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Transmission studies of *European mountain ash ringspot-associated virus* (EMARaV) to putative new host plants

H. L. Dieckmann[1], J. Roßbach[1], H.-P. Mühlbach[2], S. von Bargen[1], C. Büttner[1]
[1]*Humboldt Universität zu Berlin, FG Phytomedizin, Berlin, Germany*
[2]*Universität Hamburg, Biology, Hamburg, Germany*
dieckmann.luisa@yahoo.de

Introduction: Since more than 50 years European mountain ash trees (rowans, *Sorbus aucuparia*), display chlorotic ringspots and leaf mottling. These symptoms are associated with the *European mountain ash ringspot-associated virus* (EMARaV), a (-)ssRNA virus with 4 RNAs which is the type member of the genus *Emaravirus*. In 2013, EMARaV was detected in whitebeam (*Sorbus aria*) and Swedish whitebeam (*Sorbus intermedia*) for the first time. Other potential host species for the virus remain unknown. EMARaV was so far only transmitted successfully between rowan trees by grafting.
Objective: The known host species of EMARaV belong to the family *Rosaceae*. It was attempted to infect herbaceous *Rosaceae* and *Nicotiana* plants with the virus by mechanical inoculation.
Materials and methods: Leaves of EMARaV infected *S. acuparia* were collected locally. *Nicotiana rustica*, *N. benthamiana*, strawberry plants (*Fragaria sp*.), Lady's mantle (*Alchemilla vulgaris*) and *Potentilla megalantha* were mechanically inoculated with the diseased plant material.
EMARaV infected plant material was homogenized with an abrasive and Norit buffer, 2 % nicotine solution or phosphate inoculation buffer with 2 % nicotine and applied manually onto the leaves of the potential host plants. Alternatively, the infected material was rubbed onto the leaves using an abrasive only. Serial passaging was performed after 7 days. For detection RNA was isolated from the leaf material using the *Invitrap* Spin Plant RNA kit (STRATEC). Reverse transcription was conducted using random hexamer primer. EMARaV detection via PCR was processed according to Mielke et *al*., 2008.
Results: The majority of plants showed neither virus-specific nor stress symptoms after mechanical inoculation. One *N. rustica* plant displayed leaf mottling after dry inoculation as well as one *N. benthamiana* after inoculation with phosphate inoculation buffer with 2 % nicotine. An infection with EMARaV could not be confirmed in analysis in symptomatic as well as symptomless plants.
Conclusion: It was not possible to transmit EMARaV to a new host plant species testing various inoculation methods and a diverse set of herbaceous biotest plants. This confirms that transmission of the virus from infected rowans by mechanical means, even to related plant species is not possible, which may be due to the narrow host range of the pathogen.

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Translation initiation studies of the polyproteins encoded by RNA1 and RNA2 of *Cherry leaf roll virus*

M. Breuhahn, S. von Bargen, M. Rott, J. Langer, C. Büttner
Humboldt Universität zu Berlin, FG Phytomedizin, Berlin, Germany
mathias.breuhahn@gmx.de

Introduction: *Cherry leaf roll virus* (CLRV) is a nepovirus of the family *Secoviridae*. The bipartide genome of CLRV consists of positive single stranded RNA serving as mRNA and each encoding one polyprotein P1 and P2, respectively. The pathogen displays various genetic polymorphisms due to its worldwide distribution and wide host range including deciduous and fruit tree species. Sequence variabilities also occur within the 5' terminal regions of the viral RNAs. Some CLRV isolates possess a single startcodon whereas others contain a 2nd in frame ATG in both genome segments which could also serve as translation initiation sites.
Objective: The objective of this study was to ascertain which start codon is utilized to initiate translation of P1 and P2 from the genome segments of CLRV.
Materials and methods: The 5´ terminal regions of RNA1 and RNA2 of CLRV isolates originating from 2 differing phylogenetic groups were amplified, cloned into pJet1.2 and sequenced prior to subcloning into the expression vector pET28a(+) containing a T7 promoter region. The pJet1.2 constructs served as templates to insert point mutations by overlap extension PCR disrupting either the first, second or both startcodons in the 5´ terminus of RNA1 and RNA2 of the virus strains. Mutated 5´ terminal fragments were also cloned into pET28a(+) and sequenced. A coupled transcription/translation system in combination with a non-radioactive detection system (Promega) was used to express peptides from the wild type constructs as well as from the mutated 5´ termini of CLRV-RNA1 and RNA2 lacking one or two functional ATG sites. Samples were further analyzed by Western Blot after size separation by SDS PAGE.
Results: Constructs for analyses of translation initiation of CLRV-RNA1 and -RNA2 from different virus isolates were successfully prepared with the described cloning strategy. Sequencing confirmed the correct insertion of mutations without alterations of the reading frame. Results of peptide expression from the CLRV-5´ terminal constructs are presented and interpreted in regard to the identification of ATG sites used for expression of P1 and P2.