

Characterisation of *Cherry leaf roll virus* (CLRV) isolates from different host plants



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Introduction

Cherry leaf roll virus (CLRV) is a widespread pathogen of woody plants in Germany with a worldwide distribution (Fig. 1). It has been detected also in a limited number of herbaceous plants, e.g. rhubarb (*Rheum rhabarbarum* L.). The nepovirus with isometric particles (Fig. 2) can be transmitted by mechanical inoculation, but natural mode of transmission is through seed or pollen.

CLRV has been reported to be of economical importance in European walnut plantations. In recent years it has caused reduced yields in cherry trees in the United States, if occurring as mixed infections with *Prunus necrotic ringspot virus* (PNRSV) or *Prune dwarf virus* (PDV) (Fig. 3).



Fig. 1: CLRV infection of woody and herbaceous host plants worldwide

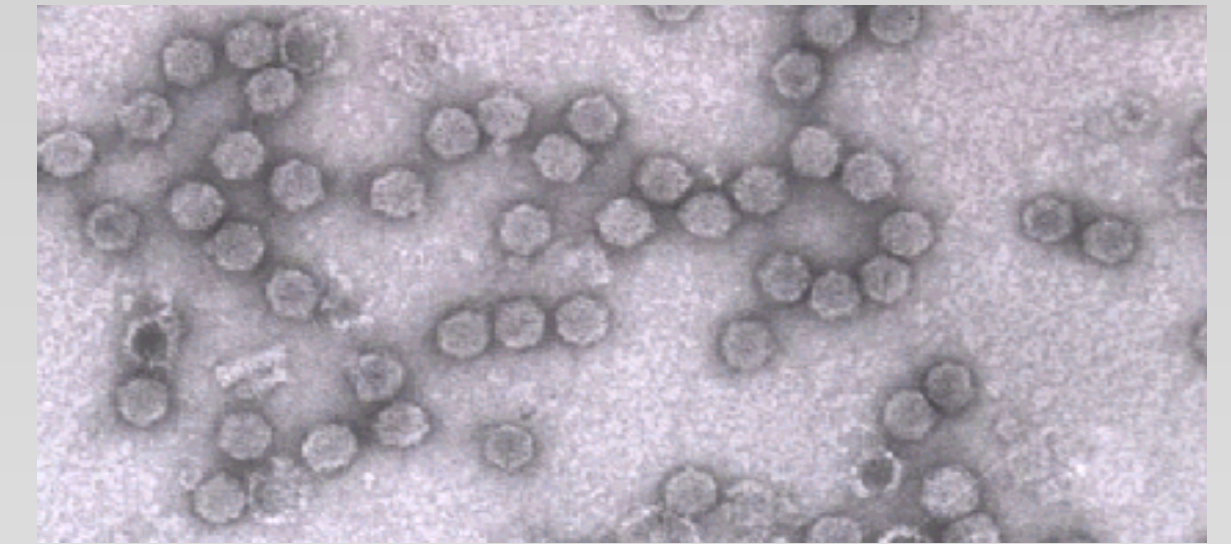


Fig. 2: CLRV, rhubarb isolate, TEM picture of negative contrasted particles (diameter 28 nm) magnification 40.000 x



Fig. 3: CLRV infected Cherry tree (left) and symptoms of a mixed infection with CLRV and PDV (right) (<http://nrsp5.prosser.wsu.edu/>)

Results

CLRV isolates from different host plant species were characterized on molecular and serological level. Phylogenetic analysis of a 375 bp fragment in the 3' non-coding region (3'NCR) of the viral genomic RNA revealed significant sequence variability up to 15.3 %, leading to different isolate groups corresponding to their original host plant species. Arrangement of CLRV isolates upon 3'NCR sequence similarity established a birch/cherry, a walnut and an elderberry class. This clustering of CLRV variants upon non-coding viral sequence parts, resembled arrangement according to serological reactivity of isolates, using a set of polyclonal and monoclonal antibodies (Fig. 4). Grouping was also confirmed by comparison of coat protein (CP) sequences of CLRV strains (Fig. 5). On RNA level CLRV sequences of the 5' proximal region of the CP (378 nucleotides) differed between 92 % (birch), 95-99 % (walnut) and 99-100 % (elderberry) within eleven grouped isolates. Nucleic acid variability of cp-coding sequence resulted in lower divergence on protein level between 96 % (walnut) and 99 % identity (elderberry) in CLRV groups as shown in table 1. In general conservation of RNA sequence seems to be higher in the 3'NCR than in the 5' proximal part of the cp coding region in isolates under investigation.

Serological and sequence data of cp-coding as well as non-coding regions obtained a strong correlation, grouping isolates according to their primary host plants.

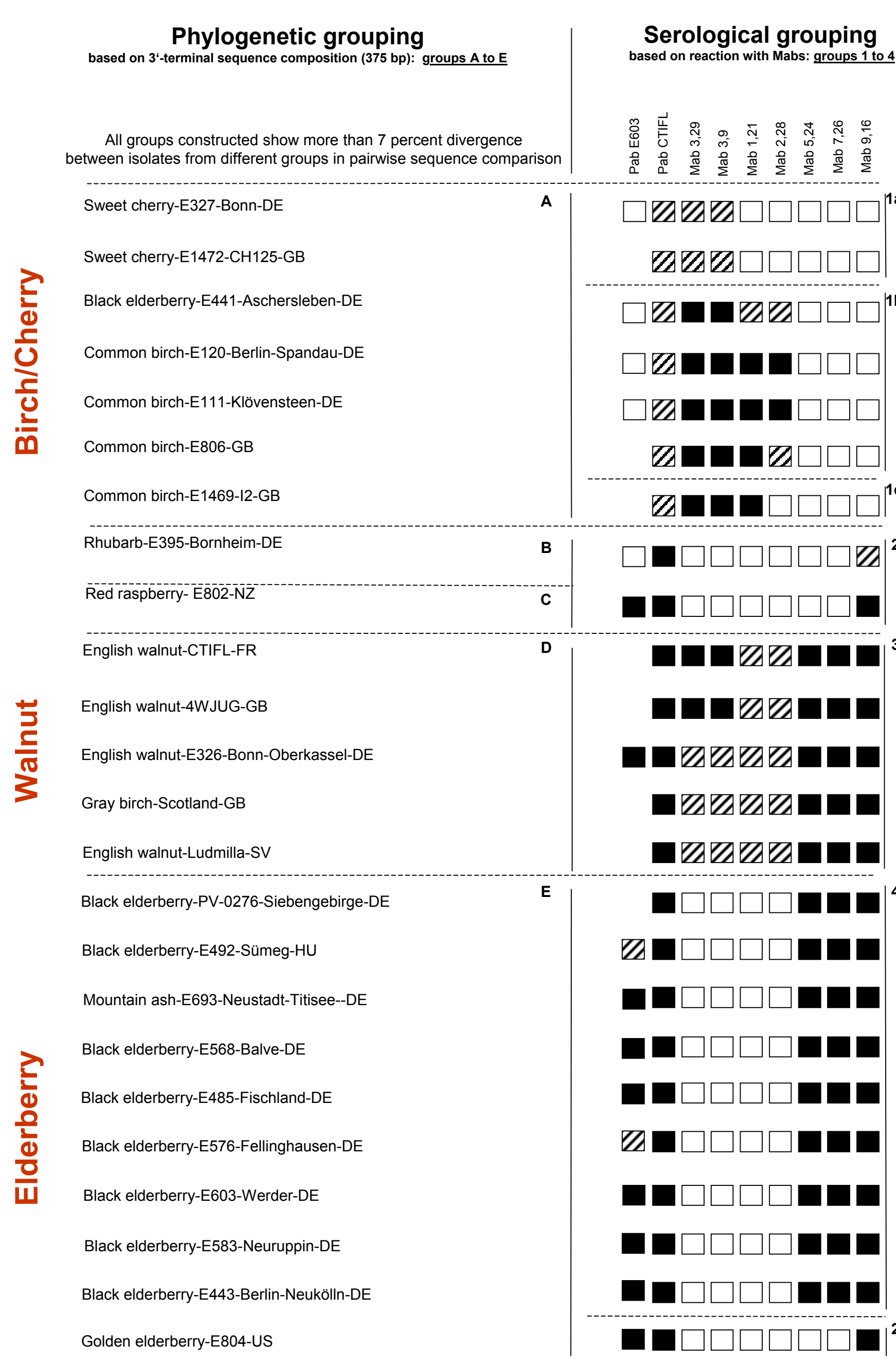


Fig. 4: Clustering of CLRV isolates according to sequence divergence of 375 bp of the 3' non coding region (left) and serological reactivity with a set of polyclonal and monoclonal antibodies (right). (PabE603 = polyclonal antiserum developed against CLRV-E603 strain from elderberry, Pab CTIFL = polyclonal antiserum against CLRV-CTIFL strain from walnut, Set of monoclonal antibodies (mab) developed against CLRV CTIFL strain).

■ ELISA values, > double values from healthy controls
 ▨ elevated values, but < double values from negative controls
 □ no serological reaction in ELISA

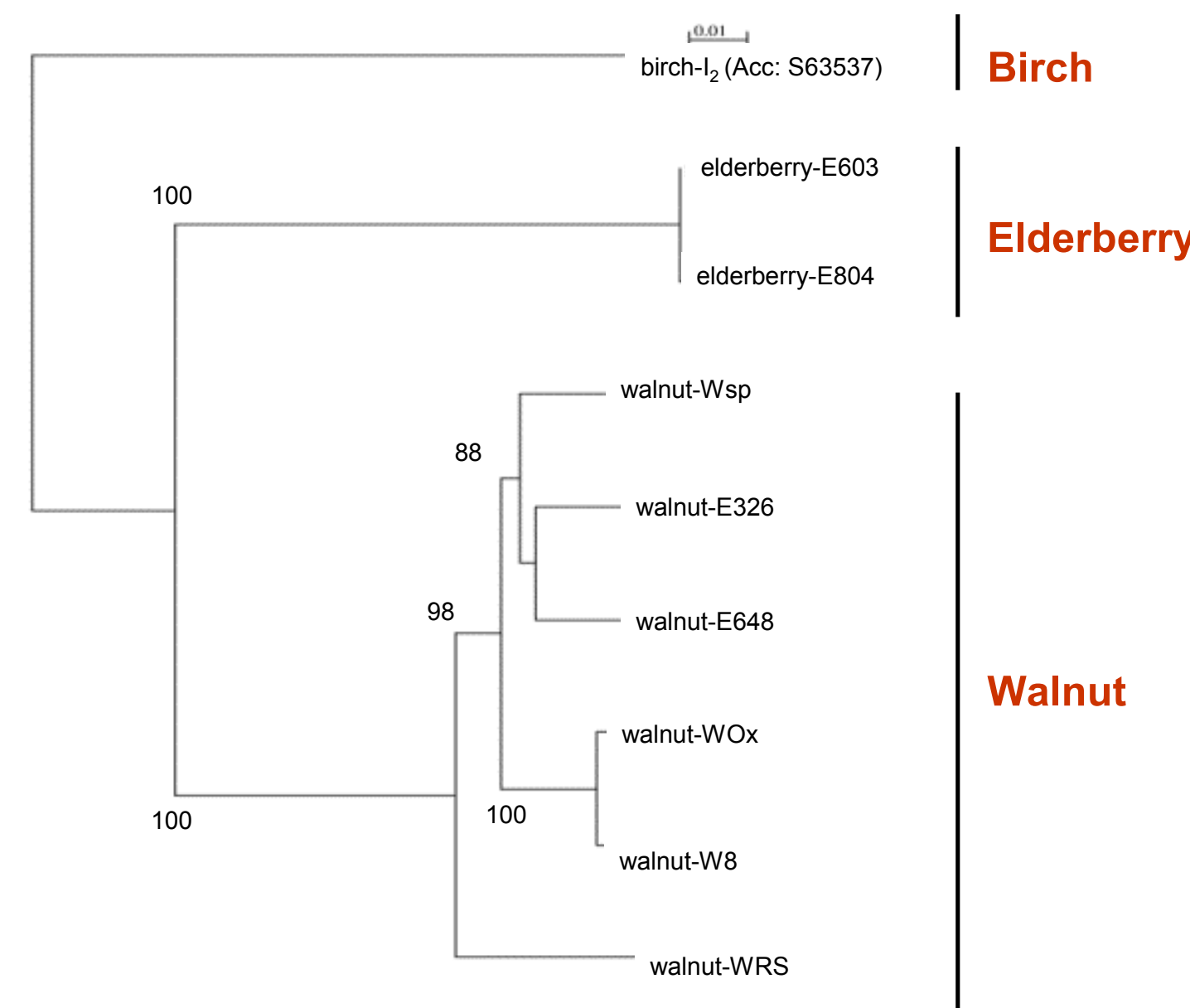


Fig. 5: Phylogenetic tree of coat protein coding nucleotide sequences (1542 nucleotides, Reference Acc.: S63537) of nine CLRV isolates from different host plants. Bootstrap values (n = 1000) larger than 50 % are indicated at branch nodes.

Table 1: Comparison of nucleic acid and amino acid sequence identities and similarities in percent of the 5' proximal cp-coding region (378 nucleotides = 126 amino acids) of eleven CLRV isolates

NA AA	W-8	W-Ox	E648	E326	W-RS	W-SP	E804	E603	E802	B-12	E441	Reference	Host plant
W-8		99.7	96.5	96.8	96.0	95.5	85.7	85.7	85.4	80.7	78.1	Zhou et al. 1998	Walnut
W-Ox	99		96.8	97.0	96.3	95.7	86.0	86.0	85.7	81.0	78.3	Zhou et al. 1998	Walnut
E648	96/99	97/100		97.0	96.3	95.7	84.6	84.6	84.4	81.5	79.9	this study	Walnut
E326	98/99	98/100	98/100		96.5	96.0	86.5	86.5	86.2	81.7	80.2	this study	Walnut
W-RS	97/99	98/100	98/100	99/100		95.7	85.7	85.7	85.4	81.5	79.1	Zhou et al. 1998	Walnut
W-Sp	98/99	98/100	98/100	100	99/100		85.4	85.4	85.2	81.5	79.4	Zhou et al. 1998	Walnut
E804	94/98	94/99	94/98	95/98	94/98	95/98		100	99.7	81.2	80.2	this study	Golden elderberry
E603	94/98	94/99	94/98	95/98	94/98	95/98	100		99.7	81.2	80.2	this study	Black elderberry
E802	93/98	94/98	93/98	94/98	94/98	94/98	99/99	99/99		81.0	79.9	this study	Red raspberry
B-12	84/92	85/93	85/92	86/92	85/92	86/92	87/92	87/92	87/92		92.3	Scott et al. 1993	Birch
E441	85/94	86/94	86/94	87/94	86/94	87/94	88/94	88/94	87/93	98/98		this study	Black elderberry

Identities of viral nucleic acid (NA) sequences (%) shown in upper grey shaded fields, corresponding to reference sequence S63537, nucleotides 263-640 (Scott et al., 1998). Comparison of amino acid (AA) identity/similarity of CLRV isolates in lower white fields, corresponding to amino acid 88-213 of reference sequence AAB27443.

Conclusion

The significant variability of CLRV strains and their association with certain host plant species may be due to the natural mode of transmission of the virus by pollen and seed which presumably limits efficient cross-species transmission, leading to rapid genetic isolation and adaptation of CLRV variants to particular host species.



References

Scott NW, Cooper JI, Edwards ML (1993) The identification, cloning, and sequence analysis of the coat protein coding region of a birch isolate (I2) of cherry leaf roll nepovirus. *Arch. Virol.* 131, 209-215; Zhou Y, Civerolo EL, Melvin JA (1998) Comparative nucleotide sequences of the coat protein-encoding region of the genomes of Cherry leafroll virus strains infecting walnut. *Acta Horticulturae* 472, 321-329

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