# Biological and molecular characteristics of different *Cherry leaf roll virus* (CLRV) isolates

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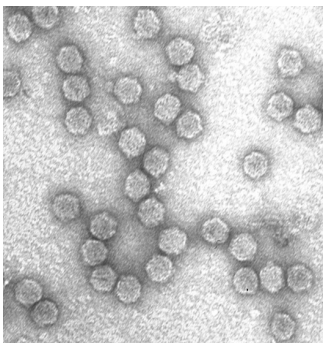

**Fig. 1** Purified CLRV particles of isolate E395
TEM picture taken at 80000x magnification

*Cherry leaf roll virus* (CLRV)

| | |
|---|---|
| Taxonomy: | Family *Comoviridae*, Genus *Nepovirus*, Subgroup C |
| Morphology: | icosahedral virions, 28 nm in diameter |
| Genome: | bipartite genome organisation of linear positive-sense ssRNA |
| Hosts: | woody plants like cherry (*Prunus avium*), walnut (*Juglans regia*), elderberry (*Sambucus spec.*) and some herbaceous plants like rhubarb (*Rheum rhabarbarum*) |
| natural Transmission: | mainly through pollen and seeds |

## Introduction

CLRV strains from different woody host species vary in RNA sequences and serological characteristics. (JONES, 1985; GIERSIEPEN, 1993)
By the analysis of a 280 bp long sequence of the 3'-non-coding region (3' NCR), CLRV isolates from 17 host species could be divided into six groups.
From these groups, ten isolates were selected and studied regarding their biological, serological and molecular characteristics. (Fig. 2)

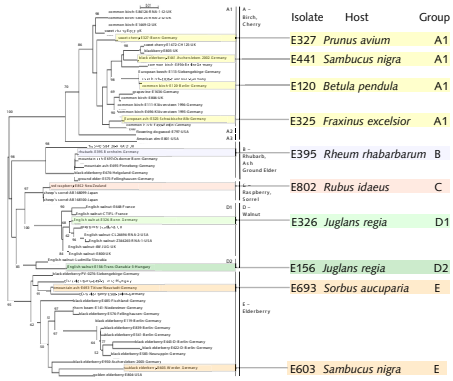


**Fig. 2** CLRV phylogenetic tree (based on differences in the sequence of the 3' NCR) and ten selected isolates

## Morphological analysis

Test plants (*Chenopodium quinoa)* were mechanically inoculated with ten selected CLRV isolates (Fig. 2) to compare symptom exhibition and development.
Furthermore virus particles were purified from infected plants and examined by transmission electron microscopy (TEM).

Nine of ten virus isolates caused a systemic infection. Typical symptoms such as chlorotic and necrotic local lesions (Fig. 4 and 5) appeared 3-5 days after inoculation. In younger leaves chlorosis started at the leaf stalk and spread across the lamina (Fig. 3). In contrast isolate E156 from walnut induced only local lesions on inoculated leaves without systemical spread.
All purified isolates showed virus particles of typical morphology in TEM (Fig. 1).

**Fig. 3** *Chenopodium quinoa* with an extensive chlorosis beginning at the leaf stalk , CLRV isolate E802

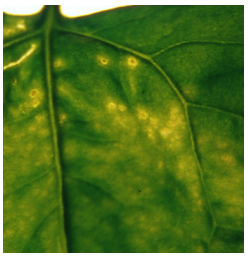

**Fig. 4** *C. quinoa:* chlorotic and necrotic local lesions induced by CLRV isolate E693

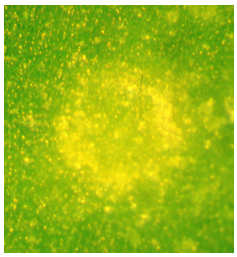

**Fig. 5** Detail of *a* chlorotic local lesion with incipient necrotic ring;
CLRV isolate E693 on *C. quinoa*

## Serological analysis

A polyclonal antiserum (pAb E603) against purified CLRV particles of isolate E603 from black elderberry (group E, Fig. 2) was raised in rabbit. The ten selected virus isolates were tested in DAS-ELISA and IC-RT-PCR using this polyclonal antiserum.
Additionally viral structural proteins were analysed by applying purified virus particles to SDS-PAGE.

Not all CLRV isolates were detectable in DAS-ELISA. Only members belonging to group C, D1 and E (e.g. E326, E603, E693 and E802) produced a specific reaction with pAb E603 (Fig. 6), confirming the serological divergence of CLRV strains from different woody hosts.
Differences in the detection of the tested isolates could also be observed in IC-RT-PCR (Fig. 7), but they differed slightly from results obtained in DAS-ELISA.
In SDS-PAGE eight CLRV isolates showed a consistent coat protein size of approximately 53 kDa. Two isolates differed from that: E156 has a calculated coat protein size of 31,5 kDa and E325 exhibited protein bands with a molecular weight of 43 kDa and 20 kDa respectively (Fig. 8).

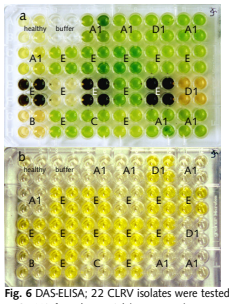


**Fig. 6** DAS-ELISA; 22 CLRV isolates were tested
a: CLRV-infected material (groups A-E)
b: 1h after addition of substrate

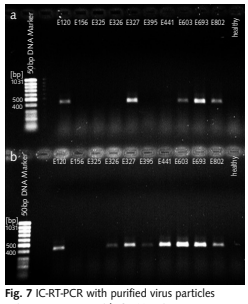


**Fig. 7** IC-RT-PCR with purified virus particles
a: immunocapture with CLRV-antiserum
b: RT-PCR without immunocapture

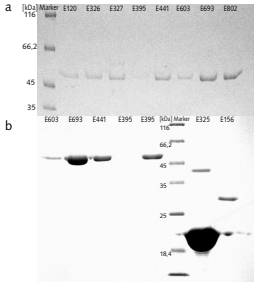


**Fig. 8** Coomassie stained SDS-polyacrylamidgel
a: seven typical CLRV coat protein bands
b: comparison of typical (left) and atypical (right) virus isolates
Marker: Protein Molecular Weight Marker (Fermentas)

## Analysis of genomic RNA

In order to compare genomes of purified virus particles, viral nucleic acids were subjected to agarose gel electrophoresis. To determine the size of RNA1 and RNA2 of purified CLRV strains, RNA preparations were separated under denaturing conditions by glyoxal gel electrophoresis and compared with an RNA standard marker.

In native RNA gels (Fig. 9) six virus isolates revealed consistent RNA1 and RNA2 bands whereas the RNAs of isolate E395 migrated slightly faster in agarose gels. RNA bands of isolates E156 and E325 varied noticeably from typical CLRV patterns, indicating considerable smaller size of genomic RNAs.
The sizes of typical CLRV RNA1 and RNA2 under denaturing conditions (Fig. 10) could be calculated for isolate E693 to be 8,3 kb (RNA1) and 6,9 kb (RNA2).

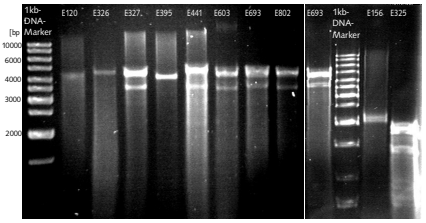


Fig. 9: RNA gelelectrophoresis of purified virus isolates under native conditions
GeneRuler™ DNA-Marker (Fermentas)

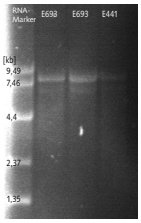


**Fig. 10** RNA gel with selected isolates under denaturating conditions
RNA-Marker (Invitrogen™)

**Results point out that seven of the ten isolates under investigation are typical CLRV isolates.**

**Isolate E395 shows atypical RNA bands, but it has a CLRV typical coat potein size and CLRV specific sequences could be amplified in RT-PCR.**

**The purified particles of isolates E156 and E325 are not CLRV, because of atypical patterns of viral coat proteins and genomic nucleic acids.**

References
Giersiepen, R (1993): Untersuchungen zur Charakterisierung des Kirschenblattrollvirus (CLRV) als Ursache einer Eschenvirose. Dissertation, Rheinische Friedrich-Wilhelms-Universität zu Bonn
Jones, AT (1985): Cherry leaf roll virus CM/AAB, Description of Plant Viruses, 306