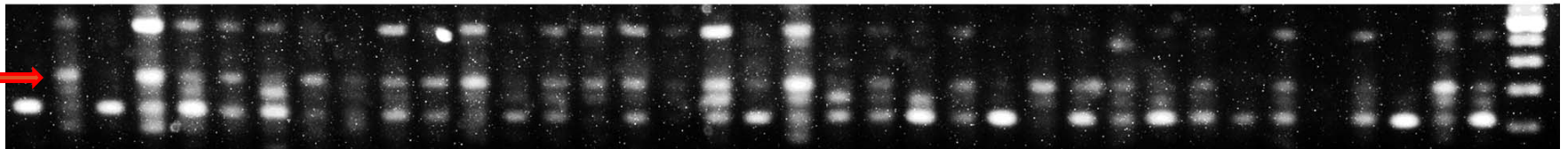
- Homozygous Allele 1/Allele 1
- Homozygous Allele 2/Allele 2
- Heterozygous Allele 1/Allele 2
- × Undetermined

# Development of intron-spanning marker derived from ABC transporter



S30 R31 S31 R32 S32 R33 S33 R34 S34 R35 S35 R36 S36 R37 S37 R38 S38 R39 S39 R40 S40 R41 S41 R42 S42 R43 S43 R44 S44 R45 S45 RO SO RS SS VR VR Ladder

R



**MAS for parents with disease resistance**

**Selected variety  
(Resistant)**

**Elite variety  
(susceptible, high  
yield and quality)**

X

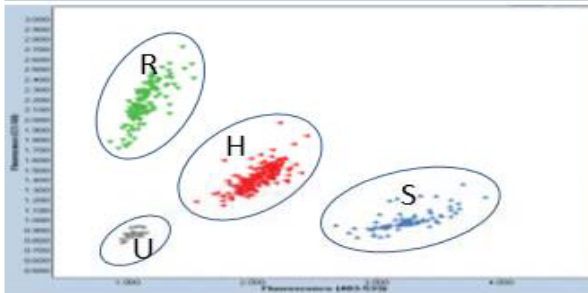
**F1**

X

**Elite variety**

**BC1**

**MAS recurrent selection**



**Improved disease resistance with  
high yield and quality**

# Acknowledgement

## **Prosser team:**

Dr. Charles Hawkins  
Dr. Xiangping Liu  
Dr. Tiejun Zhang  
Dr. Xinchun Liu  
Ms. Dinara Oshanova  
Ms. Martha Rivera  
Mr. Bill Boge  
Mr. Jose-Luis Godinez

## **Collaborators:**

Drs. Mark Smith, Modan Cas, S & W Seeds  
Drs. Jon Reich, Nicolas Enjalbert, Alforex seeds  
Dr. Julie Ho, Forage Genetics International  
Dr. Debby Samac, USDA-ARS PSRU, St. Paul  
Drs. Dorie Main, Ping Zhang, WSU  
Drs. Maria Monteros, Suresh Bhamidimarri, Noble  
Research Institute  
Dr. Qingchuan Yang, CAAS, China

