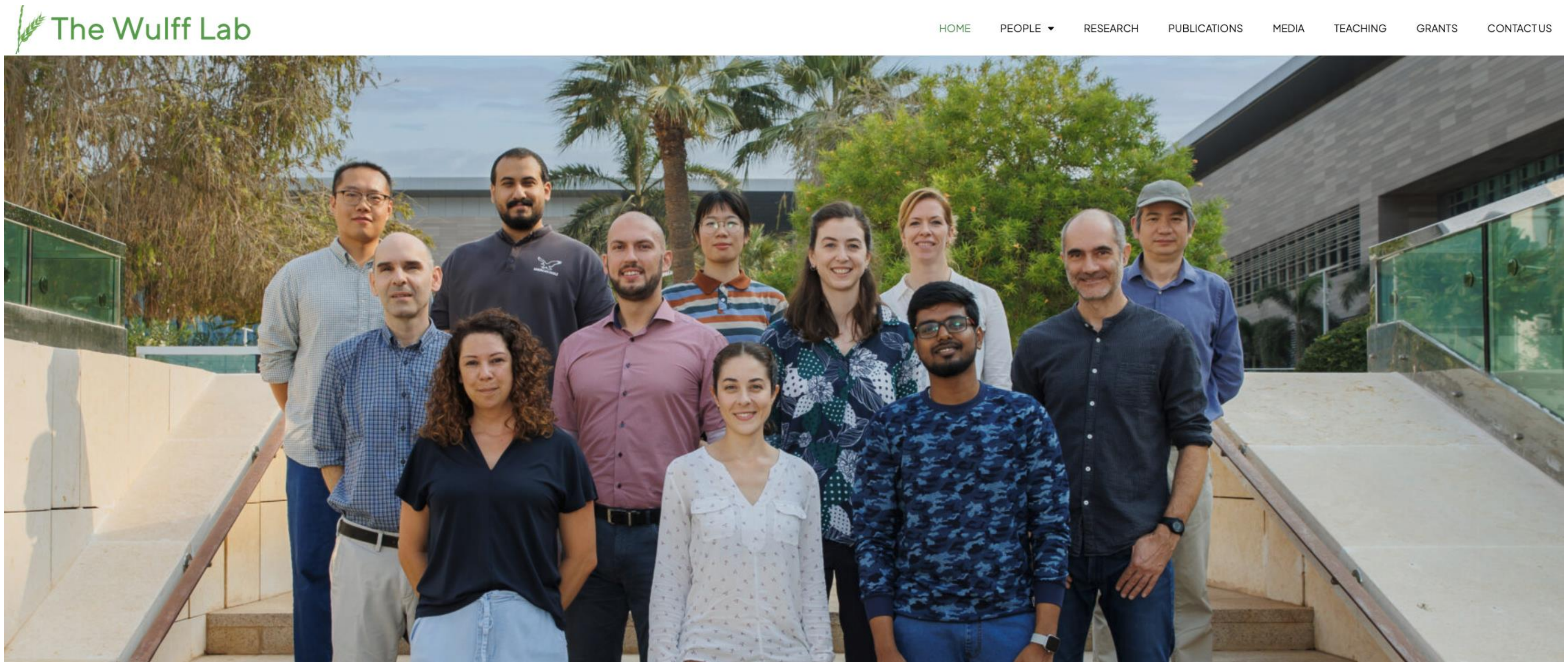
The wheat stem rust resistance gene *Sr43* encodes an unusual protein kinase

Guotai Yu, PhD



https://wulfflab.org

Stem rust in wheat

One of the major diseases affecting wheat worldwide.

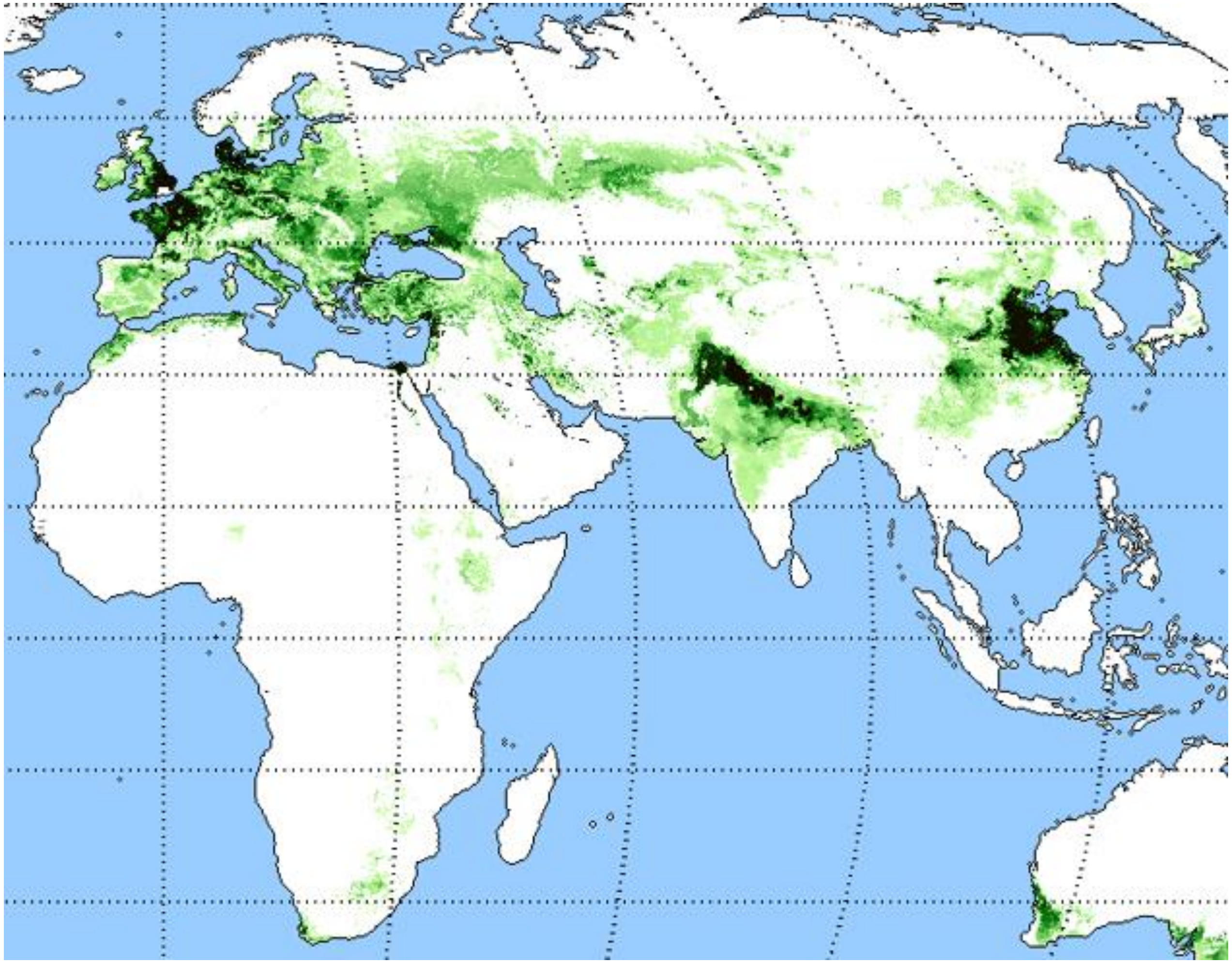
Causal fungal pathogen: *Puccinia graminis* f. sp. *tritici* (*Pgt*).

www.sciencedaily.com

In extreme epidemics, can cause severe lodging.

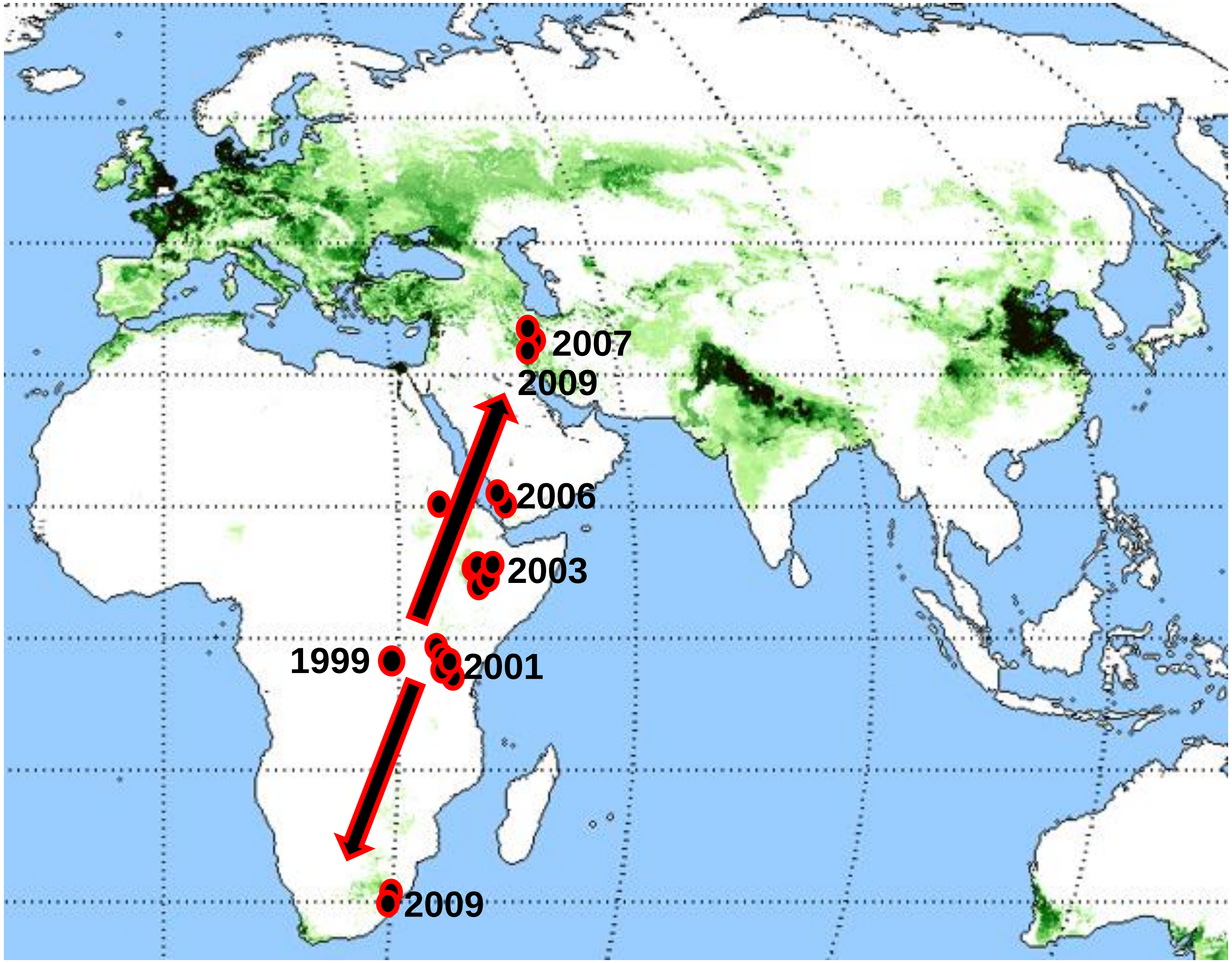
Resistant cultivar Susceptible cultivar

Wheat production map

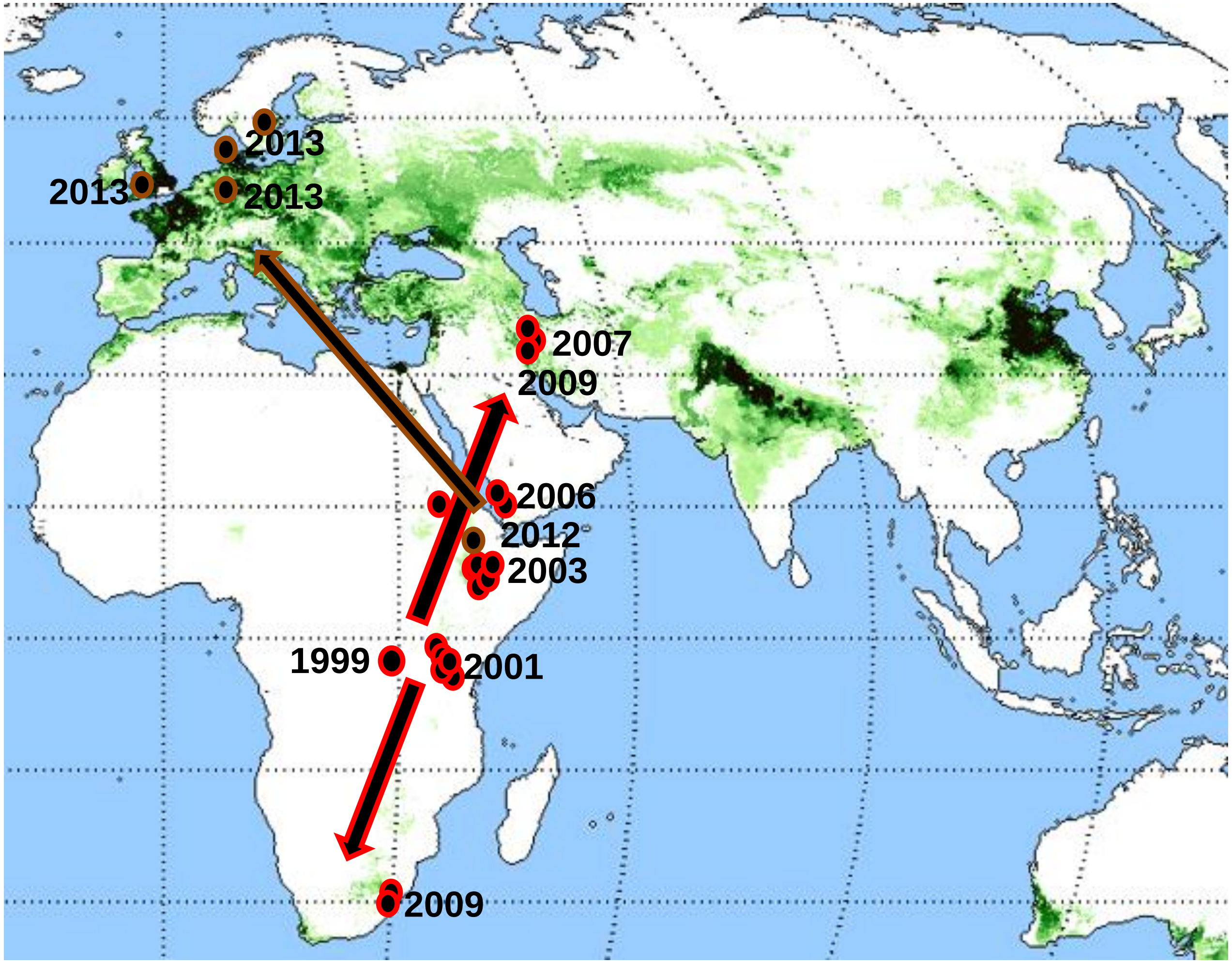


Stem rust in wheat

- ● Ug99 (TTKSK) sites



Stem rust in wheat



- ● Ug99 (TTKSK) sites
- ● TKTTF

Sr genes overcome by new races

Sr31 defeated by Ug99 lineage TTKSK (1999)

Sr24, defeated by Ug99 lineage TTKST (2006)

Sr36 defeated by Ug99 lineage TTTSK (2007)

SrTmp in Digalu defeated by race TKTTF (2013)

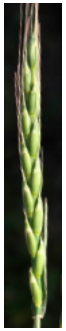
General Goal: multi-resistance gene stacking

Wheat

2007 David Monniaus

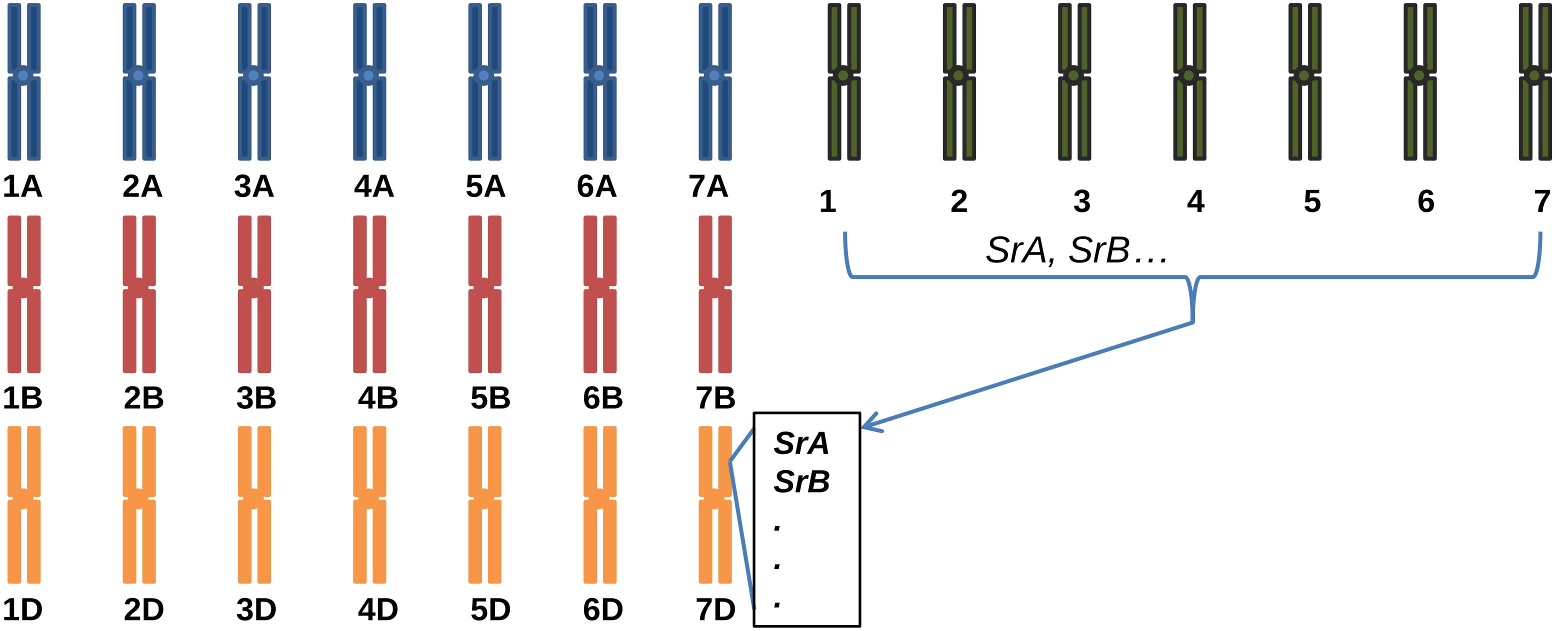
Wild relatives

Ae. longissima



Ae. sharonensis

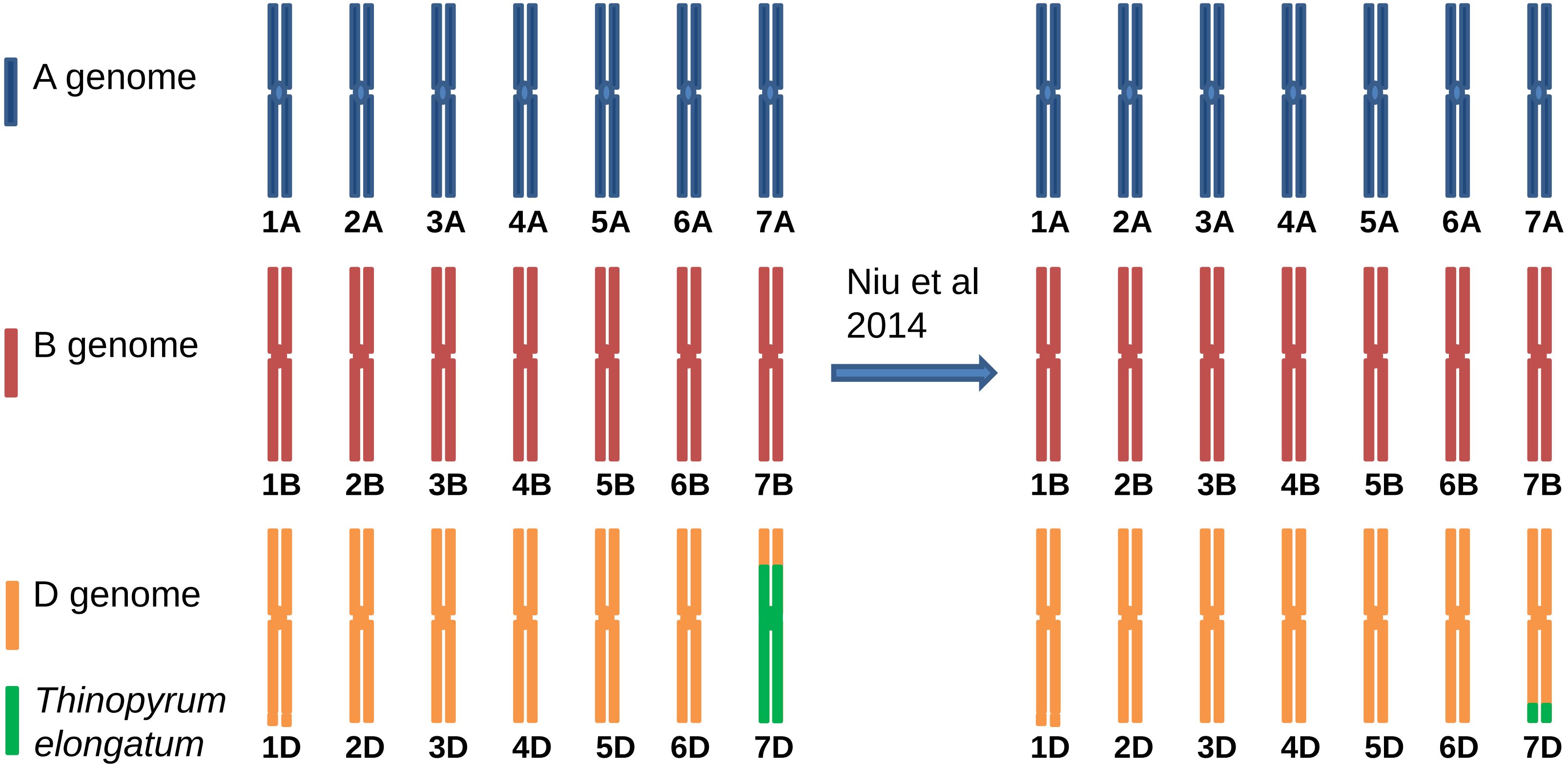
Ae. tauschii



SrA, SrB…

SrA
SrB
.
.
.

Sr43 introgression line



🟦 A genome

🟥 B genome

🟧 D genome

🟩 *Thinopyrum elongatum*

1A 2A 3A 4A 5A 6A 7A

1B 2B 3B 4B 5B 6B 7B

1D 2D 3D 4D 5D 6D 7D

Niu et al 2014

1A 2A 3A 4A 5A 6A 7A

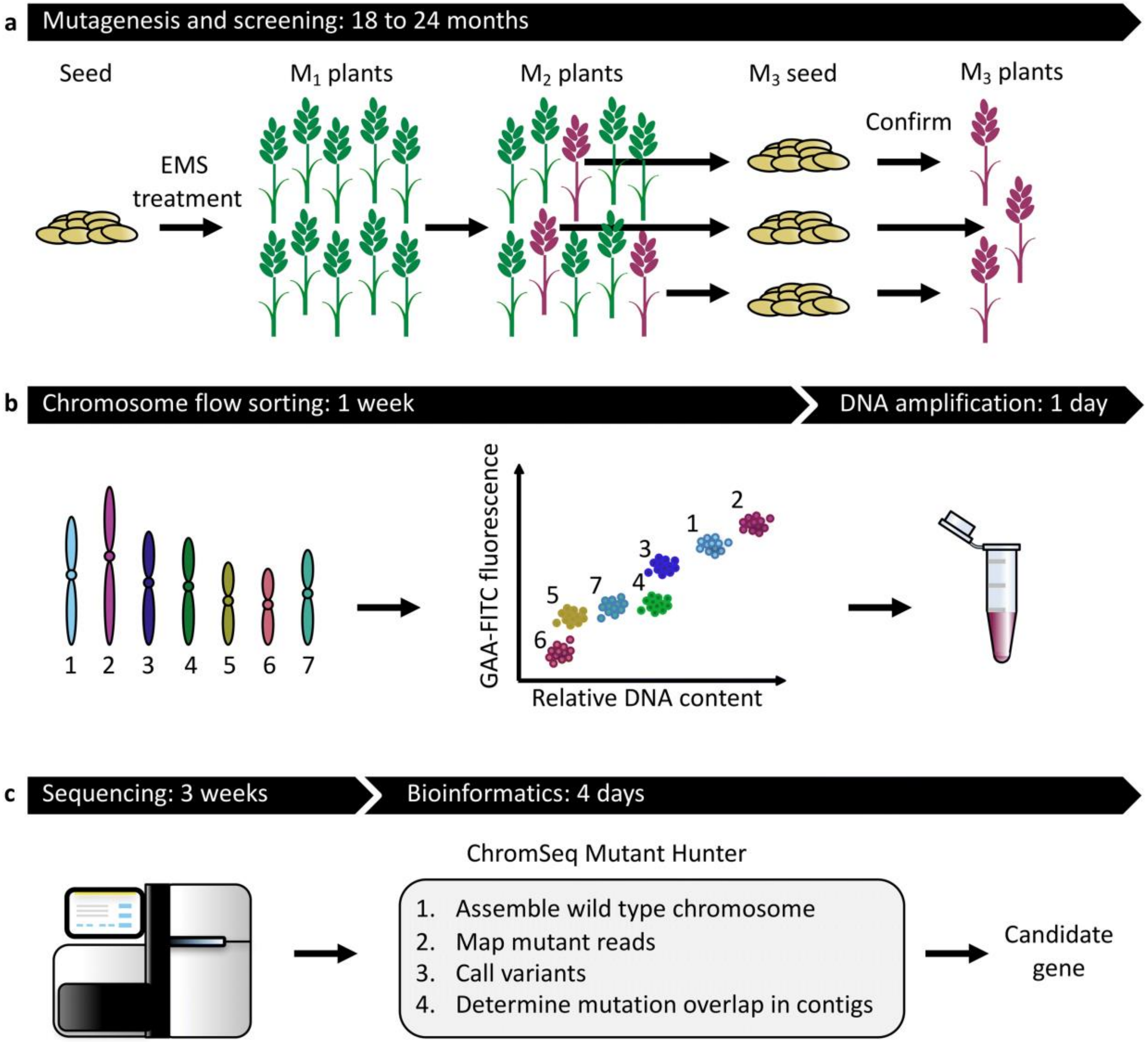
1B 2B 3B 4B 5B 6B 7B

1D 2D 3D 4D 5D 6D 7D

$7DS\cdot 7el_2S\text{-}7el_2L$

$7DS\cdot 7DL\text{-}7el_2L$

MutChromSeq method



1. Assemble wild type chromosome
2. Map mutant reads
3. Call variants
4. Determine mutation overlap in contigs

Sánchez-Martín et al 2016

Sr43 mutant development

Mutagenized 2,700 *Sr43* introgression line seeds

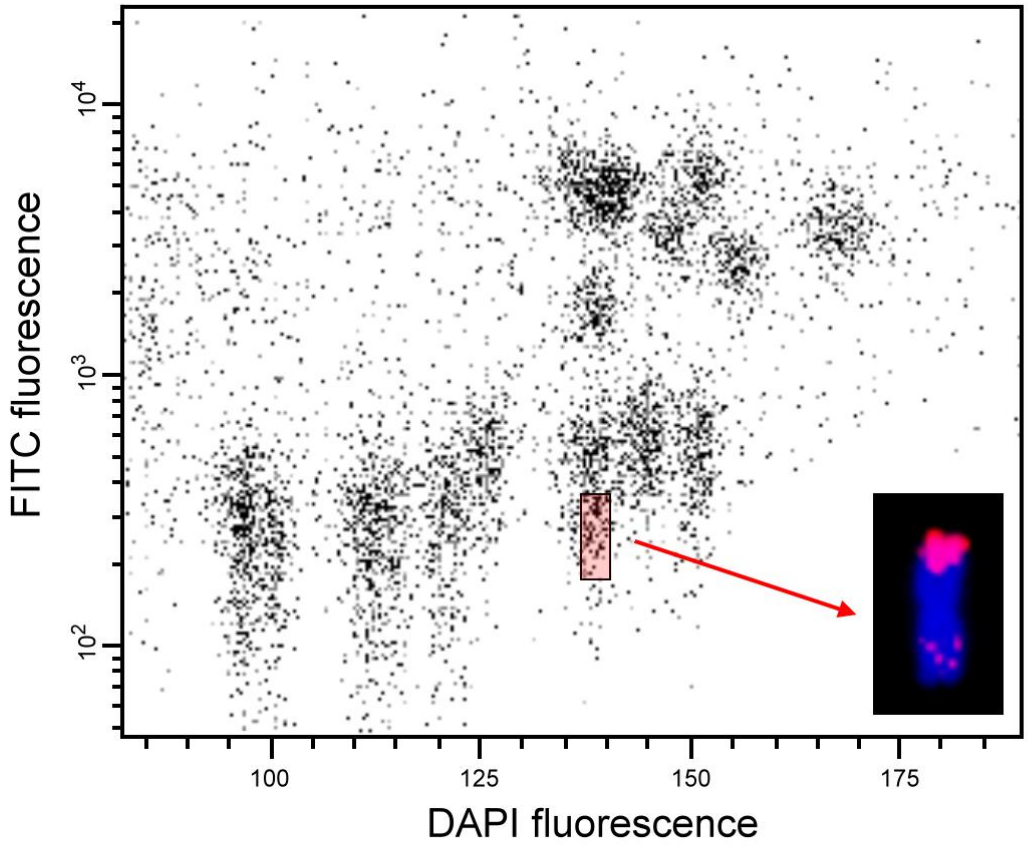
0.80% EMS

1,843 M_2 families with *Pgt* race TPMKC

11 susceptible mutants checked with GBS

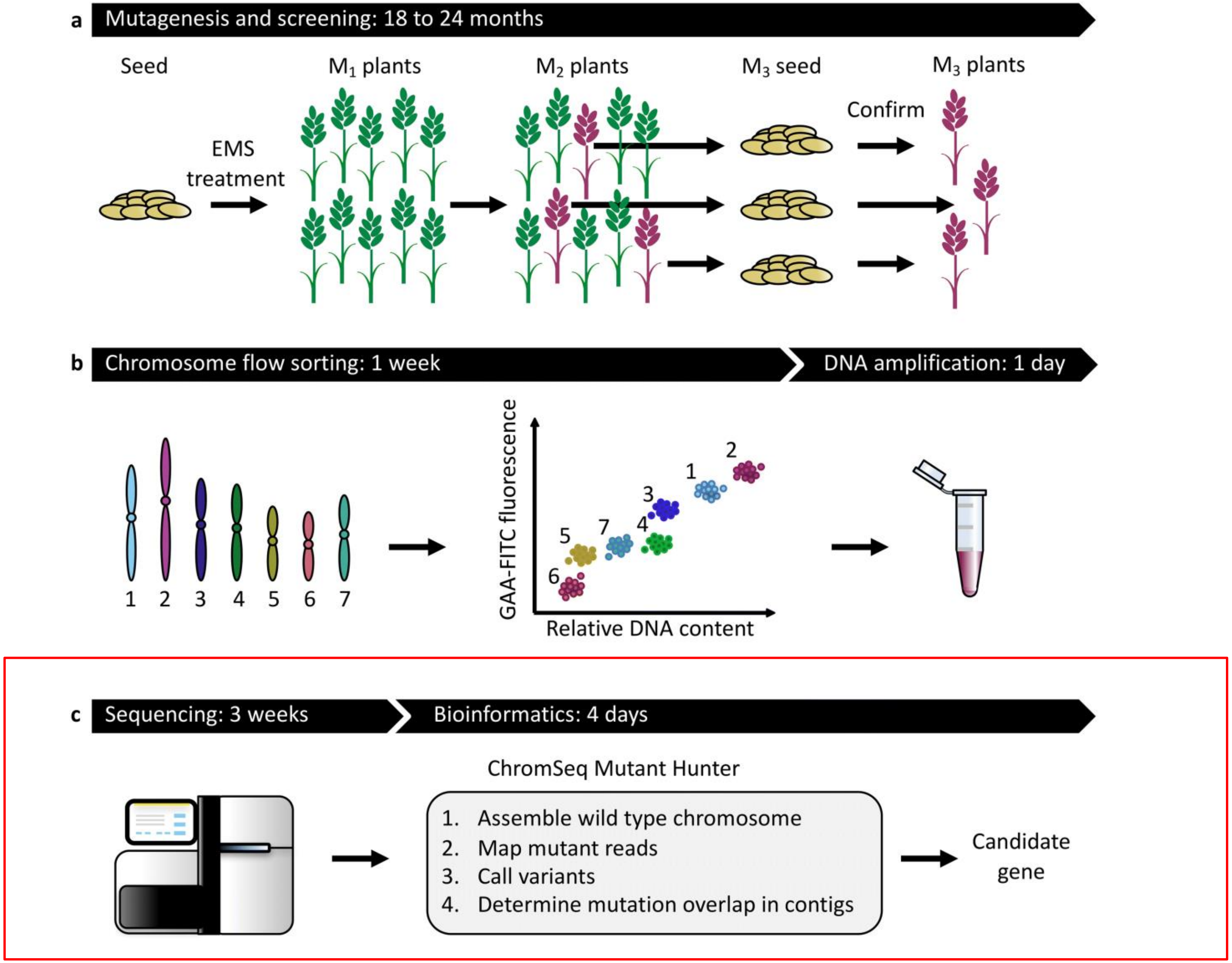
8 mutants used for chromosome flow sorting

Chromosome flow sorting of *Sr43* mutants



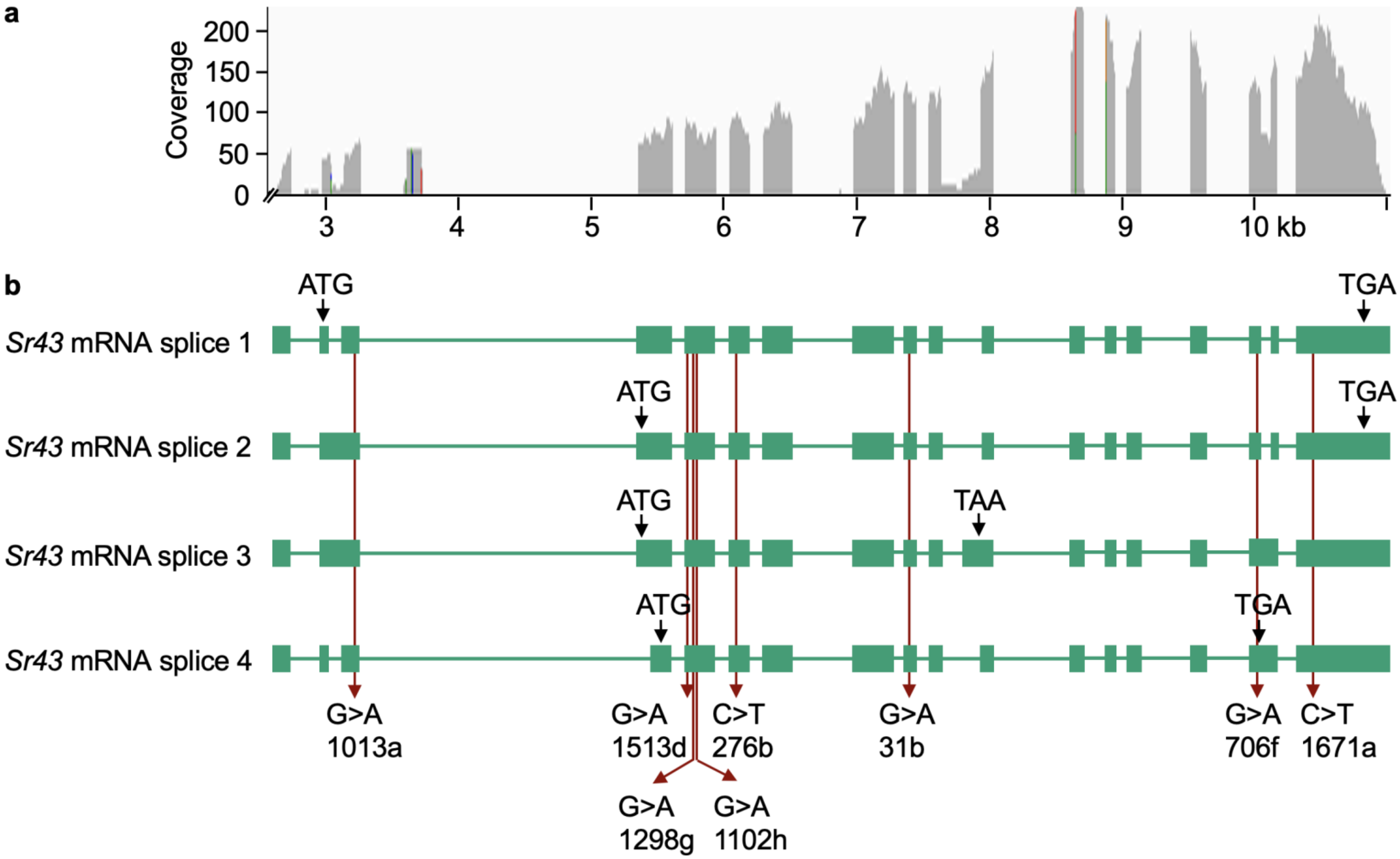
DAPI (x-axis) vs. FITC (y-axis) dot plot was obtained after the analysis of DAPI-stained chromosome suspensions labeled by FISHIS with FITC-conjugated probes for GAA and ACG microsatellites. The 7D/7el2 translocation chromosomes were sorted from the sorting window shown as red rectangle at purities of 60–65%. Inset: 7D/7el2 translocation chromosome after FISH with probes for pSc119.2 repeat (green), Afa family repeat (red) and 45S rDNA (yellow) that was used to identify chromosomes in the sorted fraction. Chromosomes were counterstained by DAPI (blue).

MutChromSeq method

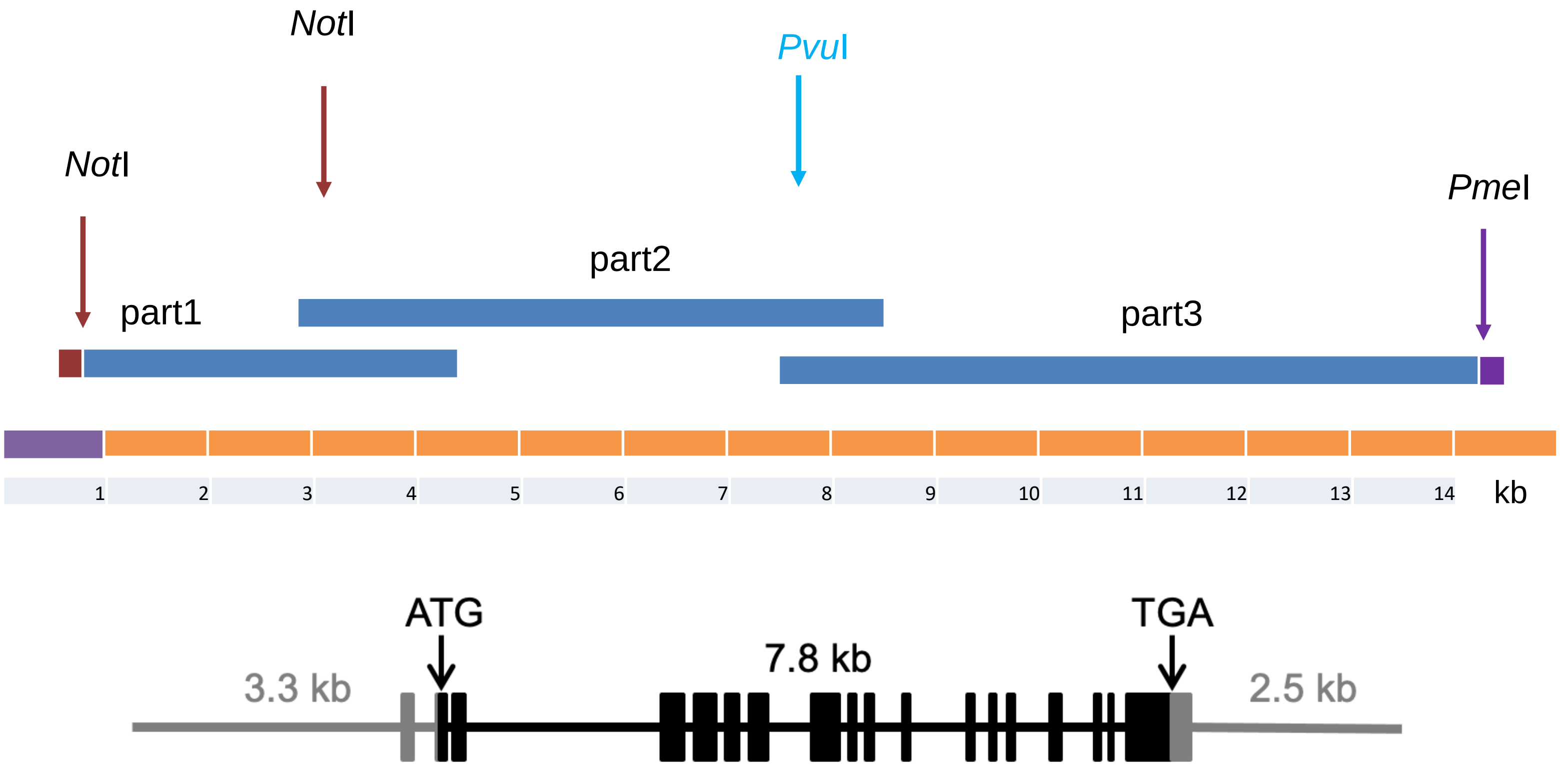


Sánchez-Martín et al 2016

Sr43 transcripts and alternative splicing

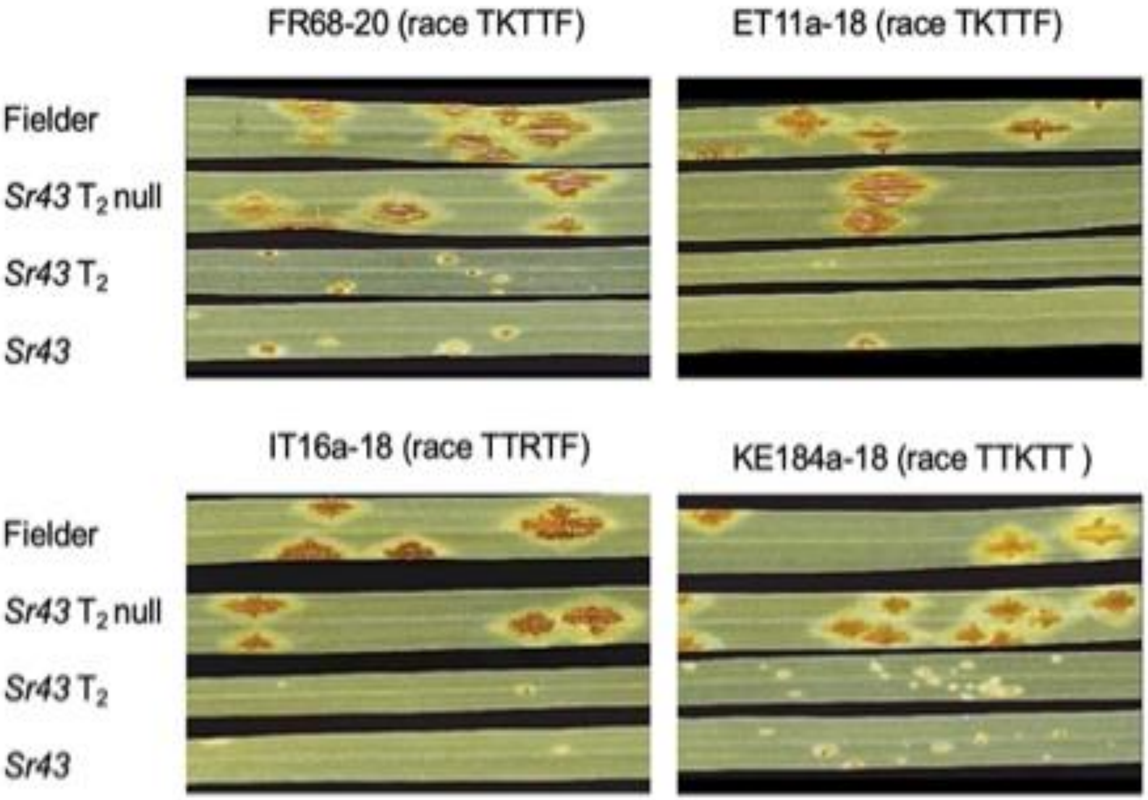


Sr43 construct for transformation

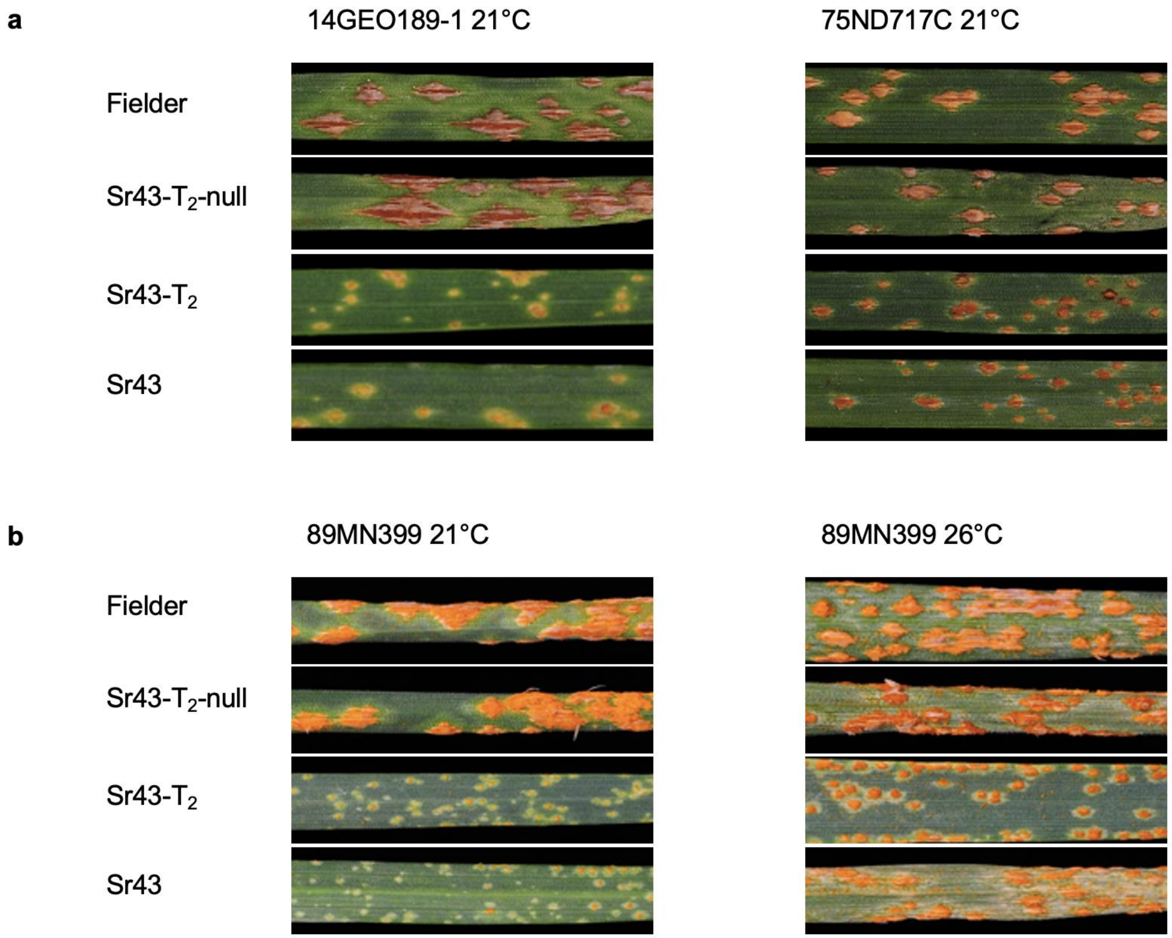


Ligated into vector pGGG and transformed into Fielder

Confirmation of *Sr43* function



Confirmation of *Sr43* race specificity and temperature sensitivity

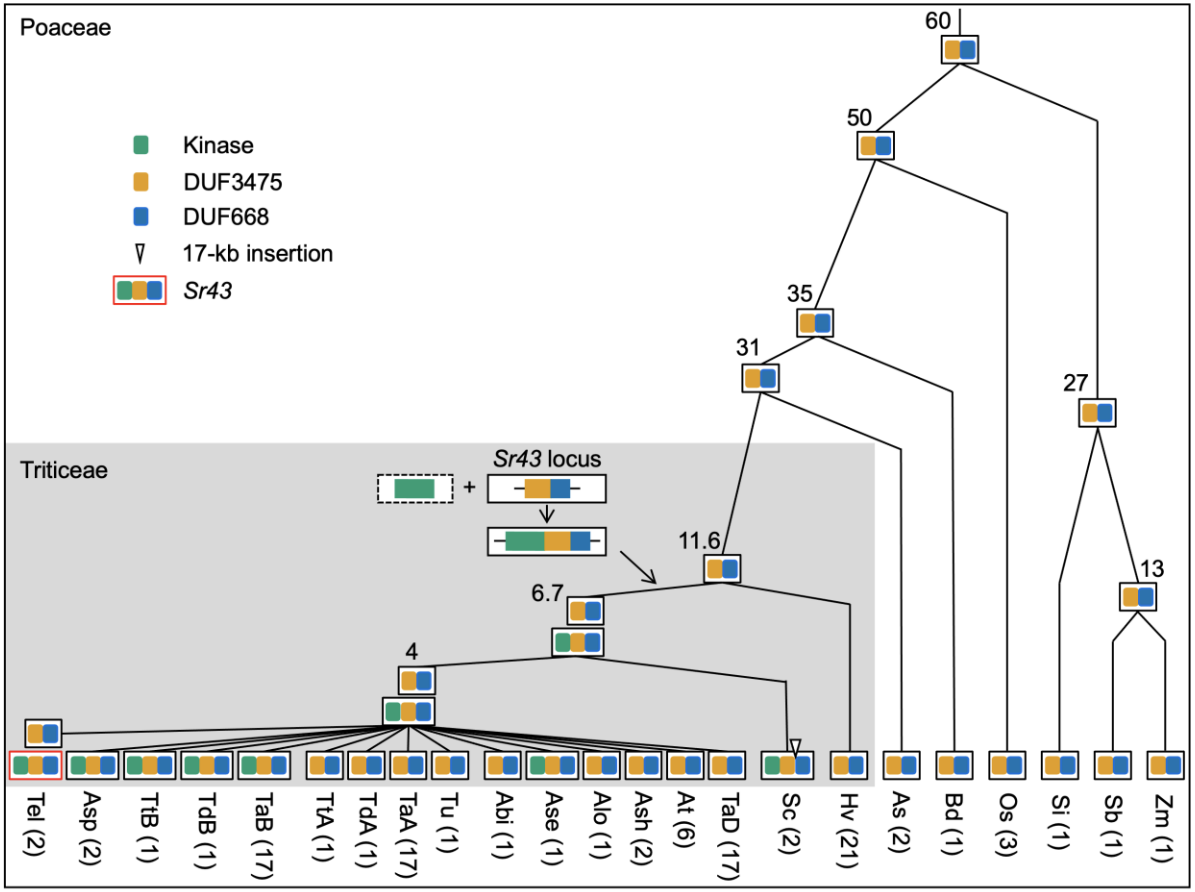


Sr43 has broad spectrum efficacy

Entry	Isolate	Race	Origin	Year	Use in study
1	74MN1409	TPMKC	USA	1974	Screening for mutants*
2	75ND717C	QTHJC	USA	1975	Phenotyping T2
3	69MN399	QTHJC	USA	1969	Phenotyping T2
4	ET11a-18	TKTTF	Ethiopia	2018	Phenotyping T2
5	KE184a/18	TTKTT	Kenya	2018	Phenotyping T2
6	IS#2079	TKTSC	Israel	2022	Phenotyping T1
7	IS#2127	TTTTF	Israel	2022	Phenotyping T1
8	IS#2135	TTTTC	Israel	2022	Phenotyping T1
9	FR68-20	TKTTF	France	2020	Phenotyping T2
10	IT16a-18	TTRTF	Italy	2018	Phenotyping T2
11	UK-01	TKTTF	United Kingdom	2018	Phenotyping T1
12	14GEO189-1	TTRTF	Georgia	2014	Phenotyping T2

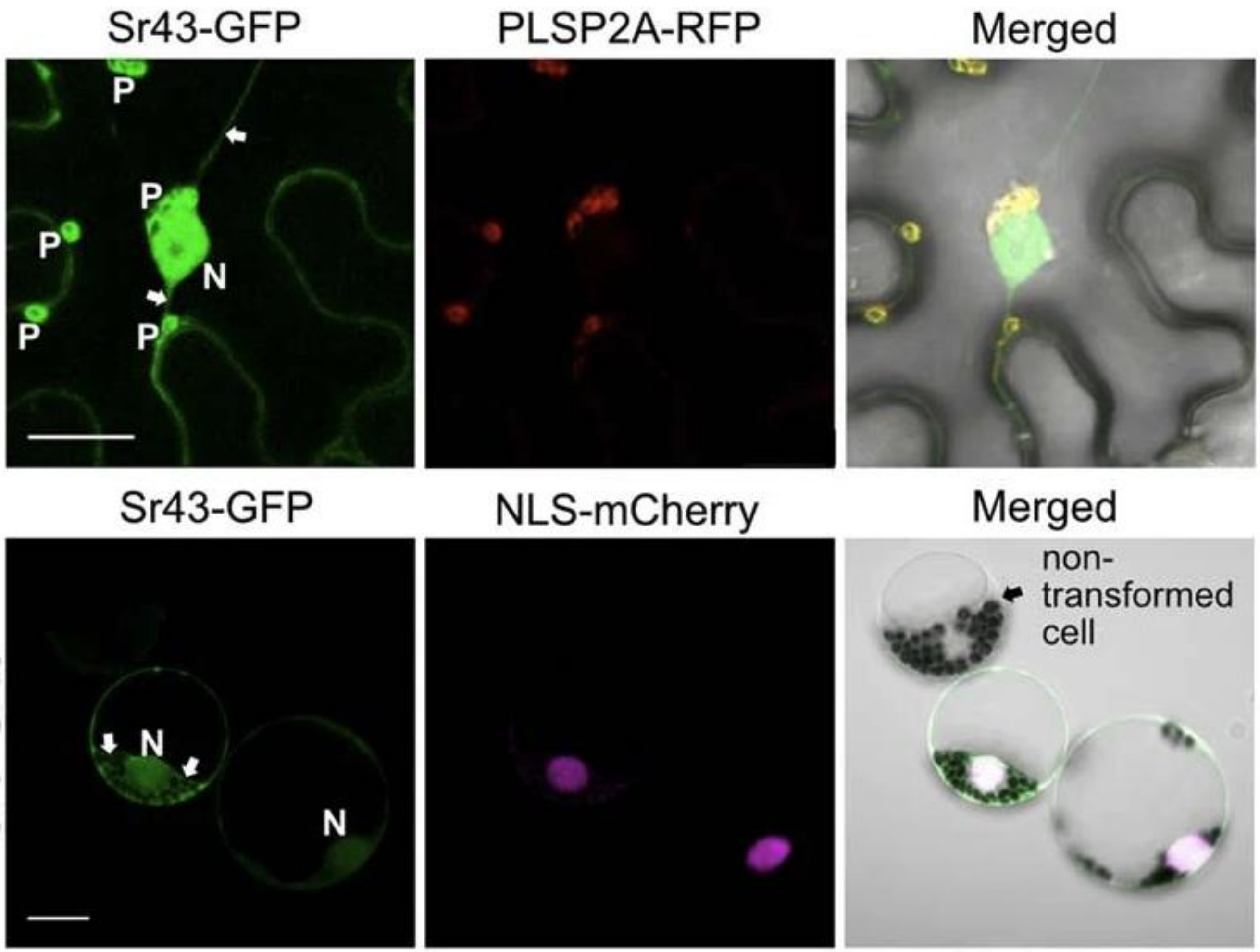
*Isolate 74MN1409 was not used to phenotype the transgenics because it is not virulent on wheat cv. Fielder.

Origin and distribution of *Sr43* orthologs



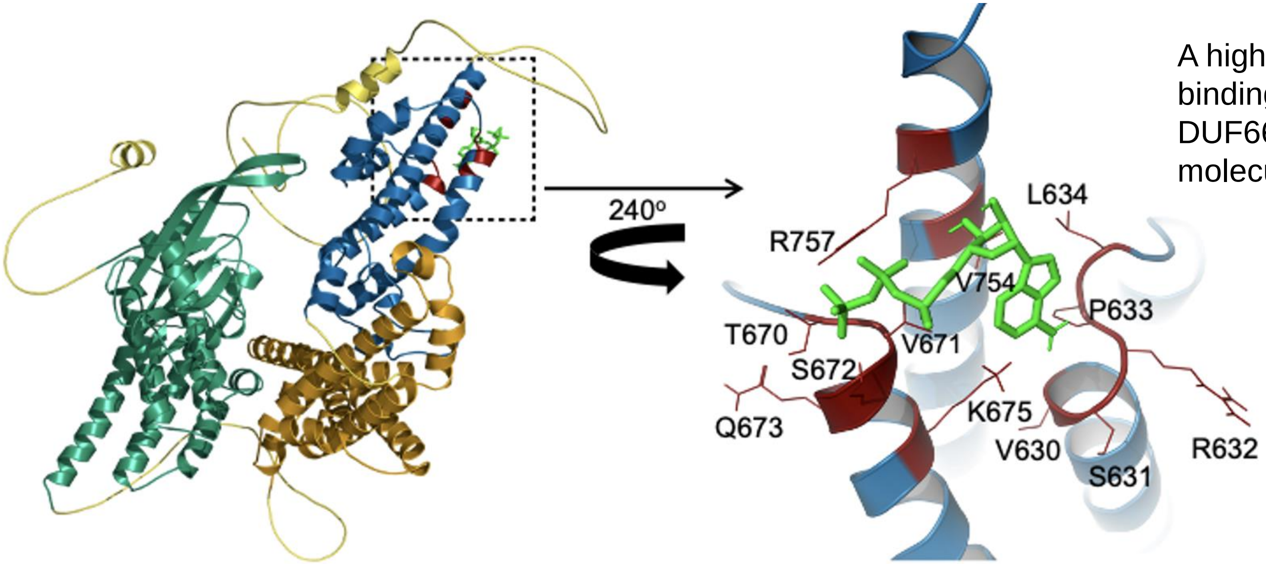
Species are indicated at the bottom and abbreviated as follows: Tel, *Thinopyrum elongatum*; Asp, *Ae. Speltoides*; TtB, *Triticum turgidum* spp. *Durum* B genome; TdB, *T. dicoccoides* B genome; TaB, *T. aestivum* B genome; TtA, *T. turgidum* ssp. Durum A genome; TdA, *T. dicoccoides* A genome; TaA, *T. aestivum* A genome; Tu, *T. urartu*; Abi, *Aegilops bicornis*; Ase, *Ae. searsii*; Alo, *Ae. longissima*; Ash, *Aegilops sharonensis*; At, *Ae. tauschii*; TaD, *T. aestivum* D genome; Sc, *Secale cereale* (Rye); Hv, *Hordeum vulgare*; As, *Avena sativa*; Bd, *Brachypodium distachyon*; Os, *Oryza sativa*; Si, *Setaria italica*; Sb, *Sorghum bicolor*; Zm, *Zea mays*. The number of genomes analyzed for each species is indicated in parentheses.

Sr43 localizes to the nucleus and plastids

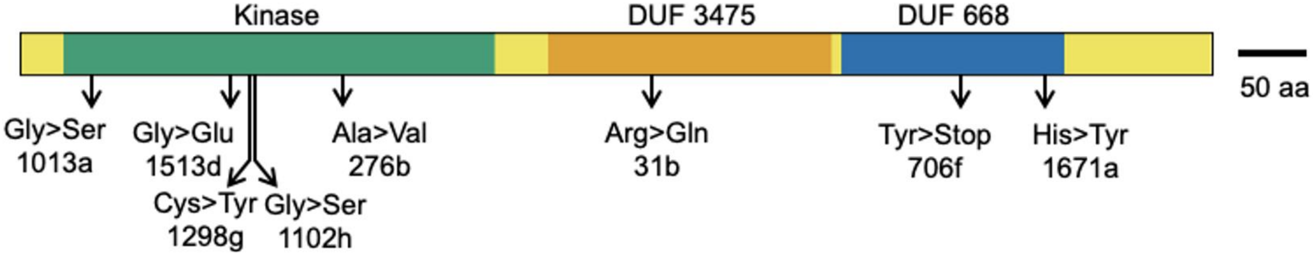


PLSP2A-RFP: plastid marker NLS-mCherry: nuclear marker

Sr43 encodes a protein kinase fused to two domains of unknown function

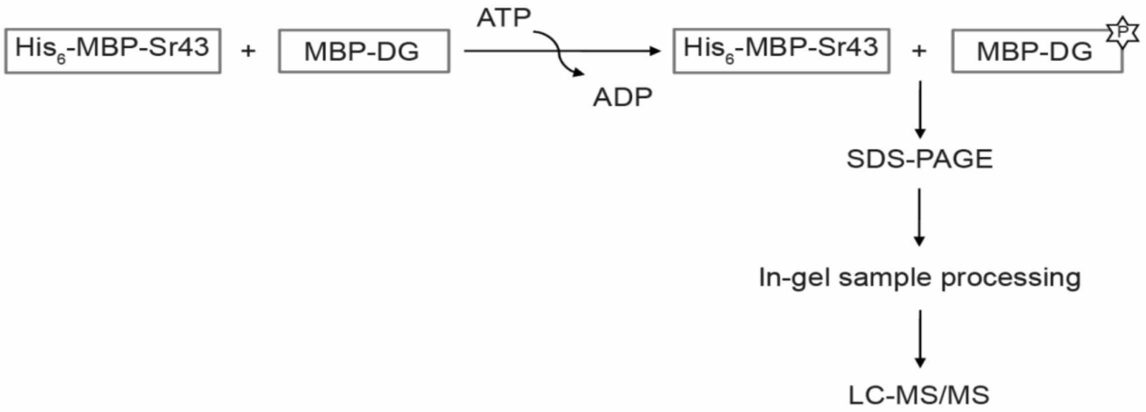


A high-confidence ATP-binding site (red residues) in DUF668 bound to an ATP molecule.



Activity of Sr43 encoded kinase

a



His_6-MBP-Sr43 + MBP-DG $\xrightarrow[\text{ADP}]{\text{ATP}}$ His_6-MBP-Sr43 + MBP-DG

SDS-PAGE

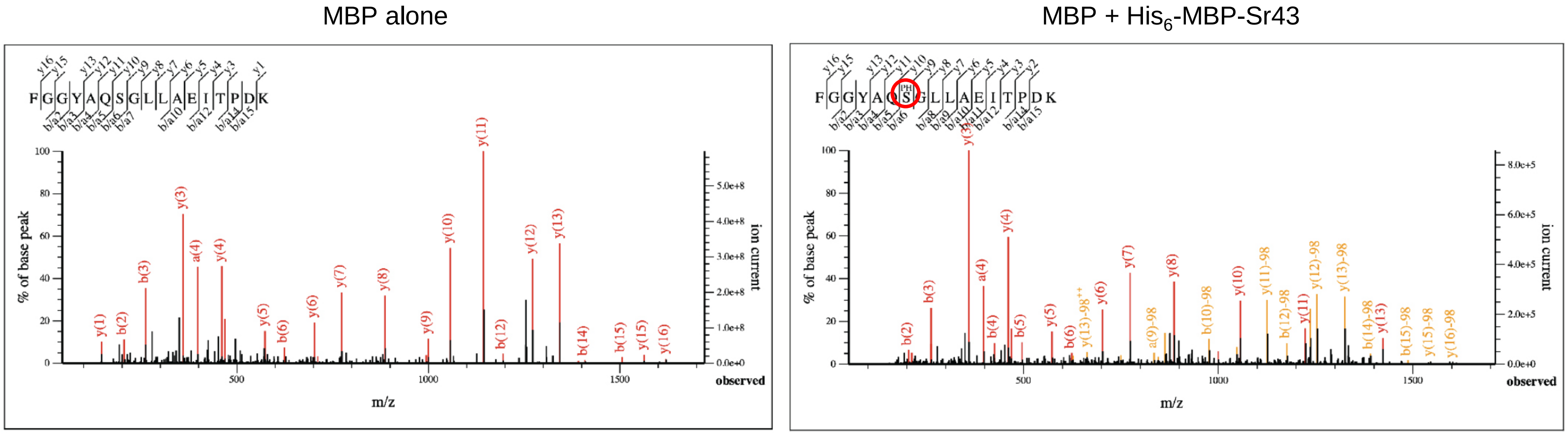
In-gel sample processing

LC-MS/MS

Experimental setups

b

S74

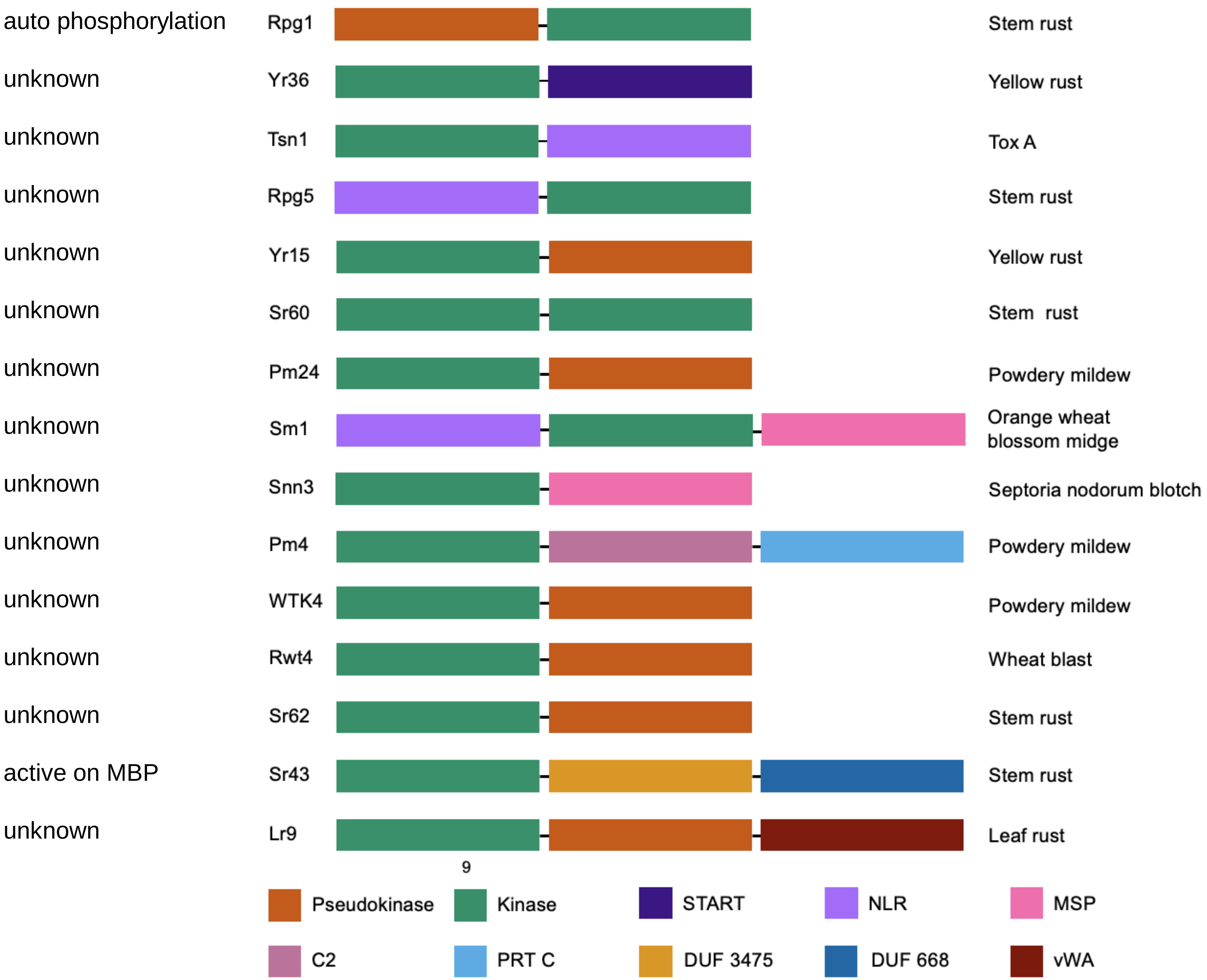


MBP alone

MBP + His_6-MBP-Sr43

S74, one of the 10 amino-acids phosphorylated by Sr43 kinase.

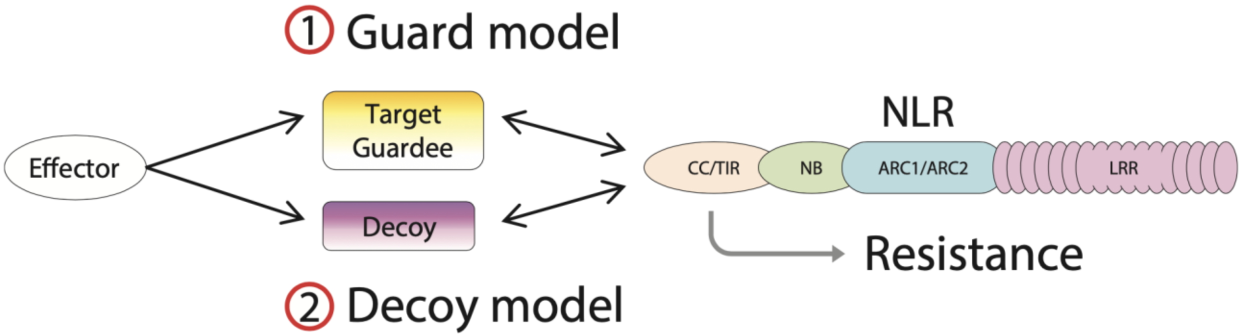
Triticeae kinase fusion proteins with disease resistance function



auto phosphorylation	Rpg1	Stem rust
unknown	Yr36	Yellow rust
unknown	Tsn1	Tox A
unknown	Rpg5	Stem rust
unknown	Yr15	Yellow rust
unknown	Sr60	Stem rust
unknown	Pm24	Powdery mildew
unknown	Sm1	Orange wheat blossom midge
unknown	Snn3	Septoria nodorum blotch
unknown	Pm4	Powdery mildew
unknown	WTK4	Powdery mildew
unknown	Rwt4	Wheat blast
unknown	Sr62	Stem rust
active on MBP	Sr43	Stem rust
unknown	Lr9	Leaf rust

Pseudokinase
Kinase
START
NLR
MSP
C2
PRT C
DUF 3475
DUF 668
vWA

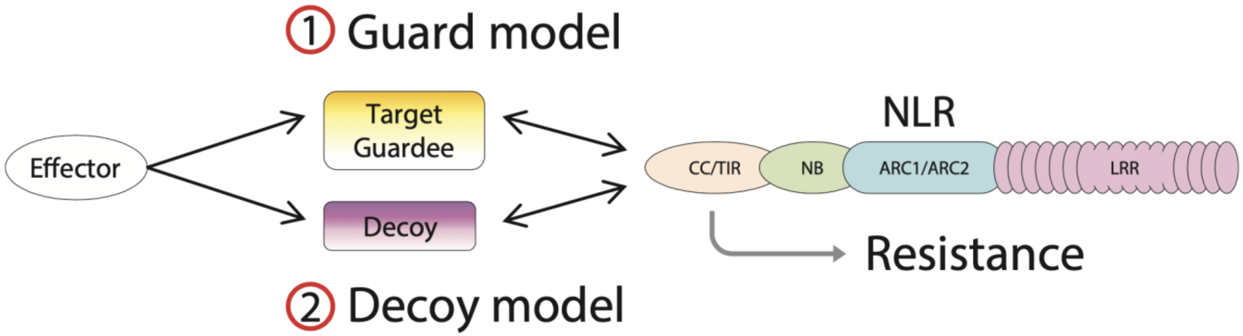
NLR models



van der Biezen and Jones, 1988

van der Hoorn and Kamoun 2008

NLR models



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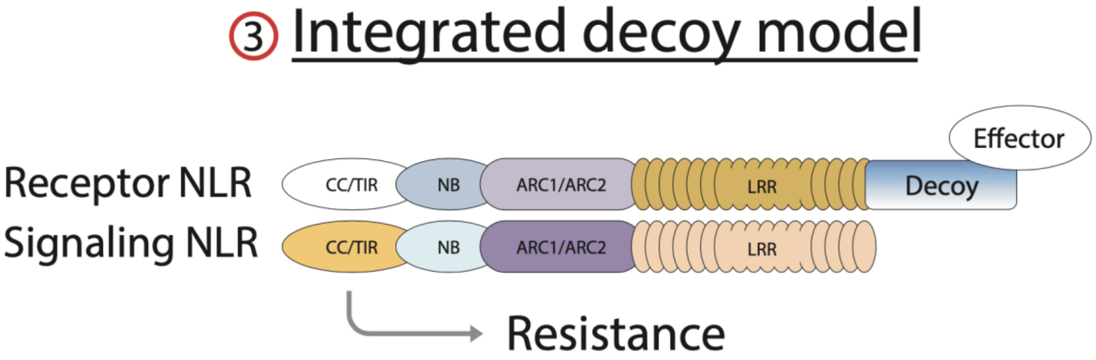
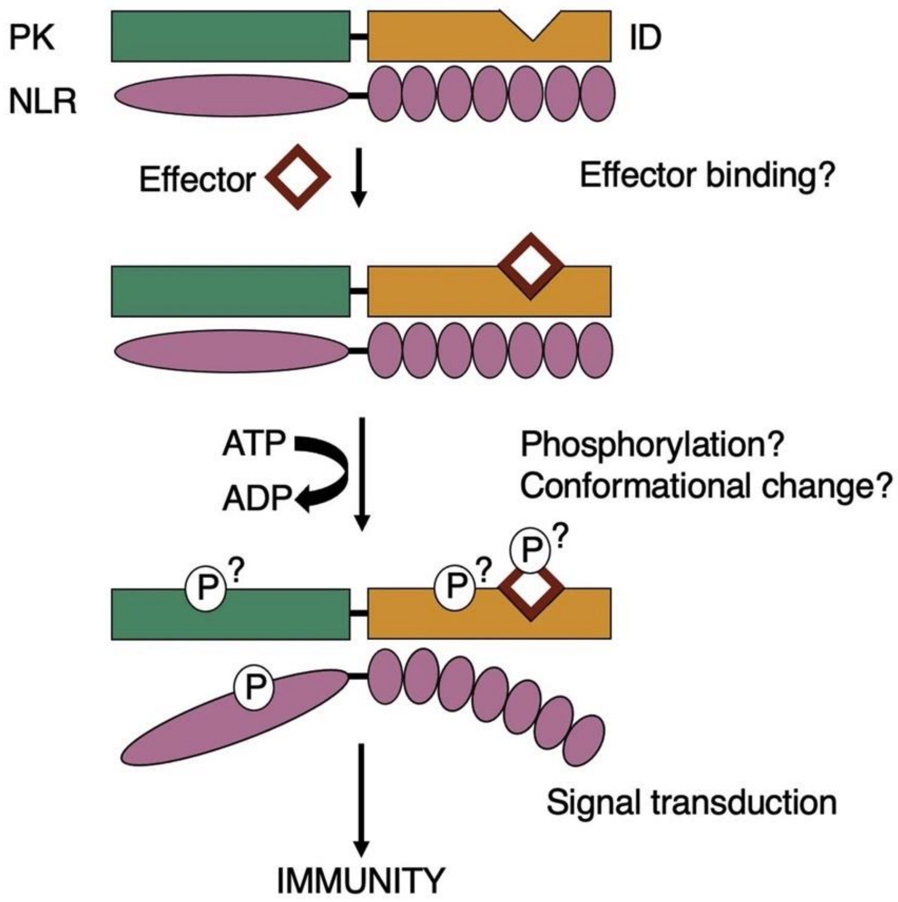


Cesari et al, 2014

Integrated decoy model for kinase fusion proteins



The protein kinase (PK) fusion protein contains an integrated decoy (ID) that traps the avirulence effector protein (red square). This triggers an autophosphorylation of either the protein kinase or decoy, the effector, or the nucleotide binding leucine-rich repeat (NLR) guard. This results in a conformational change which in turn triggers a signal cascade leading to downstream defense responses and immunity.

Summary

1. *Sr43* cloned
2. *Sr43* confers broad-spectrum stem rust resistance
3. *Sr43* encodes an unusual protein kinase
4. Kinase-fusion protein integrated decoy model

Acknowledgements

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