Virus-based delivery of interfering RNAs targeting grapevine leafroll-associated virus(es)

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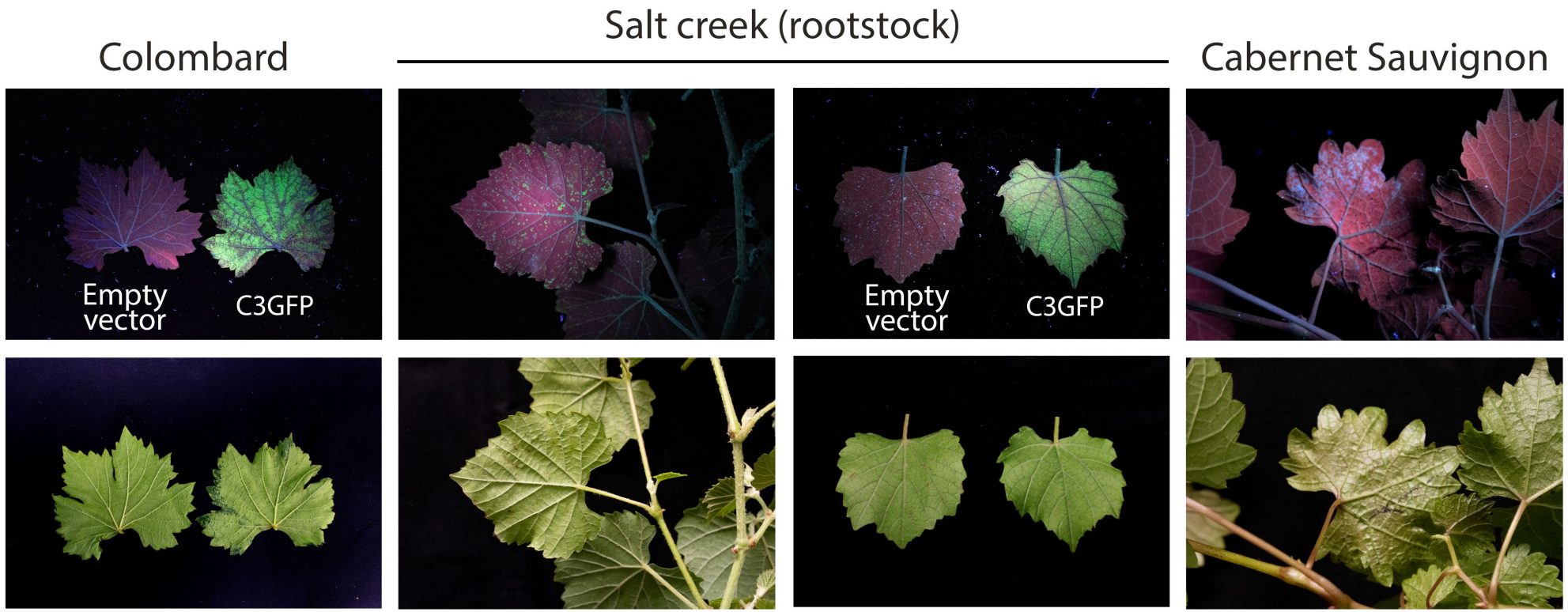
Scope of work

- Apply RNA interference (RNAi) strategy (small interfering RNA and artificial microRNA) to target grapevine leafroll-associated viruses (GLRaVs).
- Develop 2 non-pathogenic grapevine infecting viruses to deliver and induce RNAi effects targeting GLRaVs.
- Advance the virus-mediated disease control strategy in grapevine towards field applications in the future.

Greenhouse-grown grapevine plants were used for all the assays

Infiltration conditions were optimized using a GFP expressing plasmid

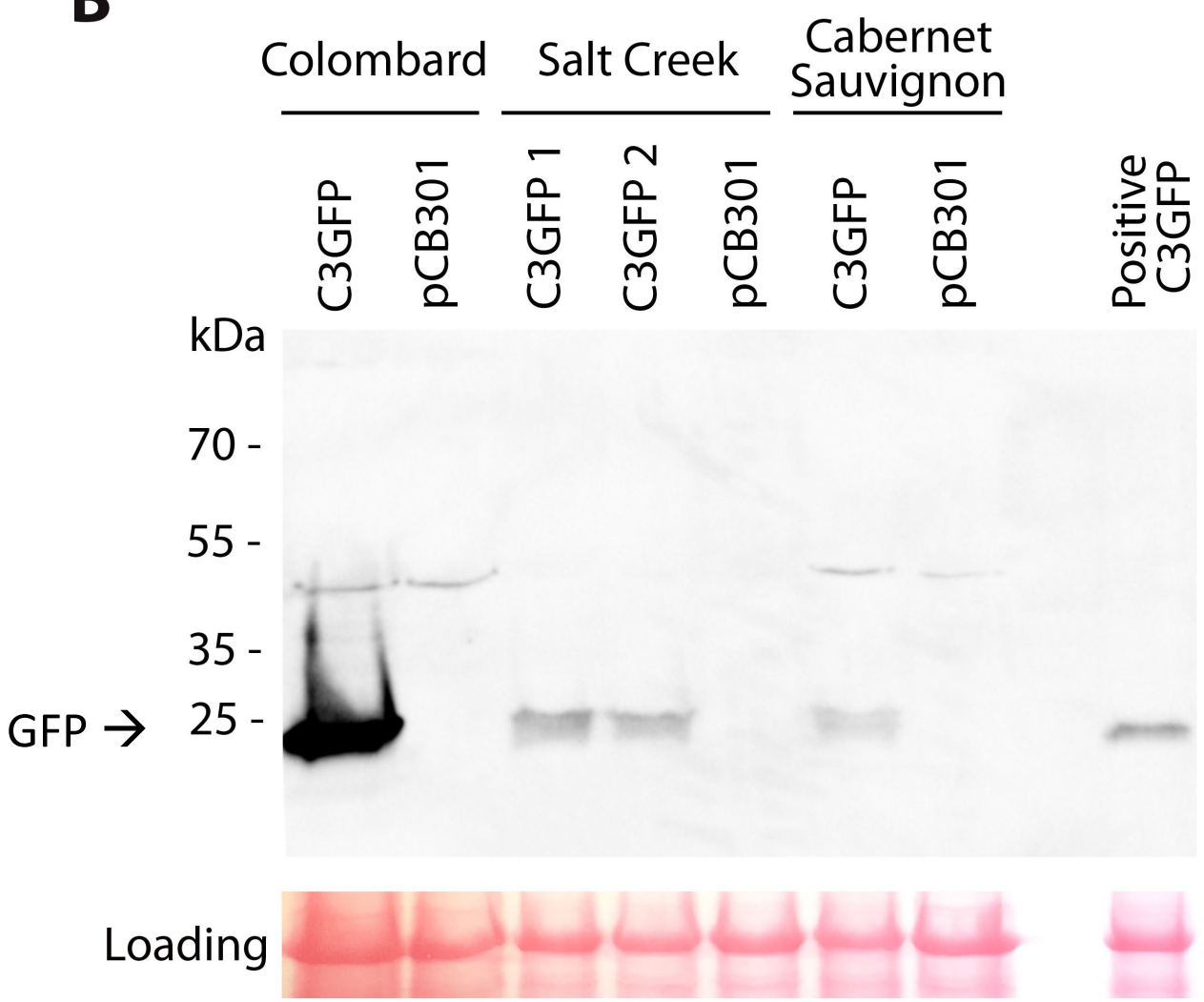
A



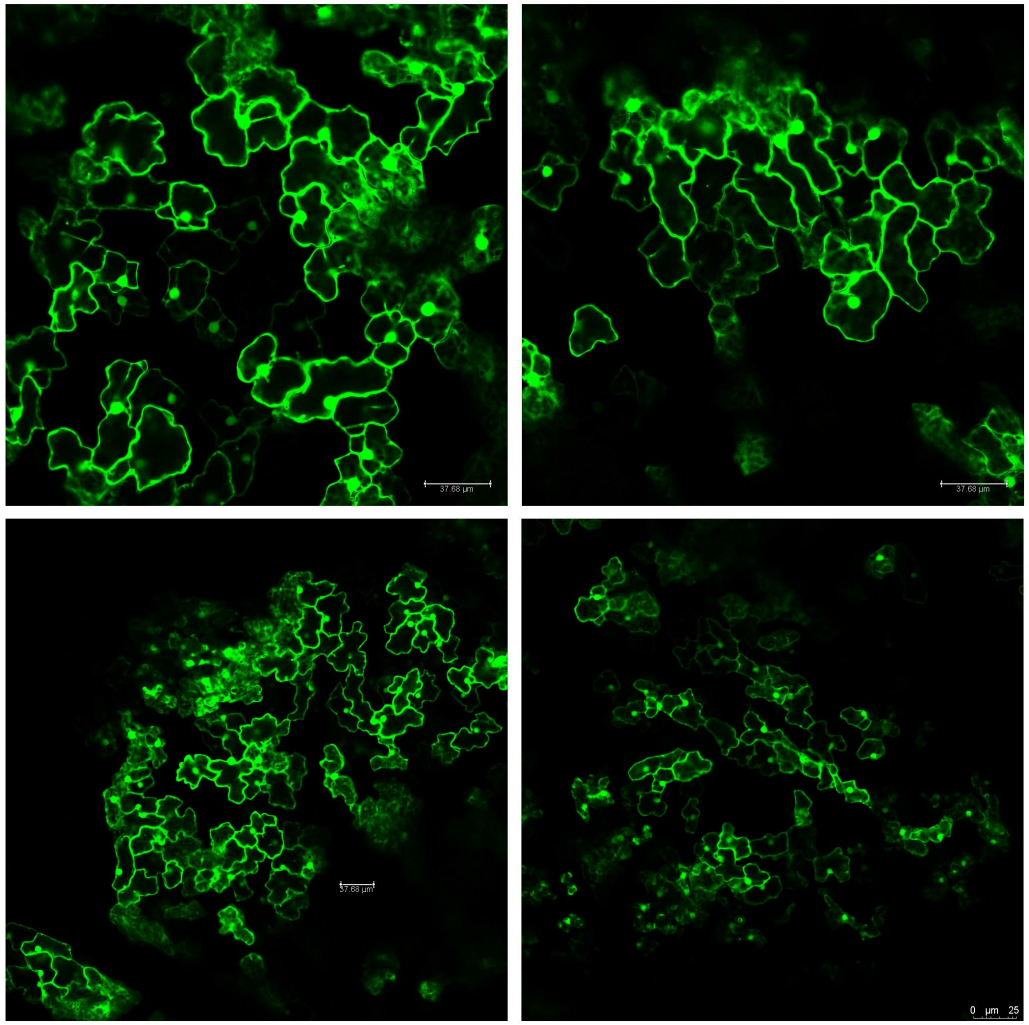
Sonication: (80 kHz)	3 sec	3 sec	3 sec	3 sec
Vacuum:	4 min	2 min	6 min	5 min

Infiltration conditions were optimized using a GFP expressing plasmid

B



C



A. Virus-induced small interfering RNA (siRNA) approach

GVA

Grapevine virus A (GVA) genome structure (~7.3 kb)

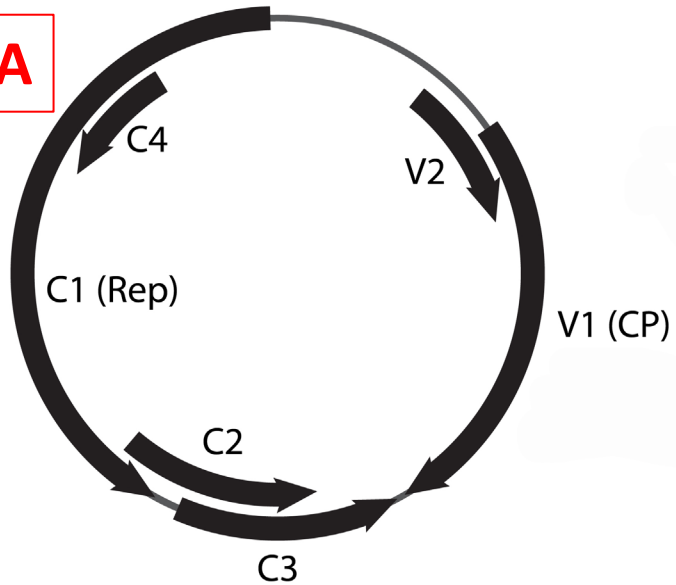
Viral vector carrying target RNAi inducer

Viral dsRNA intermeidate form during replication

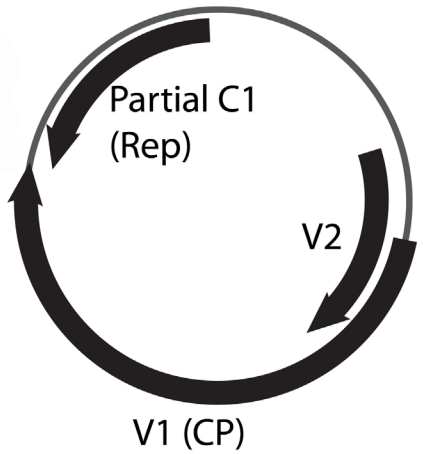
siRNAs

B. Artificial microRNA (amiRNA) approach

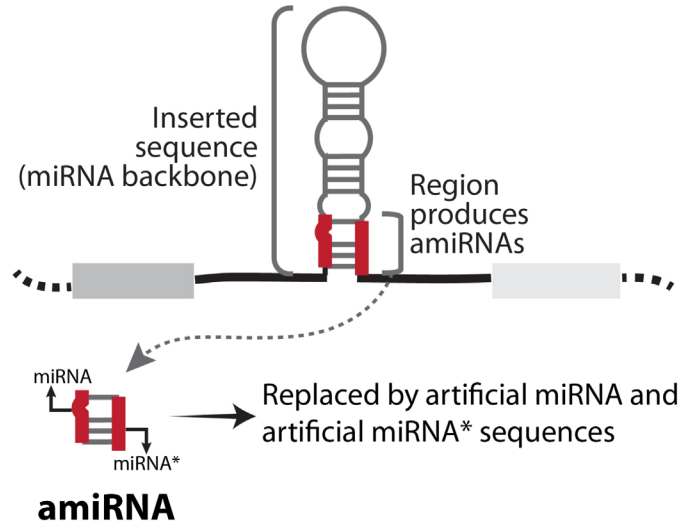
GGVA



Grapevine geminvirus A (GGVA) genome structure (~2.9 kb)



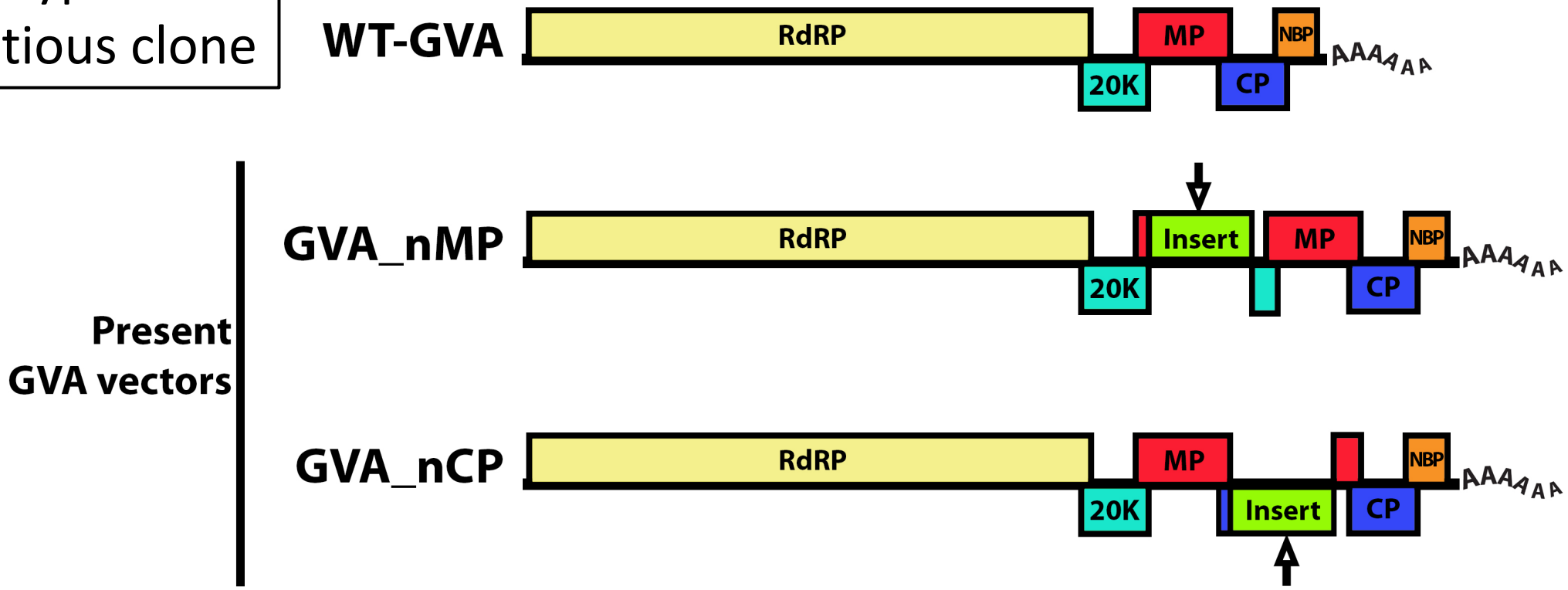
Grapevine geminivirus A defective DNA (~1.5 kb)



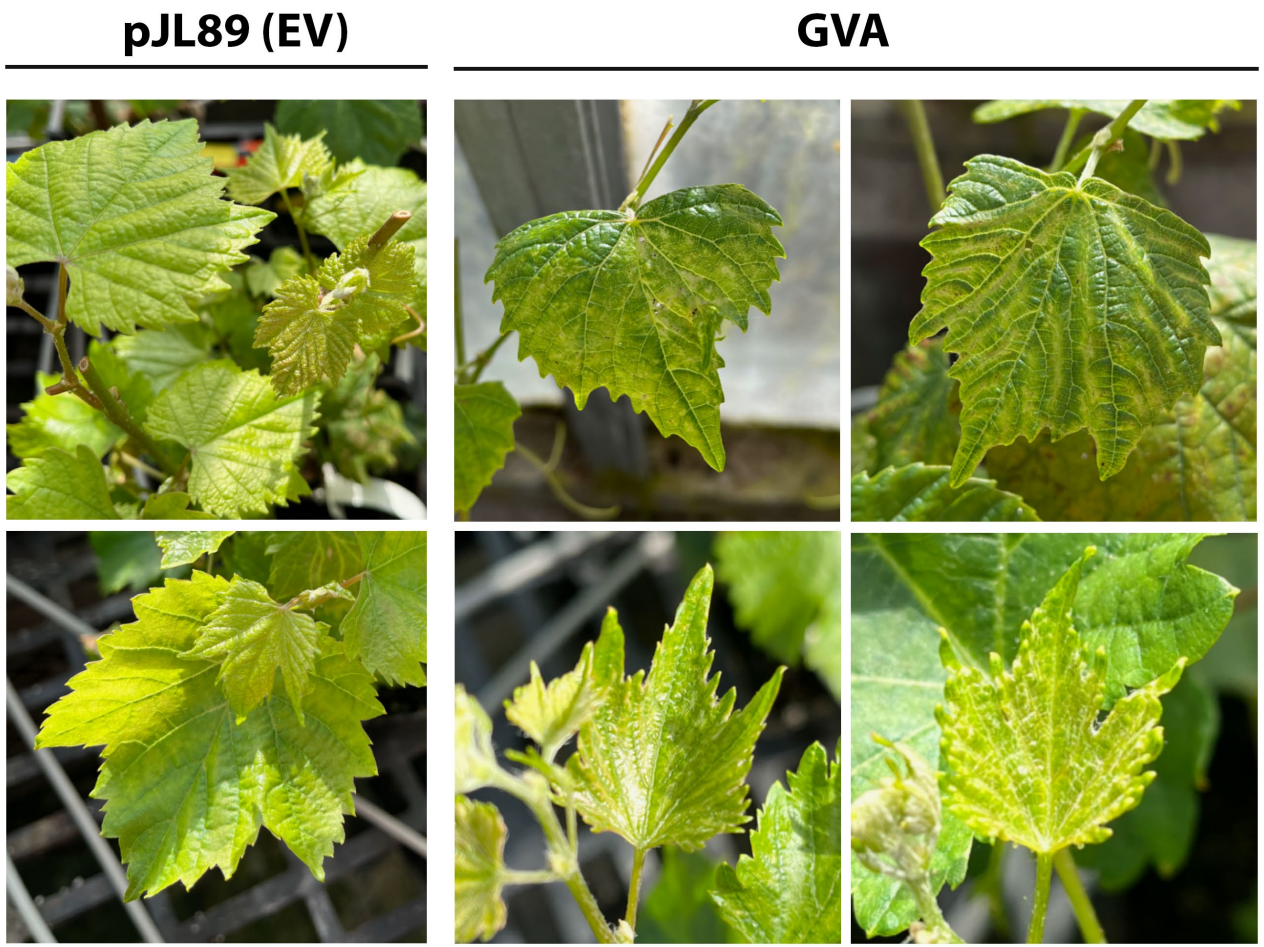
amiRNA

Grapevine virus A (GVA)

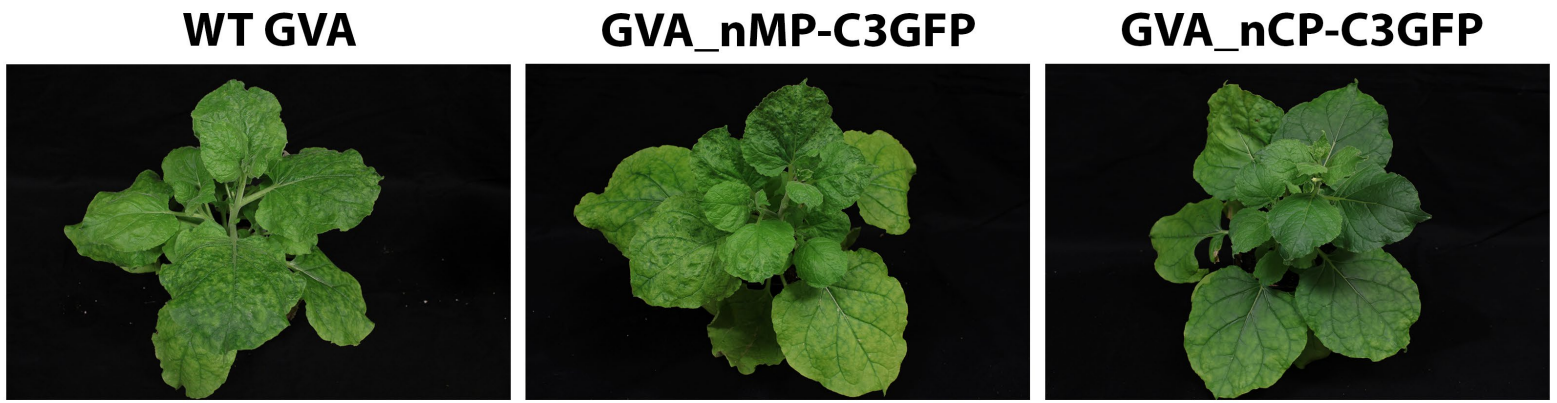
Wild-type infectious clone



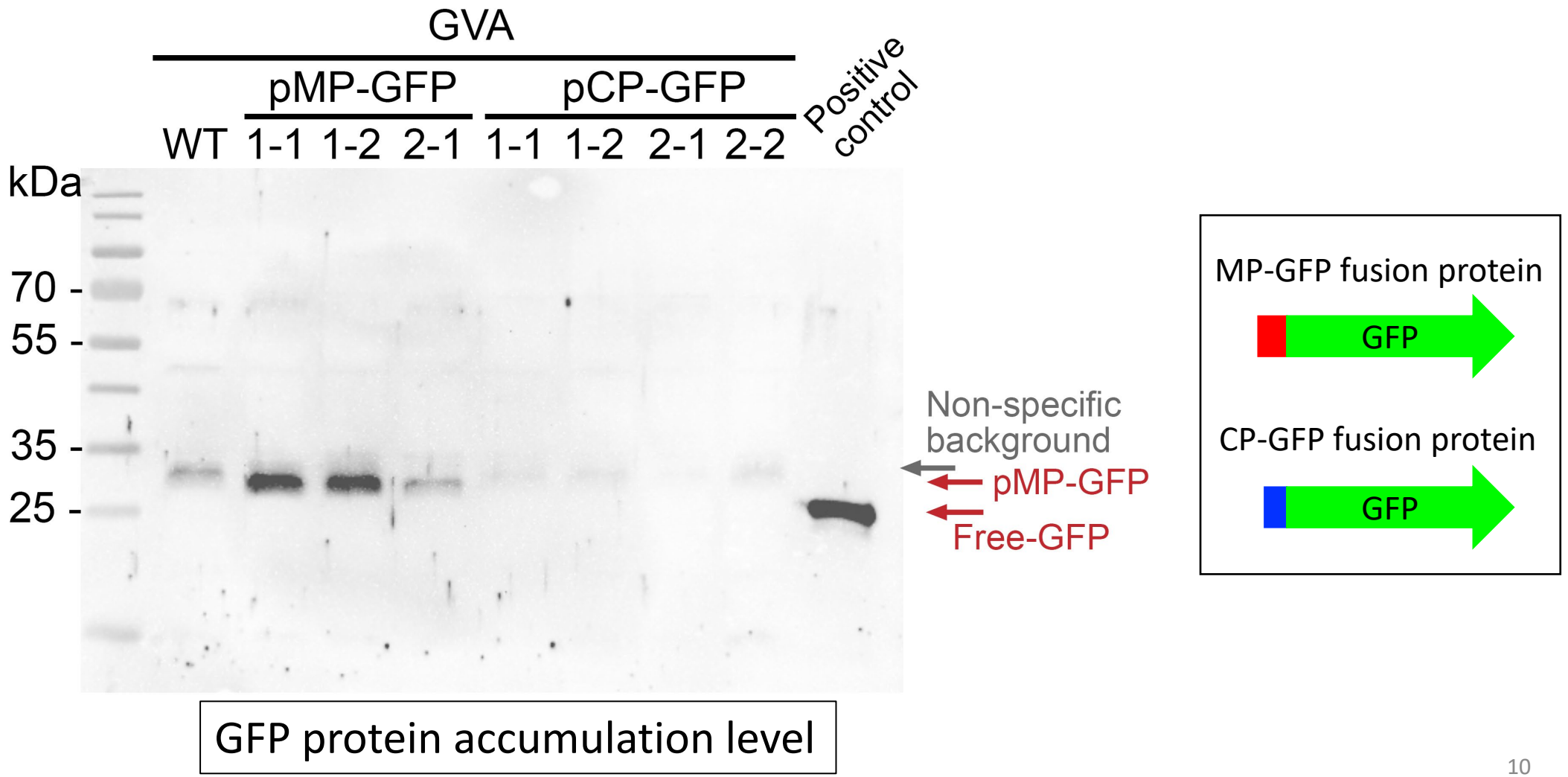
GVA caused very mild symptoms in grapevine plants, but the symptoms would disappear later in the new grown leaves



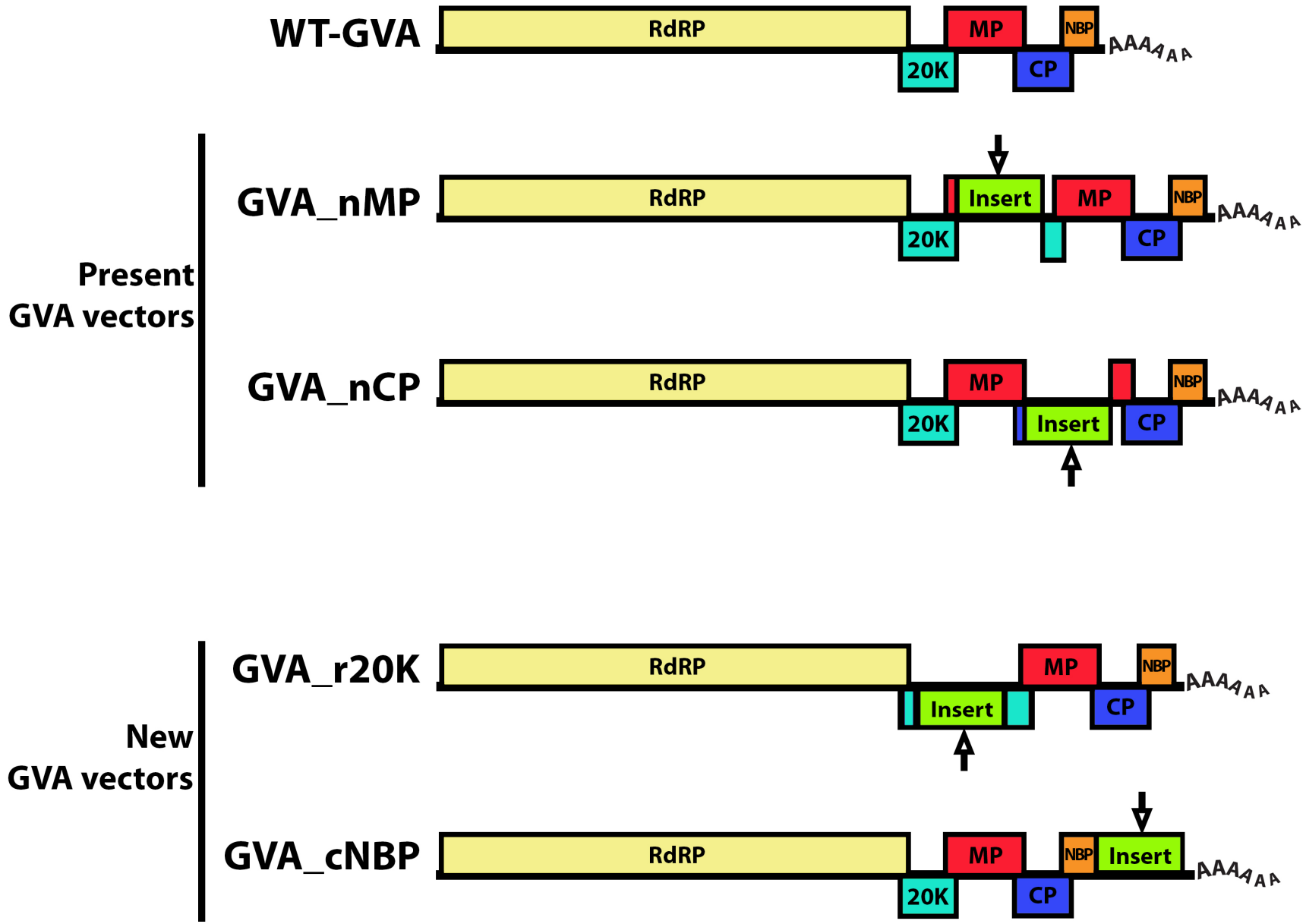
GVA caused mosaic/mottle symptoms in *N. benthamiana* plants



The insertion site at the N-terminal of MP is more stable compared to the site at the N-terminal of CP

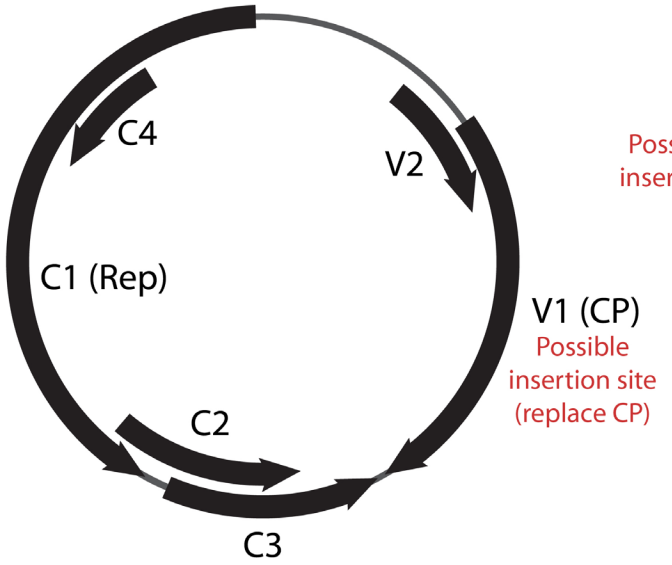


GFP protein accumulation level

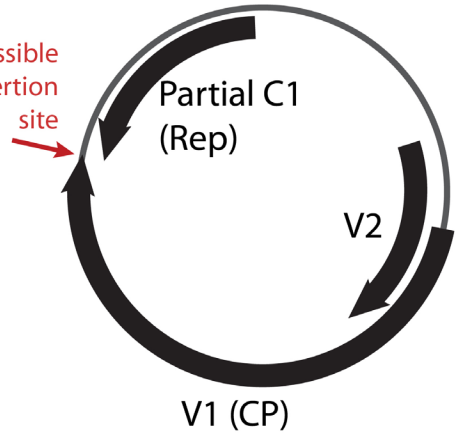

WT-GVA
RdRP
MP
NBP
20K
CP
AAAAAA
Present
GVA vectors
GVA_nMP
RdRP
Insert
MP
NBP
20K
CP
AAAAAA
GVA_nCP
RdRP
MP
NBP
20K
Insert
CP
AAAAAA
New
GVA vectors
GVA_r20K
RdRP
MP
NBP
Insert
CP
AAAAAA
GVA_cNBP
RdRP
MP
NBP
Insert
20K
CP
AAAAAA

Grapevine geminivirus A (GGVA)

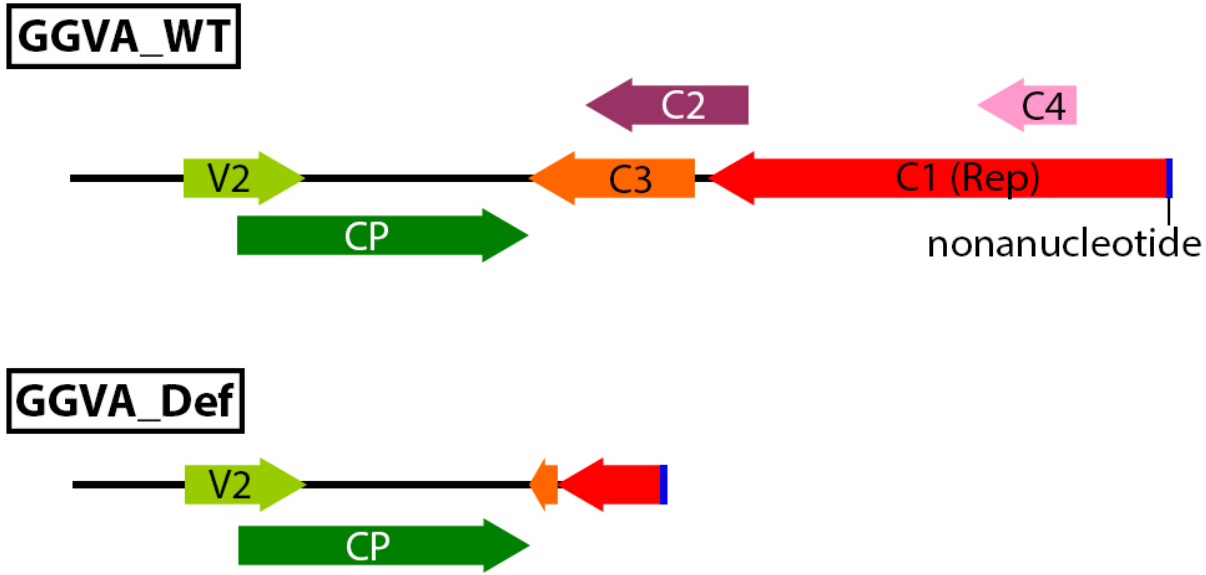
Infectious clones of the wild-type and defective genomes of 2 GGVA isolates were developed



Grapevine geminvirus A (GGVA) genome structure (~2.9 kb)

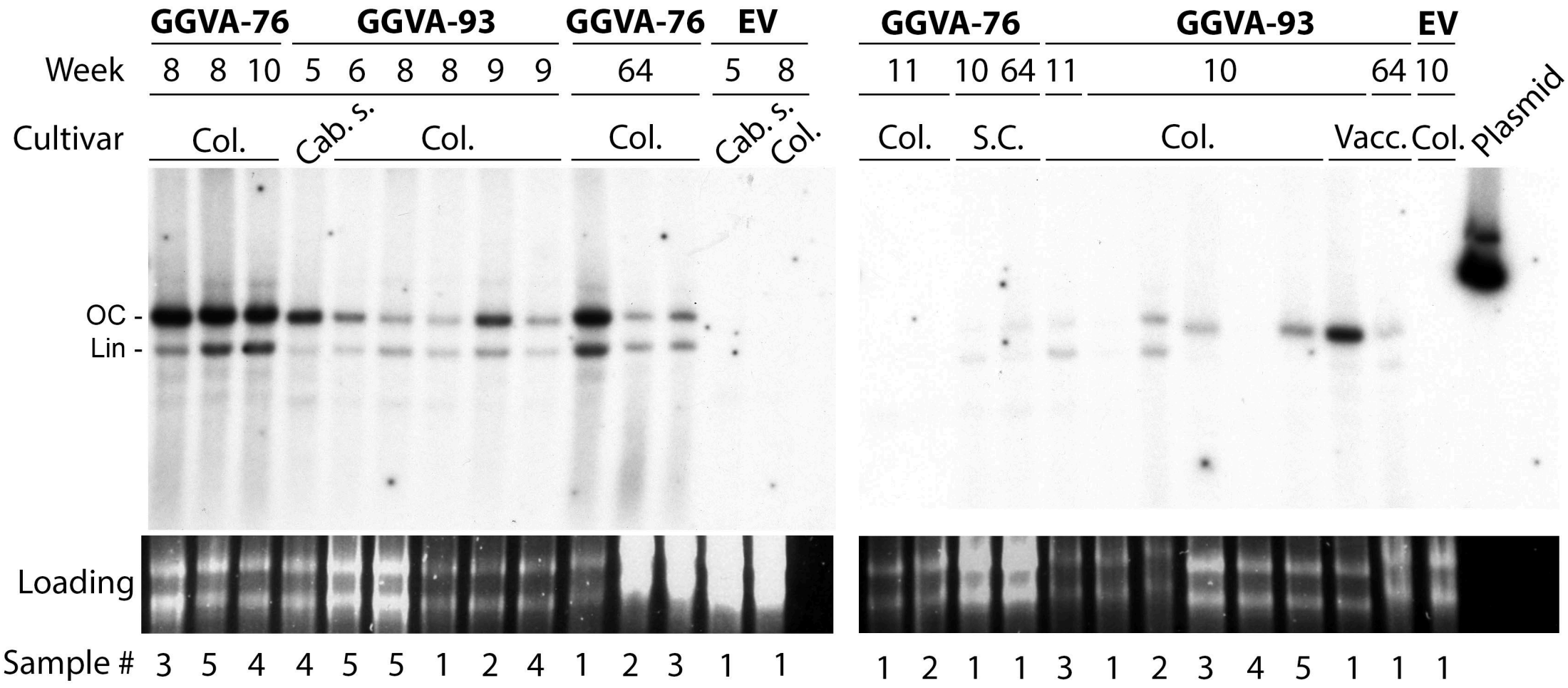


Grapevine geminivirus A defective DNA (~1.5 kb)

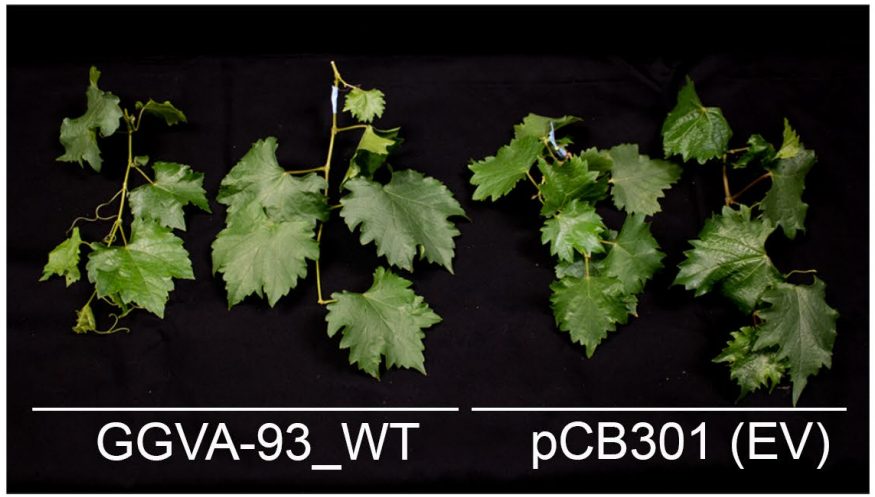


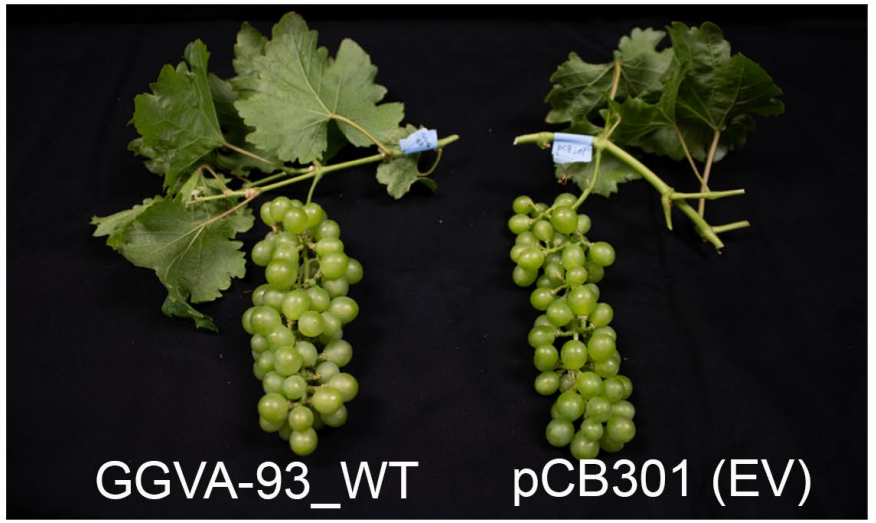
GGVA-76: Longyan – China (GenBank accession #- KX570611)
GGVA-93: Super Hamburg - Japan (GenBank accession #- KX570610)

Viral replication confirmed using Southern blot analyses in the asymptomatic grapevine plants



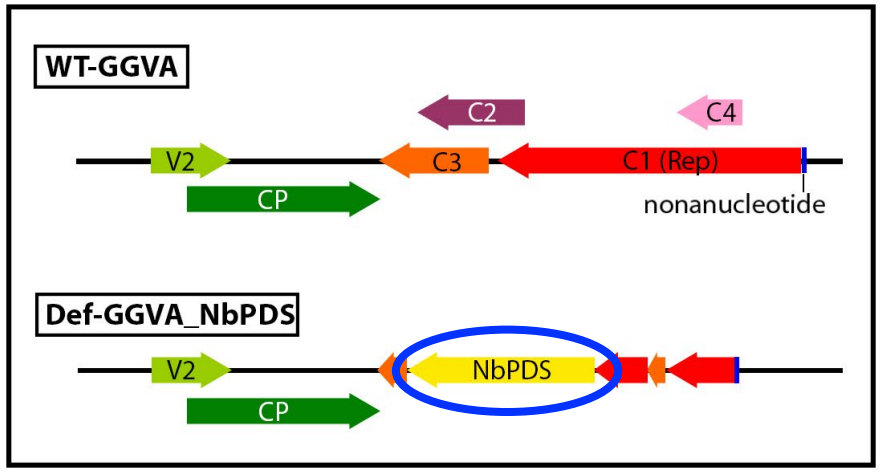
GGVA-76 or GGVA-93 did not induce observable symptoms in the infected grapevine leaves





GGVA_Def_NbPDS triggered virus-induced gene silencing (VIGS) in *N. benthamiana*

A.



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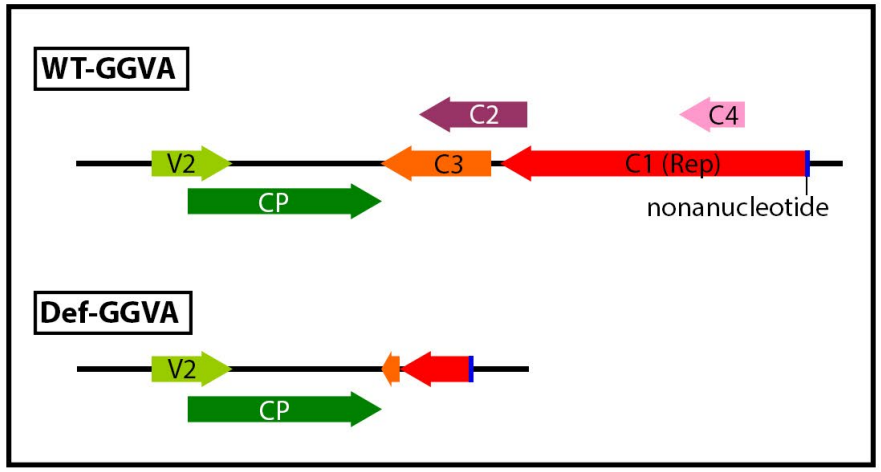
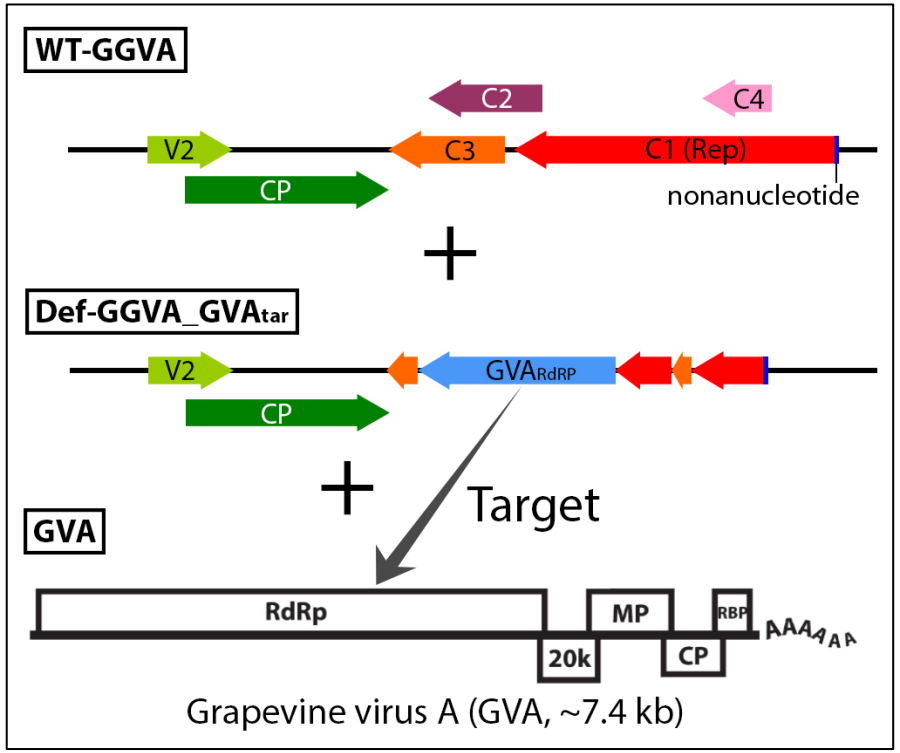
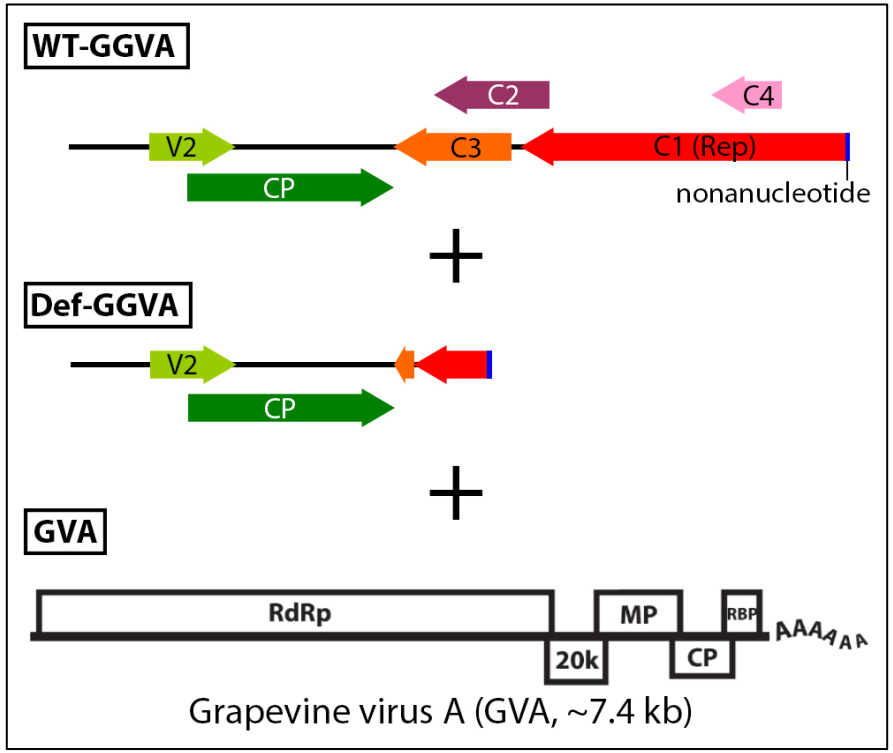
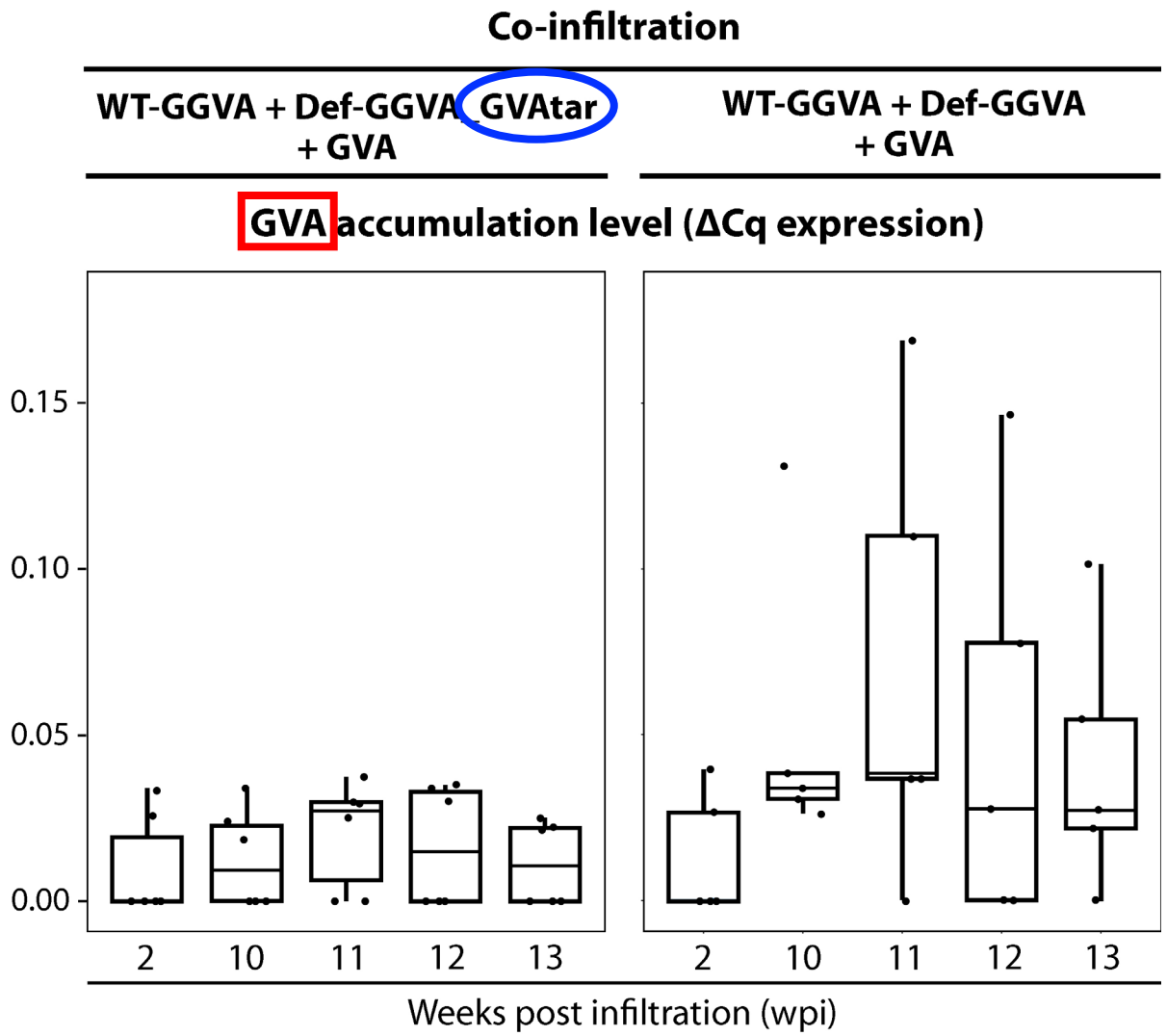


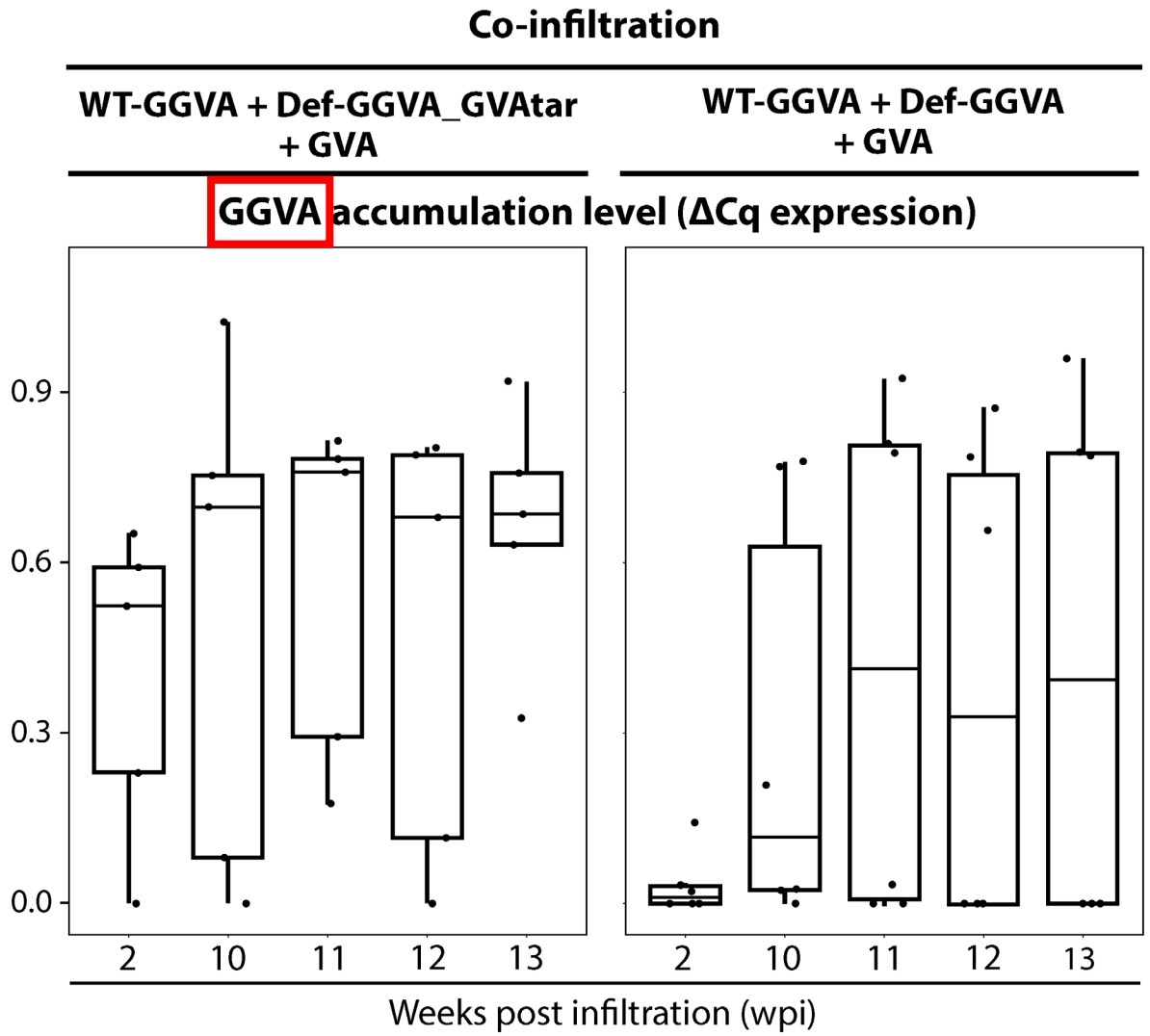


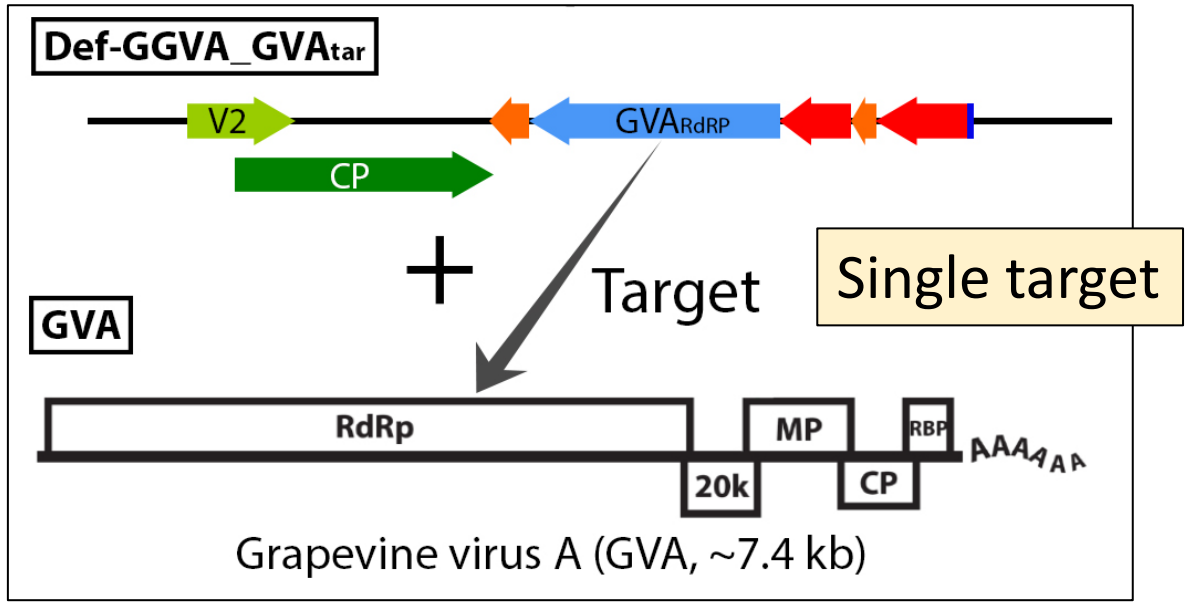


Vacuum infiltrate into grapevine plants

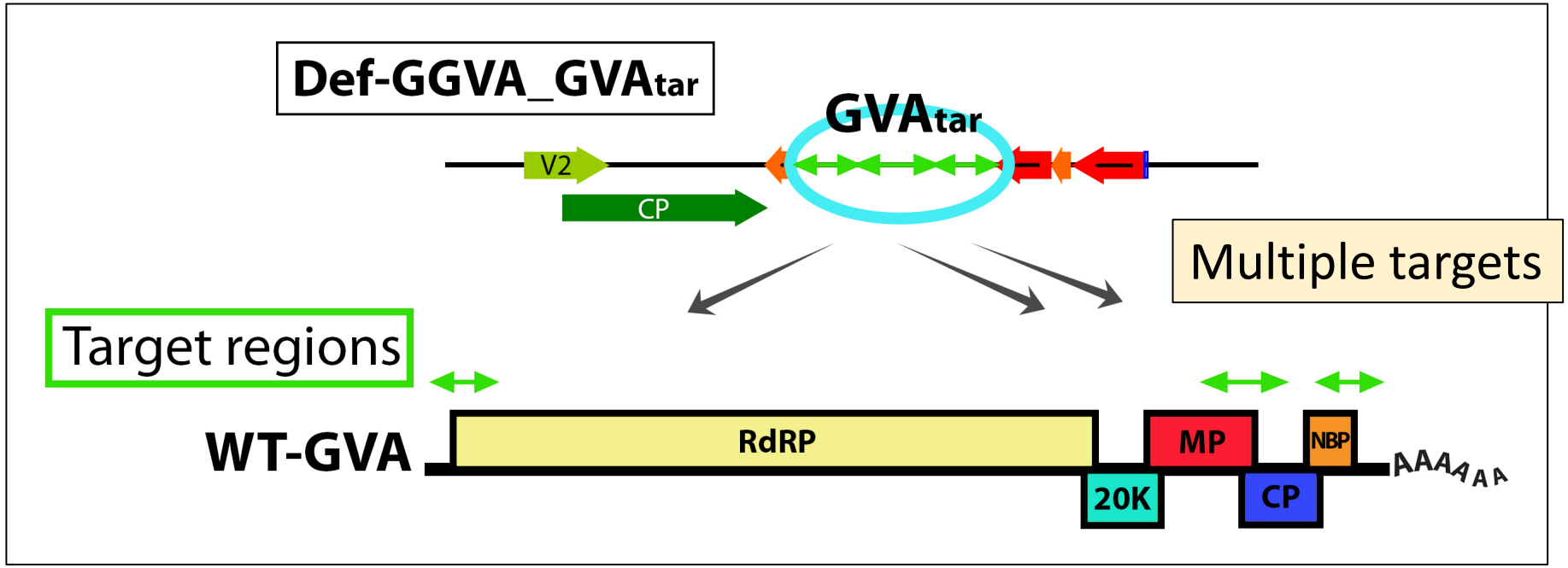
GGVA viral vector confers resistance to GVA infection in grapevine plants



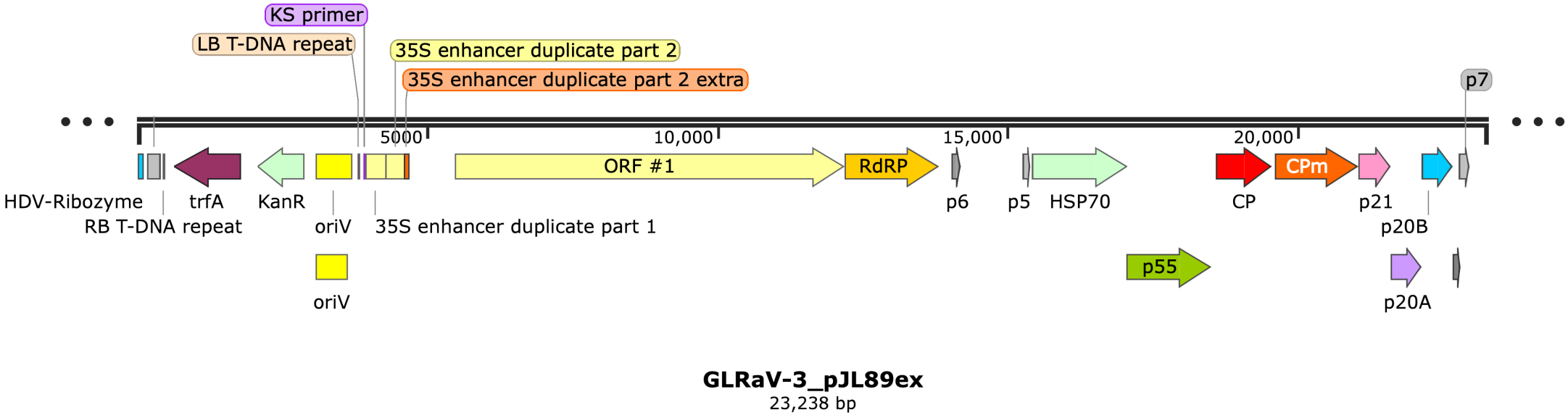




Chimeric target sequences



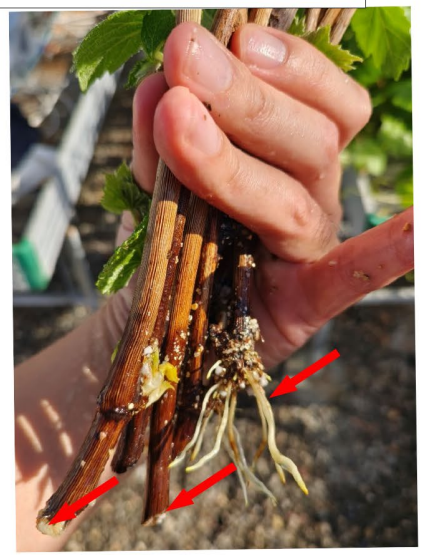
- Develop viral infectious clone (s) of a California grapevine leafroll associated virus-3 (GLRaV-3) isolate
- Apply chimeric target sequences strategy to target GLRaV-3



Improved vacuum infiltration protocol

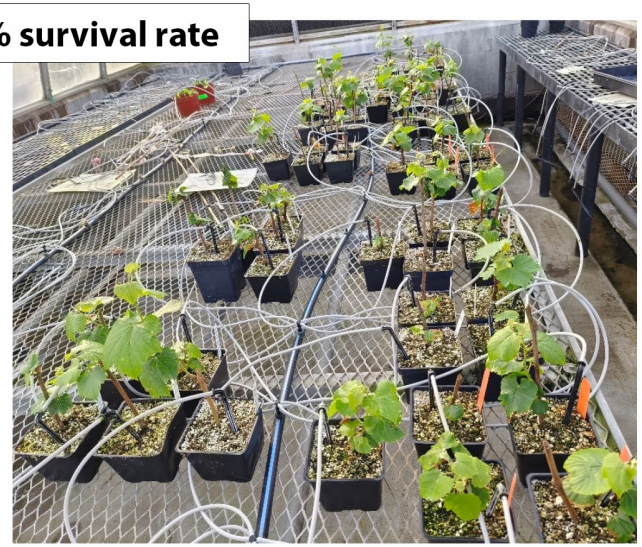
Propagation

Used for vacuum infiltration



Transplant

90% survival rate



Summary

- Developed and improved a working vacuum infiltration protocol to deliver virus infectious clones into greenhouse grown grapevine plants through roots
- Engineered three viral vectors of a DNA virus (GGVA) and an RNA virus (GVA)
- Ongoing efforts to target other grapevine viruses, GLRaVs and/or grapevine red blotch virus (GRBV) and mealybug vectors

Acknowledgement

- We would like to thank Dr. Maher Al Rwahnih and the FPS for kindly providing the grapevine cuttings/plants and the virus-infected grapevine samples.
- We would also like to thank the funding agency: the California Department of Food and Agriculture's Pierce's Disease and Glassy-winged Sharpshooter (CDFA PD/GWSS) Board.

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