**Molecular virus-plant interactions and pathogen defence**

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**SYNOPSIS**

- **Vertical transmission:** In vegetatively propagated plants, viruses are transmitted to new crops in the infected planting materials (cuttings, tubers, bulbs, etc.). Most viruses are not transmitted via true seed.

- **Horizontal transmission:** Viruses are transmitted from plant to plant by vectors (aphids, leafhoppers, whiteflies, thrips, and a few soilborne microbes and nematodes), which cannot be controlled by chemicals in most cases. Some few viruses are transmitted via pollen.
SYNOPSIS

- **Vertical transmission**: In vegetatively propagated plants, viruses are transmitted to new crops in the infected planting materials (cuttings, tubers, bulbs etc.). Most viruses are not transmitted via true seed.
  
  **Control**: Healthy plant materials and seeds

- **Horizontal transmission**: Viruses are transmitted from plant to plant by vectors (aphids, leafhoppers, whiteflies, thrips, and a few soilborne microbes and nematodes), which cannot be controlled by chemicals in most cases. Some few viruses are transmitted via pollen.
  
  **Control**: Virus-resistant cultivars

**Virus resistance**

1. Basal defence (non virus-specific): RNA silencing
2. R gene-mediated dominant resistance (virus-specific)
3. Recessive resistance due to mutations in host factors required in virus infection (possibly broad-spectrum, non virus-specific?)

**PAMPs (Pathogen-associated molecular patterns)**

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**Virus as a target of RNA silencing: Recovery from virus infection**

**Transgenic virus resistance based on RNA silencing**

Transgene messenger RNA (mRNA) (a fragment of viral gene)

small interfering RNA (siRNA)

siRNA produced from the over-expressed transgene mRNA are loaded to the RNA silencing complex (RISC). RISCs are ready to destroy the viral RNA immediately at infection.

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**Viral counter-defense**

Viruses can suppress RNA silencing-based resistance. Hence, natural or transgene-mediated resistance to a virus may be lost in mixed infection where the plant gets infected with an unrelated virus.

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**The zig-zag model of resistance evolution**

**Genome structure**

**Sweet potato chlorotic stunt virus (SPCSV)**

RNA 1 9407 kb

RNA 2 8223 kb


**Cuellar et al. 2008, Plant 106: 10354-10358**

**Transformed with a Rnase3 of SPCSV, infected with SPFMV**

**Rnase3 of SPCSV eliminates virus resistance of the sweetpotato plant.**

Plants develop a severe disease when co-infected with other viruses.
**RNase3 cleaves ds-smallRNAs**

*Synthetic siRNA*

*Total siRNA derived from SPFMV-infected sweetpotato*

Note: The proportion of siRNA derived from SPFMV was only 3.95% of total siRNA as determined by deep-sequencing of total siRNA.

Cuellar et al. 2009, PNAS 106: 10354-10358

**Mechanisms by which viruses suppress RNA silencing**

2. Unstabilize siRNA

*dsRNA: inducer*

*Cellular HEN1 methylates siRNA to increase stability*

**The zig-zag model of resistance evolution**

PTI = Pathogen-triggered immunity

ETI = Effector-triggered immunity

PR = Pathological resistance

ETS = Effector-triggered susceptibility

PAMPs (Pathogen-associated molecular patterns)


**Gene-for-gene interactions in resistance:**

recognition of viral proteins by R proteins and induction of defense

Plant | Pathogen | R Gene for Avr Gene | R Protein for Avr Protein

A system infection of *Nicotiana tabacum* cv. Turk plants showing TMV-associated mosaic.

(B) Necrotic local lesions on *N. tabacum* Glurk leaf, demonstrating Holmes’ *N*-gene resistance following inoculation with TMV.

Photo: K.-B. G. Scholthof, APSnetFeatures-2008-0408
The N protein

The C-terminal leucine-rich repeat (LRR) domain of N recognizes the helicase domain in the TMV replicase protein (p50), which is an RSS protein and hence an important viral effector. However, this is possible only after the N-proximal Toll and interleukin receptor like domain (Toll/IL-R) has bound a chloroplast protein (NRIP1), which is needed for N-p50 interaction and induction of a signal transduction cascade that activates a wide range of defence responses (see below). The hierarchical order of interactions is indicated with numbers on top of the arrows. The nucleotide binding site (NBS) in the center of N contains three kinase domains and comprises, with an ARC domain, an inhibitory pocket that regulates R protein activity.


 Potato genome sequence
(Nature 475:189-195; July 15, 2011)

CONCLUSION
Active plant defence against viruses:
R gene-mediated resistance is widely used in plant breeding programmes
RNA silencing-based resistance to viruses is utilized in transgenic plants to protect them from viral diseases.
However, improved natural RNA silencing efficiency of plants is not yet (?) an intentional target in resistance breeding.

'Passive' resistance to viruses:
It is considered that lack of compatible host factors required by the virus at any stage of the infection cycle may result in recessive resistance to the virus.

The functions needed by the virus for completion of the infection cycle
1. Replication
2. Suppression of host defence
3. Movement (transport) from cell to cell and to other parts of the plant
4. Encapsidation (plant-to-plant transmission)
Immunogold-labeled VPg at the end of particles of Potato virus A

Puustinen, Jaruve, Välikuus, & Mäkinen (2002)
Journal of Virology 76:12703–12711

The eukaryotic translation initiation complex
Robaglia & Caranta, Trends in Plant Science Vol.11 No.1 January 2006

THE ROLE OF VPg?
The VPg of potyviruses binds covalently to the 5' terminus of the viral (+)ssRNA. It is thought to substitute the 7-methylguanylate cap (m7G) that is required in mRNA.

Indeed, VPg interacts with translation initiation factors, notably eIF4E and eIF(iso)4E.

VPg enhances viral protein expression and replication on the cost of cellular mRNAs. [Skolnik et al. 2011, J. Virol. 85:5120-5131]

The role of VPg in RNA silencing

VPg is a suppressor of RNA silencing

1. VPg interferes with silencing, which requires translocation of VPg to the nucleus (why?)
2. Results reveal that nucleolus is involved in RNA silencing

Mutations to regions B or A prevent translocation of VPg to the nucleolus


Regions of the viral genome-linked protein (VPg) controlling nuclear localization

HC-Pro of potyviruses is a strong suppressor of RNA silencing (binds siRNA)


HC-Pro contains a specific eIF4E binding site.

Mutation of the IF binding site in HCpro reduces HCpro-IF interaction and greatly reduces the infectivity of PVA.

Ala-Poikela et al., unpublished

CONCLUSIONS

‘Passive’ resistance to plant viruses:

1. Disruption of the interactions between viral and host proteins reduces or inhibits virus infection.
2. Since many viruses are probably utilizing the same host factors, certain mutations in these host factors might confer broad resistance to a wide range of viruses.
3. A similar approach, including transgene-mediated RNA silencing of host factors, is also applicable in breeding for resistance to fungal pathogens.

Colour-break symptoms of virus-infected tulips

Still life with tulips – Johannes Boschaert, 1610
National Museum, Stockholm, Sweden

II. RNA silencing

dsrRNA: inducer

asaRNA: small interfering dsRNA (effector): 21, 22 or 24 nt.
- Contains 2-nt 3' overhangs and a 3' hydroxyl group.
- Prime sequence-specific RNA degradation.
- HEN1 methylates to increase stability.
- Signal molecules for local and systemic spread of silencing.

dsRNA: inducer

RNase III

Dicer (DCR; multidomain protein), endonuclease:
RNase III domains bind and cleave dsRNA to produce siRNA.

Plants have four Dicer-like proteins (DCL) for different silencing-mediated functions.

Nicotianus tabacum virus X: nts for cleavage

RISC

RISC incorporates an RNase ('Argonaute', AGO) and an siRNA duplex. AGO degrades one of the siRNA strands.

It uses the other strand as a guide to find homologous ssRNA for cleavage.

Transitivity: dsRNA generated also from outside the targeted part of the sequence.

Cellular RNA polymerase makes the cleaved ssRNA double-stranded, and it will be cleaved by Dicer.

Amplification of silencing

Secondary siRNA: nucleases for silencing

Boschaert, 1610
National Museum, Stockholm, Sweden

Gene-for-gene based recognition of viruses carried out by dominant R and N genes

Hypersensitive resistance response to virus infection on a potato leaf


Initial infection cites of GFP-tagged Potato virus A in an inoculated leaf


Long-distance transport of Potato virus A

Ala-Poikela et al. 2011, JOURNAL OF Virology 85: 6784-6794

HCpro – eIF(iso)4E interactions co-localizes with the viral replication vesicles