Improved construction of full-length cDNA clones of *Apple chlorotic leaf spot virus* and agroinoculation on woody plants by vacuum infiltration

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Apple chlorotic leaf spot virus (ACLSV) is the type member of the genus *Trichovirus* in the family *Betaflexiviridae*. It is distributed worldwide and mainly infects *Rosaceae* fruit trees. The infection generally is symptomless in most commercial apple cultivars, but causes symptoms of deformation, reduced size, chlorotic leaf spots and ring pattern mosaic on leaves of susceptible cultivars. ACLSV forms flexible filamentous-shaped particles, containing a positive single-stranded RNA genome of ca. 7,500 nucleotides excluding the 3'-poly (A) tail. In the present work we constructed full-length cDNA clones of ACLSV using an improved method of In-Fusion assembly, and infected apple seedlings with the constructed clones using vacuum-assisted agroinoculation.

Total RNA of infected apple leaves were extracted for generating viral cDNA full-length fragments. The low copy plasmid vector of pV297 (Prof. Dr. Edgar Maiss, Hannover) was linearized by PCR. The gel-purified fragments of linear vector and DNA fragments of ACLSV were then ligated using the In-Fusion assembly commercial kit. *Agrobacterium tumefaciens* strain ATHV was used in agroinoculation. Vacuum infiltration wasconducted at 500 hPa for 10 min using the vacuum system consisted of a Büchi V-500 vacuum pump and Büchi vacuum controller B-721 (Sigma-Aldrich GmbH, Munich, Germany) attached to vacuum desiccators.

Four full-length cDNA clones were screened from transformed *E. coli* cells by colony PCR. Two clones were infectious on *Nicotiana occidentalis* 37B. The viruses could be further transmitted to healthy *N. occidentalis* 37B by sap-inoculation. By the vacuum-assisted agroinoculation method, apple seedlings were infected with constructed clones in an infection rate of above 90%. ACLSV in inoculated plants was detected by PCR and transmission electron microscopy.

This was the first description of construction of full-length cDNA clones of ACLSV using In-Fusion assembly method. The method is suitable to study ACLSV and likely related filamentous viruses. Apple seedlings were infected using vacuum-assisted agroinoculation. The method is highly efficient and time-saving.