UNIVERSITAT POLITÈCNICA DE VALÈNCIA

MATE MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

COMAV Instituto de Conservación y Mejora de la Agrodiversidad Valenciana

INVESTIGATION OF POSSIBLE VIRAL SILENCING SUPPRESSOR ACTIVITY OF CHERRY VIRUS A

MASTER IN PLANT BREEDING

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Content

Introduction

Plant diseases → **Viruses**

strictly intracellular obligate parasites that depend on the host's protein synthesizing machinery

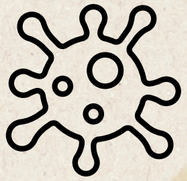
Transportation

One cell to another by plasmodesmata (short distance)

Phloem (long distance or systemic movement)

Prunus genus

- Family *Rosaceae*, order *Rosales*, 230 species
- Edible drupes
- Originated in Central Asia, now cultivated worldwide
- Viral infections → economic loss



- Apple mosaic virus (ApMV)
- Plum pox virus
- Little cherry virus 1 (LChV-1)
- Cherry virus A (CVA)

Cherry virus A (CVA)

- Family *Betaflexiviridae*, genus *Capillovirus*
- Discovered in Germany in 1995, now spread all over the world

7,383 nucleotides
+ poly (A) tail

- +ssRNA virus with two open reading frames

- RNA-dependent RNA polymerase (RdRp)
- Methyltransferase (MT)
- Helicase (HEL)
- Coat protein (CP)
- Movement protein (MP)

RNA silencing in plants

- Evolutionary conserved process of sequence-specific regulation of genes

  - endonucleotic cut and degradation
  - repression of the translation

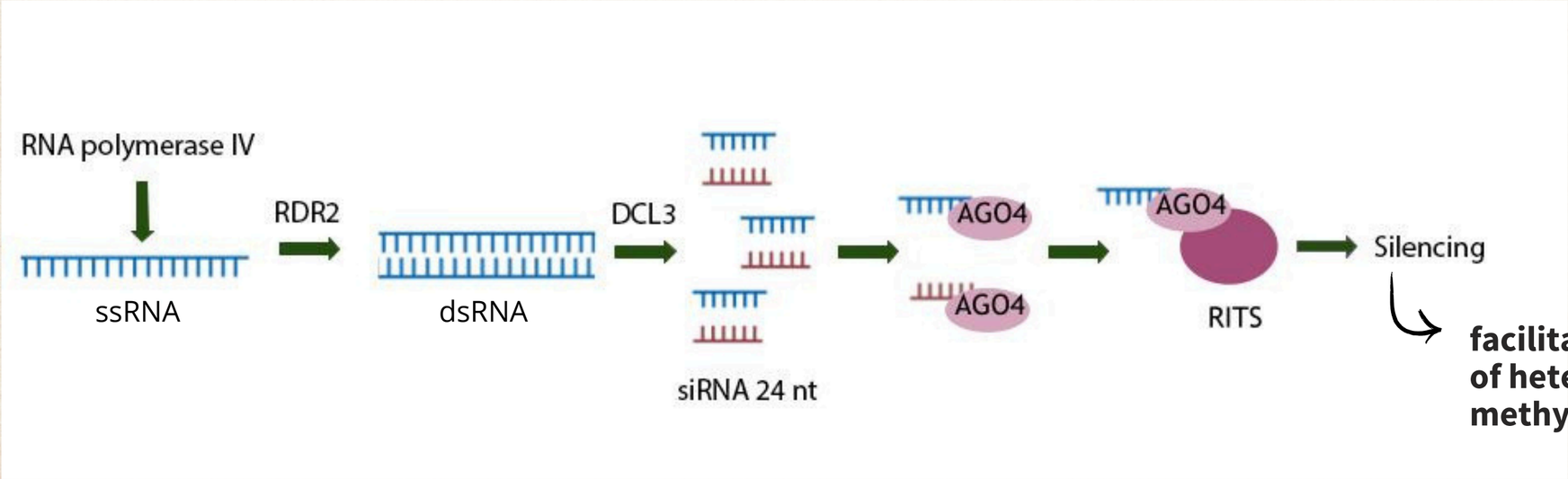
- One function of this gene-silencing mechanism is to prevent, restrict, and eliminate viral infections

- Types of RNAi

  - Transcriptional gene silencing (TGS)
  - Post-transcriptional gene silencing (PTGS)

Transcriptional gene silencing (TGS)

Regulation of genes by blocking the transcription

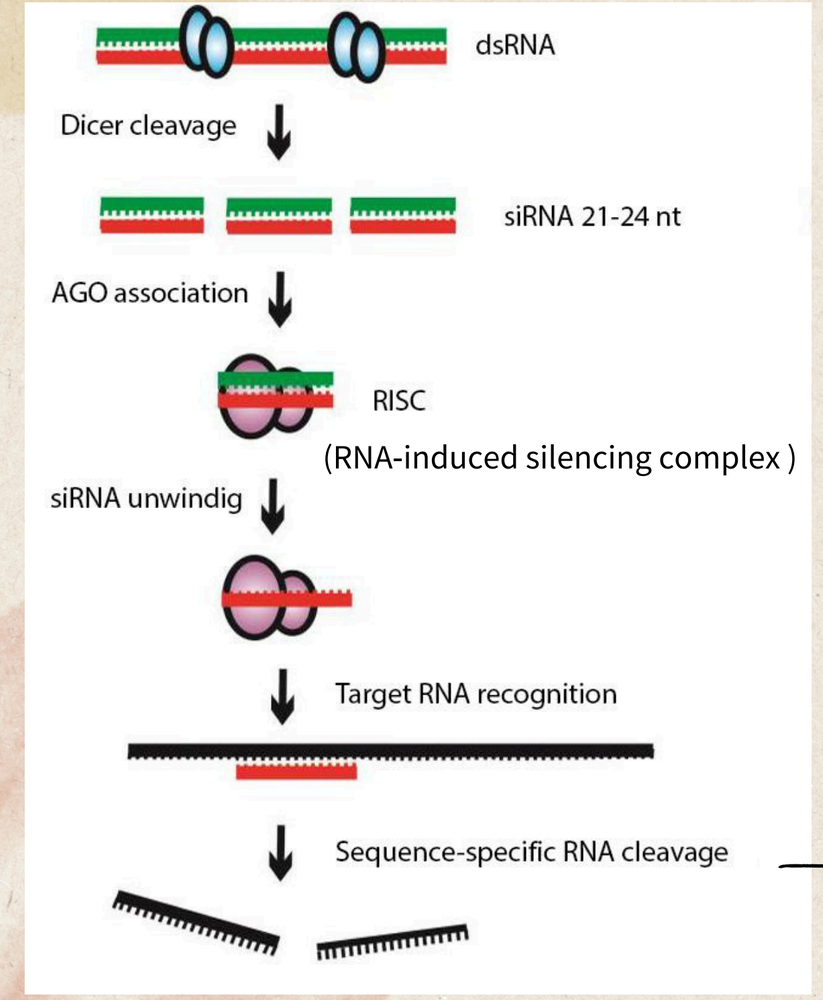


Barrientos *et al.* (2021)

- **RNA polymerase dependent on RNA 2 (RDR2)**
- **Dicer-like proteins (DCLs)**
- **RNA-induced transcriptional silencing complex (RITS)**

Post-transcriptional gene silencing (PTGS)

RDR or RNA pol II

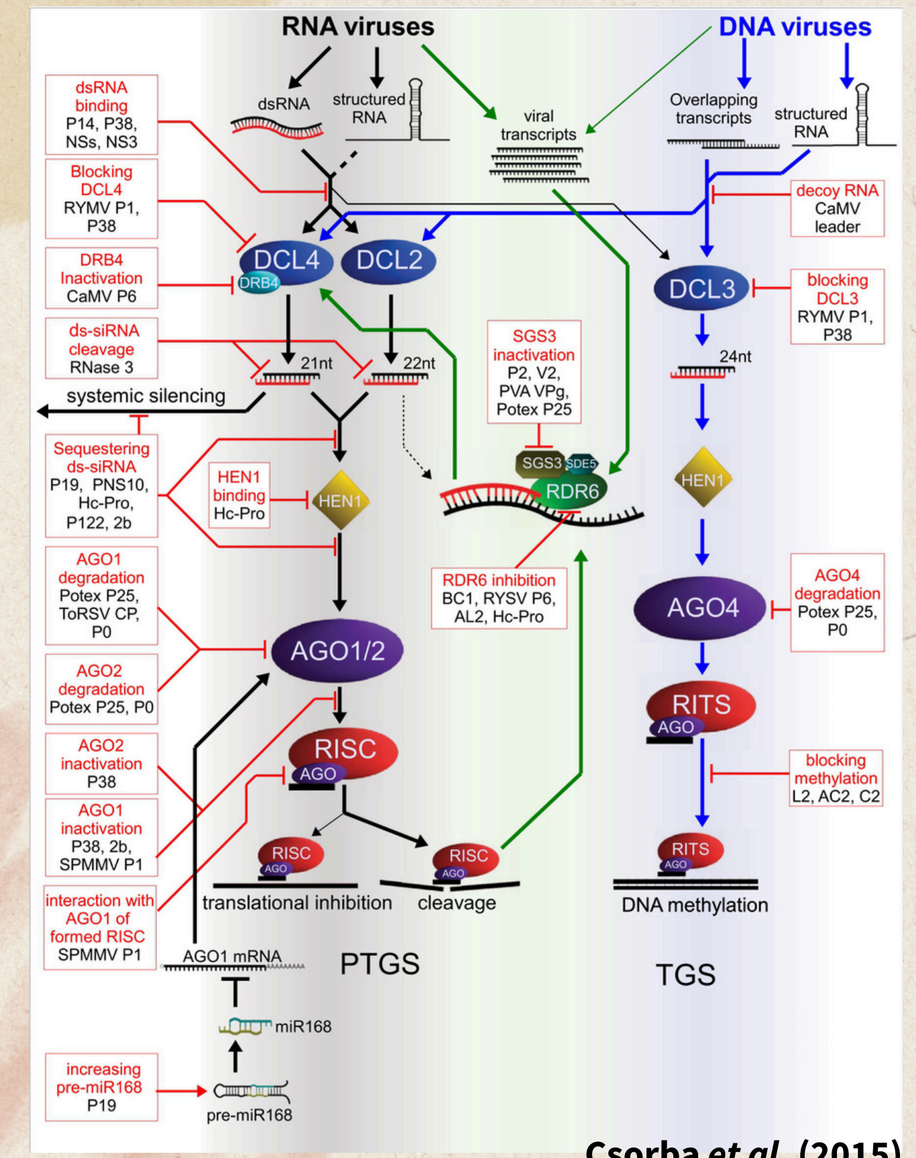


Koeppe *et al.* (2023)

- **Inactivation of genes through the cleavage of the RNA or by translational repression**
- **RNA-dependent RNA polymerase for RNA viruses or the cellular RNA polymerase II for DNA viruses**

→ **Cleavage of viral mRNA by binding complementary siRNA**

Viral suppressors of RNA silencing (VSRs)



Csorba *et al.* (2015)

- **Viral strategy to evade effective plant defense mechanism**
- **Viral proteins with an additional function of supressing RNA silencing**

- **coat protein**
- **replicases**
- **movement protein**

General objective

Amplify two proteins, MP and CP, from different strains of CVA infecting different host species that might be acting as VSRs; and compare the potential activity of these slightly different variants

Specific objectives

1. Study differences in the CVA encoding MP in different strains originating from different hosts.

2. Test and compare the possible local VSR activity of them

3. Determine it can act as a VSR of the systemic silencing signal.

4. Identify the position of the CP in the genome of CVA, and study the differences in the CP in different strains originating from different hosts.

Hyphotesis

The MP of CVA exhibits VSR activity both locally and systemically, with variations in activity among different strains from four Hungarian host species.

The CP of CVA originating from four host species will show differences at the sequence level.

Methodology

Four Hungarian *Prunus* host cultivars

Virus strains

Sweet cherry - Szomolyai fekete
Plum – Besztercei
Sour cherry – Érdi bőtermő
Apricot – Pannónia

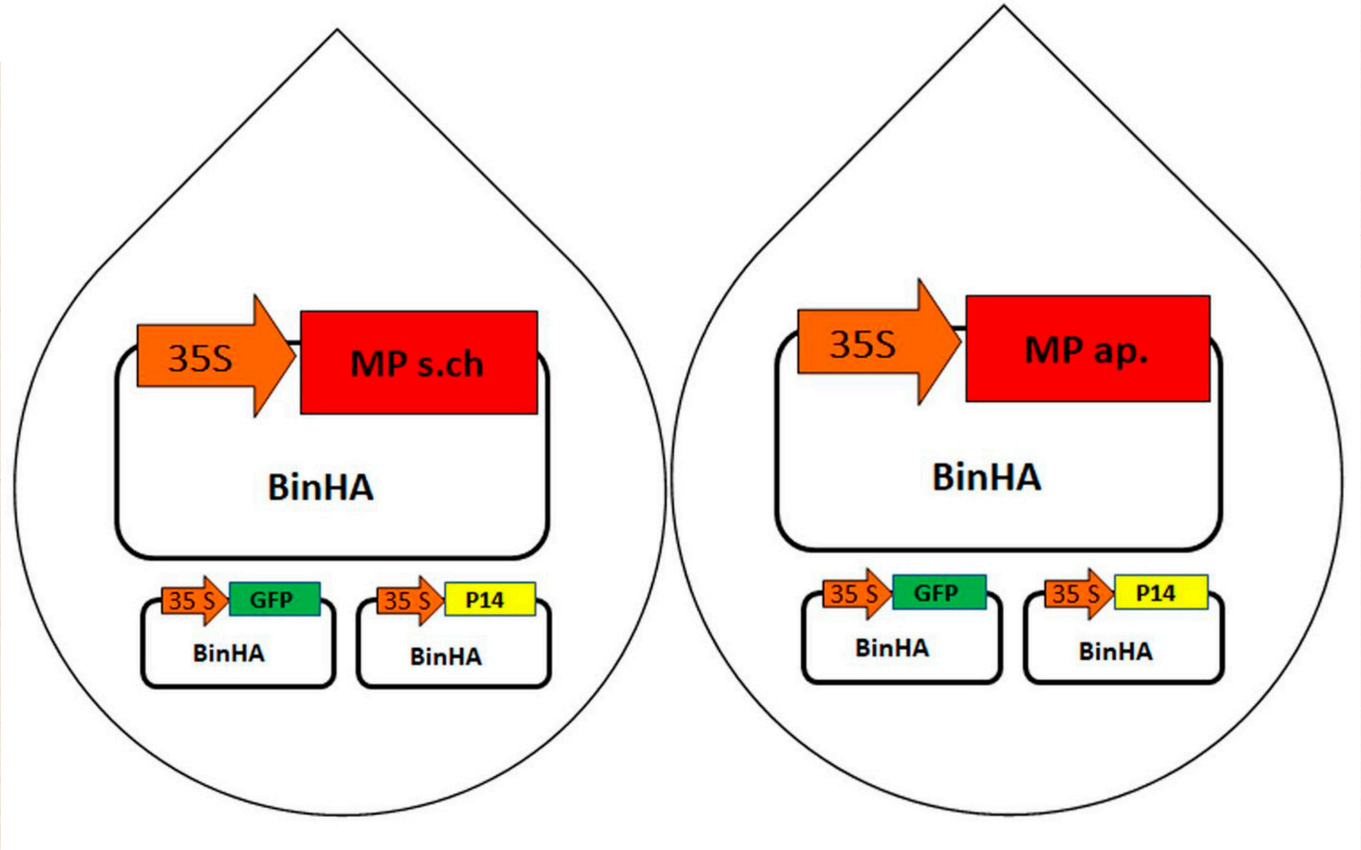



Methodology

- Building of expression vector containing CVA MP or CP coding sequences
- Sequencing by Sanger method
- Bioinformatic analysis for MP and CP (multiple alignment and creation of phylogenetic tree)
- Directional cloning of MP into the BinHASanyi binary vector by in-fusion protocol
- Triparental mating to transfer the vector to *Agrobacterium tumefaciens*

MP protein expression test

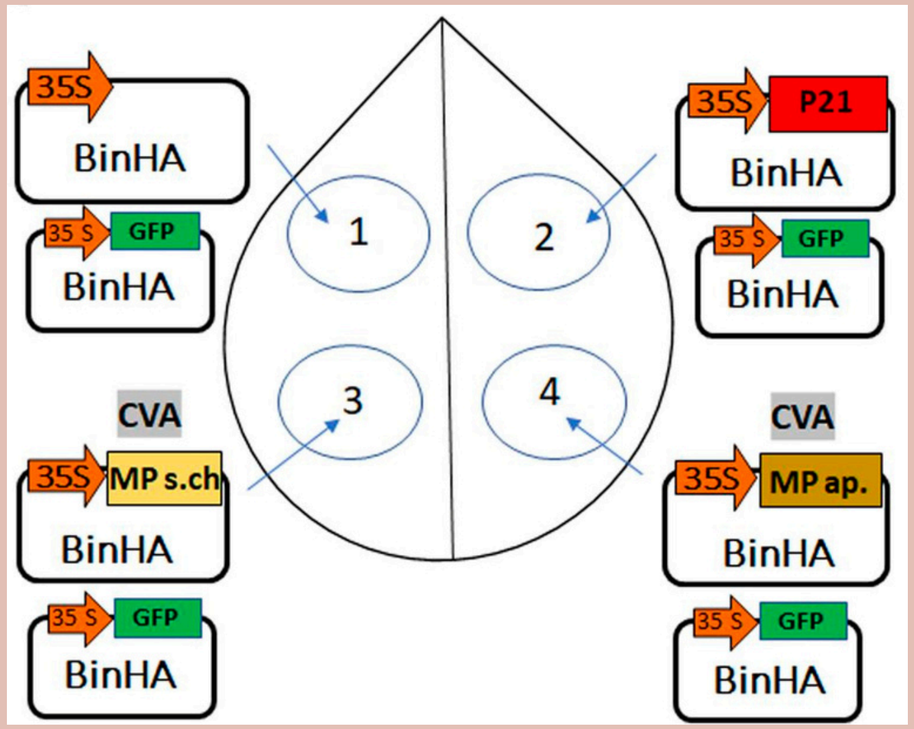
Mixing of *Agrobacterium* containing the GFP, P14 and potential VSR → **Protein extraction – Western Blot**



Evaluation of potential local VSR activity of the MP

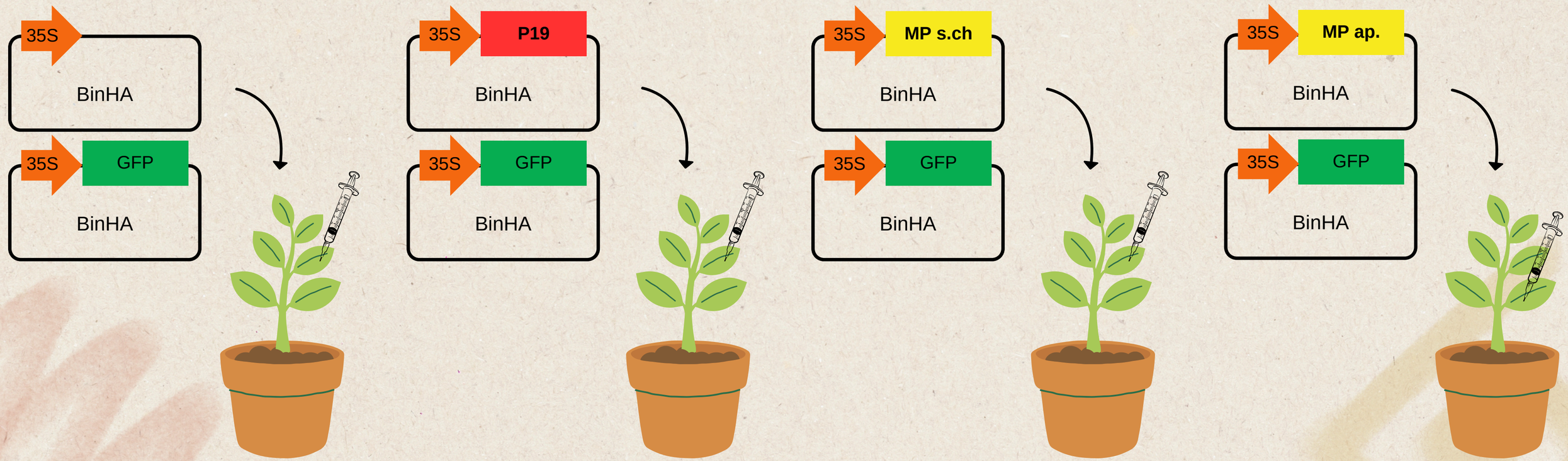
Co-infiltration of GFP-expressing *Agrobacterium* and the potential VSR coding regions in wild-type *Nicotiana benthamiana*

Total nucleic acid extraction – qPCR
Protein extraction – Western Blot



Evaluation of potential systemic VSR activity of the MP

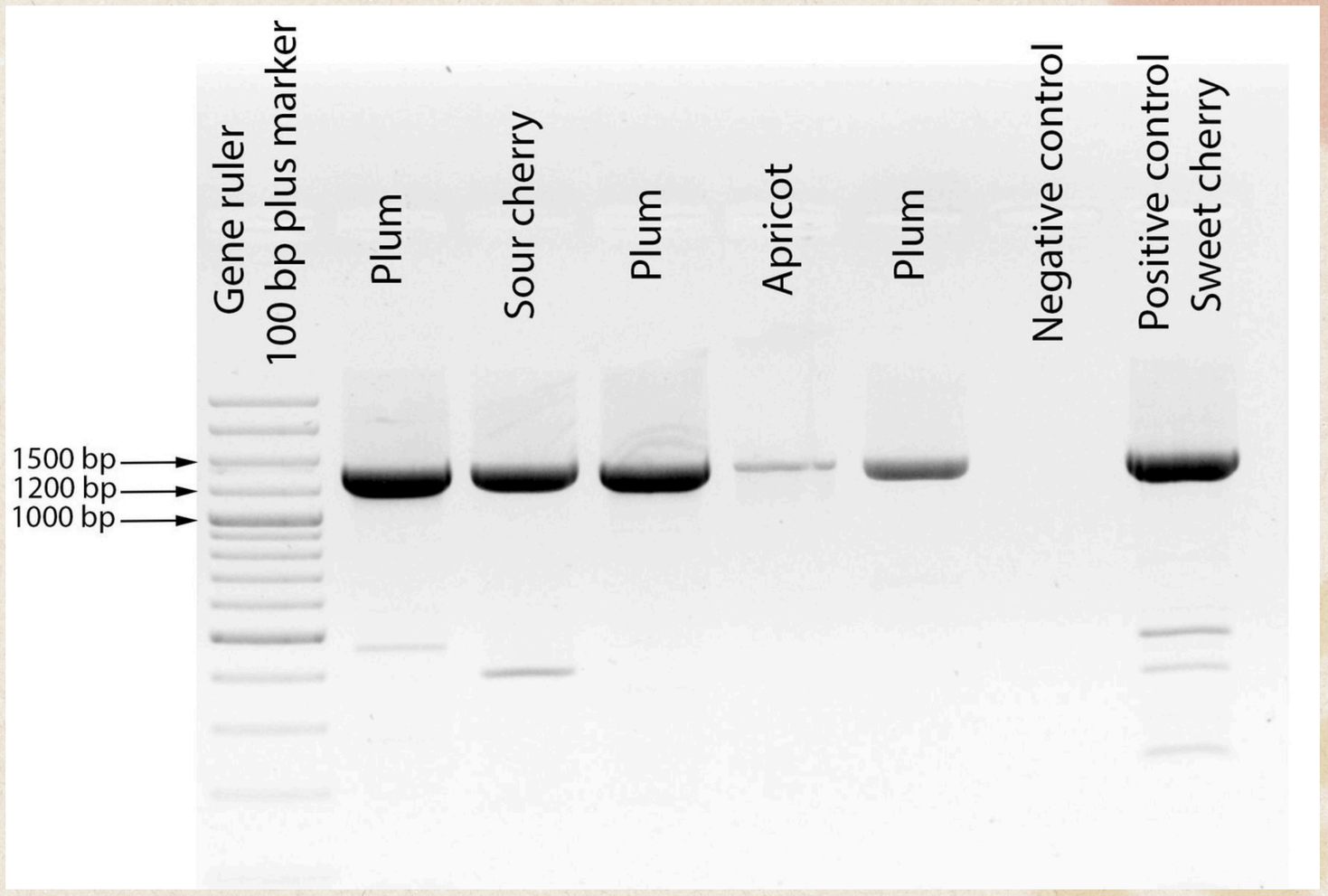
Co-infiltration of GFP-expressing *Agrobacterium* and the potential VSR coding regions in GFP transgenic *N. benthamiana* (line 16c)



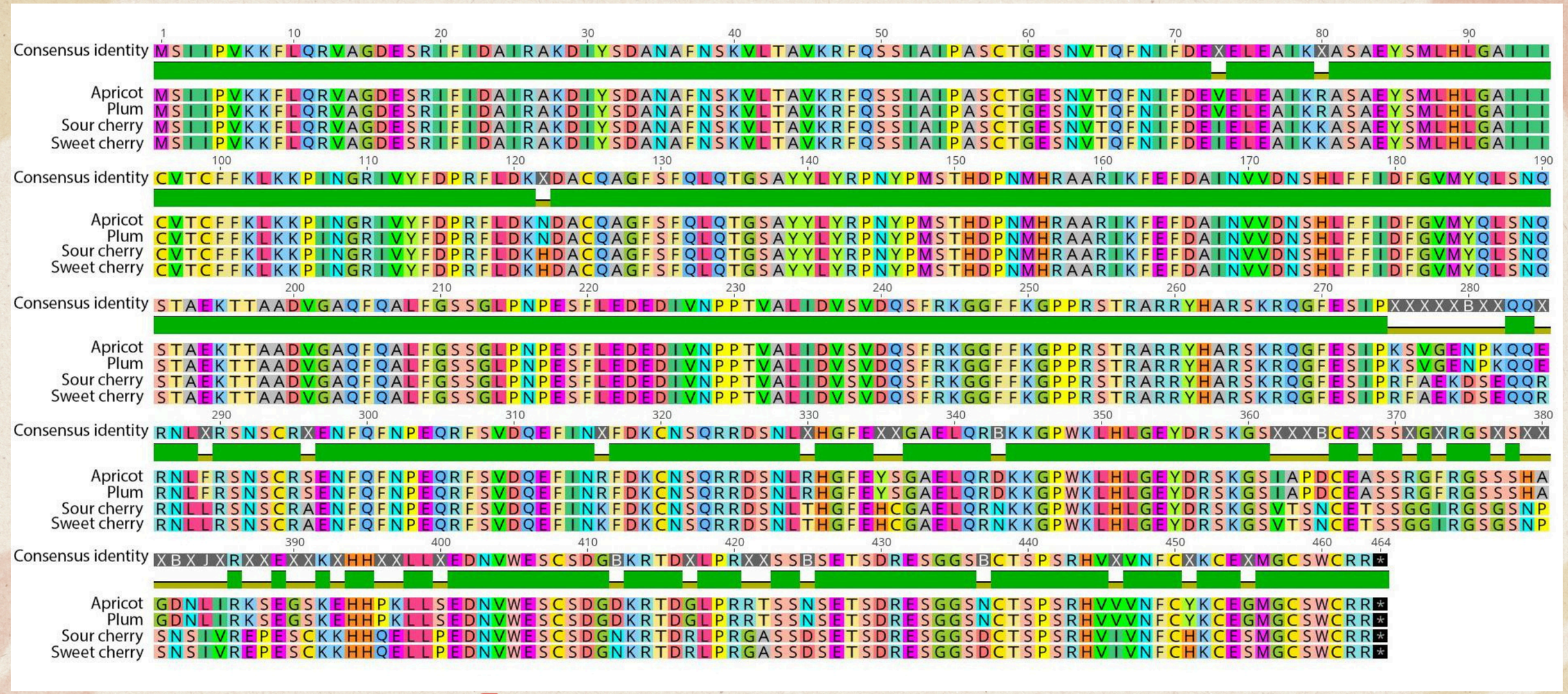
Results and discussion

The MP coding region was successfully amplified

Size of movement protein coding sequences ~1300 bp



Big similarity between sour cherry strain and sweet cherry strain, and between plum and apricot strain



Non-conservative substitutions

Aspartic Acid (D) to Asparagine (N) (acidic to amine amino acid)
Serine (S) to Glycine (G) (nucleophilic to small amino acid)
Lysine (K) to Glutamic Acid (E) (basic to acidic amino acid)

Non-conservative substitutions

- Acidic to amine amino acid
- Acid nucleophilic to small amino acid
- Basic to acidic amino acid

P0 protein

Poleroviruses
Pea mild chlorosis virus

→ **Variability in their ability to suppress RNA silencing across different virus strains**

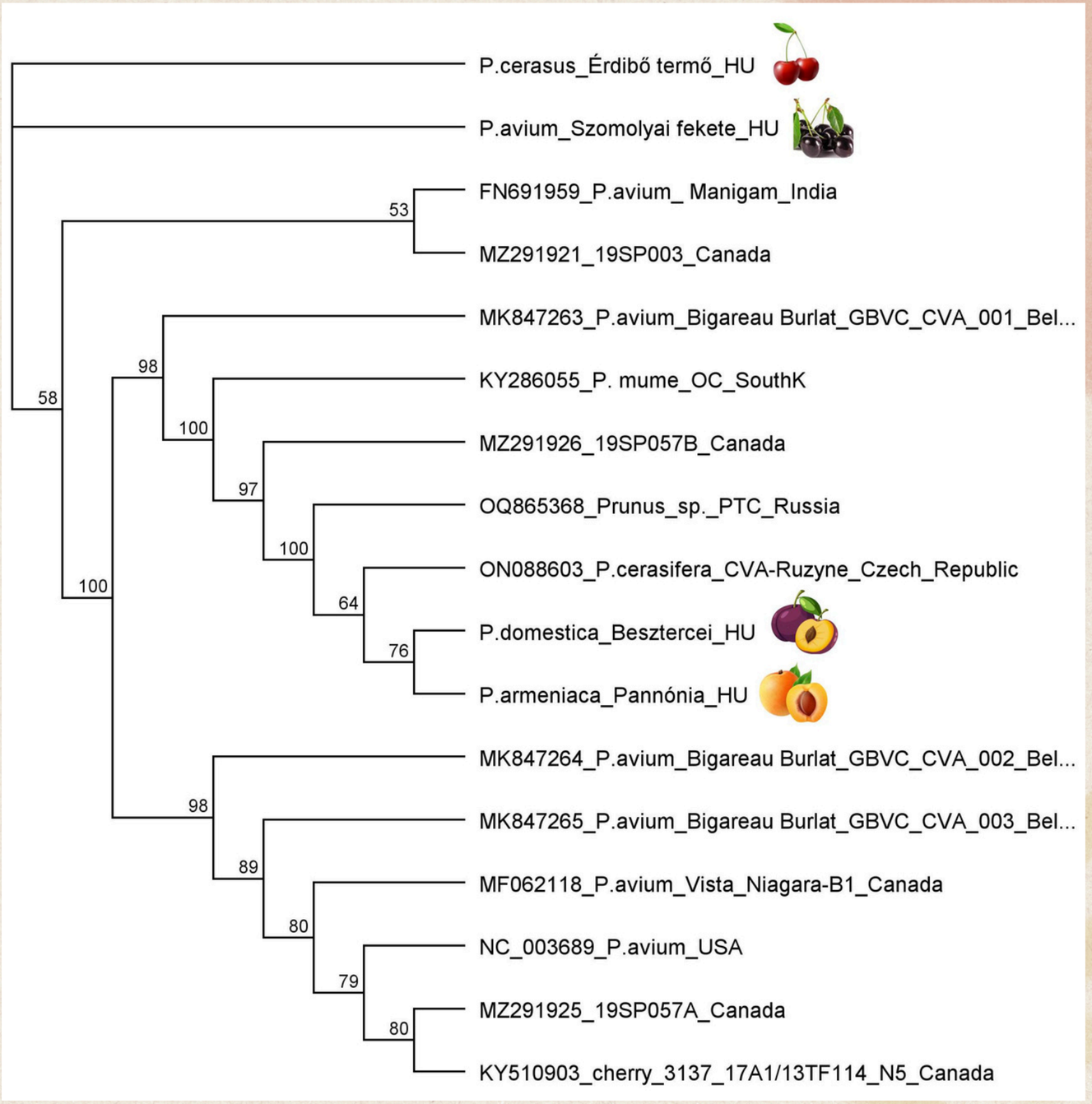
- **Alteration of structure and function of the protein**
- **Potentially alteration its ability to bind RNA or interact with other proteins**
- **Alteration its stability**

→ **Impact in VSR activity**

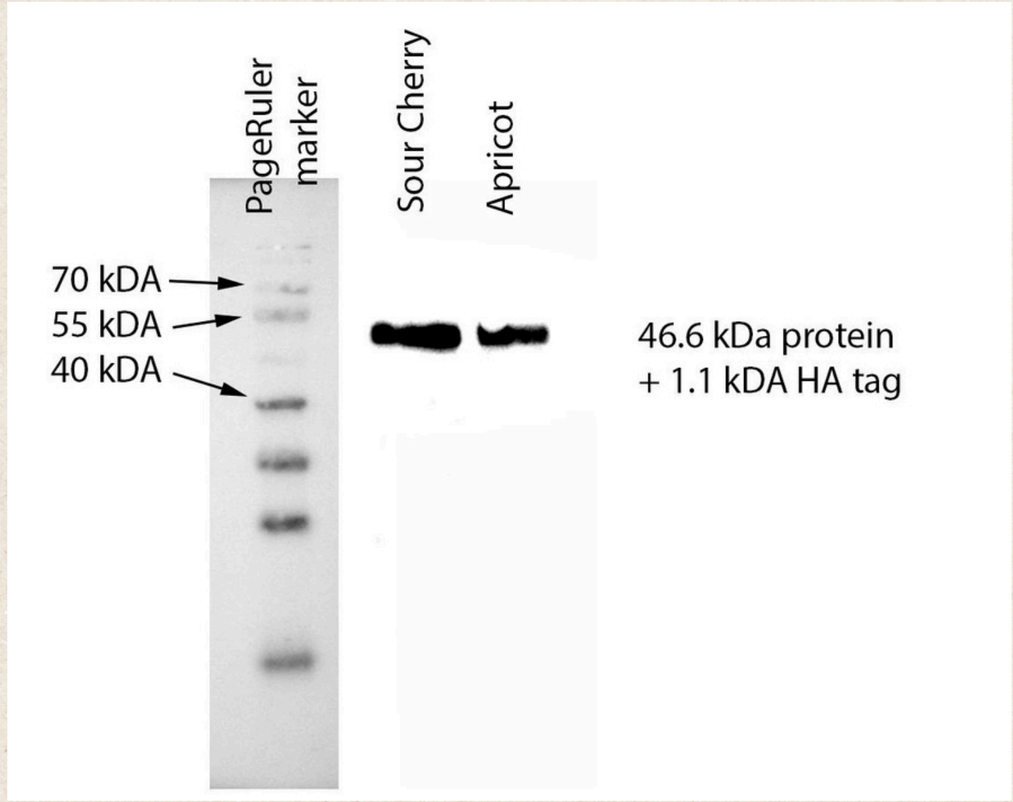
Two different clusters for the MP of CVA strains originating from Hungarian hosts

Sour cherry and sweet cherry

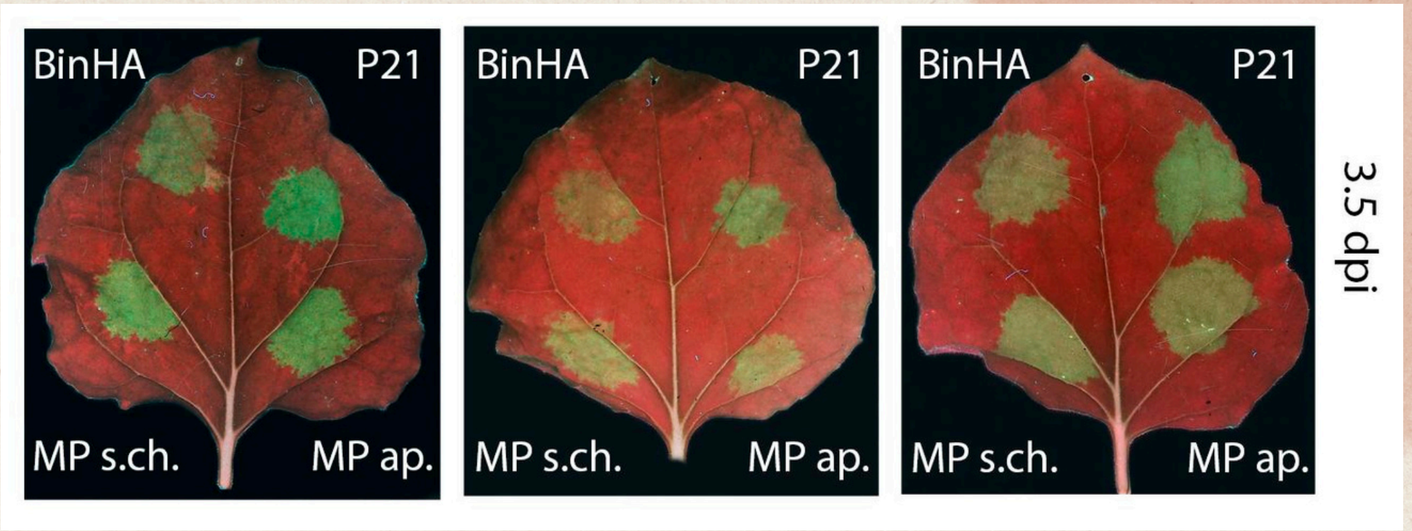
Apricot and plum

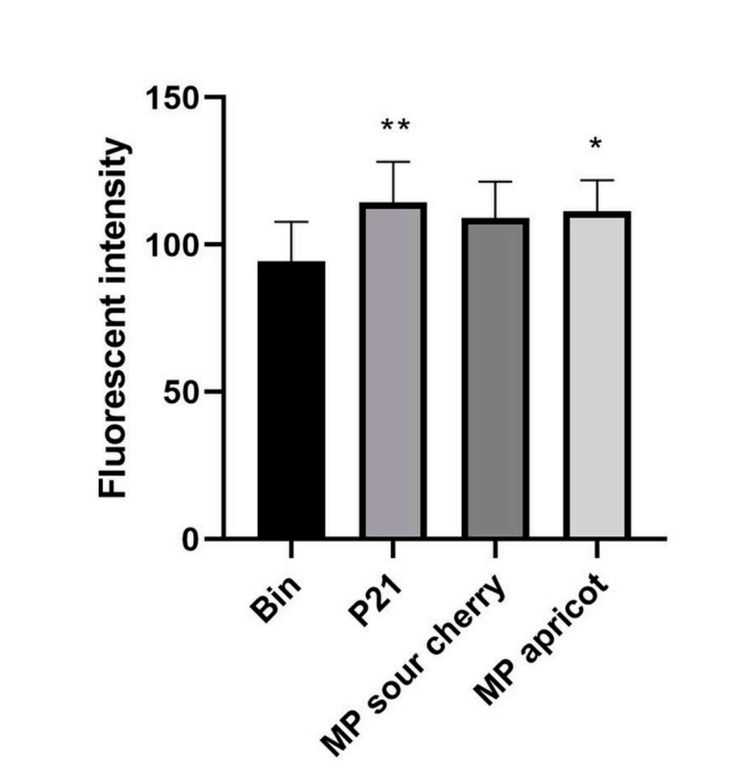


HA-tagged protein was successfully expressed with a size of 46.6 kDa for the protein and an additional 1.1 kDa for the HA tag



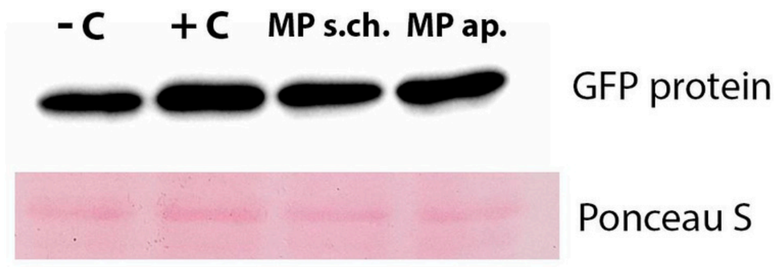
Local silencing

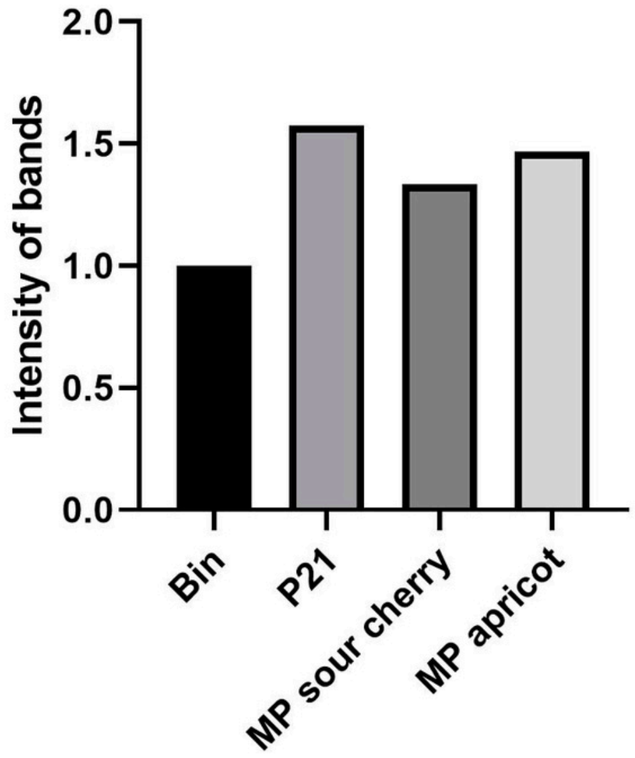




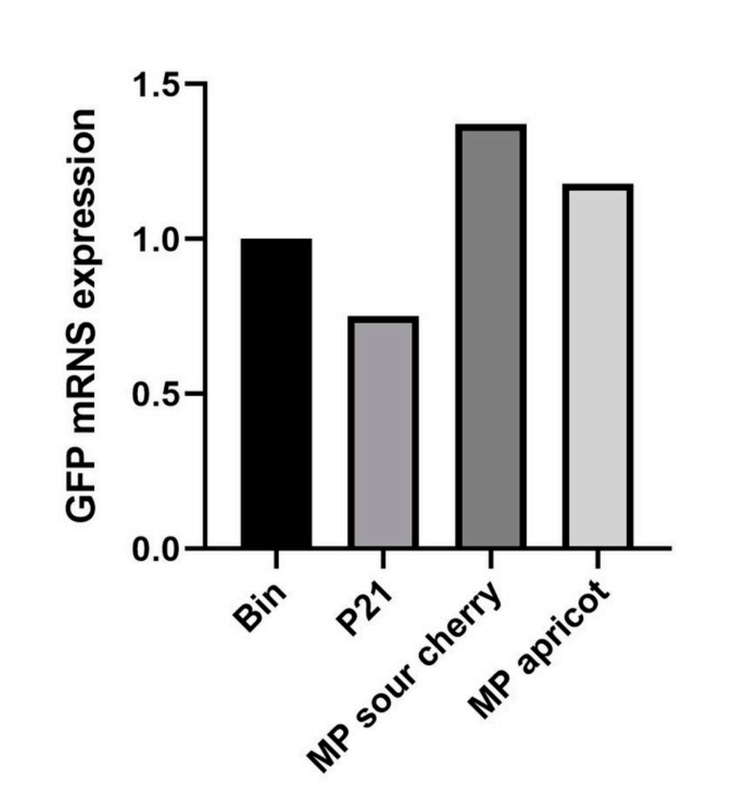
For MP from both strains, fluorescence signal is stronger than that of the negative control but weaker than that of the positive control

GFP protein expression level analysis supported the results observed previously





GFP mRNA levels did not supported the previous results



- **Technical problem?** → **RNA is sensible**
- **Biological problem?** → **Necrosis induced by P21**

Fluorescence intensity

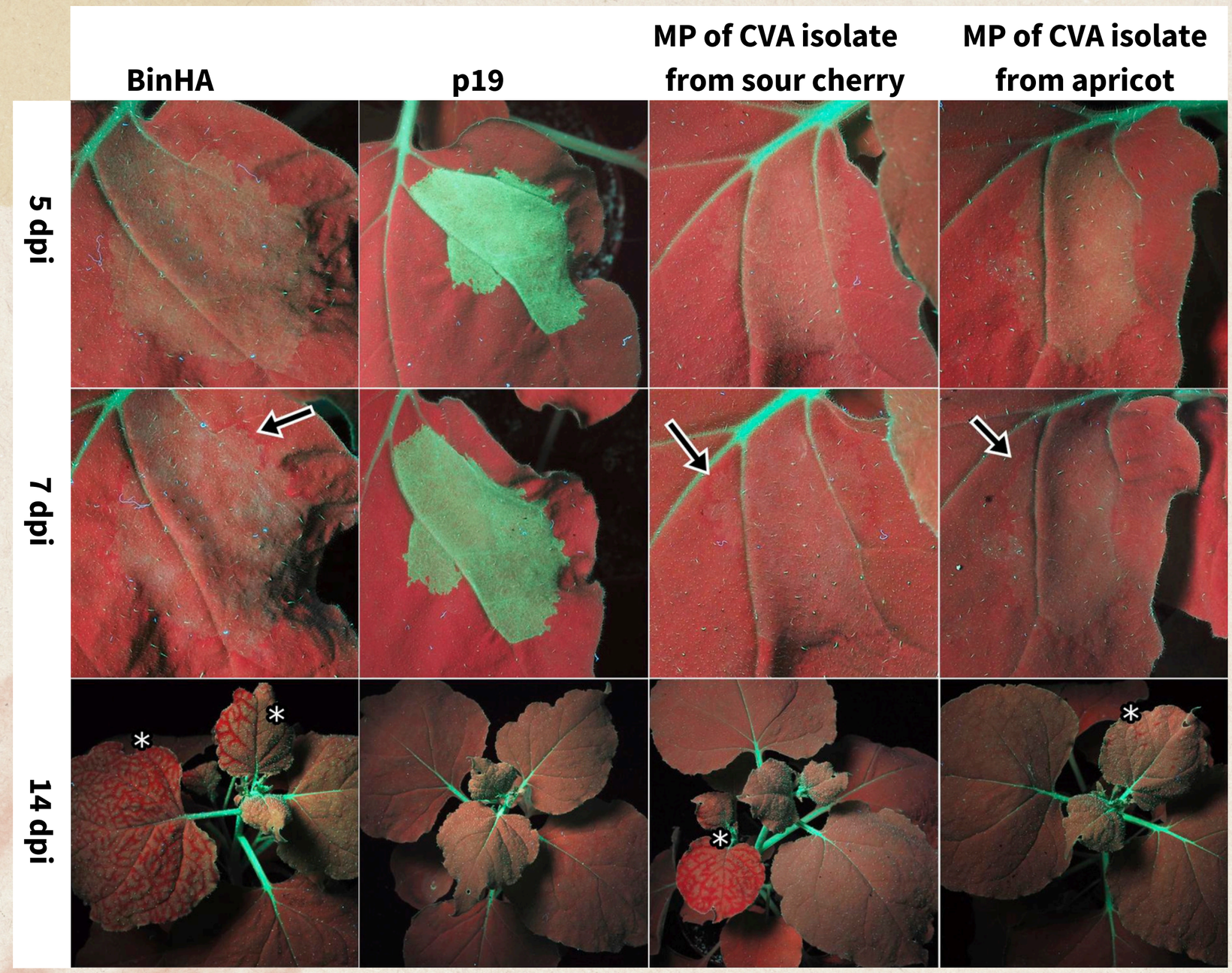
Signal is stronger than that of the negative control but weaker than that of the positive control

GFP protein levels

MPs of CVA strains from sour cherry and apricot = lower than p21, but higher than empty BinHA

Weak local VSRs

Systemic VSR activity



GFP fluorescence signal
Not as strong as + control, comparable to - control

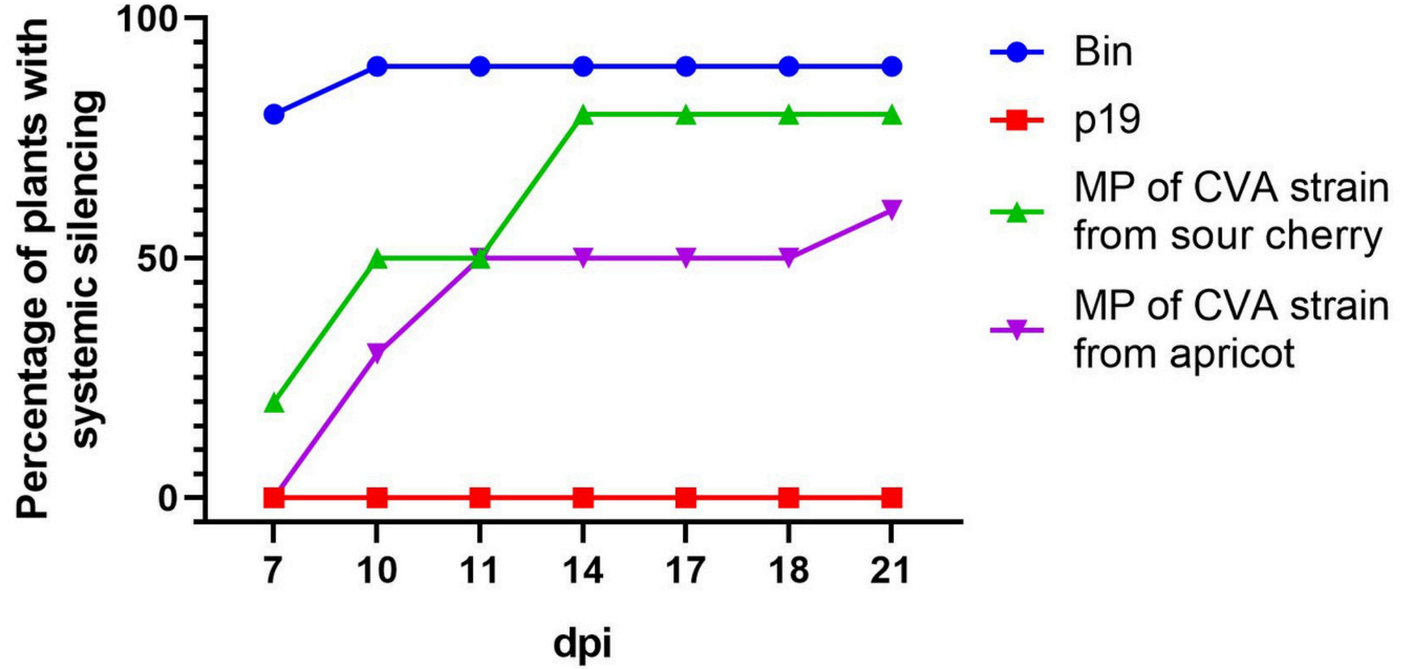
Red halo in apricot, sour cherry and - control

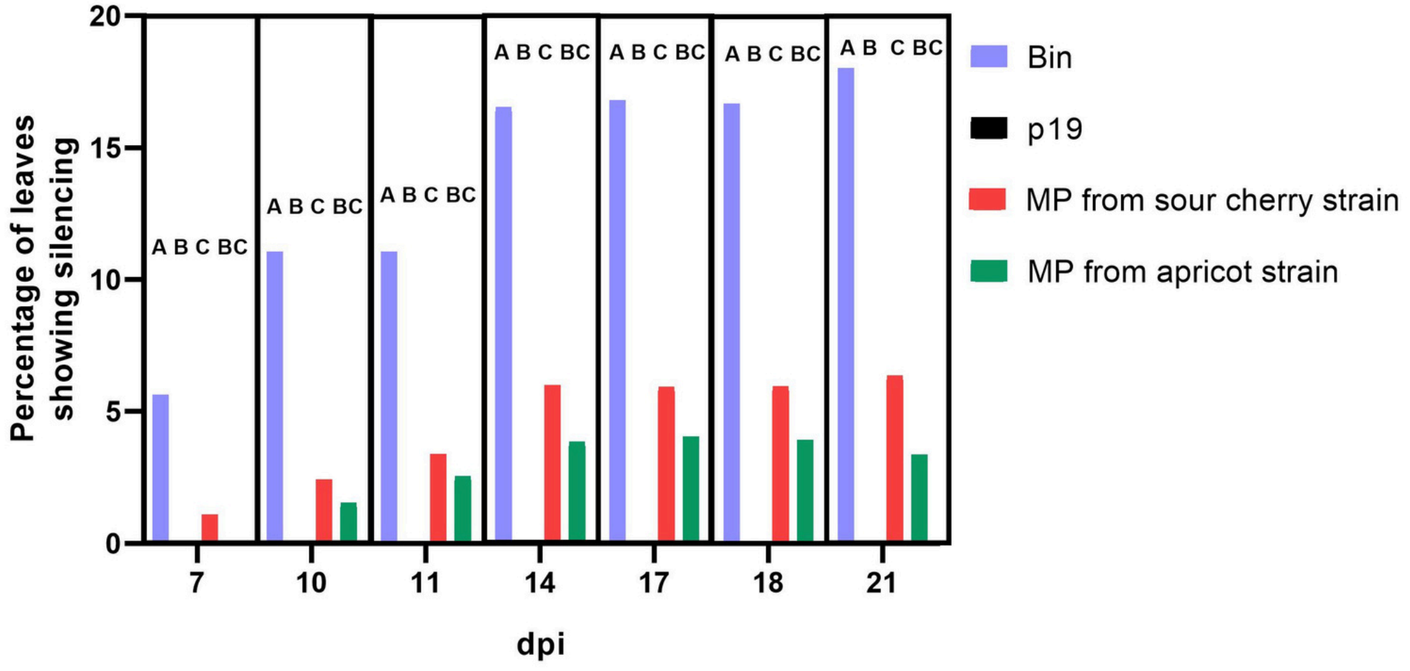
Red veins present in - control and in smaller amount in apricot and sour cherry

MP from sour cherry strain shows high systemic silencing from the 14 dpi

MP from apricot strain remains as a middle percentage between both controls

Higher percentage in - control comparing to + control or with MP from both strains





Silencing mechanism

↓

GFP degrades

→ **Visually detectable by a red fluorescence emitted by chlorophyll molecules**

Red halo around infiltrated patches or red veins in newly emerging leaves

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Sour cherry →

Low percentage of plants with systemic silencing
Higher from the 14 dpi

Low percentage of leaves that showed red veins

Weak suppressor of the short and long systemic silencing response

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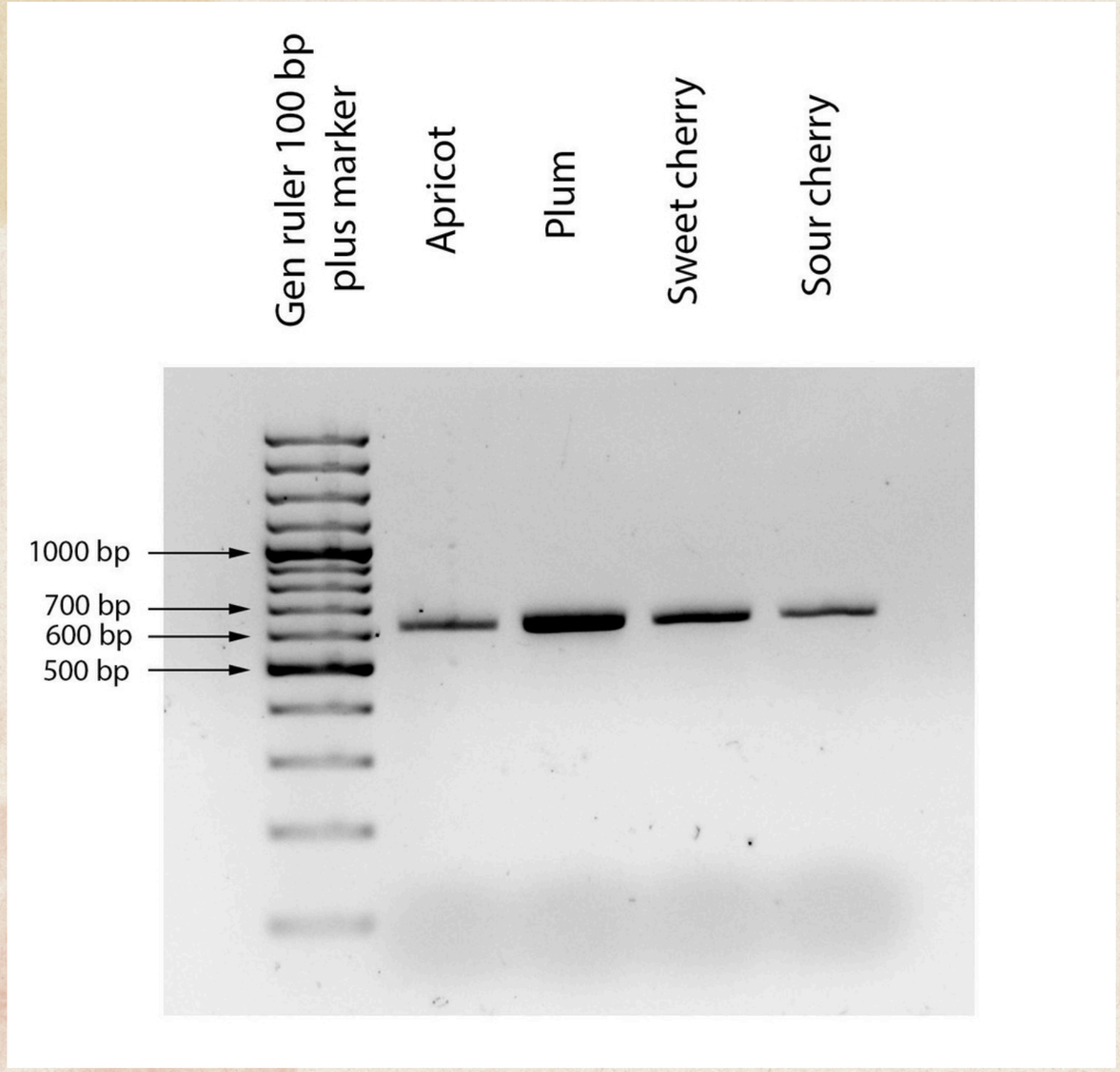
Apricot →

Percentage of plants showing systemic silencing mean between both controls

Low percentage of leaves that showed red veins

Weak suppressor of the short distance systemic silencing response, but moderate suppressor at long distance (stronger than sour cherry strain)

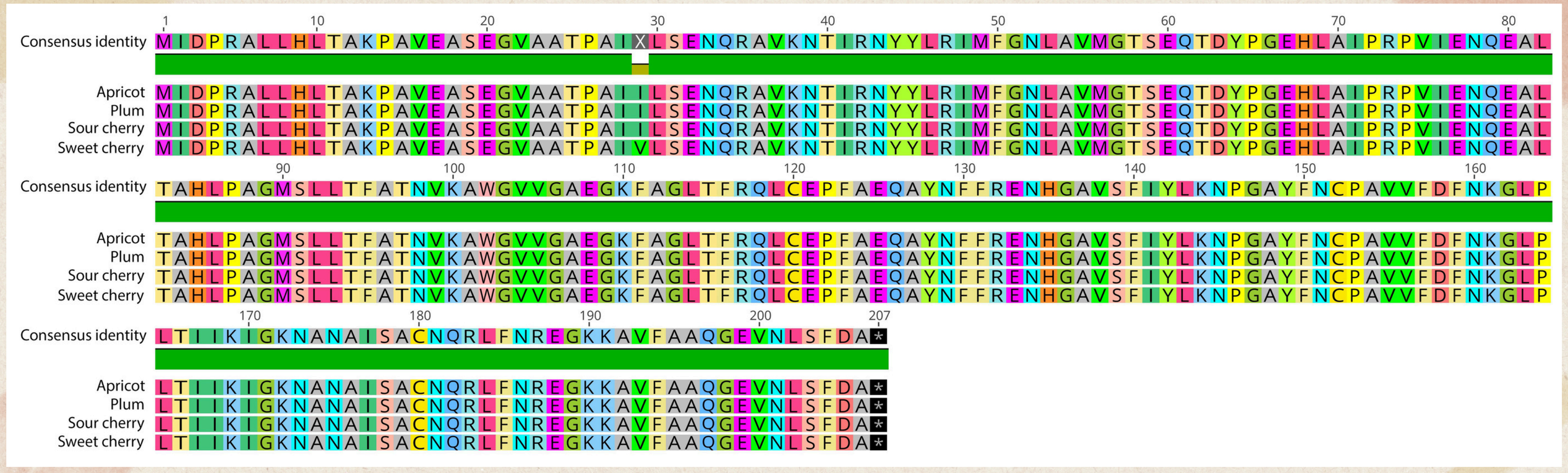
CP analysis



The CP coding region was successfully amplified

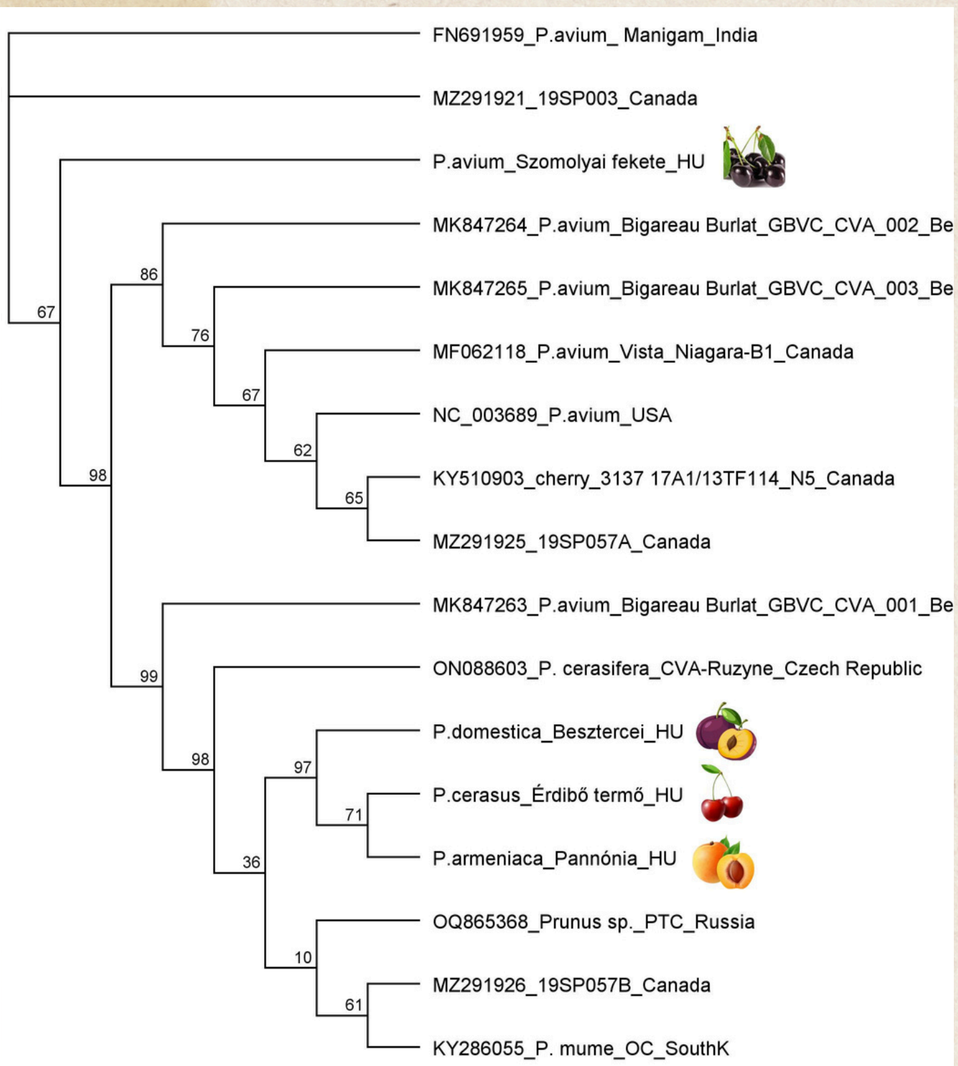
The size of the coat protein is around 600 bp

Sequences of CVA CP obtained from four Hungarian hosts are highly similar



One substitution in sweet cherry

Isoleucine (I) to Valine (V)



Distance Matrix

	P.armeniac...	P.avium_Sz...	P.cerasus_É...
P.armeniaca_Pannónia_...		86.312%	100%
P.avium_Szomolyai feket..	86.312%		86.312%
P.cerasus_Érdibő termő...	100%	86.312%	
P.domestica_Besztercei_...	99.678%	86.312%	99.678%

Plum, sour cherry, and apricot form a single cluster and sweet cherry is located in a distinct and distant cluster

Only one substitution at the amino acid level, but differences at the nucleotide level

Conclusions

MPs from sour cherry and apricot infecting CVA strains act as weak local VSRs

MP from CVA apricot strain showed stronger suppressor activity of the systemic signaling than the one of sour cherry strain, but not as strong as p19

- ✗ short distance spread of the silencing signal
- ✓ moderate suppressor of the long-distance spread

MP from sour cherry strain may act as a weak suppressor of the systemic response

- ✗ complete inhibition of mechanism of silencing
- ✓ delaying of spreading of silencing signal

Conclusions

MP sequences → sour cherry and sweet cherry strains
apricot and plum strains → **differences at amino acid level**

CP sequences → plum, sour cherry and apricot
sour cherry in different distant cluster → **differences at nucleoacid level**

Amino acid substitutions in MP sequences of CVA strains may potentially influence their different VSR activity.

Further research →

- **Characterization of steps of plant silencing pathway affected by MPs**
- **Exploring potential suppressor activity of CPs**

Conclusions

This study provides valuable insights into the role of MP proteins from CVA strains in the suppression of RNA silencing in plants, and these findings contribute to our understanding of viral adaptation and the mechanisms of RNA silencing in plants

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THANK YOU FOR YOUR ATTENTION

Gödöllő, July 2024