Webinar: Wheat genomics facilitates the discovery of kinase fusion proteins as major players in rust resistance

An unusual tandem kinase fusion protein confers leaf rust resistance in wheat

05/10/2023

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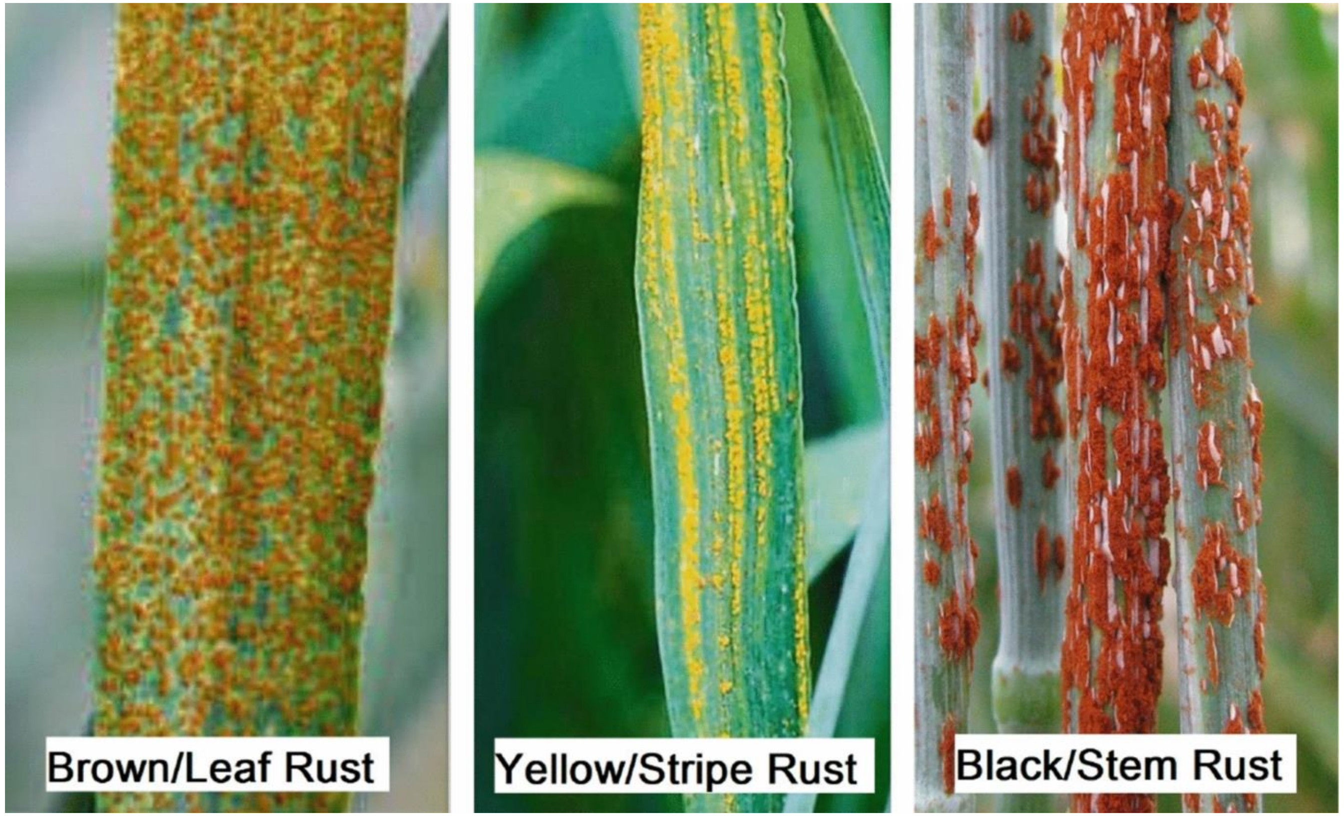
Introduction

- About **60 million tons**, account for **6%** of the global wheat production are lost to rust diseases every year

83 billion

230 million people for one year



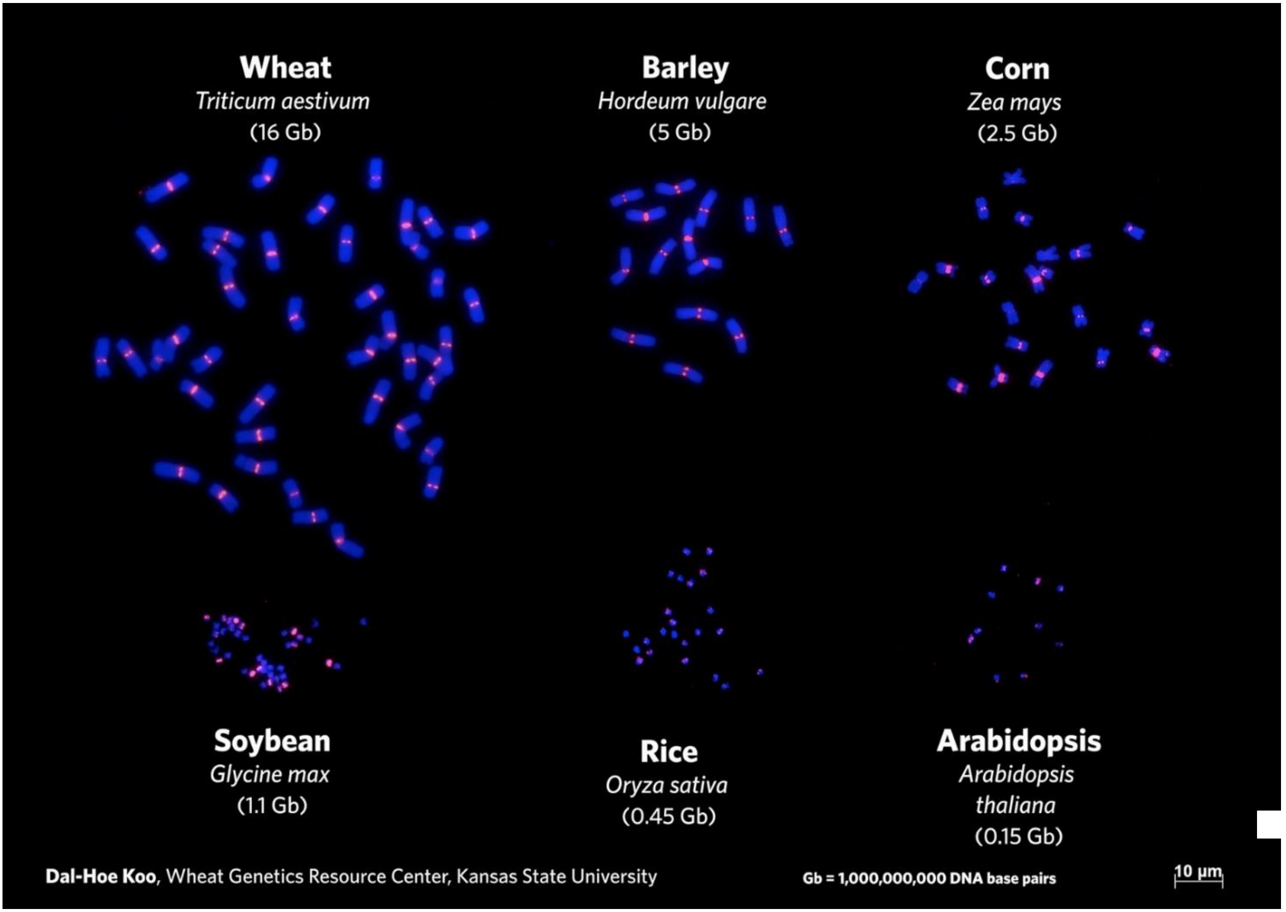
- Annual energy lost due to the to diseases and pests is **120 billion kWh**

12–112 million households for 1 year

- Using resistance genes and breeding resistant cultivars are the most economical and environment-friendly ways

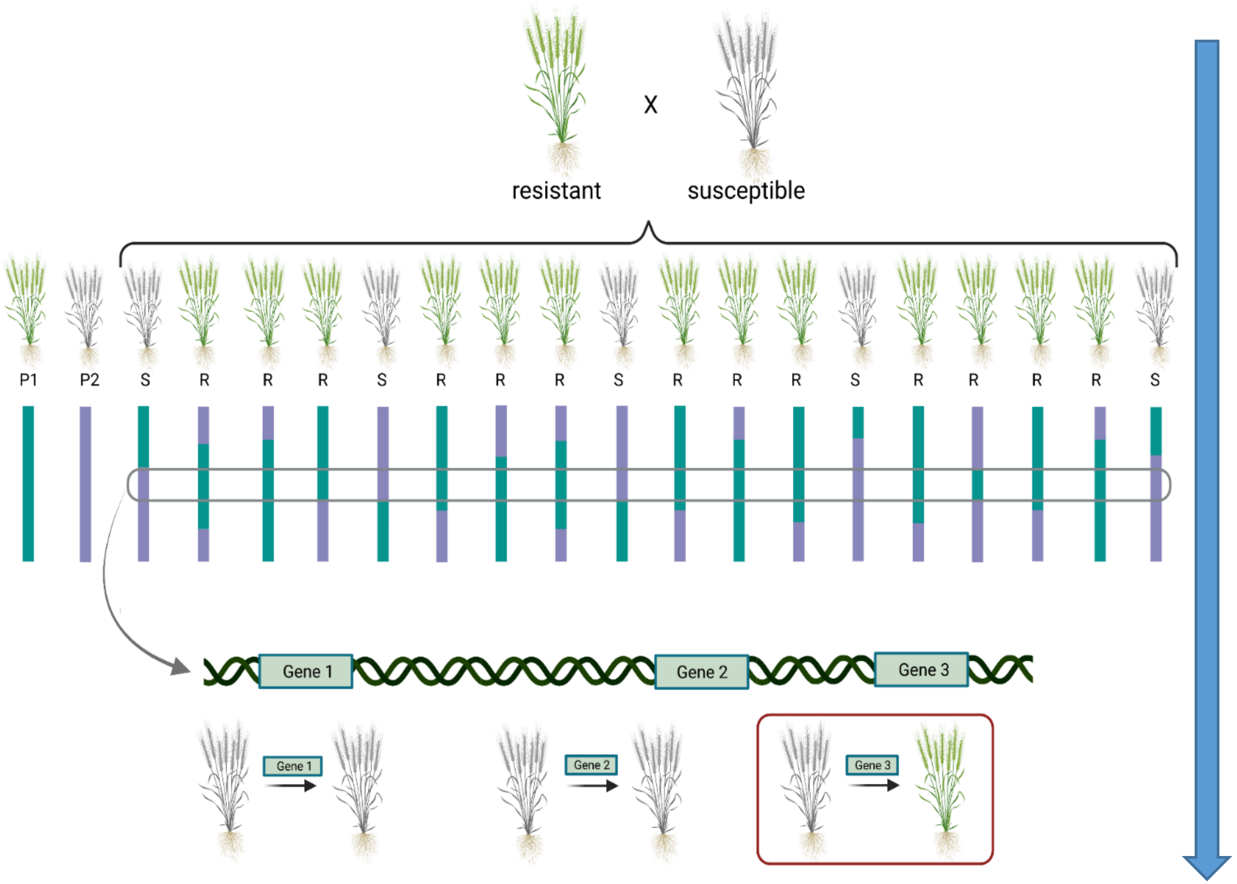
Hafeez, A.N. *et al. Molecular Plant* , 2021 Wulff, B. B., & Krattinger, S. G. *Current Opinion in Biotechnology*, 2022

Challenges in gene cloning from wheat



Dal-Hoe Koo, Wheat Genetics Resource Center, Kansas State University

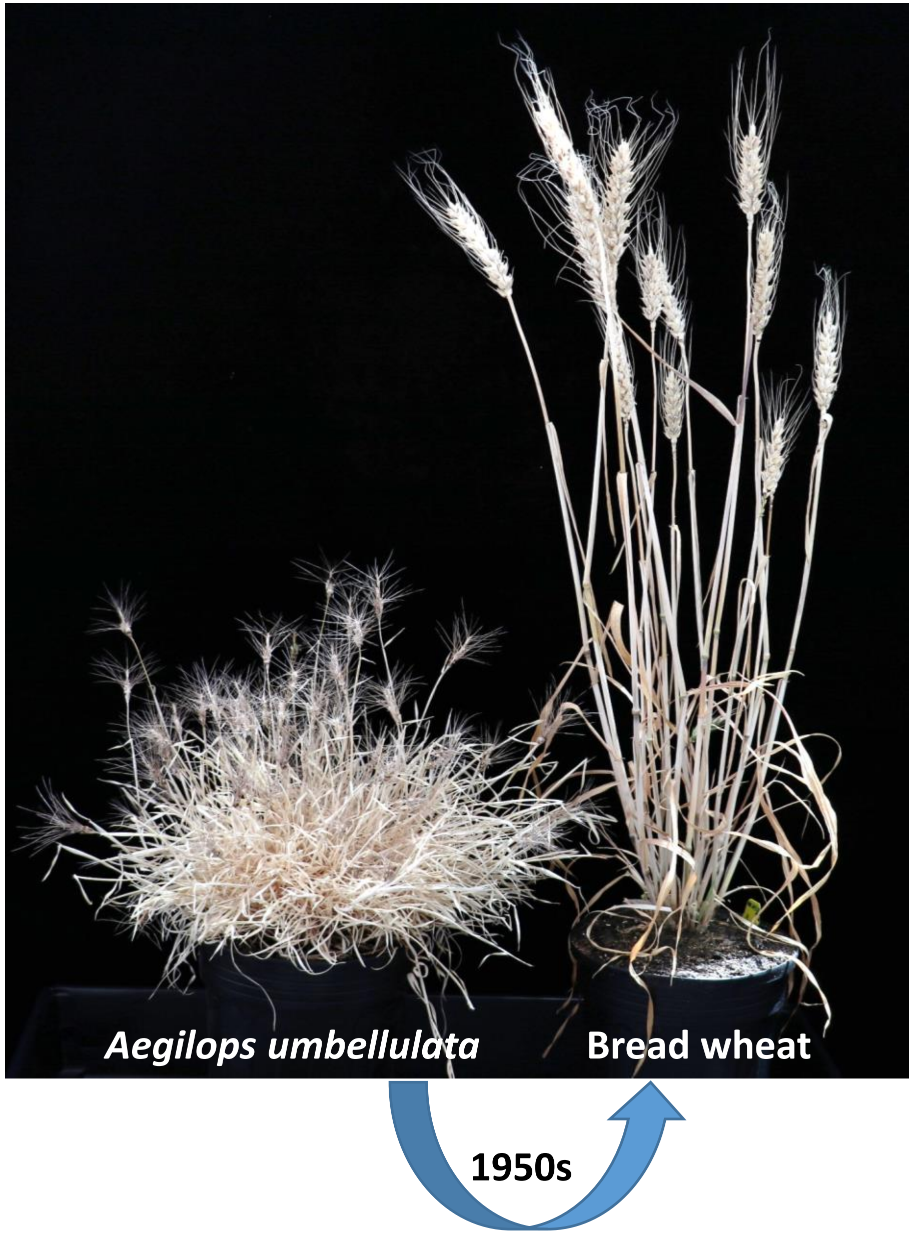
Gb = 1,000,000,000 DNA base pairs



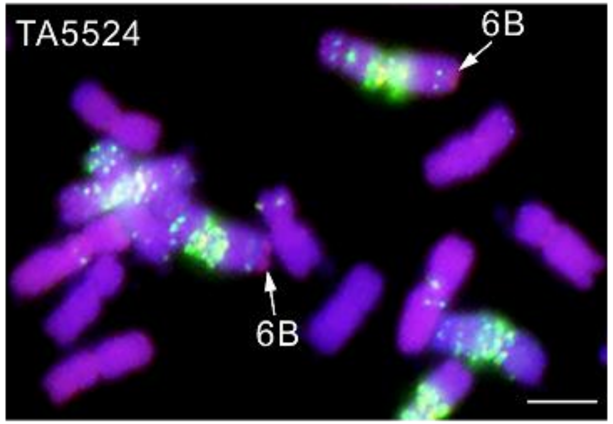
>3-5 years

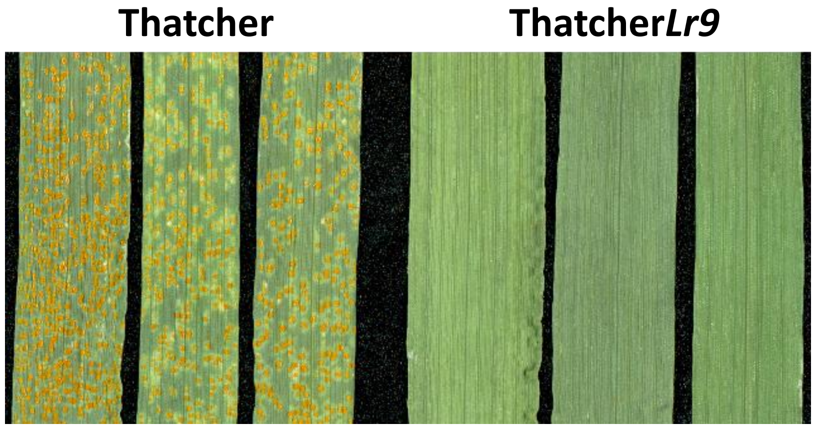
Challenges in gene cloning from wheat

- The bread wheat gene pool contains **450** disease resistance genes, most of which are not cloned
- More than **40%** of the disease resistance genes used in bread wheat breeding come from outside the bread wheat gene pool.
- The cloning of genes located on alien introgressions has been extremely challenging, because of the lack of recombination
- In a pioneering experiment conducted by Dr. Ernest Sears in the 1950s, a chromosome fragment contains wheat leaf rust resistance gene Lr9 was introgressed into bread wheat from *Ae. umbellulata*



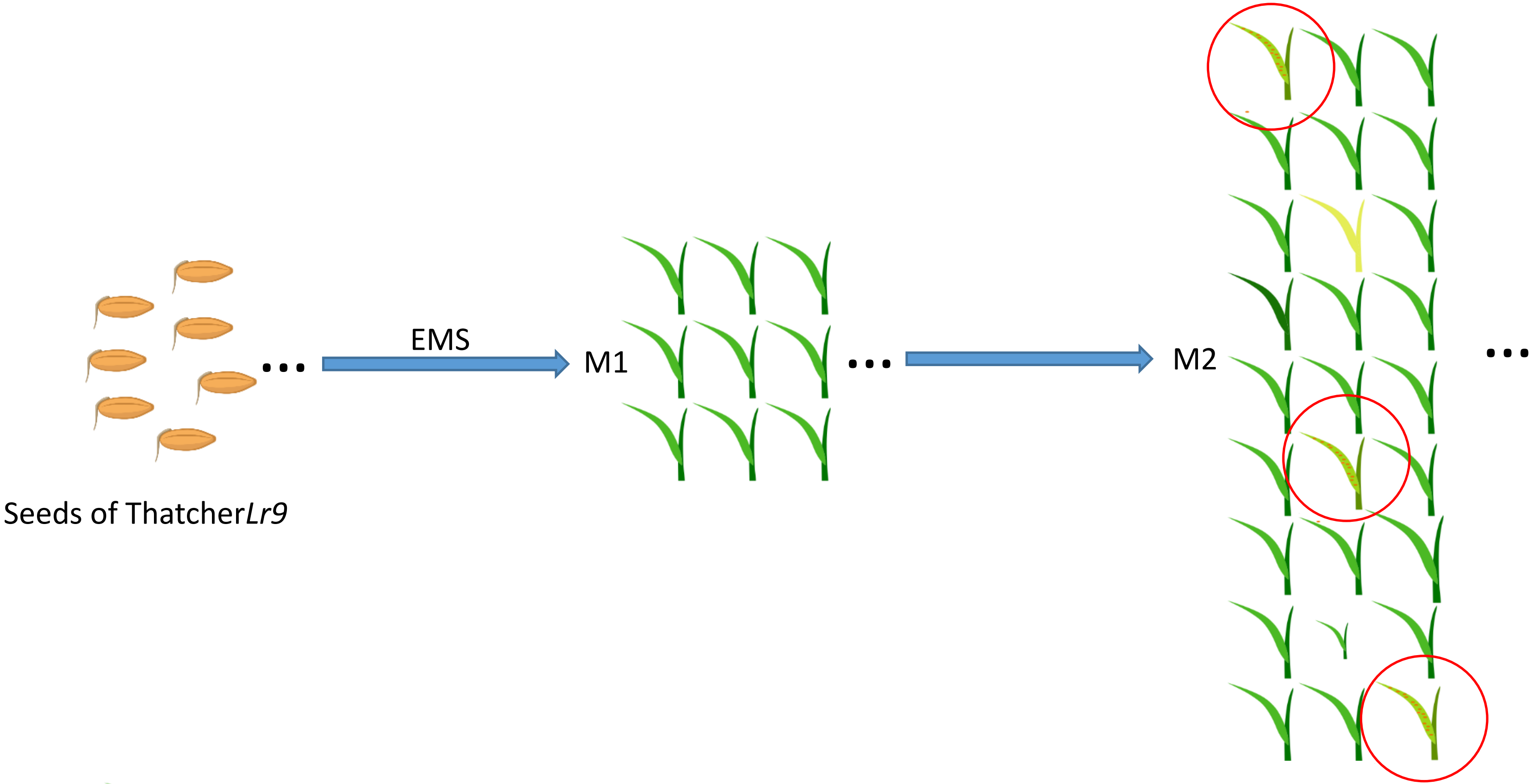
X-ray irradiation

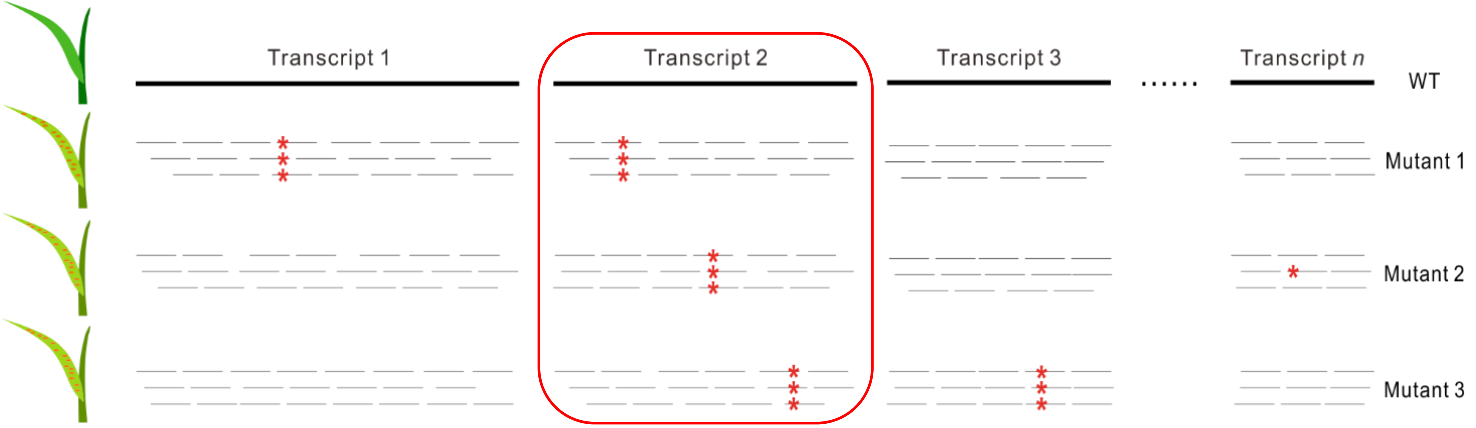




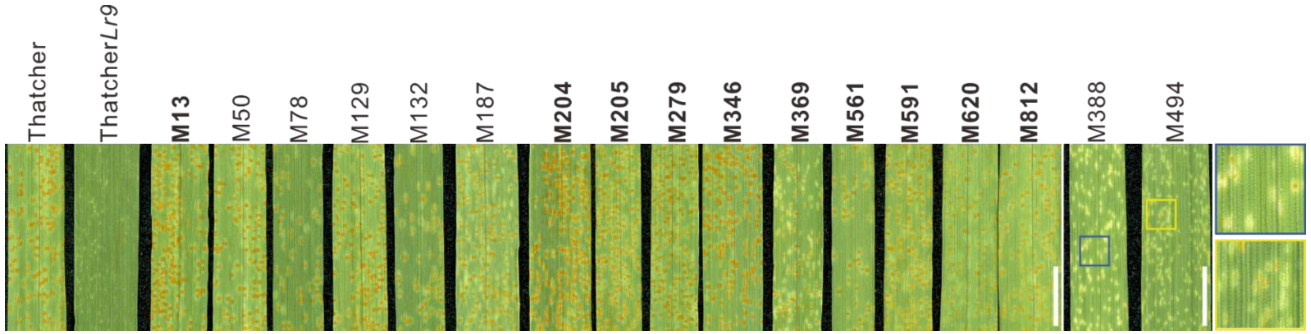
Sears, E.R. *Brookhaven Symposia in Biology* 9 (1956). 1-21

MutIsoSeq allows rapid cloning of *Lr9*





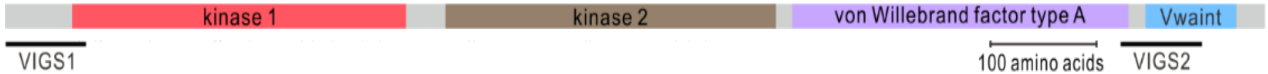
MutIsoSeq allows rapid cloning of *Lr9*

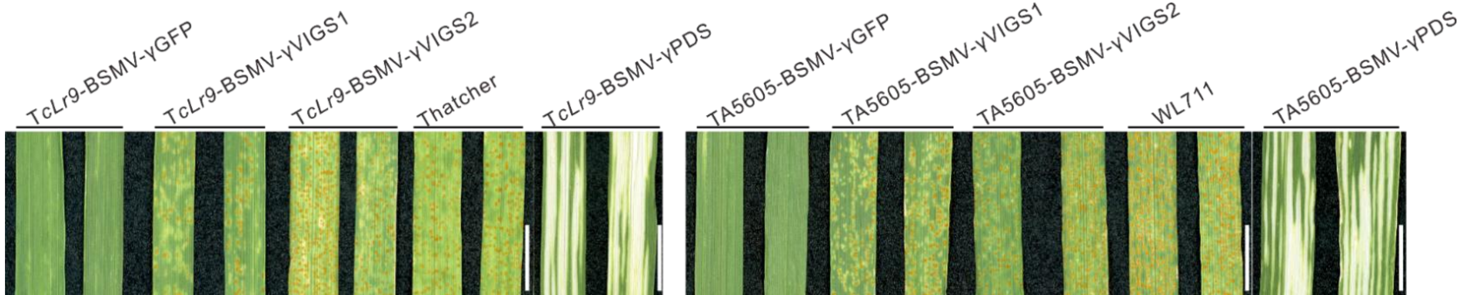


Number of transcripts with n SNPs in the **ten** sequenced

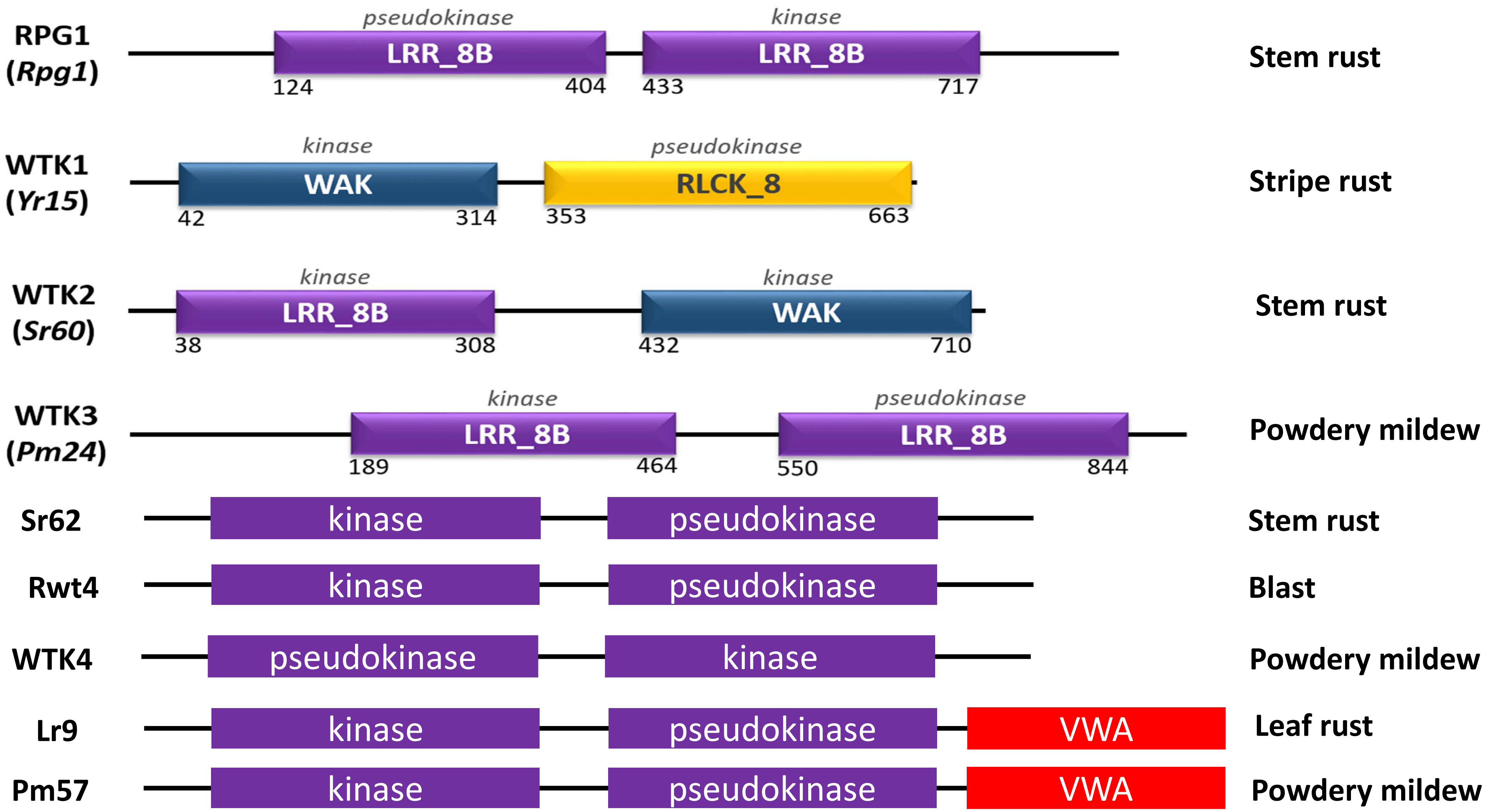
	Thatcher*Lr9*
Number of transcripts with no mutation compared to wild-type	177,216
Number of transcripts mutated in 1 out of 10 mutants	9,072
Number of transcripts mutated simultaneously in 2 out of 10 mutants	756
Number of transcripts mutated simultaneously in 3 out of 10 mutants	68
Number of transcripts mutated simultaneously in 4 out of 10 mutants	2
Number of transcripts mutated simultaneously in 5 out of 10 mutants	1
Number of transcripts mutated simultaneously in 6 out of 10 mutants	0
Number of transcripts mutated simultaneously in 7 out of 10 mutants	0
Number of transcripts mutated simultaneously in 8 out of 10 mutants	0
Number of transcripts mutated simultaneously in 9 out of 10 mutants	0
Number of transcripts mutated simultaneously in 10 out of 10 mutants	1

WTK6-vWA





Cloned tandem kinase resistance genes



RPG1 (*Rpg1*) — pseudokinase LRR_8B 124–404; kinase LRR_8B 433–717 — **Stem rust**

WTK1 (*Yr15*) — kinase WAK 42–314; pseudokinase RLCK_8 353–663 — **Stripe rust**

WTK2 (*Sr60*) — kinase LRR_8B 38–308; kinase WAK 432–710 — **Stem rust**

WTK3 (*Pm24*) — kinase LRR_8B 189–464; pseudokinase LRR_8B 550–844 — **Powdery mildew**

Sr62 — kinase, pseudokinase — **Stem rust**

Rwt4 — kinase, pseudokinase — **Blast**

WTK4 — pseudokinase, kinase — **Powdery mildew**

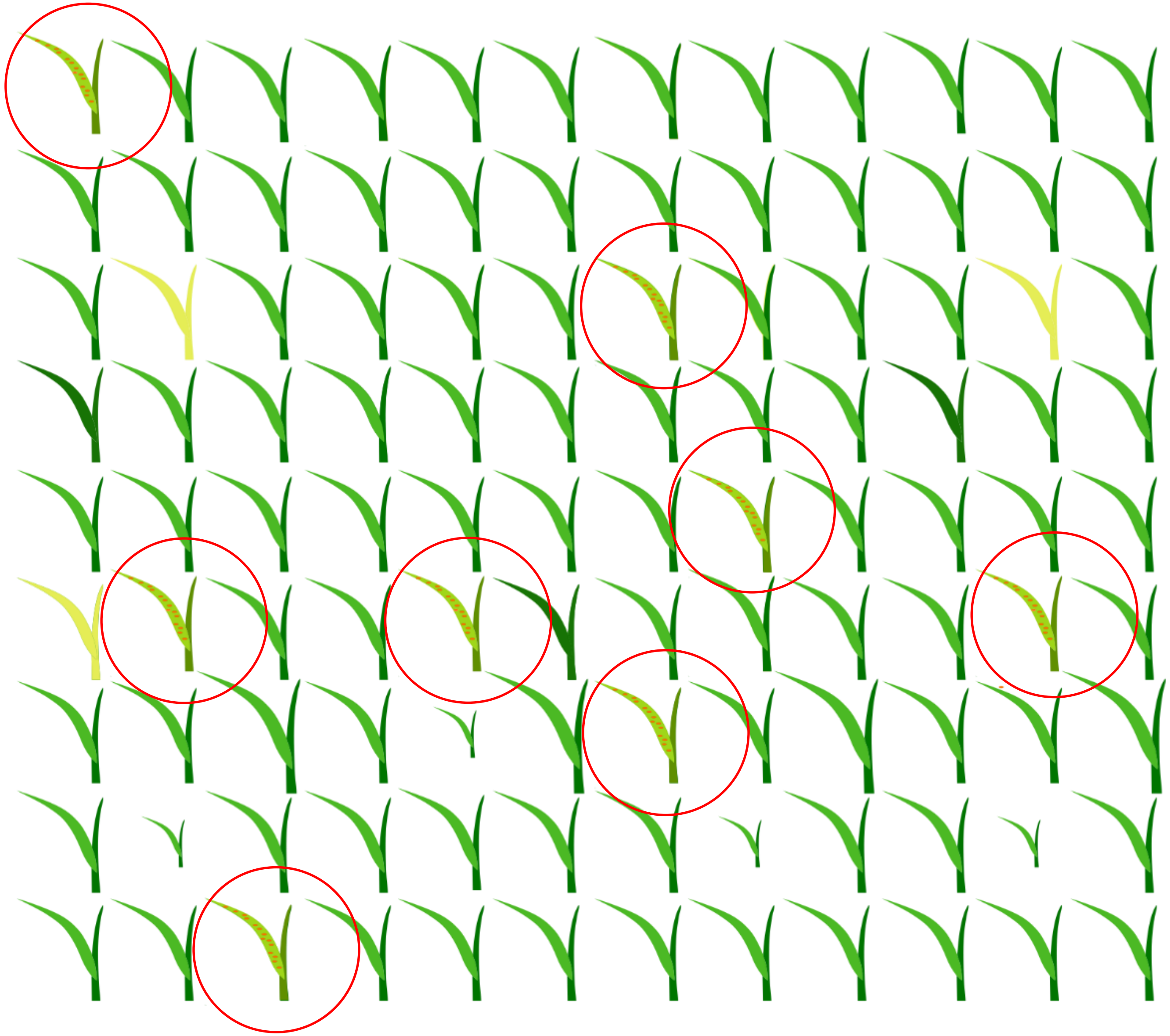
Lr9 — kinase, pseudokinase, VWA — **Leaf rust**

Pm57 — kinase, pseudokinase, VWA — **Powdery mildew**

Klymiuk et al. *Molecular Plant-Microbe Interactions*, 2021 Gaurav, Kumar, et al. *Nature biotechnology*, 2021

Yu et al. *Nature Communications*, 2022 Arora et al. *Nature Plants*, 2023 Zhao et al. *Research Square*, 2023

Large-scale of mutagenesis and mutants screening



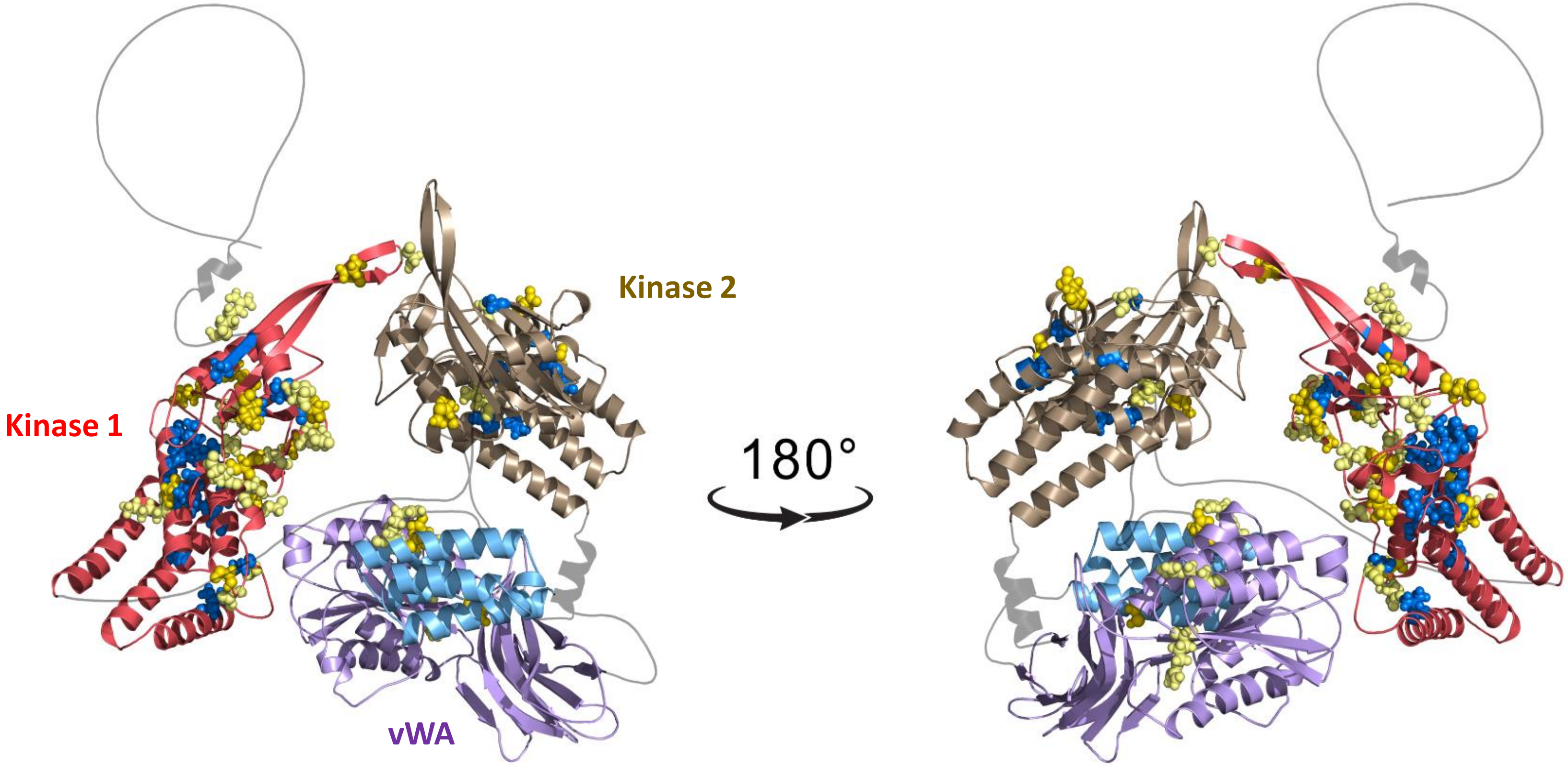
More than 8000 M2 families

……

121 susceptible mutants

Protein structure prediction and mutational analysis of Lr9

- 120 of the 121 susceptible mutants carry SNPs in *Lr9*, resulted in 67 non-redundant amino acid (AA) substitutions
- Protein modeling revealed a marked difference in the effects of AA substitutions between the two kinase domains

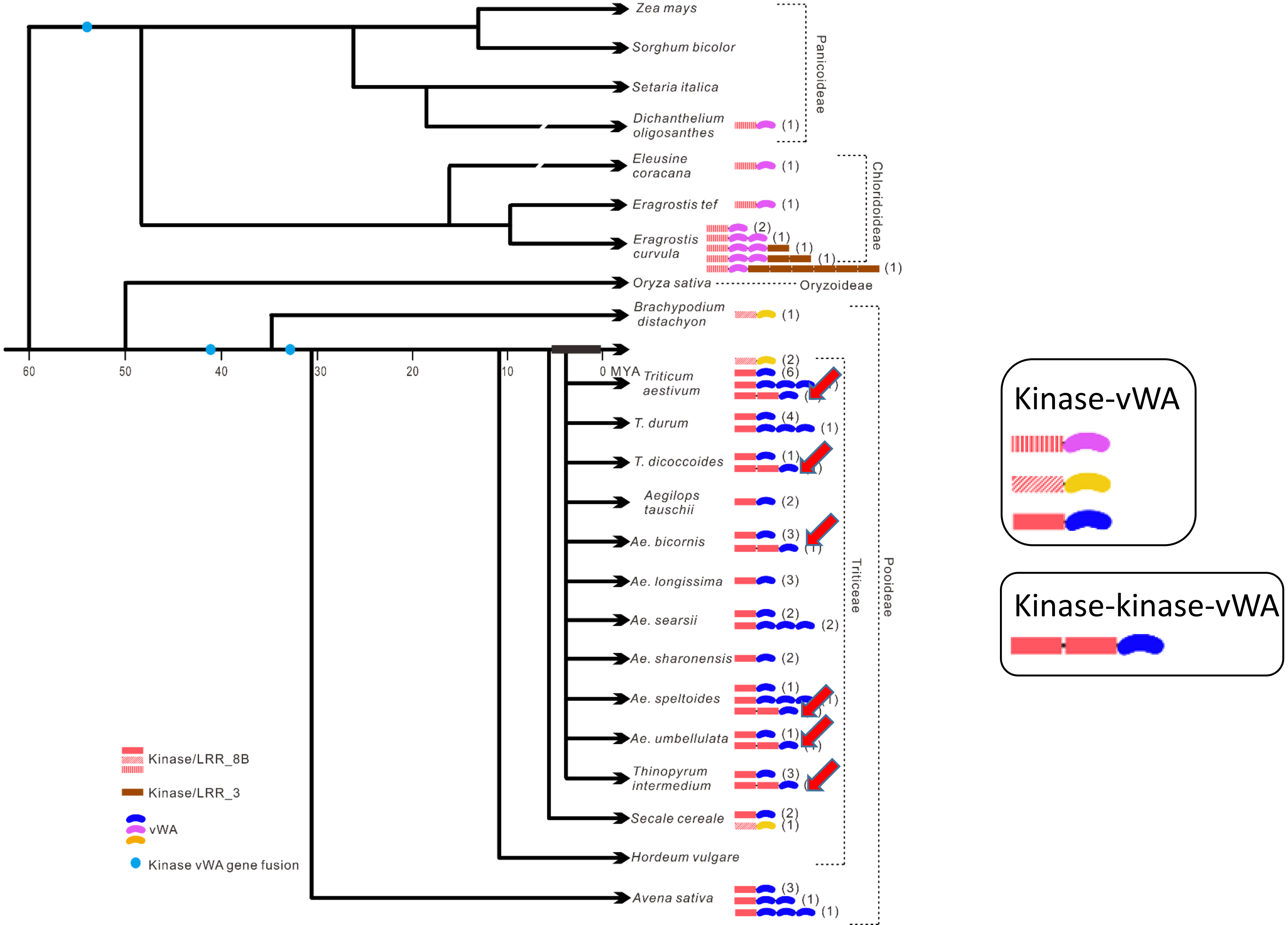


Kinase 1: 31 surface-localized (yellow spheres)
17 internal (green spheres)
48

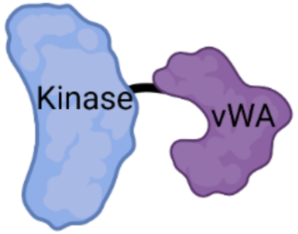
Kinase 2 : 5 surface-localized
8 internal
13

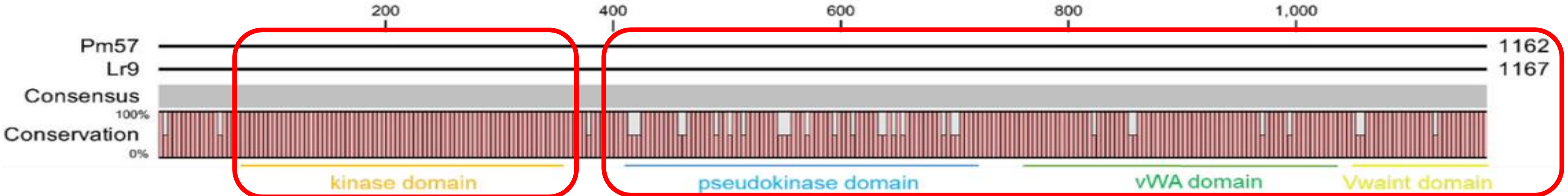
vWA : 5 surface-localized
0 internal
5

Evolution analysis showing the origin and distribution of WTK6-vWA gene family in Poaceae



The integrated decoy model





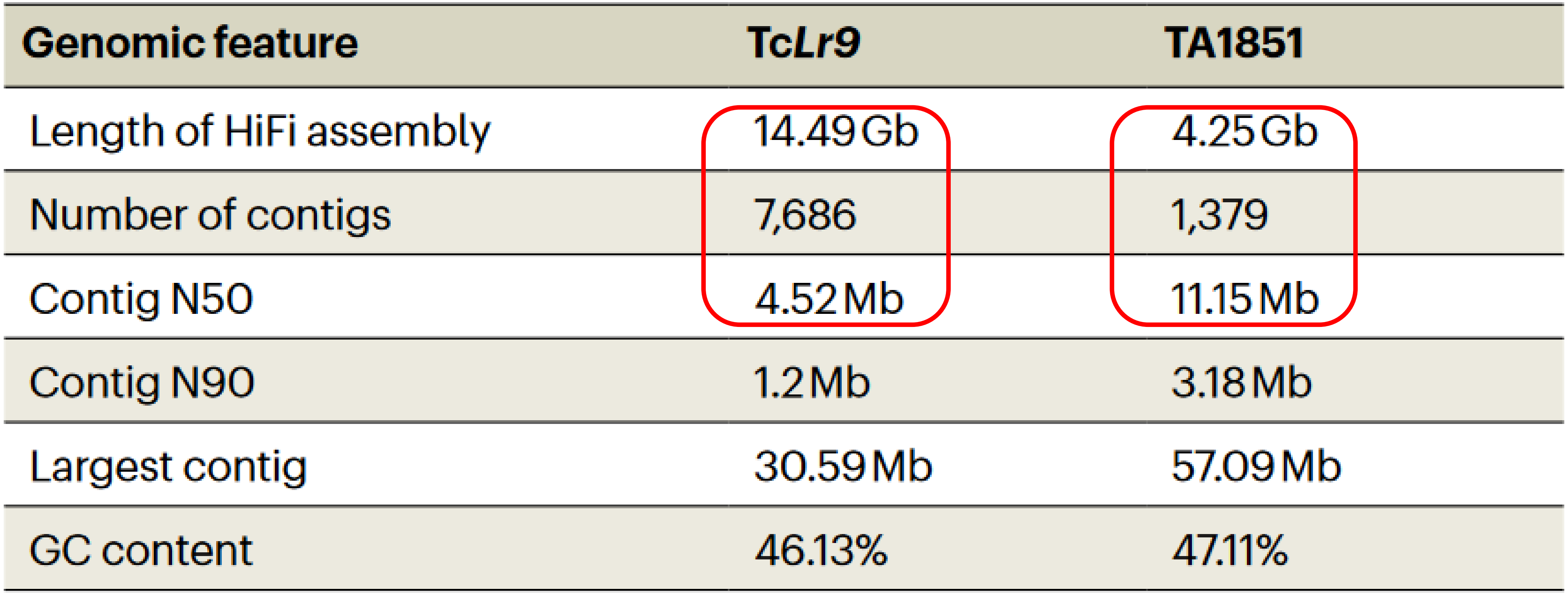
van der Hoorn, R.A. & Kamoun, S. *Plant Cell* 20 (2008): 2009-2017

Klymiuk, Valentyna, et al. *Molecular Plant-Microbe Interactions* 34.10 (2021): 1094-1102.

Zhao, Yue, et al. *Research Square* (2023).

Assembly of the *Lr9* translocation

- The *Lr9* translocation is historically important, as it is likely the first example where a gene was transferred into bread wheat from a wild wheat relative using irradiation
- We constructed PacBio HiFi assemblies of Thatcher*Lr9* (11-fold coverage) and the *Lr9* putative donor *Ae. umbellulata* TA1851(30-fold coverage)

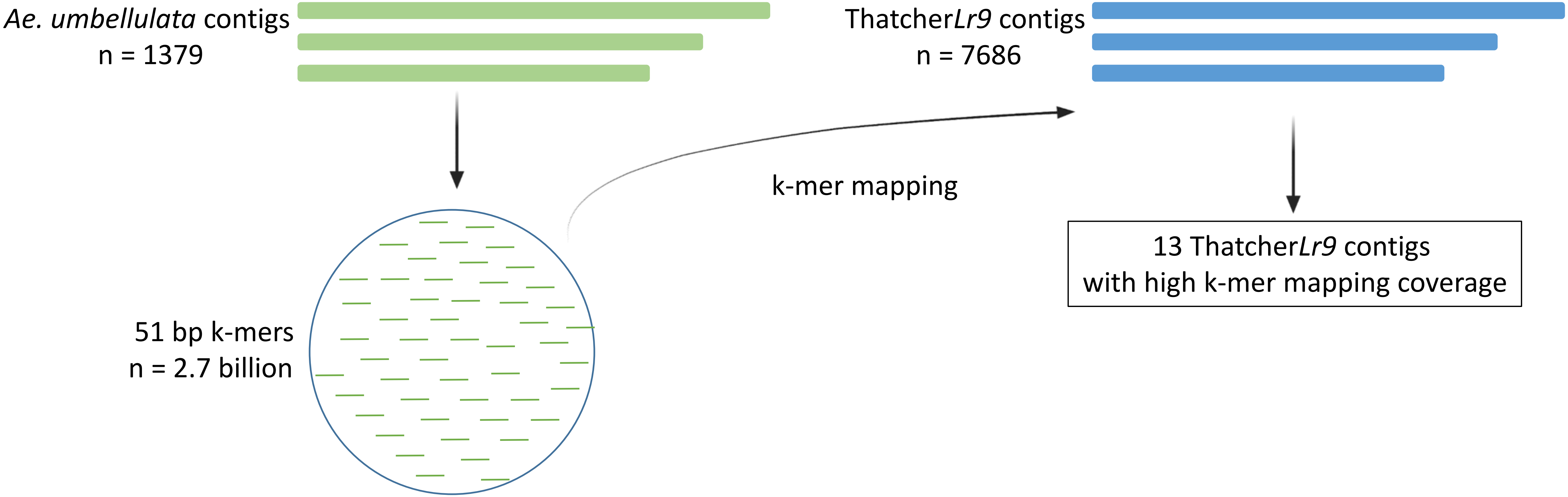


Genomic feature	**Tc*Lr9***	**TA1851**
Length of HiFi assembly	14.49 Gb	4.25 Gb
Number of contigs	7,686	1,379
Contig N50	4.52 Mb	11.15 Mb
Contig N90	1.2 Mb	3.18 Mb
Largest contig	30.59 Mb	57.09 Mb
GC content	46.13%	47.11%

- Chromosome-scale assembly *of Ae. umbellulata* : https://web.persephonesoft.com/?data=genomes/TA1851

Abrouk et al. *Scientific Data, in press*

Assembly of the *Lr9* translocation



Ae. umbellulata contigs
n = 1379

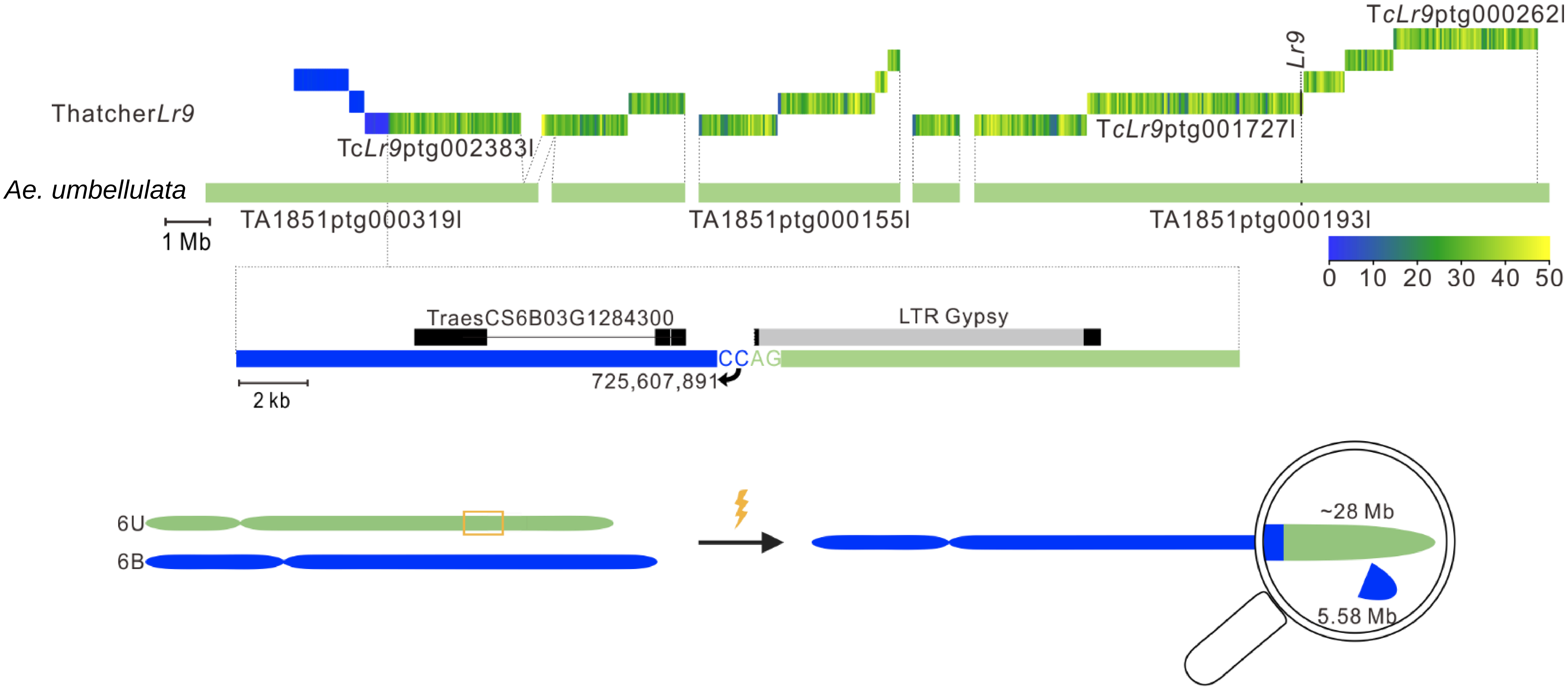
ThatcherLr9 contigs
n = 7686

k-mer mapping

51 bp k-mers
n = 2.7 billion

13 Thatcher*Lr9* contigs
with high k-mer mapping coverage

Assembly of the *Lr9* translocation



Summary

- MutIsoSeq allowed rapid cloning of *Lr9* without genetic mapping

- Large scale of mutagenesis and protein modelling enabled protein domain functional interpretation

- Long-read sequencing helped to reconstruct the historical *Lr9* translocation

Acknowledgement

KAUST:
Simon G. Krattinger
Michael Abrouk
Spyridon Gourdoupis
Naveen Athiyannan
Emile Cavalet-Giorsa
Lingli Zou
Yong Zhou
Hanin Ahmed
Jesse Poland
Łukasz Jaremko
Brande Wulff

Cereal Genetics & Genomics Lab

Elisabet Poquet I Faig
Kymbat Zhakupova
Natalia Arango Lopez

Kansas State University:
Dal-Hoe Koo
John Raupp

Institute of Experimental Botany,
Czech Academy of Sciences:
Miroslava Karafiátová
István Molnár
Kateřina Holušová
Jaroslav Doležel

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CEMPS

中国科学院分子植物科学卓越创新中心

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Thank you!