Localization of EMARaV proteins *in planta* by *Agrobacterium*-mediated transformation

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Inferences about the function of the p4 protein of EMARaV are expected by investigating the localization of the protein *in planta*.

Introduction

European mountain ash ringspot-associated virus (EMARaV) infects European mountain ash (*S. aucuparia* L.) and causes chlorotic ringspots and mottling of leaves (Figure 1). The virus is suspected to influence the decline of branches and even the entire tree (Benthack et al., 2005). EMARaV is composed of four ss(-)RNA genome segments and is assigned to the genus *Emaravirus* (Mühlbach & Mielke-Ehret, 2011). Each of the four viral RNAs is coding for one protein (p1-p4). Currently it is unknown, which function the RNA4 encoded p4 protein adopts in the infection process. For many plant viruses the existence of a gene silencing suppressor and a movement protein is essential. These functions could not be associated with the proteins encoded by RNA1-RNA3. It can be assumed that EMARaV RNA4 encodes a gene silencing suppressor and/or a movement protein (MP).

Figure 1: EMARaV infected rowan with symptoms of mottling and chlorotic ringspots on leaves.

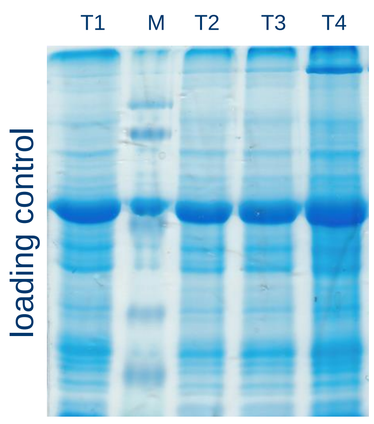
M & M

For localization purposes the p4 protein of EMARaV were C-terminal fused with gfp. Additionally, the MP of the *Tomato spotted wilt virus* (TSWV) was cloned as a reference for putative movement function of EMARaV p4. Agrobacteria were transformed with gfp constructs and *Nicotiana benthamiana* leaves were subsequently agroinfiltrated. Expression of the gfp-fused construct were checked by western blot analysis by use of an anti-gfp antibody (Roche).

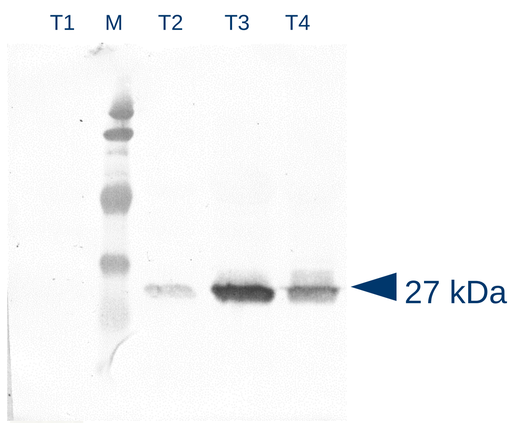
For Co-localization a construct of the MP of *Cucumber mosaic virus* fused with a red fluorescence protein (CMV_MP::RFP) was used. Plasmolysis treatments were conducted by incubation of small leaf sections (diameter of 0.5 cm) in 4% NaCl for 20 min according to Oomen et al., 2011. Confocal laser scanning microscopy was used for localization of viral proteins.

Results & Discussion

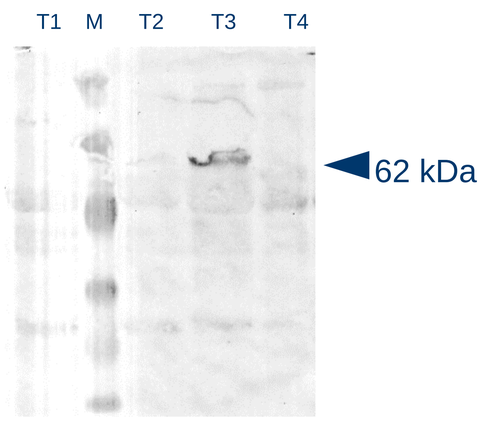
Western Blots



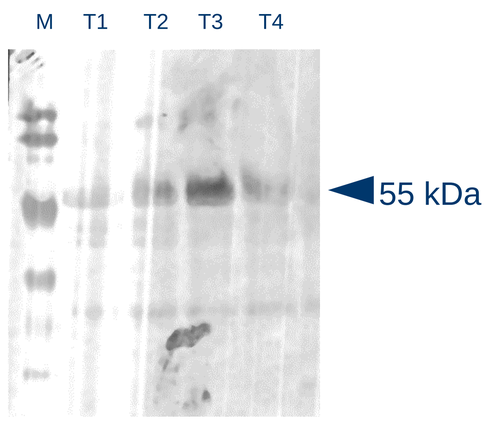
agroinfiltrated *N. b.* leaves



gfp infiltrated *N. b.* leaves



NSm::gfp infiltrated *N. b.* leaves



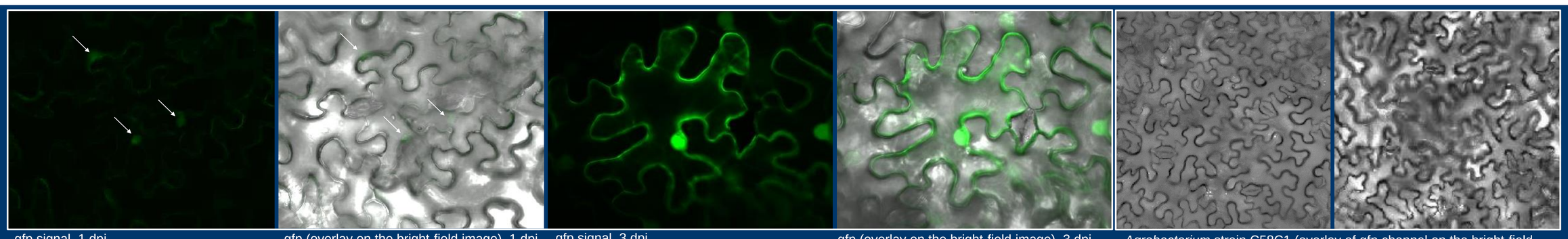
p4::gfp infiltrated *N. b.* leaves

N. benthamiana leaves sampled:

T1…24 h
T2…48 h
T3…72 h
T4…6 days

after agroinoculation

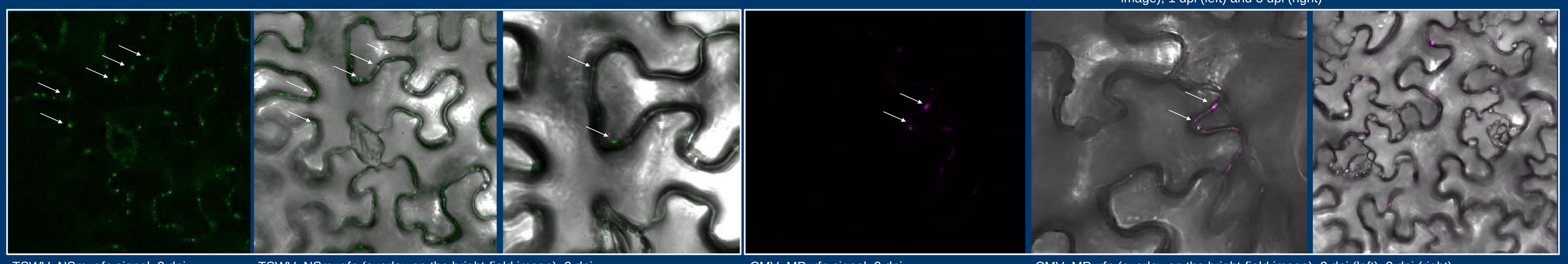
Controls



gfp signal, 1 dpi | gfp (overlay on the bright-field image), 1 dpi | gfp signal, 3 dpi | gfp (overlay on the bright-field image), 3 dpi

Agrobacterium strain C58C1 (overlay of gfp channel on the bright-field image), 1 dpi (left) and 3 dpi (right)

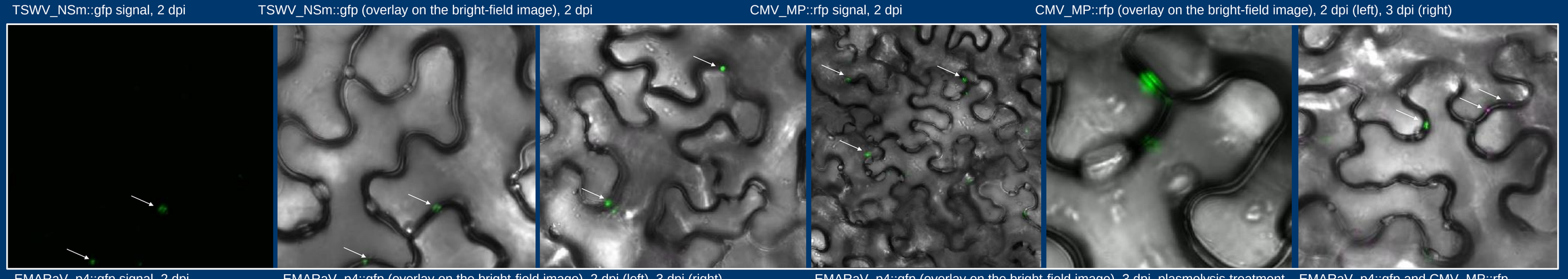
Known MPs



TSWV_NSm::gfp signal, 2 dpi | TSWV_NSm::gfp (overlay on the bright-field image), 2 dpi

CMV_MP::rfp signal, 2 dpi | CMV_MP::rfp (overlay on the bright-field image), 2 dpi (left), 3 dpi (right)

EMARaV p4



EMARaV_p4::gfp signal, 2 dpi | EMARaV_p4::gfp (overlay on the bright-field image), 2 dpi (left), 3 dpi (right) | EMARaV_p4::gfp (overlay on the bright-field image), 3 dpi, plasmolysis treatment | EMARaV_p4::gfp and CMV_MP::rfp coinfiltration (overlay of all channels), 2 dpi

- Expression in *N. b.* of gfp fusion constructs of EMARaV p4 protein and TSWV NSm protein were confirmed with an anti-gfp antibody in western blot analysis
  - p4 protein was expressed 24 hours after agroinoculation
  - A significant expression of NSm was observed after three days
- Both proteins could be localized after two days by CLSM
- TSWV NSm and CMV MP showed a regular distribution of punctuated spots
  - along the cell wall, which is typical for virus MPs
- The p4 protein is located as punctuated spots at the cell wall, too
  - But it is not regular distributed along the whole cell wall
- Plasmolysis treatment did not affect localization of p4 at the cell wall
- Co-localization of EMARaV p4 or TSWV NSm with the MP of CMV was not successful
  - Co-infiltrated constructs were found solely in different cells

References

Benthack W, Mielke N, Büttner C, Mühlbach H-P. 2005. Archives of Virology 150: 37-52.
Mühlbach H P, Mielke-Ehret N. 2011. Emaravirus. Elsevier Academic Press, San Diego/USA: 767-770.
Oomen R, Séveno-Carpentier E, Ricodeau N, Bournaud C, Conéjéro G, Paris N, Berthomieu P, Marquès L. 2011. New Phytologist 192:140-150.

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