Dissecting the Molecular Pathogenesis of Barley Yellow Dwarf Virus to Aid Engineering of Novel Antiviral Resistance

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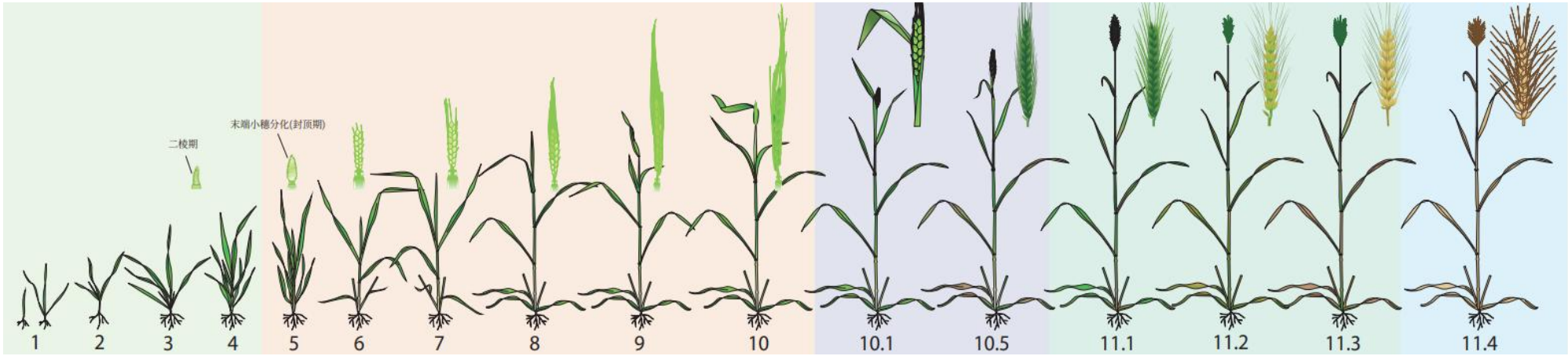
Main contents

- Introduction to the research
- Questions being investigated
- Main progress and problems
- Prospects for further studies

Global wheat production is constantly threatened by multiple diseases




Yellow dwarf


Yellow mosaic


Bacterial steak


Powdery mildew

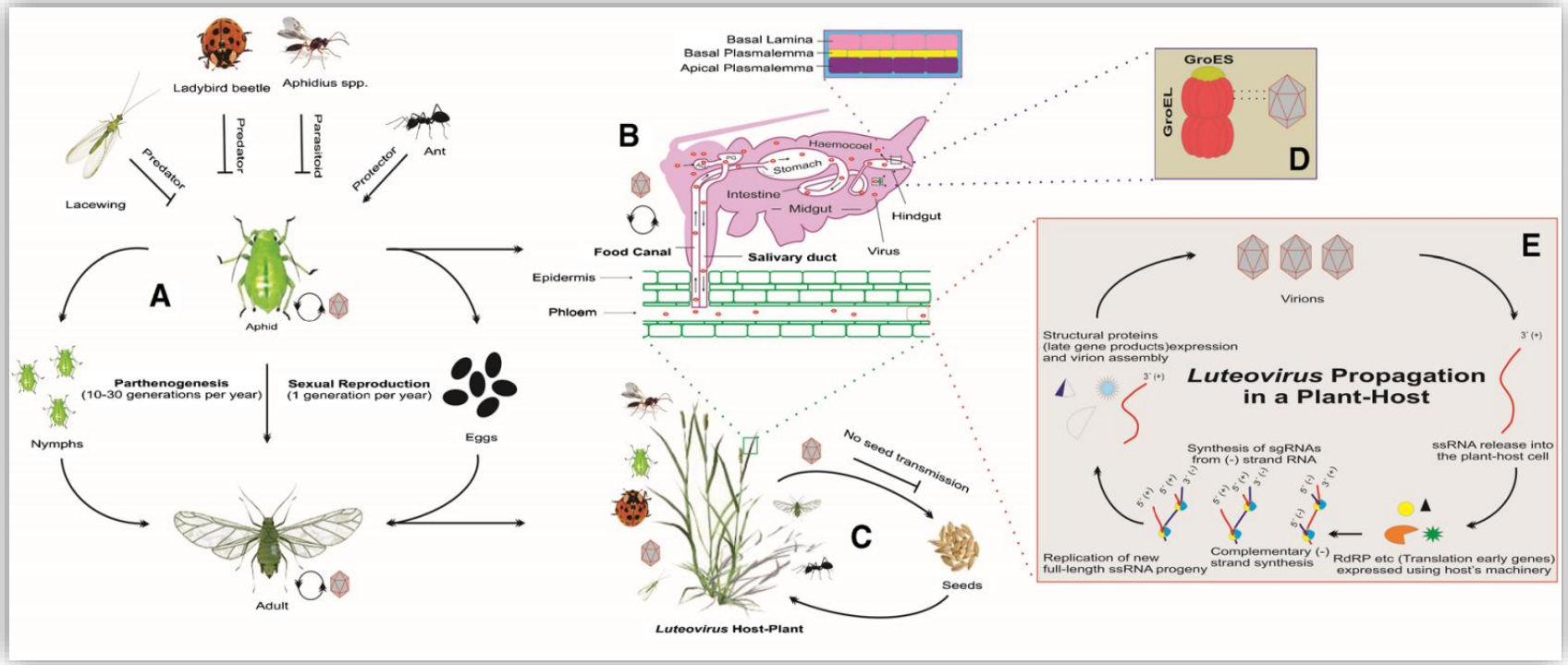

Three rusts

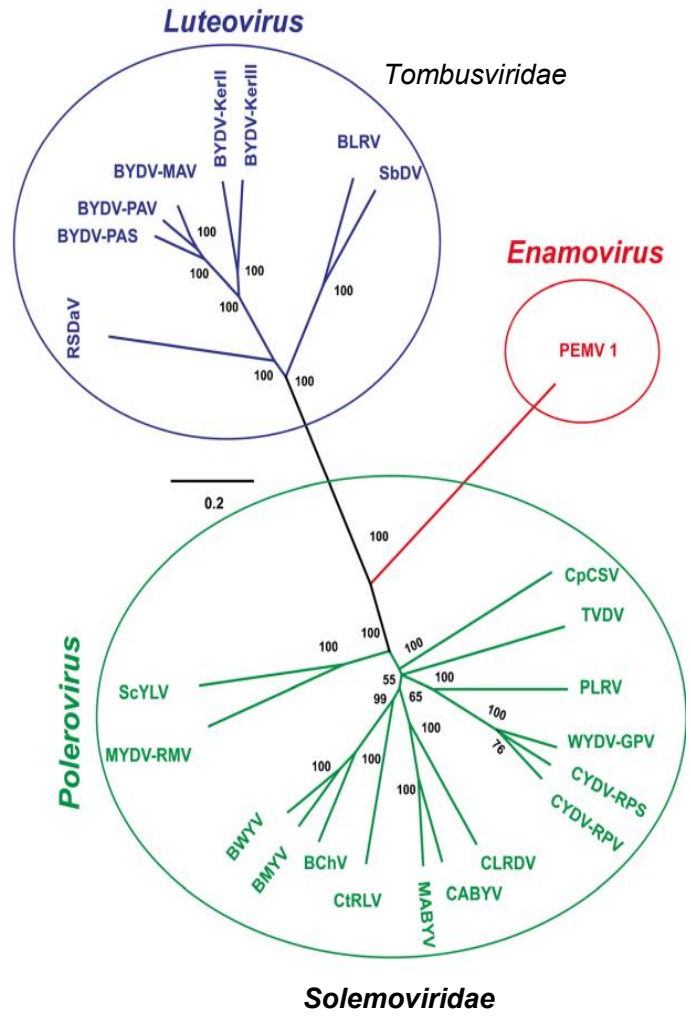

Head scab

As high as 6 billion kg of wheat yield losses annually by diseases

Barley yellow dwarf viruses (BYDVs)

- BYDVs cause serious diseases in wheat, barley, maize, oat, forages.
- Typical luteoviruses, belonging to *Tombusviridae*.
- Carrying a single-stranded positive sense RNA genome.
- Transmitted by aphids, limited to phloem tissues, present in low amounts.
- BYDV infections tend to increase as global warming worsens.
- No host resistant genes against BYDVs have been cloned.





(Ali et al., 2014, 2018; Aradottir, et al., 2021)

Barley yellow dwarf diseases in Australia

Department of Primary Industries and Regional Development
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1-10-2024

Barley leaf diseases and their management

Department of Primary Industries and Regional Development, Western Australia

Barley yellow dwarf virus and cereal yellow dwarf virus

Barley yellow dwarf (BYD) and cereal yellow dwarf (CYD) viruses (yellow dwarf viruses) are present in WA and can cause yield losses if not managed. Barley yellow dwarf virus is the more prevalent, at a ratio of 2:1.

The viruses are transmitted by aphids and the symptoms can be confused with those caused by nutrient deficiencies, waterlogging, or other stresses that cause yellowing, reddening, and striping.

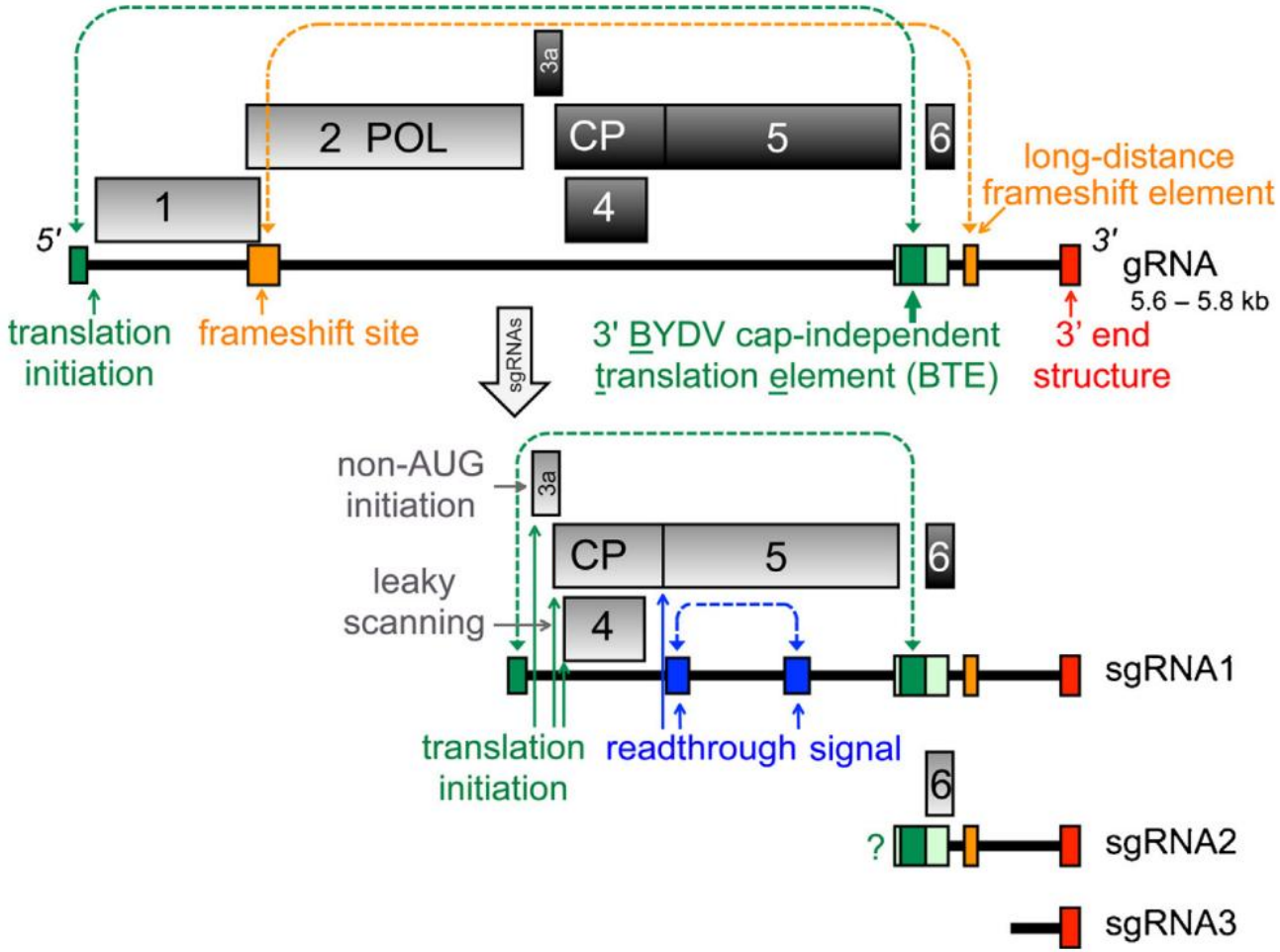
Barley yellow dwarf virus causes stunting of plants and subsequent loss of yield and quality. Early infection at the seedling stage can cause yield loss of up to 80%, while late infection can result in 20% yield loss. The virus can be damaging in high and very high rainfall areas and, more rarely, in medium rainfall areas.

Cereal aphids spread barley yellow dwarf virus to barley crops from infected grasses and volunteer cereals. The greatest risk of significant yield and quality effects is from early infection (during the first 8 to 10 weeks of crop growth). The risk of early aphid arrival depends on the amount of summer and autumn rainfall and the availability of green vegetation before the growing season.

Managing yellows dwarf viruses

- Control the green bridge, since both viruses need living plants to survive.
- In high-risk locations, sow varieties that are less susceptible to barley yellow dwarf virus.
- Each season, assess the barley yellow dwarf virus risk for your location and time of sowing to determine the most appropriate insecticide application strategy. Apply a seed dressing containing imidacloprid or spray the crop with a registered insecticide in the first 3 to 7 weeks of crop growth to substantially reduce infection.

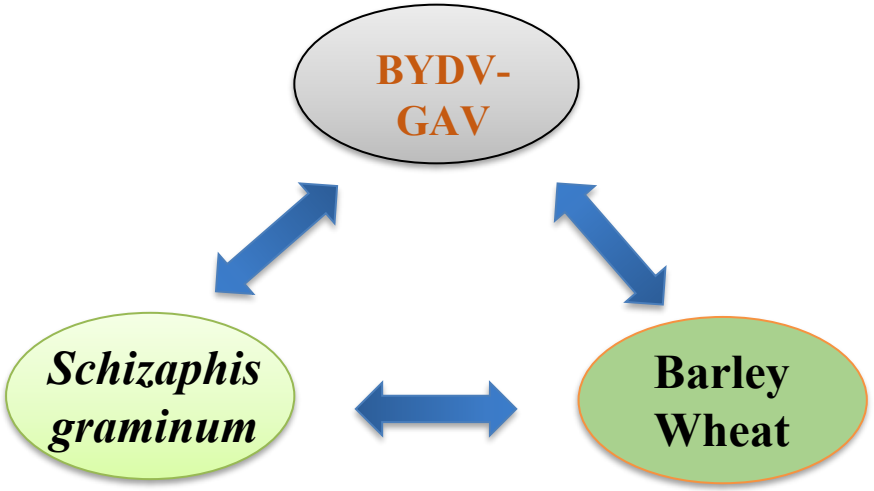
Proteins encoded by BYDVs



- P1 and P2, required by viral replication
- P3, the viral coat protein
- P3a, required for long distance movement
- **P4 (17K), movement protein, VSR, inhibitor of host mitosis**
- P5, functioning in aphid transmission
- P6, showing VSR activity

(Miller and Rasochova, 1997; Wang et al., 2013; Miller et al, 2015; Smirnova et al., 2015; Fusaro et al., 2017; Ali et al., 2018; Jin et al., 2020)

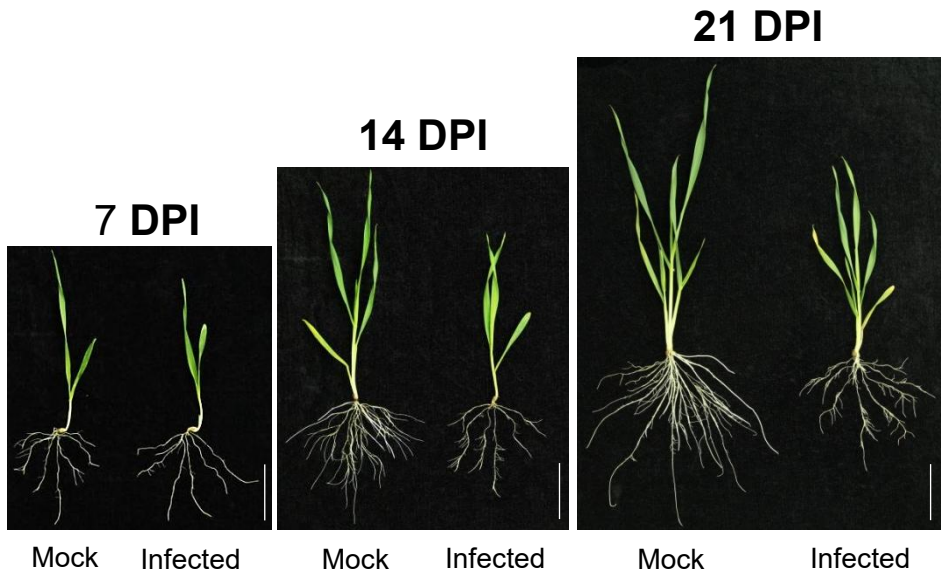
Pathosystem we used for BYDV studies



+ BYDV-GAV
- BYDV-GAV

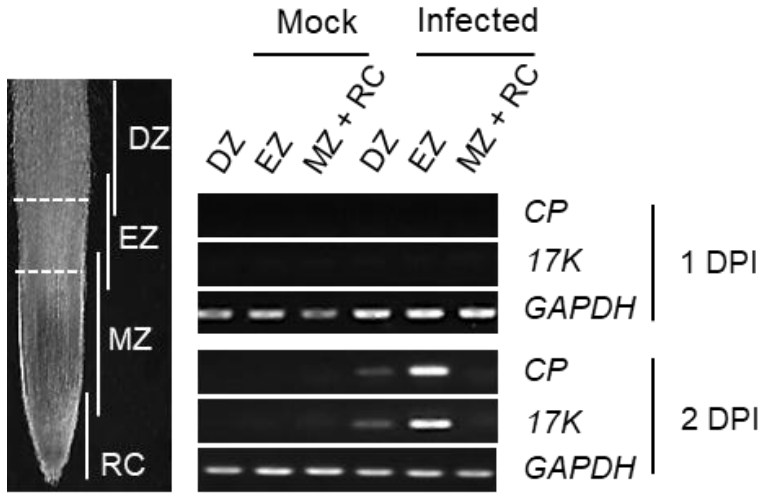
Hydroponically cultured host

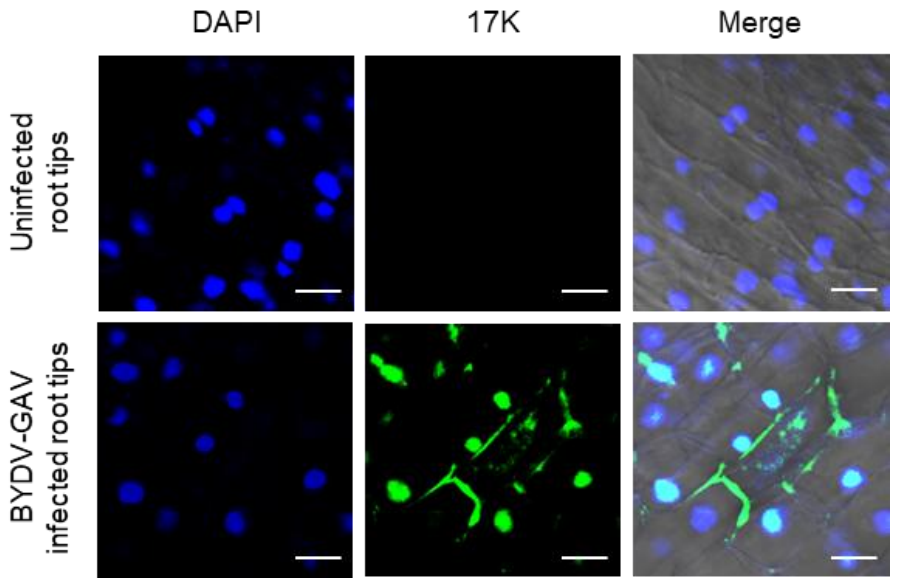
Phenotypical and molecular analyses

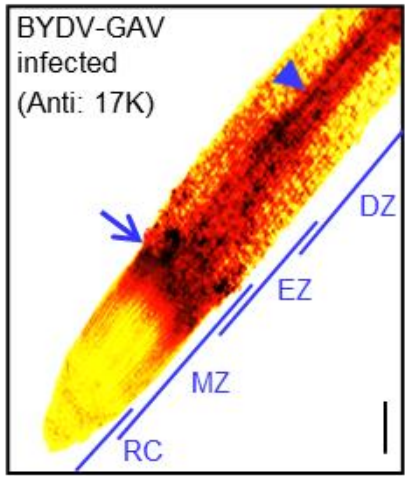


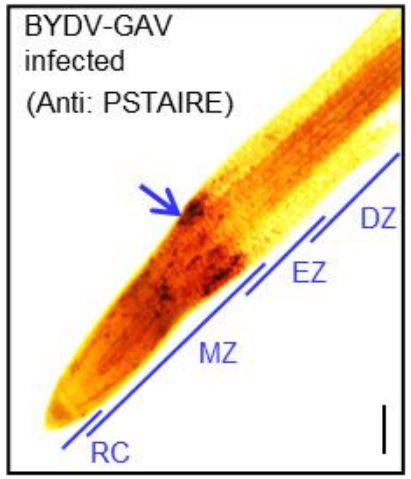
Pathosystem we used for BYDV studies

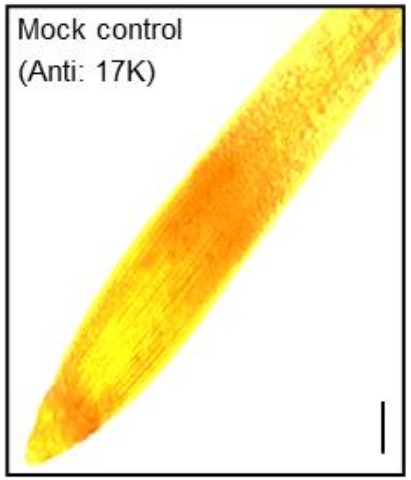
Detection of BYDV gene (*17K*, *CP*) expression in the roots by RT-PCR and *in situ* immunostaining

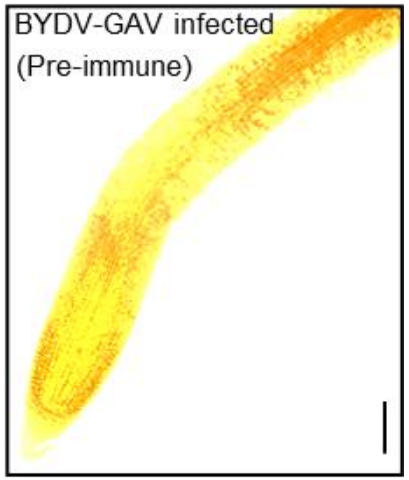












Does BYDV infection inhibits of host mitosis? If so, what are the mechanisms?

(Jin et al., 2020)

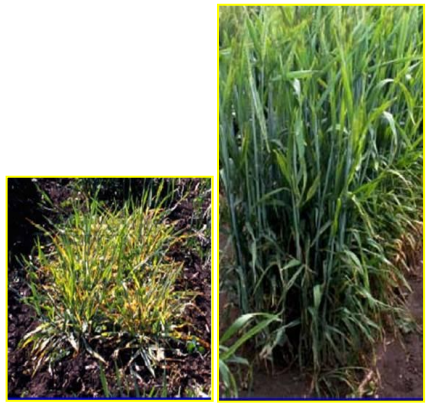
Scientific questions merit more and deeper studies in BYDV-host interactions

- The mechanisms of symptom induction by BYDV infection
- Why are BYDVs limited to host phloem tissues
- Molecular mechanisms of natural host resistance to BYDVs
- Can biotechnological resistance to BYDV be engineered

Main progress and problems

- Inhibition of host mitosis as a factor contributing to the yellow dwarf symptoms elicited by BYDVs.
- Host subversion of a viral virulence protein to aid its antiviral defence.
- Dissecting the interactomes of BYDV proteins as a key route to understand viral pathogenesis and host defense against the viruses.

BYDV 17K inhibits G2/M arrest in fission yeast



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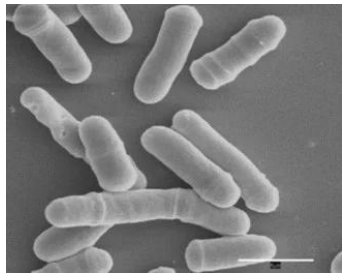
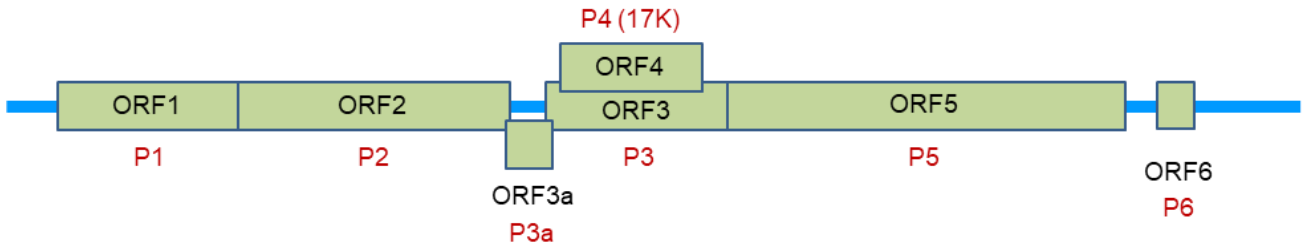
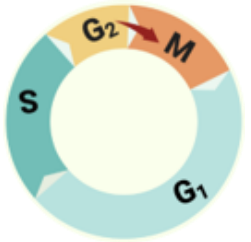
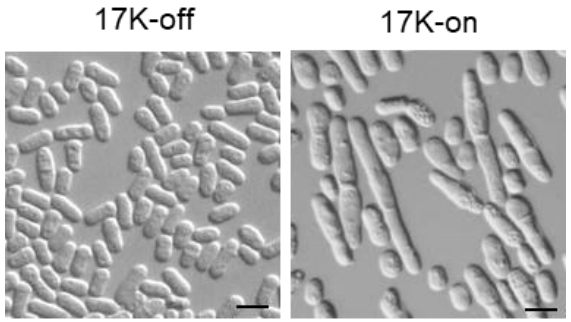
Cell mitosis

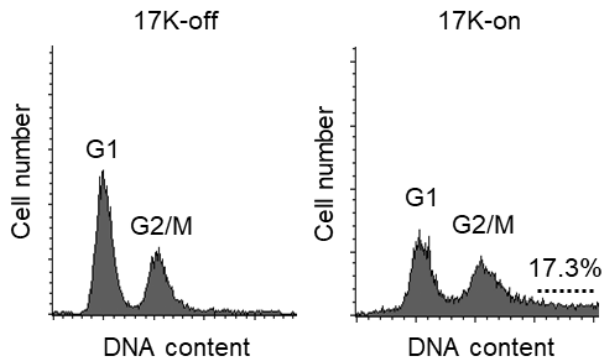


Fission yeast



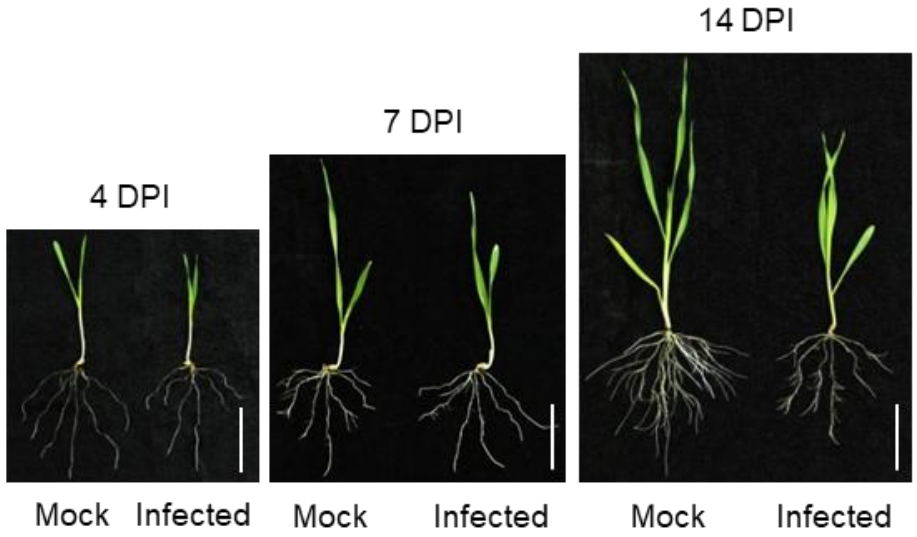


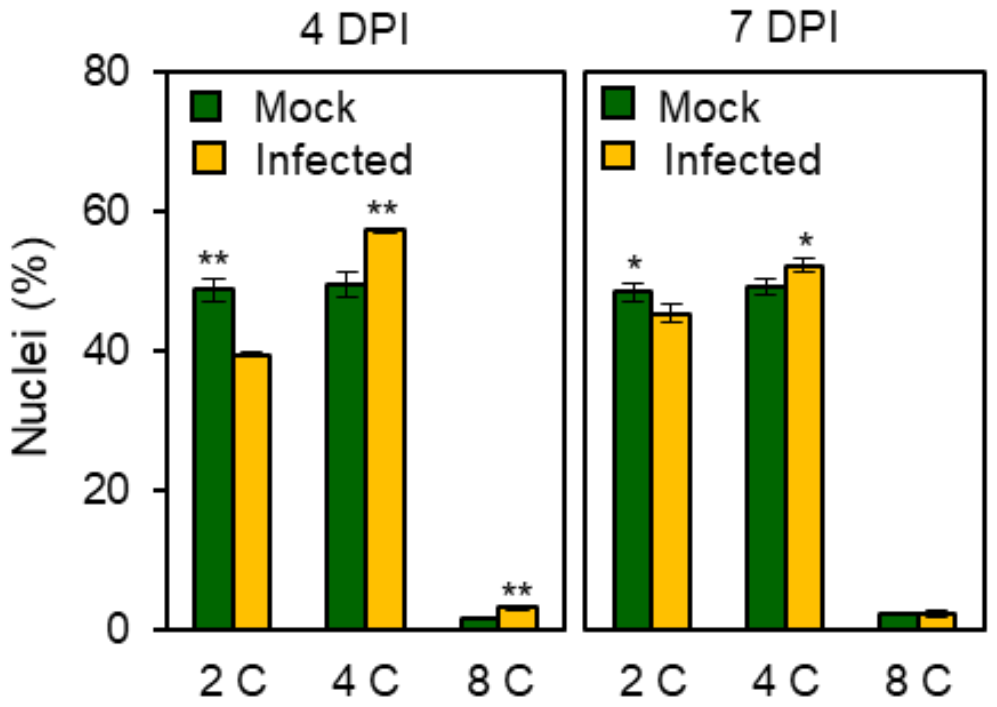




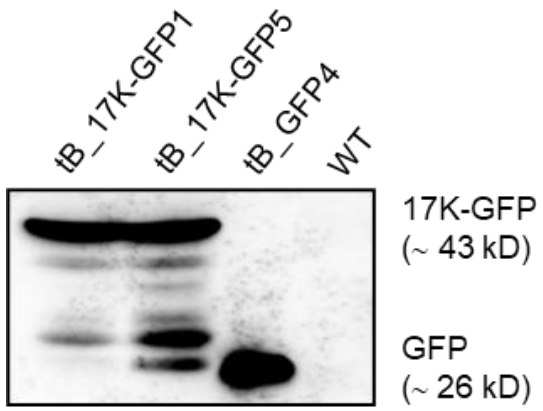
(Jin et al., 2020)

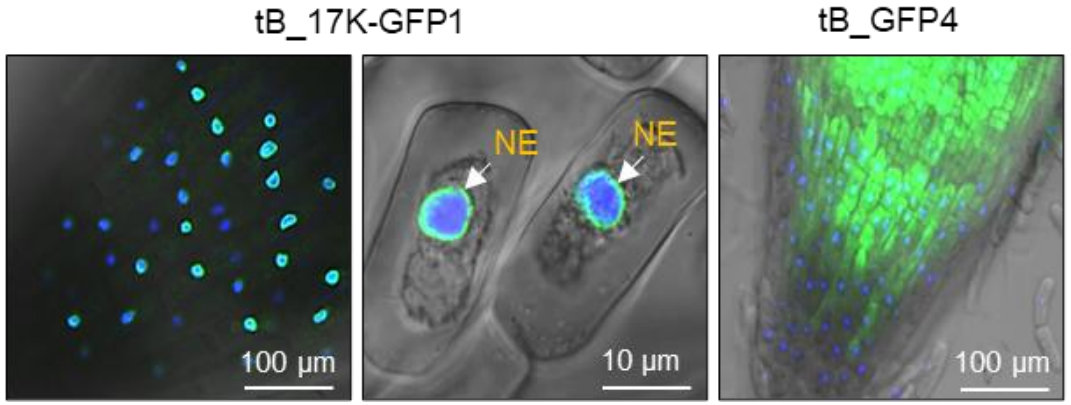
Mitosis is disrupted in the barley roots infected by BYDV

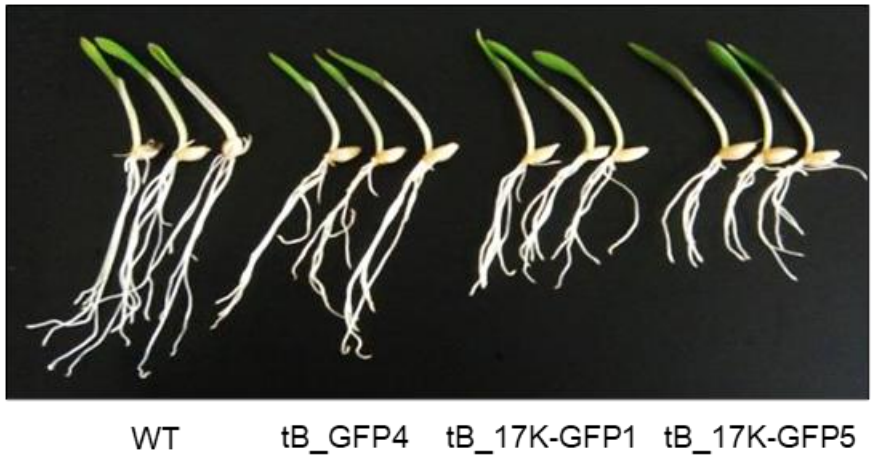


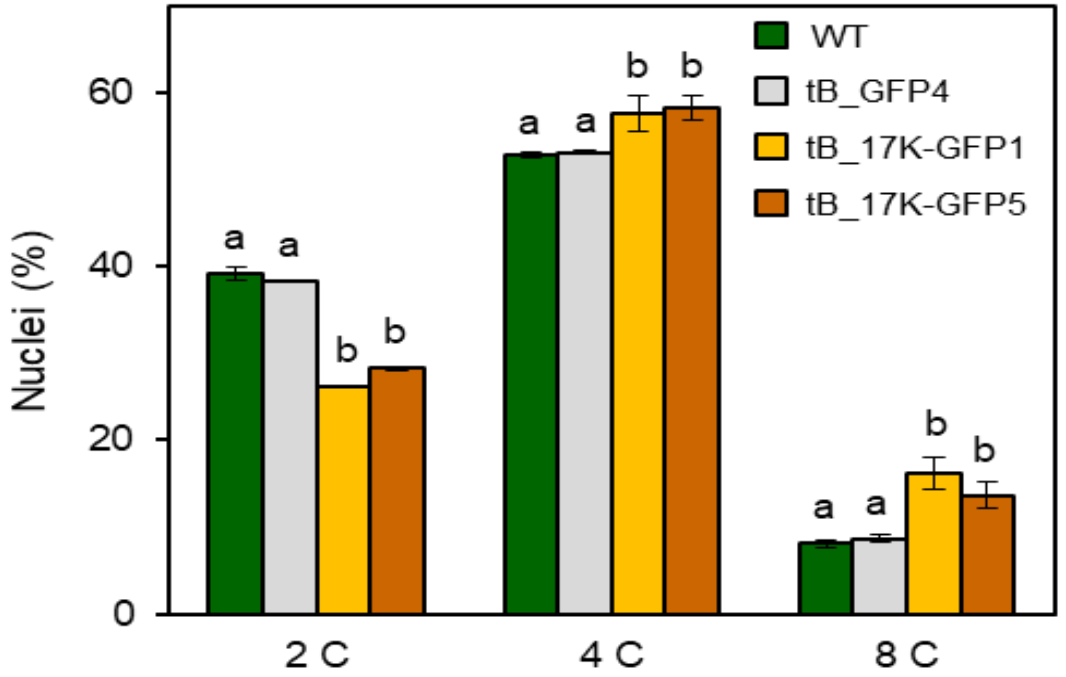


Transgenically expressed 17K disrupts barley mitosis

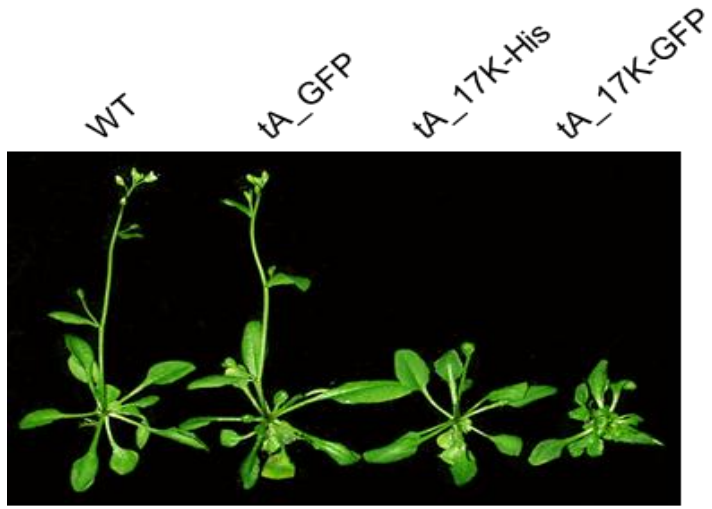


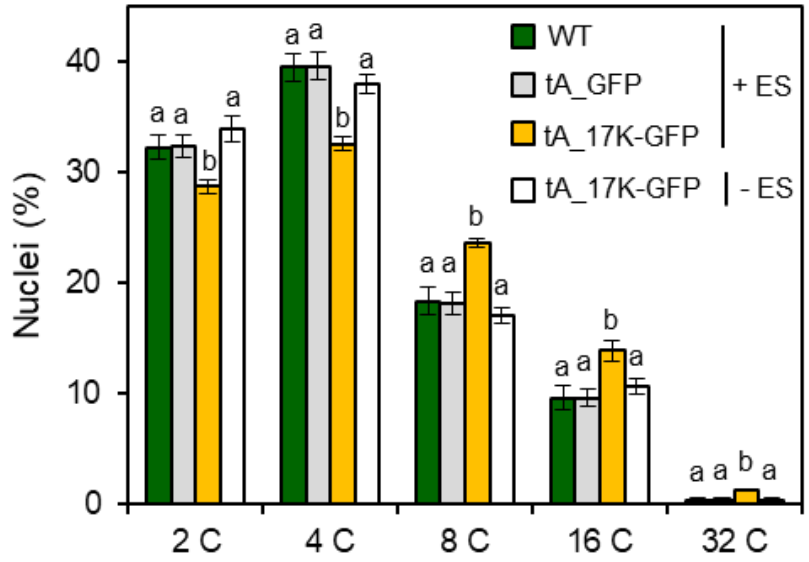


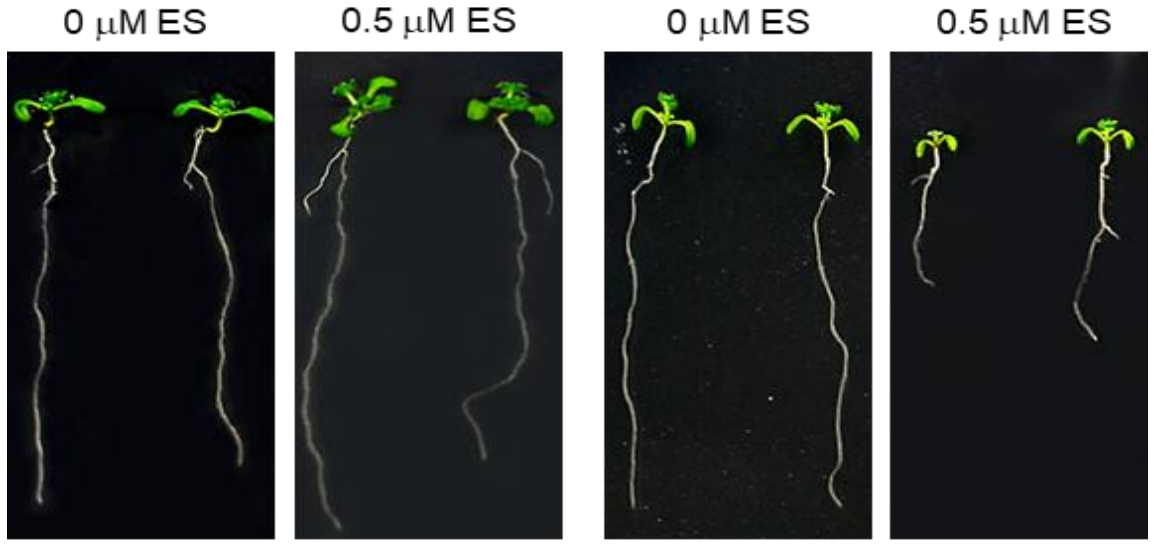


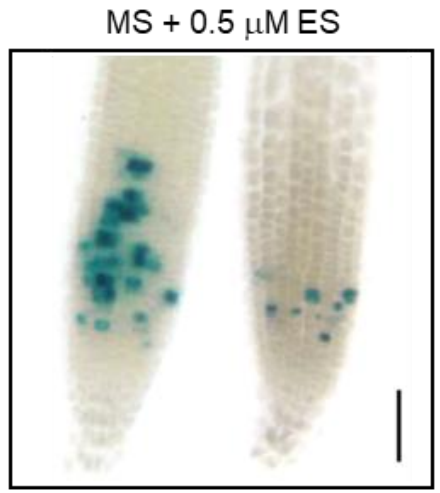


Inducible expression of 17K disrupts *Arabidopsis* growth and root mitosis

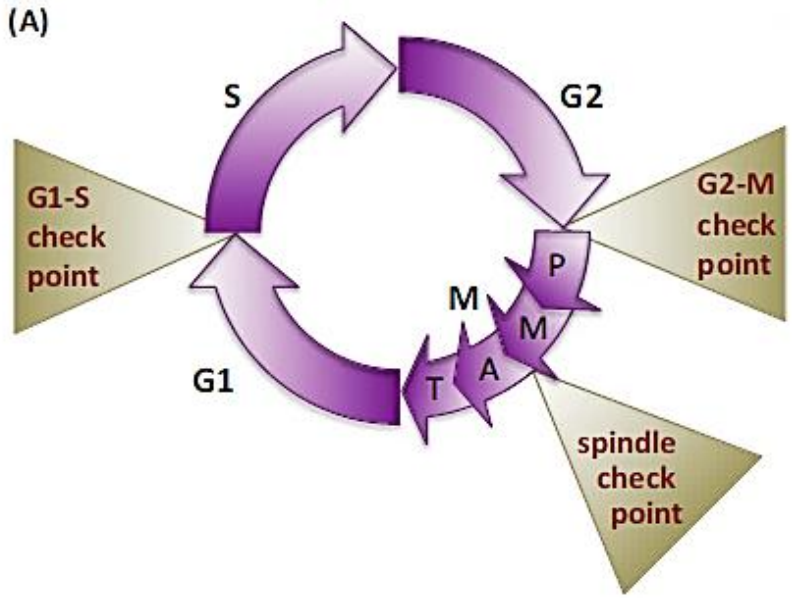


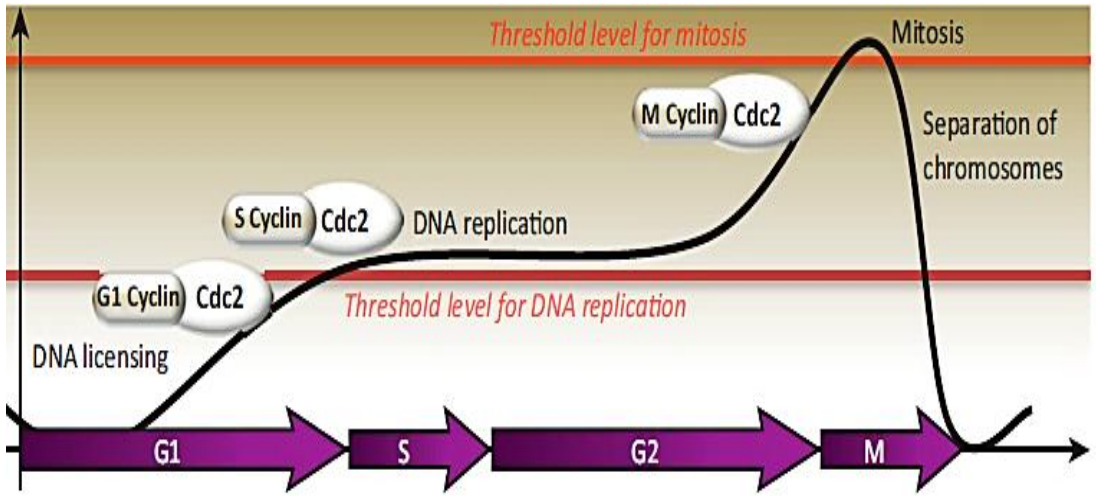






Does 17K affect members of the key mitotic switch Wee1-Cdc25-CDKA/Cdc2?





17K-AtCdc25	●	●			
17K-HvCdc25	●	●			
17K-CDKA;1	●	●	●		●
17K-HvCDKA1	●	●	●	●	
17K-HvCDKA2	●	●	●	●	
17K-AtCdc25-CDKA;1					●
17K-HvCdc25-HvCDKA1					●
17K-HvCdc25-HvCDKA2					●
AtCdc25-CDKA;1	●	●			
HvCdc25-HvCDKA1	●	●			
HvCdc25-HvCDKA2	●	●			

● Yeast-two hybrid

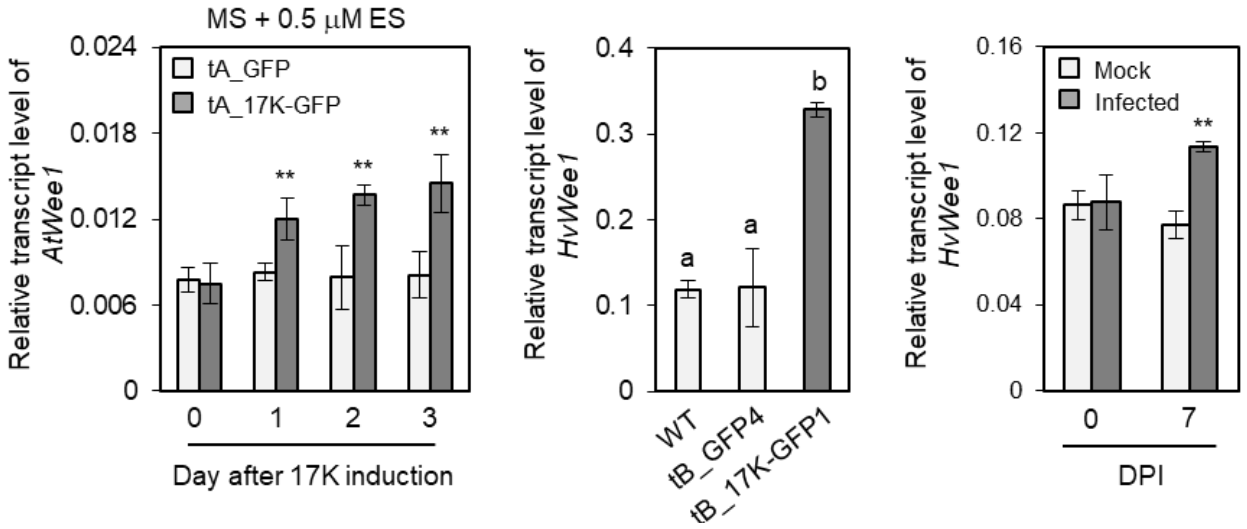
● Luciferase complementation

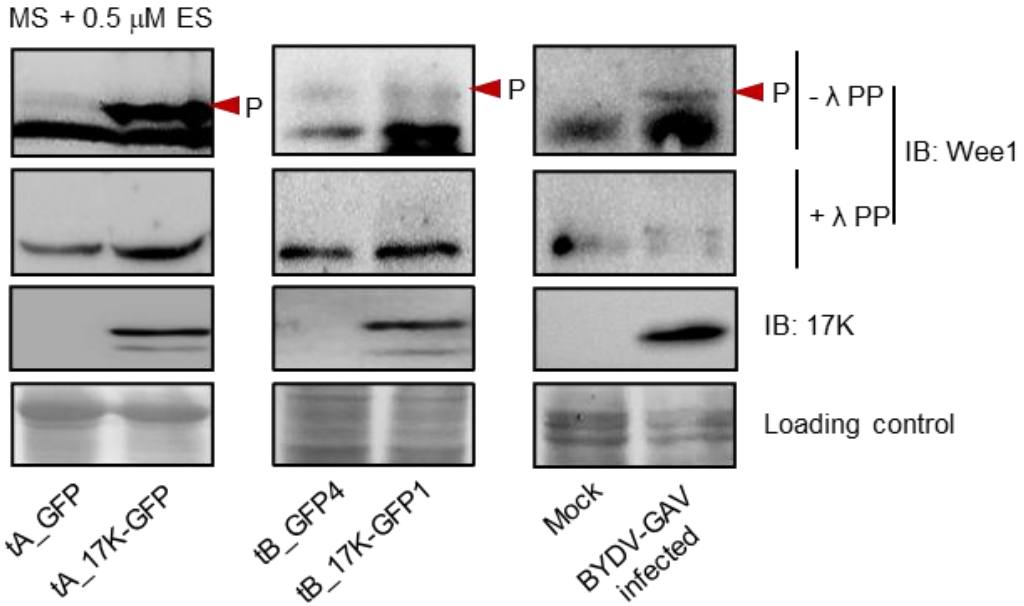
● Co-IP using transgenic lines expressing 17K-GFP

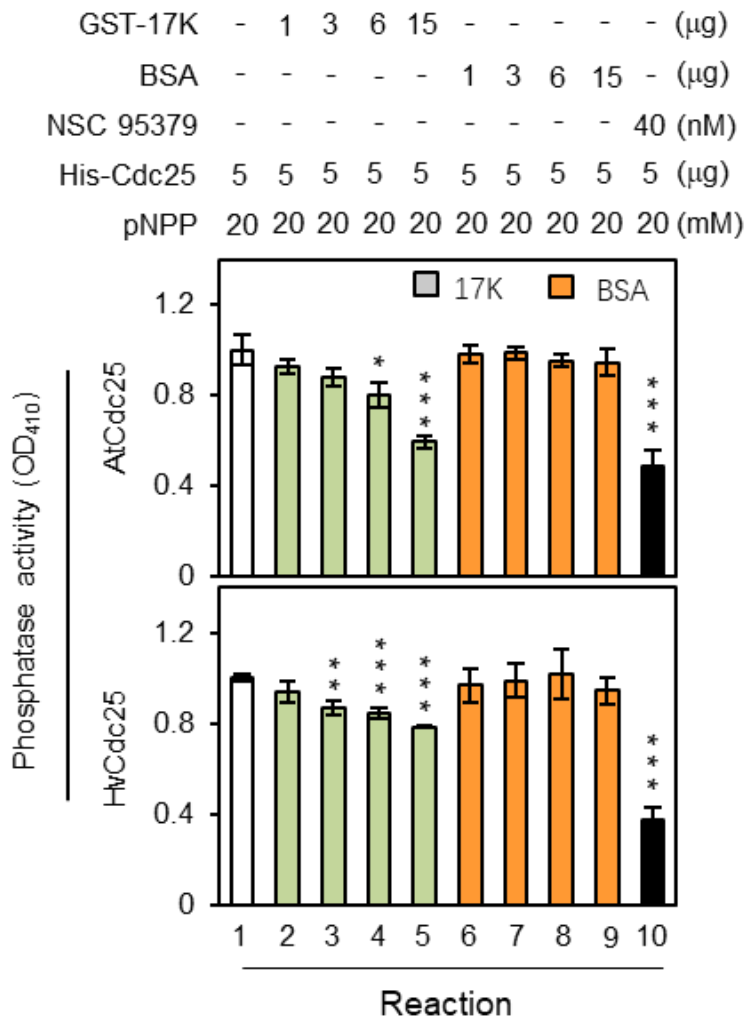
● Co-IP using barley root tips infected by BYDV-GAV

● Co-IP using tobacco leaves expressing tagged proteins

17K enhances *Wee1* expression but inhibits Cdc25 activity





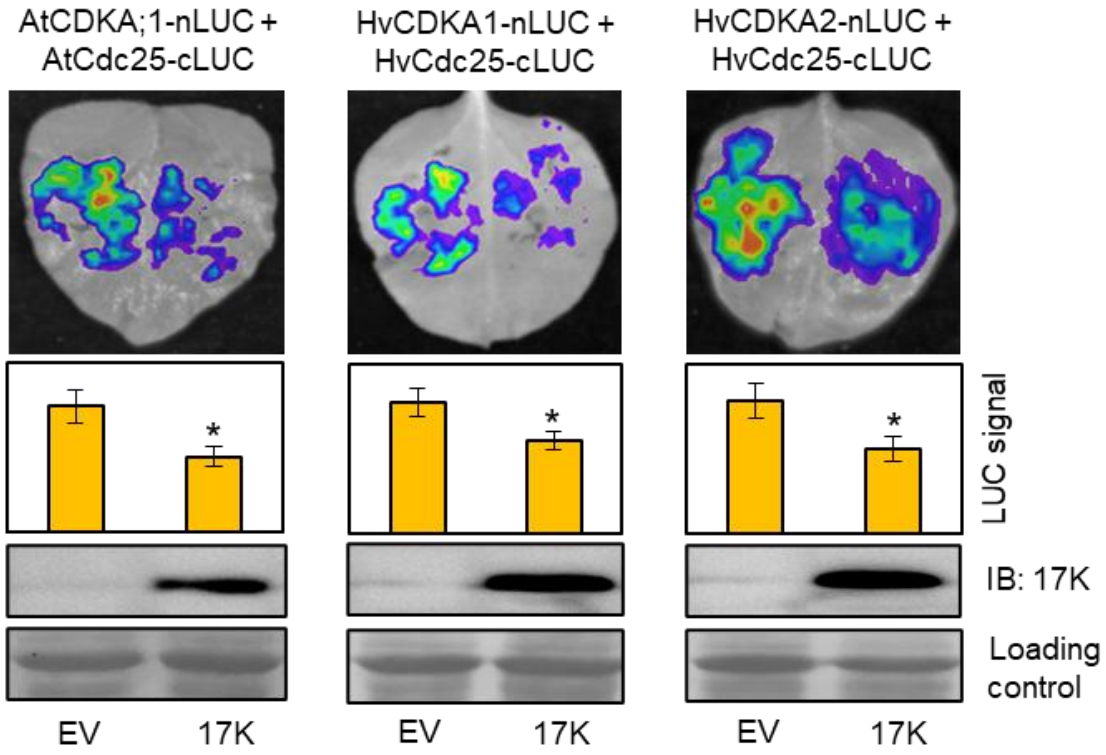


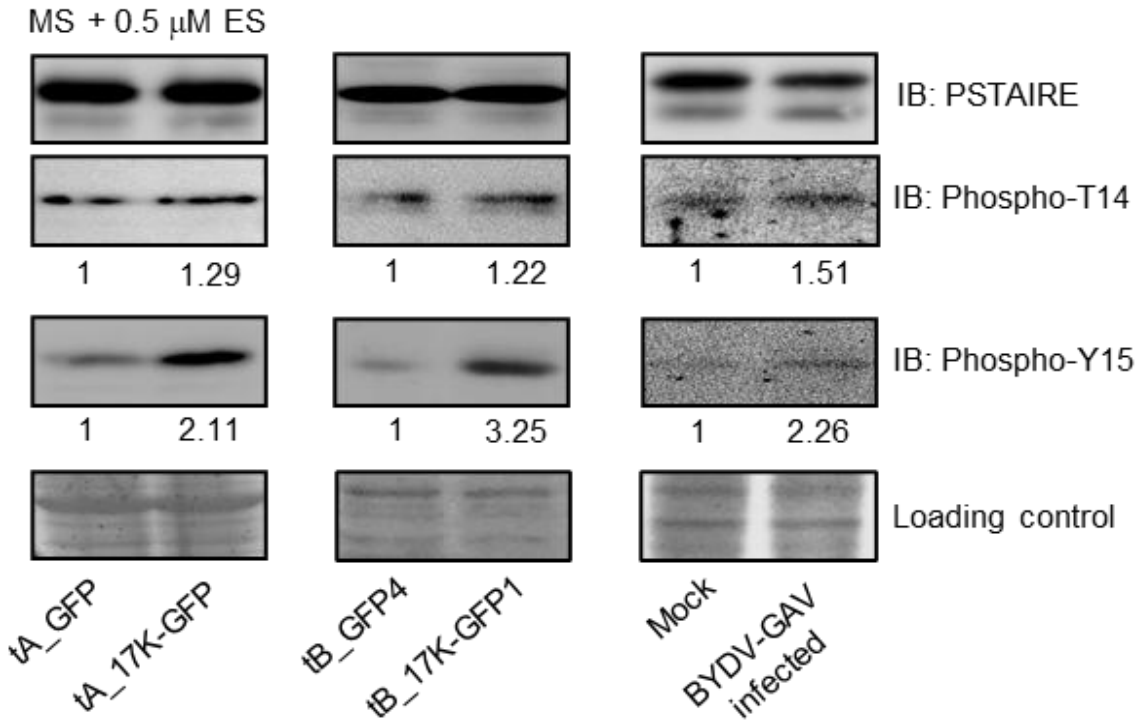
17K also weakens CDKA1-Cdc25 interaction，resulting in hyperphosphorylation of CDKA1

AtCdc25-CDKA;1		
HvCdc25-HvCDKA1		
HvCdc25-HvCDKA2		

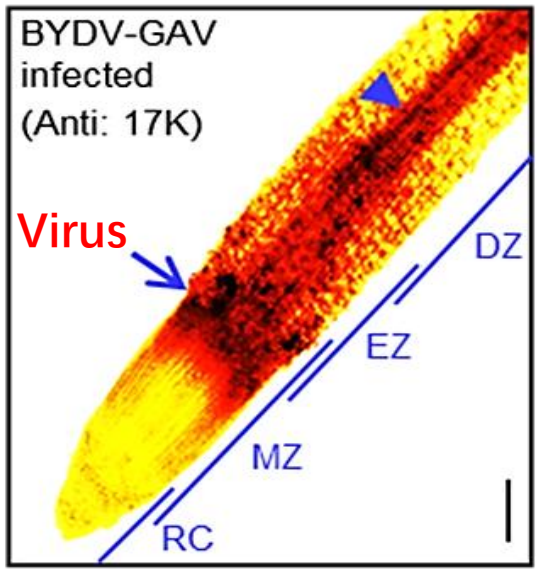
Yeast-two hybrid

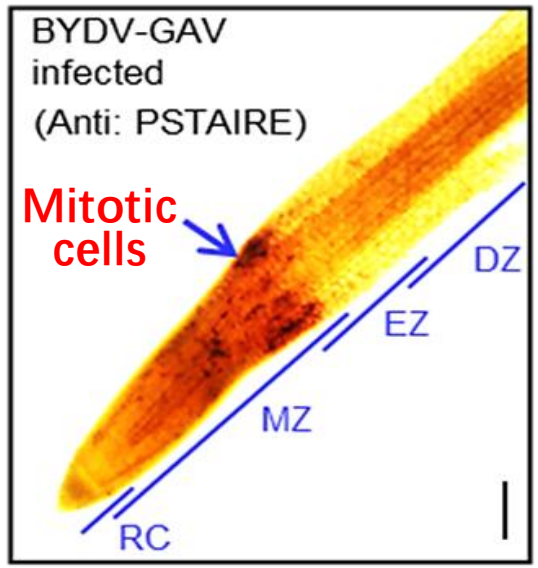
Luciferase complementation

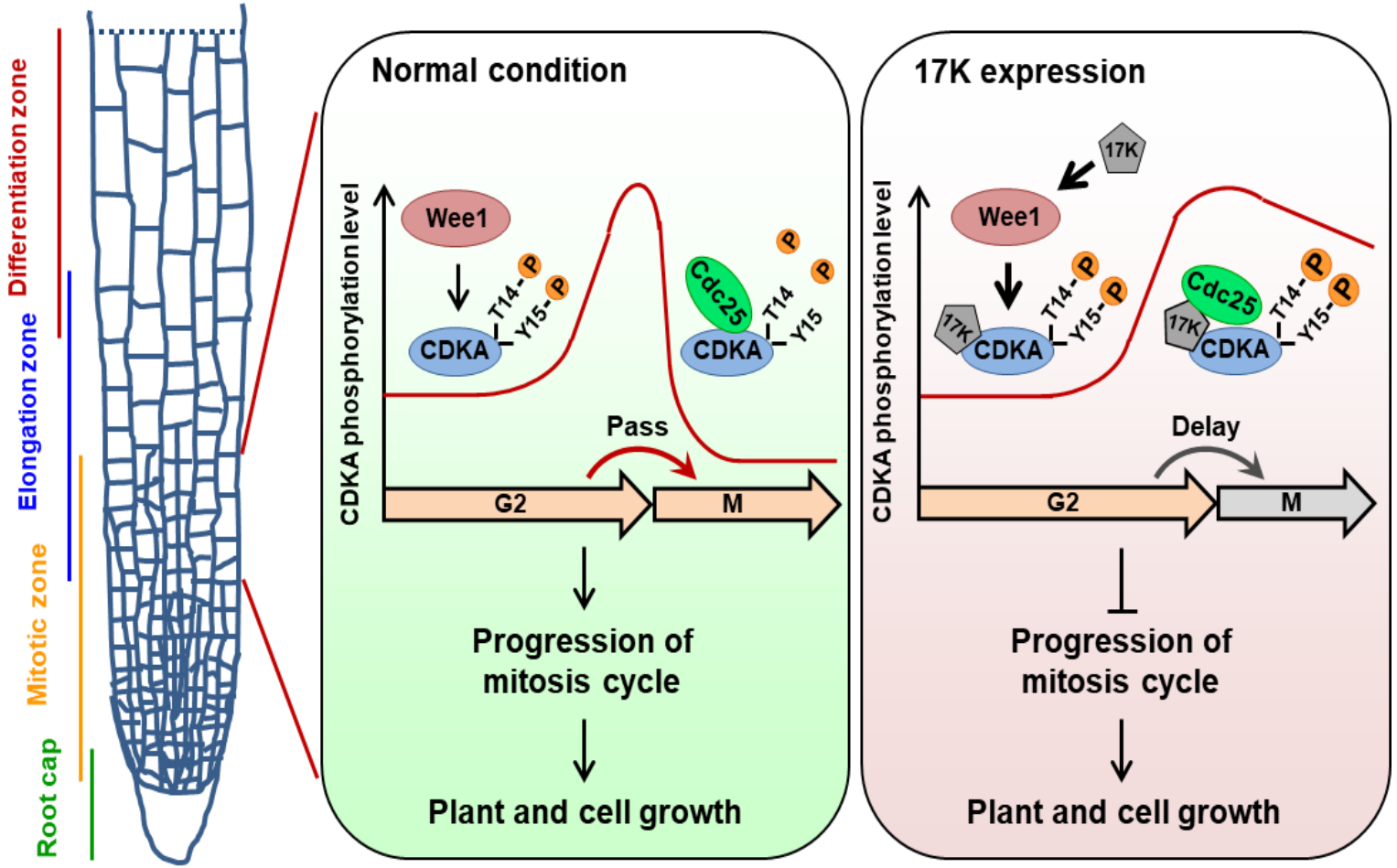




A working model on the mechanism underlying 17K's inhibition of plant mitosis

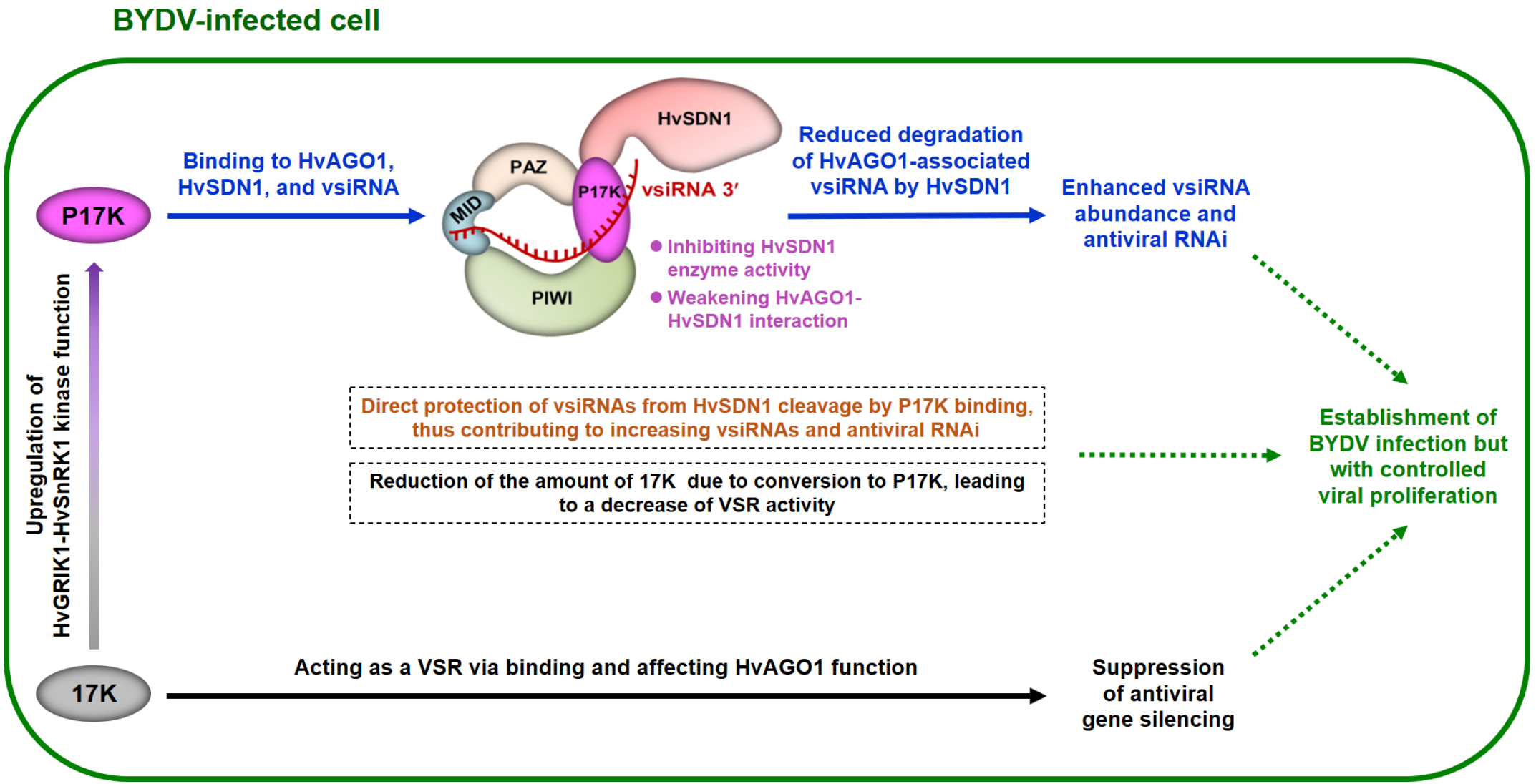






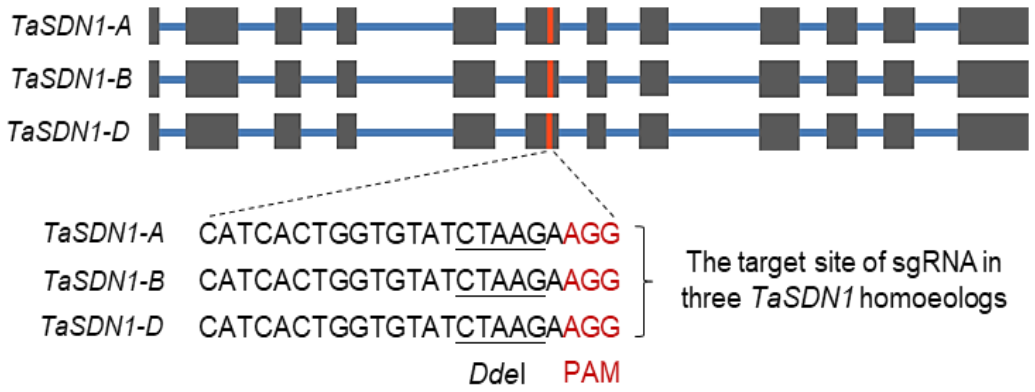
(Jin et al., 2020)

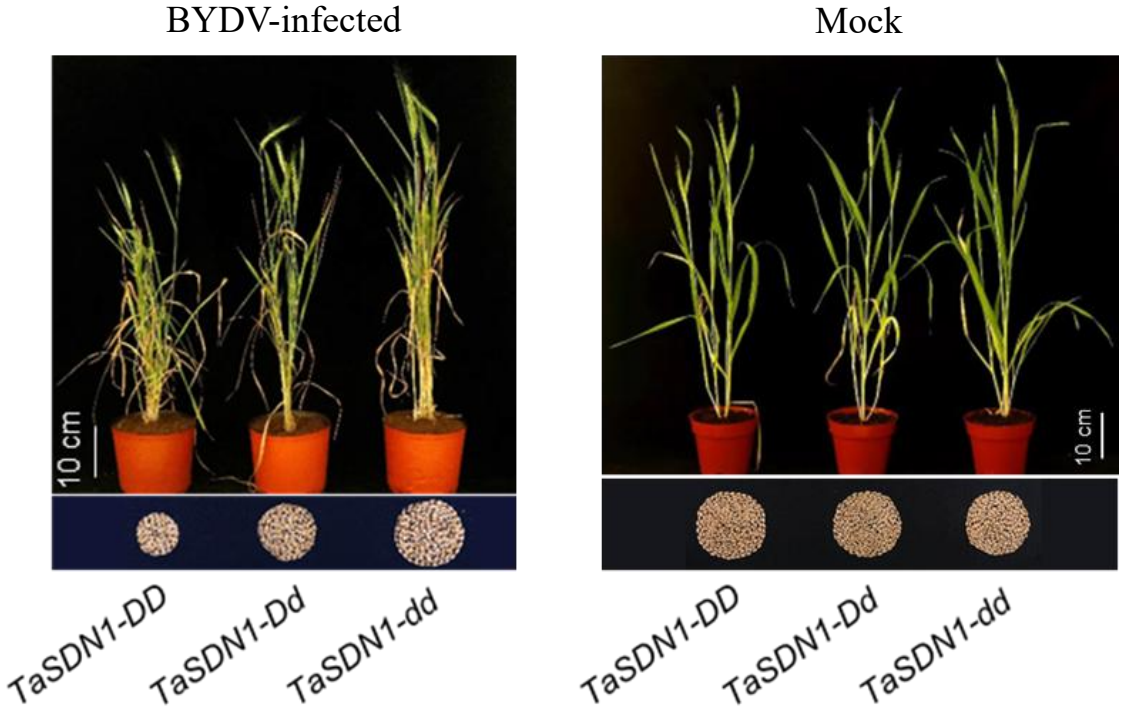
Host subversion of BYDV 17K by phosphorylation boosts antiviral defence



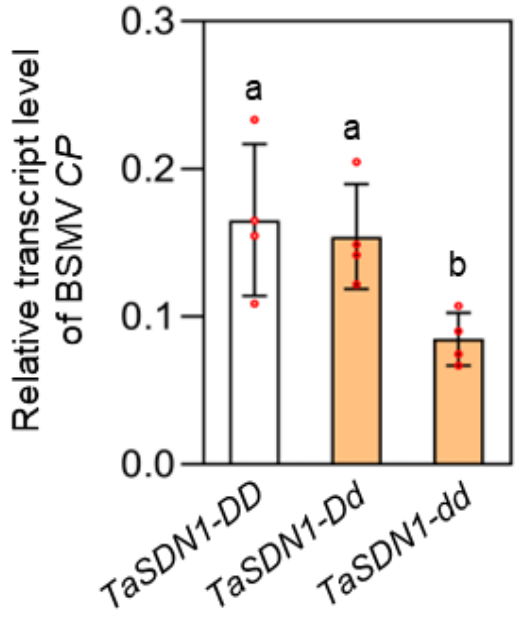
(Jin et al., 2022, EMBO J)

Knockout of *TaSDN1-D* enhances wheat resistance to both BYDV and BSMV

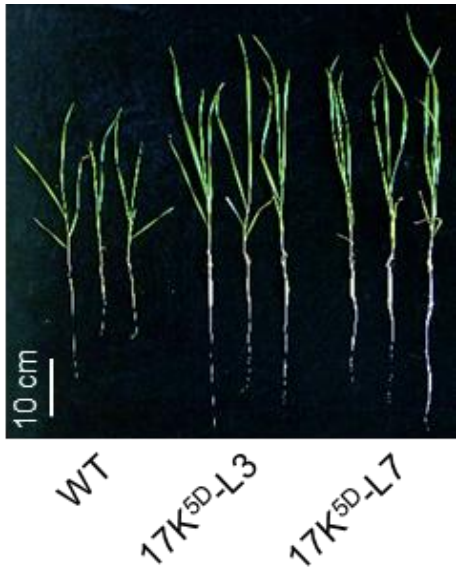


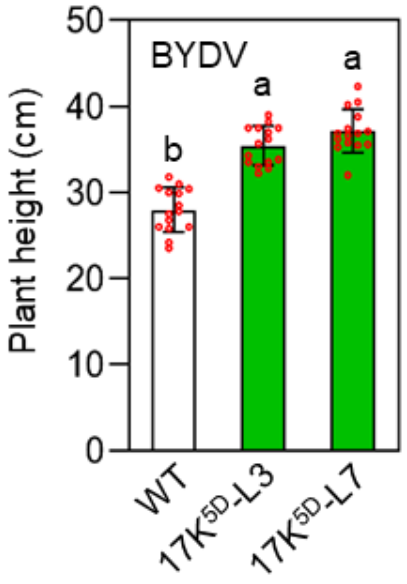


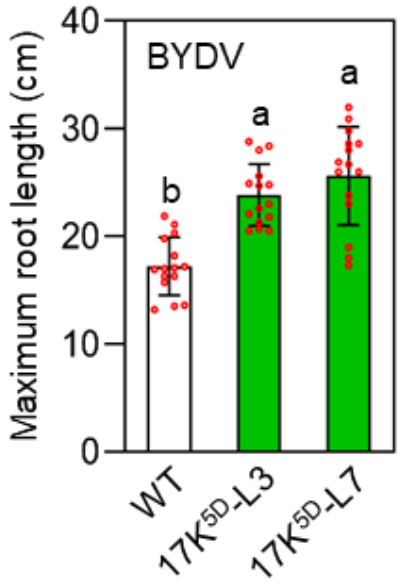
TaSDN1-DD ***TaSDN1-Dd*** ***TaSDN1-dd***

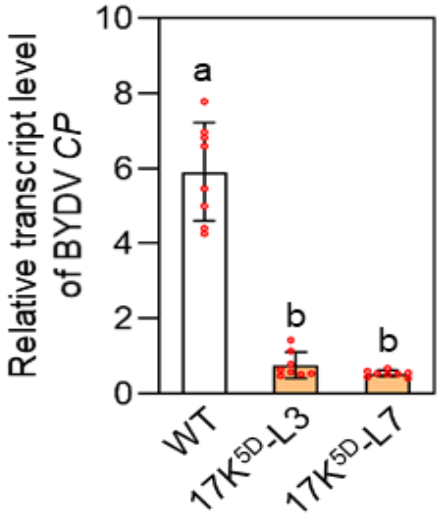


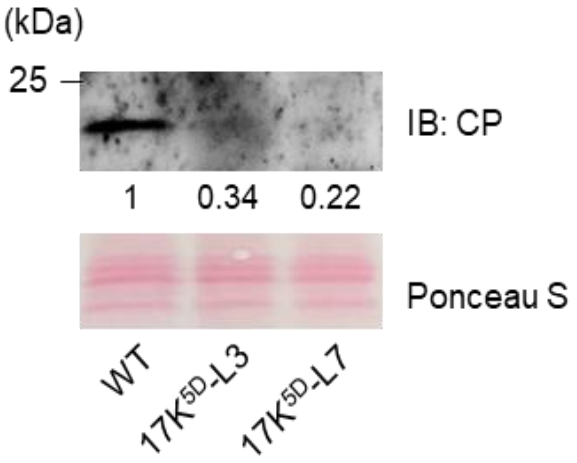
Transgenic expression 17K^{5D} (mimicking P17K) confers strong resistance to BYDV

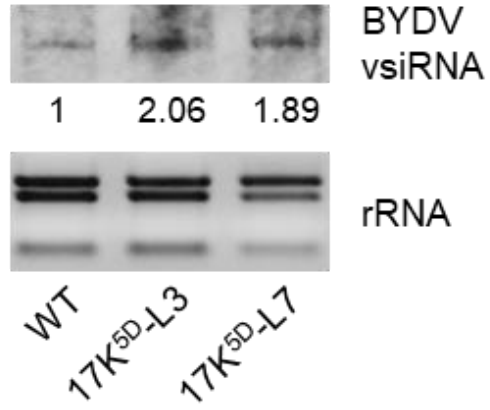










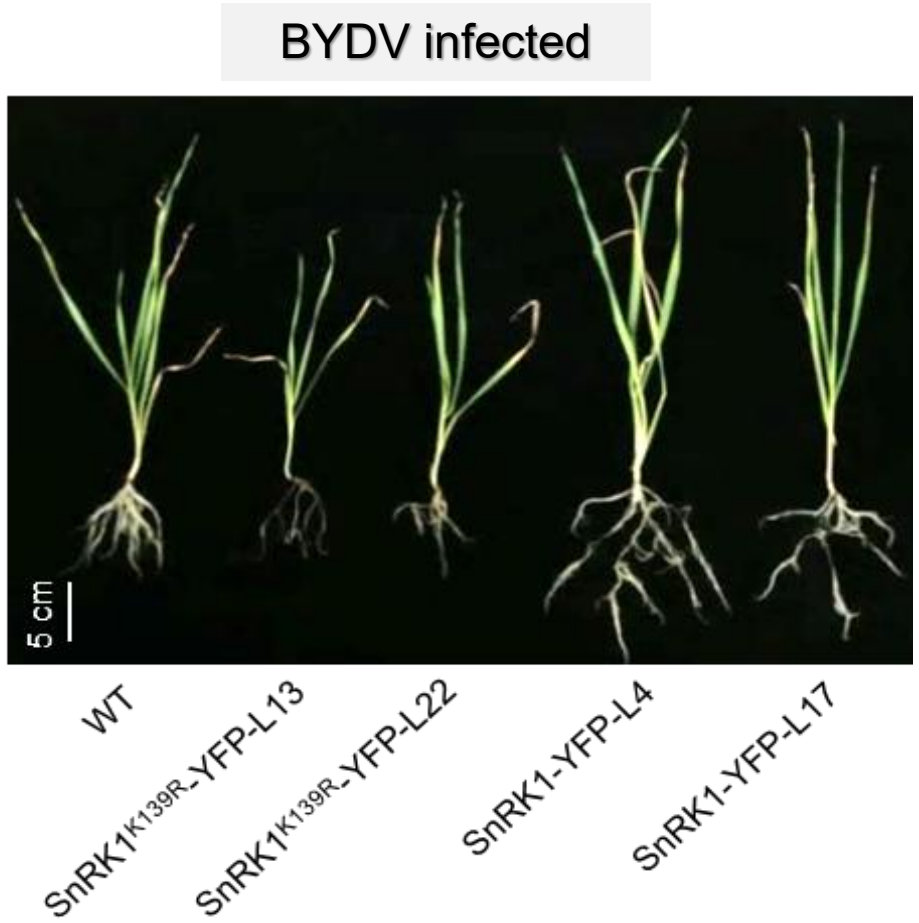


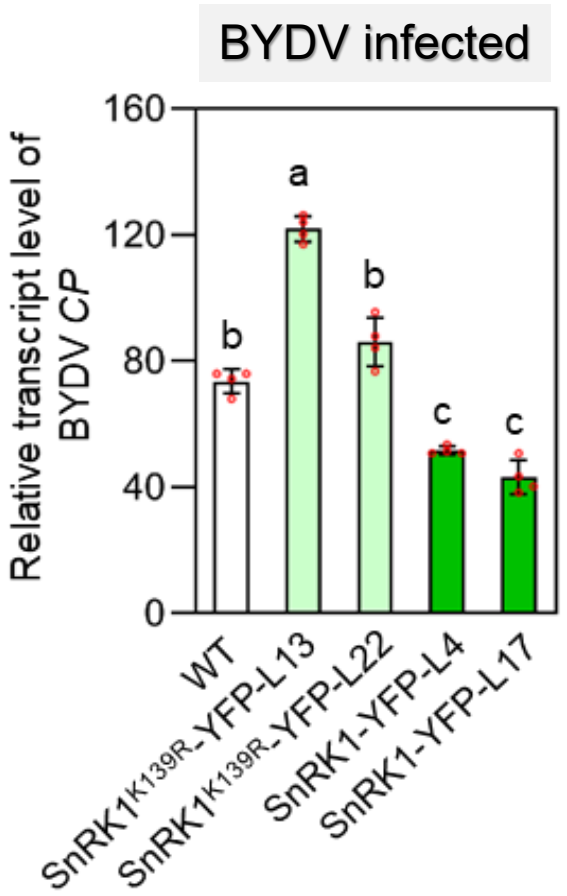
(Jin et al., 2022)

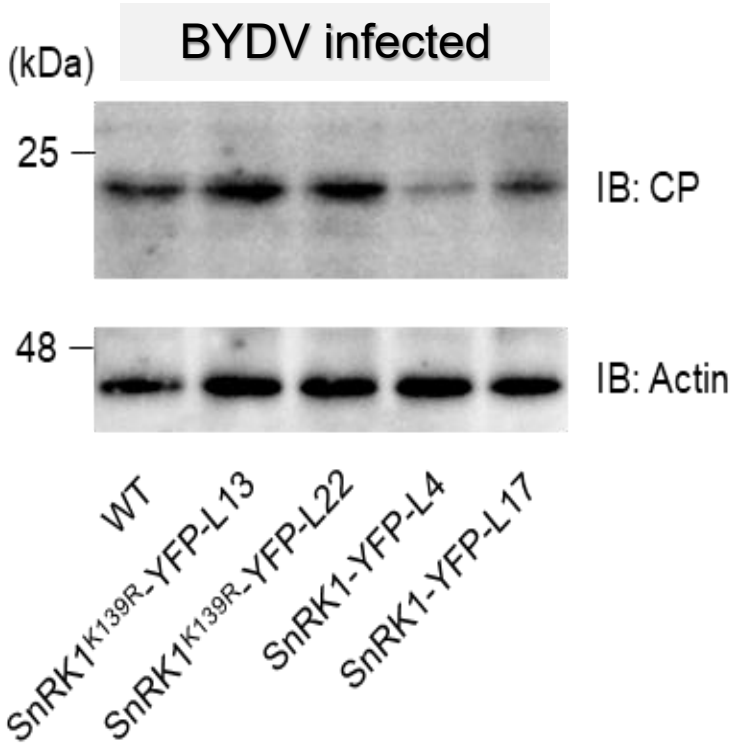
Transgenic expression of SnRK1 elevates barley resistance to BYDV

SnRK1: WT SnRK1 of barley

SnRK1^{K139R}: dominant negative mutant







Problems affecting the efficiency of BYDV studies

- Interaction of BYDV proteins with host factors is very complex and very important, needing more efficient resources to speed up the studies.
- Lack of an infectious BYDV clone capable of efficiently infecting barley/wheat, hindering the identification of important viral determinants.
- Lack of sufficient host and viral mutants, making it difficult to deeply dissect the molecular basis of host/virus interactions.

Recently developed resources for expediting BYDV research

- Several studies have reported hundreds of host proteins interacting with BYDV 17K .
- Wheat genetic transformation and genome editing have both been improved, thus benefiting gene functional analysis.
- A GFP-tagged BYDV full-length clone was inserted into the genome of *Brachypodium*, likely useful for generating both host and viral mutants in a large scale.

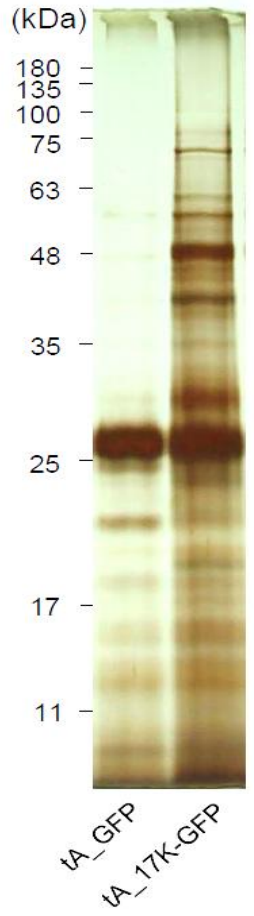
Chen S, et al. Sci Rep. 2021; 11(1): 8453.

Tian S, et al. Mol Plant. 2024; 17(4): 614-630.

Wang L, et al. Plant Biotechnol J. 2024; 22(3): 572-586.

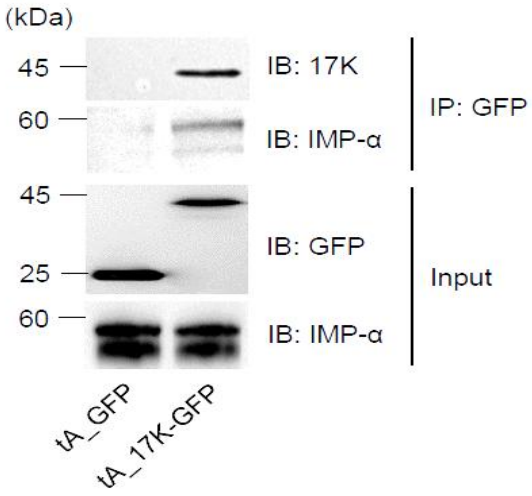
Wang Z, et al. Plant Biotechnol J. 2026; doi: 10.1111/pbi.70618.

IP-MS using 17K-GFP transgenic line: ≥ 180 putative interacting proteins



Peptide identified by MS	NATWTLSNFCR
Number of times identified	2
P-value	0.00022
Arabidopsis protein	AtIMP-α1
Arabidopsis locus	*AT3G06720*

Validation by Co-IP



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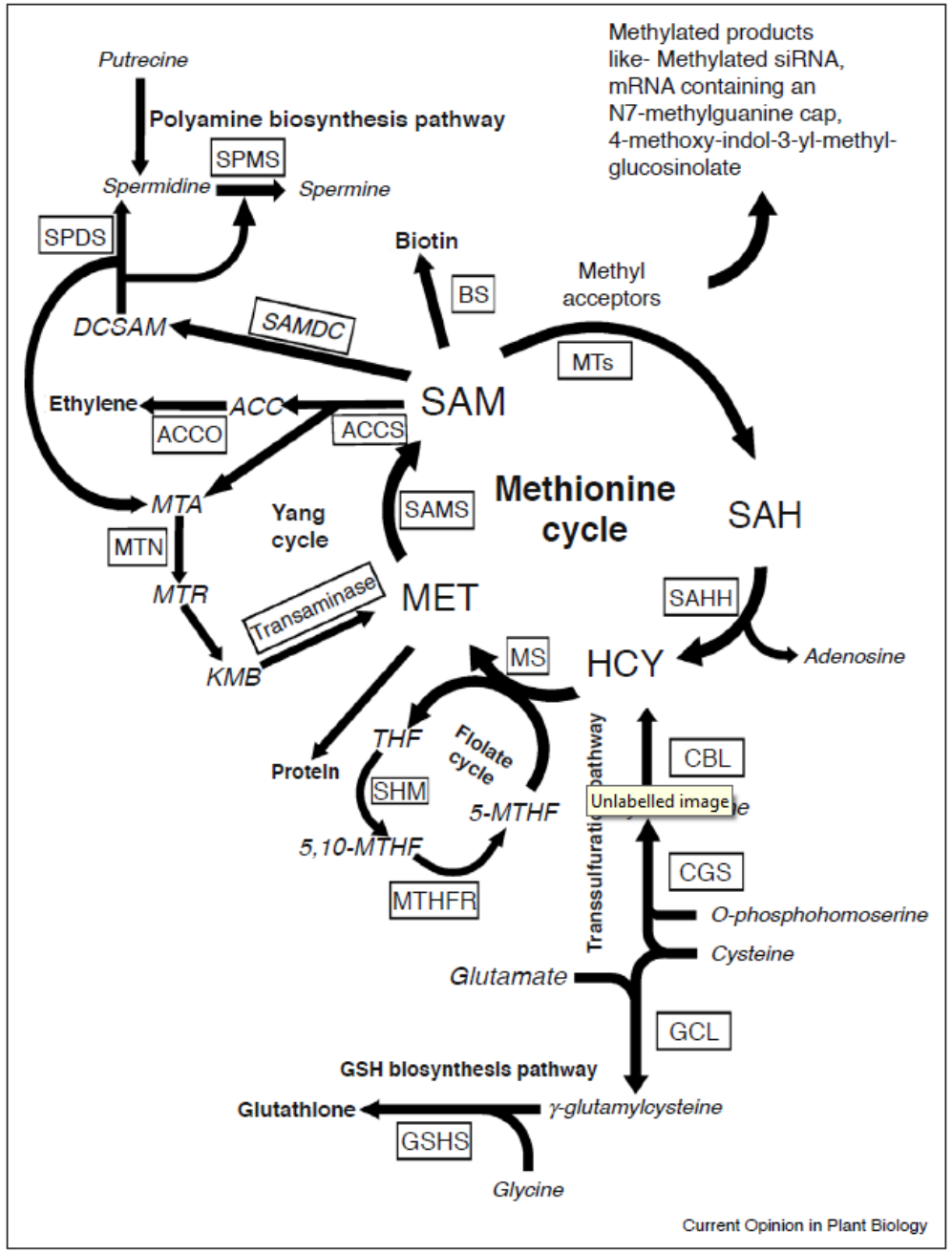
RESEARCH ARTICLE | Open Access

Methionine Synthase Positively Regulates Plant Defence to Both RNA and DNA Viruses and Is Useful for Developing Broad-Spectrum Antiviral Resistance in Crops

Zhaohui Wang, Kunpu Zhang, Chi Zhang, Jin Yang, Bei Li, Lina Wang, Zhenghao Shi, Rui Guo, Shuai Zhang, Kaiqi Gao, Jianing Li, Xiaohuan Jin, Xiang Ji, Huihui Bi, Liyuan You, Huaibing Jin, Daowen Wang

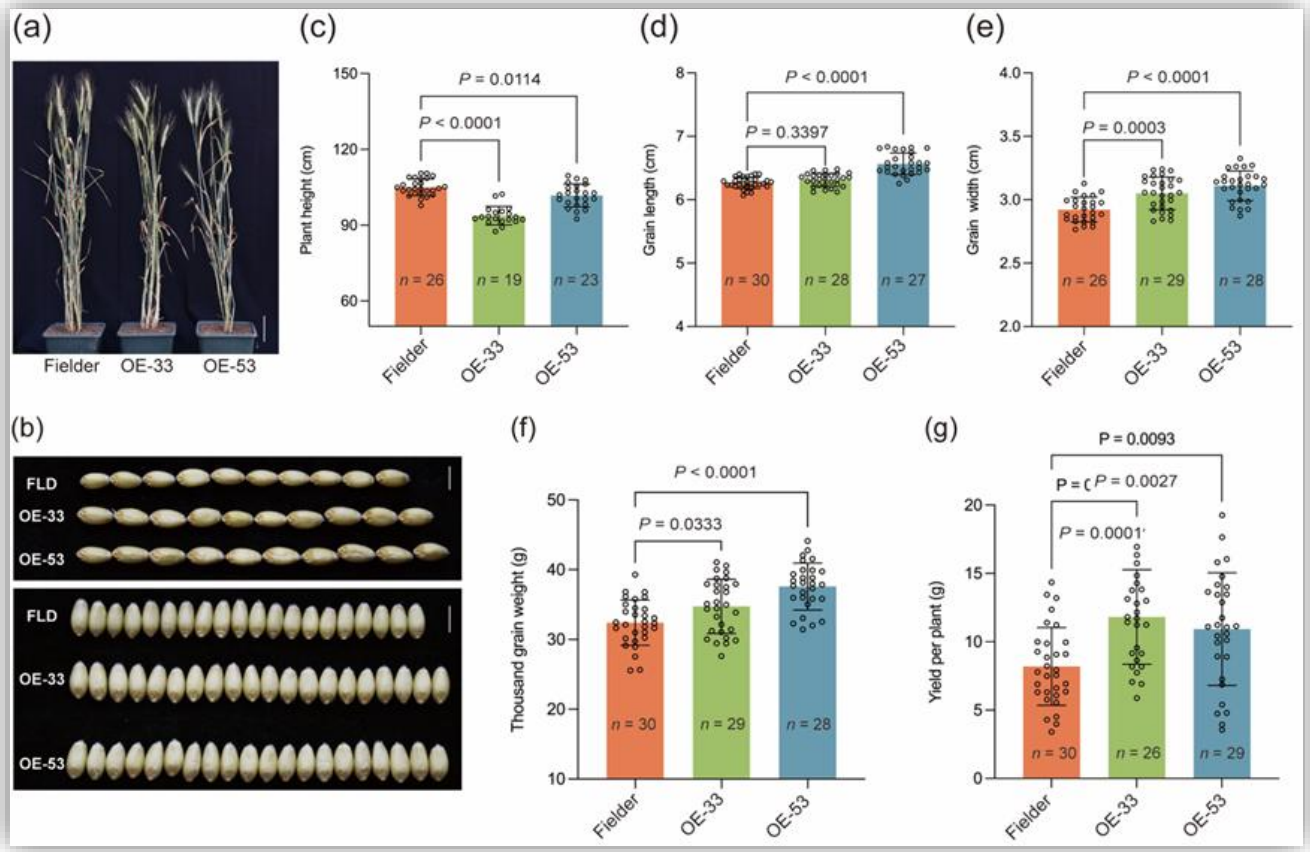
First published: 23 February 2026 | https://doi.org/10.1111/pbi.70618 | VIEW METRICS

- BYDV 17K protein interacts with plant methionine synthase (MS), the last enzyme in the methionine cycle.
- Silencing *MS* gene expression weakened host defense against multiple RNA and DNA viruses, whereas overexpressing *MS* gene expression boosts broad-spectrum antiviral resistance.
- MS1 counteracts the VSR function of 10 distinct RNA and DNA viruses, acting broadly in disrupting the anti-gene silencing activities of VSRs.

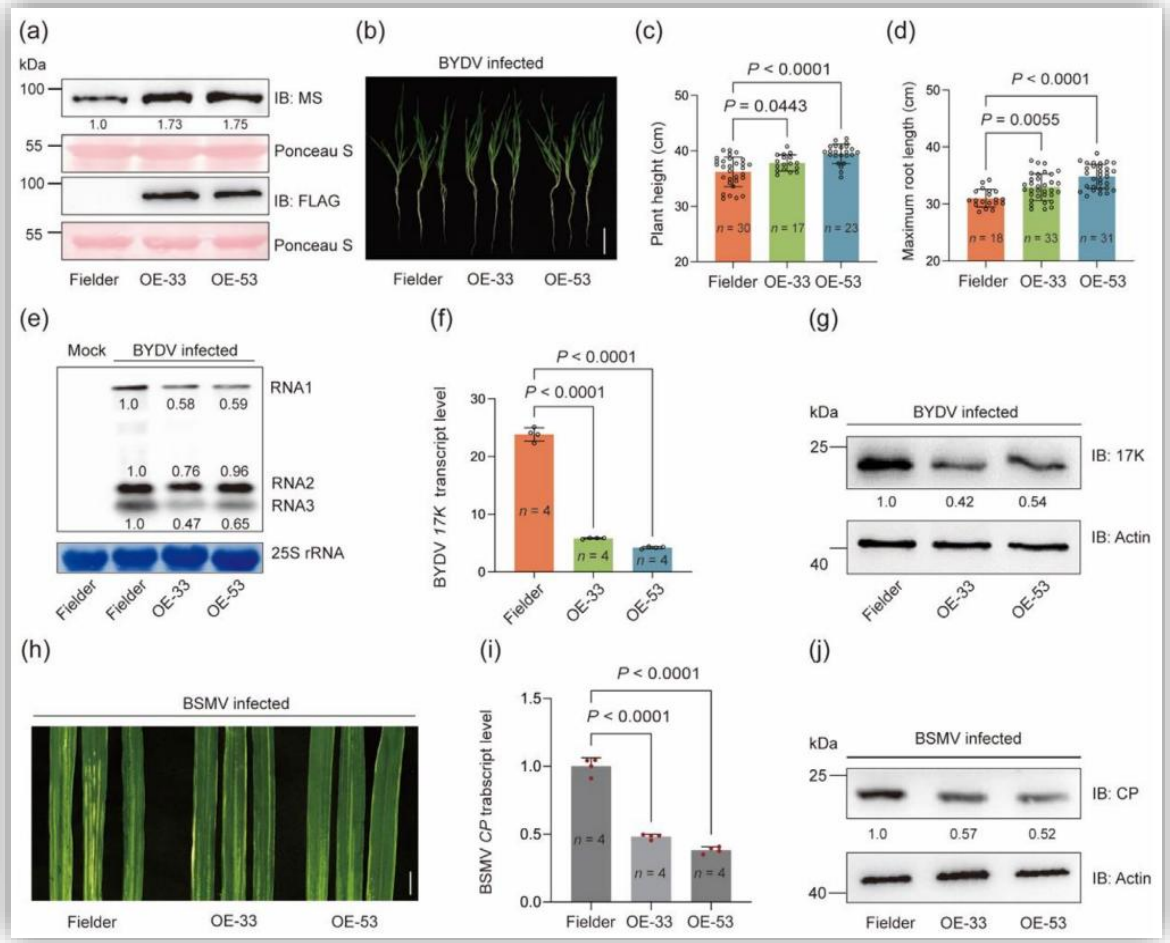


Overexpressing *HvMS1* boosts wheat yield and antiviral resistance

Comparison of WT Fielder and two derivative HvMS1 OE lines grown under normal conditions

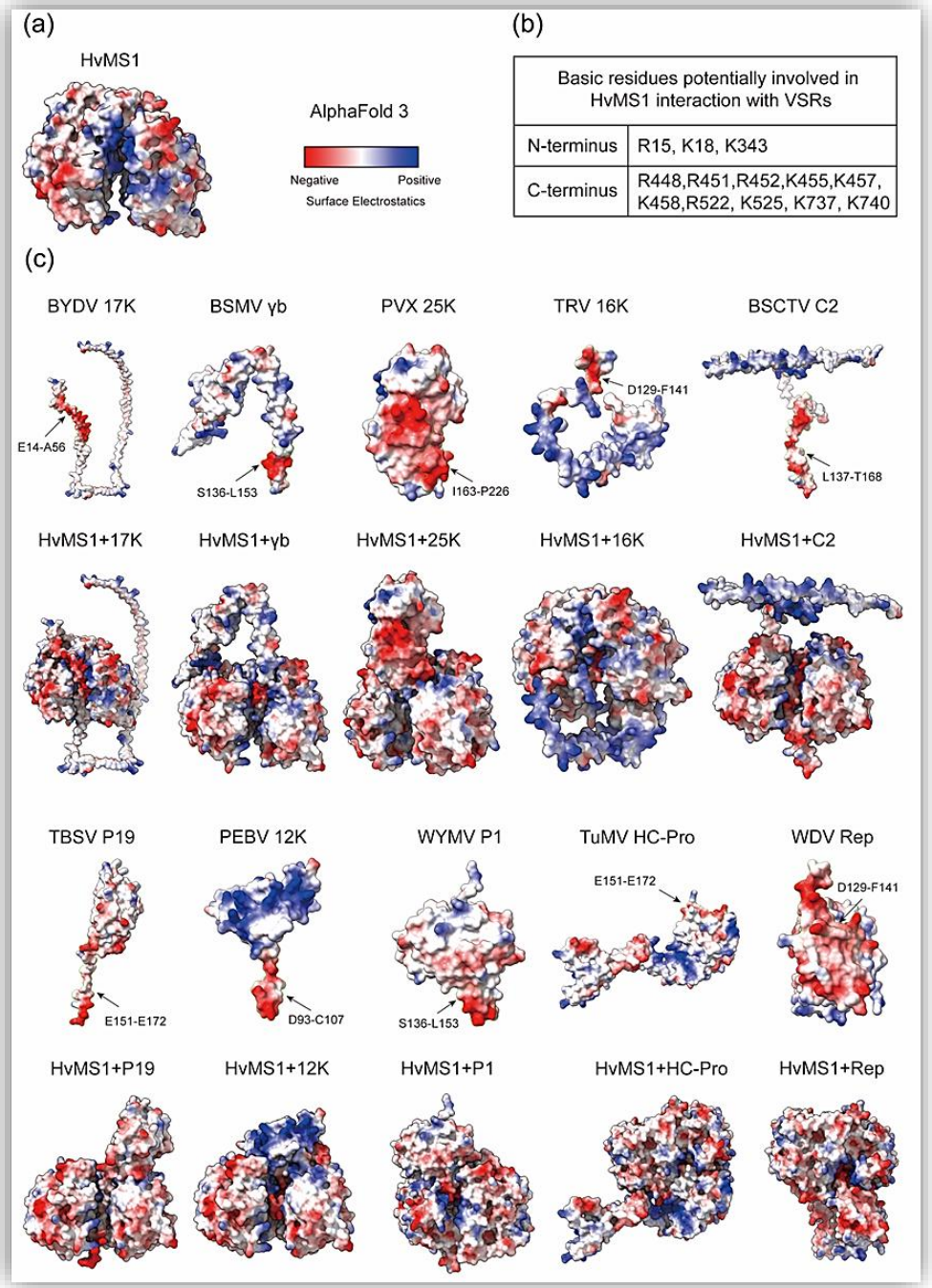


HvMS1 OE lines show enhanced resistance to both BYDV and BSMV



How can MS1 interact with ten divergent viral VSR proteins?

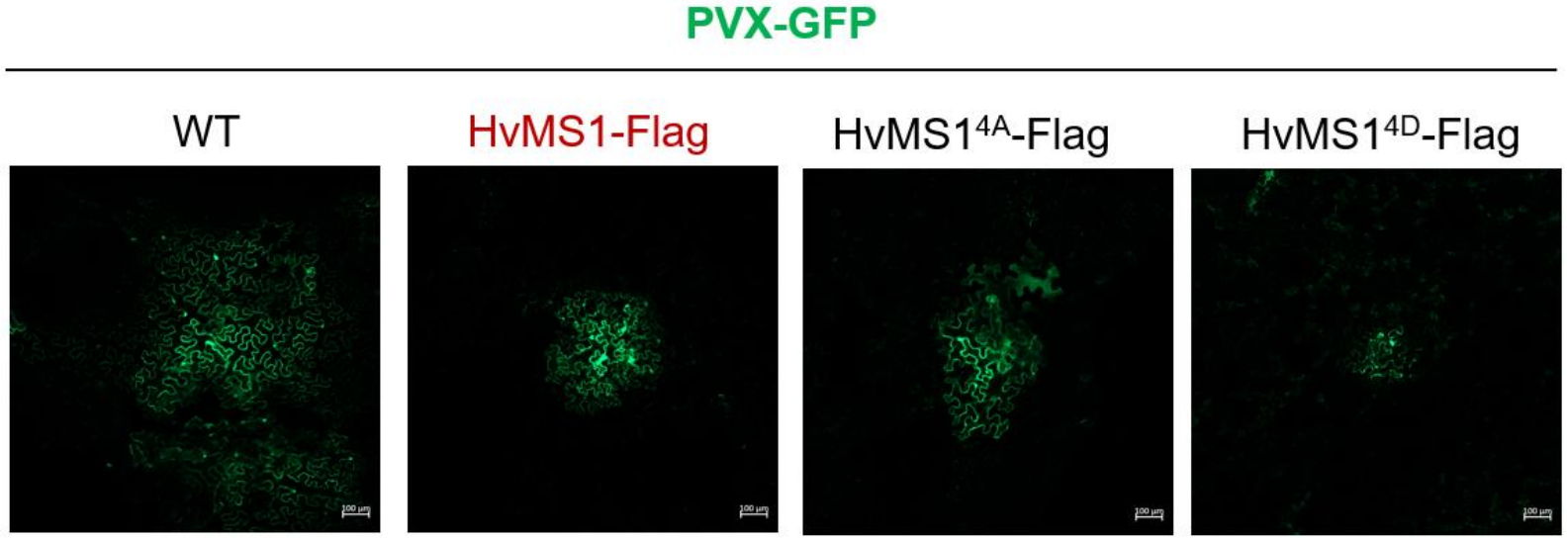
- HvMS1-VSR interaction is possibly facilitated by electrostatic complementarity at protein–protein interface.
- A positive electrostatic patch, which involves many basic amino acid residues located in the N- or C-terminal domains of HvMS1.
- The predicted structures of the10 VSRs all possess the surface with prominent negative elec-trostatic potential, which could interact with the positive elec-trostatic patch of HvMS1 in modelled HvMS1-VSR complex.

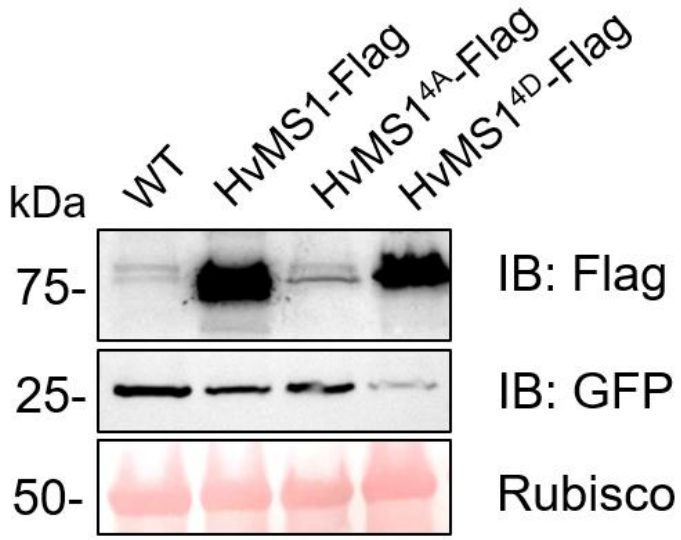


How to develop useful BYDV resistant germplasm materials using the identified host genes?

- A combination of AI driven protein engineering and precision genome editing holds the key to the question.
- AI mediated protein evolution can be used to obtain functionally further enhanced protein variants.
- Precision genome editing tools can be employed to elevate target gene (with or without AI-mediated functional enhancement) expression at desired scales.

Phosphorylation of MS enhances its antiviral potency



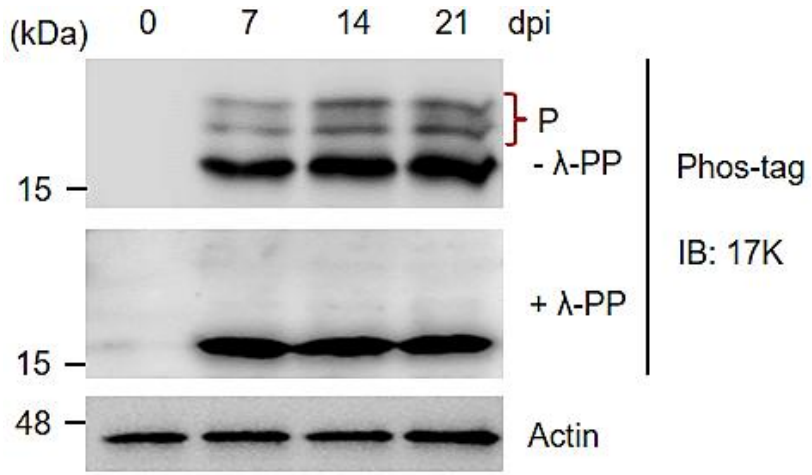


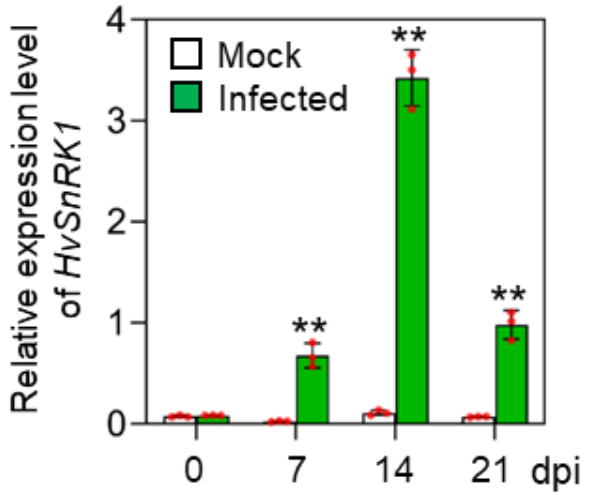
Acknowledgements

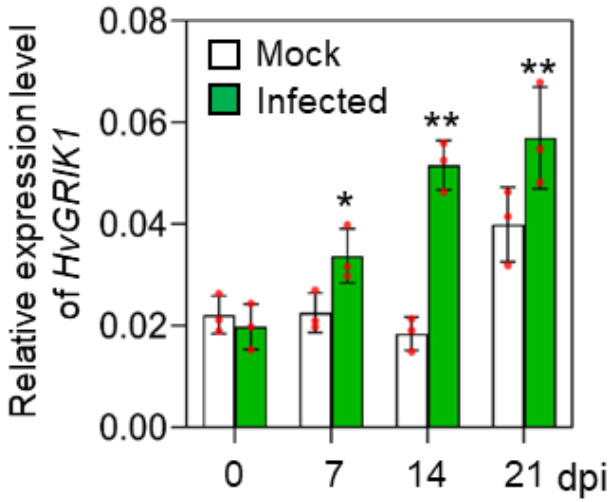
- We thank many international and national colleagues for helps and collaborations.
- We are grateful to Ministry of Science and Technology of China, National Natural Science Foundation of China, and Henan Provincial Government for financial support.

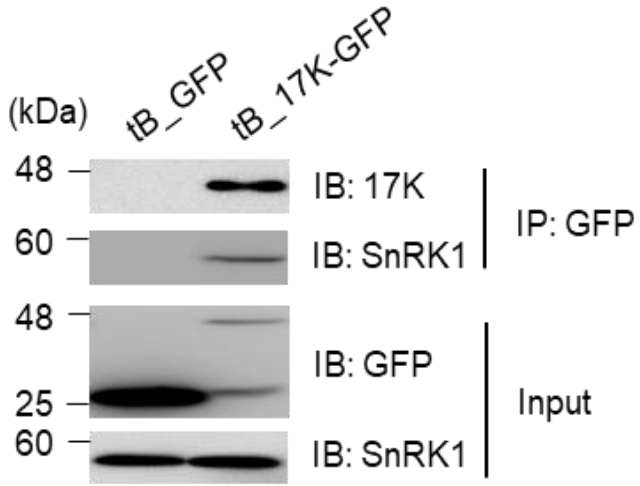
Thank you !

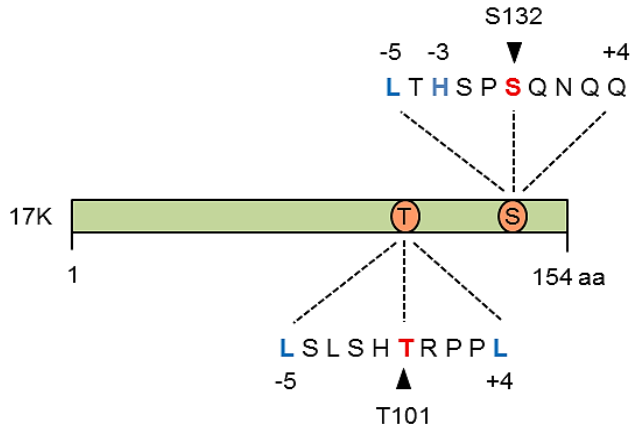
SnRK1/GRIK1 cascade phosphorylates BYDV-GAV 17K *in vivo* and *in vitro*

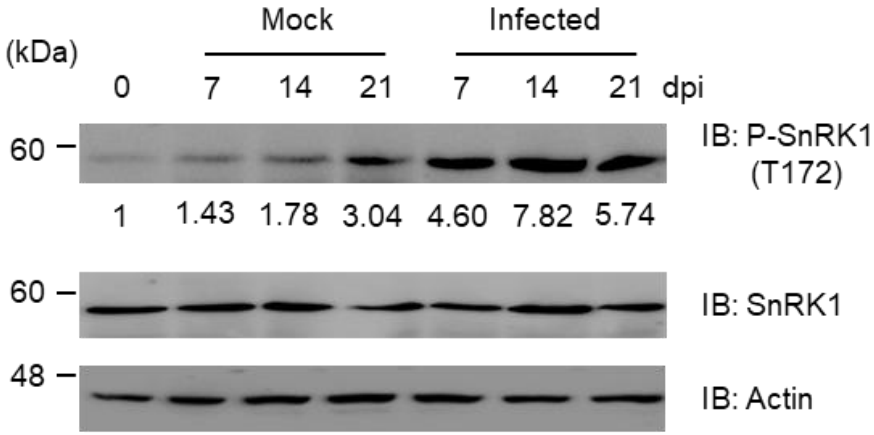






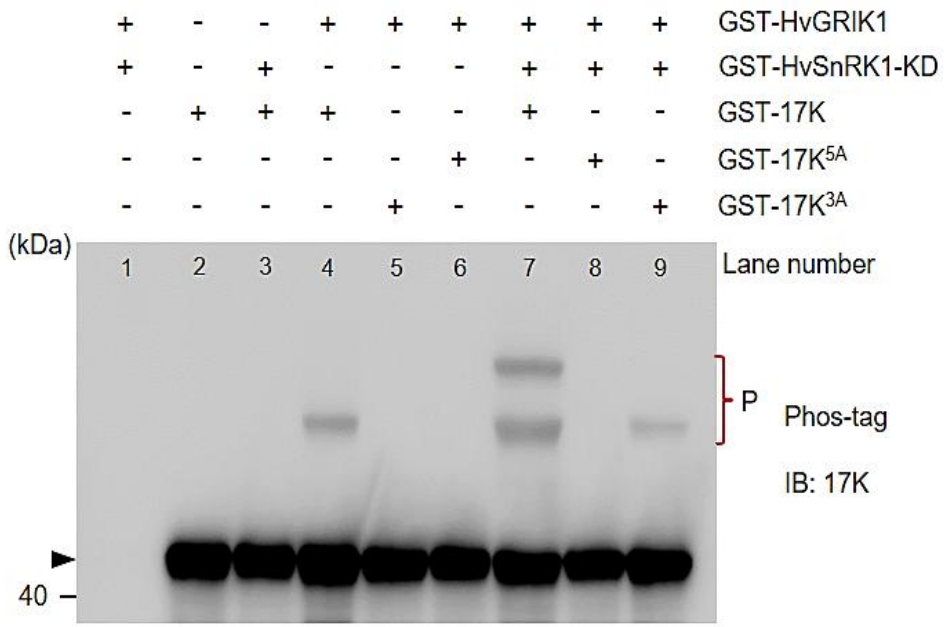


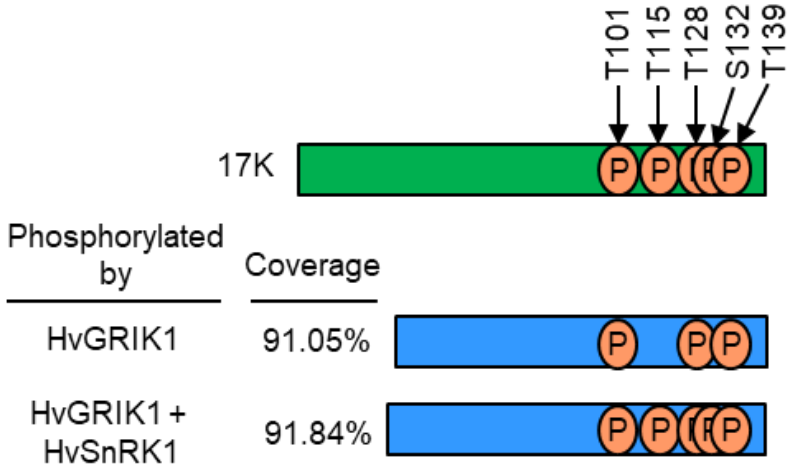


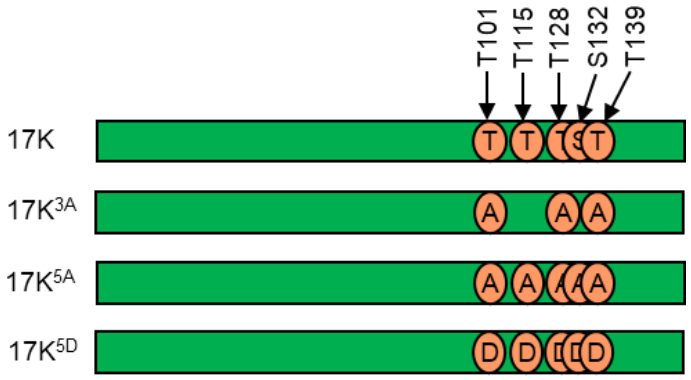


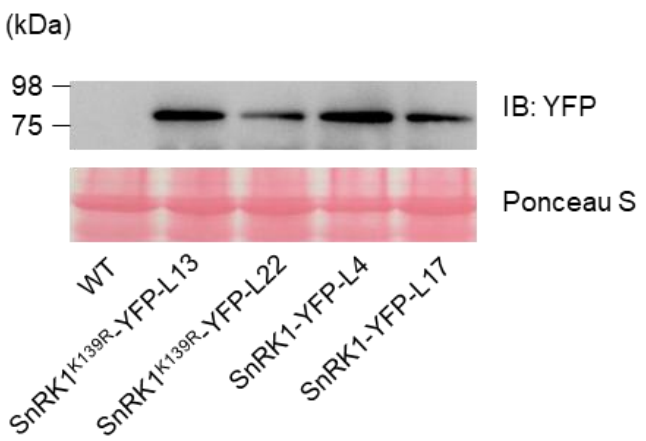
(Jin et al., 2022)

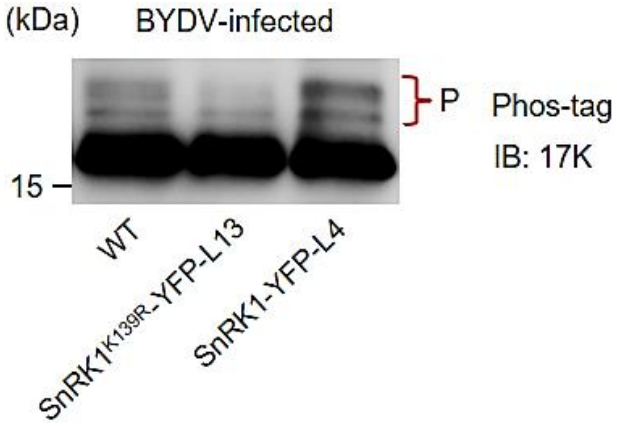
SnRK1/GRIK1 cascade phosphorylates BYDV-GAV 17K *in vivo* and *in vitro*









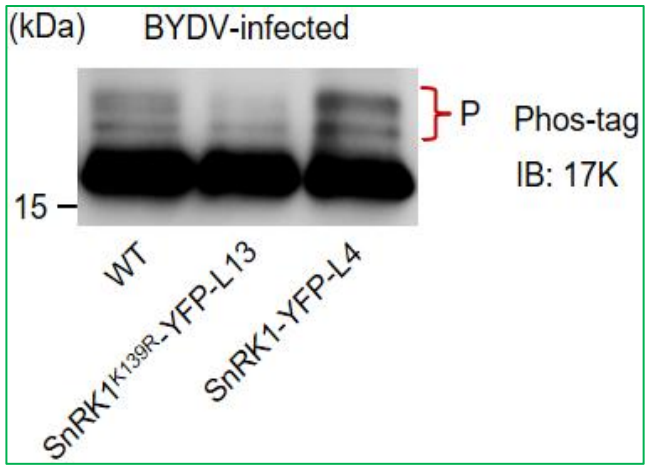


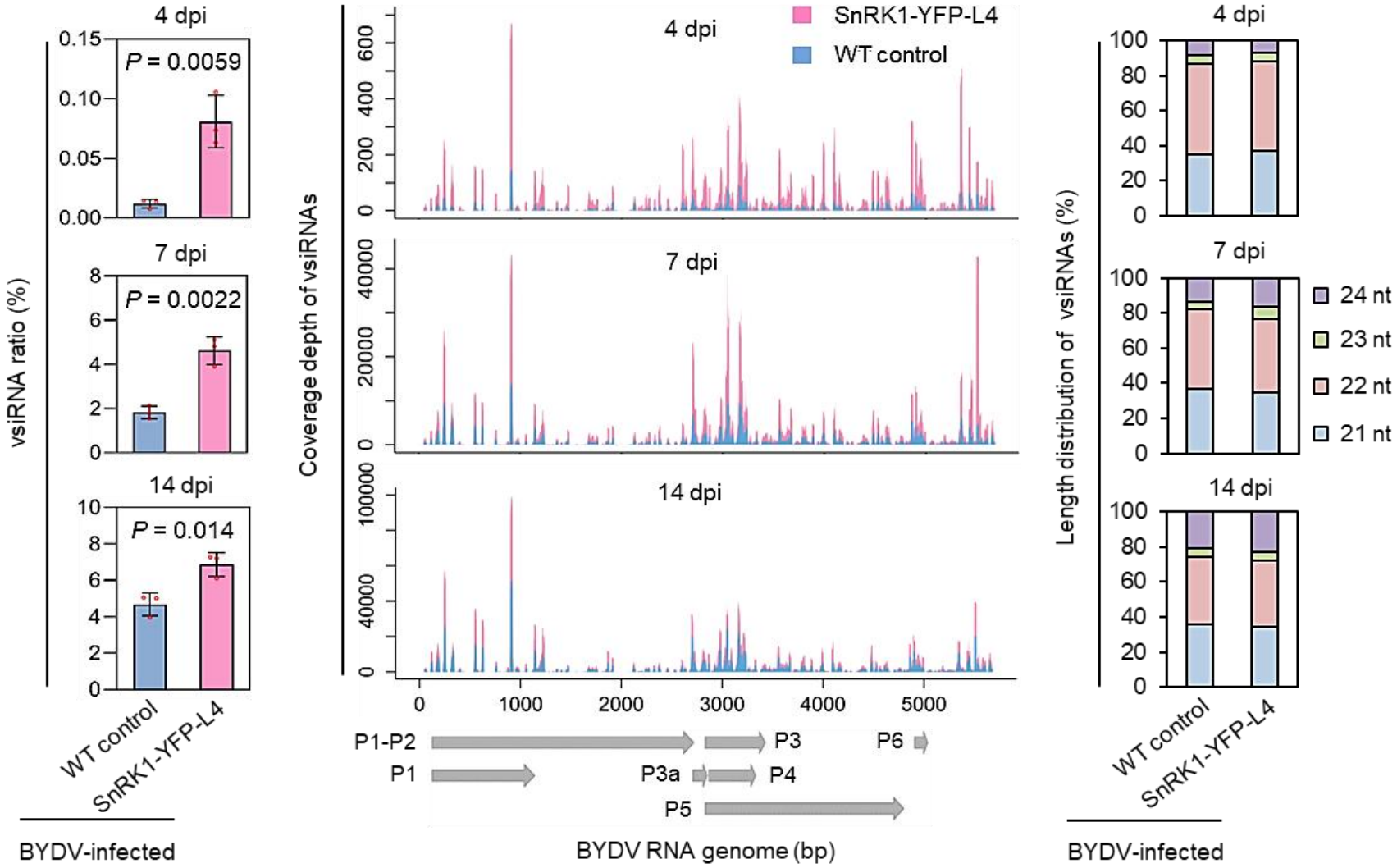
(Jin et al., 2022)

Overexpression of SnRK1 increases P17K and vsiRNA level in BYDV infection

SnRK1: WT SnRK1 of barley

SnRK1^{K139R}: dominant negative mutant



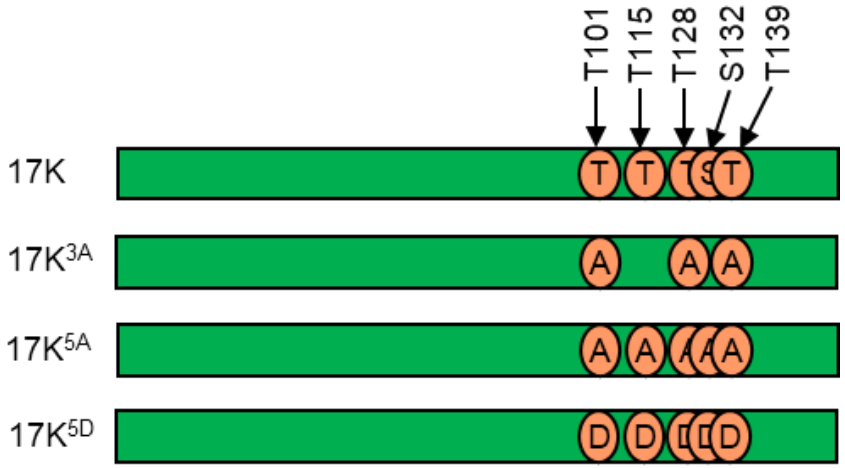


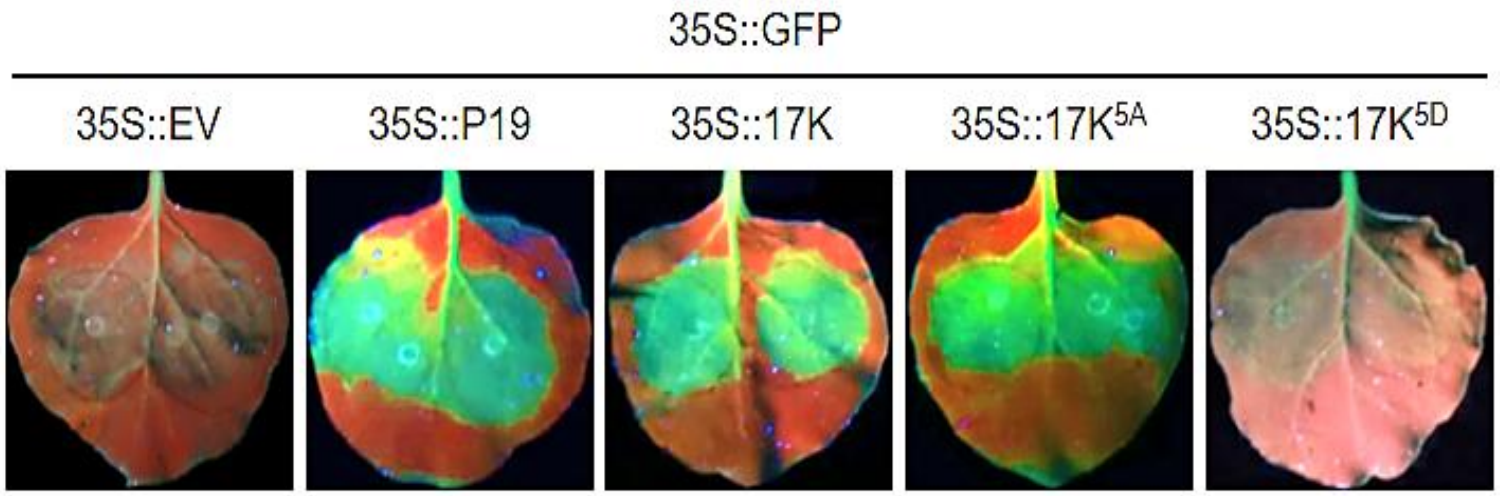
P17K (mimicked by 17K^{5D}) loses VSR activity but

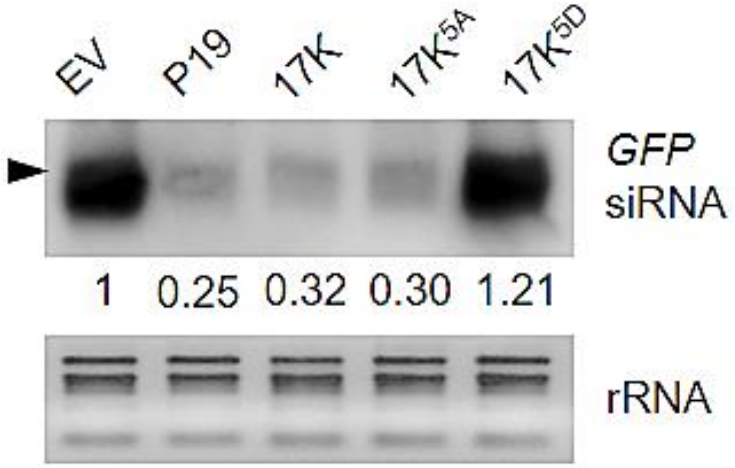
- **Does P17K has VSR activity?**

17K^{5D}: P17K mimic

17K^{5A}: dephosphorylation mimic

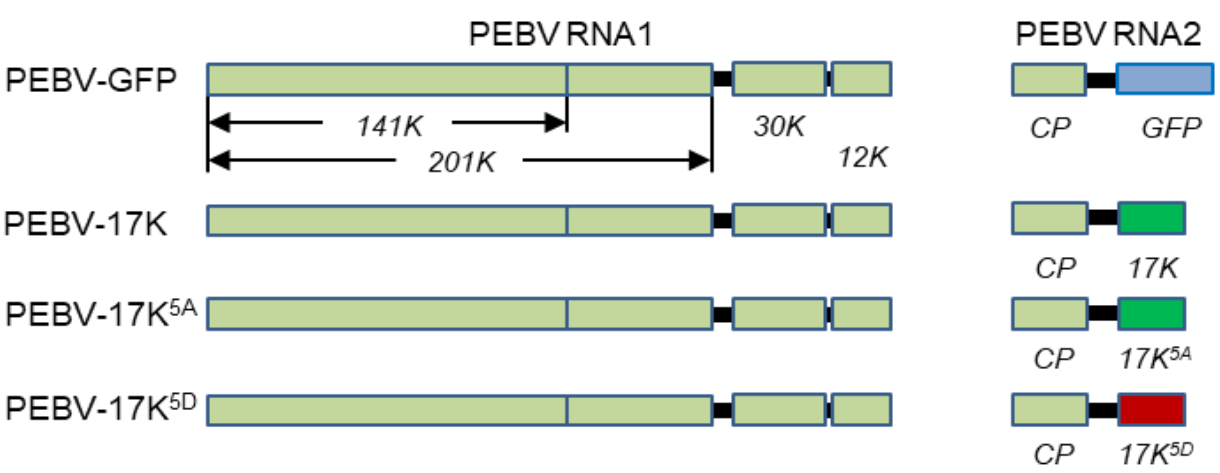


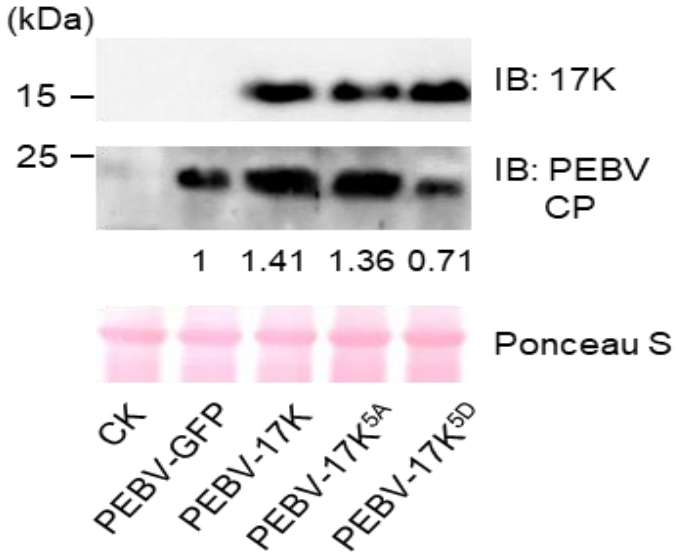


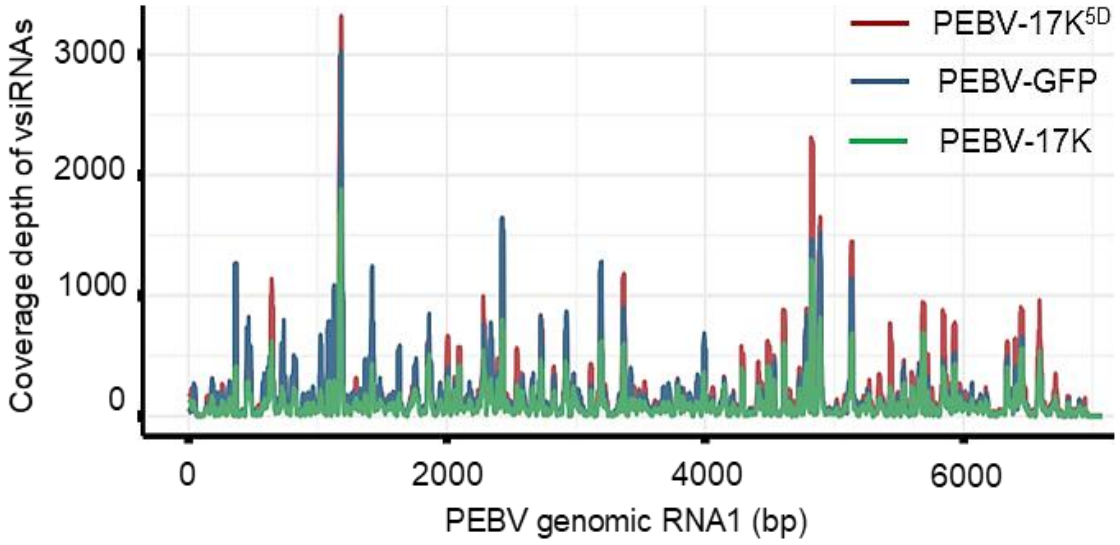


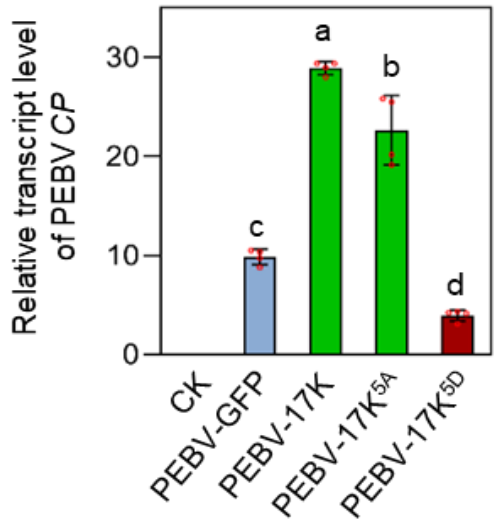
(Jin et al., 2022)

17K^{5D} (mimicking P17K) elevates vsiRNA level and enhances antiviral defence



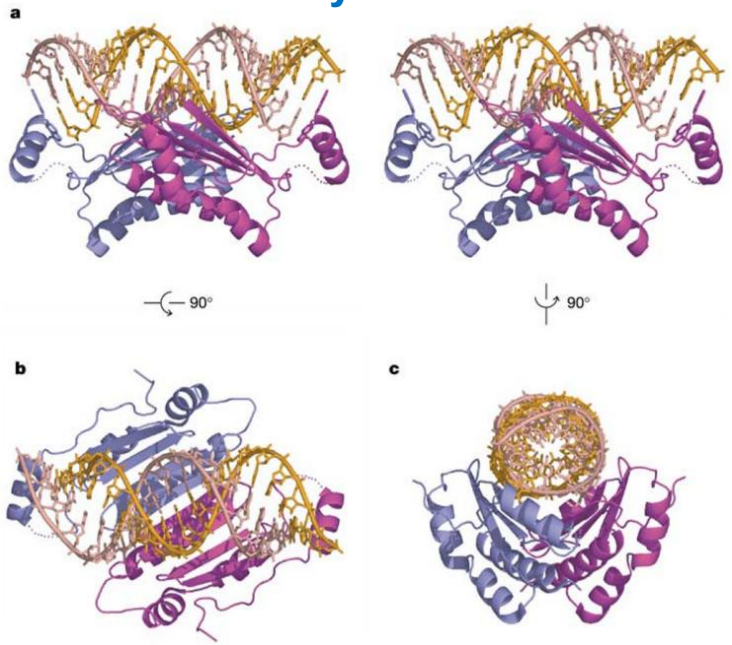




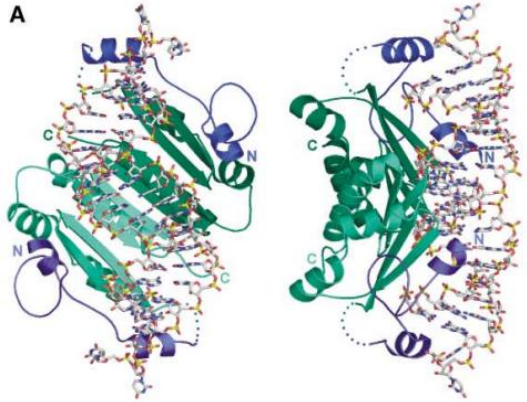


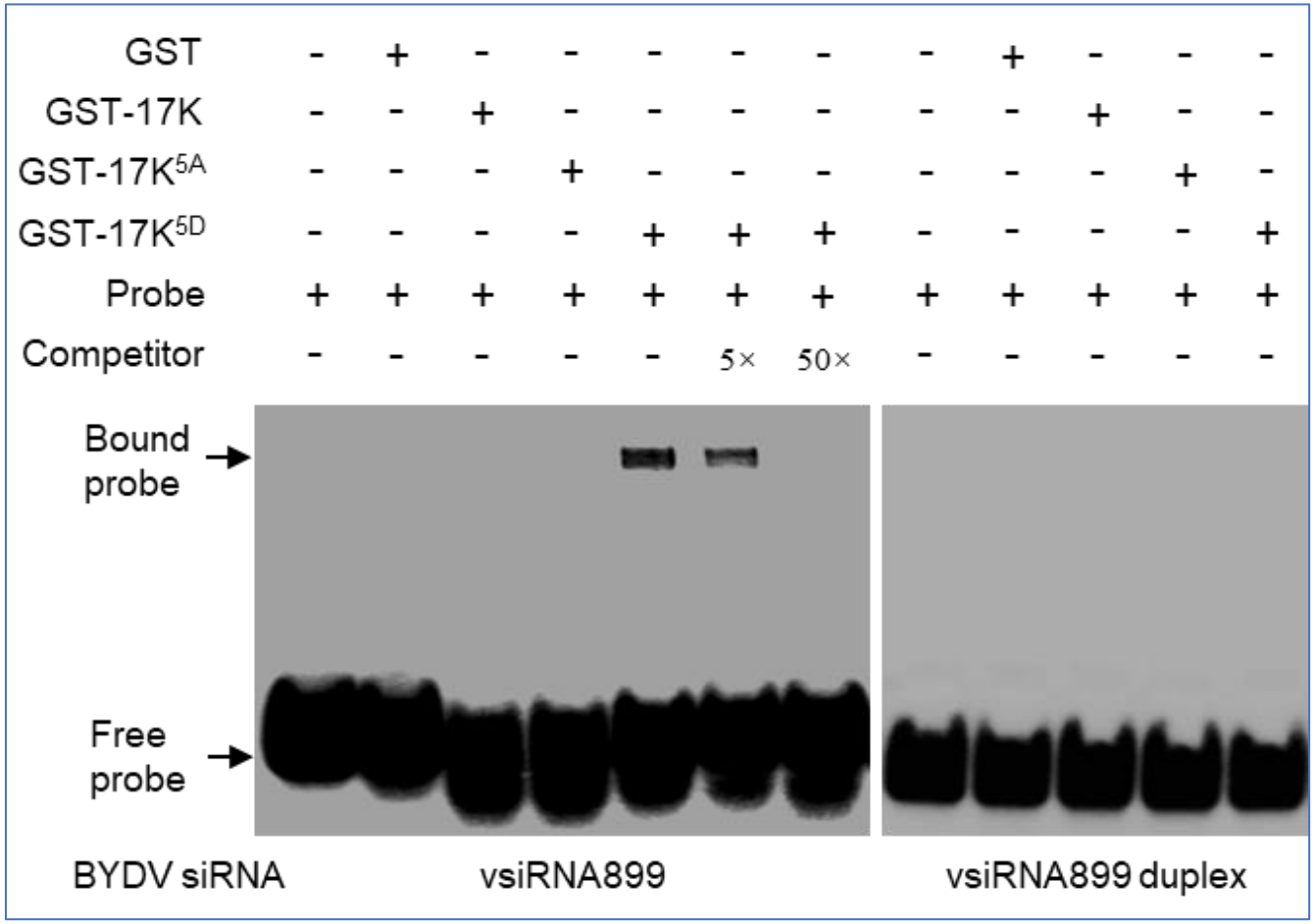
P17K (mimicked by 17K^{5D}), but not 17K, binds vsiRNA

Tomato bushy stunt virus P19



Carnation Italian ringspot virus P19





(Vargason et al., 2003; Ye et al., 2003; Jin et al., 2022)

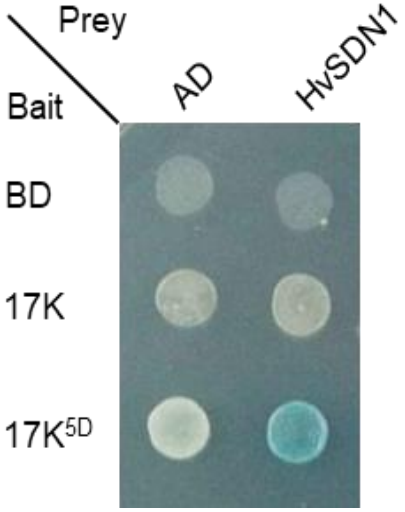
P17K (mimicked by 17K^{5D}) binds to HvSDN1 and inhibits its cleavage of vsiRNA

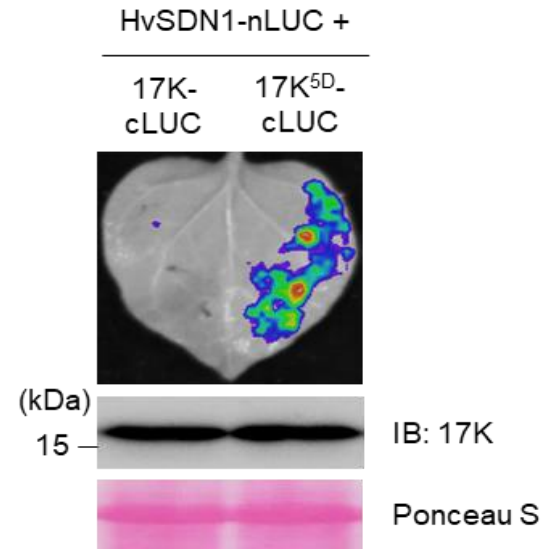
Barley RISC components studied

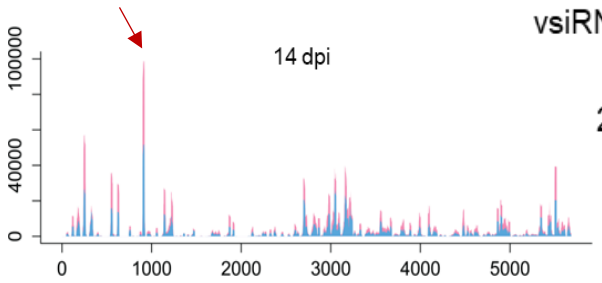
HvDCLs
(HvDCL3a, HvDCL3b, HvDCL4)

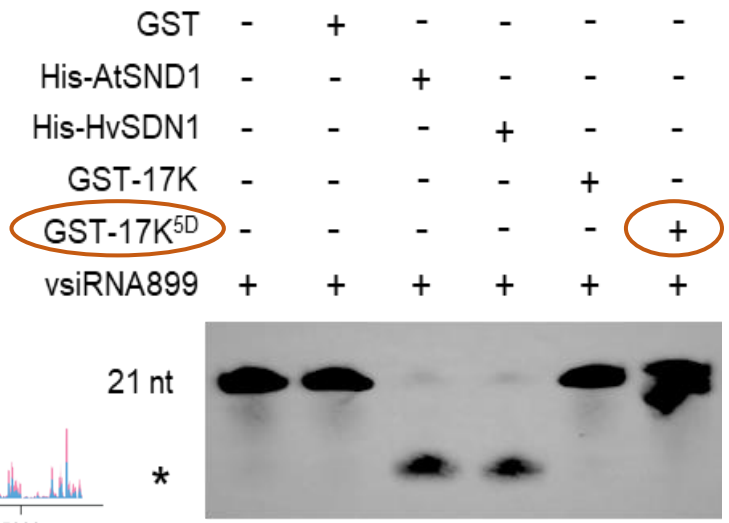
HvAGOs
(HvAGO1, HvAGO2, HvAGO4)

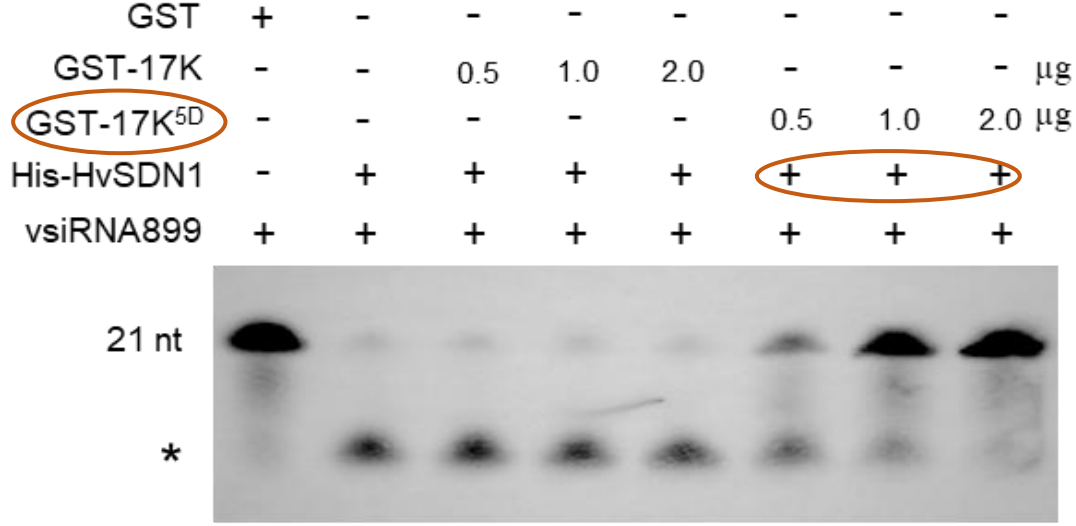
HvSDN1



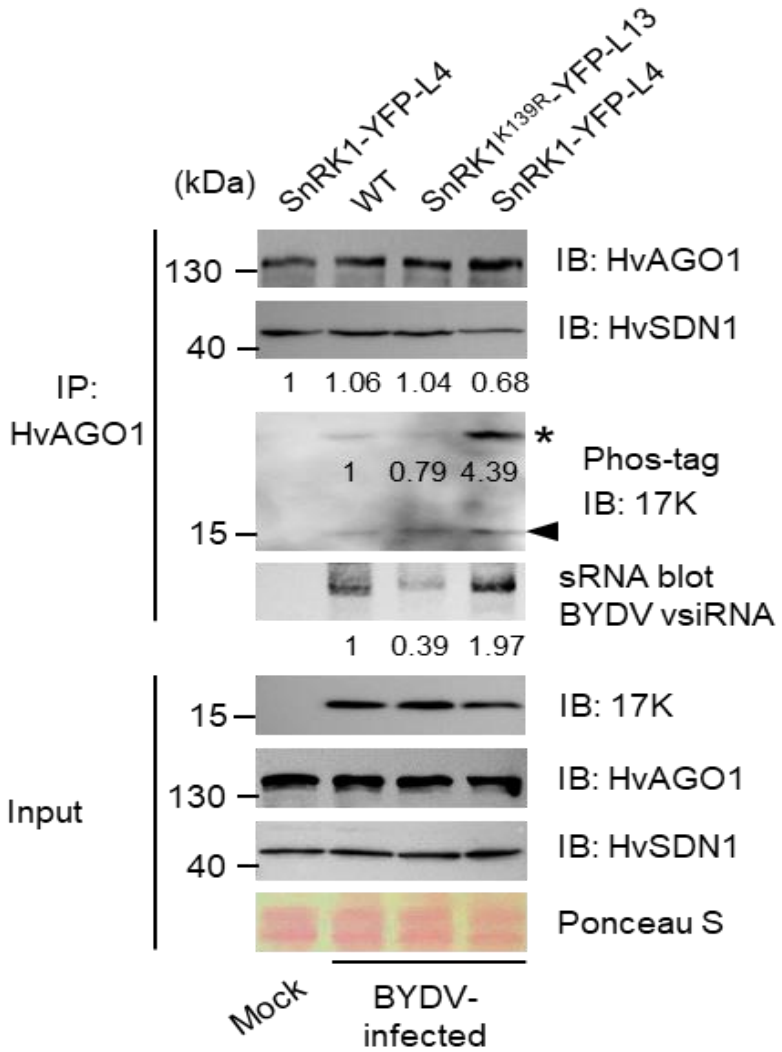


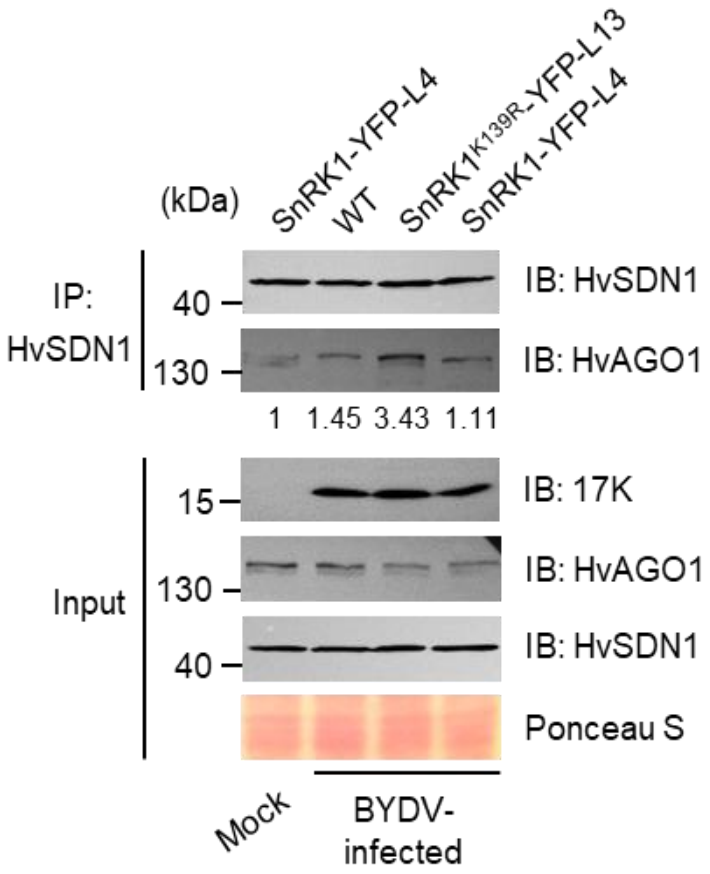


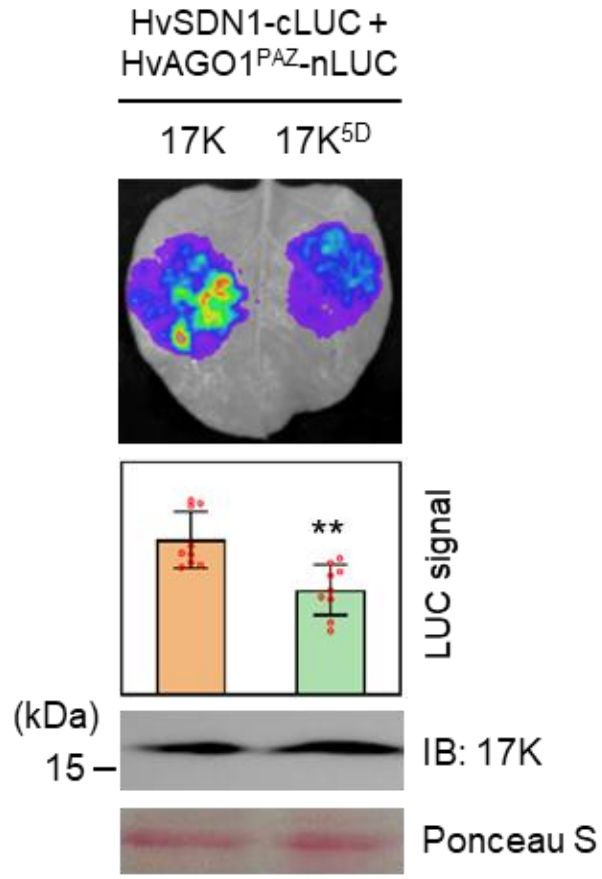




Increase of P17K enhances BYDV vsiRNA level by 1) inhibiting SDN1 activity and 2) weakening AGO1 and SDN1 interaction

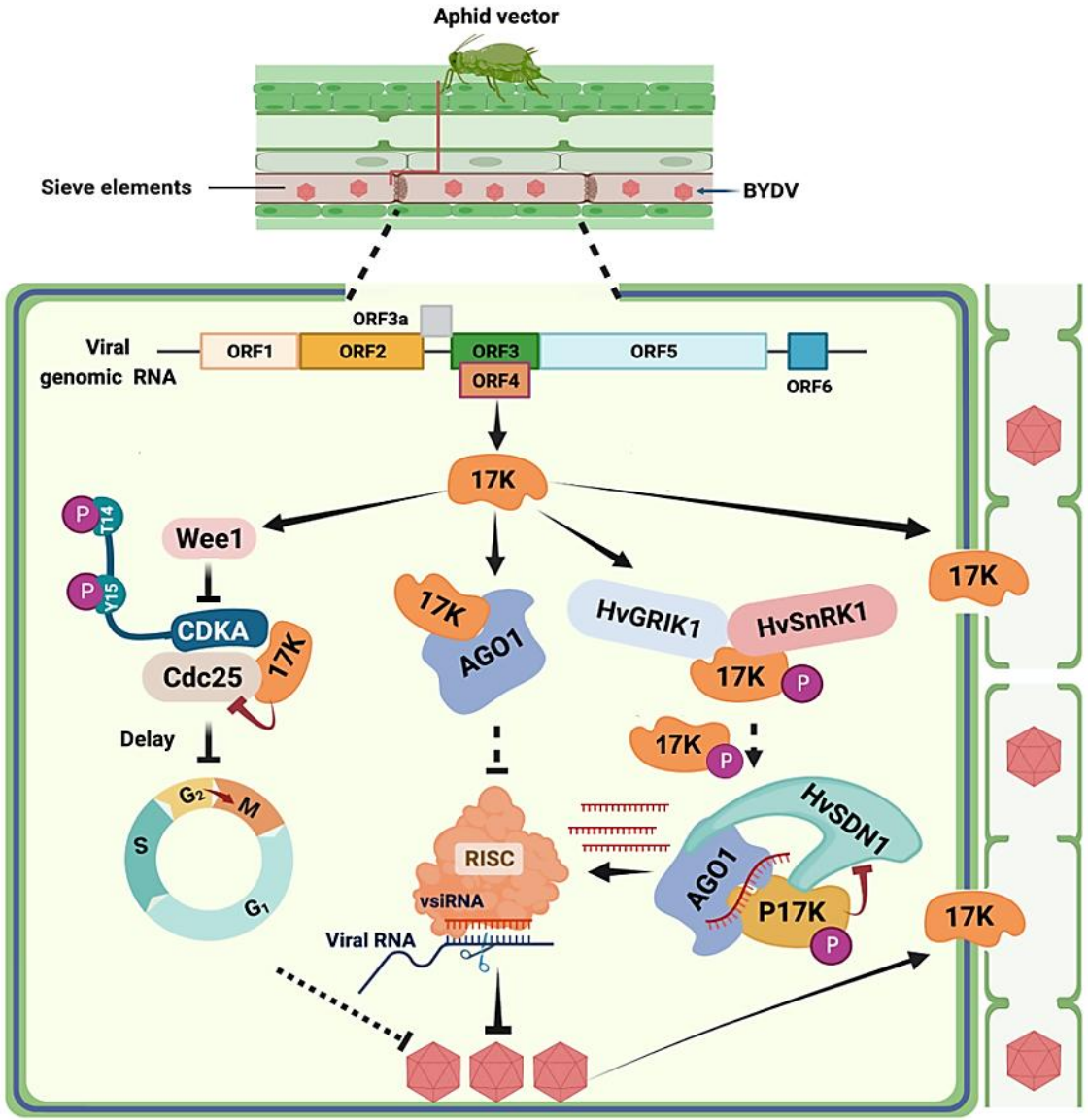






(Jin et al., 2022)

BYDV-GAV 17K protein: inhibitor of host mitosis, regulator of vsiRNA-based host defence



Molecular Plant

CellPress

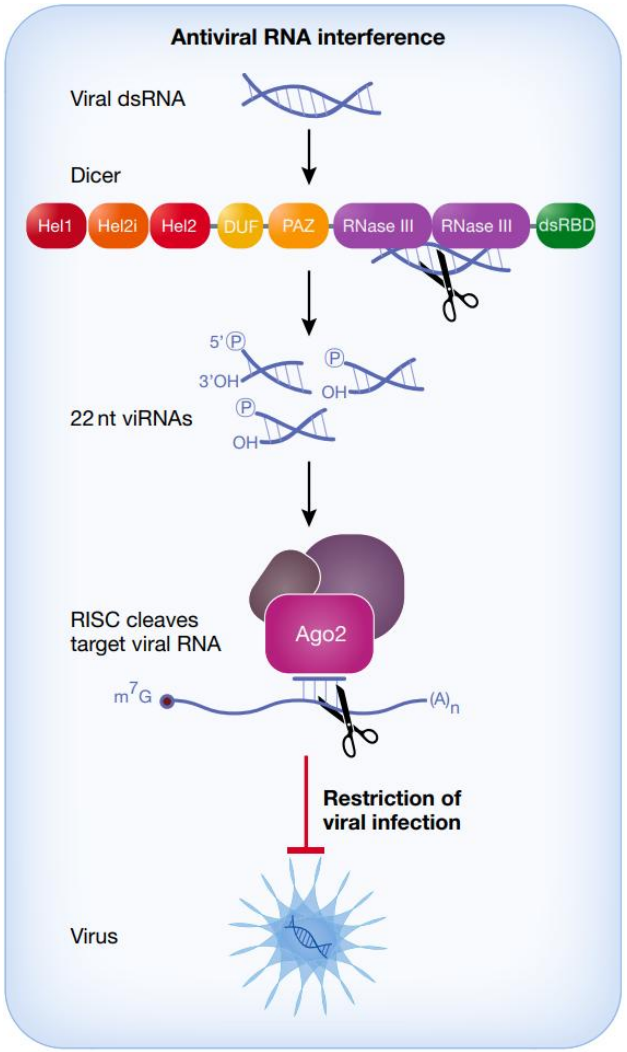
Volume 15, Issue 10, 3 October 2022, Pages 1514-1516

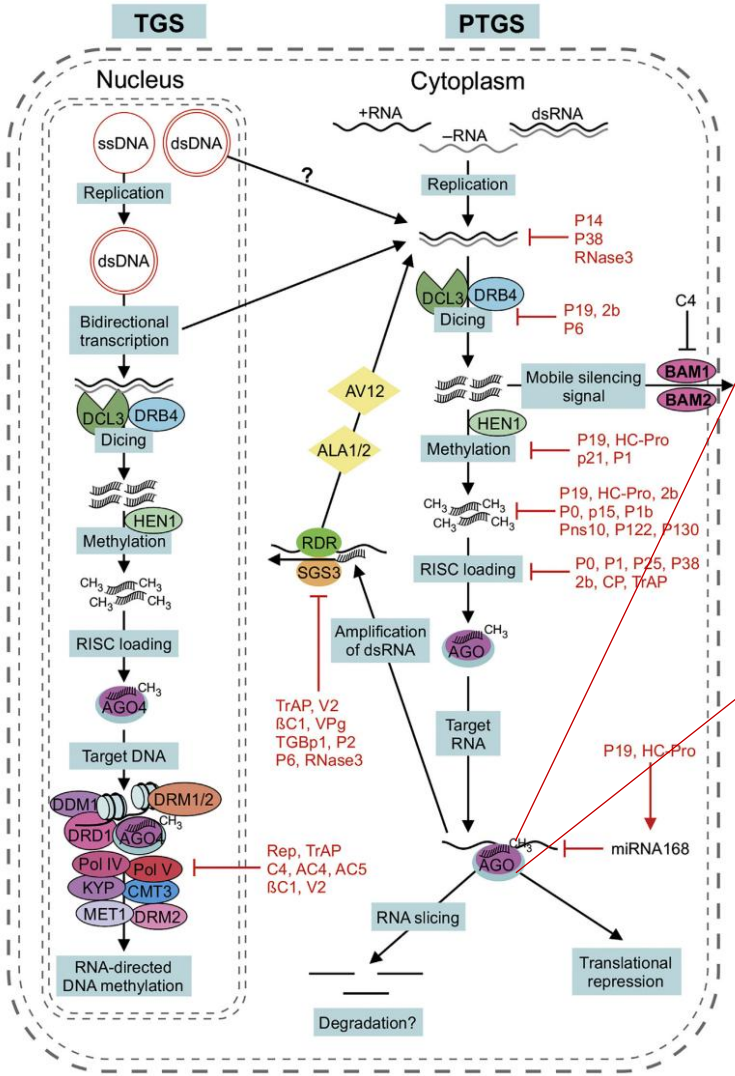
Spotlight

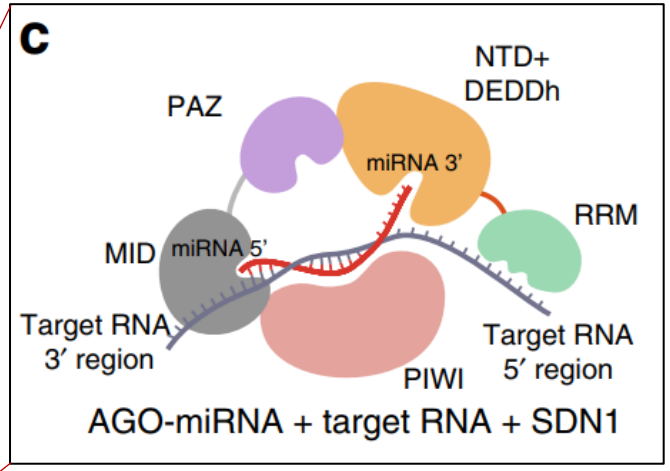
Achieving a more robust antiviral RNAi via subverting a viral virulence protein

Liyuan You [1], Ruize Zhang [2], Zheng Qing Fu [2]

RNAi抵御病毒的感染和VSR蛋白功能







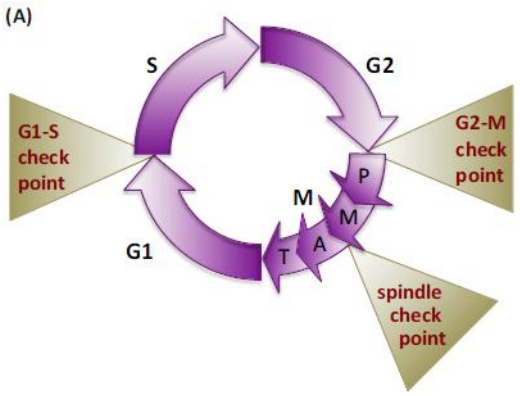
HvDCLs
(HvDCL3a, HvDCL3b, HvDCL4)

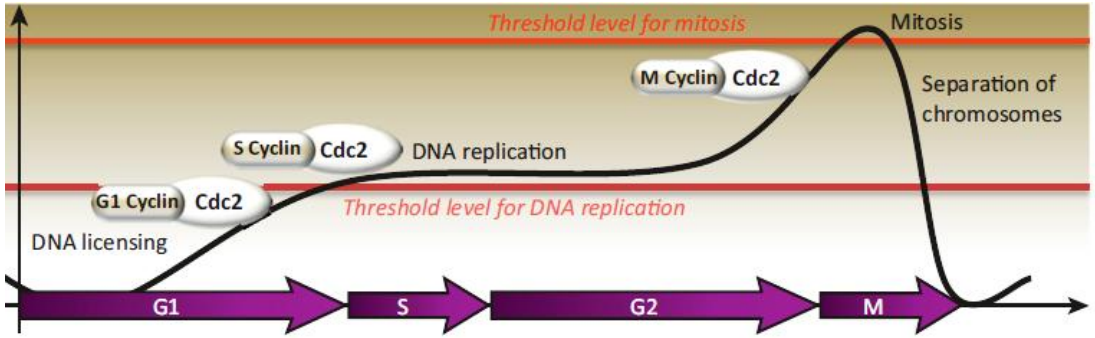
HvAGOs
(HvAGO1, HvAGO2, HvAGO4)

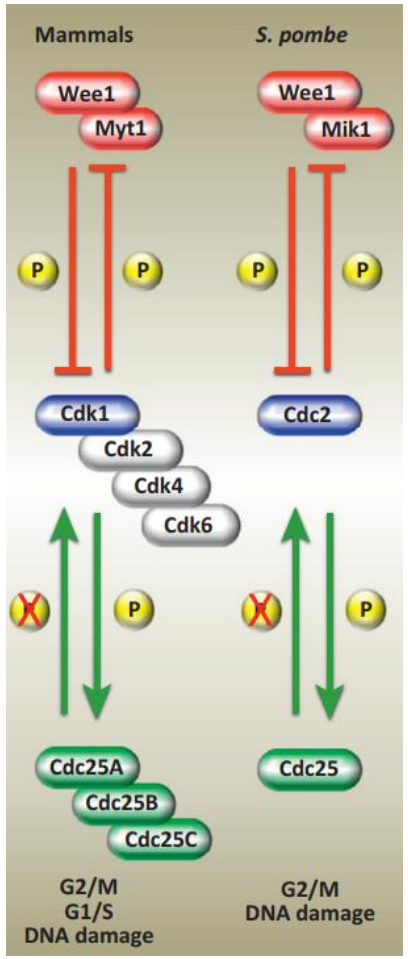
HvSDN1

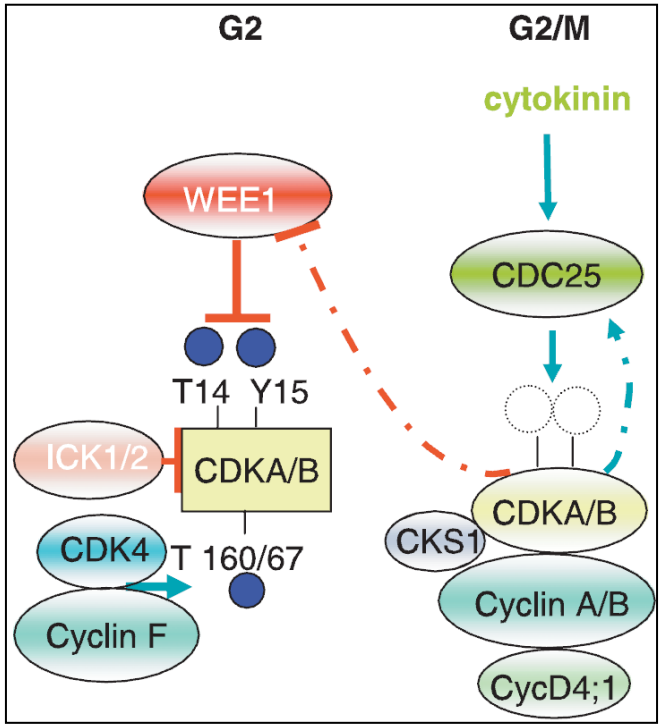
(Fire et al., 1998; Ramachandran and Chen, 2008; Yu et al, 2017; Chen et al, 2018; Rosa et al., 2018; Guo et al., 2019; Li and Wang, 2019; Maillard et al., 2019; Hung and Slotkin, 2021; Jin et al., 2021)

有丝分裂细胞周期调控





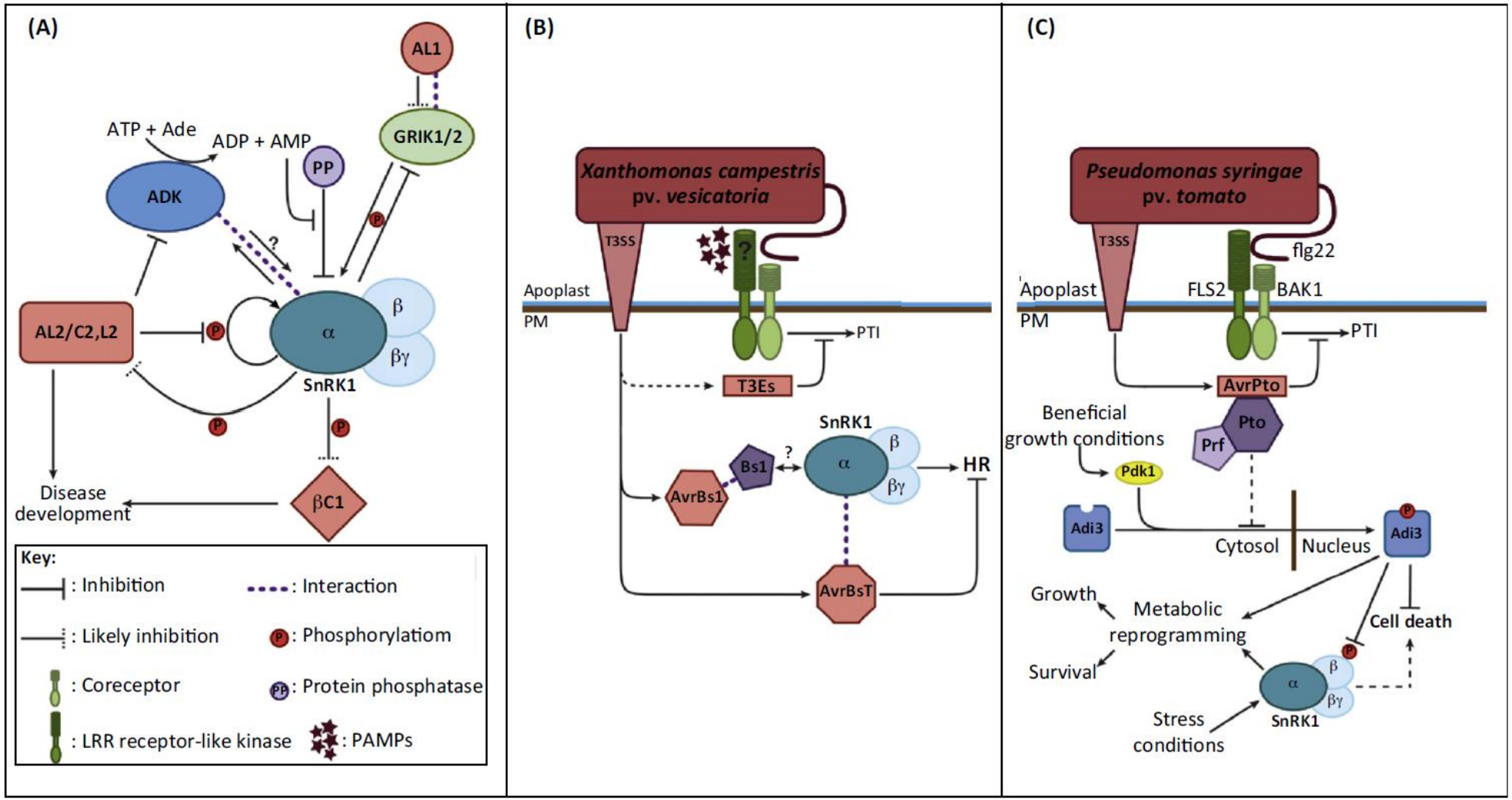




Wee1-Cdc25-CDKA/Cdc2分子开关调控细胞周期G2/M转换

(Landrieu et al., 2004; Francis, 2007; Dissmeyer et al., 2009; Nowack et al, 2012; Harashima et al., 2013; Gutierrez-Escribano and Nurse, 2015; Hégarat et al., 2016; Hutter et al., 2017; Atkins and Cross, 2018)

SnRK1在病原微生物侵染宿主过程中的调控功能



(Emanuelle et al., 2016; Hulsmans et al., 2016; Shen W and Hanley-Bowdoin L, 2020)