University of Helsinki

Faculty of Agriculture and Forestry

PLANT BREEDING FOR THE FUTURE

THE 50TH ANNIVERSARY OF UPOV
GENEVA, OCTOBER 21, 2011

Molecular virus-plant interactions and pathogen defence

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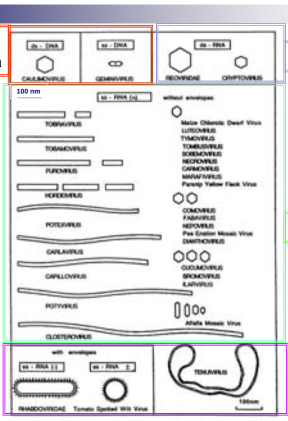
dsDNA (rare)
ssDNA common

- Over 1000 plant viruses (represent ca. 25 % of all known viruses)



dsRNA (rare)

(+)ssRNA –common!

(-)ssRNA (rare)

Figure 5.1 The families and groups of viruses infecting plants. Outline diagrams are drawn approximately to scale. (Courtesy R. I. B. Francki.) One additional group, the commelina yellow mottle virus group with dsDNA and bacilliform particles, was approved by the I.C.T.V. in August 1990.

Matthews 1991

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.

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SCRI

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:** 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.

Photos: Richard Harrington

LEAFHOPPERS

WHITEFLIES

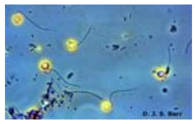
THRIPS

Photos: MTT, Växtskyddscentralen, Peter Mullin, E.R. Ingham, J.S. Barr

SOILBORNE VECTORS:

Nematodes

Xiphinema
Longidorus
Trichodorus
Paratrichodorus



Zoospores and resting spores

Olpidium
Polymyxa
Spongospora

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

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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?)

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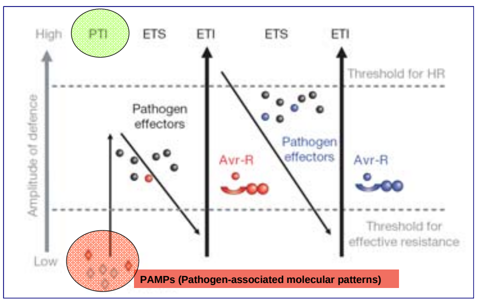
The zig-zag model of resistance evolution

PTI = Pathogen-triggered immunity ETI = Effector-triggered immunity
ETS = Effector-triggered susceptibility



Jones & Dangl (2006) Nature 444:323-329

Basal defence recognizes molecular patterns caused by virus infection

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

=> Double-stranded RNA induces basal defense

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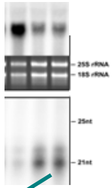
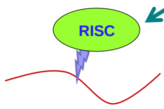
Virus as a target of RNA silencing: Recovery from virus infection

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

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.

The zig-zag model of resistance evolution

PTI = Pathogen-triggered immunity ETI = Effector-triggered immunity
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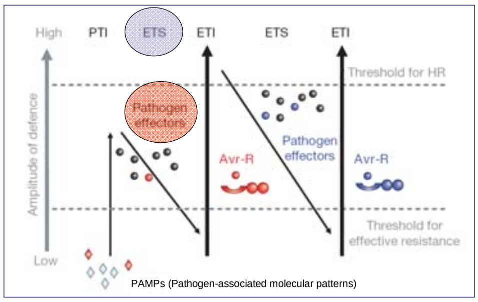
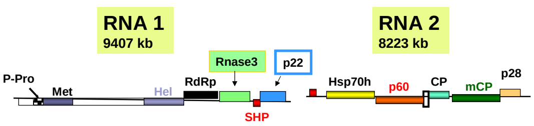
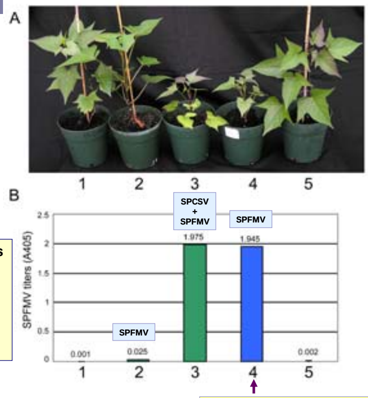


Jones & Dangl (2006) Nature 444:323-329

Genome structure
Sweet potato chlorotic stunt virus (SPCSV)



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



Rnase3 of SPCSV eliminates virus resistance of the sweetpotato plant.

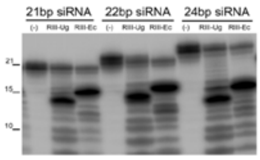
Plants develop a severe disease when co-infected with other viruses.

Transformed with a RNase3 of SPCSV, infected with SPFMV

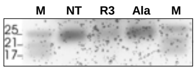
Cuellar et al. 2009, PNAS 106: 10354-10358

RNase3 cleaves ds-smallRNAs

Synthetic siRNA



Total siRNA derived from SPFMV-infected sweetpotato



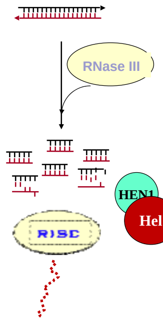
EtBr-stained gel

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 interferring 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

The zig-zag model of resistance evolution

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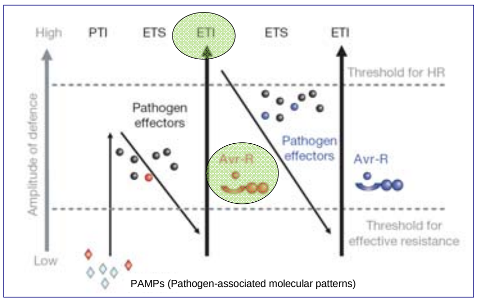
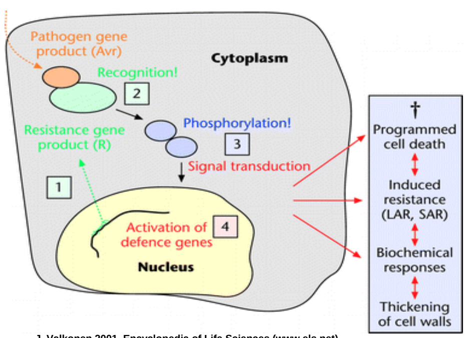


Jones & Dangl (2006) Nature 444:323-329

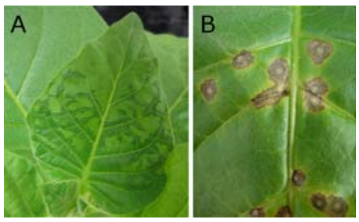
Gene-for-gene interactions in resistance:

recognition of viral proteins by R proteins and induction of defense

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



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

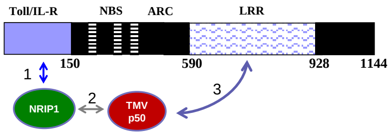


(**A**) Systemic infection of *Nicotiana tabacum* cv. Turk plants showing TMV-associated mosaic.

(**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



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

◄ **Fig. 1** Schematic representation of the genome-wide integrated genetic map of RGHs and resistance trait loci in potato. Chromosomes or linkage groups are represented by *broad vertical bars* with the bin signatures (van Os et al. 2006) separated by *white horizontal bars*. To the right of each linkage group, genetic markers are indicated in *black*. Previously described *R* genes which reside at syntenic loci are indicated in *red* and previously described *R* genes for which no syntenic loci is shown are indicated in *black*. Resistance trait loci that map approximately to an RGH locus are indicated in *green* and otherwise in *blue*. Horizontal *dashed bars* indicate that the marker used to map a previously described resistance trait locus is also mapped in the UHD map. To the left of each linkage group, RGH (TNL and CNL) loci are indicated in *red* if they are syntenic to previously described *R* genes, in *green* if they map approximately at the same location as a resistance trait locus and in *blue* if they do not. *Thin vertical bars* represent genetic intervals and *thin horizontal bars* a genetic map position

Bakker et al. (2011) Theor Appl Genet 123:493–508

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopathol 39:79–102

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.

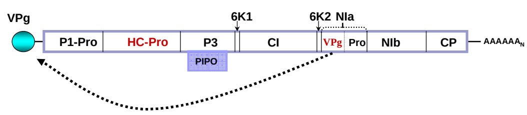
’Passive’ resistance to viruses:

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.

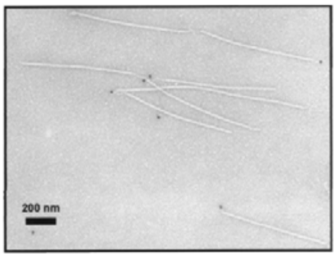
The functions needed by the virus for completion of the infection cycle

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)

The viral genome-linked protein, VPg



Potato virus A



Immunogold-labeled VPg at the end of particles of *Potato virus A*

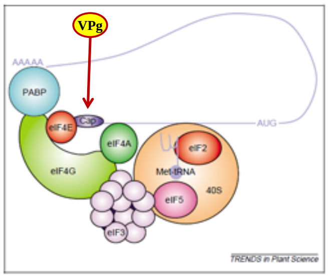
Puustinen, Rajamäki, Ivanov, Valkonen & Mäkinen (2002)
JOURNAL OF VIROLOGY 76:12703–12711

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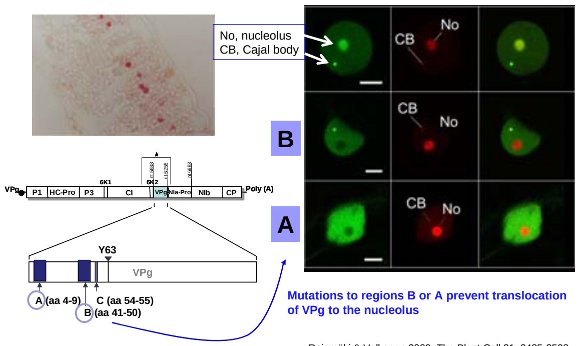
Mutated eIF4E (and eIF4G) genes function as recessive resistance genes

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]
	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]
	CMV	*Arabidopsis*	*cum2*	EMS-induced mutations	eIF4G	[34]
Carmovirus	TCV	*Arabidopsis*	*cum2*	EMS-induced mutations	eIF4G	[34]
	MNSV	*Cucumis melo*	*nsv*	Naturally occurring mutations	eIF4E	
Bymovirus	BaYMV, BaMMV	*Hordeum vulgare*	*rym4/5*	Naturally occurring mutations	eIF4E	[31,32]

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



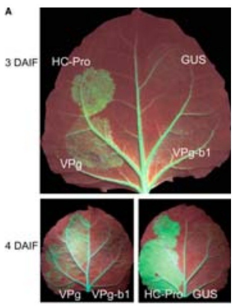
Mutations to regions B or A prevent translocation of VPg to the nucleolus

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

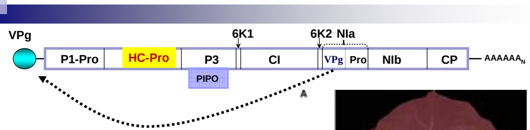
VPg is a suppressor of RNA silencing

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

2. Results reveal that nucleolus is involved in RNA silencing

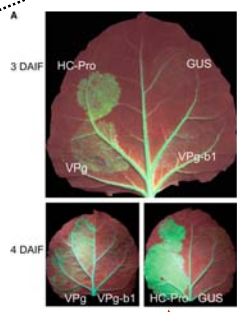


Rajamäki & Valkonen 2009, The Plant Cell 21: 2485-2502.



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

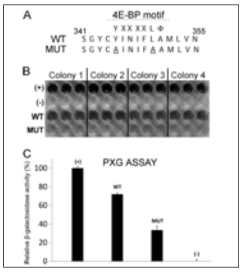
Kasschau & Carrington (1998) Cell 95:461–470.
Shiboleth et al. (2007) J. Virol. 81:13135–13148.
Torres-Barcelo et al. (2008) Genetics 180: 1039–1049.



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

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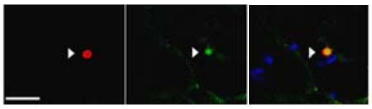
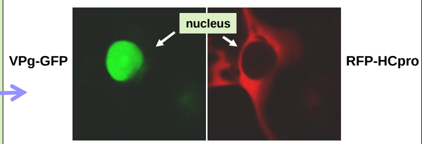
1. VPg and HC-Pro are synthesized in the same place on the cellular membranes and co-localize there.

2. Later, VPg (and its precursor NIa) and HCpro are separated to different subcellular compartments:
VPg - nucleus
HCpro – cytoplasm

HCpro and VPg interact with translation initiation factors, which exist cytoplasm and nucleus!





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.

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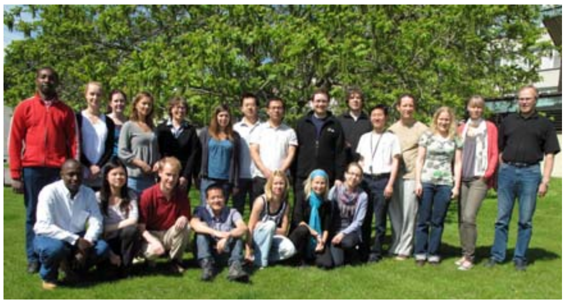
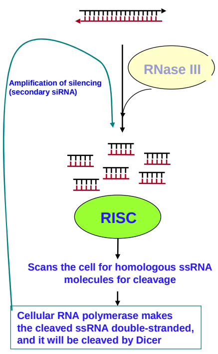


Colour-break symptoms of virus-infected tulips

Still life with tulips – *Johannes Bosschaert*, 1610

National Museum, Stockholm, Sweden

II. RNA silencing



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.)

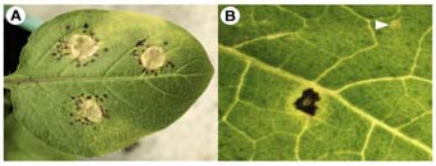
siRNA - small interfering dsRNA (effector): 21, 22 or 24 nt.
- Contain 2-nt 3’ overhangs and a 3’ hydroxyl group.
- Prime sequence-specific RNA degradation.
- HEN1 methylates to increase stability
- Signal molecules for local and systemic spread of silencing

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.

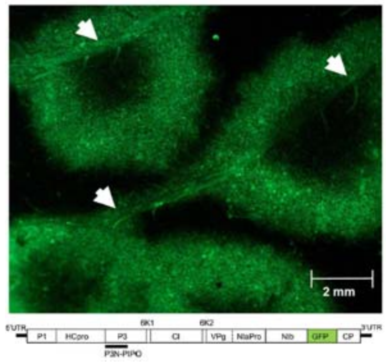
Gene-for-gene based recognition of viruses
carried out by dominant *R* and *N* genes

Hypersensitive resistance response to virus infection on a potato leaf



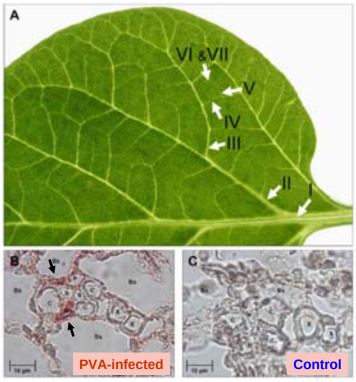
Hämäläinen et al. (2000) Mol. Plant-Microbe Interact. 13: 402-412

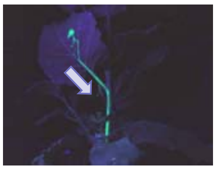
Initial infection cites of GFP-tagged Potato virus A in an inoculated leaf



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

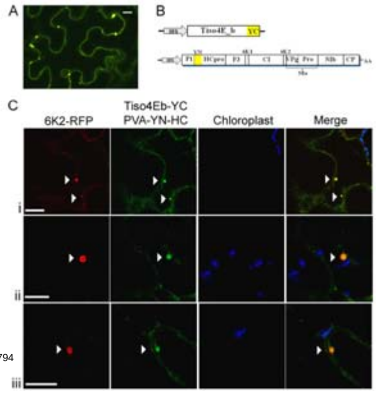
Long-distance transport of Potato virus A





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

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



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