

Exploration and precision editing of host factors to develop bymovirus-resistant cereal crops



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BaYMV disease, Langenstein Germany, kindly provided by Dr. Frank Rabenstein, JKI

Where are we?



National Crop Genebank of China (long-term)

Beijing, P.R. China

(種, 种, Seed)

Barley Genetic Resources (BGR), CAAS

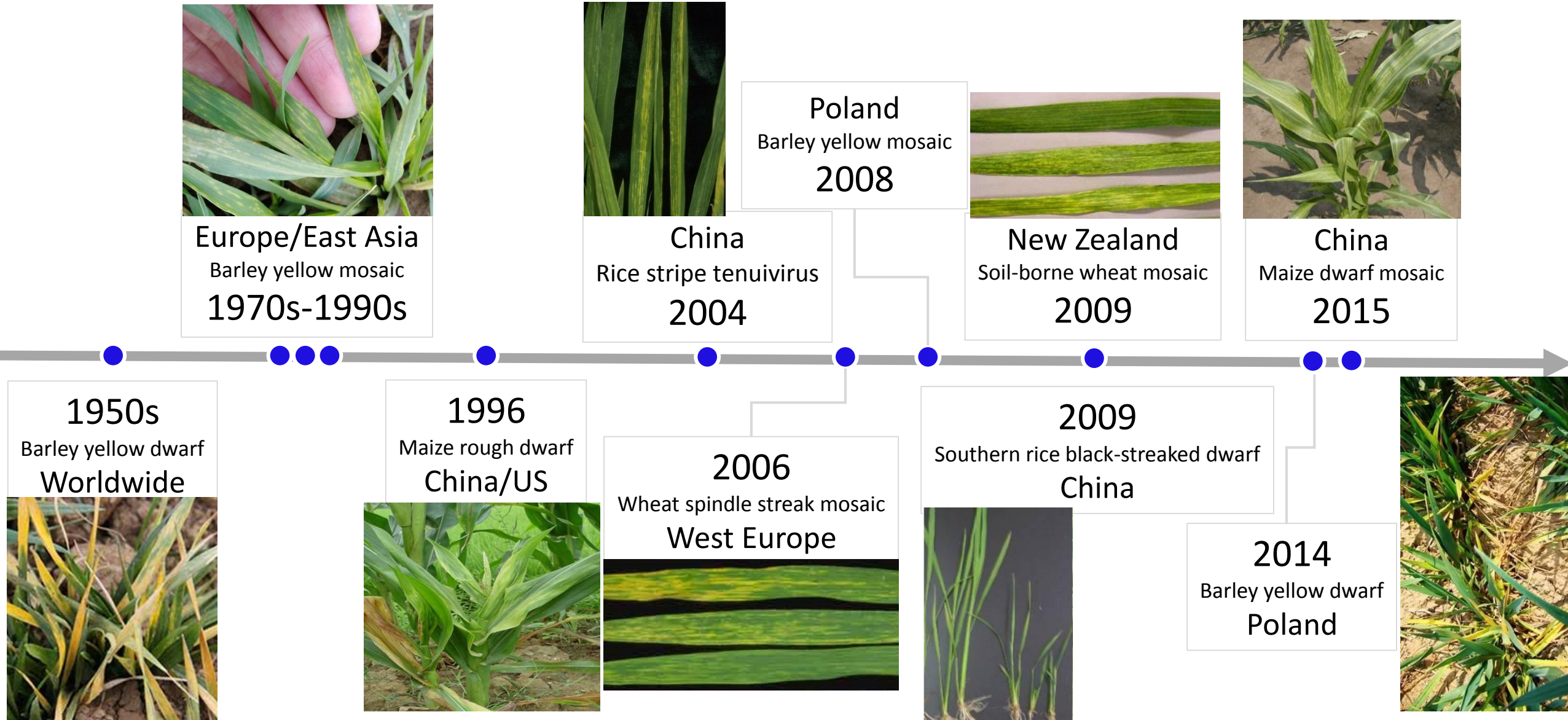
Mission: Characterization and innovation of barley germplasm resources



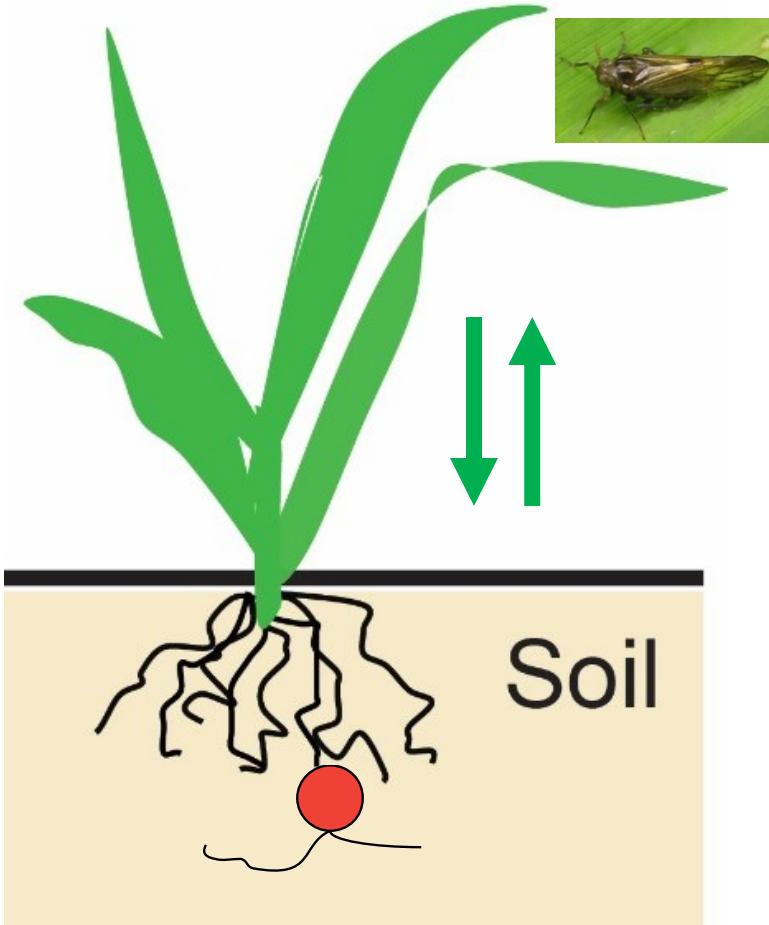
Content

- (1) Introduction of soil-borne bymovirus diseases**
- (2) Uncovering hidden S genes in bread wheat**
- (3) Future perspective to explore novel S genes**

Viruses are challenging production in cereal crops

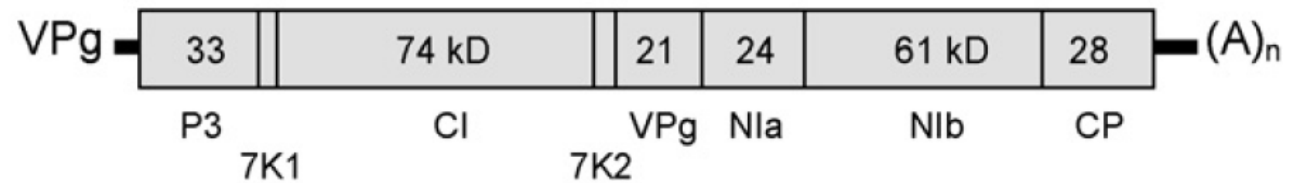


Virus infection relying on hijacking of host factors



BaYMV, the typical virus strain of the genus *Bymovirus*

RNA 1 (7263 nt)



RNA 2 (3524 nt)



Feature:

(A) Small genome; (B) Obligate; (C) Chemical treatment useless

Soil-borne *P. graminis* transmitted viruses infect cereals

Taxonomy	Type of viruses	Wheat	Barley	Oat	Triticale	Rye	Rice	Sorghum
<i>Bymovirus</i> of the family <i>Potyviridae</i>	<i>Barley yellow mosaic virus (BaYMV)</i>		X					
	<i>Barley mild mosaic virus (BaMMV)</i>		X					
	<i>Oat mosaic virus (OMV)</i>			X				
	<i>Wheat spindle streak mosaic virus (WSSMV)</i>	X			X	X		
	<i>Wheat yellow mosaic virus (WYMV)</i>	X						
	<i>Rice necrosis mosaic virus (RNMV)</i>						X	
<i>Furovirus</i> of the family <i>Virgaviridae</i>	<i>Soil-borne wheat mosaic virus (SBWMV)</i>	X	X		X	X		
	<i>Soil-borne cereal mosaic virus (SBCMV)</i>	X			X	X		
	<i>Chinese wheat mosaic virus (CWMV)</i>	X	X					
	<i>Oat golden stripe virus (OGSV)</i>			X				
	<i>Sorghum chlorotic spot virus (SrCSV)</i>							X

Symptoms of bymovirus diseases in wheat and barley

BaYMV-infected barley



Langenstein, Germany

kindly provided by Dr. Frank Rabenstein, JKI

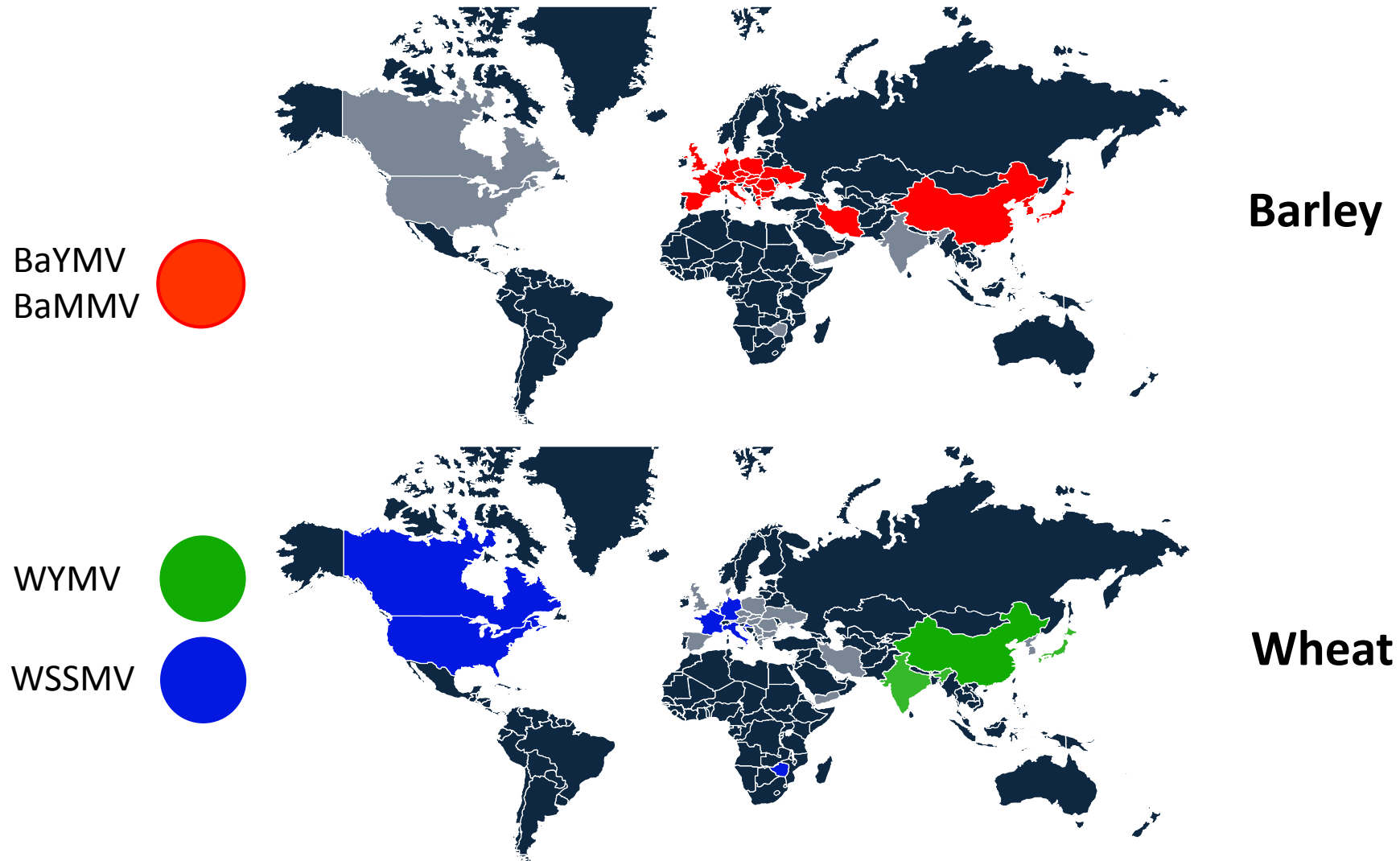
WYMV-infected wheat



Luohe city, China

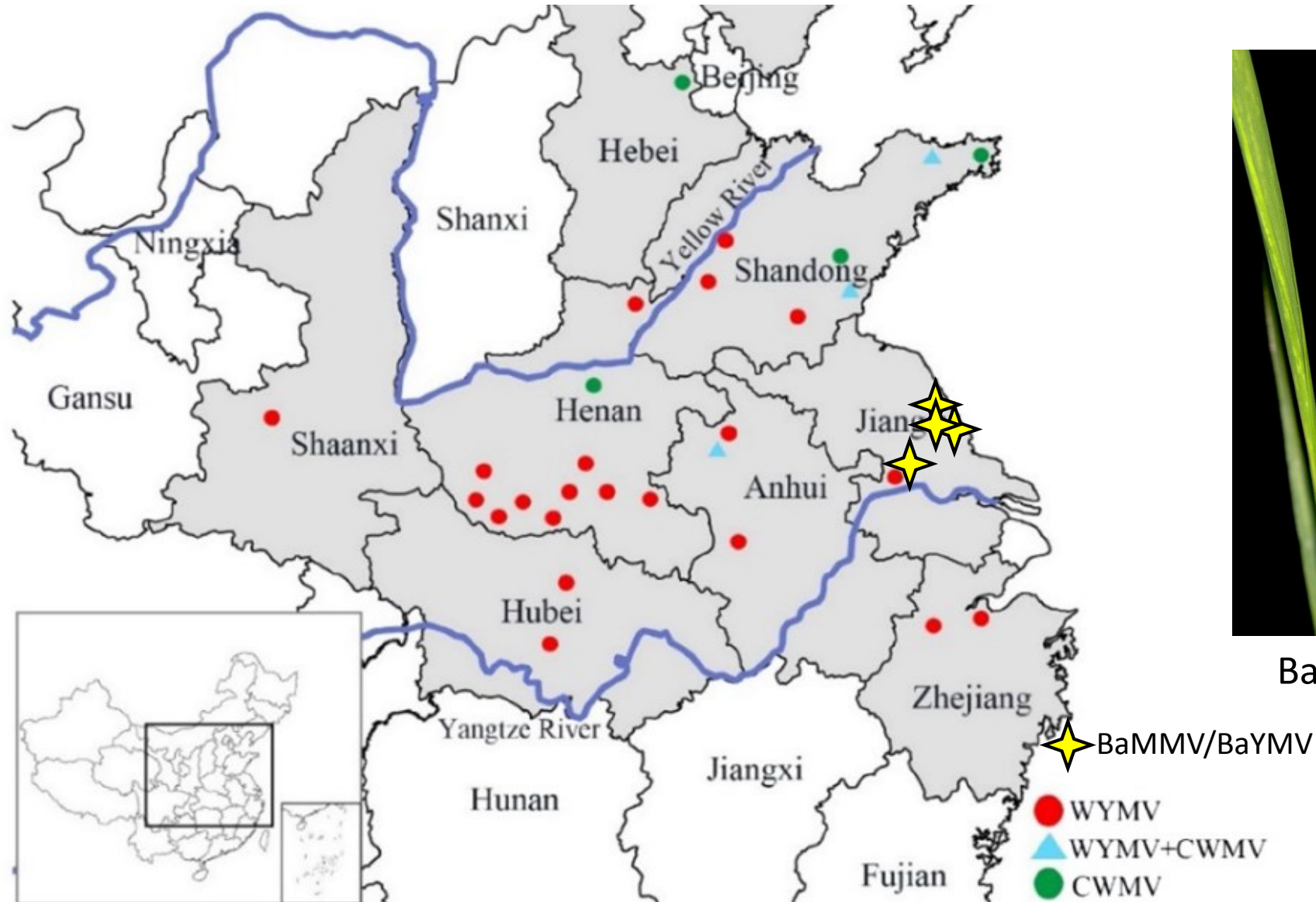
March 19, 2025

Bymoviruses constantly threaten winter barley and wheat



Kanyuka *et al.* 2003; Kuhne, 2009; Jiang *et al.* 2020

Soil-borne *P. graminis* transmitted viruses in China



BaMMV

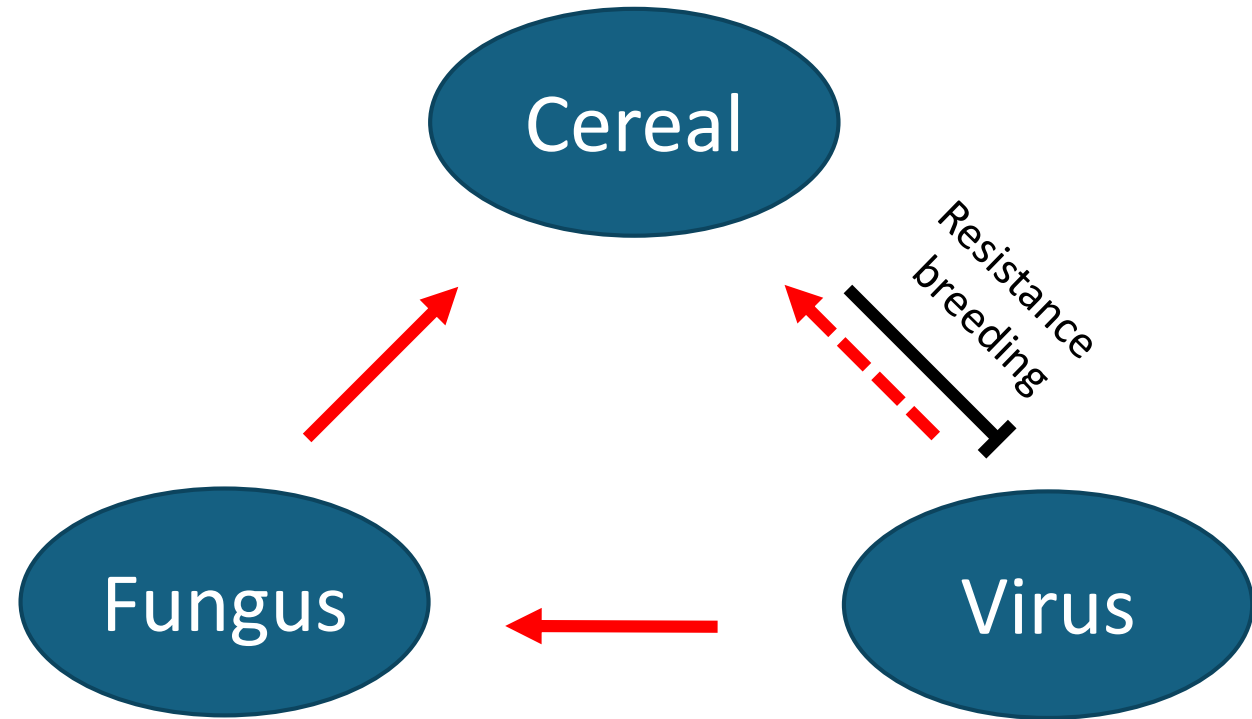
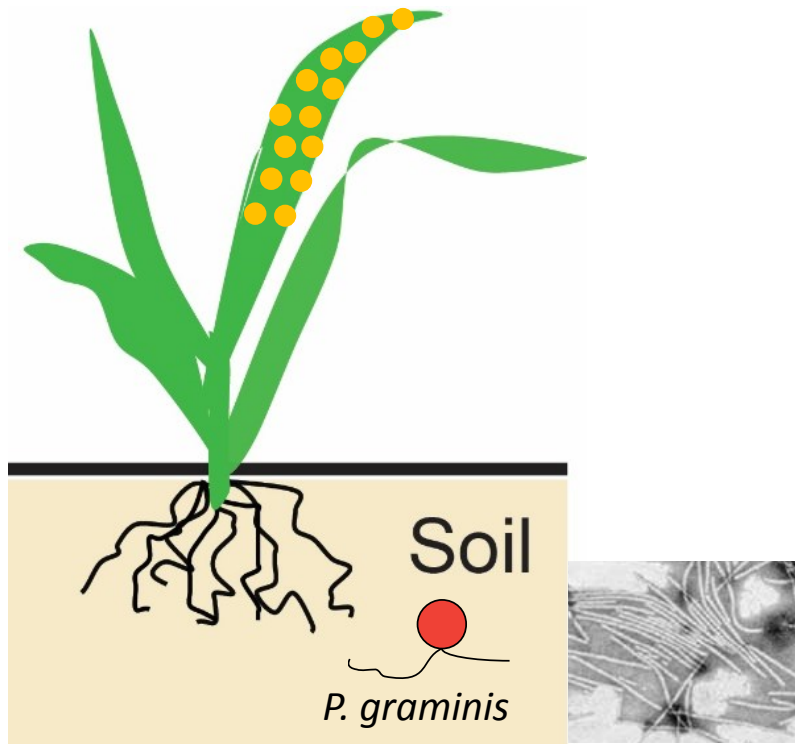
BaYMV

WYMV

Yellow star: BaMMV/BaYMV

Jiang et al., 2022 Plant disease
Yang et al., 2022

Virus infection relying on hijacking of host factors



Loss of susceptibility to BaMMV/BaYMV in barley

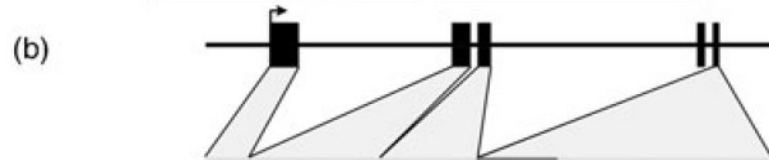
Amino acid substitutions at *HveIF4E*

R S

(a) *>Hv-eIF4E*

```

1  ATGGCGGAGG ACACGGAGAC GAGGCCGCGG TCGGCGGCGG CGGAGGAGAG
51  GGAGGAGGGG GAGATCGCGG ACGACGGAGA CCGGTCGCGG GCGGCGGCGG
101 CCGGCGGCGG GAGGCGGCGG CCTCTGGAGA ACGGCTGGAG CTCTCGGCTG
151 GACACCGCGG AGGGCAAGT CCGGCGGCGG GCGTGGGGGA GCACCATCCA
201 CCCCATCCAC ACCTCTCCA CCGTCGAGGA CTCTGGAGC CTTTACAACA
251 ATATTATCA CCTAGCAAG TTGAATGTTG GAGCGGACIT CCATTGCTTC
301 AAGGATAAGA TTGAGCCAAA ATGGGAAGAC CCCATTGTG CCAATGGCGG
351 TAAATGGA C ATCAGTTGTG GCAAAGGAA ATCTGACACA TTTTGGTTGC
401 ATACTTIGCT GGCATTGATT GGTGAACAAT TCGACTTTGG TGATGAAATT
451 TGCGGAGCAG TCGTCAGCGT GCGTAAGAAC CAGGAAAGAG TAGCTATCTG
501 GACTAAAAAT GCTGCCAATG AAAGTCTCTA GATAAGCATC GGTAAGCAGT
551 GGAAGGAGTT TCTGGACTAC AAGGACTCCA TTGGATTCTG CGTTCATGAG
601 GATGCTAAGA GGTCCCAAA AGGCGCAAG AACCGCTACA CGGTT
    
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(c)

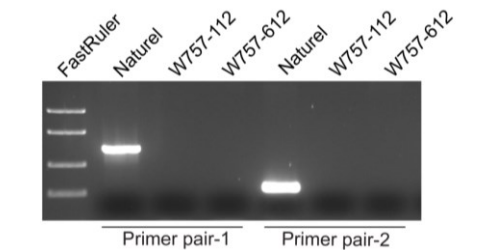
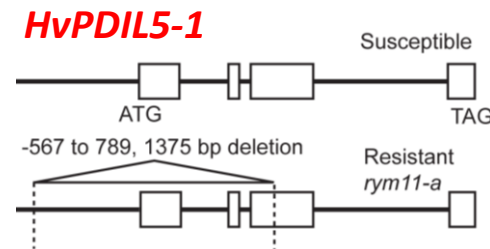
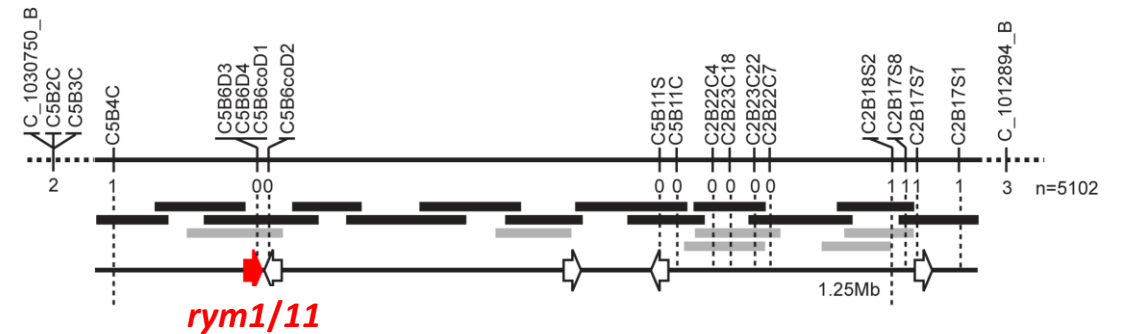
	T	C/T	C	A	C	C/T	G	G	C
<i>rym4</i>	T	C/T	C	A	C	C/T	G	G	C
sus	C	A	C	A	C	C	A	G/A	C/G
<i>rym5</i>	C	A	G	G	A	C	A	G	G
bp-pos	170	353	359	478	481	614	617	622	623

(d)

<i>rym4</i>	Phe	Thr/Ile	Thr	Asn	Gln	Ser/Phe	Gly	Ala
<i>SUS</i>	Ser	Lys	Thr	Asn	Gln	Ser	Asp	Ala/Ser/Gly
<i>rym5</i>	Ser	Lys	Ser	Asp	Lys	Ser	Asp	Gly
AA-pos	57	118	120	160	161	205	206	208



loss of function at *HvPDIL5-1*



PDIL5-1 = Protein disulfide isomerase like 5-1

Editing for BaMMV/BaYMV resistance in barley

elf4E edits (BaMMV-inoculated)

Primary mutants	Mutation	No. of M ₂ plants tested	BaMMV detection by ELISA
P1	+A	13	0/13
P3	+T	9	0/9
P4	+T	16	0/16
Igri wt	none	8	7/8

Hoffie *et al.*, 2021 (Jochen Kumlehn's group)

PDIL5-1 edits (BaMMV-inoculated)



GP

Edited #1

Edited #2

Cheng *et al.*, 2023 Frontiers in Plant Sciences

Resistance genes from wheat wild relatives to WYMV

Wheat *Ym2* originated from *Aegilops sharonensis* and confers resistance to soil-borne *Wheat yellow mosaic virus* infection to the roots

Mishina *et al.*, 2023 PNAS

Ym2/Qym.njau-2B.1
(3BS)

Aegilops sharonensis

A wheat CC-NBS-LRR protein *Ym1* confers WYMV resistance by recognizing viral coat protein

Liu *et al.*, 2023 Nat. Commun.

YmYF2/Ym1/Ym1b/Q.Ymym
(2DL)

Aegilops uniaristata

A papain-like cysteine protease-released small signal peptide confers wheat resistance to wheat yellow mosaic virus

Chen *et al.*, 2025 Nat. Commun.

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Genetic resistance genes/loci in wheat and barley

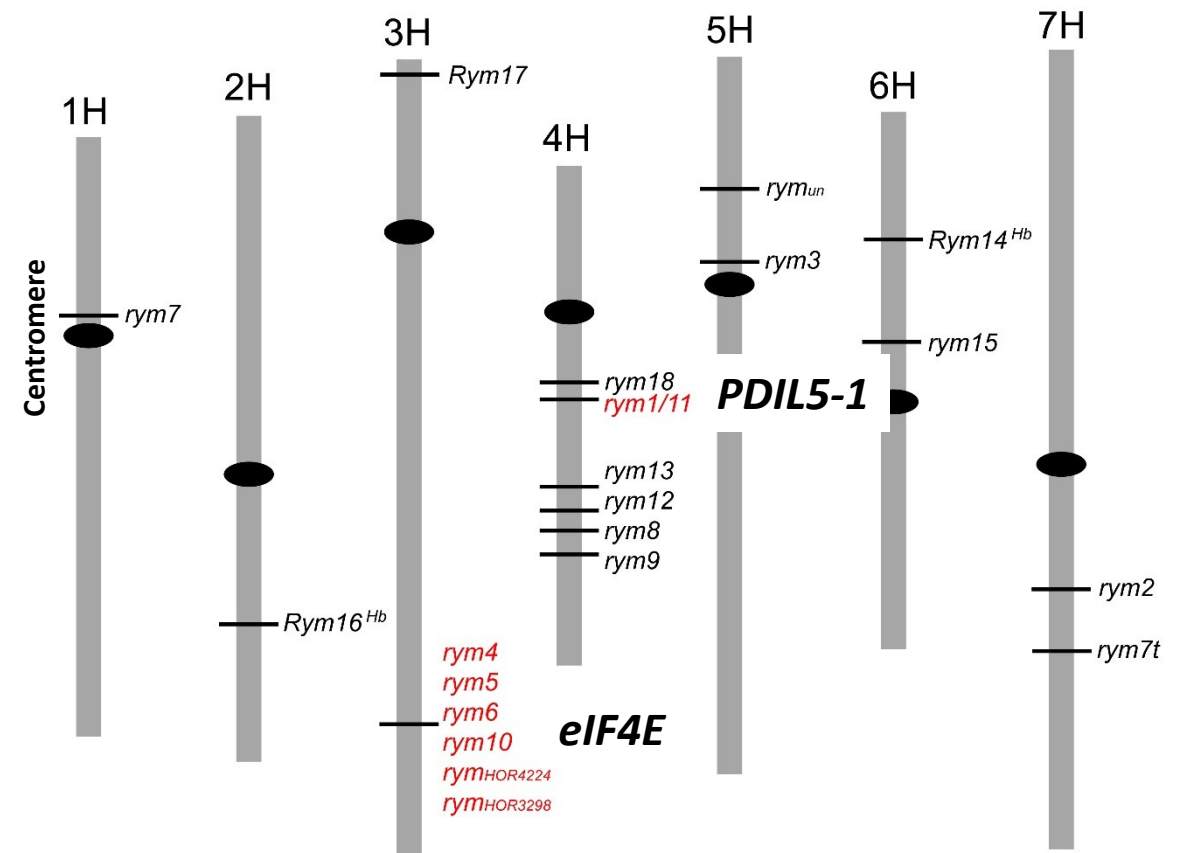
Wheat

12 genes/7 loci, recessive genes = **0** of 12

Genes	Chr.	
<i>YmYF</i>	2DL	NBS-LRR RD21A
<i>YmIb</i>	2DL	
<i>Qym1</i>	2DL	
<i>Un-designated</i>	2DL	
<i>Q.Ymym</i>	2DL	
<i>YmNM</i>	2A	NBS-LRR
<i>Qym2</i>	3BS	
<i>Qym.njau-2B.1</i>	3BS	
<i>Wss1</i>	4DS	
<i>Qym.njau-5A.1</i>	5AL	
<i>Qssm-mtpsa-7A</i>	7A	
<i>Qssm-mtpsa-7BS</i>	7BS	

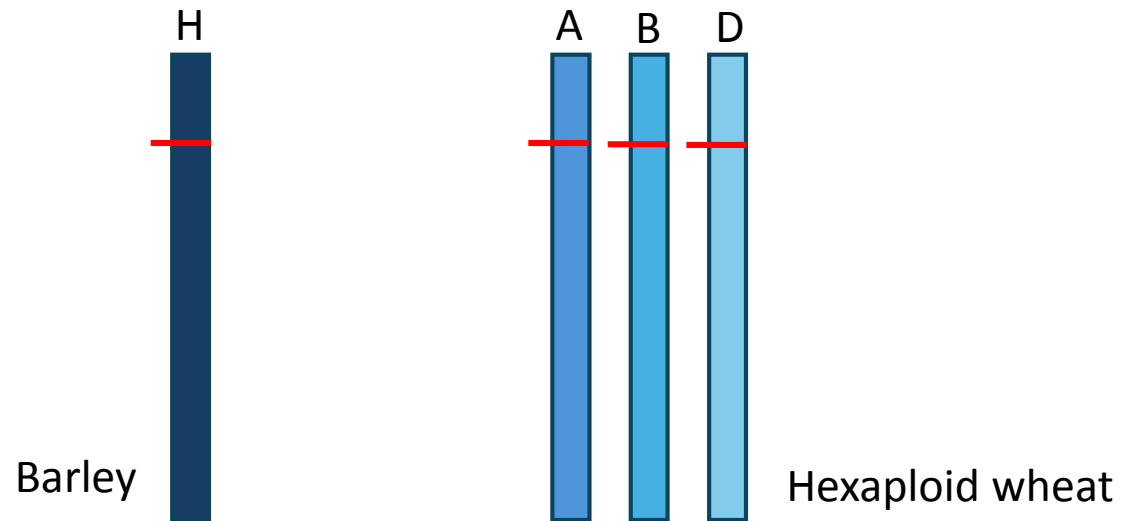
Barley

22 genes/16 loci, recessive genes = **19** of 22

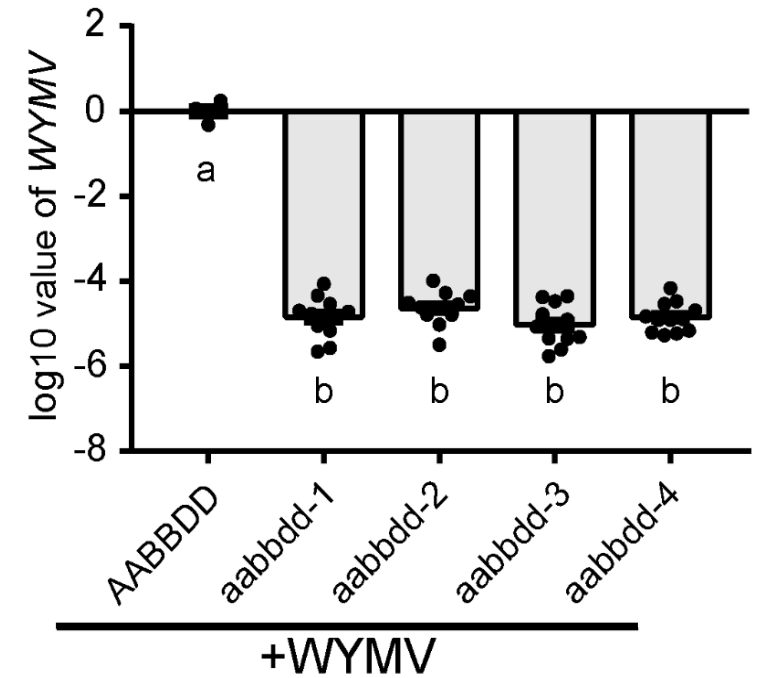
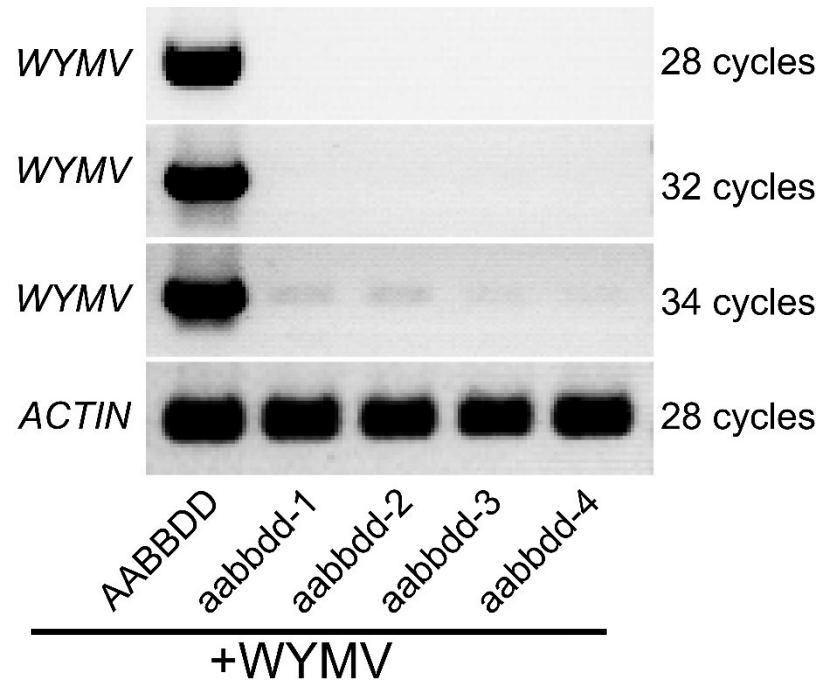
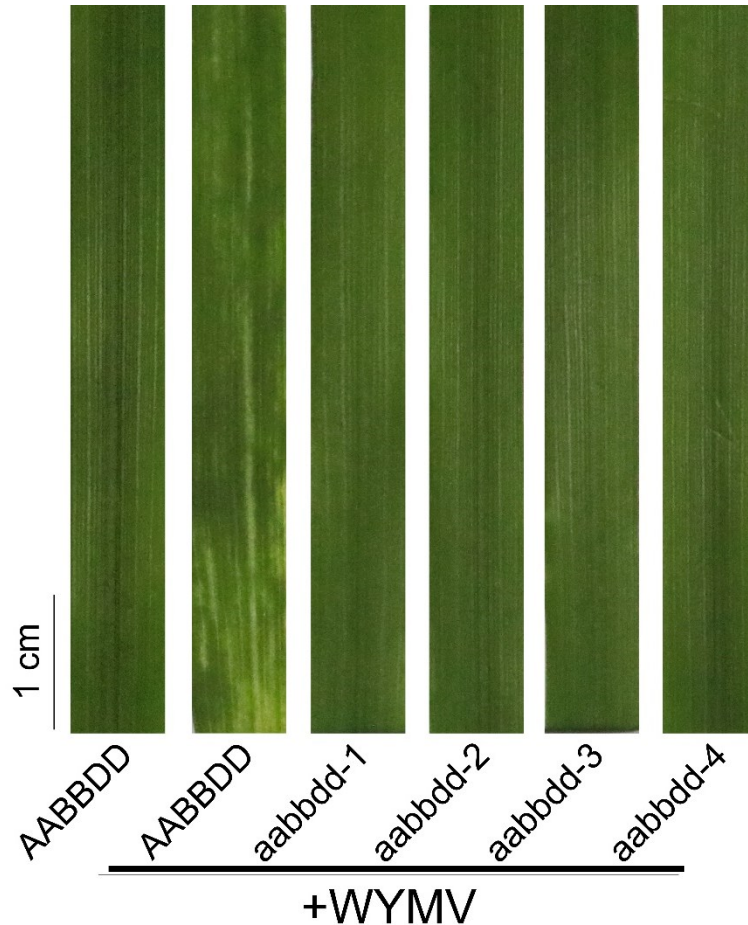


Hypothesis

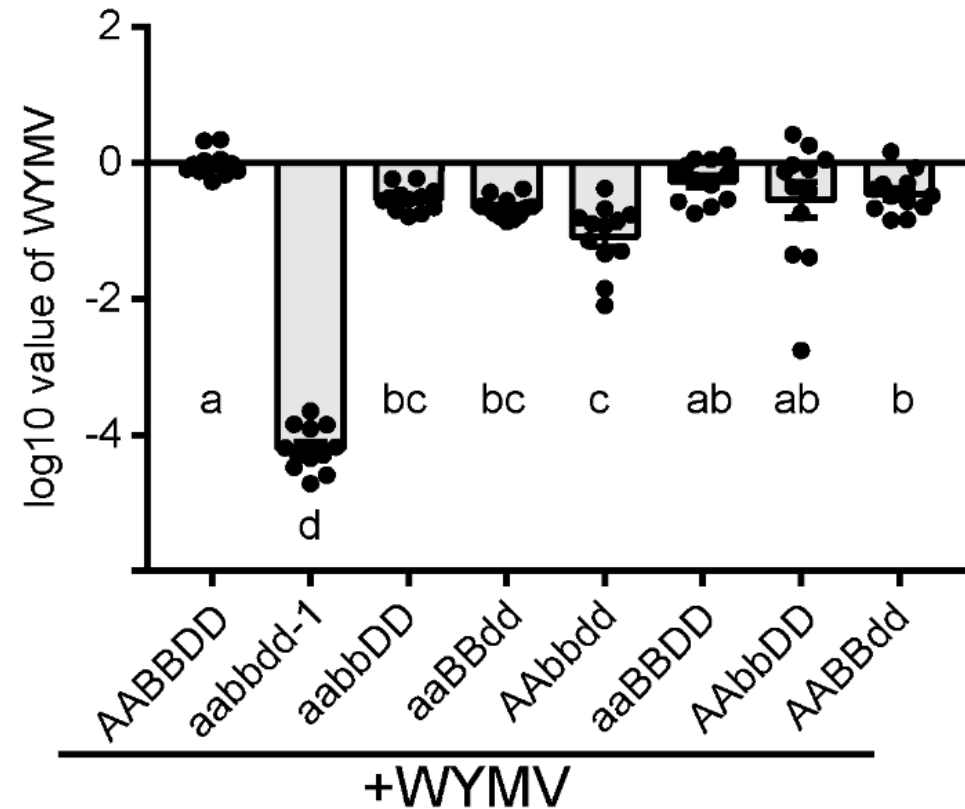
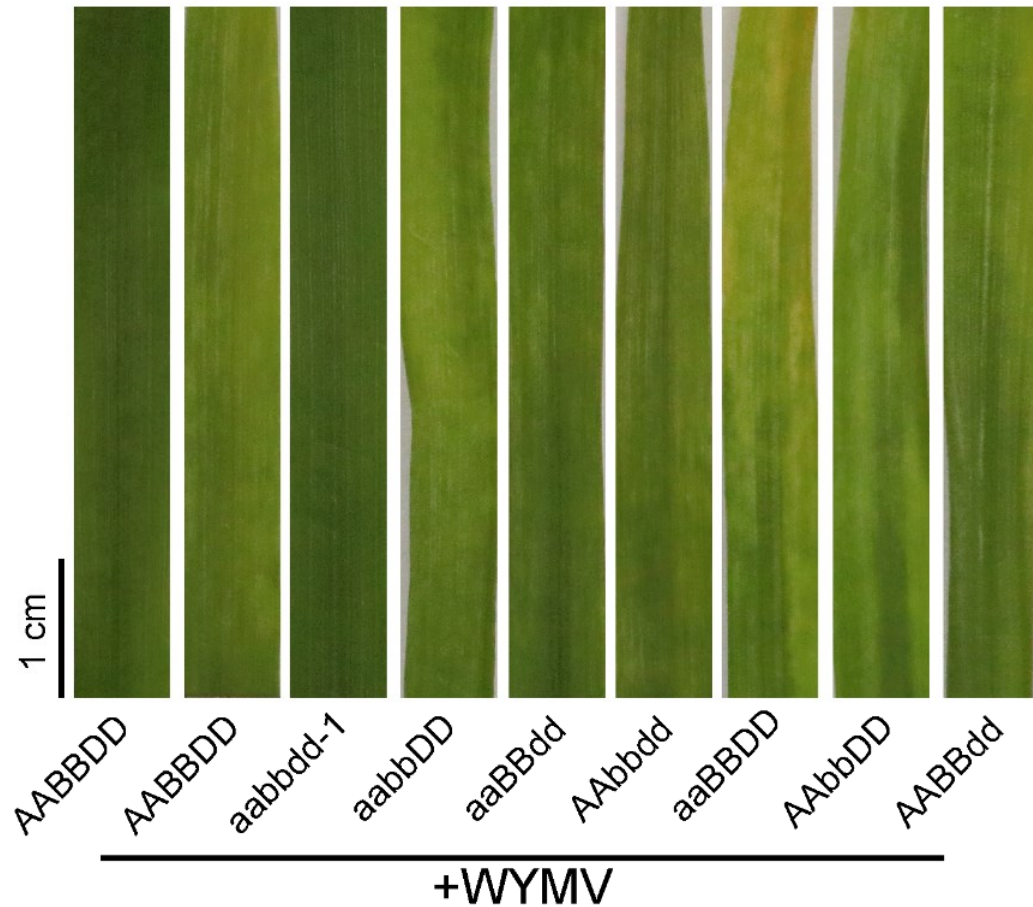
Polyploidization in wheat has resulted in functionally redundant homoeologous genes, blocking the identification of recessive resistance (loss of susceptibility genes, S).



Triple-editing *TaPDIL5-1* leads to WYMV resistance



TaPDIL5-1 homoeologous genes show redundant



TaelF4E is a susceptibility (S) host factor to WYMV

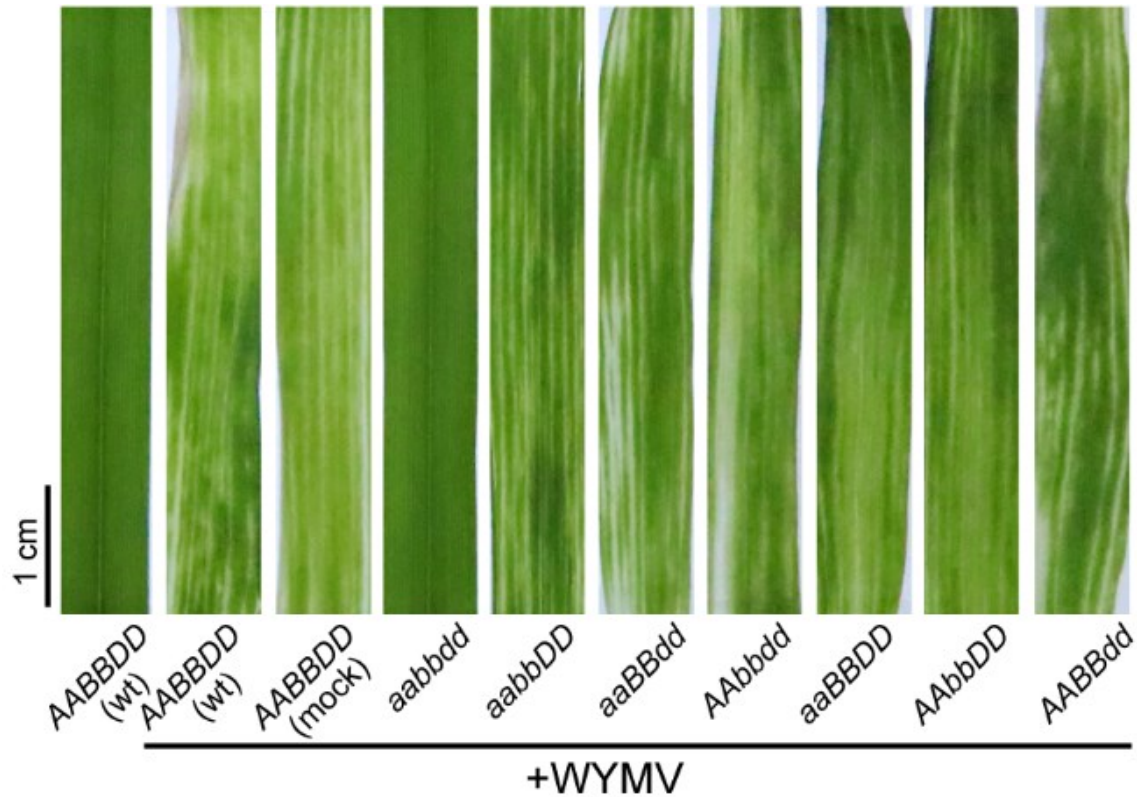
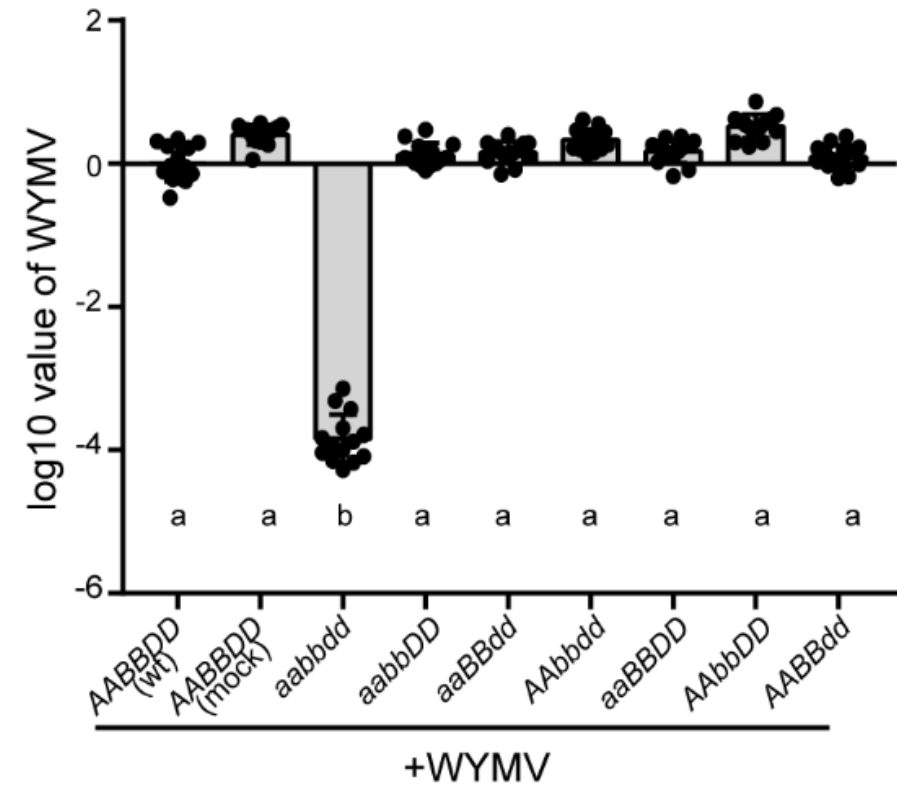
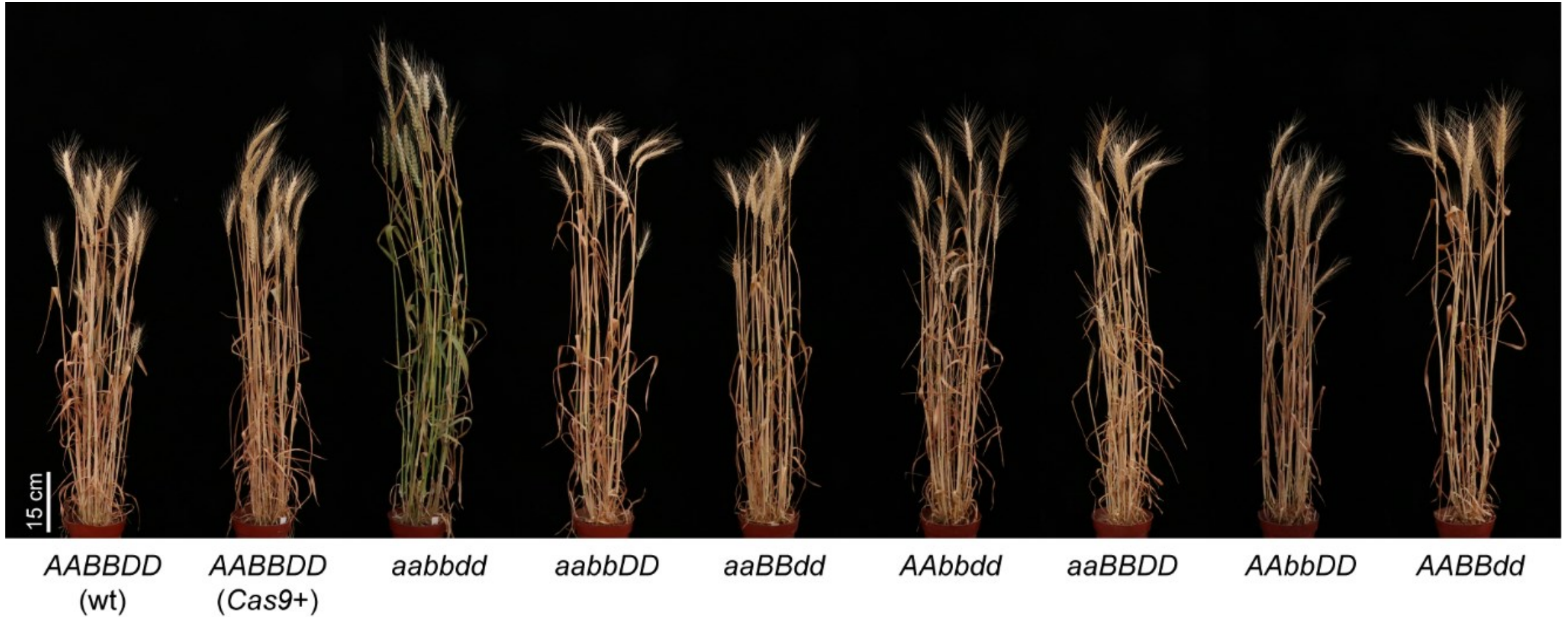


Photo was taken using translucent plate



Kan *et al.*, 2023 Plant Biotechnology Journal

Triple-editing *eIF4E* plant is taller with delayed maturity



Garden experiment without virus infection, Beijing, 2022

Triple-editing *TaPDIL5-1* without yield penalty

Fielder



ABBDD aabbdd-1 aabbdd-2 aabbdd-3 aabbdd-4

Garden experiment without virus infection, Beijing, 2021

Kan *et al.*, 2022 New Phytologist; Patent, CN114292852B

Mutagenesis and stacking of three *TaPDIL5-1* alleles

Mutagenesis + Target identification + MAS stacking + Two-backcrossing + One-selfing

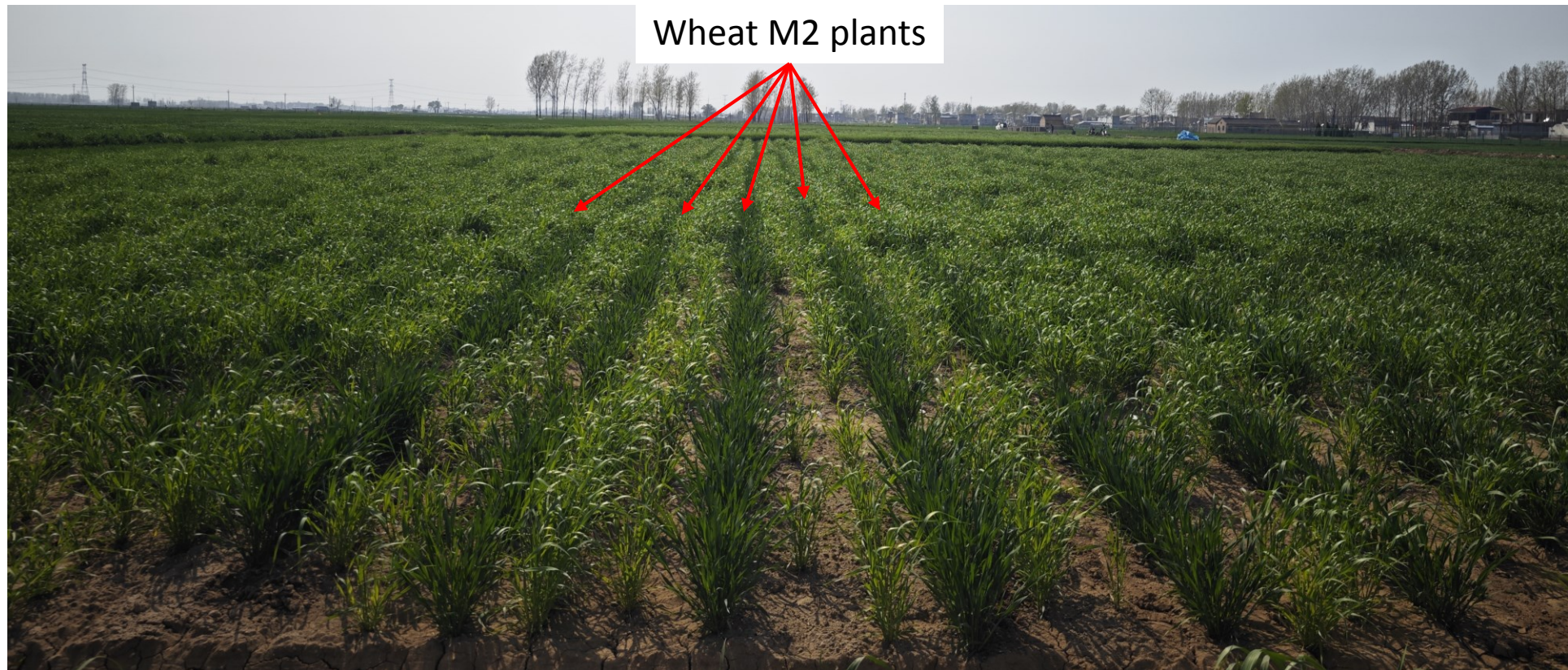
1 year

0.5 year

0.6 year

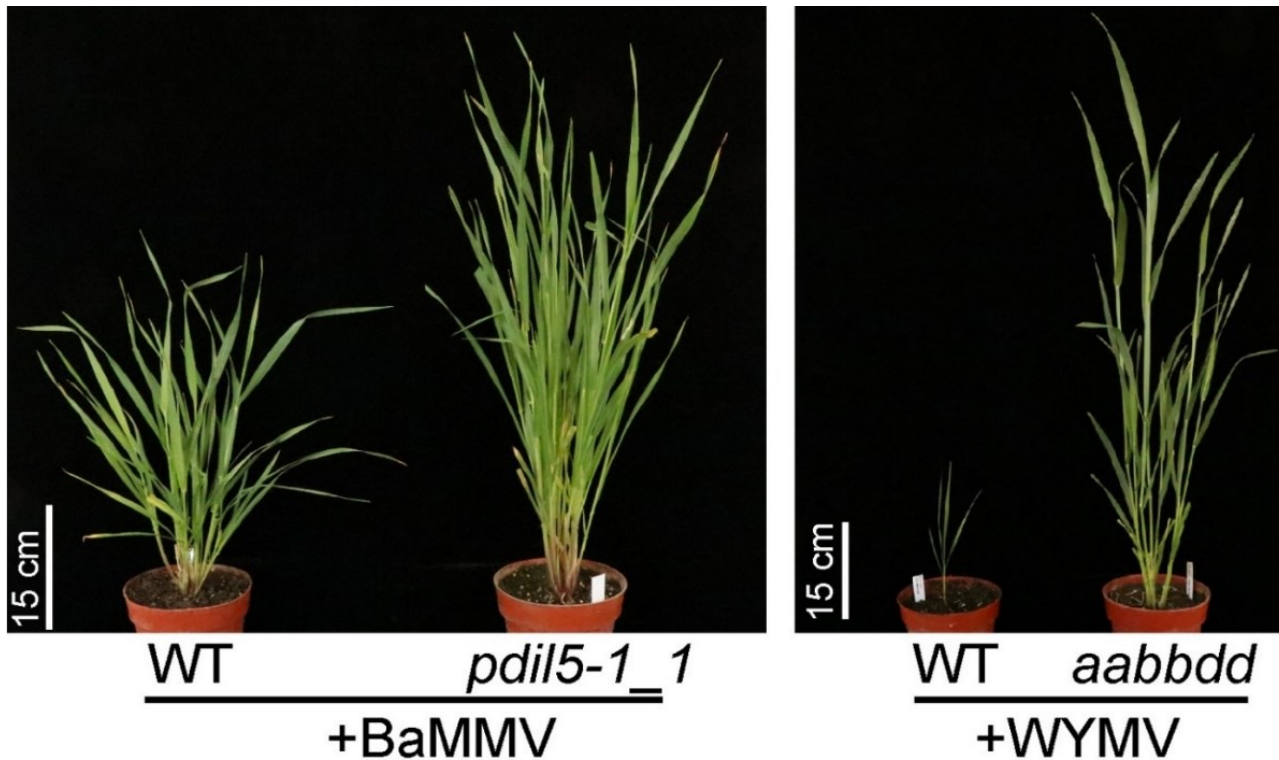
0.6 year

0.3 year



Xinxiang Research station of ICS-CAAS (March 23, 2023)

Targeting *PDIL5-1* for WYMV/BaMMV/BaYMV resistance



Editing for complete resistance

No plasmid fragments detected

No off-targets detected

No penalties observed

1 year for improved lines

MAS for complete resistance

>2 years for improved lines

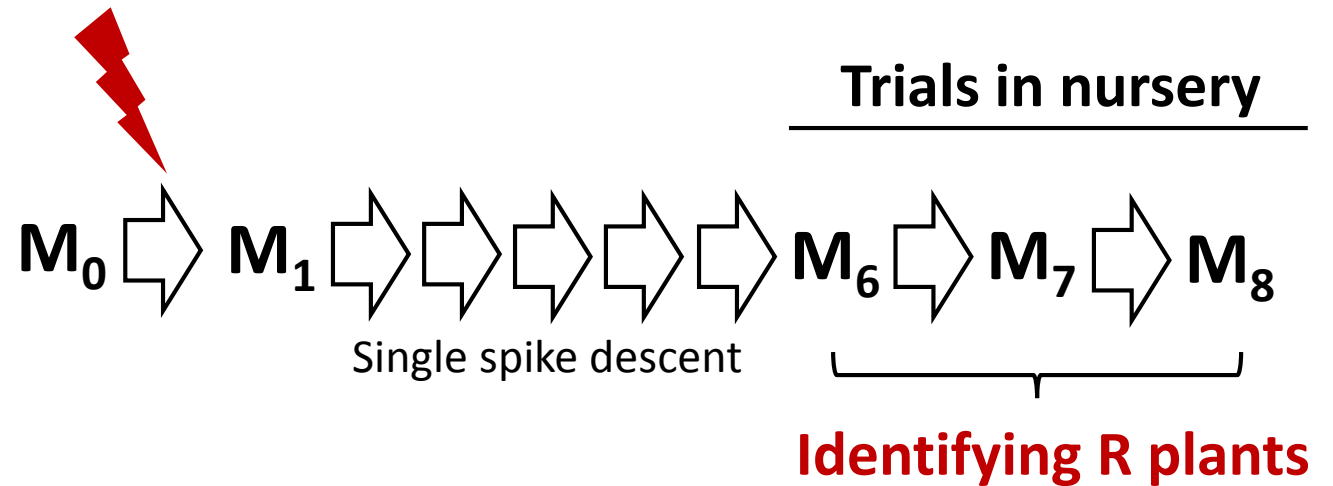
Linkage drag (genotype dependent)

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Strategy to identify S genes in barley/wheat

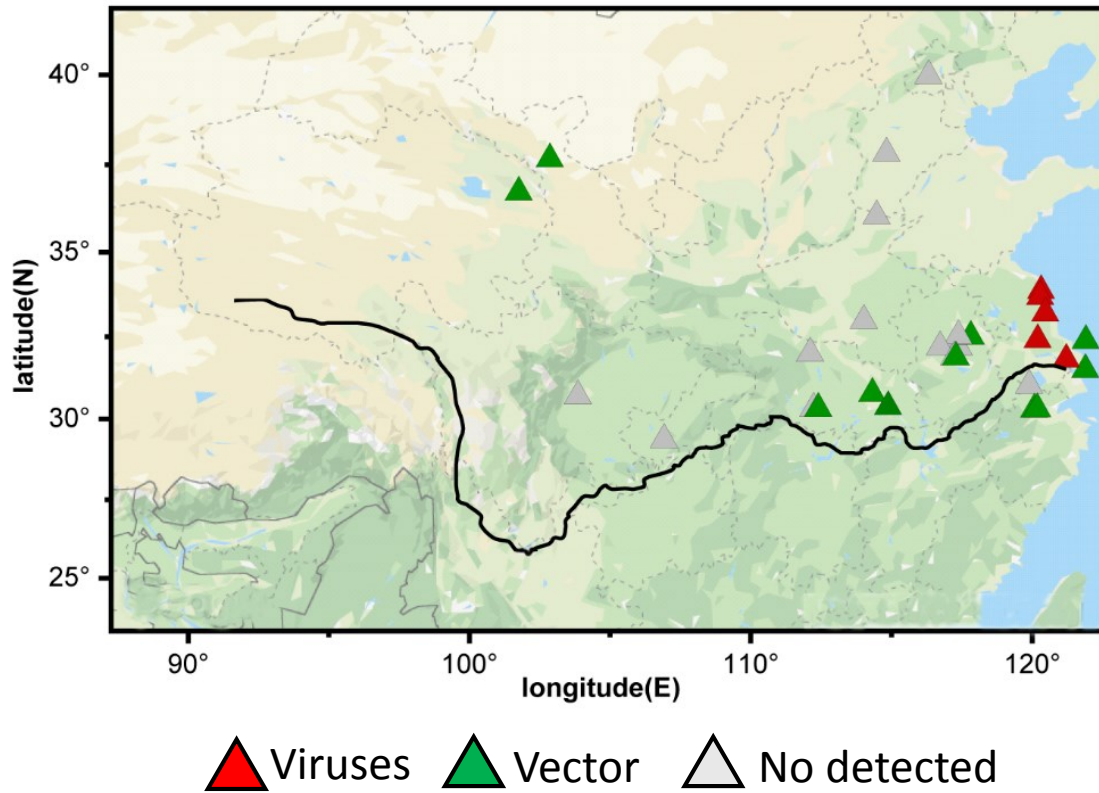
Hatiexi (HTX)



- (1) Excluding *eIF4E/PDIL5-1* alleles to obtain novel variants
- (2) WGS/MutMap strategy to isolate new S genes
- (3) Knockout of wheat orthologous genes by editing

Identifying BaYMV-infested site for field trials

Dazhong national farm with BaYMV only



Jiang *et al.*, 2022 Plant Disease

Growing S cultivar in 2022 to 2024



Da-Zhong national farm, March of 2024

Screening for BaYMV-resistant lines

Preliminary result: 234[?] of 8372 M₆ lines showed BaYMV resistance based on scoring



Dazhong national farm, Nov. 27, 2024



Dazhong national farm, Feb. 27, 2025

Summary

- *PDIL5-1* and *eIF4E* are host factor genes for soil-borne WYMV/BaYMV/BaMMV, indicating a common mechanism for bymovirus infection.
- *PDIL5-1* is a desirable modification target for enhancing WYMV resistance in wheat and BaYMV/BaMMV resistance in barley, without yield penalty.
- Future cloning host factors to BaYMV and deciphering their functional basis are of interest in barley and wheat.

Acknowledgements



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Frank Ordon (JKI Quedlingburg)



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