Generation of interspecies recombinants of sugar beet infecting poleroviruses *Beet chlorosis virus* (BChV) and *Beet mild yellowing virus* (BMYV)

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Introduction:

Two of the economic relevant virus species of the sugar beet virus yellows complex in Northern Europe are *Beet chlorosis virus* (BChV) and *Beet mild yellowing virus* (BMYV). These species belong to the genus *Polerovirus* and are transmitted by aphids in a persistent, non-propagative manner, *Myzus persicae* being the most important vector. The single stranded RNA viruses are restricted to the phloem tissue. Sequence homology analysis revealed that highest variability appears in the 5´ end of the genome and the C-terminus of ORF5 leading to different adaptions to their environment (Tab. 1). Thus, the host plant range is clearly distinguishable. The model plant *Nicotiana benthamiana* represents a host for BMYV, but not for BChV. This suggests that the factors of host specificity are located in the variable regions of the genome.

Aim of the project:

To investigate factors of host specificity, recombinant cDNA full-length clones should be generated by exchanging viral open reading frames (ORFs) between BMYV and BChV.

Materials and methods:

Infectious cDNA full-length clones of BMYV (Stephan and Maiss, 2006) and BChV (Wetzel *et al*., 2018) were used to produce interspecies recombinants. Recombination was achieved by exchange of BMYV ORF 0-2 and ORF3a-5 into the BChV plasmid backbone (Fig. 1). The cDNA full-length clones were constructed by Gibson Assembly for *Agrobacterium tumefaciens* mediated infection in *N. benthamiana*. Viral replication was analyzed by ELISA, systemic spread additionally by RT-PCR.

Tab. 1: Sequence homologies of BMYV (DQ132996.1) and BChV (MH271171.1) open reading frames (ORF).

Sequence	ORF0	ORF 1+2	ORF 3a	ORF 3	ORF 4	ORF 5
Nucleotide level (%)	21.1	41.3	91.9	92.9	93.2	85.1
Amino acid level (%)	15.9	43.8	62.5	92.1	88.6	86.9

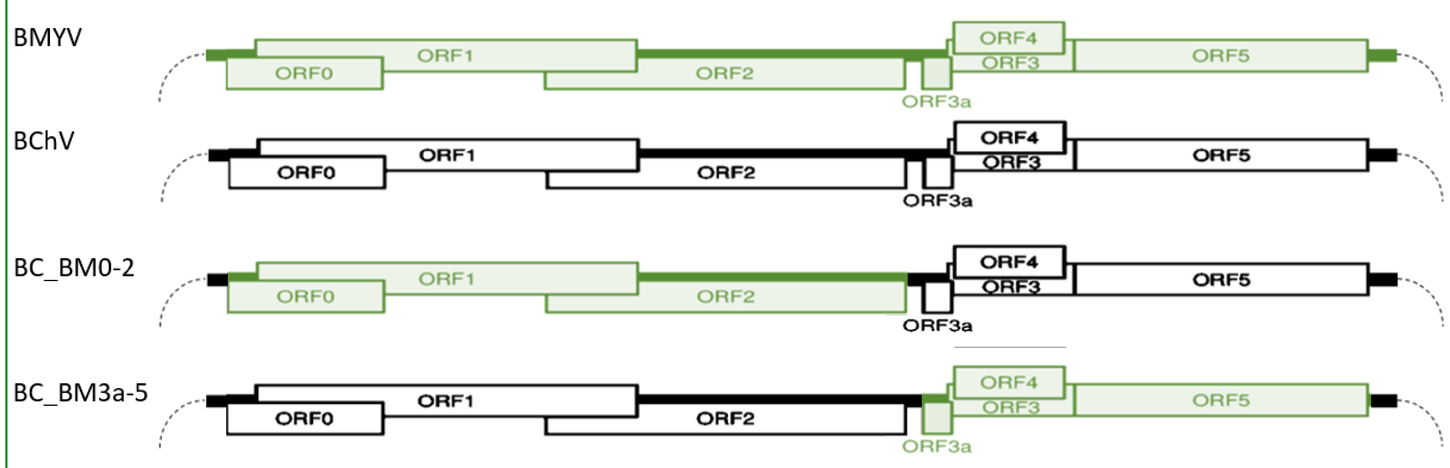

Fig. 1: Cloning strategy for the construction of BMYV and BChV interspecies recombinants.

Results:

BMYV, BC_BM0-2 and BC_BM3a-5 caused local plant response in the infiltrated leaf patch (Fig. 2). It could be shown, that beside the infectious cDNA full-length clones of BMYV and BChV, both recombinant viruses are able to replicate at the infiltration site (Fig. 3). Systemic infection could be detected by ELISA in BMYV, but only very low OD values could be shown for BC_BM0-2. For BC_BM3a-5 values were close to 0, as it was also shown for BChV. Using the more sensitive RT-PCR analysis, systemic infection could only be shown for BMYV and for BC-BM0-2 single plants (Fig. 4).

Fig. 2: Local plant response of BC_BM0-2 in *N. benthamiana* 9 days after agrobacterium inoculation compared to a non-infected control.

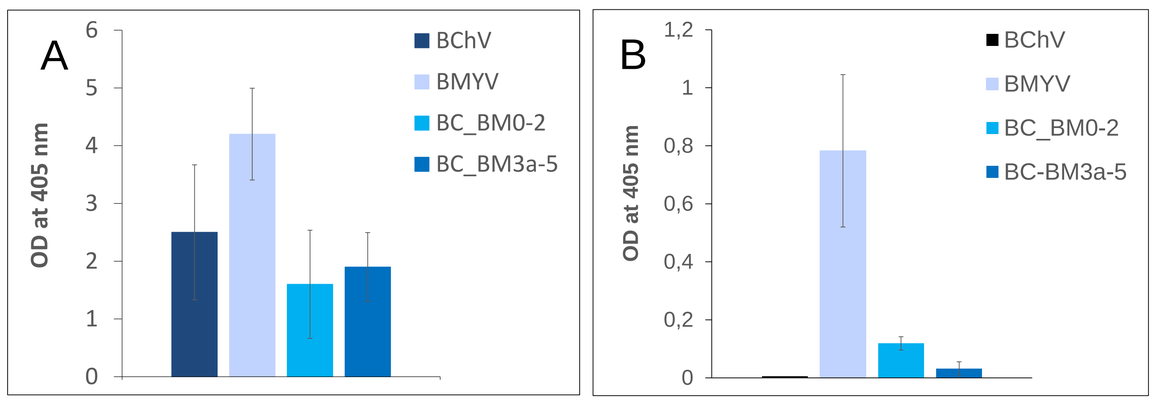

Fig. 3: ELISA analysis of viral replication in infiltrated leaf patches of *N. benthamiana* 14 dpi (A) and of systemic spread in leaves 35 dpi (B).

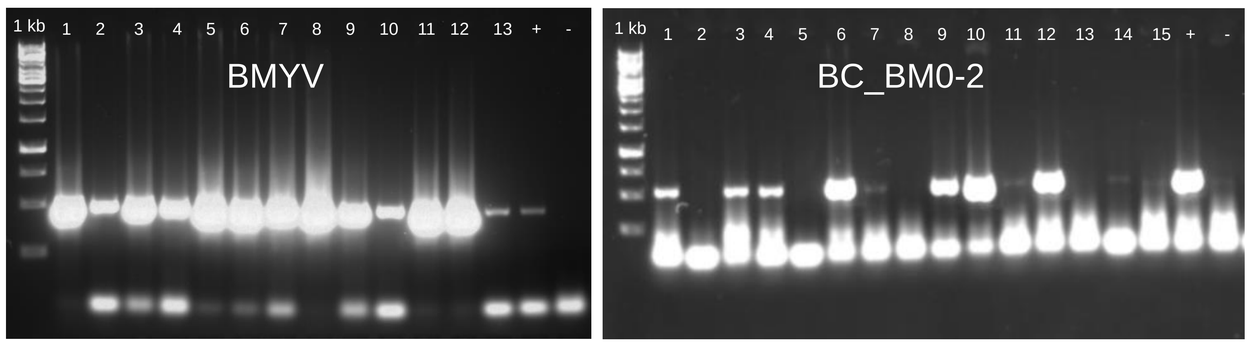

Fig. 4: RT-PCR systemic spread analysis 35 days after infection.

References:

Wetzel, V., Brault, V., & Varrelmann, M. (2018). Production of a Beet chlorosis virus full-length cDNA clone by means of Gibson assembly and analysis of biological properties. *Journal of General Virology*, *99*(11), 1522-1527.

Stephan, D., & Maiss, E. (2006). Biological properties of Beet mild yellowing virus derived from a full-length cDNA clone. *Journal of General Virology*, 87(2), 445-449.

Conclusions:

The infectious BChV cDNA clone is able to systemically infect its non-host *N. benthamiana* when ORF0-2 is exchanged by BMYV ORF0-2. The exchange of ORF3a-5 has no effect on the systemic spread. This suggests that host specificity lays on the genome region ORF0, 1 or 2, respectively. Further investigations are needed to completely understand the underlying mechanism and exact location of host specificity.