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9-6 Genome organization of *Cherry Leaf Roll Virus* and Comparative analyses of RNA2-encoded proteins

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INTRODUCTION

Cherry leaf roll virus (CLRV) infects many deciduous trees and shrubs including important species of European forests such as several birch species (*Betula* spp.), rowan, (*Sorbus aucuparia*), beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*), elderberry (*Sambucus* spp.). Moreover, several stone fruits such as cherry (*Prunus avium*), walnut (*Juglans* spp.), olive (*Olea europaea*) are affected by the virus (Büttner *et al.* 2011, 2013). Recently, the complete genome was determined for a rhubarb isolate (von Bargen *et al*. 2012) and a cherry strain (Eastwell *et al*. 2012) revealing the genome organization of the bipartite positive stranded plant RNA virus (*Secoviridae*, *Nepovirus*).

MATERIAL AND METHODS

CLRV isolates originating from rhubarb (Bornheim, Germany) and sweet cherry (Bonn, Germany) were propagated in *Chenopodium quinoa*. A combination of methods was employed for generation of full length sequences of virus isolates. Either random primed cDNA libraries were produced from virus purifications according to Froussard (1992) or purified viral RNA was subjected to illumina´s sequencing by synthesis protocol. Short reads with an average size of 35 b were assembled and internal gaps were closed by sequencing of RT-PCR products employing virus specific primers applying total RNA prepared from *C. quinoa* infected with the respective virus isolate. The 3´ untranslated regions were amplified using RNA1 or RNA2 specific forward primers in combination with M4 primer and a M4T-primed cDNA targeting the polyA-tail as described by Chen *et al.* (2001). 5´ termini were determined by inverse PCR (Ochman *et al.* 1990).

RESULTS AND DISCUSSION

RNA1 encodes a polyprotein (P1) containing domains characteristic for a proteinase cofactor (PCo), a nucleotide-binding helicase (Hel), a genome-linked protein (VPg), a proteinase (Pro), and a RNA dependent RNA polymerase (Pol). The RNA2-encoded polyprotein (P2) comprises the putative movement protein (MP) and the coat protein (CP) of CLRV. The genome region upstream of the MP has a coding capacity of 77 kDa, however the function of the encoded peptide is unclear. Comparative sequence analyses of CLRV isolates from rhubarb and cherry revealed that the RNA2 is less conserved than the RNA1 of the virus. Among isolates the region encoding the putative X4 protein on RNA2 shows maximal 44 % identity on amino acid level. The putative X4 and MP encoded by the cherry isolates also differ in size from the rhubarb strain. In contrast to the rhubarb isolate, both genomic segments of cherry isolates contain a 2nd in frame ATG located 69 nucleotides downstream of the 1st ATG.

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