

The p20 and p23 silencing suppressors from Citrus tristeza virus are synergetic

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Citrus tristeza virus and Tristeza symptoms

a) Mild

b) seedling yellows

c) Quick decline



Photo from Lee RF (by Roistacher, C.N.)

d) Stem pitting

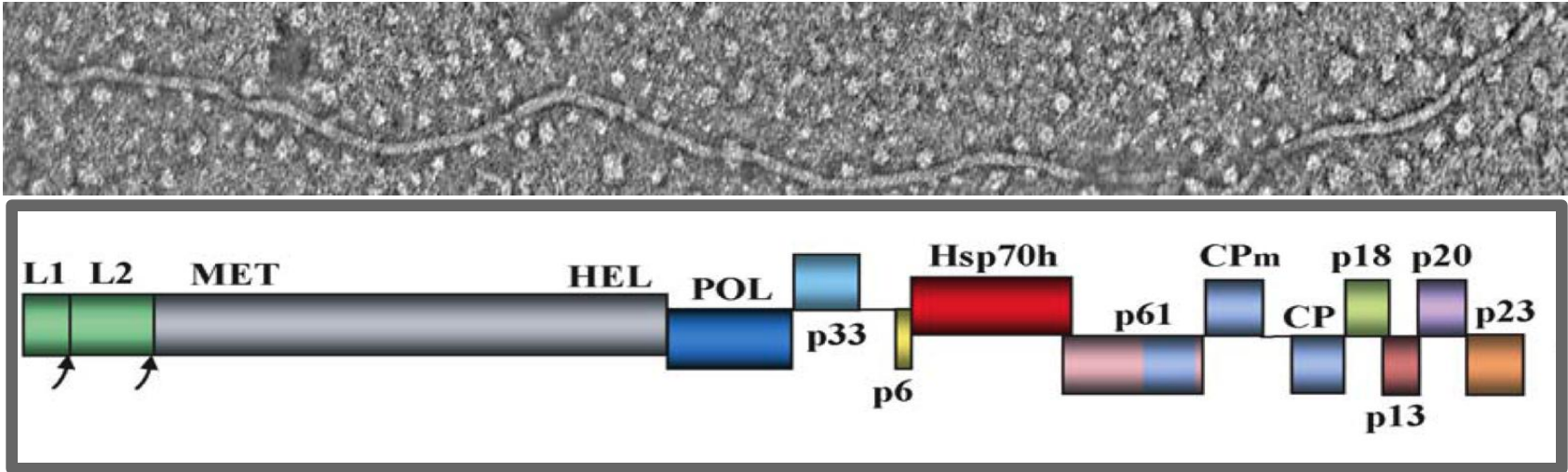


Images from Florida Division of Plant Industry Archive



Photo from Roistacher,

Introduction



***Citrus tristeza virus* (CTV) is a filamentous virus with \cong 20 Kb**
Closteroviridae family

Coat protein is formed by p25 (CP) and p27 (CPm)

CTV is transmitted by **man and by **aphids**:**

- *Toxoptera citricida* (Kirk.) is the most efficient

- *Aphis gossypii* (Glover).

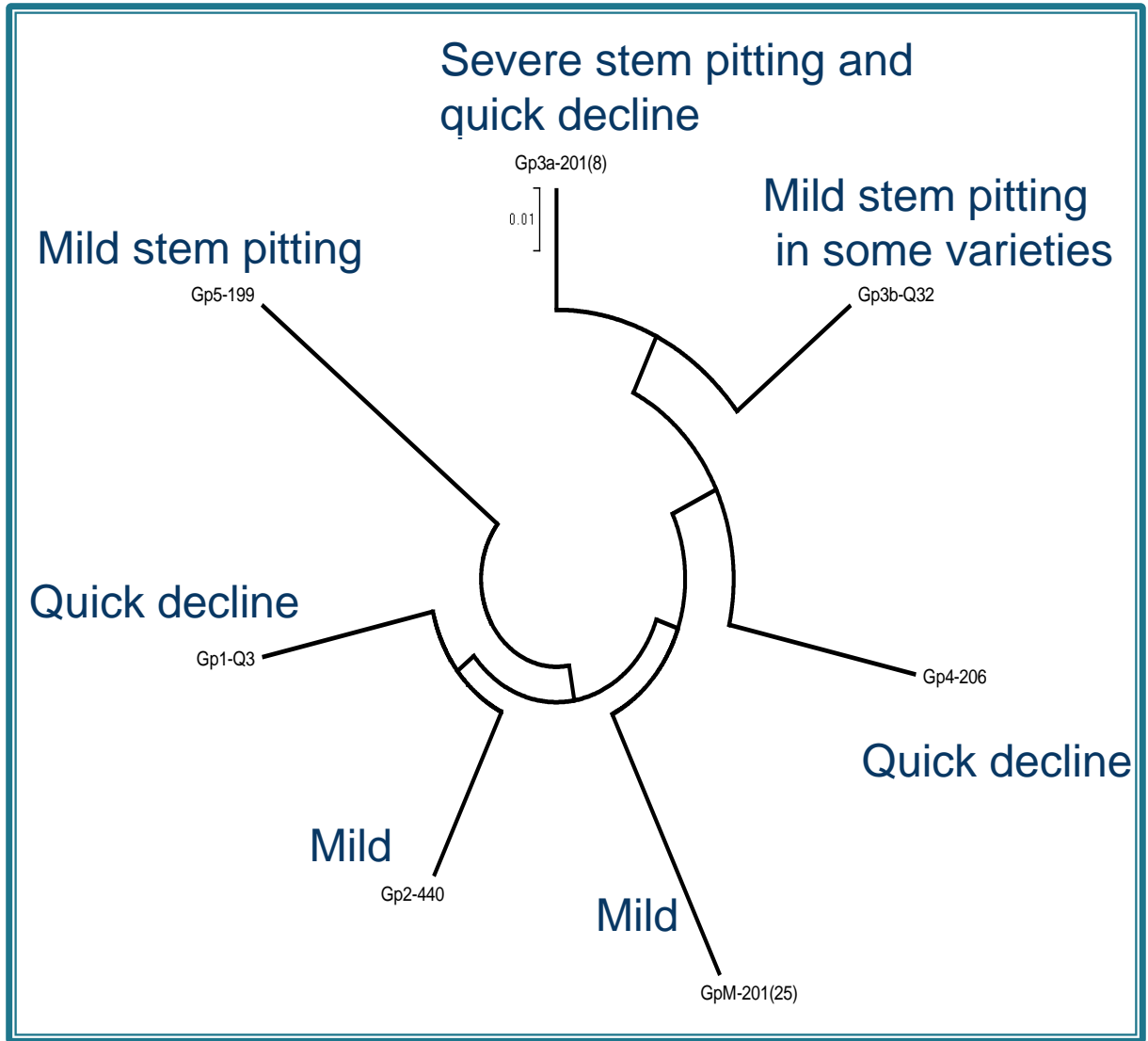
- *Aphis spiraecola*



Citrus tristeza virus

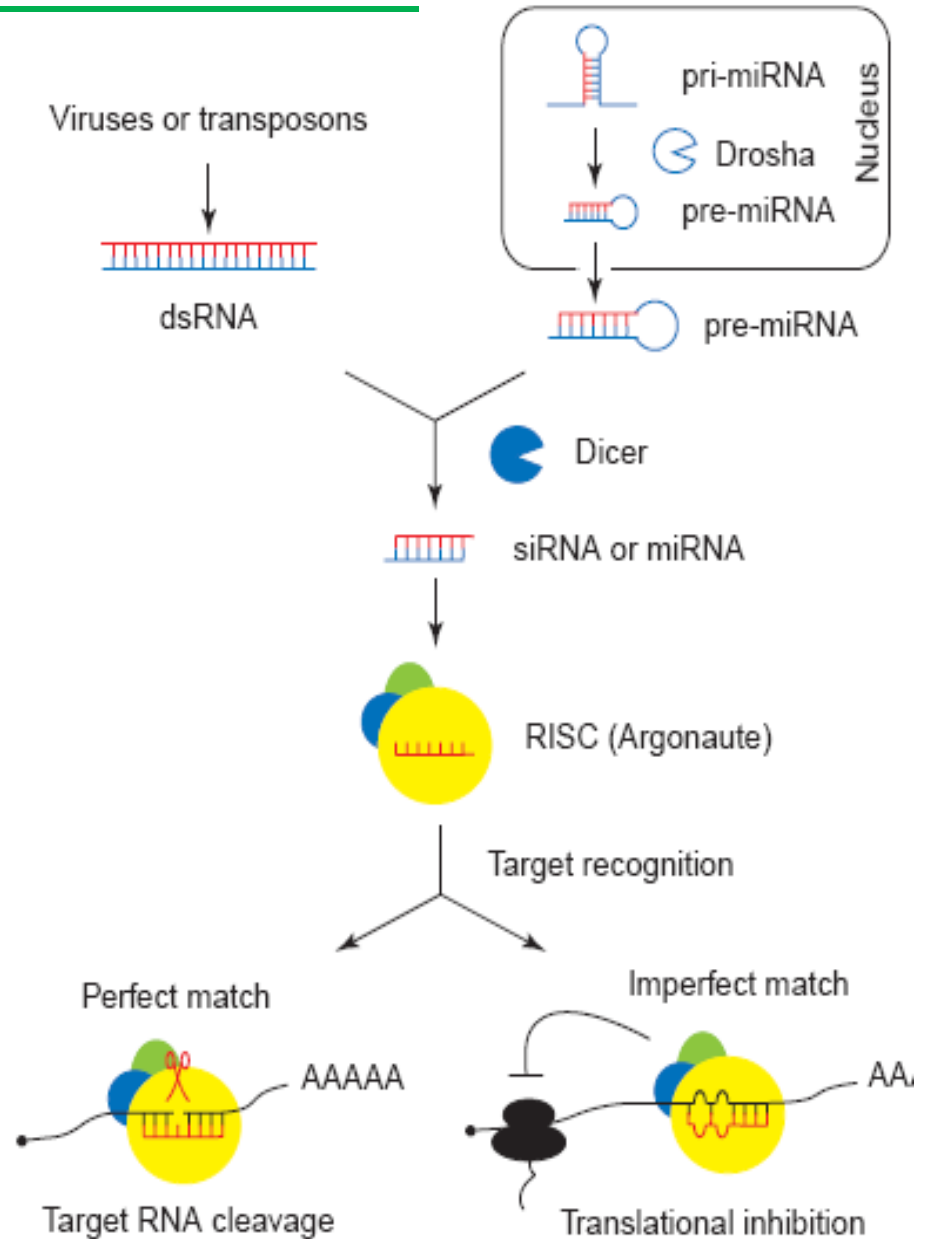
Seven phylogenetic distinct groups based on:

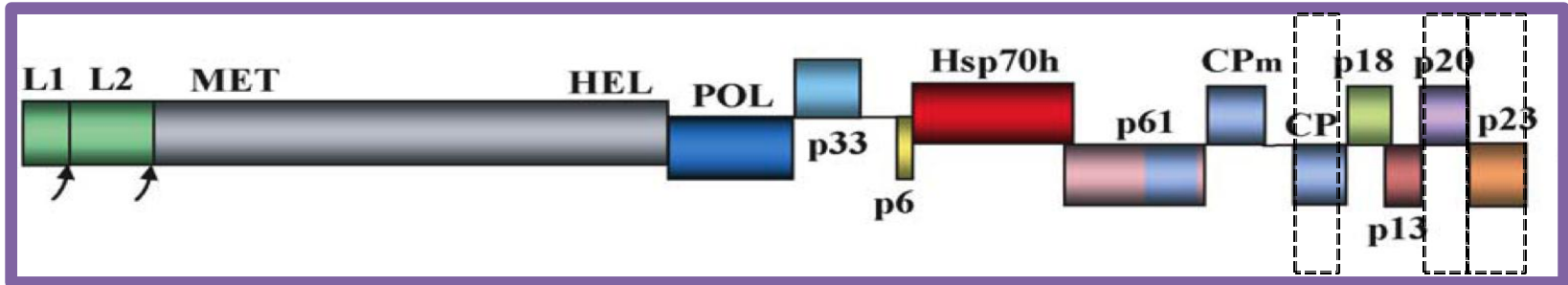
- a) nucleotide variability of the CP (p25) gene
- b) the same phylogenetic structure is maintained throughout the 3' region (p20, p23)
- c) distinct CTV groups are also related with different plant syndromes



RNA silencing mechanism

- a) Induced by dsRNA
- b) RNA is processed to small RNA fragments of 21-24 nt - - siRNAs -
- c) siRNA incorporates RISC complex.
- d) Complementary RNA sequences are degraded/ translation is blocked.





CTV genes with a special function: suppressors of the RNA silencing mechanism

CTV encodes at least three distinct suppressors of the RNA silencing mechanism : p25 (CP), p20 and p23 proteins

Protein CP (p25) – suppresses at the intercellular level
Lu et al., 2004, PNAS, vol.101, n°44, 15742–15747)

Protein p23 and p20 – suppresses at the intracellular level

The success of a viral infection results from the competition between the plant RNA silencing mechanism and the ability of the virus to suppress it.

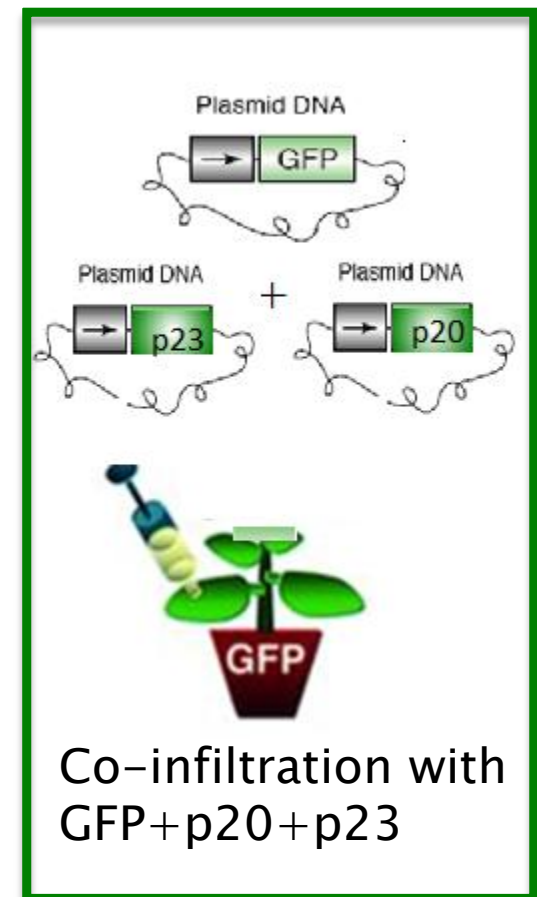
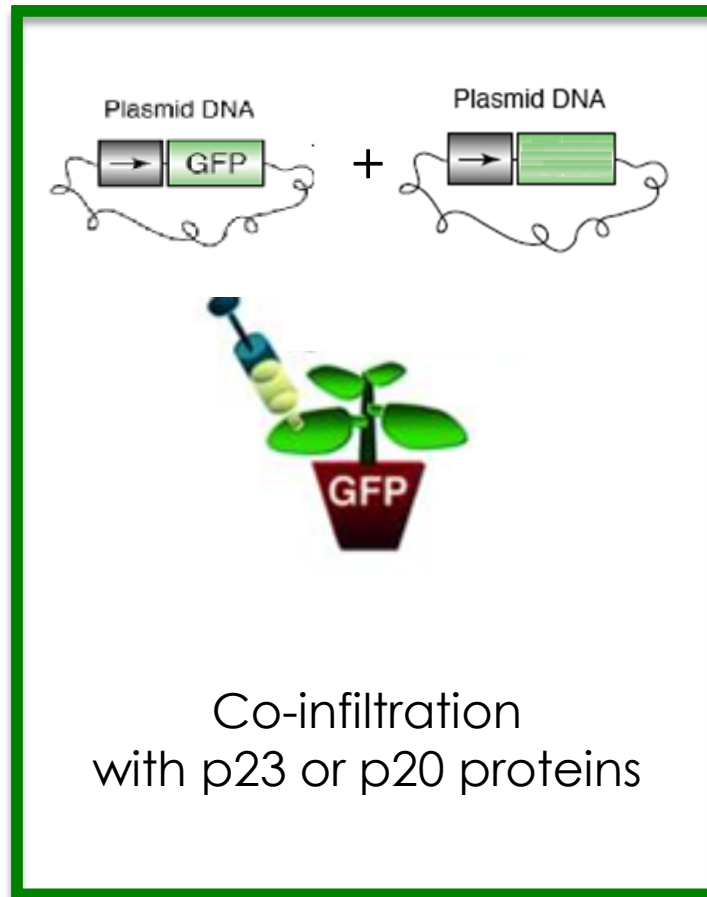
Although different p20 and p23 suppressor abilities were found between CTV groups, it was not possible to draw a relationship between the severity of the group and the ability of the suppressor protein.

Objective

To assay the suppressor activity when both p20 and p23 proteins act together

Methodology for transient expression

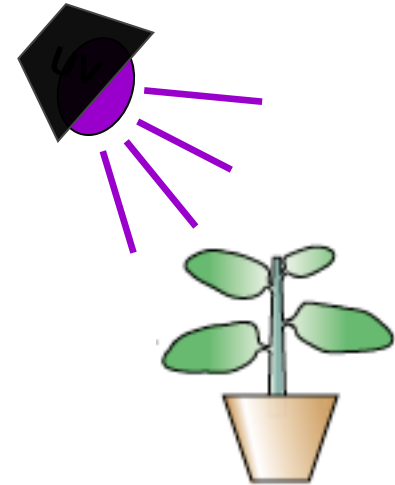
Agroinfiltration of *Nicotiana benthamina* line 16C



CTV Group	3a	M
p23 clone	11.5	25.4
p20 clone	11.4	25.11

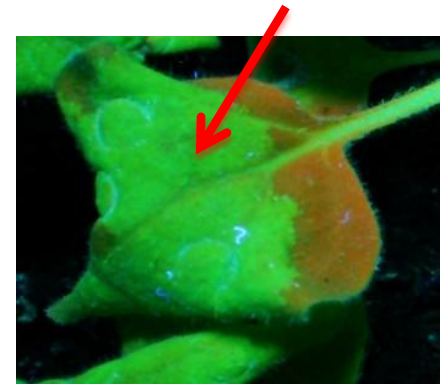
Differences on the suppressor activity were monitored by:

- UV observation
- *Northern blot*
- Real time RT-PCR



RNA was extracted from infiltrated leaf patches

5 days post-infiltration (dpi) / 9 days post-infiltration



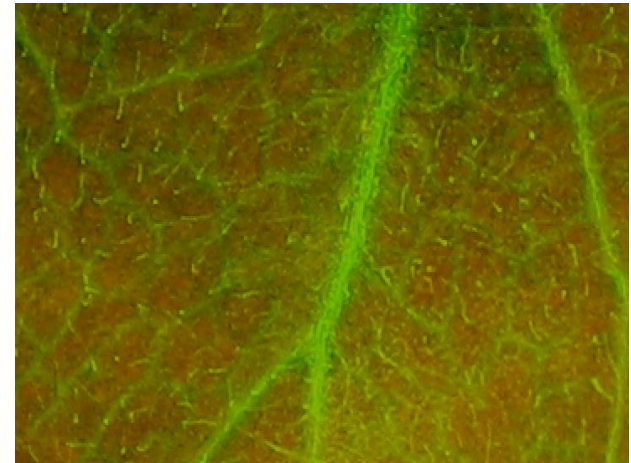
Results

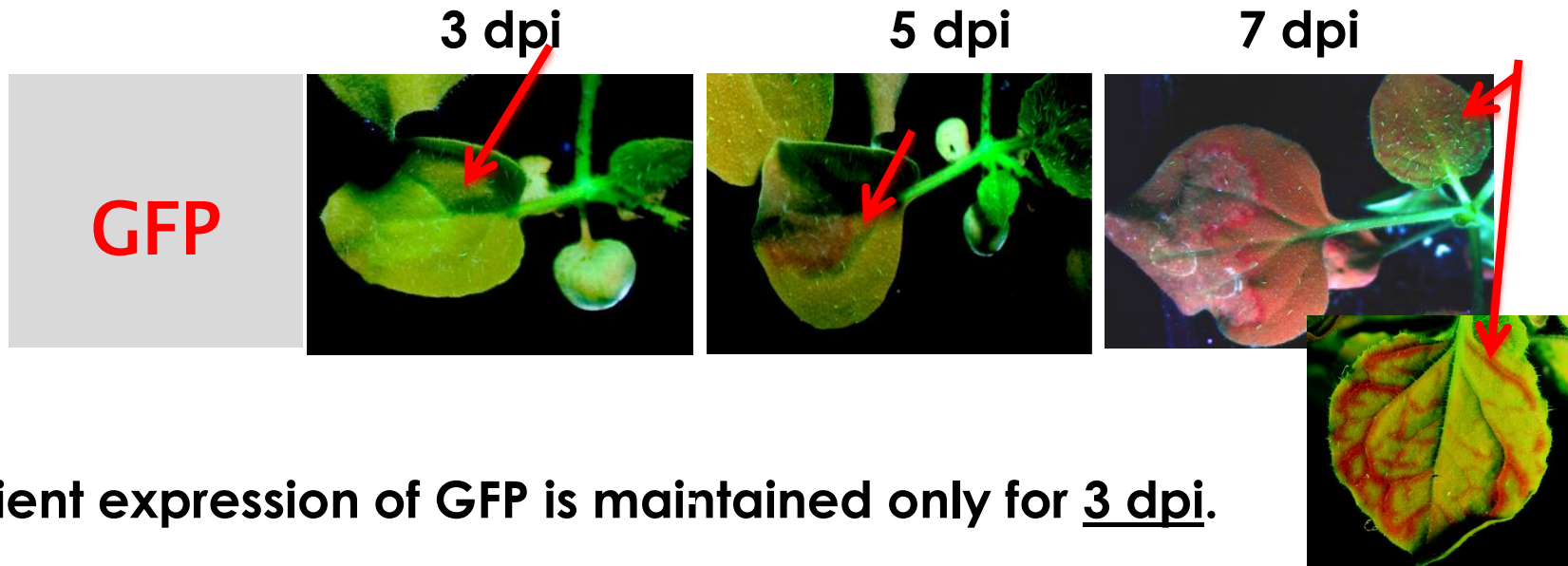
N. benthamiana plants
under UV light

WT



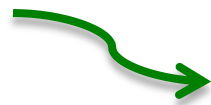
16C





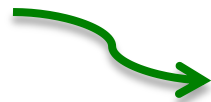
Transient expression of GFP is maintained only for 3 dpi.

At 5-6 dpi a red ring forms around the agroinfiltrated area



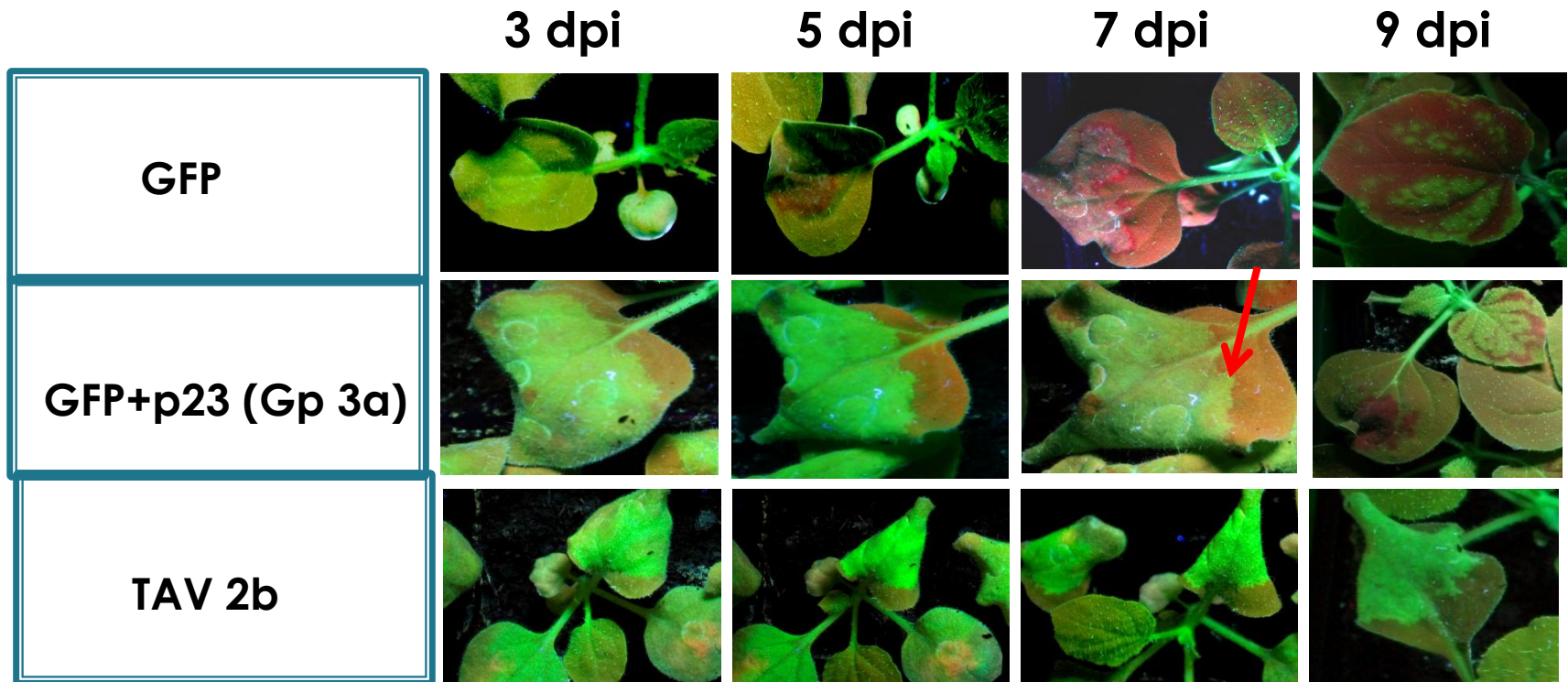
A mobile silencing signal (short-range) was formed and moves 10-15 cells around the infiltrated area.

At 6-8 dpi is observed the mobile silencing signal moving on vasculature.



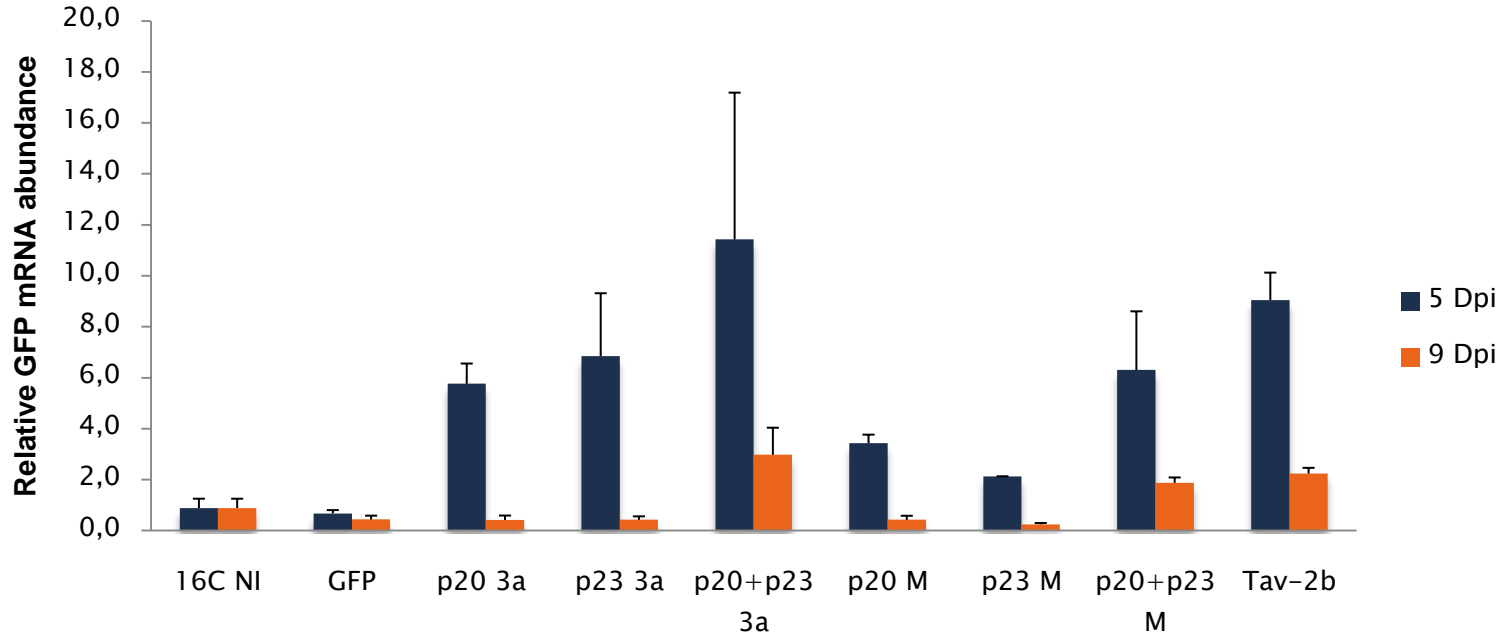
A mobile silencing signal (long-range) enters the vascular system and moves to new leaves

Results



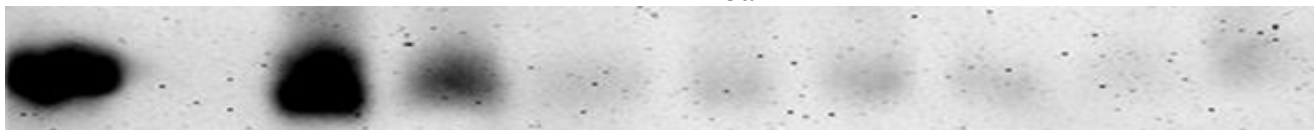
- In plants agro-inoculated with CTV suppressors is expected that transient expression of GFP is maintained longer.
- 7 dpi - local silencing was observed in all leaves co-inoculated with p20 (+GFP) or p23 (+GFP).

Real-time RT-PCR



Northern blot 5dpi

24 nt marker



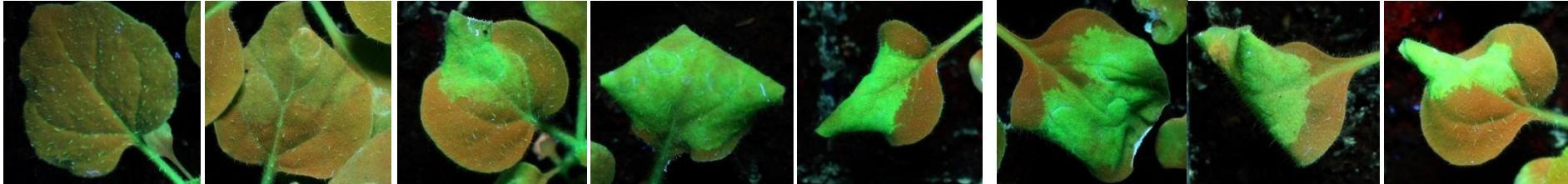
GFP siRNAs



5S RNA

- Non-inoculated 16C plants shows the normal level of GFP mRNA in plants; values obtained were used as a reference to determine the relative level of GFP mRNA in samples.
- Reference gene: ubiquitin.

Results



16C NI

GFP

p20_3a

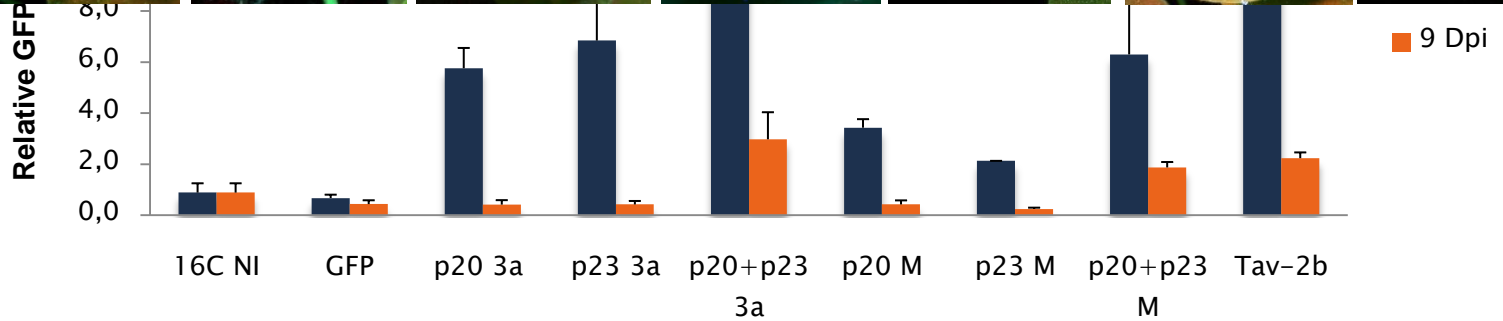
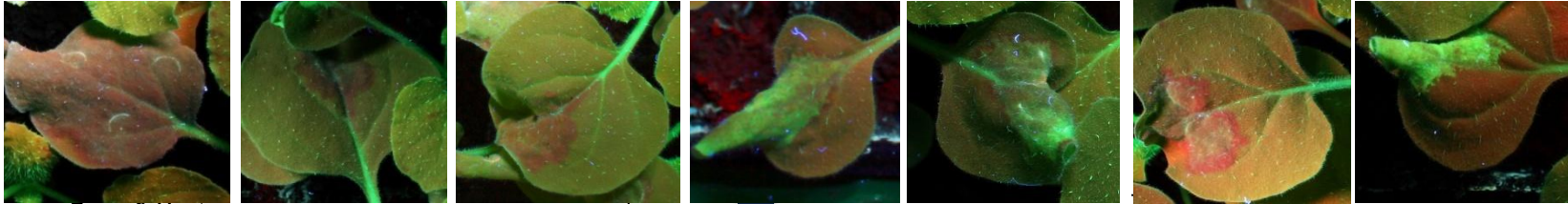
p23 3a

p20+p23_3a

p20M

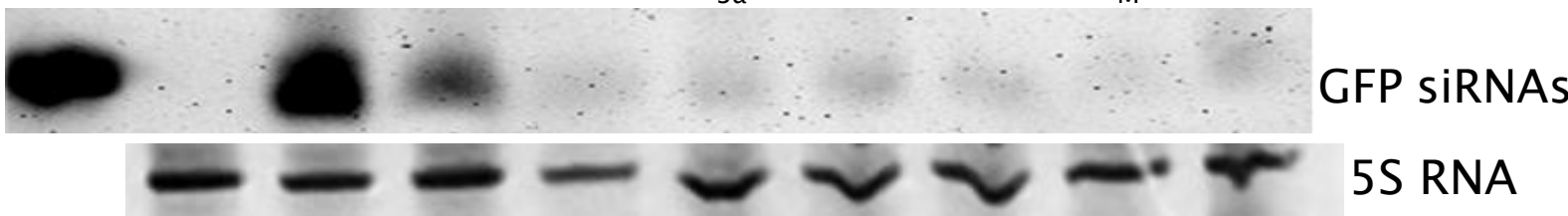
p23M

p20+p23M



Northern blot 5dpi

24 nt marker



• Observation of co-inoculation of GFP with p23 + p20 at 5 dpi and 9 dpi under UV light

Conclusions

Results point to a synergetic action when both p23 and p20 suppressors from CTV act together.



Acknowledgements



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