Biological and molecular characteristics of *Cherry leaf roll virus* (CLRV) isolates from different host plants

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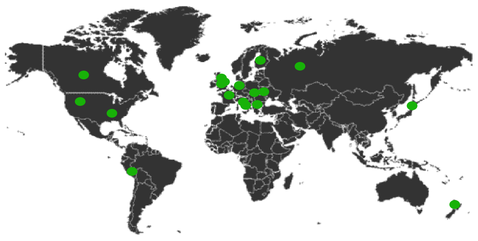

Fig. 1: Reports of naturally CLRV infected plants worldwide

Fig. 2: Symptoms on leaves of CLRV infected birch (left) and elderberry (right).

Introduction

Cherry leaf roll nepovirus (CLRV) is a widespread pathogen of woody plants especially in Germany and throughout the European Community (Fig.1). It is also spread in several herbaceous plants, such as rhubarb, but the origin of this virus is unclear. CLRV often induces symptoms in ash, birch, cherry, elderberry, walnut and others, including delayed leaf development, chlorotic leaf streaks or spots as well as dieback of branches or whole trees (Fig.2). In nature, CLRV is mainly transmitted through seed or pollen, leading to viral spread within one plant species; mechanical transmission, including grafting procedures, is also possible. Genomic organisation of CLRV is still unknown, but morphology and particle composition is typical for nepoviruses. A phylogenetic analysis of a 280 base pair fragment of the 3´non-coding-region of viral RNA demonstrated that CLRV isolates were clustered on a genomic level that accorded with the original host plant (Fig.3).

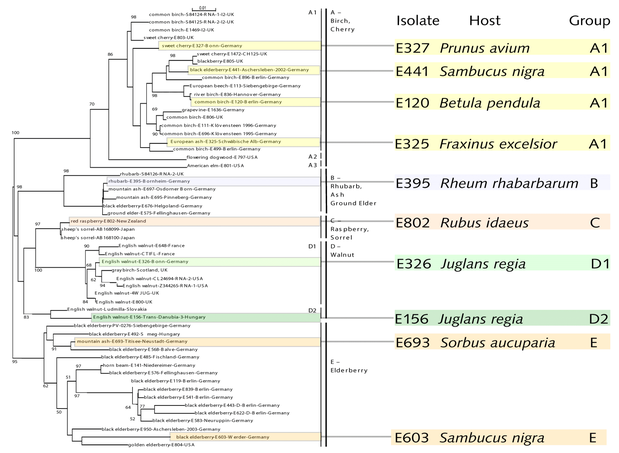

Fig. 3: Phylogenetic analysis of CLRV isolates from different plant species, based upon sequence differences of a 280 bp fragment of the 3´NCR. Highlighted are CLRV isolates included in investigations described below.

Field study

One-year-old seedlings of nine different plant species were mechanically inoculated and cultured in the field (Berlin, Germany, Fig. 4), to evaluate the role of transmission via wounding as a source of horizontal virus spread between different plant species under natural environmental conditions. Ten selected CLRV isolates, originating from different host plants, were used for inoculation. Six months after inoculation no characteristic symptoms of CLRV infection were observed. However, buds from inoculated trees were sampled and analysed for CLRV infection by a sensitive IC-RT-PCR assay with specific primers, according to Werner *et al.* (1997). In none of the samples could an infection be confirmed.

Fig. 4: Field experiment in the second year - one year after infection of nine woody host plant species (birch, hornbeam, elderberry, cherry, beech, Mountain ash, ash, buckthorn, Flowering dogwood) with ten different CLRV-isolates.

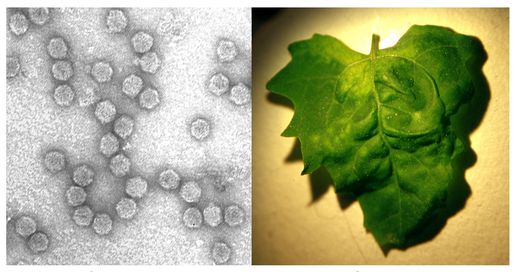

Fig. 5: Electron microscopic picture of isometric CLRV particles (isolate E395) (left) and symptoms on *C. quinoa* leaf after infection with CLRV (right)

Biological characteristics of CLRV

After infection of the experimental host *Chenopodium quinoa* all ten CLRV isolates under investigation induced chlorotic spots on inoculated leaves 3–5 days after inoculation (Fig. 5 right). A systemic infection, exhibiting leaf distortion and necrosis of expanding leaves, was established 9 days after inoculation in all cases, with the exception of CLRV-walnut strain from Hungary; this particular isolate induced necrotic local lesions on inoculated *C. quinoa* leaves without systemic symptoms. Electron microscopic control of purified virus preparations revealed isometric virus particles (28 nm in diameter, Fig. 5 left) typical for nepoviruses in all investigated isolates.

Molecular characteristics of CLRV

Virus particles were purified in order to compare viral components (structure-proteins and nucleic acids) of CLRV isolates included in the field experiment. Protein analysis of purified viruses by SDS-PAGE displayed a structure-protein of expected size (MW is approximately 53 kDa) from eight CLRV isolates, displayed in Fig. 6 (top). In two isolate purifications different proteins of significantly smaller size were present, suggesting an infection with another virus. In RNA-gels (Fig. 6, bottom), seven virus isolates showed typical RNA patterns, with a larger genomic RNA1 (molecular weight around 8,2 kb) and a slightly shorter RNA2 (~ 6.9–7.3 kb). The CLRV isolate derived from European ash (Germany) as well as the CLRV-walnut strain from Hungary, which were different in protein analysis, also exhibited atypical RNA patterns. CLRV detection by RT-PCR, using particle purifications of these two isolates as templates, also failed (data not shown).

To date it remains unclear, whether or not CLRV isolates appear as mixed infections with other viruses of similar morphology in certain woody host plants.

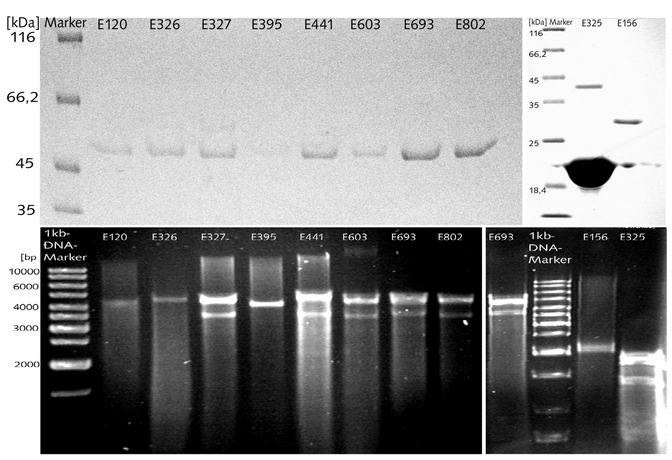

Fig. 6: Purified virus particles of different CLRV isolates were analysed by SDS-PAGE to visualize viral coat proteins (top) and separated by RNA gelelectrophoresis to compare pattern of viral genomes (bottom)

Conclusions

Further investigations are necessary to identify the viruses responsible for unusual RNA and protein patterns. Additionally, detection of CLRV in the initiated field experiments has to be continued to clarify the potential of different virus isolates to infect various woody host plants through wounds and the relevance of CLRV distribution between species by other factors than through seed or pollen.

Reference: Werner R; Mühlbach, HP; Büttner C (1997). Detection of cherry leaf roll nepovirus (CLRV) in birch, beech and petunia by immunocapture RT-PCR using a conserved primerpair. *European Journal for Pathology* 27, 309-318