EVALUATION OF THE RNA SILENCING SUPPRESSION ABILITY OF THREE CHERRY VIRUS F ENCODED PROTEINS

L. Lotos*, A. Katsiani*, N.I. Katis and V.I. Maliogka

Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, 54124, Thessaloniki, Greece
(vmaliogk@agro.auth.gr)
*Both authors contributed equally

Introduction

RNA silencing is a natural defense mechanism of plants against viruses that degrades RNA in a sequence-specific manner. As a counter-defense, plant viruses encode one or more suppressor proteins interfering with the silencing pathway with different mechanisms (Baulcombe, 2004). Cherry virus F (CVF) (family *Secoviridae*, genus *Fabavirus*) is a recently identified sweet cherry (*Prunus avium*) and Japanese plum (*Prunus mume*) infecting virus (Koloniuk et al., 2018; Jo et al., 2021). CVF has a bipartite genome with each RNA coding for a polyprotein which, after translation, gets proteolytically processed into functional proteins. Studies on other fabaviruses and comoviruses have indicated that the three proteins (movement protein - MP, large coat protein - CPL, small coat protein - CPS) produced from RNA2 could act as possible RNA silencing suppressors (RSSs).

To screen proteins CPL, CPS and MP of CVF for a putative RNA silencing suppressor activity and to study the mechanisms they interfere with this defense pathway.

Materials & Methods

Molecular constructs and bacterial strains

- PCR amplification of CVF CPL, CPS and MP with primers carrying in the 5' and 3' prime ends the recognition sequence of the restriction enzymes *EcoRI* and *BamHI*, respectively. An ATG start codon was included in the forward primers of CPL and CPS and a TAA stop codon in the reverse primers of MP and CPL. Polyprotein cleavage sites were estimated based on genetically close viruses.
- Digestion of the amplicons and the plasmid vector pART7 with the restriction enzymes and cloning.
- Subcloning of the constructs 35S-CPL, 35S-CPS and 35S-MP into the binary plasmid vector pART27.
- Transformation of *Agrobacterium tumefaciens* C58C1 cells with the recombinant vectors [pART27-CPL, pART27-CPS and pART27-MP; Figure 1.A (III), (IV), (V)].

Plant material and GFP imaging

The Agrobacterium-mediated transient expression assay was performed on *Nicotiana benthamiana* wild type (WT) and genetically modified *N. benthamiana* line 16c plants, which express the green fluorescent protein (GFP). Infiltrated plants were observed daily for GFP fluorescence under UV lamp and photographed using a digital camera.

Techniques of identification and RNA analyses

- Co-infiltration of *Agrobacterium* cultures containing each construct and the controls with 35S-GFP (Figures 1.A and 2.A).
- Total RNA extraction from infiltrated patches of *N. benthamiana* collected 4, 5, 6 and 8 dpi were subjected to RT-qPCR for the presence of GFP mRNAs using L23 as the reference gene (Orfanidou et al., 2019).

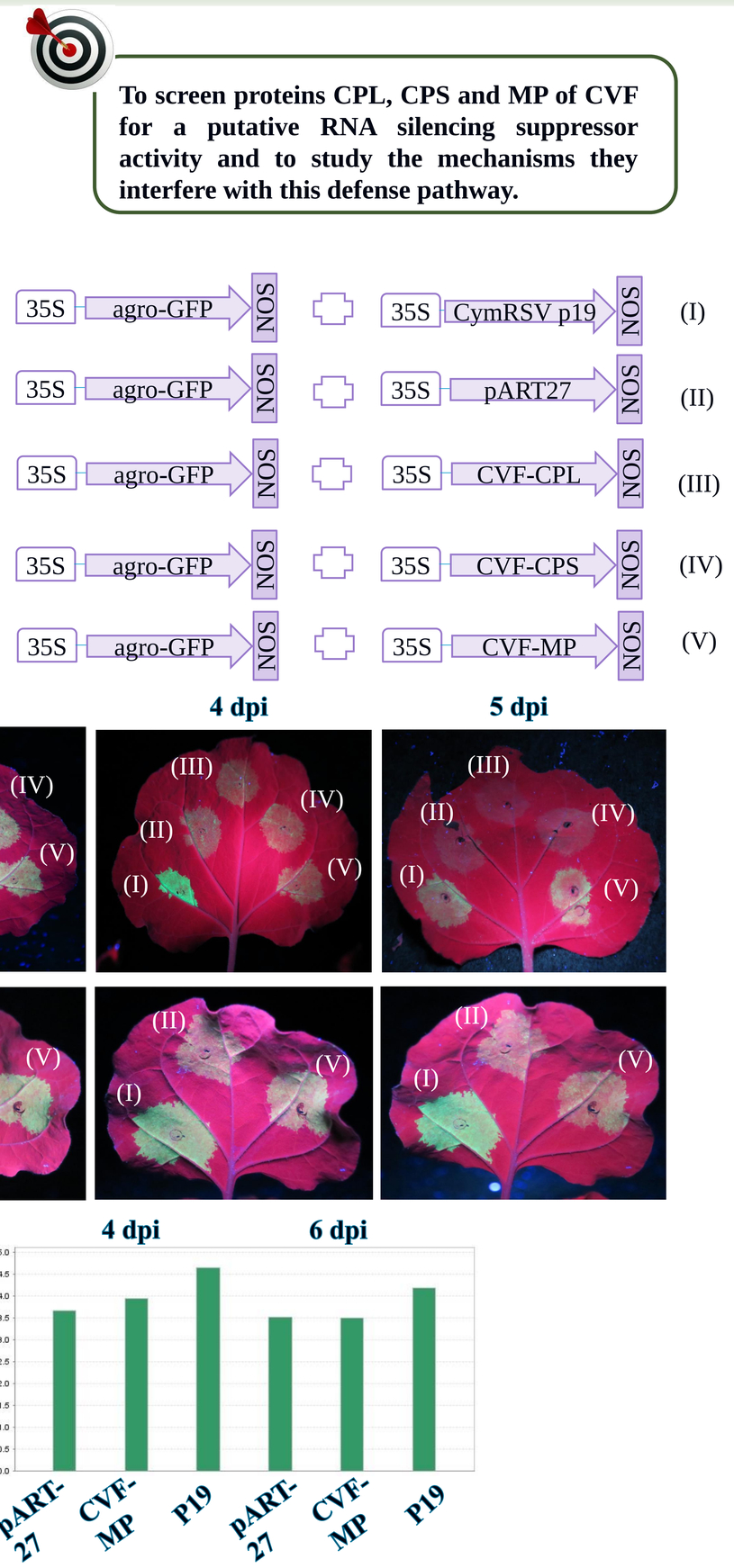

Figure 1. Evaluation of CVF-CPL, CPS and MP as suppressors of ssRNA-induced RNA silencing of GFP in *Nicotiana benthamiana* wild type plants. **A:** *N. benthamiana* leaves infiltrated with mixtures of *A. tumefaciens* cultures harboring 35S-GFP in combination with CymRSV p19 (positive control) (I), pART27 empty vector (II) or with constructs expressing CVF-CPL (III), CVF-CPS (IV), CVF-MP (V). **B:** GFP fluorescence in agroinfiltrated *N. benthamiana* WT plants. The constructs and the 35S-GFP were diluted to 0,45-0,55 O.D. and were mixed in a 1/1 ratio. UV light images were taken 3, 4 and 5 days post-infiltration (dpi). **C**: Relative quantitation values (log10) for the expression of GFP in 4 and 6 dpi. Only a few replicates infiltrated with CVF-MP exhibited increased GFP levels relative to pART27 at 4dpi. After 6dpi GFP levels of pART-27 and CVF-MP were the same.

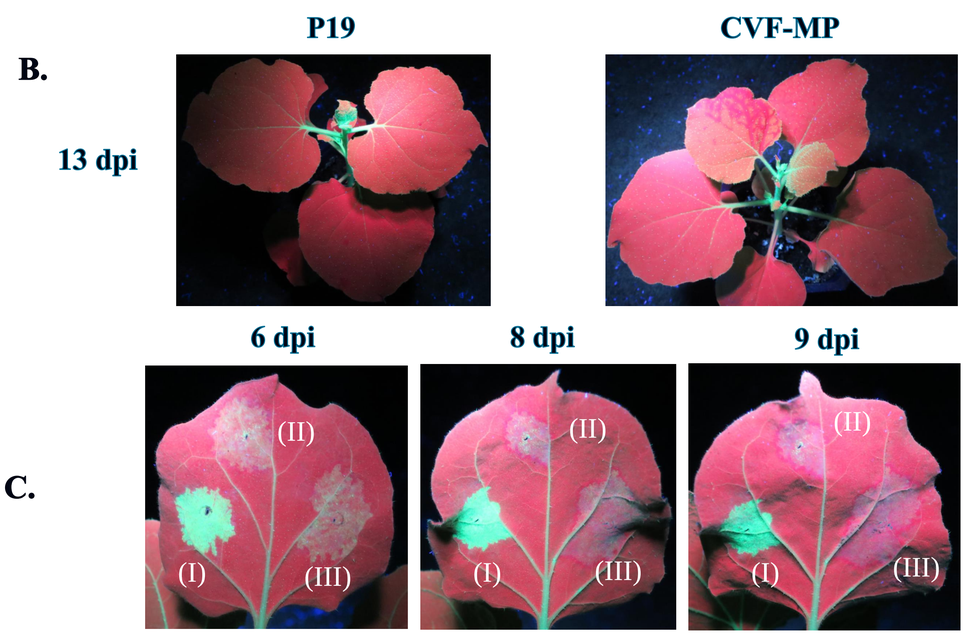

Figure 2. Impact of CVF-MP in cell-to-cell and systemic movement of the GFP silencing signal in *N. benthamiana* 16c line. **A.** Co-infiltration of the leaves with mixtures containing the molecular constructs for the expression of GFP and P19 (I), the empty vector pART27 (II) or CVF MP (III). **B.** Systemic sense-PTGS results. Three *N. benthamiana* 16c plants infiltrated with each construct. UV light images were taken 13 dpi. Systemic silencing was present in CVF-MP infiltrated plants **C.** Cell-to-cell movement of silencing signal results. Eight plants were infiltrated with all three constructs. UV light images were taken at 6, 8 and 9 dpi. Infiltration with CVF MP resulted in the development of a red halo around the infiltration spot.

Results, Discussion & Future work

- ✓ **MP** of CVF **is a potential suppressor** of the RNA silencing pathway (Figure 1B, C). However, GFP expression was maintained only in a limited number of plants. CVF-CPL and CPS do not exhibit a silencing suppression capacity.
- ✓ MP **was not able to prevent the cell-to-cell or long-distance spread** of the RNA silencing signal of GFP (Figure 2B/C)
- ✓ Screening for additional CVF encoded proteins that might possess RNA-mediated silencing suppressor activity

References

- Baulcombe, D., (2004). Nature 431, 356-363.
- Jo, Y. et al., (2021). Plant Dis. 105(4), 1232.
- Koloniuk, I. et al., (2018). Viruses 10(4), 204.
- Orfanidou C. G. et al., (2019). Arch. Virol. 164:2747–2759

FUNDING
This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Identification of proteins implicated in the suppression of RNA silencing and study of their mode of action for two new sweet cherry viruses" (MIS 5047873).

ACKNOWLEDGMENTS We would like to thank prof K. Kalantidis and Dr. K. Katsarou for providing the GFP, pART27 and P19 constructs as well as seeds for the 16c plants.