

# Cherry leaf roll virus (CLR-V): genome organisation of the RNA1



S. von Barga<sup>1</sup>, J. Langer<sup>1</sup>, A. Rumbou<sup>1</sup>, J. Gentkow<sup>2</sup>, C. Büttner<sup>1</sup>

<sup>1</sup> Humboldt-Universität zu Berlin, Institute of Horticultural Sciences, Section Phytomedicine, Lentzeallee 55/57, 14195 Berlin, Germany, susanne.von.barga@agr.ar.hu-berlin.de  
<sup>2</sup> present address: Leibniz-Institute of Plant Biochemistry, Weinberg 3, 06120 Halle, Germany

## Introduction

The complete organisation of the *Cherry leaf roll virus* genome, a virus which affects many fruit trees and other woody hosts, has not been determined to date. However, partial sequence information of the bipartite virus which is available of the 3' proximal portion including the complete 3' non-coding region (NCR) of the genomic RNA1 and RNA2 has led to the classification as a subgroup c nepovirus.

## Methods and Results

Sequences of the RNA1 of two CLR-V isolates from different host plants (CLR-V-E395 originating from *Rheum rhabarbarum* and CLR-V-E326 from *Juglans regia*) were obtained. The genomic structure of the CLR-V-RNA1 coding for a polyprotein (PP1) was compared with other established subgroup c nepoviruses like *Tomato ringspot virus* (ToRSV), *Blackcurrant reversion virus* (BRV) and *Peach rosette mosaic virus* (PRMV). The polyprotein of the rhubarb isolate (ORF<sub>12-6350 nt</sub>; 2112 amino acids) contains a N-terminal protease cofactor (PCo), adjacent is a nucleotide-binding protein-domain (NTBp), followed by the sequences coding for the genome-linked viral protein (VPg), a protease (Pro) and the viral replicase (RdRp). Putative protein functions were predicted by identification of characteristic sequence motifs. The region coding for the CLR-V-VPg protein was identified with the computer programs NetPicoRNA V1.0 and NetCorona V1.0., and exhibited highest similarities with the corresponding ToRSV-VPg. Predicted specific protease recognition sequences in the CLR-V isolates (Q<sub>1121</sub>/S<sub>1122</sub> and Q<sub>1150</sub>/S<sub>1151</sub>) also corresponded to ToRSV.

## Conclusion

The genome organisation of the RNA1 of CLR-V strains E395 from rhubarb and E326 from the woody host walnut corresponds with other subgroup c nepoviruses.

## References

Argos (1988) NAR 16, 9909-9919; Gorbalenya et al. (1989a) J. Mol. Evol. 24, 256-268; Gorbalenya et al. (1989b) FEBS letters 243, 103-114; Rott et al. (1995) JGV 76, 465-473; Wang et al. (1999) JGV 80, 799-809.

## Genome organisation of CLR-V-E395

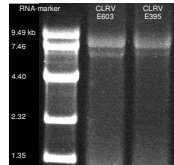
RNA1, 7918 nt (ORF nucleotides: 12-6350; polyprotein 1: 2112 amino acids)



RNA2, approx. 6800 nt



## viral RNAs



Viral RNAs of CLR-V strains E603 and E395 separated by denaturing agarose gel electrophoresis.

## protease-cofactor (PCo) motif

	490	500	510	520	530	540	550	560
CLR-V-E395	FVRLRDEF	SNSVGKYLNAVSEYKAQIENFWAWAVRW	SNNLMNKVDVSLRALQGSAPFAAALVIVGGIVY	LLENLPPAA	467			
CLR-V-E326	FVRLRDEF	SNSVGKYLNAVSEYKAQIENFWAWAVRW	SNNLMNKVDVSLRALQGSAPFAAALVIVGGIVY	LLENLPPAA	467			
BRV-NC003509	FFQTLKSKF	FEFLKPYIQHAIYASAEIEKYWAFIHGWATKMN	NVNVGLQALGDAAWVAIGITMVCGIVL	VEKLLVYLG	463			
ToRSV-NC003840	FVKLLRHEF	DDTIKWKVPLKLGATQKIEELWRWSEW	QAQMSKLDVSLRVLRSALVGVGLLVG	GLVYFELQLRSLRF	553			
PRMV-AF06626	ILDCKCYF	MSTFSFFLASLQSAKAEIEKFWQNCMS	WARNLWSKAHLALQGLYAIWALVLTIL	CGIVYLL	455			
Consensus	.....	.....	.....	.....	.....	.....	.....	.....

**FXXXXXXXXXXXXXXXXXXXXXXXXXWXXXXXXXXXXLXXXXXXXXXXXXXXXXXXXXLX**

**F-X<sub>27</sub>-W-X<sub>11</sub>-L-X<sub>21</sub>-L-X<sub>1</sub>-E** (Rott et al., 1995)

## NTB protein (NTBp) motif

	810	820	830	840	850	860	870	880
CLR-V-E395	YFGKART	GKTI	FANNFKNTMVQYLGITSENIF	YKNARDQFWPKYRQQAIVIV	DDLSAVENPPS	--LESEF	IQLMSTMPY	778
CLR-V-E326	YFGKART	GKTI	FANNFKNTMVQYLGITSENIF	YKNARDQFWPKYRQQAIVIV	DDLSAVENPPS	--LESEF	IQLMSTMPY	778
BRV-NC003509	YIYGKSHC	GKSL	FMEDVSRALLENKENGHPNDI	YKNAKNSDFWSGYLQACVQ	VDDLSACVTRPS	--LESEF	IQLVGSKMY	773
ToRSV-NC003840	YLIFGPRC	GKSL	FAQSFMAAIVDFMGITVDN	CFKNARDDFWSGYRQEAIC	CVDDLSLSCSTQPS	--IESEF	IQLLITMRY	870
PRMV-AF06626	YIFGASQC	GKSL	TLANYLCPLLALHMGWDADHDV	SKDPTGEYWSGGYQQCLKM	DDLSAVVPKQVSPLEQQLIPLISTEEK			772
Consensus	.....	.....	.....	.....	.....	.....	.....	.....

**Gxx(G)xGKS/T-x<sub>n</sub>-D/ED** (Gorbalenya et al., 1989a)

## putative VPg (M: 3.28 kDa) with flanking 3C-like protease (Pro) recognition sites

	1240	1250	1260	1270	1280	1290	1300	1310
CLR-V-E395	RQIFCGVG	-AGSAGAMKMSV	QS-----	TIPSGSDV	QSYASRNLRVRYR	TRTLGL	QSAMNPVETV	SQAQLM
CLR-V-E326	RQIFCGVG	-AGSAGAMKMSV	QS-----	TVPSGSDV	QSYASRNLRVRYR	TRTLGL	QSAMNPVETV	SQAQLM
BRV-NC003509	QTFVAGT	SALVSAGMIVQLS	ANAGSVCTSANPS	GAASVSSNIP	IHRWRNSYNSERS	YALNSNLE	DKYLLD	LVLWLQI
ToRSV-NC003840	QYIFCGA	GGTVSMAAVFMT	QSG-----	TIPSGSYADV	NARNMTRVFP	RPQSV	QSSLA	EAQFNSHAYNMLVRIDL
PRMV-AF06626	KTFLSGV	GFSGSALAKNLQ	LDVHSSVAVSS	GSITQSYARS	IPVIAKVAR	YANVHSQ	VQESSHF	NFPEDGLAHLVLRVVG
Consensus	.....	.....	.....	.....	.....	.....	.....	.....

predicted by NetPicoRNA V1.0 (0.546) NetCorona V1.0 (0.545)  
 NetCorona V1.0 (0.677)  
**Q/E X G/S** (Wang et al., 1999)

## viral cysteine protease (Pro) motif

	1290	1300	1310	1320	1330	1340	1350	1360
CLR-V-E395	.....	.....	.....	.....	.....	.....	.....	.....
CLR-V-E326	.....	.....	.....	.....	.....	.....	.....	.....
BRV-NC003509	.....	.....	.....	.....	.....	.....	.....	.....
ToRSV-NC003840	.....	.....	.....	.....	.....	.....	.....	.....
PRMV-AF06626	.....	.....	.....	.....	.....	.....	.....	.....
Consensus	.....	.....	.....	.....	.....	.....	.....	.....

**H-X<sub>30-46</sub>-E-X<sub>69-101</sub>-CG-X<sub>6-8</sub>-G-X<sub>3-5</sub>-G-X-H/LXXG**  
 (Gorbalenya et al., 1989b)

## viral replicase (RdRp) core motif

	1850	1860	1870	1880	1890	1900	1910	1920
CLR-V-E395	.....	.....	.....	.....	.....	.....	.....	.....
CLR-V-E326	.....	.....	.....	.....	.....	.....	.....	.....
BRV-NC003509	.....	.....	.....	.....	.....	.....	.....	.....
ToRSV-NC003840	.....	.....	.....	.....	.....	.....	.....	.....
PRMV-AF06626	.....	.....	.....	.....	.....	.....	.....	.....
Consensus	.....	.....	.....	.....	.....	.....	.....	.....

**DxxxD-x<sub>n</sub>-DxxxTxxxN-x<sub>n</sub>-GDD**  
 (Argos, 1988)

H-X<sub>47</sub>-E-X<sub>104</sub>-CG-X<sub>6-8</sub>-G-X<sub>3-5</sub>-N-X-H/LXXG CLR-V, Nepovirus Subgruppe C

## Acknowledgements

Research was funded by the DFG projects Bu890/8-1 and Bu890/8-2.