Barley *rpg4*/*Rpg5* Integrated Decoy Resistance to Ug99; Towards Effector Identification

Robert Brueggeman, Roshan Sharma Poudel, Shyam Solanki and Jonathan Richards

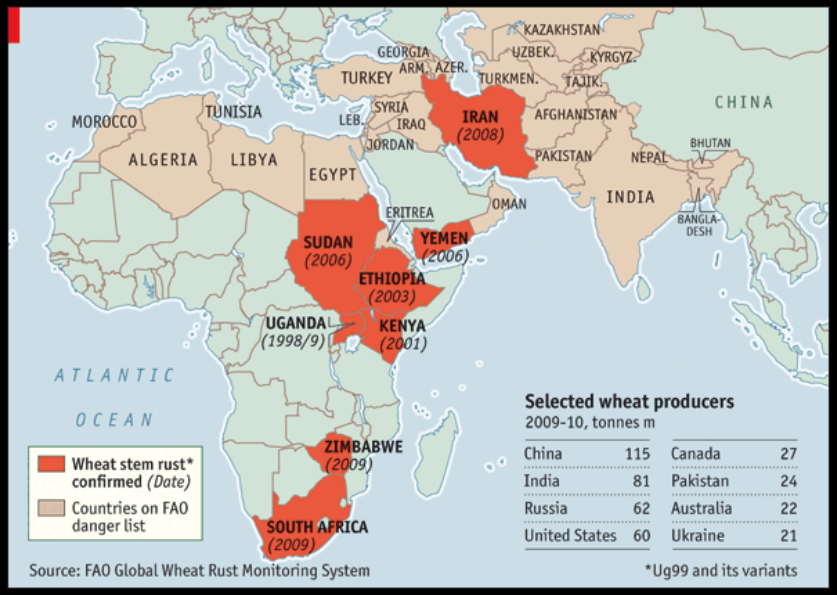


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Introduction

- Stem rust is caused by the obligate biotrophic fungal pathogen *Puccinia graminis*
- Wheat stem rust – *Puccinia graminis f. sp. tritici (Pgt)*
- Ug99 race of *Pgt*-Virulent on 97 %_of Barley (Steffenson et al., 2012) and 70% of Wheat cultivars (Singh et al., 2008).



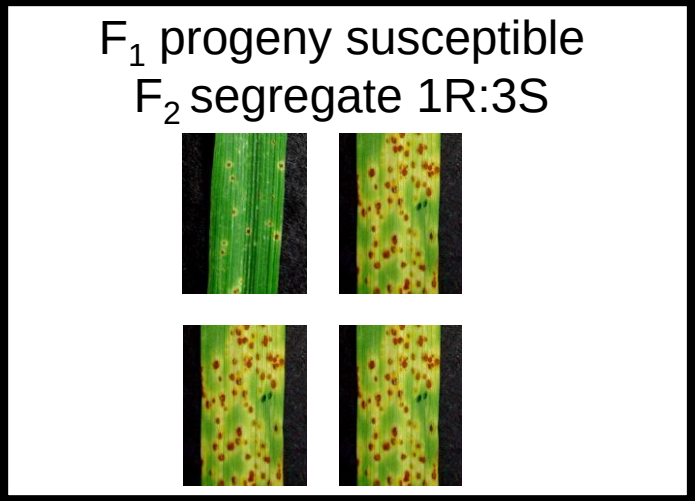
Emergence of *Pgt* Race TTKSK (Ug99)



Barley disease handbook, NDSU

rpg4/Rpg5-mediated resistance

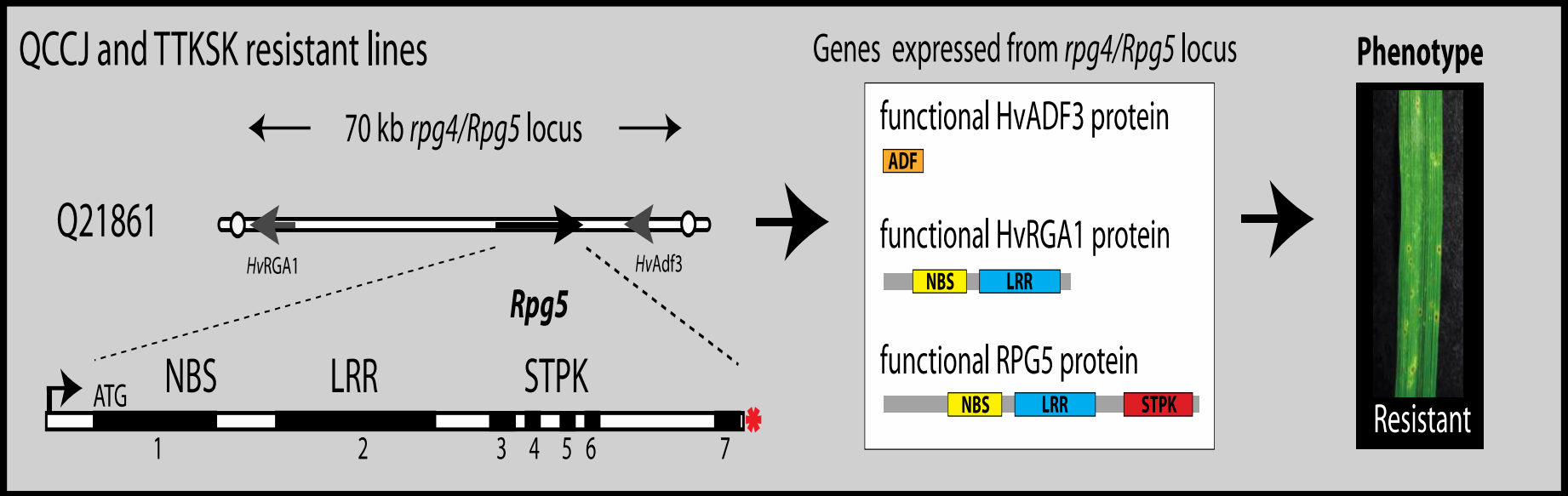
- The best source of resistance identified in the unimproved barley line Q21861.
- The recessive resistance was designated as *rpg4*.
- *rpg4* confers resistance to a broad range of *Pgt* (TTKSK (Ug99), QCCJ, MCCF, …..).



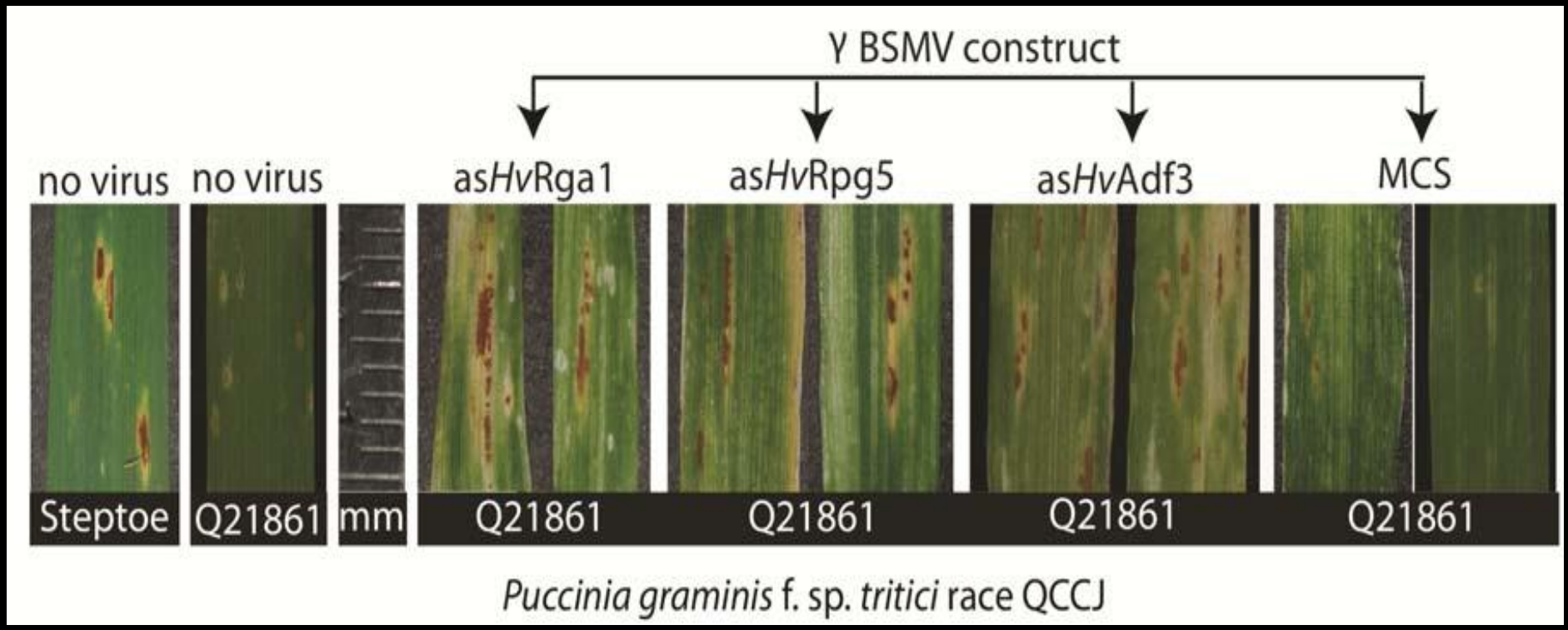
Brueggeman et al., 2009. *Cell Cycle*
Jin et al., 1994. *Phytopathology*

rpg4/Rpg5 locus in Q21861

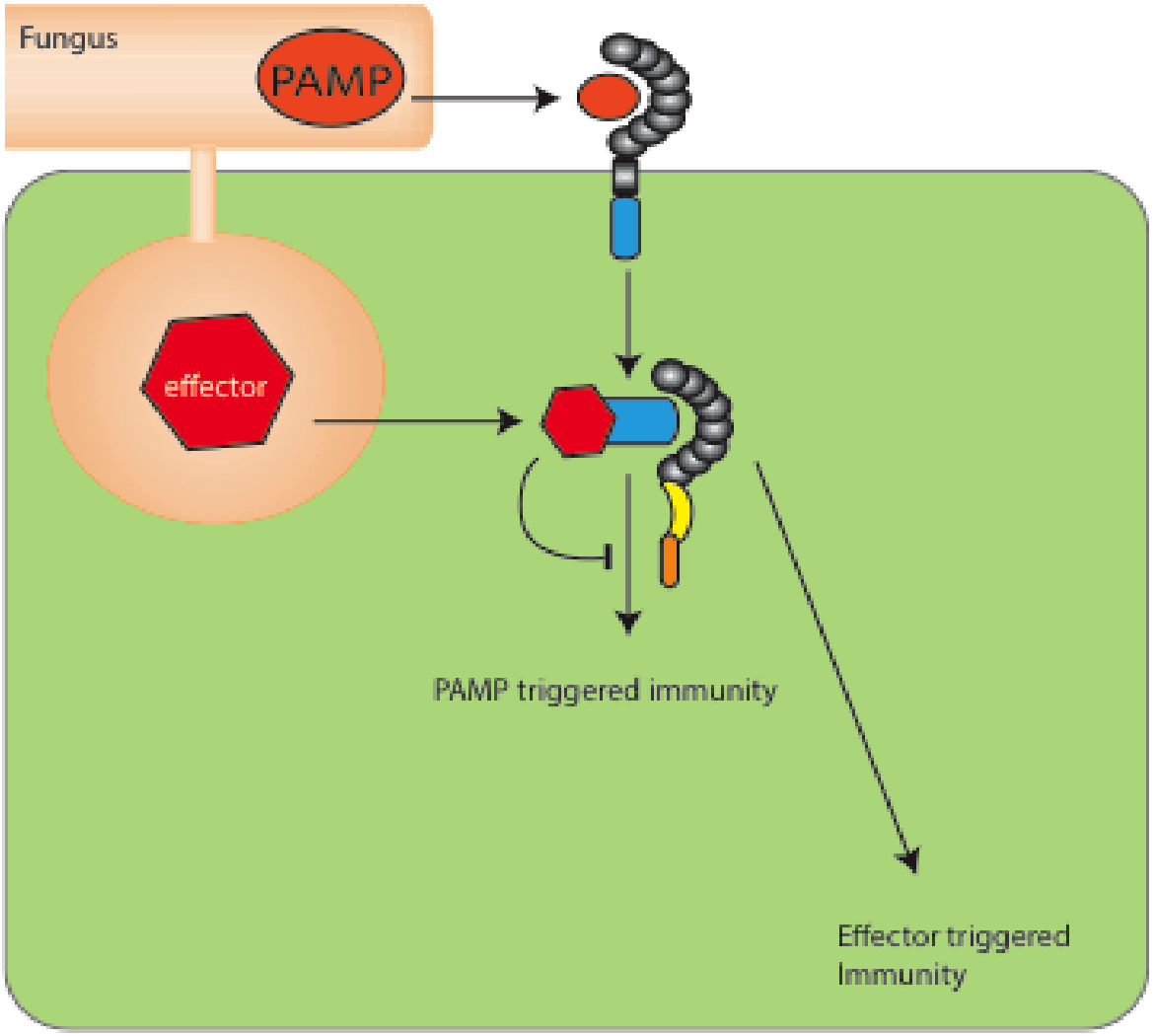
Predicted proteins encoded by candidate *rpg4* genes



All three candidate genes are required for resistance

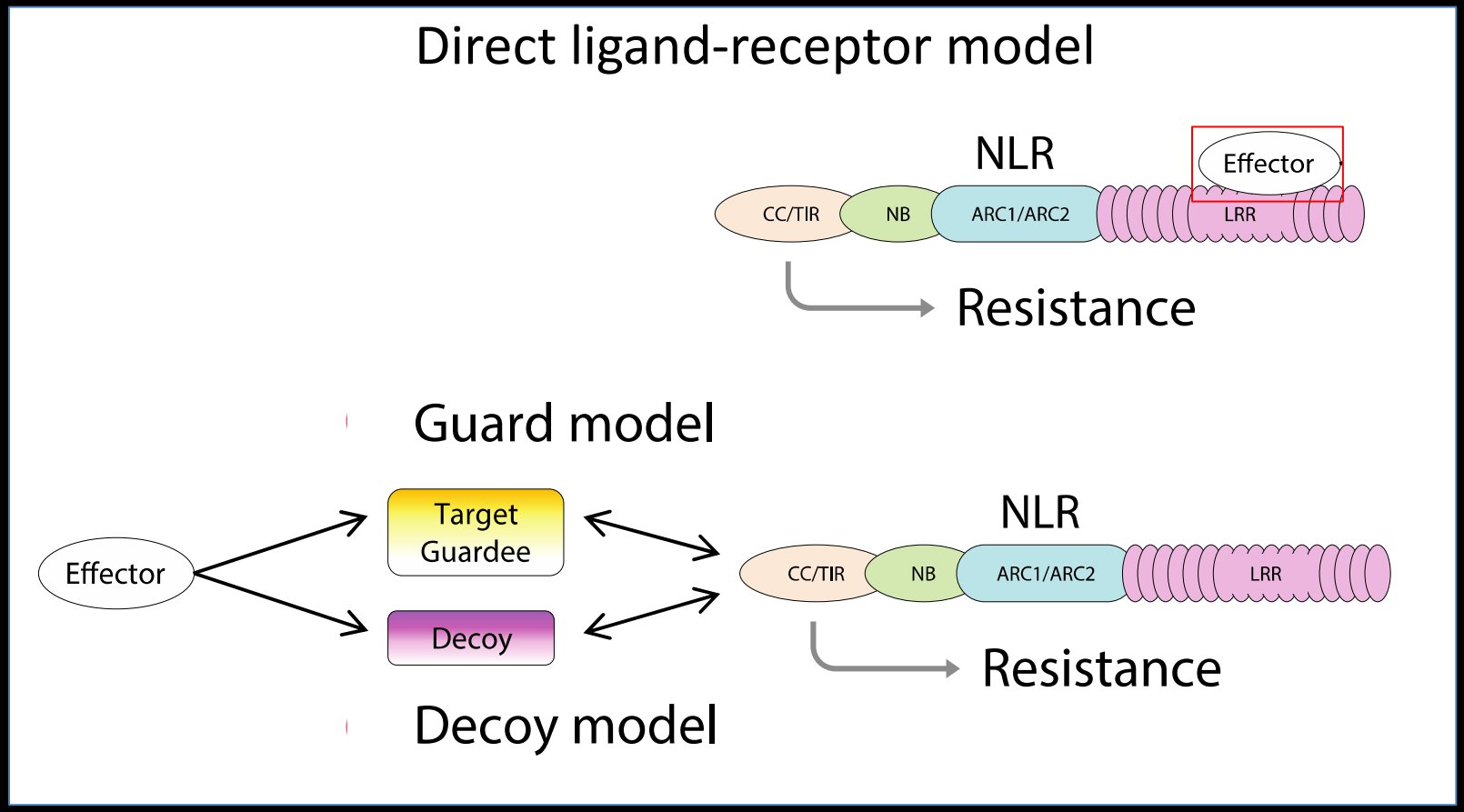


Principals of Plant Immunology



Dodds and Rathjen et al., 2010

Effector triggered immunity models



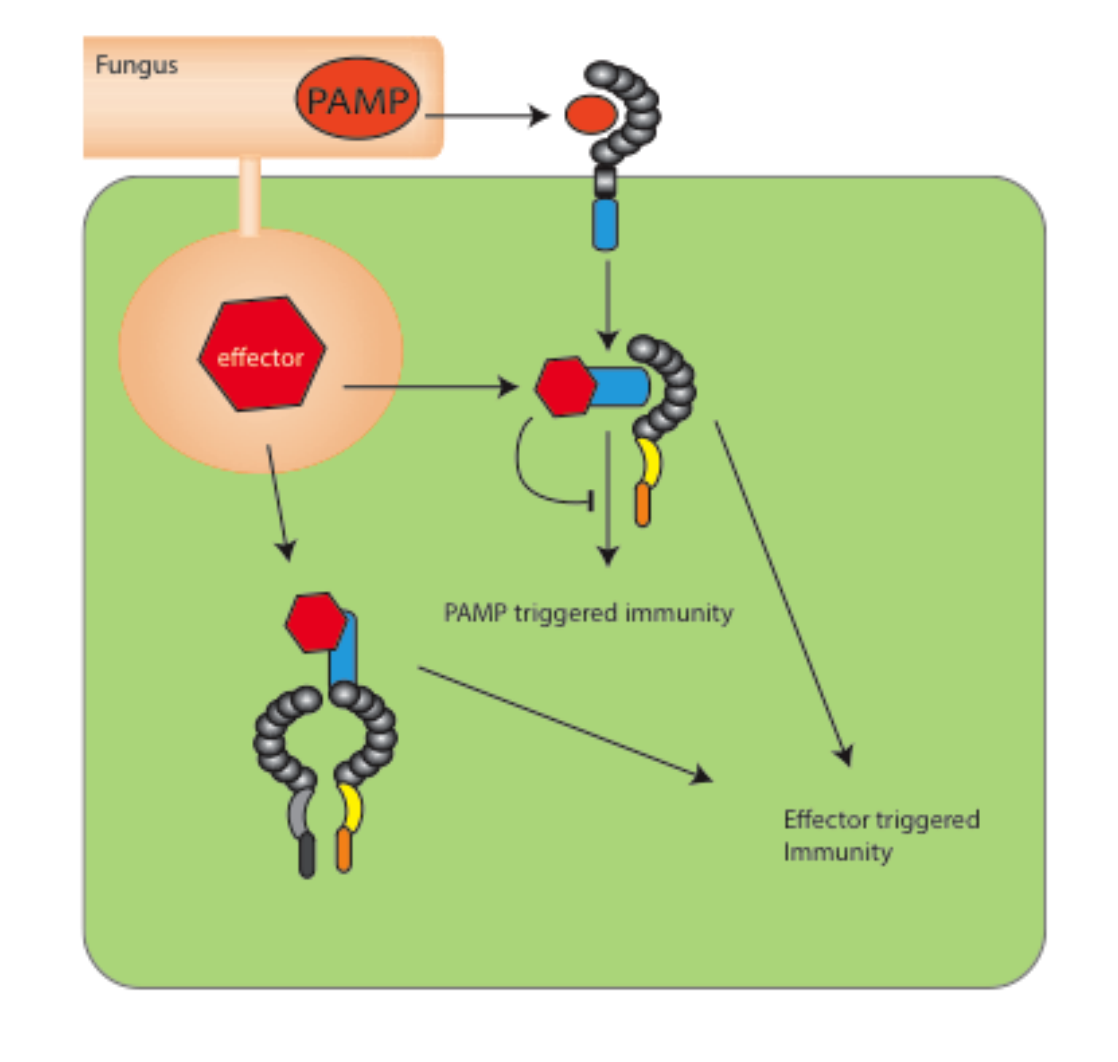
Unusual variable domain in paired NLRs

Table 1 | Unusual domains in paired NLR R proteins.

NLR pair	Pathosystem (pathogen/host)	NLR with unusual domain	Unusual or additional domain	Location of the domain	References
RRP2A RPP2B	*H. arabidopsidis*/Arabidopsis	RPP2A	TIR-NB-DUF640	N-terminus	Sinapidou et al., 2004
RRS1 RPS4	*R. solanacearum, P. syringae, C. higginsianum*/Arabidopsis	RRS1	WRKY	C-terminus	Gassmann et al., 1999; Deslandes et al., 2003; Birker et al., 2009; Narusaka et al., 2009
RGA4 RGA5	*M. oryzae*/rice	RGA5	RATX1	C-terminus	Okuyama et al., 2011; Cesari et al., 2013
Pik-1 Pik-2	*M. oryzae*/rice	Pik-1	RATX1	Between the CC and NB	Ashikawa et al., 2008; Yuan et al., 2011; Cesari et al., 2013; Zhai et al., 2014
Pi5-1 Pi5-2	*M. oryzae*/rice	Pi5-2	AvrRpt2-cleavage site	C-terminus	Lee et al., 2009
Fom-1 Prv	*F. oxysporum*, Papaya ring-spot virus/melon	Prv	NB	C-terminus	Brotman et al., 2012
RGA1 Rpg5	*P. graminis*/barley	Rpg5	Protein kinase	C-terminus	Wang et al., 2013
Lr10 RGA2	*P. triticina*/wheat	RGA2	NB	Between the CC and NB	Loutre et al., 2009

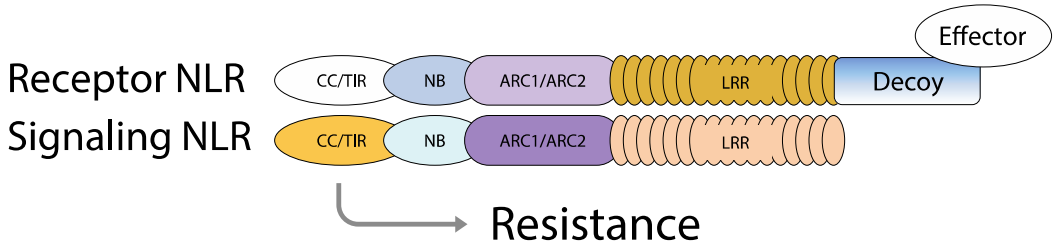
Cesari et al., 2014

Integrated Decoy Model

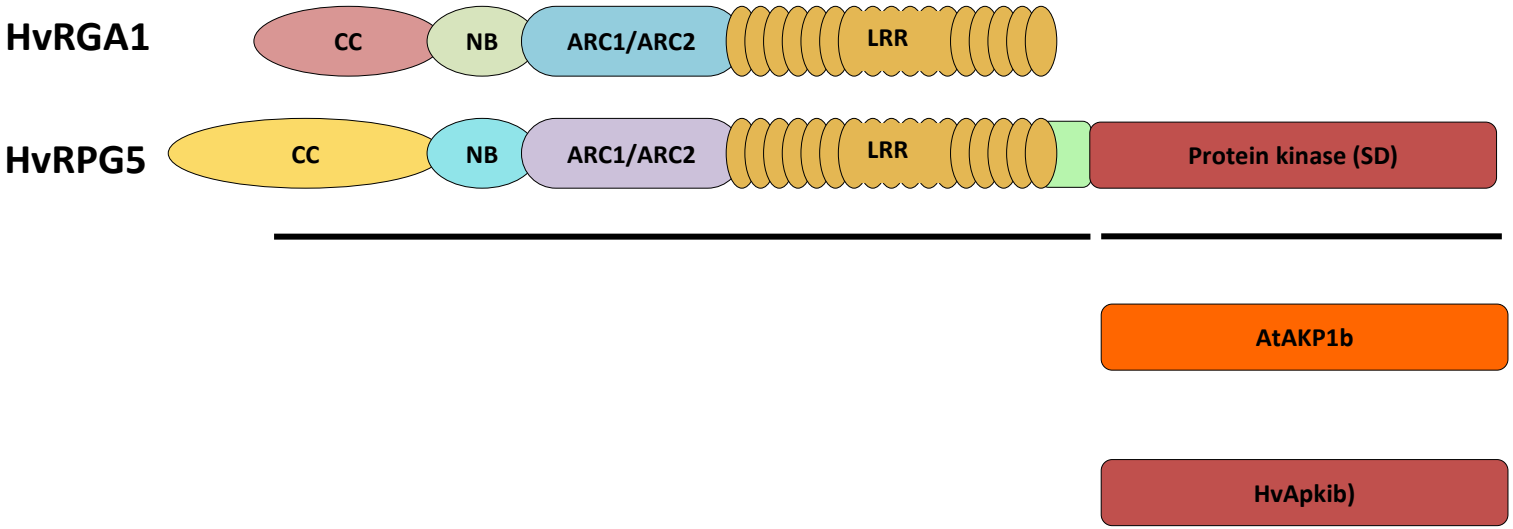


Integrated decoy Model

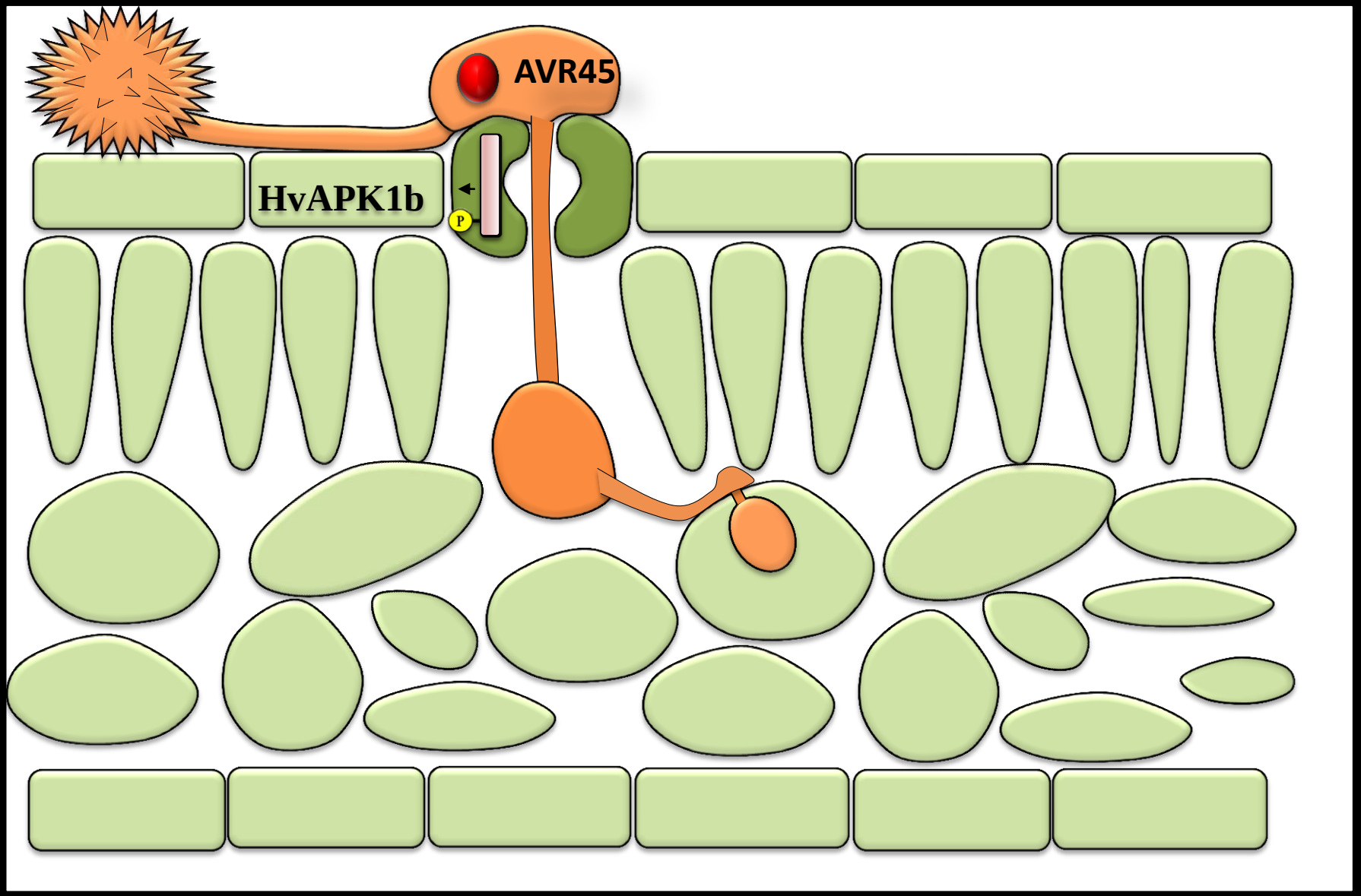
Integrated decoy model



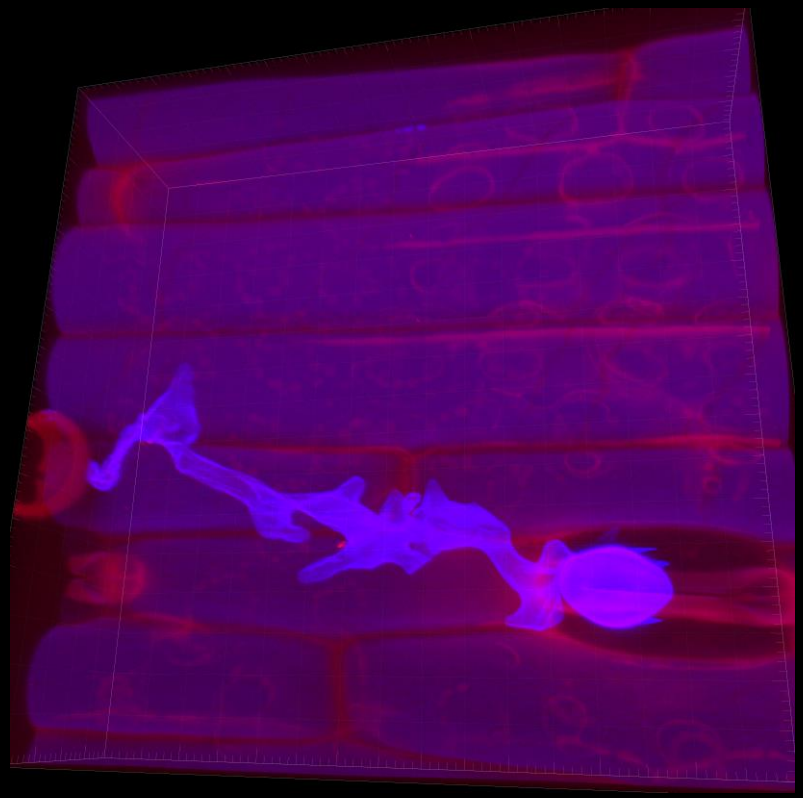
Cesari et al., 2014, Frontiers in Plant Science

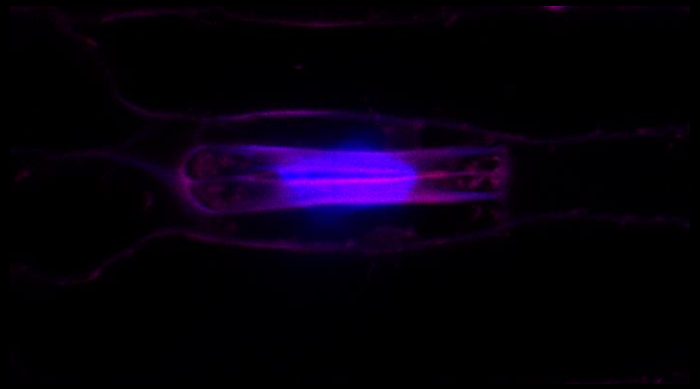


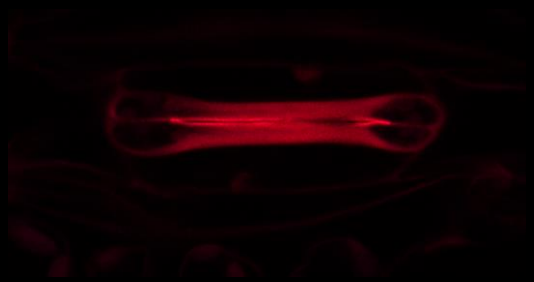
Stomata Opening Model



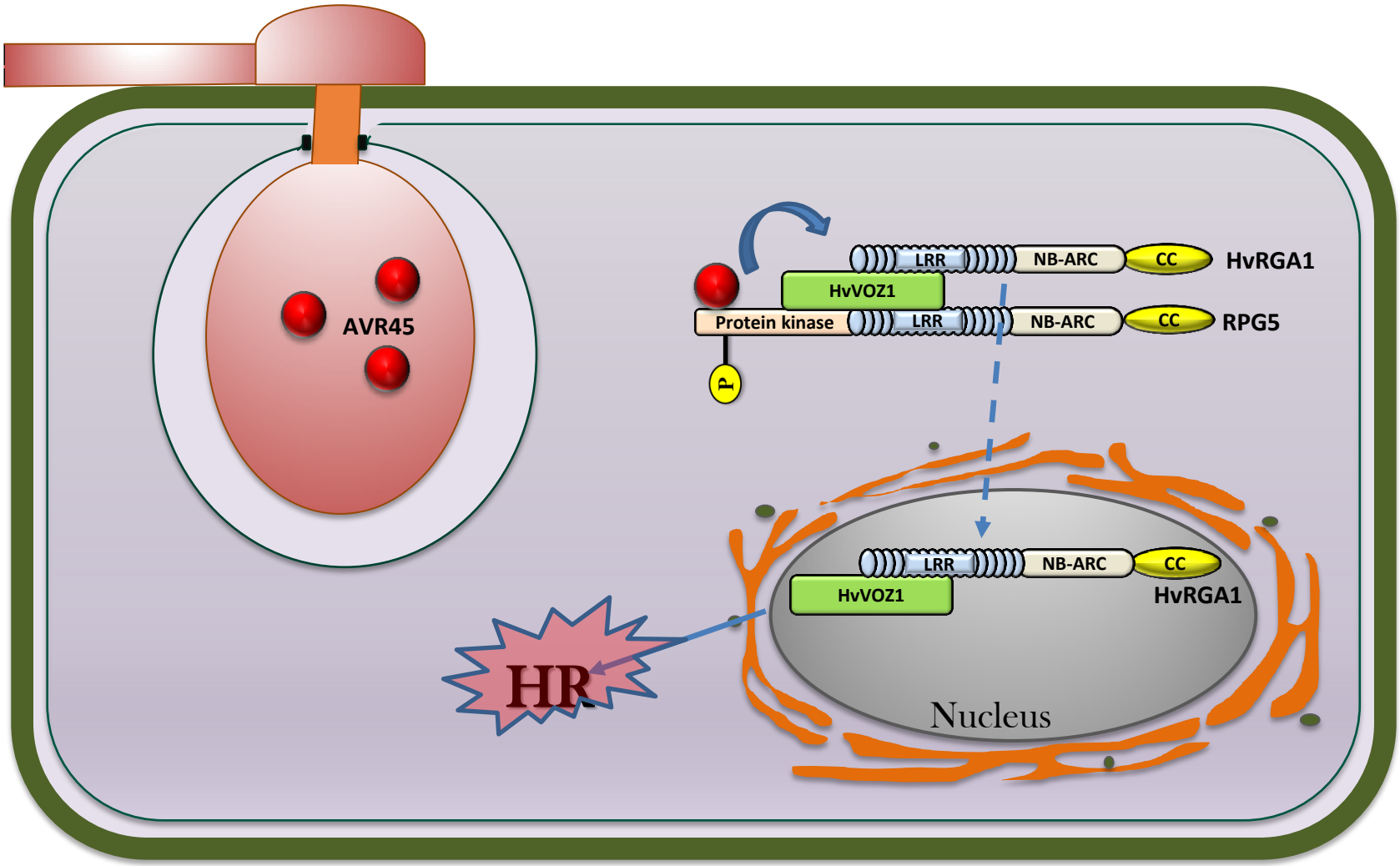
Histology







Rpg5/HvRga1 protein complex model



Avr-r4/5 identification

- Barley differentials; Q21861 (*rpg4*/*Rpg5*+, *Rpg1*+), HQ1 (*Rpg5*+, *rpg1*-), Chevron & Morex (*Rpg4/rpg5*-/*Rpg1*+) and Harrington (*Rpg4/rpg5*-,*Rpg1*-) were used as barley differentials.
- Phenotyping data were collected by inoculating these barley differentials with 37 diverse *Pgt* isolates, collected in ND since 1970.
- Genotyping data for these 37 isolates were generated using a RAD-GBS protocol designed for Ion Torrent PGM™ platform.

RAD-GBS identified 4919 SNPs

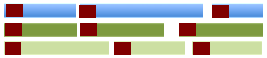
Sample Preparation → Sequencing and SNP's Calling → Candidate Gene Identification

Sample Preparation

DNA isolation from 37 isolates



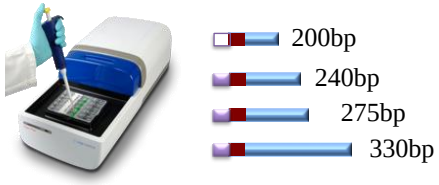
Restriction digest with ApekI and HhaI



Ligate Adaptors



Pool samples and 4 size selections using Pippin Prep



PCR amplification of each size selected library (14 cycles)

Sequencing and SNP's Calling

Sequencing in Ion Torrent PGM™



Sequence alignment and Variant calling

Burrows-Wheeler Aligner-Mem and GATK Pipelines

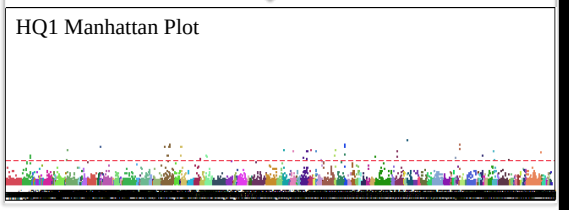
SNP

Isolate 1: AGTACCATCATGACAAGTGC
Isolate 2: AGTACCAGCATGACAAGTGC
Isolate 3: AGTACCATCATGACAAGTGC

Candidate Gene Identification

Association Mapping

11,367 SNPs + Q Model in JMP® Genomics



RNAseq Analysis

RNAseq

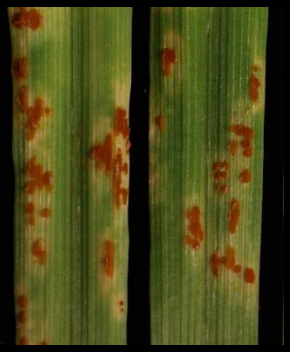
Isolate 81AC-28



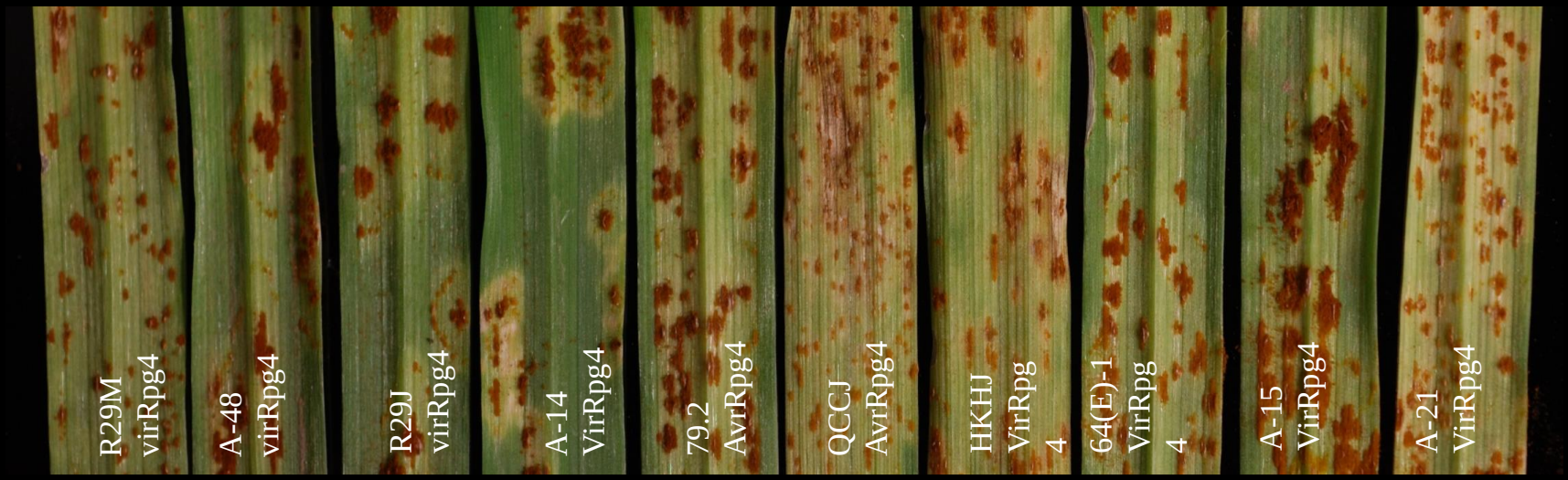
Avr-r4/5



Isolate 72-41 (*Avr-r4/5*)

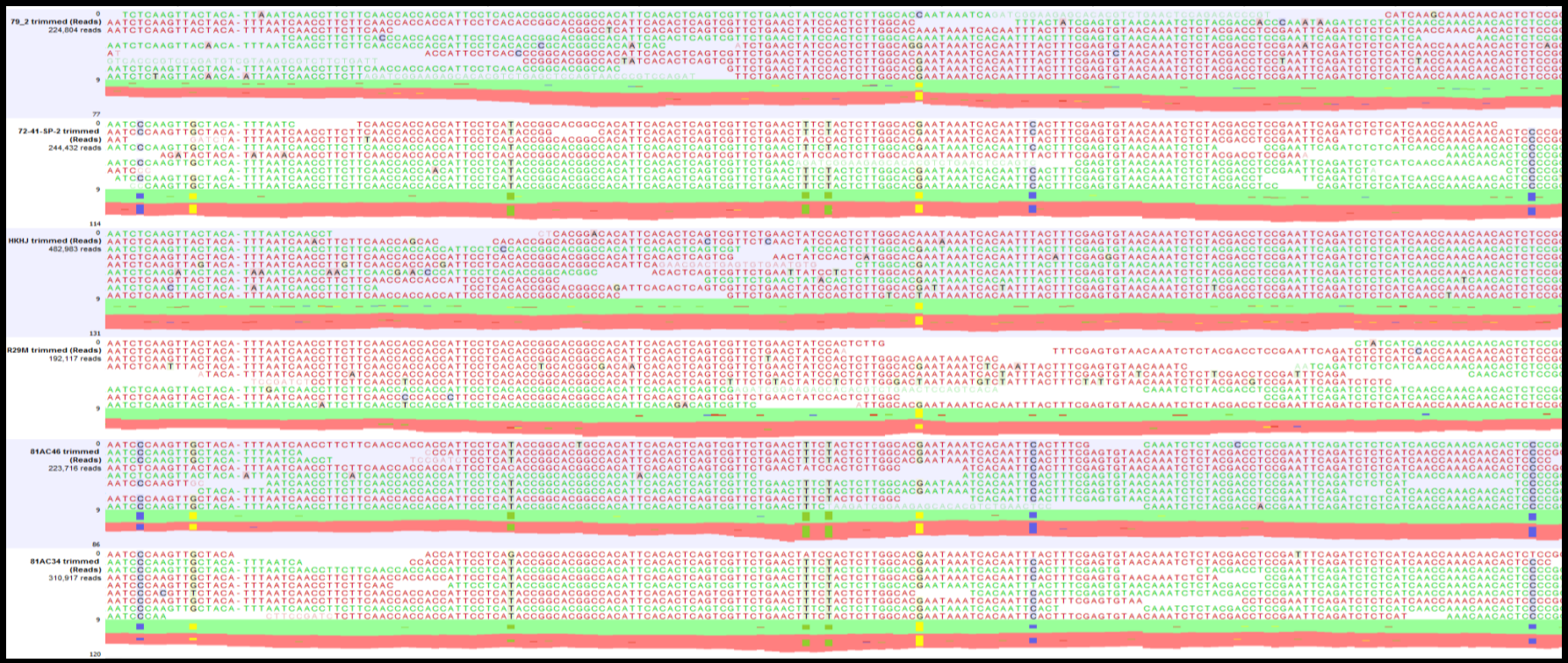


Isolate A-21 (*avr-r4/5+*



14DPI of Harrington leaf inoculated with PGT isolates. Samples for RNAseq were collected 5DPI

SNP identification



Puccinia graminis gene expression patterns

Avr-r4/5 cg1

Avr_rpg4/5

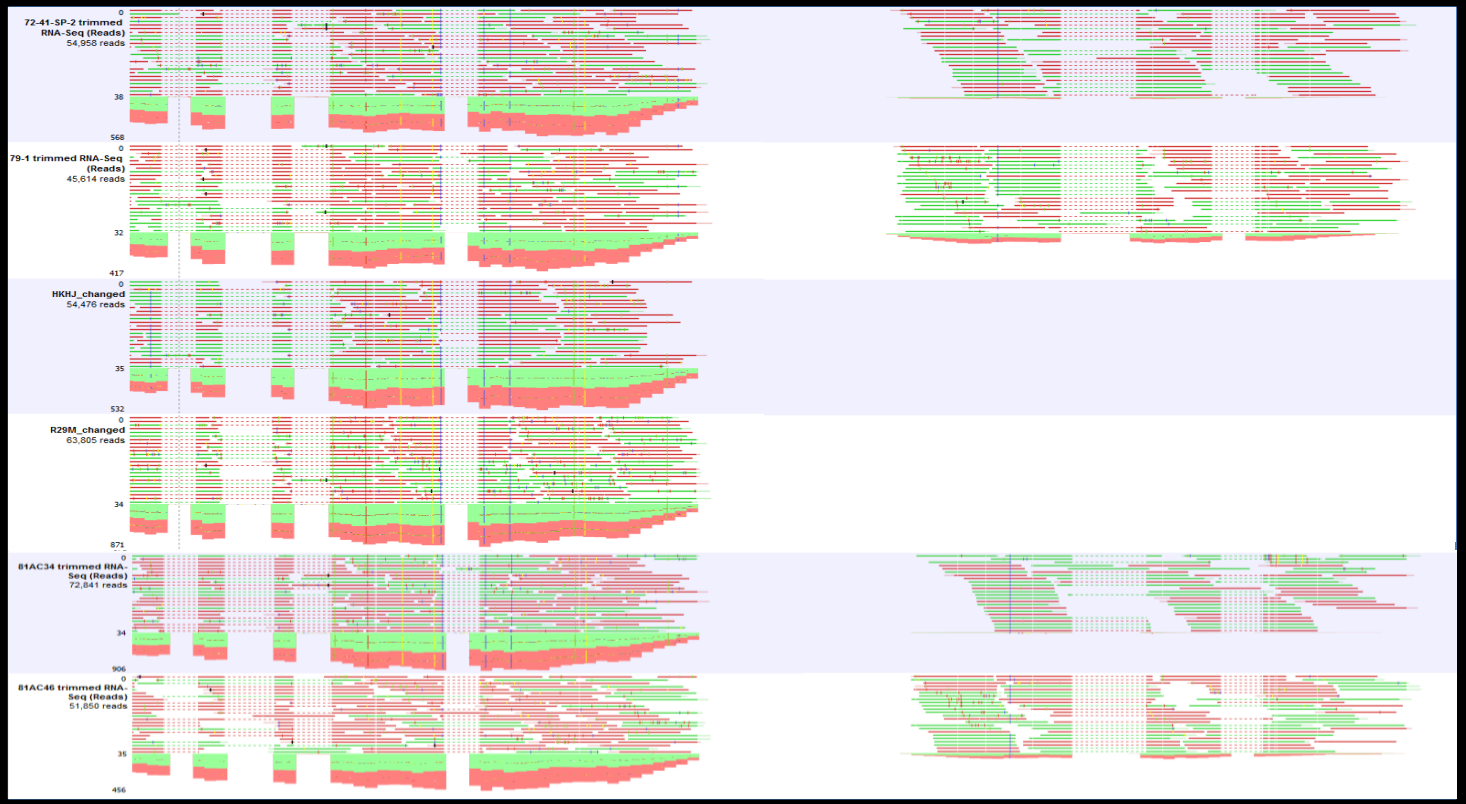
Avr_rpg4/5

Vir_rpg4/5

Vir_rpg4/5

Avr_rpg4/5

Avr_rpg4/5



Candidate Avr-r4/5 genes

		cg1	cg2	cg3	cg4	cg5	cg6	cg7
A-48	Vir_rpg4	1	0	1	59	5	121	3
R29J	Vir_rpg4	0	0	0	29	0	10	0
370C	Vir_rpg4	1	0	0	36	8	78	1
A-14	Vir_rpg4	0	0	1	57	1	41	1
R29M	Vir_rpg4	0	0	4	33	5	33	0
64E(1)-1	Vir_rpg4	1	2	4	28	3	16	0
HKHJ	Vir_rpg4	4	0	0	16	16	12	0
P84-16	Avr_rpg4	2451	895	394	5287	572	2734	1828
79-1	Avr_rpg4	990	364	139	3207	303	479	1596
72-41-SP-2	Avr_rpg4	768	147	55	2872	317	1234	1327
79.2	Avr_rpg4	768	178	153	2837	351	2228	1261
81AC28	Avr_rpg4	1476	763	281	6412	1021	1315	2555
A-12	Avr_rpg4	285	15	141	295	6	16	7
AC-12	Avr_rpg4	540	29	209	1353	147	96	0
81AC34	Avr_rpg4	284	98	236	216	347	2921	163
81AC46	Avr_rpg4	879	249	233	4216	845	5	1032

Concluding remarks

- Plant genomes can rearrange to attach virulence targets to immune receptors as “bait” to trap pathogens

- Understanding the mechanisms underlying integrated decoy mechanisms may present opportunities to engineer novel resistance mechanisms

Acknowledgements

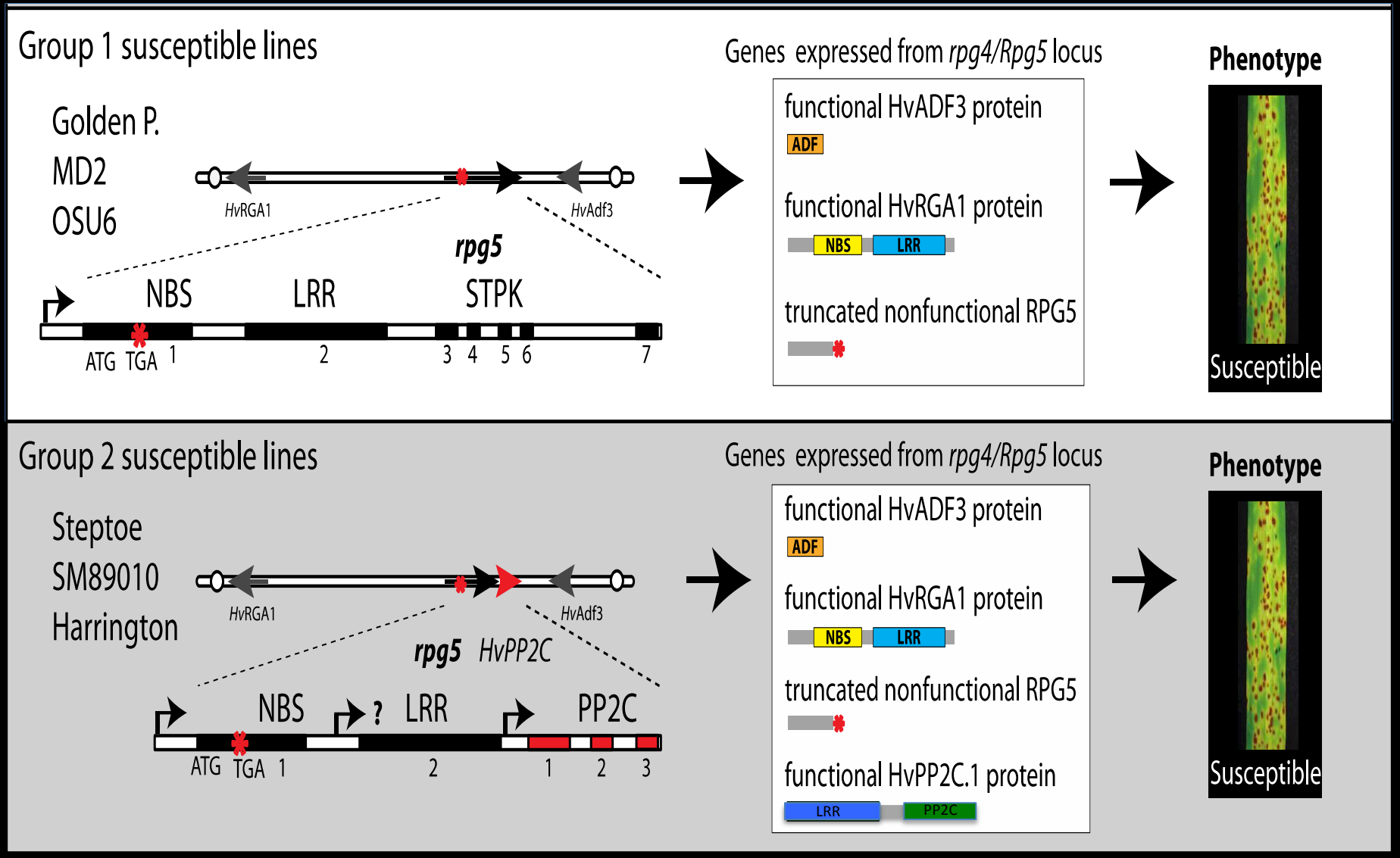
Shyam Solanki
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Jon Richards
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Deepika Arora
Gazala Ameen

NDSU
Barley Pathology

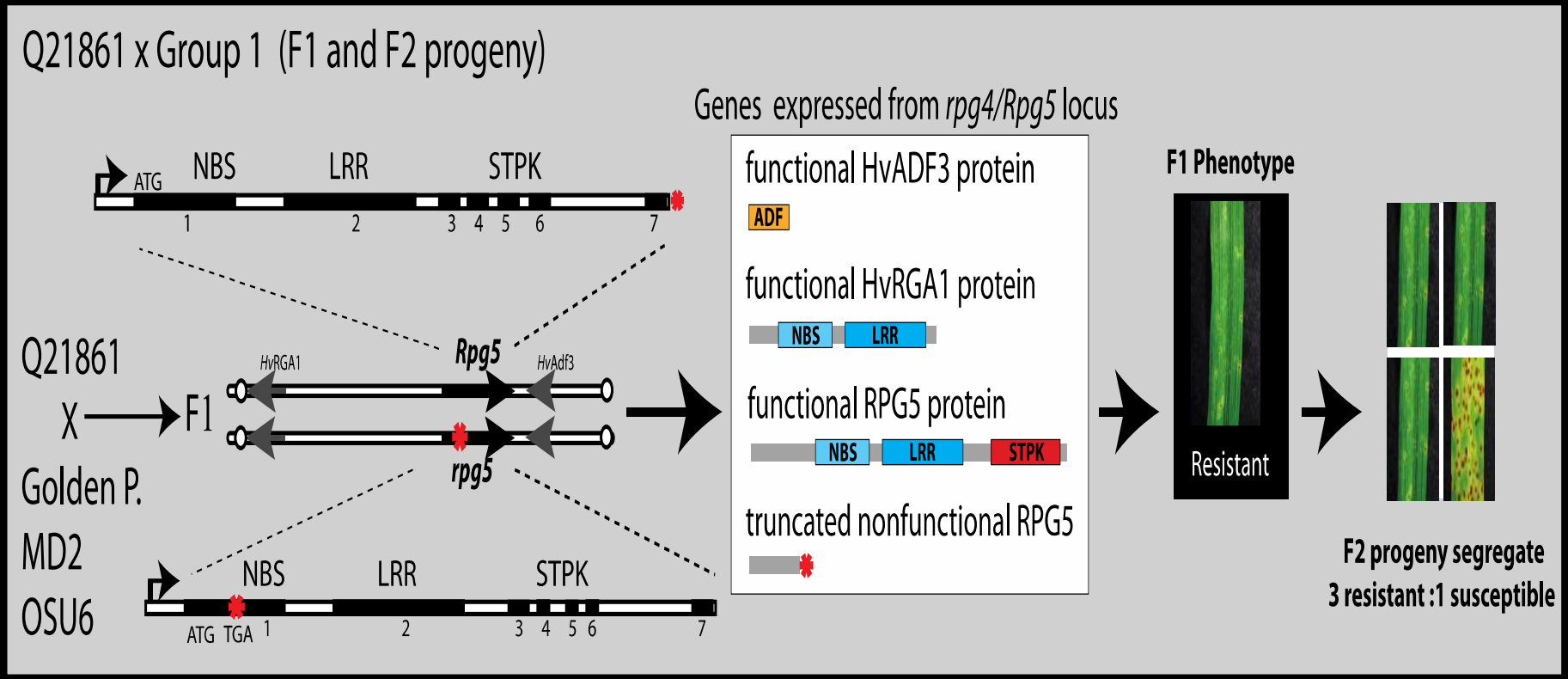
North Dakota State University

NSF

Allele analysis revealed clue to recessive resistance



Genetics suggest that *rpg5* alleles that do not contain the *PP2C* domain result in heterozygous resistant individuals (dominant resistance)



Genetics suggest that *rpg5* alleles containing *PP2C* suppress resistance (recessive resistance)

