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Detection of alien viruses and viroids in plants by siRNA deep-sequencing

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RNA silencing mechanism is activated by double-stranded RNA (dsRNA)

Viruses form dsRNA

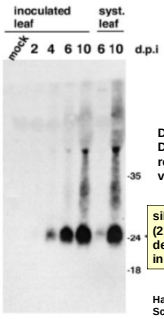
- replicative forms of RNA viruses
- ds secondary structures of single-stranded RNA virus genomes
- ds secondary structures of RNA transcripts produced by DNA viruses

RNA silencing is an antiviral defence mechamism:

Viruses are always detected by RNA silencing in infected plants!

In virus-susceptible plants, however, RNA silencing cannot prevent infection.

Viral RNA is detected and cleaved to small RNA by the RNA silencing mechanism



Northern blot, virus-spefic probe

Dicer-like (DCL) enzymes DCL2 and DCL4 are responsible for detecting viral RNA in plants

siRNA (21, 22 and 24 nt) derived from Potato virus X in an infected plant

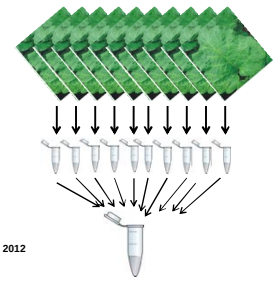
Hamilton & Baulcombe, 1999 Science 286:950-952

The plant detects the virus – we analyze the end products resulting from the defence response

siRNA can be isolated, sequenced, and viruses detected

Kreuze, J.F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., et al. (2009) Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. Virology 388: 1–7.

Procedure



1. Collect samples (store in freezer)

2. Exract total RNA, check quality, measure concentration, store in freezer

3. Pool similar amounts of RNA from different samples (e.g., 50 plants)

The pool of RNA (5-10 µg) will be sent to a sequencing laboratory

Data analysis ja utilization - A

5. Original RNA molecules or parts thereof are reconstructed from overlapping siRNA sequences:

Data analysis using bioinformatics may be all what is needed

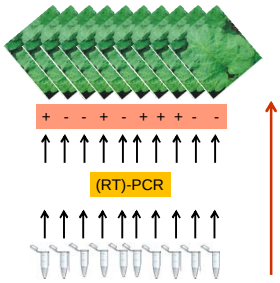
6. Reconstructed RNA molecules are identified by comparison with sequences in public databases → **7. VIRUSES DETECTED!**

8. Are the detected viruses new strains, or new viruses? Analyses can be continued by mapping siRNA reads to the genomes of viruses found in the sample (or to the closest-related viruses).

Data analysis ja utilization - B

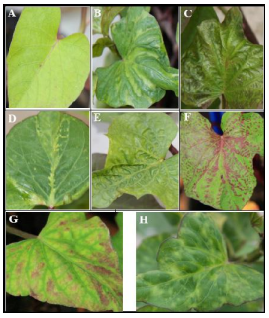
Identification of infected plants if needed

8. …. Analyses continued by mapping siRNA reads to the genomes of viruses found in the sample (or to the closest-related viruses).



9. PCR primer pairs designed based on the reconstructed viral genomes; original samples (RNA stored in freezer) tested by RT-PCR to detect the infected plants

SPPV-A	Sweet potato pakakuy virus, strain A	DNA, pararetro
SPPV-B	Sweet potato pakakuy virus, strain A	DNA, pararetro
SPLCGV	Sweet potato leaf curl Georgia virus	DNA, gemini
SPCSV-WA	Sweet potato chlorotic stunt virus, strain WA	RNA, crini
SPFMV	Sweet potato feathery mottle virus	RNA, poty
SPVC	Sweet potato virus C	RNA, poty



Kashif, Pietilä, Artola, Jones, Tugume, Mäkinen and Valkonen 2012. Detection of viruses in sweetpotatoes from Honduras and Guatemala augmented by deep-sequencing of small-RNAs. Plant Disease 96:1430-1437.

Raspberry cv. 'Glen Ample'

Raspberry leaf blotch virus **(RLBV)**
Previously known in Scotland and Serbia (McGavin et al. 2012)

Bi, Artola, Kurokura, Hytönen & Valkonen, 2012. First report of *Raspberry leaf blotch virus* in raspberries in Finland. Plant Disease 96:1231

Woolly burdock, a wild plants species (*Arctium tomentosum*)

Alstroemeria virus X **(AlsVX)**
Previously known only in Japan (Fuji et al. 2005)

Woolly burdock vein yellowing virus (a new putative emaravirus)

Bi, Tugume & Valkonen 2012. Small-RNA deep-sequencing reveals Arctium tomentosum as a natural host of *Alstroemeria virus X* and a new putative emaravirus. PLoS ONE 7: e42758.

Other plant viruses:

Hagen, C., Frizzi, A., Kao, J., Jia, L.J., Huang, M.Y., et al. 2011. Using small RNA sequences to diagnose, sequence, and investigate the infectivity characteristics of vegetable-infecting viruses. Arch Virol 156: 1209–1216. (***Tomato spotted wilt virus***)

Pallett, D.W., Ho, T., Cooper, I. & Wang, H. 2010. Detection of ***Cereal yellow dwarf virus*** using small interfering RNAs and enhanced infection rate with *Cocksfoot streak virus* in wild cocksfoot grass (Dactylis glomerata). J. Virol. Methods 168: 223–227.

Zhang, Y., Singh, K., Kaur, R. & Qiu, W. 2011. Association of a **novel DNA virus** with the grapevine vein-clearing and vine decline syndrome. Phytopathology 101:1081–1090.

Viroids:

Li, R., Gao, S., Hernandez, A.G., Wechter, W.P., Fei, Z., et al. 2012. Deep sequencing of small RNAs in tomato for virus and viroid identification and strain differentiation. PLoS ONE 7: e37127.

Animal viruses:

Ma, M.J., Huang, Y., Gong, Z.D., Zhuang, L., Li, C., et al. 2011. Discovery of DNA viruses in wild-caught mosquitoes using small RNA high throughput sequencing. PLoS ONE 6: e24758.

Wu, Q., Luo, Y., Lu, R., Lau, N., Lai, E.C., et al. 2010. Virus discovery by deep sequencing and assembly of virus-derived small silencing RNAs. Proc. Natl. Acad. Sci. USA 107: 1606–1611.

Human viruses (HIV-1)

Isakov, O., Modai, S. & Shomron, N. 2011. Pathogen detection using short-RNA deep sequencing subtraction and assembly. Bioinformatics 27: 2027–2030.