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Faculty of Agriculture and Forestry

PLANT BREEDING FOR THE FUTURE

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Molecular virus-plant interactions and pathogen defence

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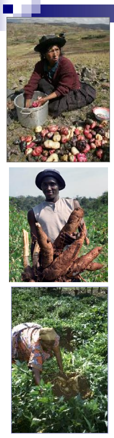

Potato, cassava and sweetpotato are vegetatively propagated food crops

The most important food crops:

Maize	817 million tn
Rice	678
Wheat	681
Potato	329
Cassava	228
Barley	136
Sweetpotato	126

FAOSTAT 2009

SYNOPSIS

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

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

Virus resistance is the main approach to control the spread of plant viruses and the diseases they cause.

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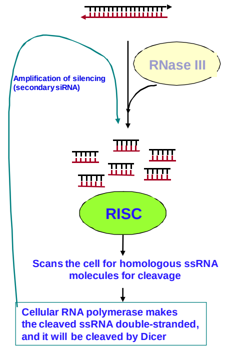
1. Basal defence (non virus-specific): RNA silencing
2. R gene-mediated dominant resistance (virus-specific)
3. Recessive resistance due to mutations in host factors required in virus infection (possibly broad-spectrum, non virus-specific?)

Basal defence recognizes molecular patterns caused by virus infection

RNA viruses replicate (multiply) via double-stranded RNA intermediates

=> Double-stranded RNA induces basal defense

II. RNA silencing



Amplification of silencing (secondary siRNA)

RNase III

RISC

Scans the cell for homologous ssRNA molecules for cleavage

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

dsRNA: inducer

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.)

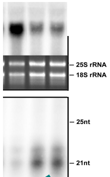
siRNA - small interfering dsRNA (effector): 21, 22 or 24 nt.
- Contain 2-nt 3' overhangs and a 3' hydroxyl group.
- Prime sequence-specific RNA degradation.
- HEN1 methylates to increase stability
- Signal molecules for local and systemic spread of silencing

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.

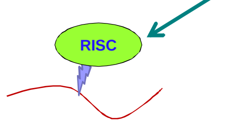
Transitivity: siRNA generated also from outside the original targeted part of the sequence. Unidirectional in animals, bidirectional in plants.

Transgenic virus resistance based on RNA silencing

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



small interfering RNA (siRNA)

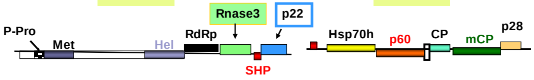


Genome structure

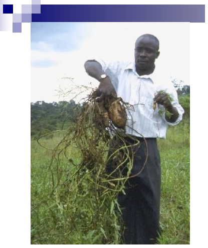
Sweet potato chlorotic stunt virus

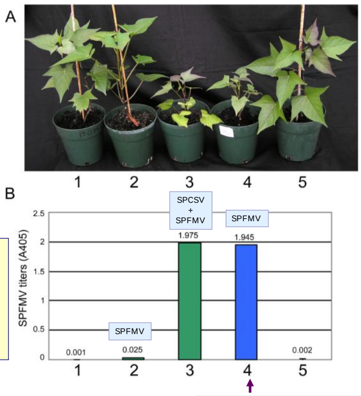
RNA 1
9407 kb

RNA 2
8223 kb



Kreuze et al. 2002, J Virol 76, 9260-9270





Rnase3 of SPCSV eliminates virus resistance of the sweetpotato plant.

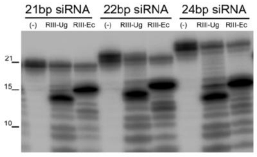
Plants develop a severe disease when co-infected with other viruses.

Transformed with a RNase3 of SPCSV, infected with SPFMV

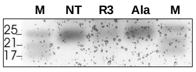
Cuellar et al. 2009, PNAS 106: 10354-10358

RNase3 cleaves ds-smallRNAs

Synthetic siRNA



Total siRNA derived from SPFMV-infected sweetpotato



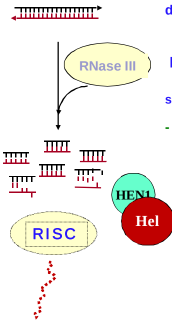
EtBr-stained gel

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

Dicer

siRNA - small interfering dsRNA (effector): 21, 22 or 24 nt

- Cellular HEN1 methylates siRNA to increase stability

**TMV helicase suppresses silencing:
It binds HEN1 and unstabilizes siRNAs, and also binds ds-siRNA and prevents them from being incorporated into RISC**

Kubota et al. (2003) J Virol 77, 11016-26
Vogler et al. (2007) J Virol 81, 10379-88
Csorba et al. (2007) J Virol 81, 11768-80

Gene-for-gene resistance:

recognition of viral proteins and induction of defense

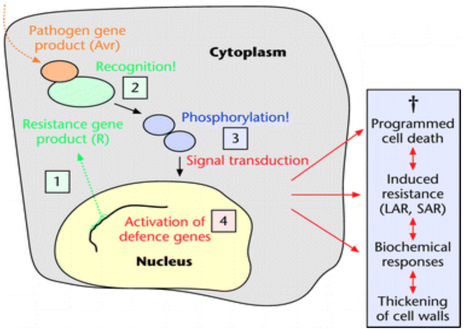
Plant **Pathogen**

gene - for - gene

↓ ↓

Protein - for - Protein

HOW DOES IT WORK?



J. Valkonen 2001. Encyclopedia of Life Sciences (www.els.net)

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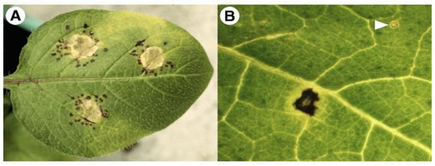
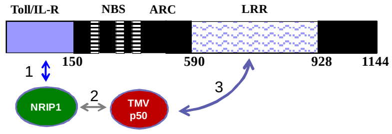
Gene-for-gene based recognition of viruses carried out by dominant *R* and *N* genes

Hypersensitive resistance response



Hämäläinen et al. (2000) Mol. Plant-Microbe Interact. 13: 402-412

The N protein



Structure of an R protein belonging to the TIR-NBS class, and recognition of the pathogen on the gene-for-gene basis.

The C-proximal 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 nt-binding pocket that regulates R protein activity.

Caplan et al. 2008. Cell 132, 449-462

CONCLUSION

Active plant defence against viruses:

The basic concept of evolution of resistance and virulence is described as a process of defence and counter-defence between plants and pathogens.

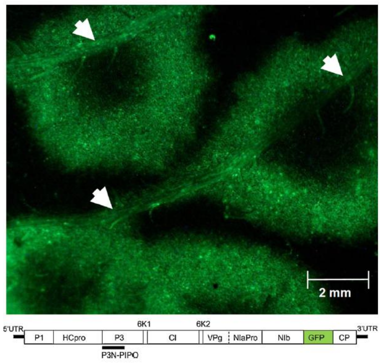
’Passive’ resistance to viruses:

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

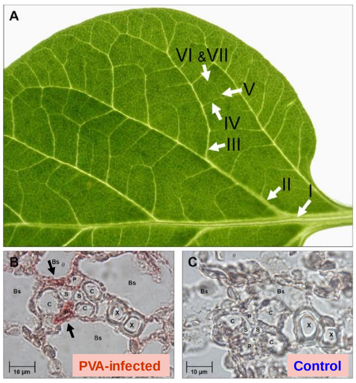
1. Replication
2. Suppression of host defence
3. Movement (transport) from cell to cell
4. Encapsidation (plant-to-plant transmission)

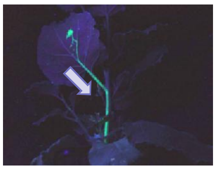
Initial infection cites of GFP-tagged Potato virus A in an inoculated leaf



Vuorinen, Kelloniemi, & Valkonen (2011) *Plant Science* **181**:355-363.

Long-distance transport of Potato virus A





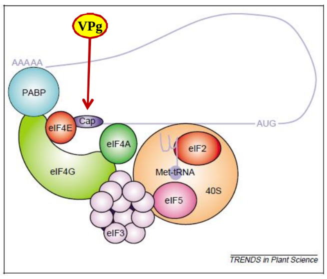
Vuorinen, Kelloniemi, & Valkonen (2011) *Plant Science* **181**:355-363.

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 (m^7G) 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 (Eskelin et al. 2011, J. Virol. 85:8210-8221)



The eukaryotic translation initiation complex

Robaglia & Caranta, TRENDS in Plant Science Vol.11 No.1 January 2006

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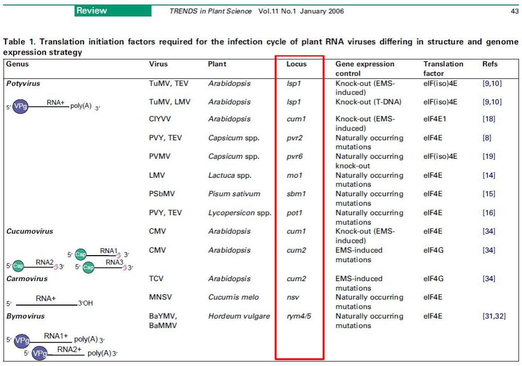
Mutated eIF4E (and eIF4G) genes function as recessive resistance genes



Review *TRENDS in Plant Science* Vol.11 No.1 January 2006 43

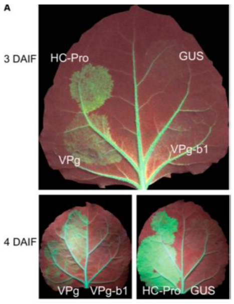
Table 1. Translation initiation factors required for the infection cycle of plant RNA viruses differing in structure and genome expression strategy

Genus	Virus	Plant	Locus	Gene expression control	Translation factor	Refs
Potyvirus	TuMV, TEV	*Arabidopsis*	*lsp1*	Knock-out (EMS-induced)	eIF(iso)4E	[9,10]
5' VPg RNA+ poly(A) 3'	TuMV, LMV	*Arabidopsis*	*lsp1*	Knock-out (T-DNA)	eIF(iso)4E	[9,10]
	ClYVV	*Arabidopsis*	*cum1*	Knock-out (EMS-induced)	eIF4E1	[18]
	PVY, TEV	*Capsicum* spp.	*pvr2*	Naturally occurring mutations	eIF4E	[8]
	PVMV	*Capsicum* spp.	*pvr6*	Naturally occurring knock-out	eIF(iso)4E	[19]
	LMV	*Lactuca* spp.	*mo1*	Naturally occurring mutations	eIF4E	[14]
	PSbMV	*Pisum sativum*	*sbm1*	Naturally occurring mutations	eIF4E	[15]
	PVY, TEV	*Lycopersicon* spp.	*pot1*	Naturally occurring mutations	eIF4E	[16]
Cucumovirus	CMV	*Arabidopsis*	*cum1*	Knock-out (EMS-induced)	eIF4E	[34]
5' Cap RNA1 3' 5' Cap RNA2 3' 5' Cap RNA3 3'	CMV	*Arabidopsis*	*cum2*	EMS-induced mutations	eIF4G	[34]
Carmovirus	TCV	*Arabidopsis*	*cum2*	EMS-induced mutations	eIF4G	[34]
5' RNA+ 3'OH	MNSV	*Cucumis melo*	*nsv*	Naturally occurring mutations	eIF4E	
Bymovirus	BaYMV, BaMMV	*Hordeum vulgare*	*rym4/5*	Naturally occurring mutations	eIF4E	[31,32]
5' VPg RNA1+ poly(A) 3' 5' VPg RNA2+ poly(A) 3'						

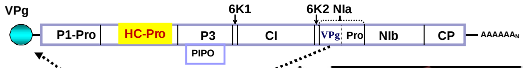
VPg is a suppressor of RNA silencing

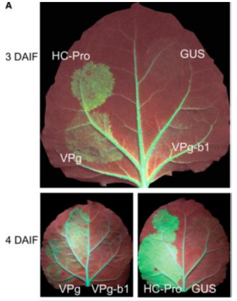
1. VPg interferes with silencing, which requires translocation of VPg to the nucleolus (why?)

2. Results reveal that nucleolus is involved in RNA silencing



Rajamäki & Valkonen 2009, The Plant Cell 21: 2485-2502.





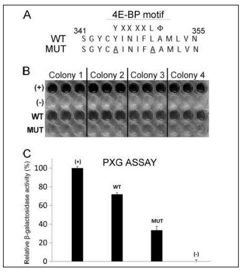
HC-Pro of potyviruses is a strong suppressor of RNA silencing (binds siRNA)

Kasschau & Carrington (1998) Cell 95:461–470.
Shiboleth et al. (2007) J. Virol. 81:13135–13148.
Torres-Barcelo et al. (2008) Genetics 180: 1039–1049.

HC-Pro, a strong silencing suppressor

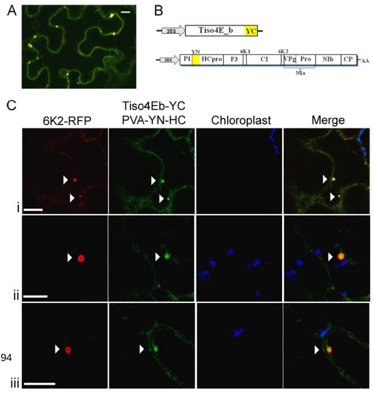
HC-Pro contains a specific eIF4E binding site

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



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

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



Ala-Poikela et al. 2011,
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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, fundamental mutations in these host factors might confer the broadest type of virus resistance.

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Plant pathology/virology group

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