Complete nucleotide sequences and genome organization of a cherry isolate of cherry leaf roll virus

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Abstract The complete nucleotide sequence of cherry leaf roll virus (CLRV, genus Nepovirus) from a naturally infected cherry tree (Prunus avium cv. Bing) in North America was determined. RNA1 and RNA2 consist of 7,893 and 6,492 nucleotides, respectively, plus a poly-(A) tail. Each RNA encodes a single potential open reading frame. The first 657 nucleotides of RNA1 and RNA2 are 99% identical and include the 5'-UTR and the first 214 deduced amino acids of the polyproteins following the first of two in-frame start codons. Phylogenetic analysis reveals close relationships between CLRV and members of subgroup C of the genus Nepovirus.

Cherry leaf roll virus (CLRV; family Secoviridae; subfamily Comovirinae, genus Nepovirus [13]) and the disease caused by it in sweet cherry (Prunus avium) were first described in Europe [4] and later in North America [5]. However, its wide host range of herbaceous and woody plants in more than 36 plant families [10, 18] suggests that CLRV has the potential to impact agricultural production in many areas.

Members of the genus Nepovirus possess genomes of two positive-sense, single-stranded RNA molecules. Each RNA molecule is encapsidated separately in an isometric particle, and both RNAs are required for virus infection [9]. Taxonomically, members of this genus are classified into three subgroups based on the relative lengths of RNA2 as well as serological and sequence relationships [9]. CLRV is a member of subgroup C. In this study, the complete genomic sequence and organization of CLRV (CLRV-Ch) from a naturally infected cherry tree (Prunus avium cv. Bing) in North America is presented and compared to those of other members of the genus Nepovirus, including the recently determined genome of a European isolate of CLRV from Rheum spp. (CLRV-Rh)(GenBank accession number FR851461; FR851462).

CLRV-Ch was mechanically transmitted from a symptomatic cherry tree to Chenopodium quinoa; the virus isolate was designated ‘Olm1’. CLRV-Ch was confirmed by ELISA (BioReba, Reinach, Switzerland) and by RT-PCR followed by sequencing of amplicons from the 3'-UTR [5]. Oligo (dT)-primed synthesis and cloning of cDNA from RNA isolated from virions yielded 1,800 nucleotides (nt) from the 3' terminus of RNA1. The 3' terminus of RNA2 was amplified from the initial cDNA synthesis reaction using primers AdPr (TAT-GACA-CGC-GTC-GACT-AGC) and degenerate primer NEPOR1 (WVDK-DRYN-WAT-GGW-GATG). Sequential 5'-RACE (Invitrogen, Carlsbad, CA) reactions yielded the remaining genomic sequences. Virus-specific primers were then designed to amplify overlapping RT-PCR segments from RNA isolated directly from cherry tissue. Since sequences at the termini of RNA1 and RNA2 are nearly identical, RNA1- and RNA2-specific primers were designed and

The nucleotide sequences presented in this report were deposited in the GenBank database under accession numbers JN104385 and JN104386.

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used in combination with 5'-RACE or AdPr primers. The resulting amplicons were sequenced to yield the genomic sequences of CLRV-Ch RNA1 and RNA2 reported herein (deposited under GenBank accession numbers JN104386 and JN104385, respectively).

The sizes of CLRV-Ch RNA1 and RNA2 are 7,894 and 6,492 nt, respectively, excluding the poly(A) tails. Sequence analysis suggests that each segment of the CLRV-Ch genome encodes a large potential polyprotein in only one sense. The first AUG start codon of RNA1 occurs at nt 13 and is followed by an uninterrupted ORF extending to nt 6,353 from the first in-frame UAG stop codon. This would yield a putative polypeptide (P1) of 2,113 amino acids (aa) with a predicted molecular mass of 236 kDa. The single ORF of RNA2 extends from nt 13 at the first AUG start codon to nt 4,938, yielding a putative polyprotein (P2) of 1,641 aa and molecular mass of 180 kDa. However, both RNAs possess a second in-frame AUG codon and potential translation initiation site at nt positions 82-84. The sequences flanking both AUG codon positions are in the context of a Kozak consensus sequence [8] for optimal translation initiation, and RNA secondary structure analysis suggests that both AUG codons are in favorable contexts to act as functional start codons. However, RNA1 and RNA2 of CLRV-Ch contain sequences in the 5'- and 3'-UTRs of CLRV-Ch to those from birch (RNA1, S84124; RNA2, S84125), walnut (RNA1, U24694; RNA2, Z34265), and Rheum spp. (RNA1, FR851461; RNA2, FR851462) reveals greater host-associated differences—up to 28%. The biological significance of the long 3'-UTRs is yet to be fully elucidated. RNA1 and RNA2 of CLRV-Ch contain a small ORF downstream of the P1 and P2 coding regions that has the potential to encode a 5.2-kDa protein. It is not known if this protein is expressed in vivo, but it has been reported that a subgenomic RNA is produced by the walnut strain of CLRV RNA1 containing a small small ORF [2]. Although small ORFs occur in the 3'-common region of most CLRV sequences analyzed, the locations and predicted aa sequences encoded by the ORFs are not conserved.

The placement of CLRV-Ch in subgroup C is supported when sequences of RNA1-encoded polyproteins are compared (Fig. 1A). The genetic distance values indicate that CLRV-Ch is more closely related to CLRV-Rh and ToRSV than to other members of the genus Nepovirus. The CLRV-Ch polyproteins P1 and P2 were examined for conserved sequence motifs (see supplemental information). The P2 sequences of CLRV-Ch and other members of subgroup C are exceptionally long compared to those of other members of the genus Nepovirus; the coat protein (CP) coding sequence occupies 30 to 33% of P2 at the C-terminus. Based on sequence analogy to the partial P2 sequence of CLRV-birch [15] and the complete P2 sequence of ToRSV [12], a predicted protease Q/S cleavage site occurs at positions 1,128 of CLRV-Ch and 1,077 of CLRV-Rh. The putative CPs of CLRV-Ch and CLRV-Rh contain 513 and 512 residues (56.3 kDa), respectively, whereas CLRV-birch has only 469 amino acid residues (51.6 kDa) [15]. The difference, as confirmed by sequences generated from multiple clones, is attributed to the presence of an additional 44 aa at the C-terminus of the CP of CLRV-Ch before the first in-frame stop codon. The molecular mass reported herein for CLRV-Ch CP is consistent with that previously determined by denaturing gel electrophoresis of purified CLRV-Ch virions (55 to 56 kDa; [18]). The CP aa sequence of CLRV-Ch is 97% and 93% similar to that of CLRV-birch and CLRV-Rh, respectively. The affinity of the subgroup C members based on the CP sequence (Fig. 1C) is noteworthy since CP determinants provide vector specificity [14]. BRV is the only nepovirus reported to be transmitted by mites [17], and ToRSV is transmitted by nematodes [3], whereas a biological vector for CLRV remains undetermined. Analysis of the complete RNA2-encoded polyprotein places BRV in a different clade (Fig. 1B).

The N-terminal region of the P2 polyprotein upstream from the CP is not yet well characterized. In this study, we observed that the first 657 nt of RNA1 and RNA2 of CLRV-Ch are nearly identical (99%). This region includes the 5'-UTR and the first 214 deduced aa residues of the polyproteins. Repetition of the coding sequence at the 5'-termini of RNA1 and RNA2 also occurs in ToRSV [11].
and CLRV-Rh. This unique property does not occur in BRV or grapevine Bulgarian latent virus (GBLV), other members of subgroup C. The function of the peptide derived from the N-terminal region of P2 of ToRSV is poorly understood, but sequence comparison suggests that the region might encode a movement protein [12]. This protein domain of ToRSV is 386 aa versus 400 aa from the corresponding region of CLRV-Ch and shows 61% sequence similarity.

This study lays the groundwork for development of full-length infectious clones of CLRV to investigate the possible roles of the 5' terminal regions in planta via mutagenesis.

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References

1. Borja M, Sanchez F, Rowhani A, Bruening G, Ponz F (1995) Long, nearly identical untranslated sequences at the 3' terminal regions of the genomic RNAs of cherry leafroll virus (walnut strain). Virus Genes 10:245–252
2. Brooks M, Bruening G (1995) A subgenomic RNA associated with Cherry leafroll virus infections. Virology 211:33–41
3. Brown DJF, Halbrendt JM, Jones AT, Vrain TC, Robbins RT (1994) Transmission of three North American nepoviruses by population of four distinct species of the Xiphinema americanum group. Phytopathology 84:646–649
4. Cropley R (1961) Cherry leaf-roll virus. Ann Appl Biol 49:524–529
5. Eastwell KC, Howell WE (2010) Characterization of Cherry leafroll virus in sweet cherry in Washington State. Plant Dis 94:1067
6. Gultyaev AP, van Batenburg FHD, Pleij CWA (1995) The computer simulation of RNA folding pathways using a genetic algorithm. J Mol Biol 250:37–51
7. Karetnikov A, Lehto K (2008) Translation mechanisms involving long-distance base pairing interactions between the 5' and 3' non-translated regions and internal ribosomal entry are conserved for both genomic RNAs of Blackcurrant reversion Nepovirus. Virology 371:292–308
8. Kozak M (1999) Initiation of translation in prokaryotes and eukaryotes. Gene 234:187–208
9. Le Gall O, Iwanami T, Karasev AV, Jones T, Lehto K, Sanfaçon H, Wellink J, Wetzel T, Yoshikawa N (2005) Comoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) Virus taxonomy: eighth report of the international committee on taxonomy of viruses. Elsevier, Academic Press, London
10. Rebenstorf K, Candresse T, Dulucq MJ, Büttner C, Obermeier C (2006) Host species-dependent population structure of a pollen-borne plant virus, Cherry leaf roll virus. J Gen Virol 80:2453–2462
11. Rott ME, Tremaine JH, Rochon DM (1991) Comparison of the 5' and 3' termini of Tomato ringspot virus RNA-1 and RNA-2: evidence for RNA recombination. Virology 185:468–472
12. Rott ME, Tremaine JH, Rochon DM (1991) Nucleotide sequence of Tomato ringspot virus RNA-2. J Gen Virol 72:1505–1514
13. Sanfaçon H, Wellink J, Le Gall O, Karasev A, van der Vlugt R, Wetzel T (2009) Secoviridae: a proposed family of plant viruses within the order Picornavirales that combines the families Secoviridae and Comoviridae, the unassigned genera Cheravirus and Sadwavirus, and the proposed genus Torradovirus. Arch Virol 154:899–907
14. Schellenberger P, Sauter C, Lorber B, Bron P, Trapani S, Bergdoll M, Marmonier A, Schmitt-Keichinger C, Lemaire O, Demangeat G, Ritzenhaler C (2011) Structural insights into viral determinants of nematode mediated Grapevine fanleaf virus transmission. PLoS Pathog 7:e1002034. doi:10.1371/journal.ppat.1002034
15. Scott NW, Cooper JJ, 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
16. Scott NW, Cooper JJ, Liu YY, Hellen CU (1992) A 15 kb sequence homology in 3'–terminal regions of RNA-1 and RNA-2 of a birch isolate of cherry leaf roll nepovirus is also present, in part, in a rhubarb isolate. J Gen Virol 73:481–485
17. Susi P (2004) Black currant reversion virus, a mite-transmitted Nepovirus. Mol Plant Pathol 5:167–173
18. Walkey DGA, Stace-Smith R, Tremaine JH (1973) Serological, physical, and chemical properties of strains of cherry leaf roll virus. Phytopathology 63:566–571