

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

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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

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

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INRES – Phytomedicine, DSMZ - Plant Viruses

The two pospiviroids PSTVd (Potato spindle tuber viroid, EPP0 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,

TCDVd and PSTVd, can be transmitted successfully from both petunia and tomato to tomato and less well to brugmansia by diluted sap, contaminated gloves and razor blades. Tomato is a very susceptible host for PSTVd and TCDVd, whereas transmissions hardly succeeded on brugmansia. Furthermore, other studies show the stability of ribonucleic acid of PSTVd and TCDVd under different storage conditions, as they were stored asinoculum in fresh leaves in unsealed bags at room temperature and at low temperatures (in a refrigerator at +40C and a deep freezer at -200C). The results show that both viroids could be detected after storage for four months under different conditions by RT-PCR. Viroid-bands could be detected in all samples stored at -200C, whereas less positive samples could be detected at +40C- and room temperature storage. These results indicate that easy mechanical transmission and high nucleic acid stability could contribute to outbreaks of viroid epidemics in practice.

156 - Phytoplasmen in Schleswig-Holstein

Phytoplasmas in the state of Schleswig-Holstein

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Ziel

Phytoplasmen werden vermehrt in gartenbaulichen Kulturen detektiert. Ist eine relative Zunahme der infizierten Pflanzen, die lebenslang in der Ertragsleistung geschwächt sind oder absterben, festzustellen?

Nachweise

Der schleswig-holsteinische Pflanzenschutzdienst hat die Erregergruppe seit 1999 mittels molekularer Methoden auf der Agenda. Die im Poster vorgestellten Ergebnisse wurden im Rahmen von Monitorings und Beratungsanfragen erhoben. Beispielhaft werden positive Nachweise mit Fotos aus den Gartenbausparten Obstbau, Gemüsebau und Baumschule vorgestellt. Es wird stichwortartig auf die Symptomatik eingegangen und dokumentiert, welche anderen Pathogene ausgeschlossen wurden.

Fazit

Durch die Fokussierung auf Phytoplasmen in den letzten Jahren wurden vermehrt Testreihen durchgeführt. Entsprechend stieg die absolute Anzahl der positiven Nachweise in verschiedenen Kulturen an. Einige Abnehmer fordern entsprechende Nachweise, so dass die Produktionssicherheit in den Betrieben nach lässt und zum Teil pflanzengesundheitliche Einschränkungen die Folge sind. In vielen Gattungen wie zum Beispiel *Carpinus betulus*, *Corylus avellana*, *Quercus rubra*, *Fraxinus pennsylvanica*, *Rosa canina*, *Prunus serrulata* und *Prunus fruticosa* aus verschiedenen Betrieben und Herkünften konnte allerdings kein Befall mit Phytoplasmen bestätigt werden. Nach den vorliegenden Ergebnissen dürfte die relative Anzahl an Phytoplasmosen im gartenbaulichen Bereich konstant sein.