154 - Analysis of mixed populations of latent viruses of apple and rubbery wood disease of apple using new generation sequencing

Analyse von Mischpopulationen latenter Apfelviren und der Gummiholzkrankheit an Apfel mittels Hochdurchsatzsequenzierung

Vladimir Jakovljevic, Patricia Otten[2], Jonathon Blake[3], Wilhelm Jelkmann

Julius Kühn-Institut für Pflanzenschutz in Obst- und Weinbau
[2]FASTERIS, SA - Ch. du Pont-du-Centenaire 109, Case postale 28, 1228 Plan-les-Ouates, Switzerland
[3]EMBL Genomics Core Facility, Meyerhofstr. 1, 69117 Heidelberg, Germany

Characterization of many fruit diseases is hampered by the fact that the causative agents or their nucleotide sequences are unknown. Majority of plant viruses belong to the class of RNA viruses which due to their mode of replication (reverse transcription) exist within genetically diverse population, often termed as “quasispecies”. Furthermore, many species of plant viruses are found within mixed natural populations making isolation and Sanger sequencing of a single species difficult.

New generation sequencing (NGS) is emerging technology with its use in phytopathology still in early development. Here we show examples how this technology can be used to analyse mixed viral populations of latent viruses of apple, apple rubbery wood (ARW) and flat limb, diseases with unknown ethiology.

- *Apple stem pitting virus* (ASPV), *apple stem grooving virus* (ASGV) and *apple chlorotic leaf spot virus* (ACLSV) are single-stranded (+) RNA viruses belonging to the family of Betaflexiviridae. They usually cause latent infections of apple but in some cases they can produce symptoms and significant economic losses, especially when found in mixed infection (Yanase, 1974). We isolated viral double-stranded RNA (dsRNA) from apple samples tested by PCR as positive for mixed infection of ASPV, ASGV and ACLSV. Some of the samples had also symptoms of ARW and/or flat limb disease. Viral dsRNA was sequenced using Illumina HiSeq technology. We show how obtained sequence reads can be used to determine the structure of the viral population and to detect new viruses and viroids of apple.
- ARW disease is known in orchards since decades (Crane *et al*., 1945) but the causative agent of it is still unknown. Here we performed NGS of total RNA (RNA-seq) and of small RNAs from ARW infected apple and a healthy control using Illumina HiSeq. After subtraction from the control, ARW-specific reads were used to search for potential pathogens using various bioinformatic approaches. Among small RNAs, several micro-RNAs (miRNAs) have been found as candidates responsible for the development of the disease symptoms.

Literatur

YANASE H. 1974 Studies on apple latent viruses in Japan. Bull. Fruit Tree Res. Stn. Jpn. Ser. C1:47-109.
CRANE MB, 1945 Origin of Viruses. Nature. 155: 115-116.

155 - Experiments on transmission of viroids under glass and longevity of viroid RNA in detached leaves under different storage conditions

Thi Thu Vo, Heinz-Wilhelm Dehne, Stephan Winter, Joachim Hamacher

INRES – Phytomedicine, DSMZ - Plant Viruses

The two pospiviroids PSTVd (Potato spindle tuber viroid, EPPO A2 List) and TCDVd (Tomato chlorotic dwarf viroid) cause economically severe diseases on many host plants in the Solanaceae family. Solanaceous ornamental plants were found to be potential sources of infection. This study shows the effectiveness of three ways of mechanical transmission between tomato (Solanum esculentum), petunia (Petunia x hybrida) and Brugmansia suaveolens. The results show both,