



Molecular diversity in the resistance interactions of wheat and its fungal pathogens

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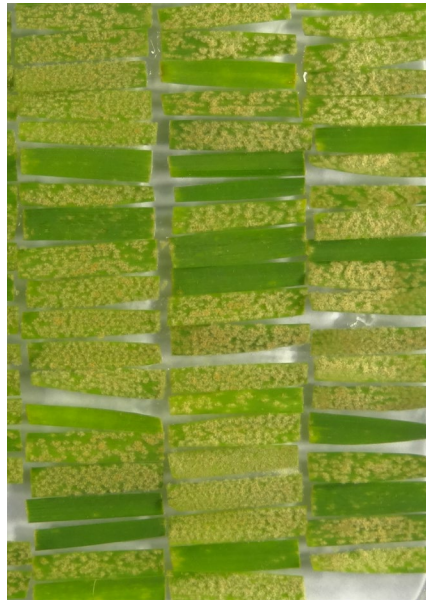
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Powdery mildew and leaf rust disease in wheat

About 10 % of the potential wheat production is lost by diseases (Oerke 2010, Savary et al. 2019)



Powdery mildew (*Blumeria graminis* f.sp. *tritici*)

Leaf rust (*Puccinia triticina*)

Obligate biotrophic ascomycete and basidiomycete pathogens, respectively.

RESEARCH ARTICLE SUMMARY

IWGSC, 2018. Science

WHEAT GENOME

Shifting the limits in wheat research and breeding using a fully annotated reference genome

International Wheat Genome Sequencing Consortium (IWGSC)*

The International Wheat Genome Sequencing Consortium (IWGSC)

IWGSC RefSeq principal investigators: Rudi Appels^{1,36*‡}, Kellye Eversole^{2,3*‡}, Catherine Feuillet¹⁷, Beat Keller⁴¹, Jane Rogers^{6‡}, Nils Stein^{4,5*‡}.



Article

Multiple wheat genomes reveal global variation in modern breeding

<https://doi.org/10.1038/s41586-020-2961-x>

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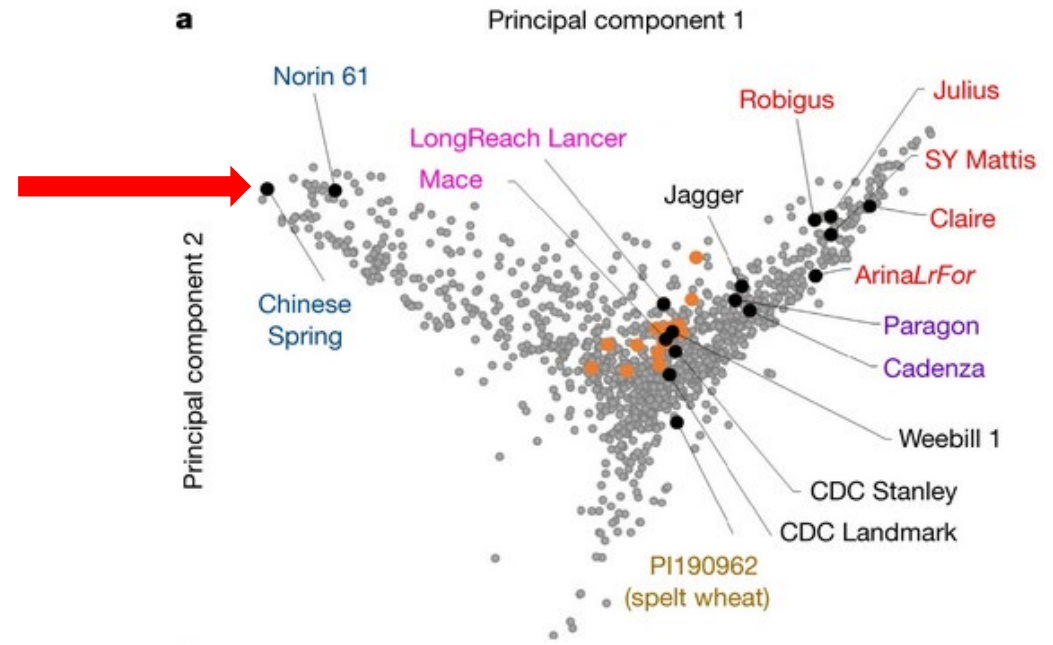
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Cereals have an enormous genetic diversity in the gene pools of wild progenitors and domesticated crops which are largely unexplored

=> A rich resource for the identification of novel resistance genes



Photo:
Rebecca Leber, UZH
Collection of wheat
landraces with a
focus on the Near
and Middle East

Gene isolation from elite material: Durable leaf rust resistance in the Swiss cultivar 'Forno'

Resistance in cv. Forno is quantitative and has been durable over many years in Europe



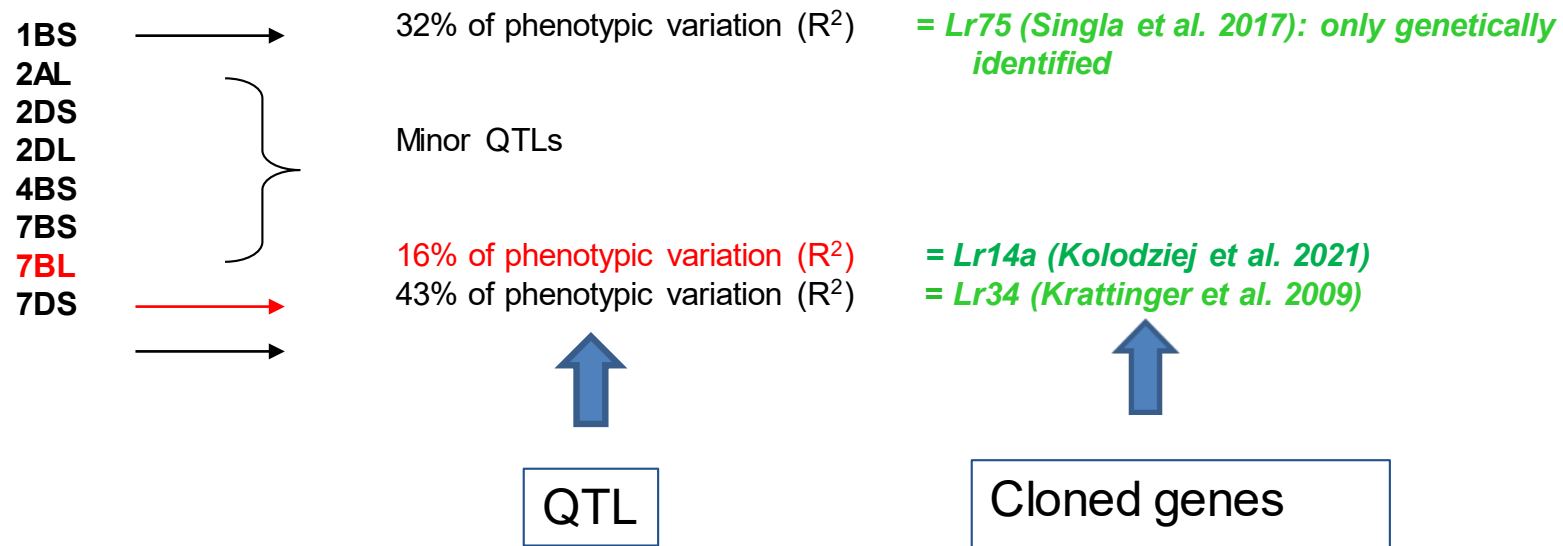
cv. 'Arina': Swiss winter wheat, susceptible



cv. 'Forno': Swiss winter wheat, durably resistant

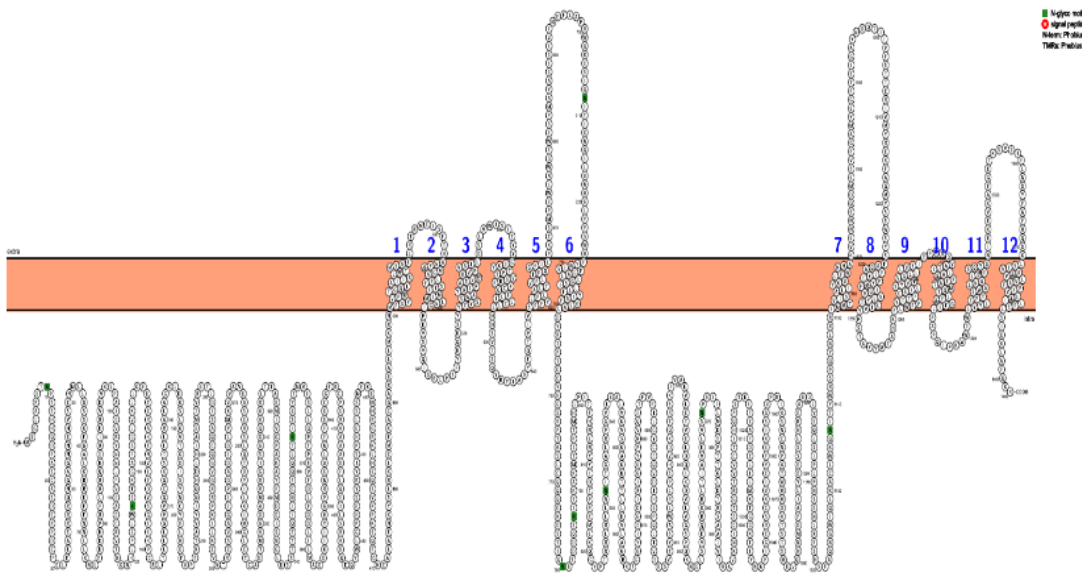
Quantitative and durable leaf rust resistance in cultivar Forno: identification of molecular components underlying resistance

QTL analysis revealed **8 chromosomal regions** contributing to the quantitative and durable leaf rust resistance in population of 240 recombinant inbred line population derived from a cross “Arina” x “Forno”:



Schematic model of Lr34 protein

Lr34: an ABCG transporter



Structural model of the LR34 protein: an ABCG-type of transporter (Krattinger et al. 2009)

Lr34 is likely an ABA (abscisic acid) transporter (Krattinger et al. 2019, Bräunlich et al. 2021)

Lr34 is functionally transferable across all major cereals



maize

maize rust
(*Puccinia sorghi*)
biotrophic

northern corn leaf blight
(*Exserohilum turcicum*)
hemi-biotrophic

Sucher et al. 2017
Plant Biotech. J.



rice

rice blast
(*Magnaporthe oryzae*)
hemi-biotrophic

Krattinger et al. 2016
Plant Biotech. J.



barley

barley leaf rust
(*Puccinia hordei*)
biotrophic

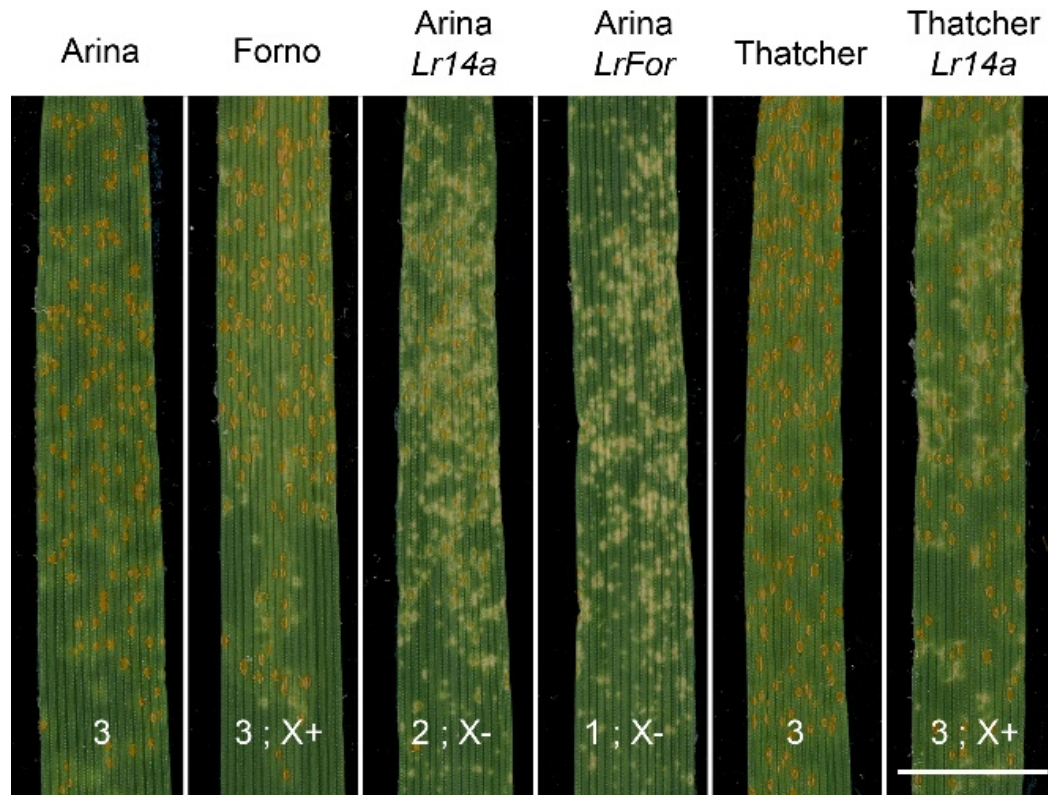
barley powdery mildew
(*Blumeria graminis* f.sp.
hordei)
biotrophic

stem rust
(*Puccinia graminis* f.sp.
tritici)
Biotrophic

Risk et al. 2013, Plant
Biotech. J.
Harsh et al. 2015, Plant J.

The QTL on chromosome 7BL confers a rust resistance phenotype very similar to the seedling resistance gene *Lr14a* described 50 years ago

Seedling infections of wheat genotypes Arina, Forno, Arina*Lr14a*, Arina*LrFor*, Thatcher, Thatcher*Lr14a*

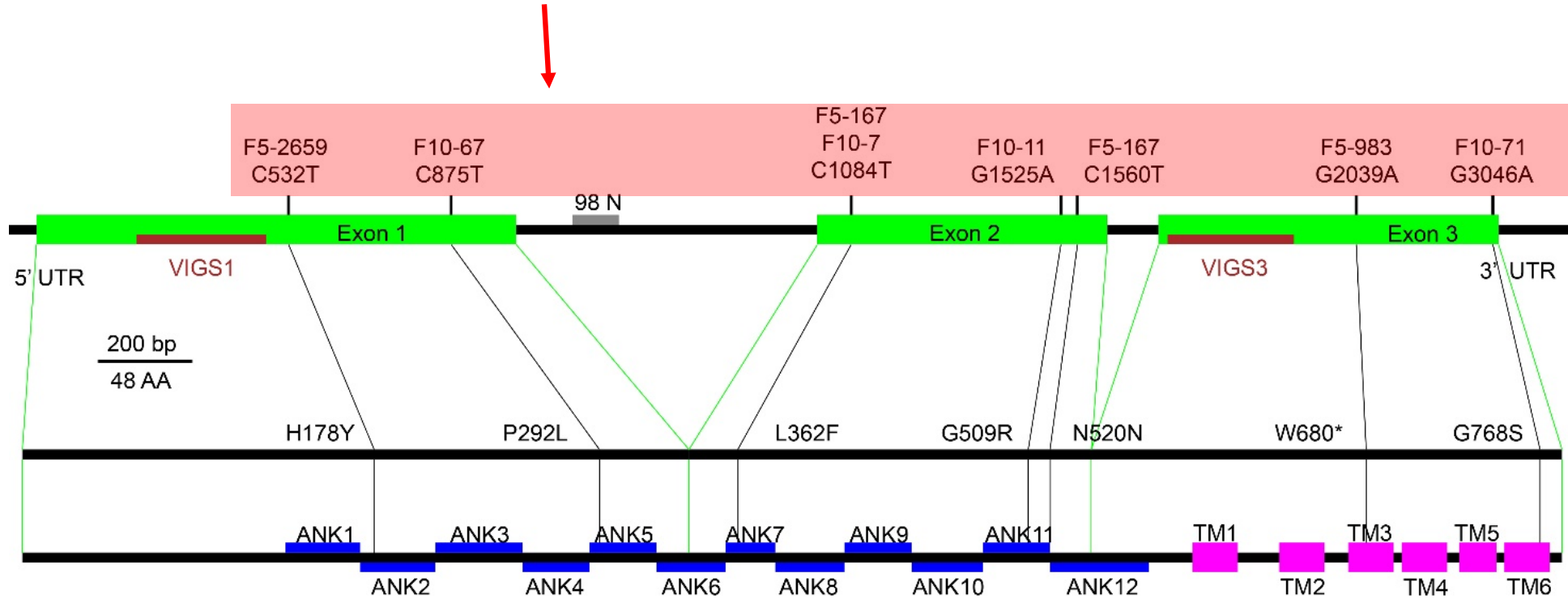


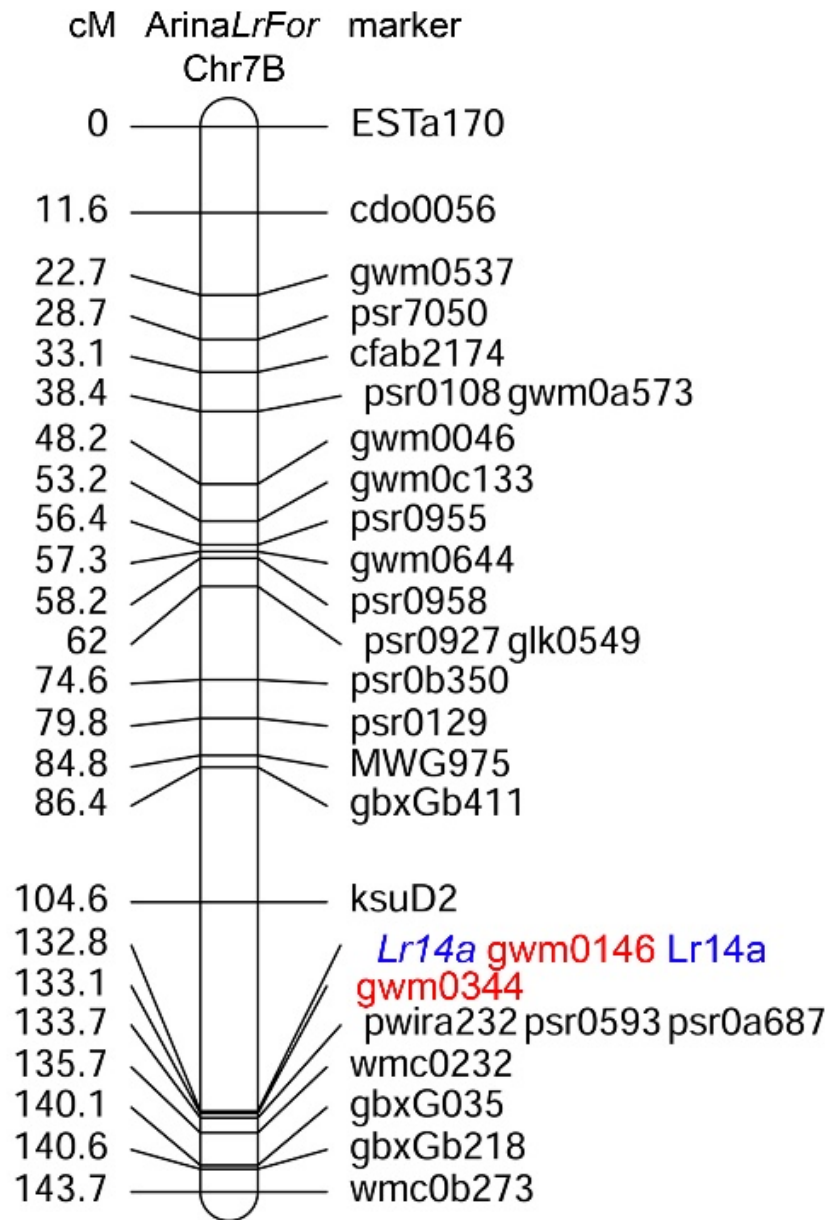
Lr14a gives race-specific resistance, i.e. there are virulent and avirulent races (gene for gene interaction according to Flor).

“X” = random distribution of variable-sized uredinia (mesothetic reaction),

Gene and protein structures of *Lr14a*

Gene isolation after **mutagenesis** and MutChromSeq



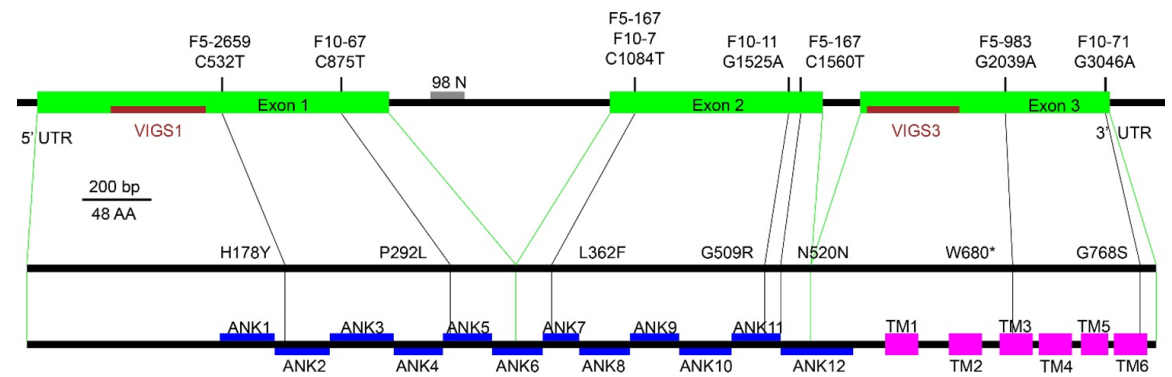
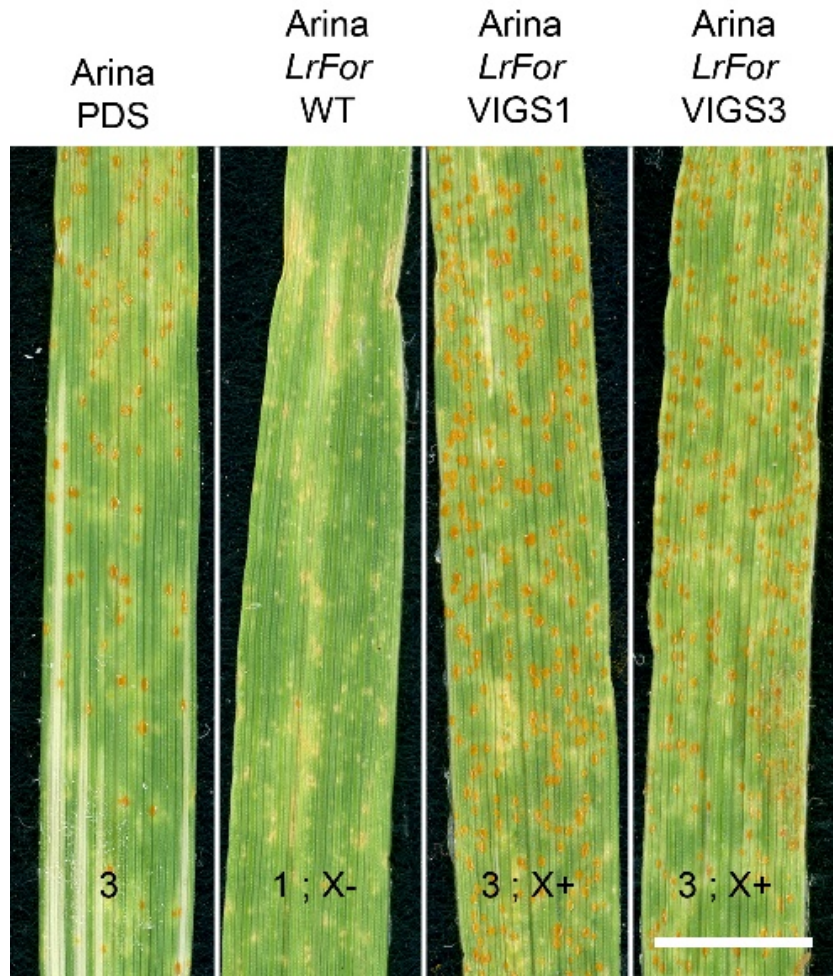


Genetic map of the *Lr14a* gene-specific marker and the *Lr14a* resistance phenotype in a RIL population consisting of 158 genotypes:

Co-localization of the Forno gene and the *Lr14a* gene as described in the literature

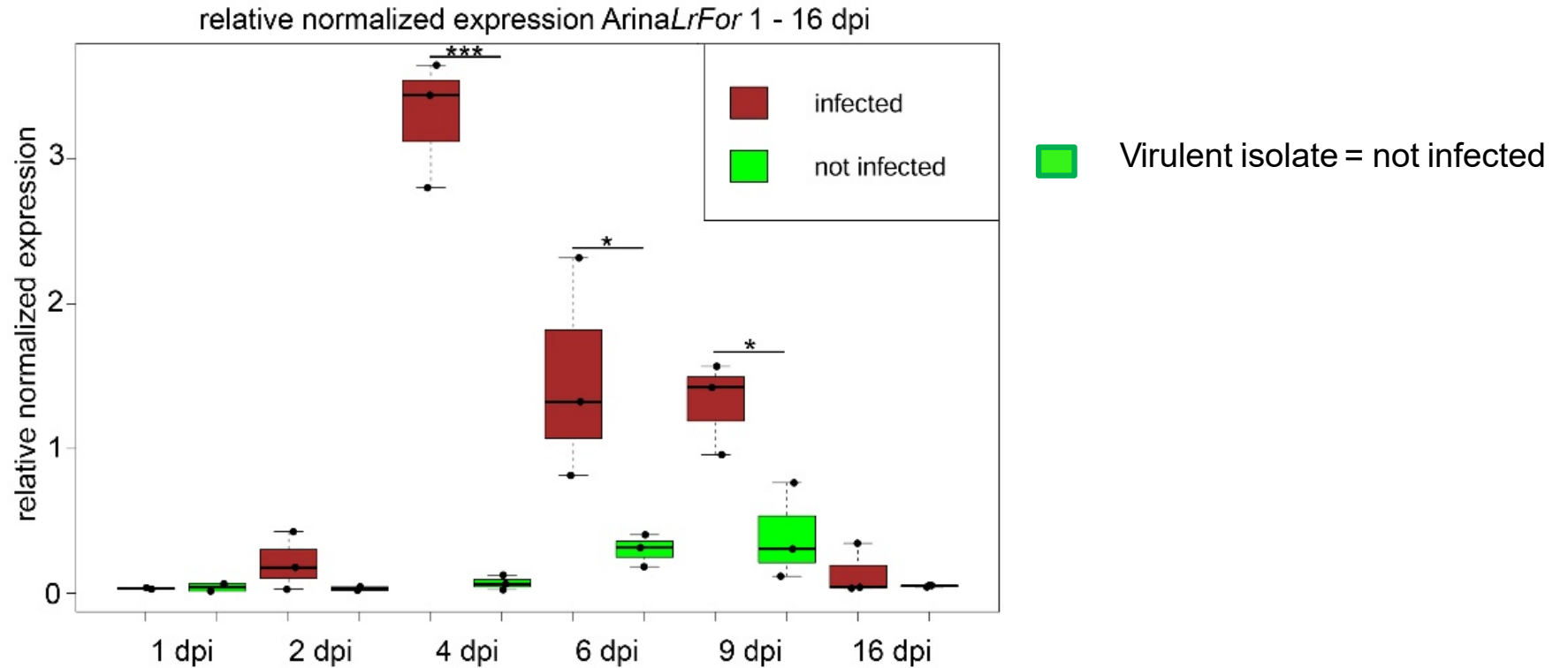
The red markers *gwm0146* and *gwm0344* are the closest markers associated with *Lr14a* according to the literature. The gene-specific marker (*Lr14a*, blue, no italics) and phenotype (*Lr14a*, blue, italics) of *Lr14a* both co-segregated with *gwm0146*.

Increased leaf rust susceptibility after silencing the *Lr14a* candidate gene through virus-induced gene silencing



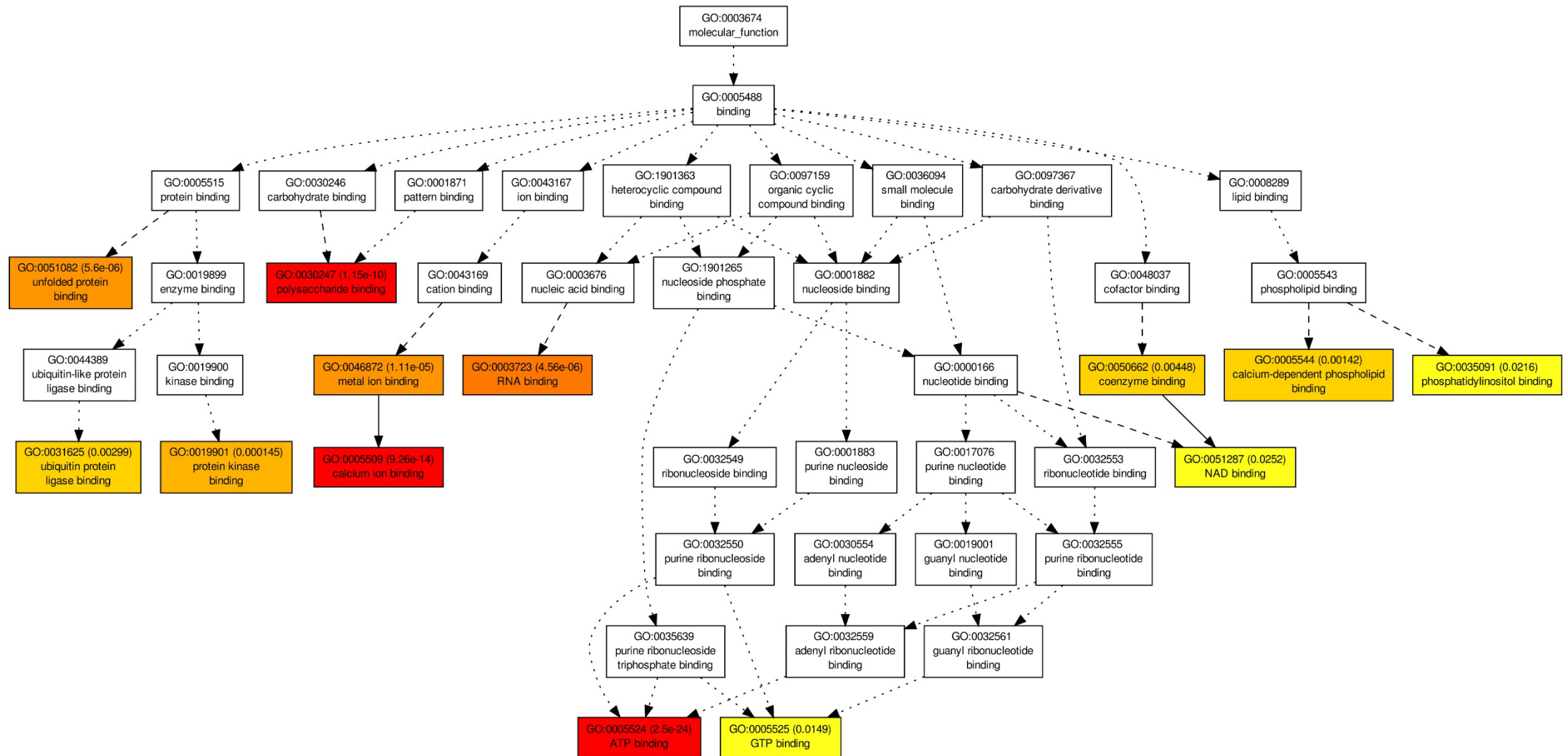
PDS = phytoene desaturase control, WT = barley stripe mosaic virus without silencing construct.

Lr14a is not expressed in the absence of leaf rust or infection with a virulent isolate, but induced by an avirulent leaf rust race



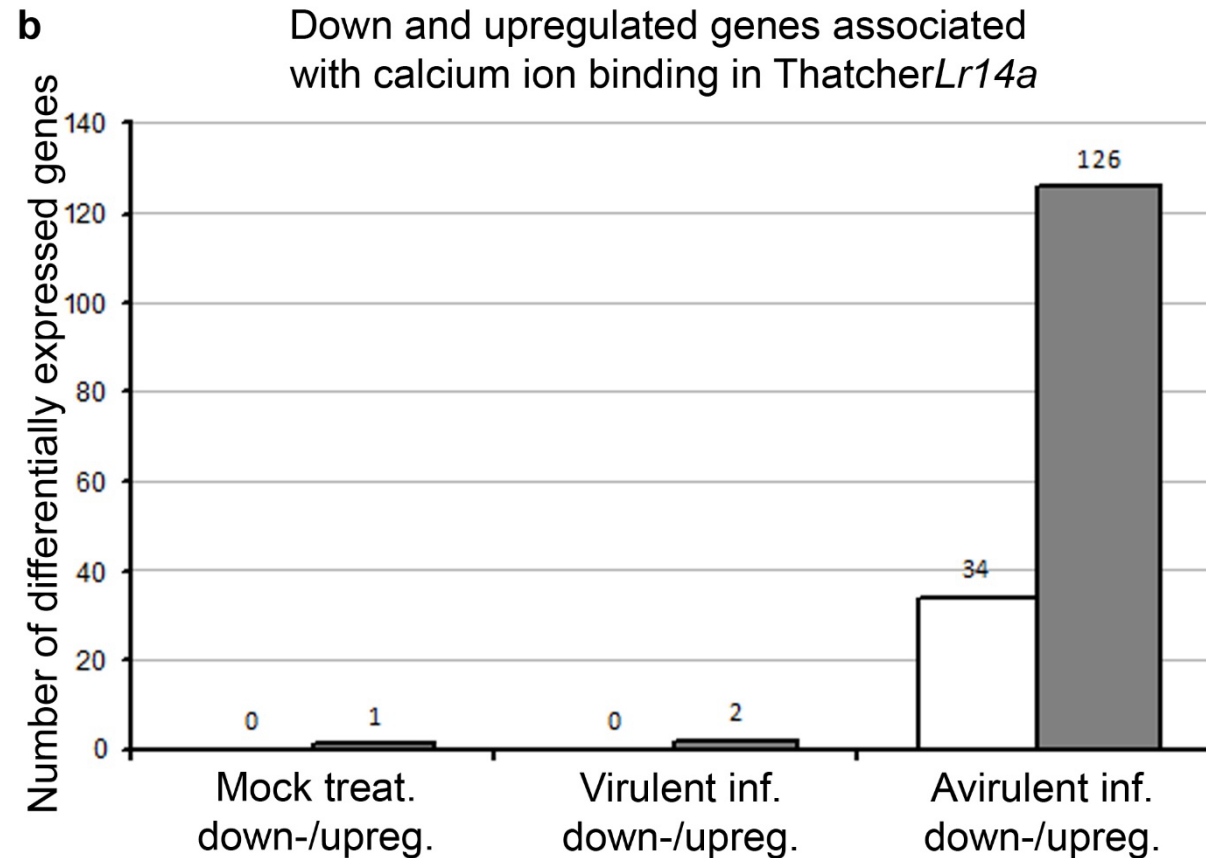
Lr14a expression in *ArinaLrFor* with and without infection (brown/green) with the avirulent leaf rust isolate 96209.

a

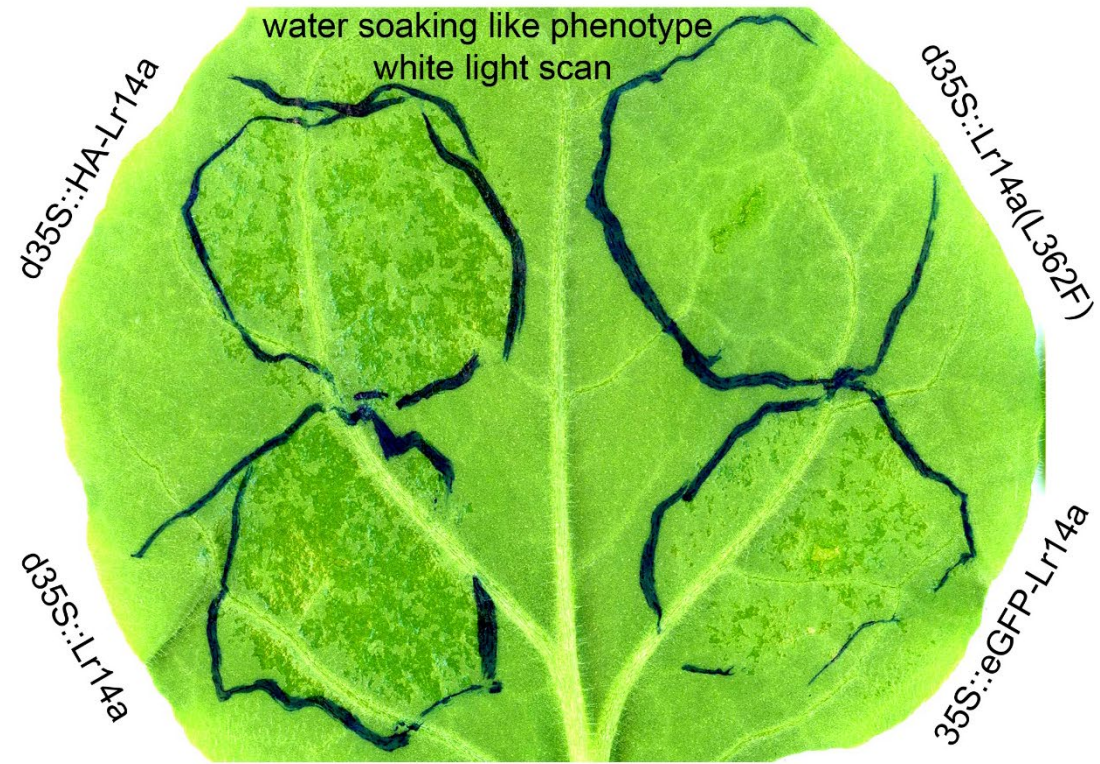


Gene ontology (GO) network graph showing that *Lr14a* causes induction of calcium ion binding associated genes 8 days post inoculation (dpi) in the wheat line *ThatcherLr14a* compared to *Thatcher* after inoculation with an avirulent *P. triticina* isolate.

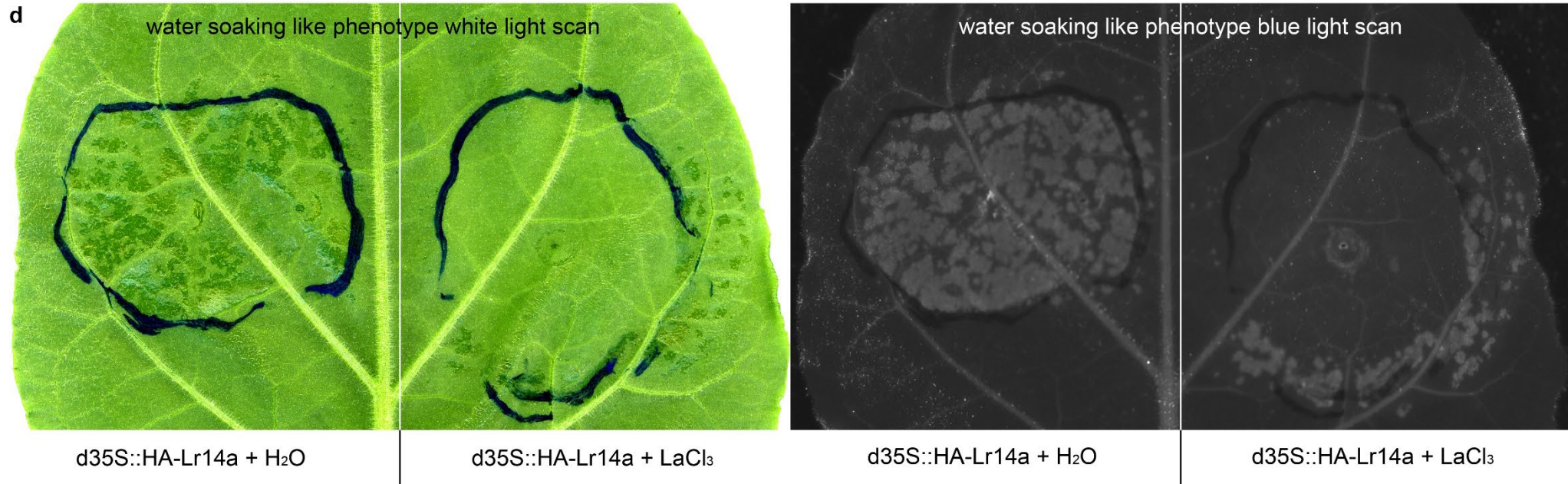
Number of differentially expressed genes that are associated with the GO term “calcium ion binding” in Thatcher *Lr14a* compared to Thatcher after mock treatment and inoculation with a virulent and avirulent *P. triticina* isolate, respectively.



N. benthamiana leaves transiently transformed with *Lr14a* coding sequences: a water-soaking-like phenotype on the lower leaf surface, 3 dpi



The water-soaking-like phenotype on the lower leaf surface of *N. benthamiana* caused by *Lr14a* overexpression was suppressed by infiltration of 2 mM Lanthanum(III) chloride (LaCl_3), a calcium channel blocker two hours after *Agrobacterium* infiltration.



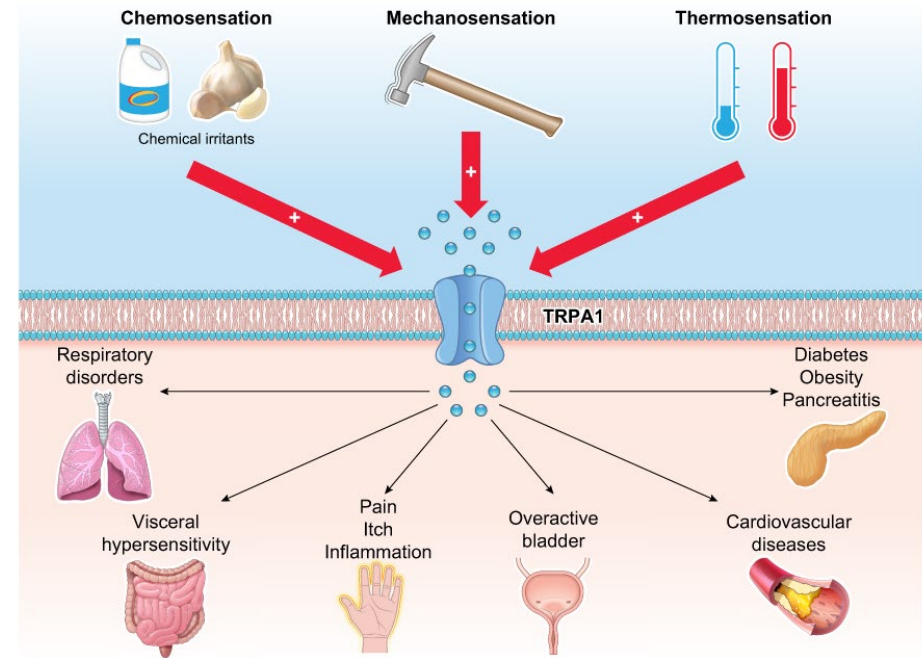
Is the Lr14a protein a non-selective Ca^{2+} channel?

Top 10 hits of HHPred analysis of the Lr14a protein

(HHPred is a fast server for remote protein homology detection and structure prediction and implements pairwise comparison of profile hidden Markov models (HMMs))

PDB entry ^a	Description	E-value	Ref ^b
5Y4D_A	ANK2, Homo sapiens	9.60E-54	1
6PQQ_D	Transient receptor potential cation channel TRPA1, Homo sapiens	4.20E-52	2
5LEB_A	DARPin DDD_D12_06_D12_06_D12, biosynthetic protein	1.50E-51	3
5Y4D_A	ANK2, Homo sapiens	3.90E-51	1
1N11_A	D34 region of Ankyrin-R, Homo sapiens	8.10E-51	4
3J9P_A	Transient receptor potential cation channel TRPA1t, Homo sapiens	6.60E-49	5
4CJ9_B	BurrH DNA-binding protein, Burkholderia rhizoxinica	5.30E-51	6
4RLV_A	ANK repeat AnkB, Homo sapiens	1.10E-48	7
6MOL_A	Monoextended DARPin M_R12, biosynthetic protein	9.50E-48	8
4CJ9_B	BurrH DNA-binding protein, Burkholderia rhizoxinica	2.20E-50	6

Kolodziej et al. 2021, Nature Communications, supplementary table 3



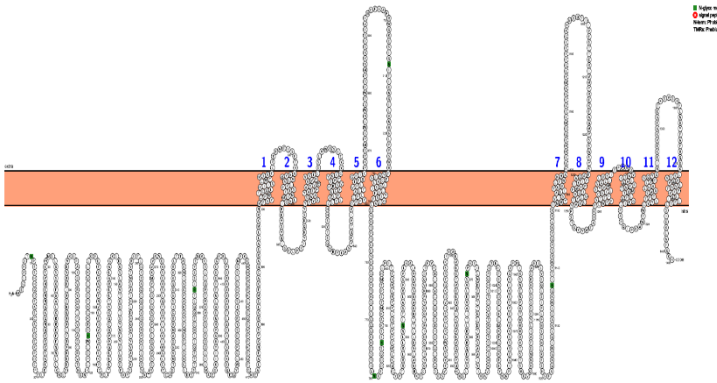
Physiological Reviews © 2020



"The transient receptor potential ankyrin (TRPA) channels are **Ca²⁺-permeable nonselective cation channels** remarkably conserved through the animal kingdom. Mammals have only one member, TRPA1, which is widely expressed in sensory neurons and in non-neuronal cells (such as epithelial cells and hair cells). TRPA1 owes its name to the **presence of 14 ankyrin repeats located in the NH₂ terminus of the channel**, an unusual structural feature that may be **relevant to its interactions with intracellular components**. TRPA1 is primarily involved in the detection of an extremely wide variety of exogenous stimuli that **may produce cellular damage**."

Additive action of the two resistance genes *Lr34* and *Lr14a* present in the durable rust resistant cultivar 'Forno'

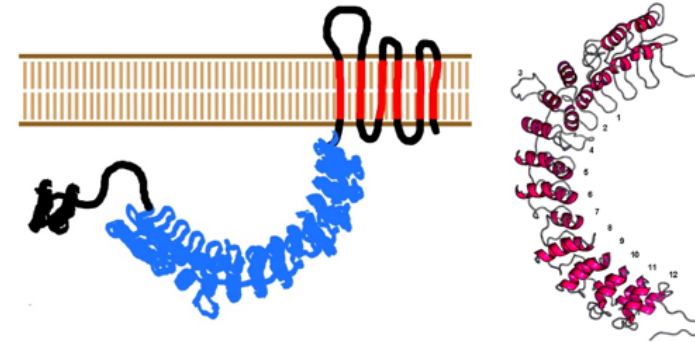
Lr34: an ABCG transporter



Structural model of the LR34 protein: an ABCG-type of transporter (Krattinger et al. 2009)

Lr34 is likely an ABA transporter (Krattinger et al. 2019, Bräunlich et al. 2021)

Lr14a: an Ank-repeat transmembrane protein



We propose that the protein is anchored to the plasma membrane with its 6 TM domains (red) while the N-terminal ANK domain (blue) is cytoplasmic.

RaptorX predicted 3-dimensional protein structure of the ANK domain of Lr14a.

Future research: What is the molecular basis of the observed additive gene action observed in the field?

Is Lr14a an executor protein?

No expression of *Lr14a* after infection with a virulent leaf rust race

Induction by *Lr14a* after infection with an avirulent leaf rust race

Presence of Lr14a in transient expression is sufficient to confer water-soaking phenotype in *Nicotiana benthamiana*

Homology to non-selective Ca²⁺ permeable cation channels

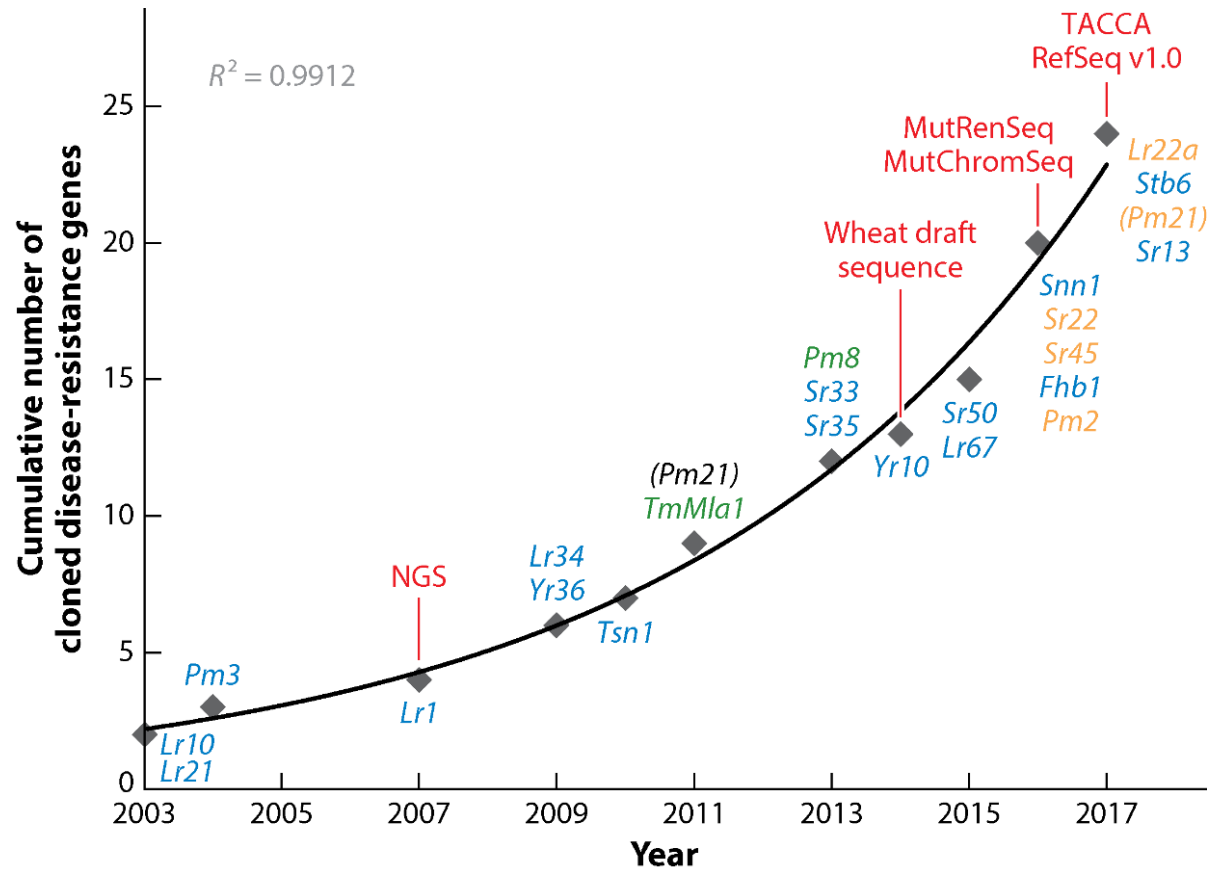
Similarity to executor genes in bacterial – plant interactions (promoter traps for TAL effectors for *Xa27*, *Xa10*, *Xa23* resistance genes in rice and *Bs3* and *Bs4C* in pepper against *Xanthomonas*)

Identified executor genes either encode for proteins with a catalytic function (*Bs3* and *Bs3-E* encode for a putative flavin monooxygenase) or for proteins with multiple putative transmembrane domains (*Xa27*, *Xa10*, *Xa23*, and *Bs4C-R*).



Markus Kolodziej

The genomic revolution in wheat: Isolation of resistance genes is accelerating:



Wheat gene catalogue:

Against powdery mildew (*Pm*):
> 65 genes

<http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp>

Our goals and selection procedure for isolating **powdery mildew** resistance genes:

Our goal: identify the proteins involved in resistance interactions both in wheat and the wheat mildew pathogen and characterize their interactions

A focus on the most interesting genes defined by, for example for powdery mildew resistance:

- Allelic diversity
- Genetic source (alien introgression, emmer wheat origin)
- Unusual phenotype
- Any hint in classical literature on unusual genetics (modifiers, suppressors)
- Good genetic material available (ideally near-isogenic lines)



Javier Sanchez-Martin



Victoria
Widrig

Geri
Herren

PD Dr.
Thomas
Wicker



Matthias
Neidhart

Helen
Zbinden

Dr. Julien
Gronier

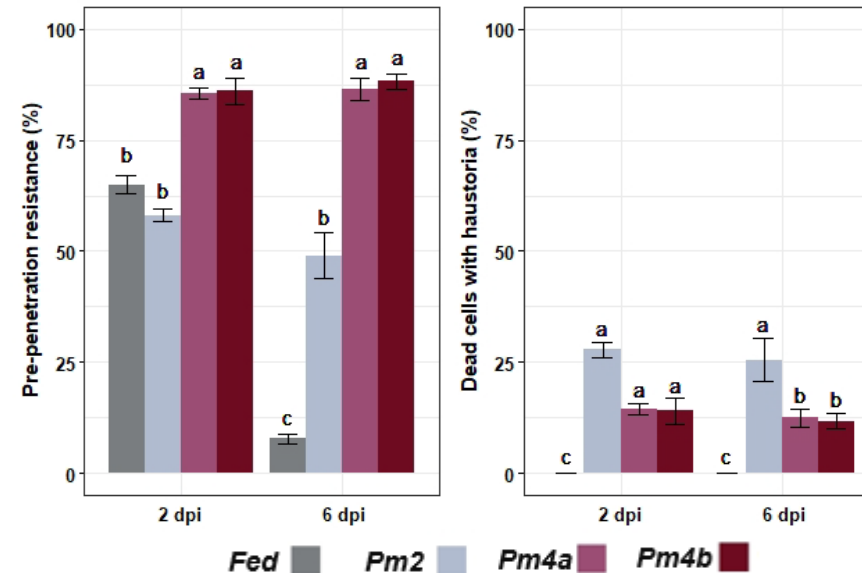
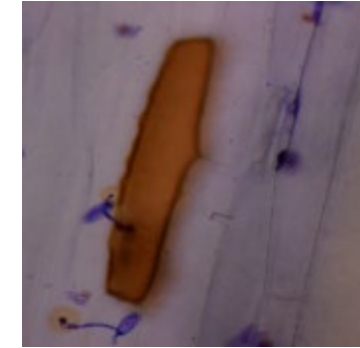
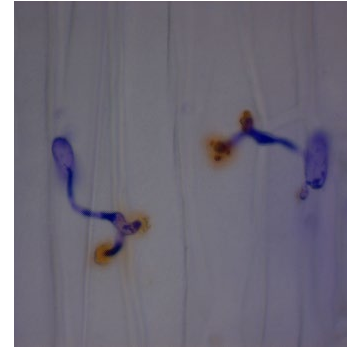
The powdery mildew resistance gene *Pm4* gene in wheat

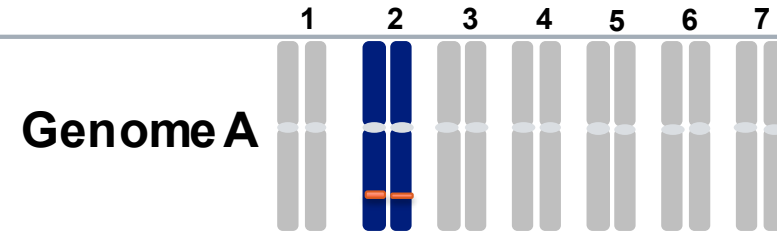
- Occurs in several allelic variants (*Pm4a – d*), of which *Pm4a* and *Pm4b* are genetically best characterized.
- *Pm4a* and *Pm4b* originate from domesticated, tetraploid emmer wheat genotypes and were introgressed into bread wheat
- Near-isogenic lines are available for *Pm4a* and *Pm4b*
- Confers classical, race-specific resistance
- From much of these data, a typical NLR-based resistance could be expected.
- However, introgressions from tetraploid emmer have revealed other gene classes involved in resistance, suggesting that emmer wheat has been an “evolutionary lab” for wheat resistance gene evolution: e.g. tandem kinases (Yr15); kinase-START domain (Yr36)



Pm4-mediated resistance is associated with hypersensitive cell death

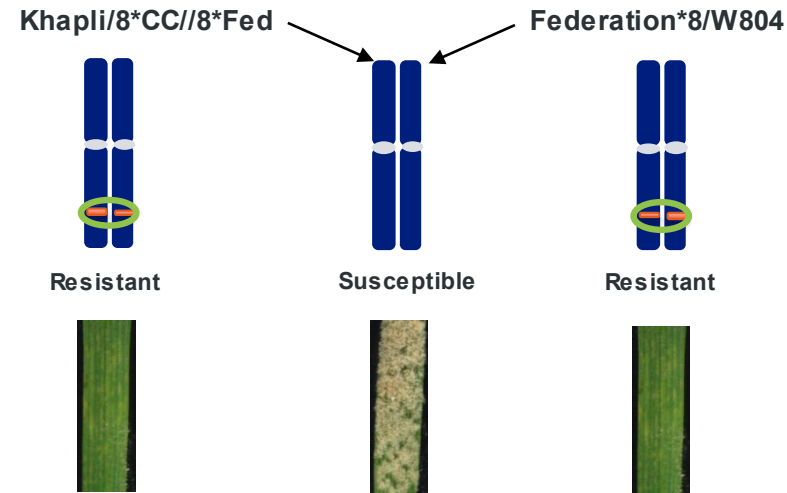
- Lower levels of HR than canonical NLR-mediated resistance from *Pm2*
- Pre-penetration component, papilla-based?





The *Pm4* locus

- Mapped on the distal part of the long arm of chromosome 2A
- Our working material are the near-isogenic lines *Pm4a* (Khapli/8*CC/8*Fed) and *Pm4b* (Federation*8/W804)

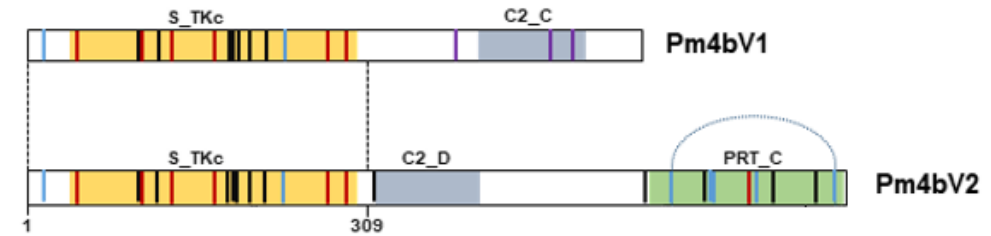
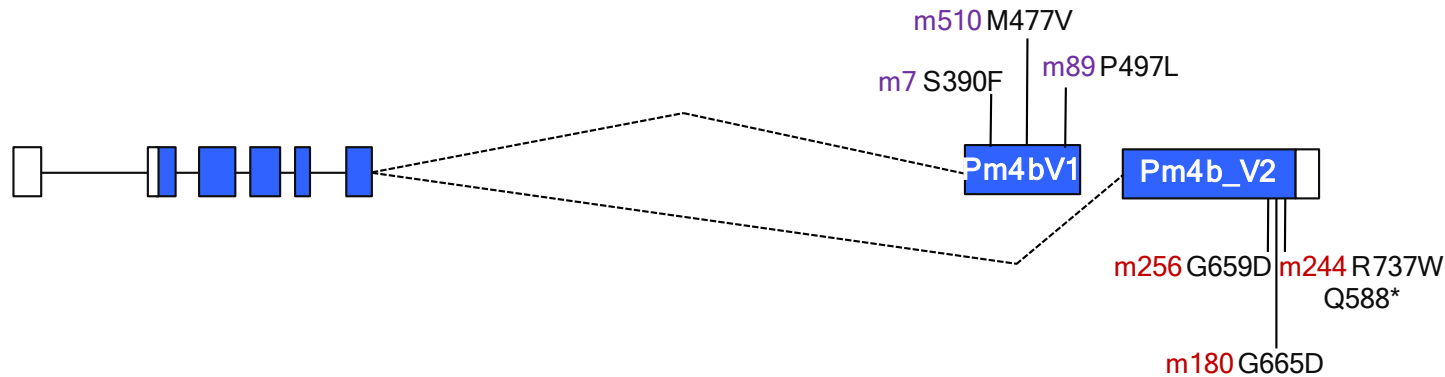


=> Seven mutants in *Pm4b* identified, and their chromosome 2A was sorted, assembled and compared to the wildtype



The *Pm4* gene

- MutChromSeq: forward genetic approach based on mutants
 - Undergoes constitute alternative splicing -> **two transcripts**
 - Mutants affecting mutually exclusive exons 6 and 7
- => Both protein isoforms are required for resistance**



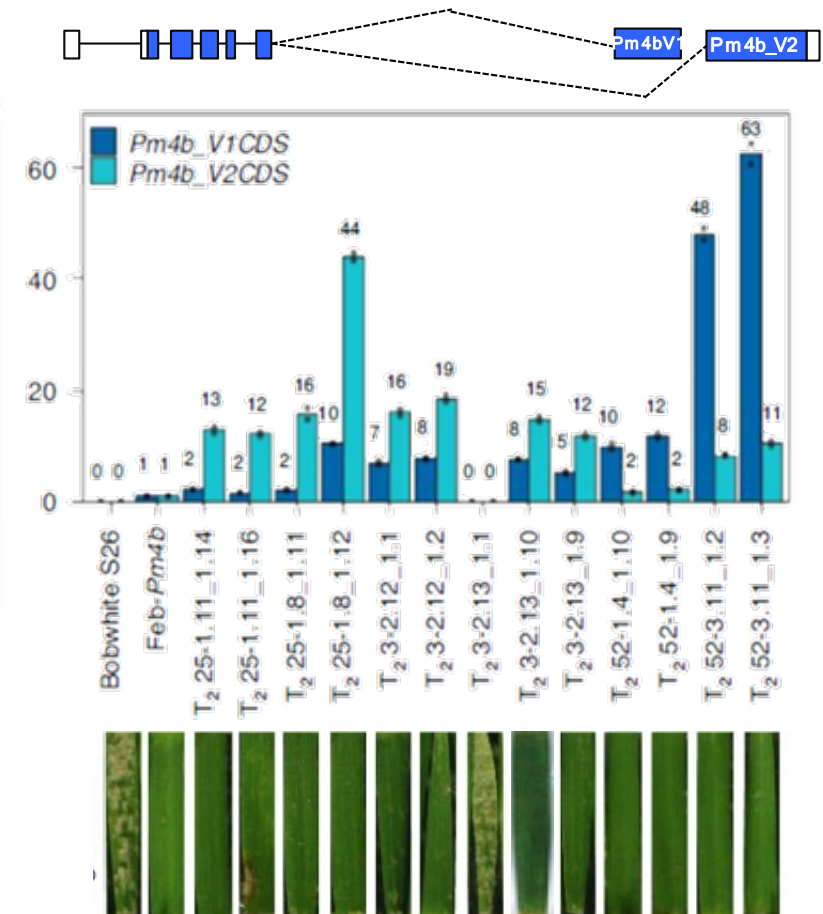
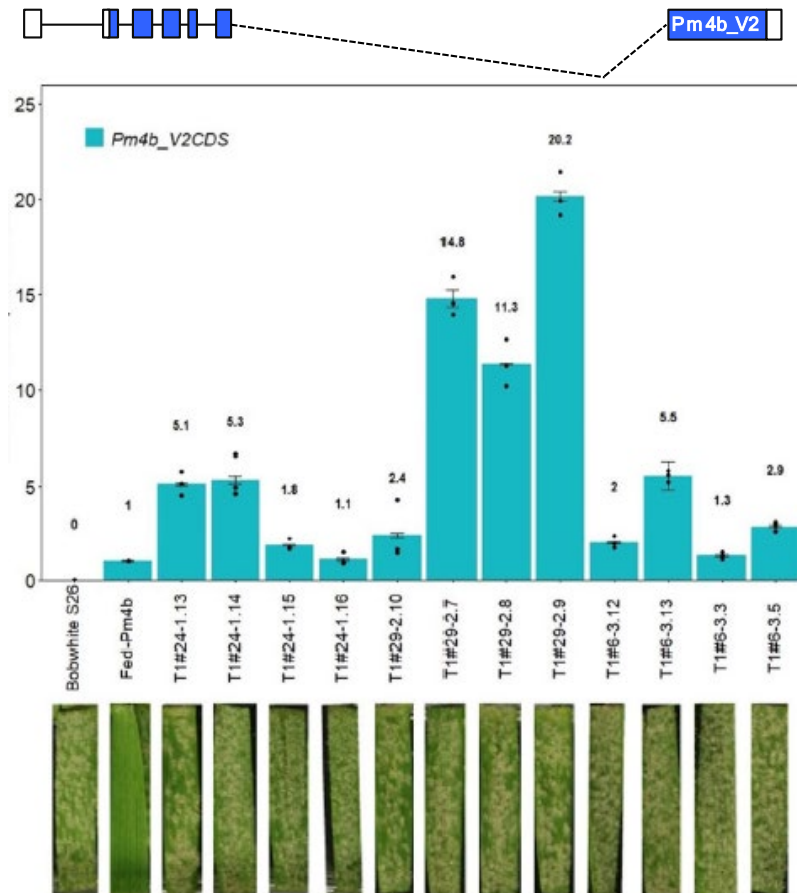
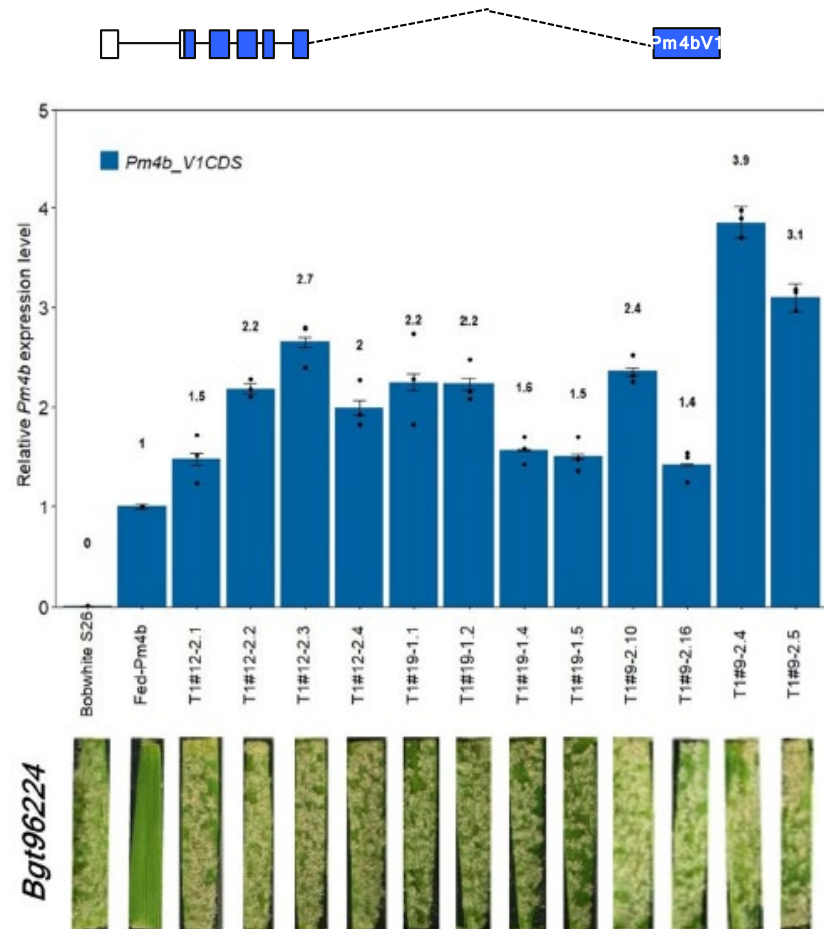
| **Seven *pm4b* EMS-derived mutants by MutChromSeq**

|| **Ten *pm4b* EMS-derived mutants by Sanger Seq**

| **Thirteen *pm4a* EMS-derived mutants by Sanger Seq**

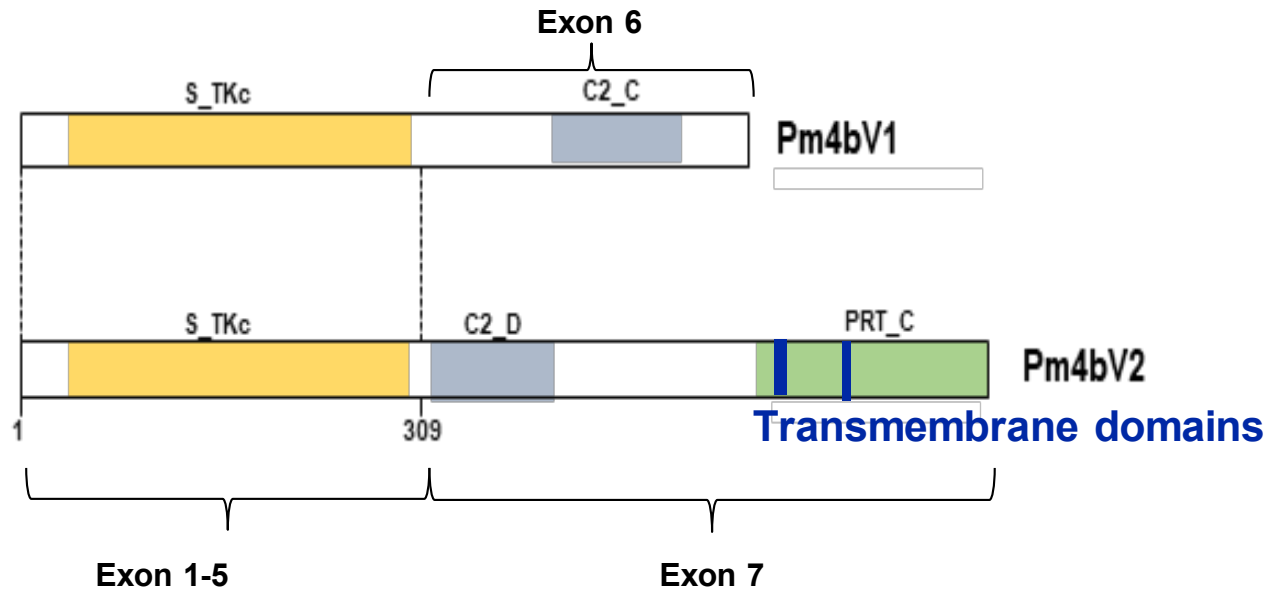
=> Total of 30 EMS mutants

Transgenic seedlings with both splicing variants restore powdery mildew resistance



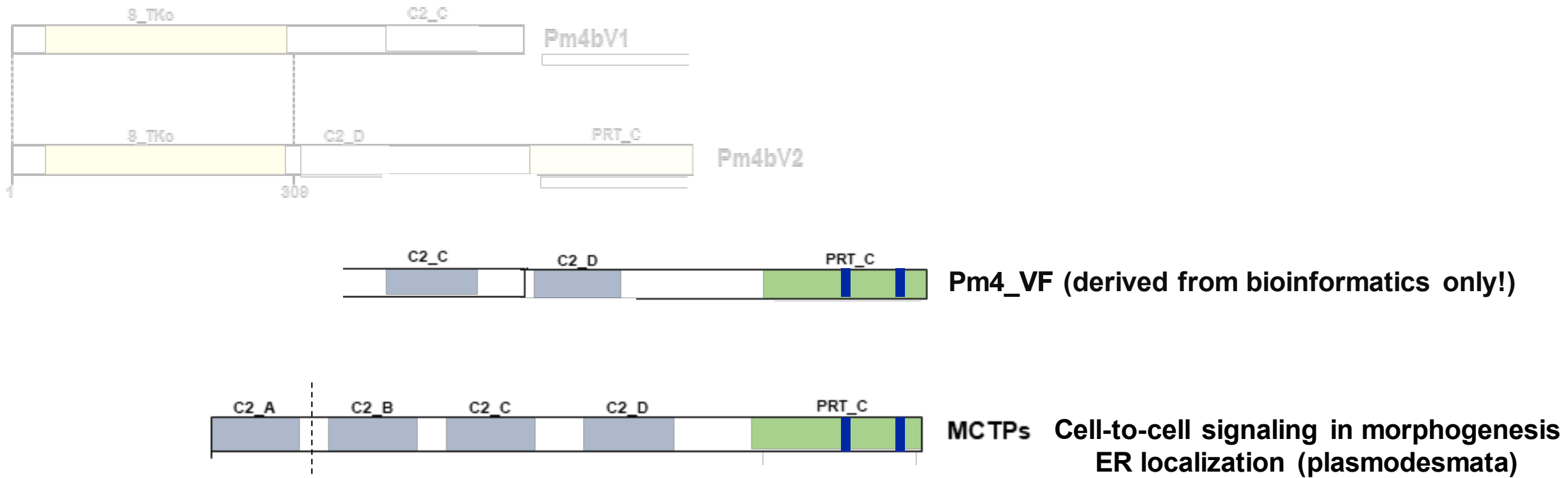
The *Pm4* gene encodes a resistance protein with a unique domain architecture

Not described in proteins involved in plant immunity



The *Pm4* gene encodes a resistance protein with a unique domain architecture

Not described in proteins involved in plant immunity

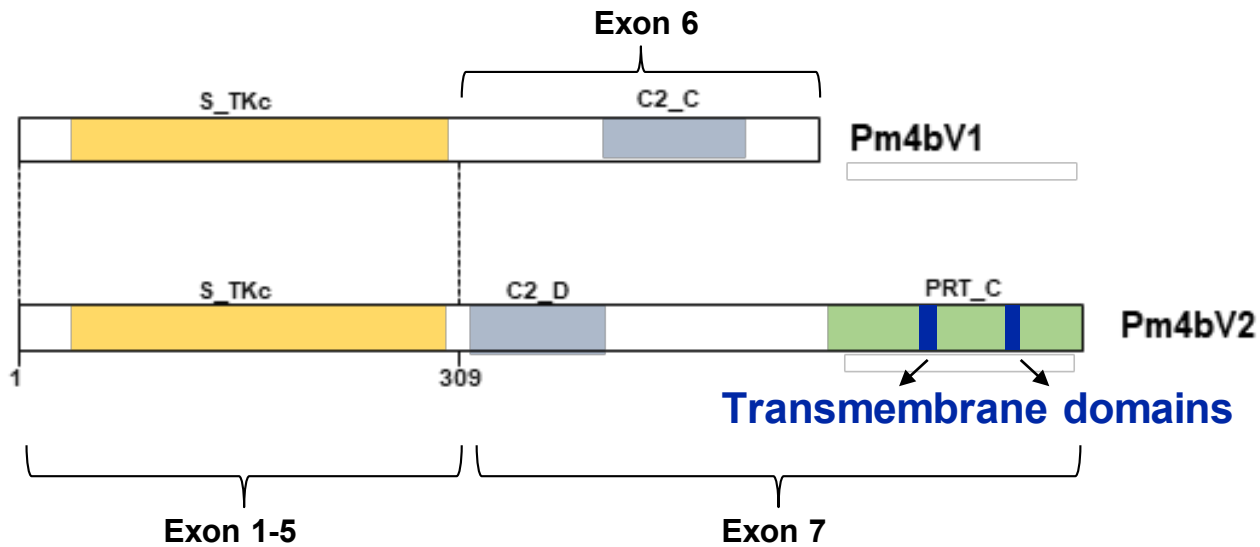


Proteins containing multiple C2-domain and transmembrane region(s) proteins (MCTPs)

The *Pm4* gene encodes a resistance protein with a unique domain architecture

Not described in proteins involved in plant immunity

Results from a gene fusion event between a S_TKc and C-terminal part of a MCTP -> Kinase-MCTP



How are these two isoforms working to confer *Pm4*-mediated resistance?

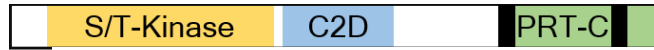


Localization studies revealed...

- Pm4b_V1 localises to the cytoplasm

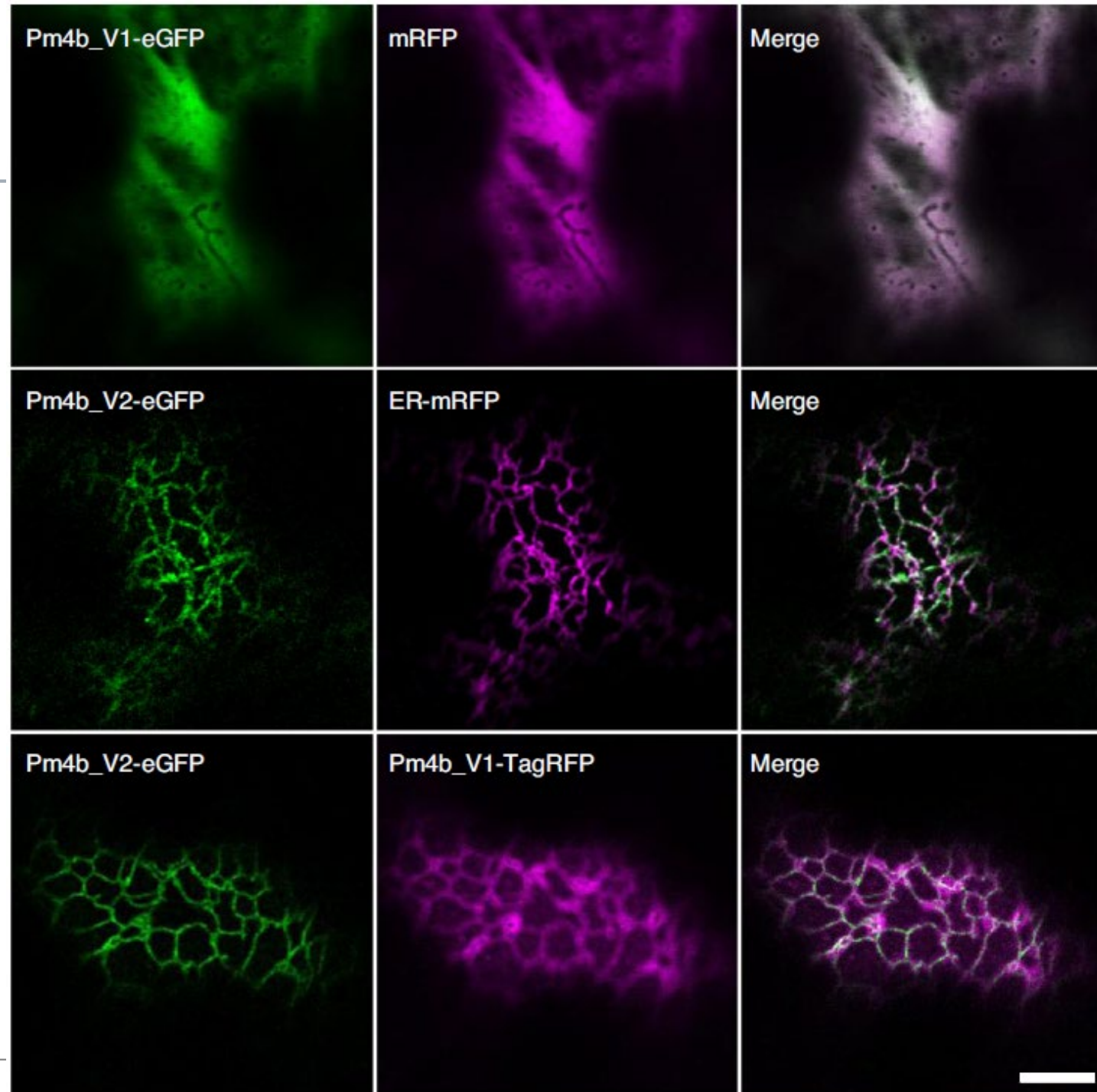


- Pm4b_V2 localises to the ER



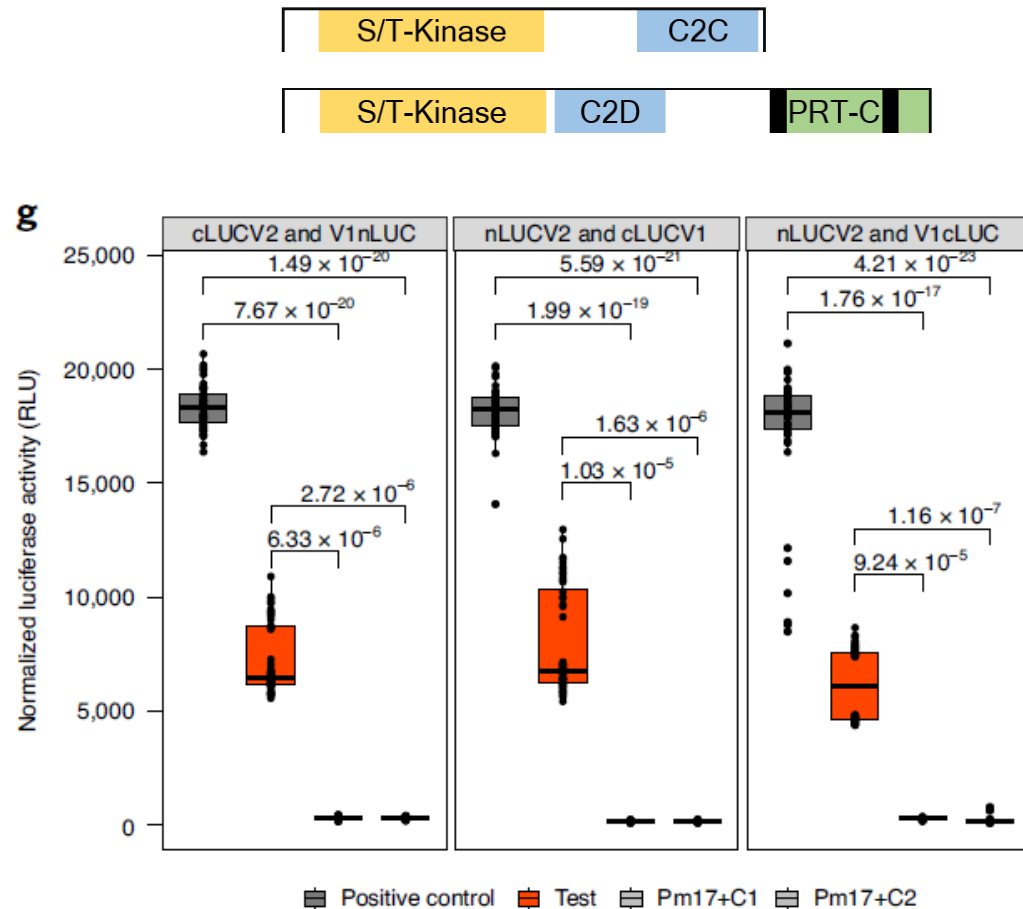
- Pm4b_V2 «recruits» Pm4b_V1 from the cytosol to the ER...

Do both protein isoforms interact?

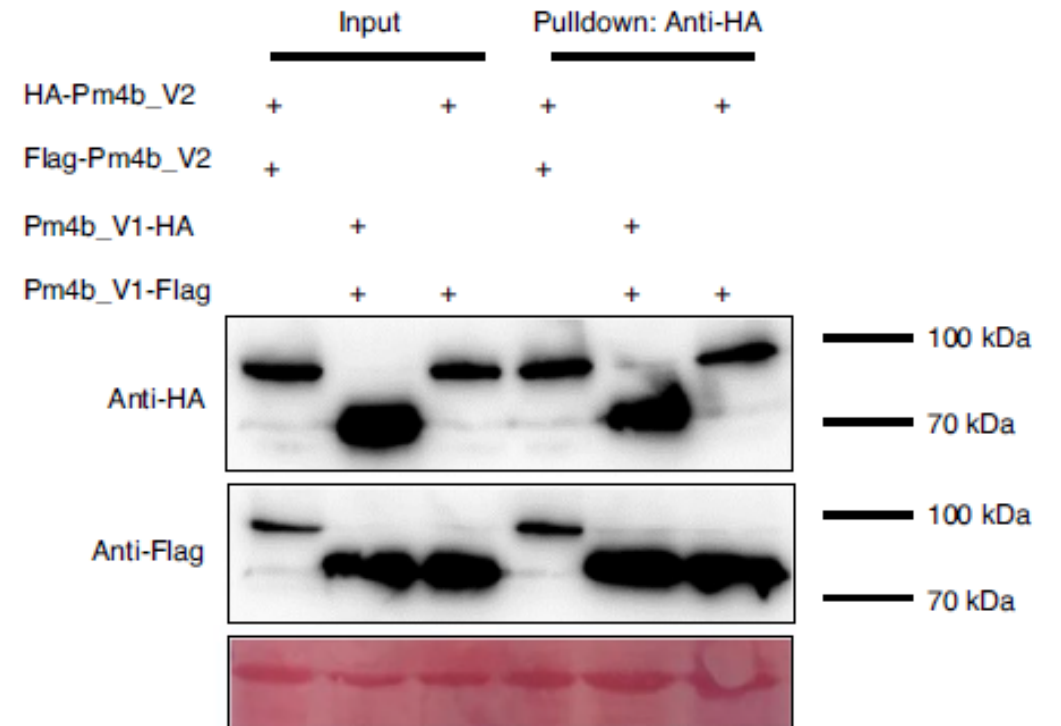




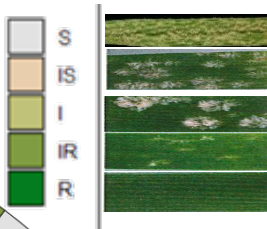
Pm4b_V1 and Pm4b_V2 establish homo- and heteromeric interactions



d



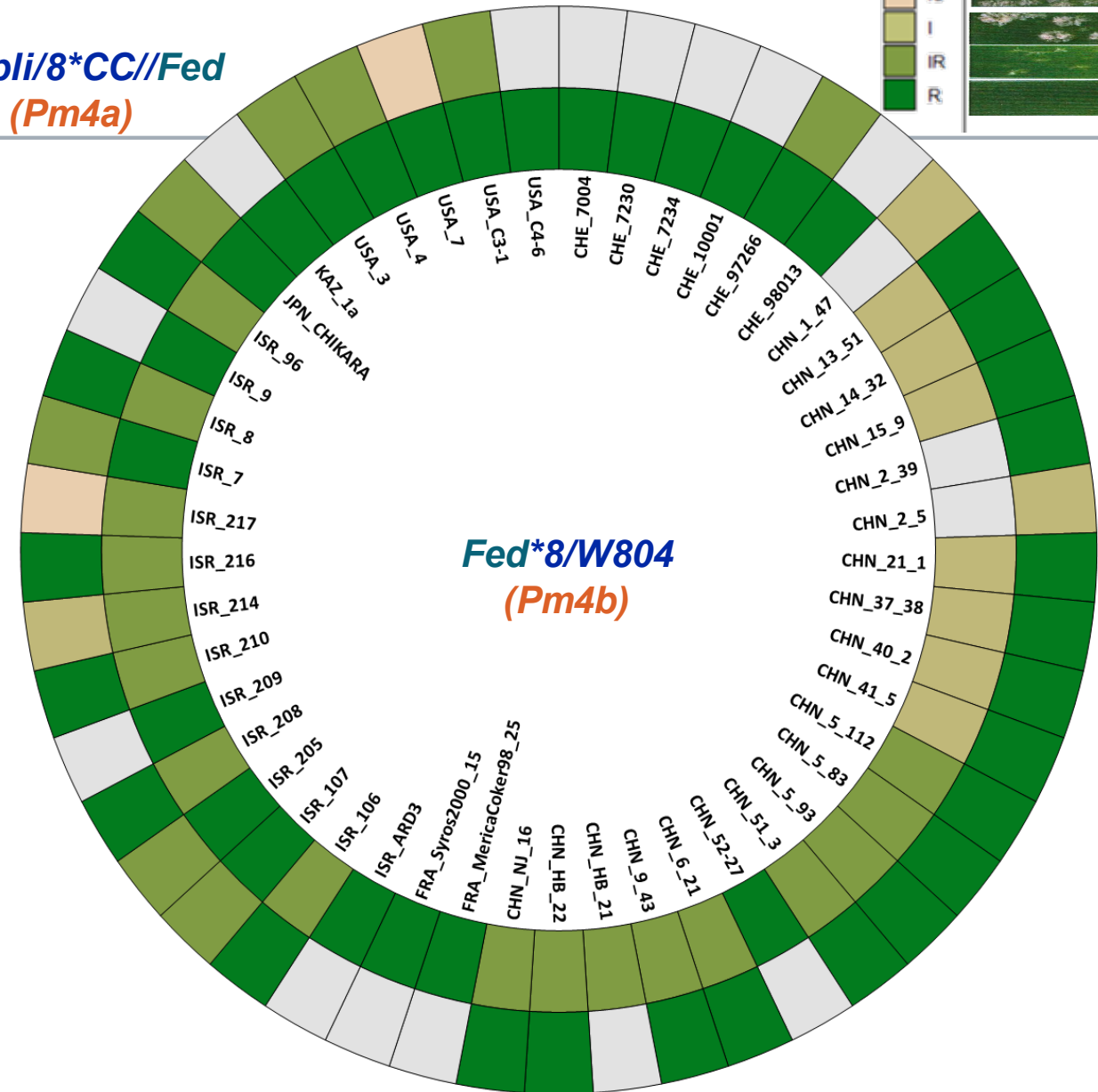
Khapli/8*CC//Fed
(Pm4a)



The *Pm4* gene

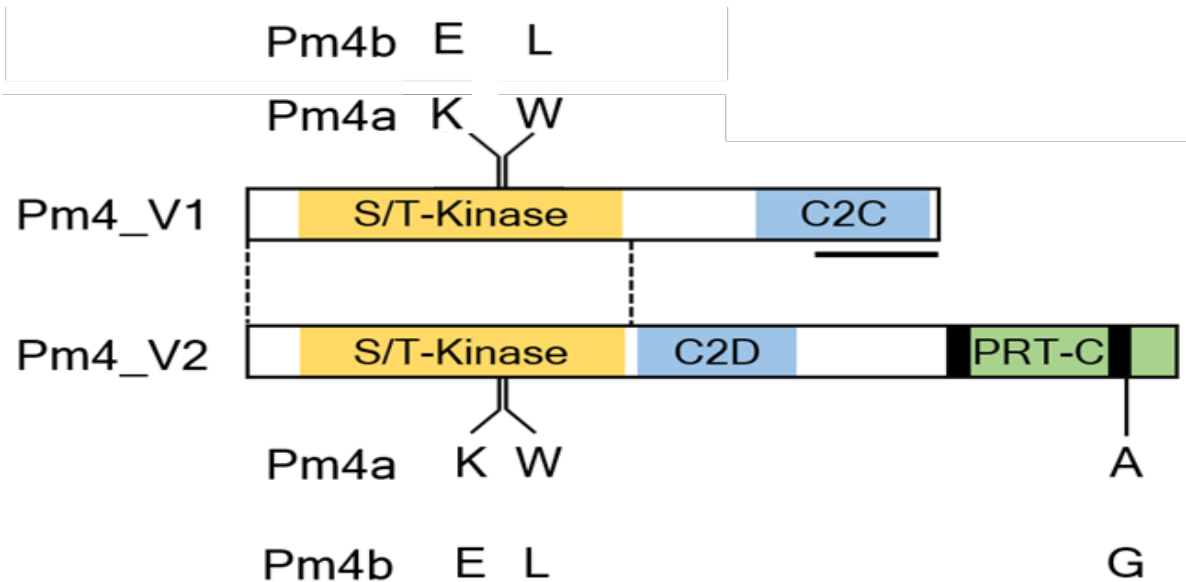
- Mapped on the distal part of the long arm of Chr2A
- *Pm4a* (Khapli/8*CC/8*Fed) and *Pm4b* (Fed*8/W804)

largely overlapping (65 isolates),
yet distinct resistance spectrum (42 isolates)





Molecular basis of Pm4a vs. Pm4b specificity



Three polymorphisms between Pm4a and Pm4b

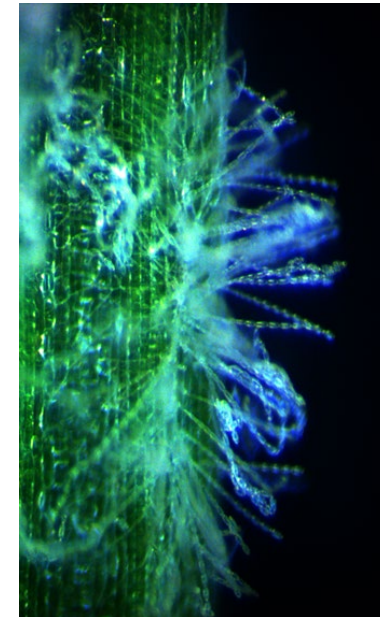
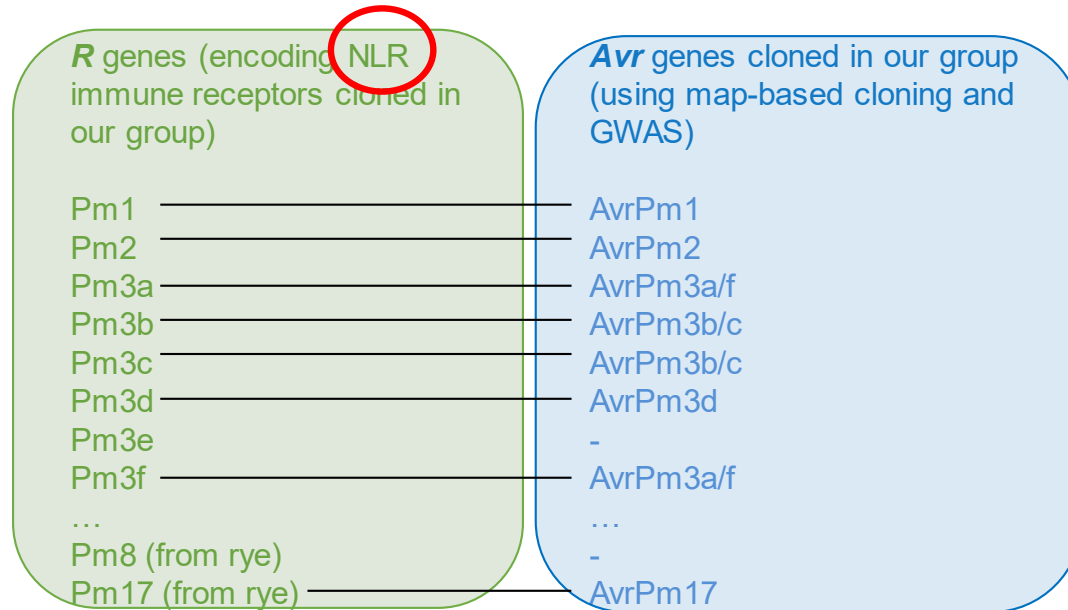
All of them in highly conserved regions of the serine/threonin kinase domain



Summary: The *Pm4* race-specific gene encodes a chimeric kinase-MCTP protein. Its two alternative splice variants interact to form an ER-associated complex and are both essential for resistance function

- The cloning of *Pm4* reveals a new type of immune receptor previously unknown.
 - Is it an exception, or is this the first member of an additional gene family that we can use for disease resistance breeding?
 - Further biochemical characterization of the *Pm4* resistance complex and possible interacting partners.
 - *Pm4a/b* originate **from tetraploid wheat**.
-

Lr14a, Pm4, WTK4 all are non-NLR, race-specific immune receptors



- Sanchez-Martin et al. 2016, Genome Biology
- Yahiaoui et al. 2006, Plant J.
- Hurni et al. 2013, Plant J.
- Singh et al. 2018, Plant J.
- Hewitt et al. 2021, New Phytologist

- Müller et al. 2021, bioRxiv, in revision
- Hewitt et al. 2021, New Phytologist
- Bourras et al. 2015, Plant Cell
- Praz et al. 2017, New Phytologist
- Bourras, Kunz, et al. 2019, Nature Communications

Other *Pm* genes cloned:

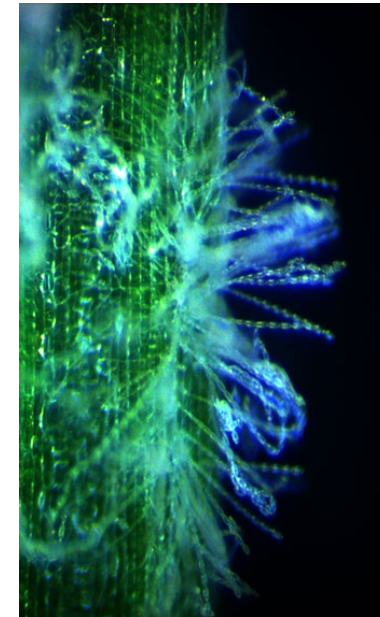
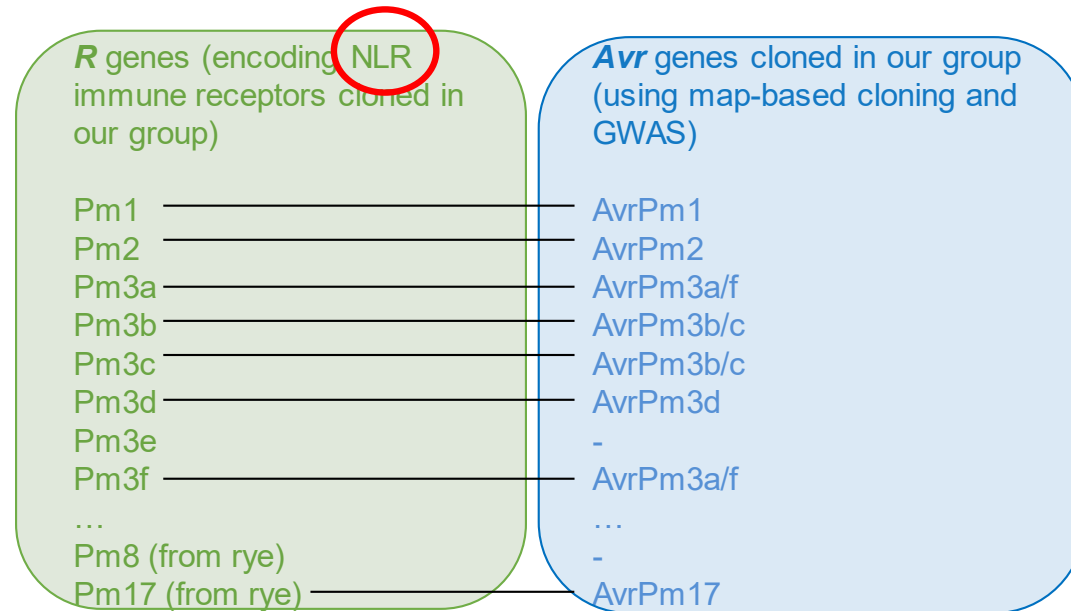
- Pm21: Xing et al./He et al. 2018; Mol. Plant;
- Pm60: Zou et al. 2018, New Phytologist
- Pm24: Nature Communications 2020

- Pm12, unpublished
- Pm5e, Pm41: New Phytologist 2020
- Pm38: Krattinger et al. 2009

Lr14a, Pm4, WTK4 all are non-NLR, race-specific immune receptors

What are the avirulence genes in the pathogens that induce race-specific resistance?

AvrLr14a
AvrPm4a,b
AvrWTK4



- Sanchez-Martin et al. 2016, Genome Biology
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Summary

Different genetic resources have allowed us to isolate three novel types of race-specific resistance genes from the wheat gene pool:

- The durable resistance in elite wheat cultivar Forno is partially based on Lr14a, an ankyrin-repeat, transmembrane protein possibly acting as a race-specific executor gene.
- Tetraploid wheat as a source for the evolutionary innovation of the Ser/Thr kinase – MCTP chimeric proteins Pm4a and Pm4b

Novel types of race-specific immune receptors reveal possibly novel biological mechanisms in plant immunity. It is important to isolate the corresponding avirulence genes.

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IWGSC

International Wheat Genome
Sequencing Consortium



From the field to the lab,
from the lab to the field



International
Wheat Genome
Sequencing
Consortium



SCHWEIZERISCHER NATIONALFONDS ZUR
FÖRDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG



2010-2015



Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Office for Agriculture FOAG

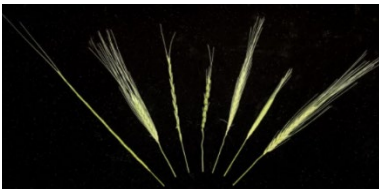


2003-2025



URPP
Evolution in
Action





OPENWILDWHEAT

openwild.org



K. Gaurav



S. Arora



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J. Sanchez



R. Horsnell



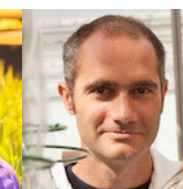
A. Bentley



B. Keller



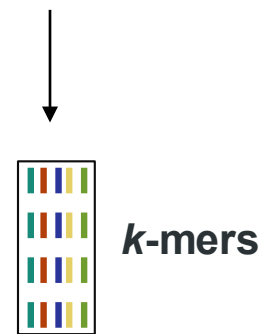
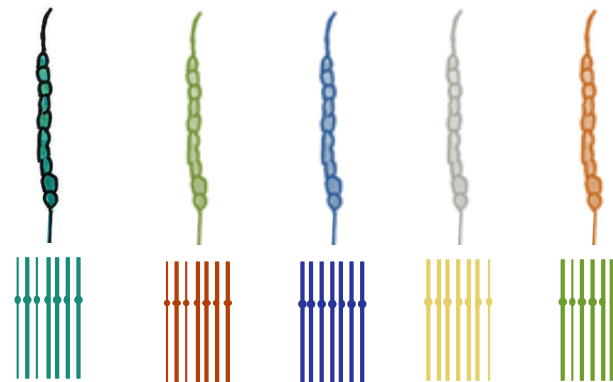
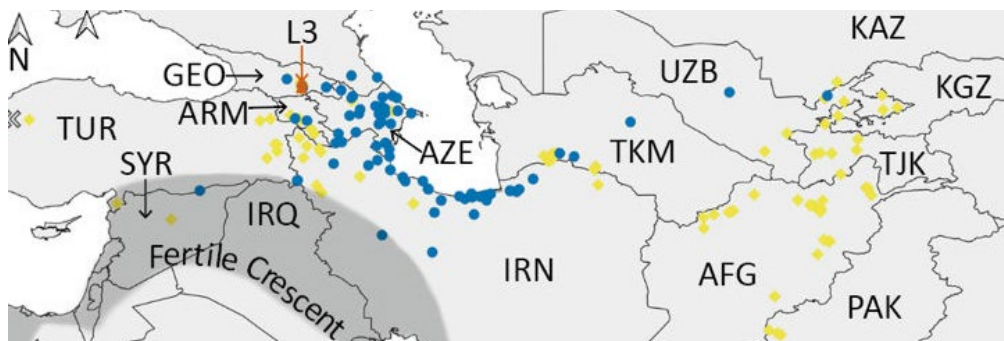
J. Poland



B. Wulff

Open Wild Wheat Consortium

• *Ae. tauschii* diversity panel (242 accessions)



k-mers

GWAS

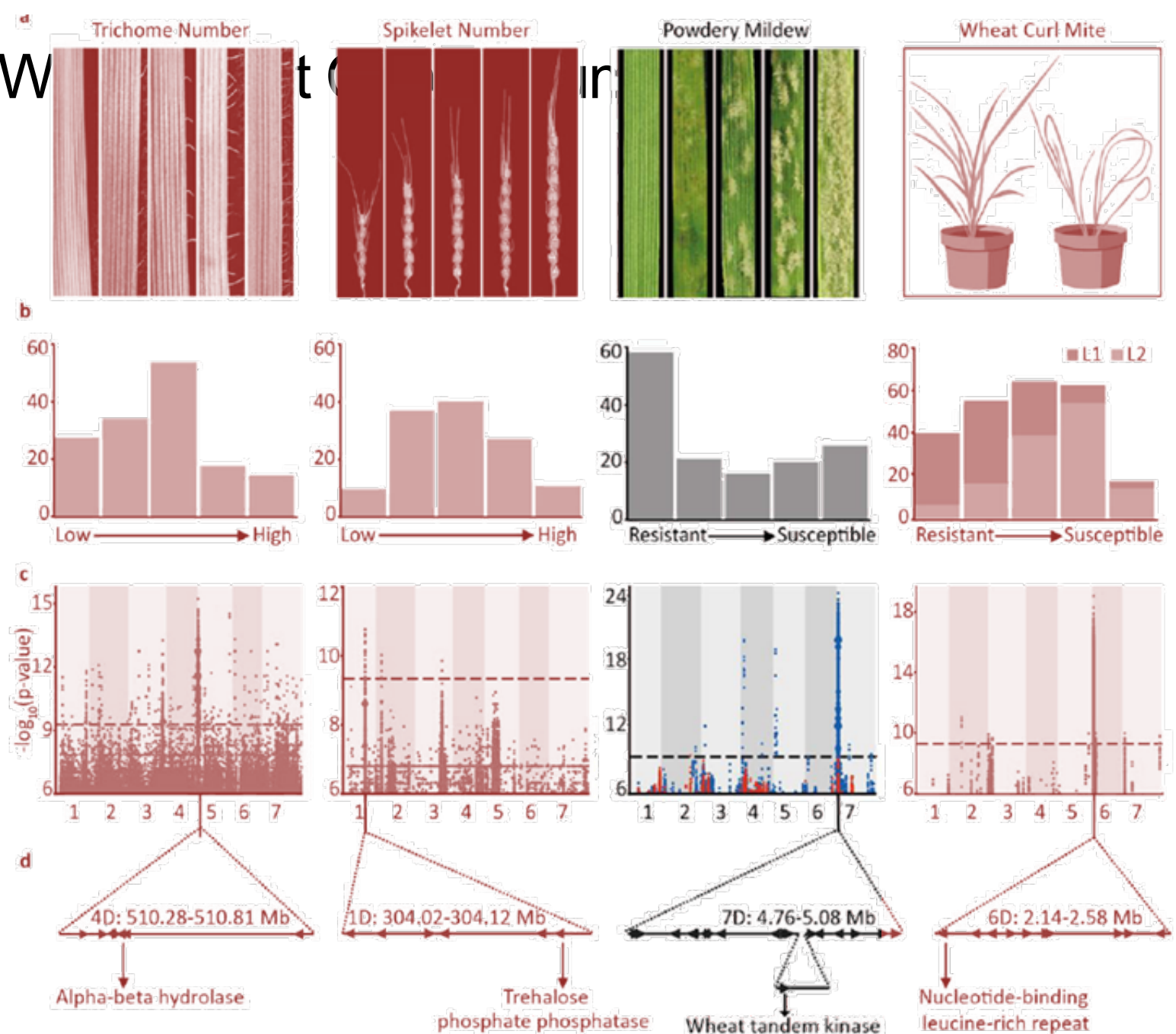
Genes of interest

- WGS short-reading-sequencing
- K-mer-based association mapping

Open Wheat Genomes

Targeted transfer of agronomic traits

- ✓ Leaf trichomes (a/biotic resilience trait)
- ✓ Spikelet number (yield component)
- ✓ Disease resistance (powdery mildew)
- ✓ Pest resistance (wheat curl mite)



WTK4 was identified as a candidate gene for powdery mildew resistance

- Powdery mildew resistance mapped to a 300 kb LD block on chromosome arm 7DS containing eight genes.
 - No NLR immune receptor-encoding gene was detected.
 - However, the interval contains a wheat-tandem kinase (*WTK*), **a gene class of the tandem kinase protein (TPK) family previously reported to confer resistance**
- Wheat stripe rust (*Yr15*), (*WTK1*) Kymiuk et al. 2018
 - Stem rust (*Rpg1*) Brueggeman et al. 2002, *Sr60* (*WTK2*) Chen et al. 2020, *Sr62* Yu et al. 2022
 - Wheat powdery mildew *Pm24* (*WTK3*), Lu et al. 2020

