Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen
Federal Research Centre for Cultivated Plants

Cross-protection: Combatting plant diseases with mild virus isolates

Heiko Ziebell

Julius Kühn-Institut, Institut für Epidemiologie und Pathogendiagnostik, Braunschweig, Deutschland

Housekeeping rules

- Please ask your questions in the chat
- Two presentations followed by Q&A session
- Please support us by signing up for the stakeholder information plattform (link at the end of the presentations)
- This webinar will be recorded and made accessible

Virtigation Project facts

- **Title:** VIRTIGATION – Emerging viral diseases in tomatoes and cucurbits: implementation of mitigation strategies for durable disease management
- **Funding program:** European Union's Horizon 2020 research and innovation
- **Call topic:** Sustainable food security – new and emerging risks to plant health
- **Type:** Research & Innovation Action
- **EU contribution:** EUR 6,998,668.34
- **Start:** 1 June 2021
- **End:** 31 May 2025
- **Duration:** 4 years
- **Number of partners:** International consortium of 25 partners from 12 countries

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

Virtigation Consortiuum

VIRTIGATION is coordinated by the Department of Biosystems at KU Leuven. The coordinator is Prof. Hervé Vanderschuren from the Laboratory for Tropical Crop Improvement:

KU LEUVEN

DCM

tecnova CENTRO TECNOLÓGICO

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NRI | Natural Resources Institute

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UNIVERSITY OF AGRICULTURAL SCIENCES, BENGALURU

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Virtigation Project challenge

- **New aggressive viruses spreading in greenhouses and fields threaten the multi-billion value chain of tomato and cucurbit** (i.e. cucumber, melon, pumpkin, zucchini and gourds):
  - **The whitefly-transmitted begomoviruses** Tomato leaf curl New Delhi virus (**ToLCNDV**) and the Tomato yellow leaf curl virus (TYLCV)
  - **The mechanically transmitted tobamovirus** Tomato brown rugose fruit virus (**ToBRFV**)

- **Due to their rapid transmission**, these plant diseases are difficult to control and can cause complete crop loss in affected areas
- **Producers often use chemical pesticides to fight these viruses**, which exposes growers and consumers to pesticide residues
- At the moment, **only limited organic solutions exist on the market** to combat these viruses

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Virtigation Project aim & objectives

- **The aim of VIRTIGATION is to develop rapid and long-lasting solutions to combat emerging viral diseases in tomato & cucurbit,** implemented through 6 specific objectives:
  1. Knowledge sharing and engagement of stakeholders in research activities
  2. Develop robust diagnostic tests, quarantine measures and identify ecological factors driving disease outbreaks
  3. Understand plant-virus-vector interactions
  4. **Develop Integrated Pest Management (IPM) solutions**
  5. Identify and pyramid natural resistance to viral diseases and vectors
  6. Train the tomato and cucurbit value chain

- **VIRTIGATION further seeks to increase the know-how of the value chain to better control viral diseases, through its multi-actor approach anchored in co-design**

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Virtigation More information:

JKI

www.virtigation.eu

Also at

zenodo

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Cross-protection
Definition

A primary infection with a virus prevents a secondary infection with a challenging homologous virus

Early mechanism hypotheses

- Formation of immunising substances
- Antibody formation
- Competition for replication materials
- Occupation of virus-specific replication sites

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MOSAIC DISEASES IN THE CANARY ISLANDS, WEST AFRICA, AND GIBRALTAR [1]

By H. H. McKINNEY [2]

Senior Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

Mechanisms involved in cross-protection

Coat protein mediated protection

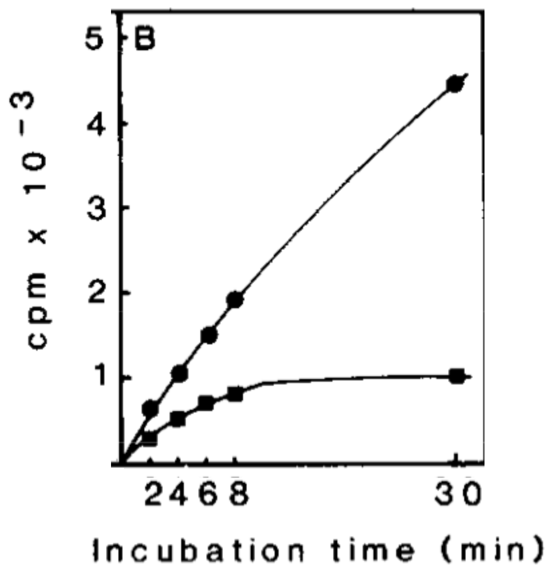
Mechanism A: CP of the protective strain partially coats the challenging strain's RNA

- Brome mosaic virus (BMV) *in vitro* test (Horikoshi et al., 1987)
- BMV replicase extract :
  - addition of BMV CP → inhibition of RNA synthesis
  - addition of TMV CP → no inhibition of RNA synthesis
- The two viruses are too distantly related to achieve cross-protection (different viral families)

Time course of BMV RNA synthesis in vitro in the presence or absence of BMV CP

● Absence of BMV CP

■ Presence of BMV CP



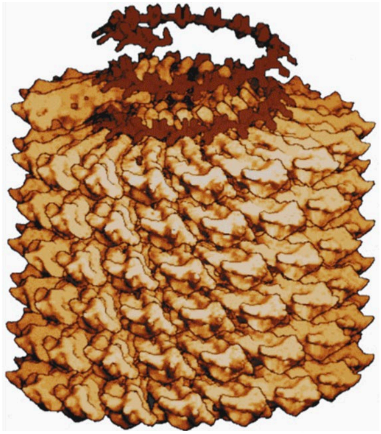
Horikoshi et al., 1987

Mechanisms involved in cross-protection

Coat protein mediated protection

Mechanism B: CP of the protective strain prevents uncoating of the challenging strain

- Protoplasts from transgenic plants expressing tobacco mosaic virus (TMV) ”protective” strain CP (Register and Beachy, 1988):
  - Challenged with purified TMV RNA → “challenging” strain can infect → no cross-protection
  - Challenged with intact TMV viral particles → no uncoating → “challenging” strain cannot infect → cross-protection



The ICTV Report on
Virus Classification and Taxon Nomenclature

Mechanisms involved in cross-protection

Coat protein mediated protection

- Viroids are single-stranded circular RNA molecules that are not coated by CP
- Viroid induced cross-protection (Horst, 1975)
  - Latent infectious agent of Chrysanthemum chlorotic mottle viroid (ChCMV-NS) identified in *Chrysanthemum morifolium*
  - ChCMV-NS leads to cross-protection against a challenge with an infectious ChCMV

Conclusion:

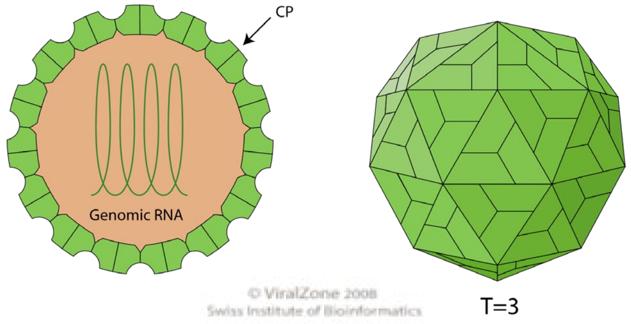
The coat protein may play a role in cross-protection but it is not the only mechanism involved

Mechanisms involved in cross-protection
RNA silencing mediated protection

Mechanism C: Primary infection activates RNA silencing of the viral mRNA of the challenging closely related virus

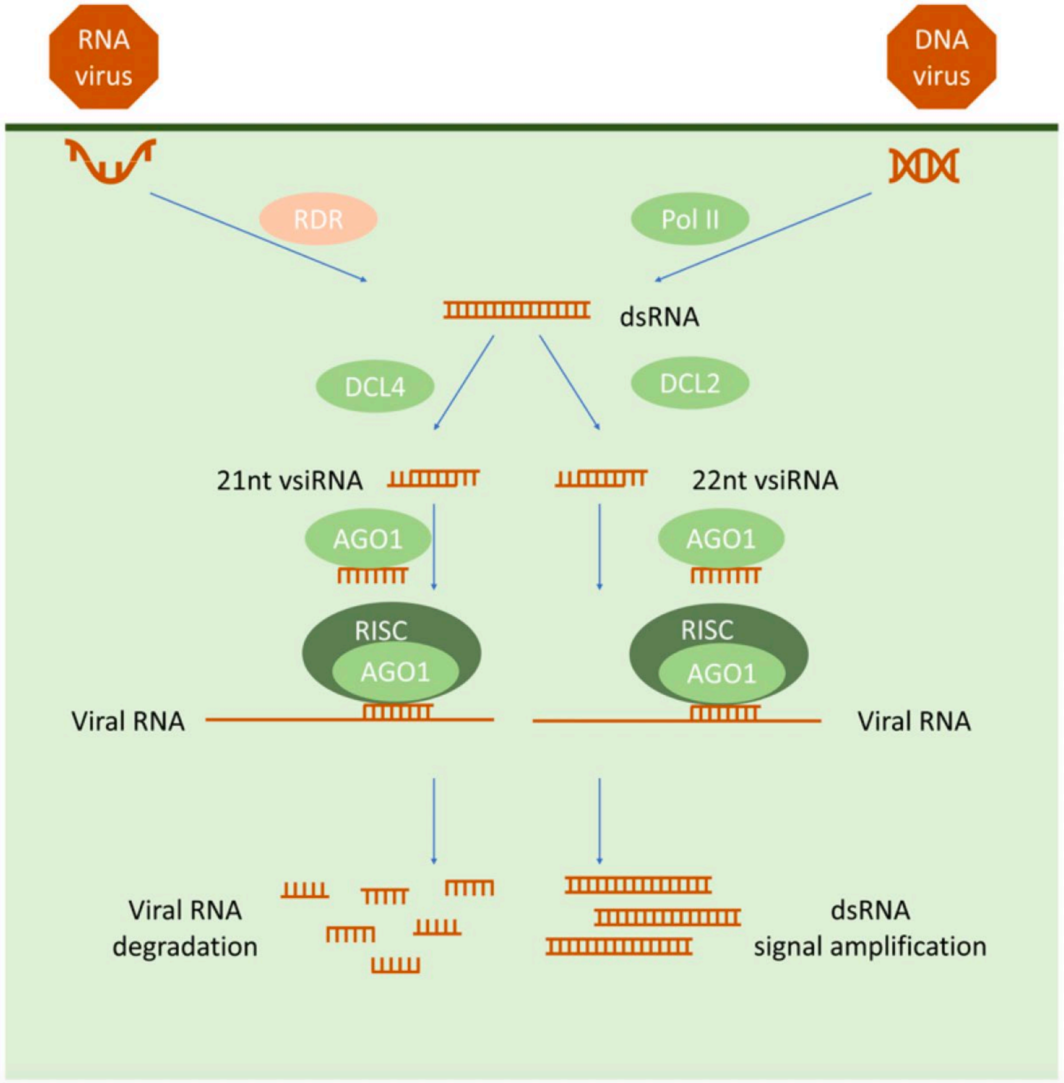
Exogenous application of virus-targeting dsRNA molecules induces RNA silencing mediated protection against a challenging strain

- Bean common mosaic virus (BCMV) (Worrall et al., 2019):
    - dsRNA molecules targeting the NIb and CP genes
    - Exogenous application of dsRNA molecules on *N. benthamiana* and *Vigna unguiculata*
    - Challenge with BCMV 5 days post application → protection against infection



Mechanisms involved in cross-protection

RNA silencing mediated protection



Ortega-Parra, N., Zisi, Z., & Hanssen, I. M. (2021). The use of mild viruses for control of plant pathogenic viruses. In J. Köhl, & W. J. Ravensberg (Eds.), *Microbial bioprotectants for plant disease management* (pp. 507-540). Burleigh Dodds Science Publishing Limited.

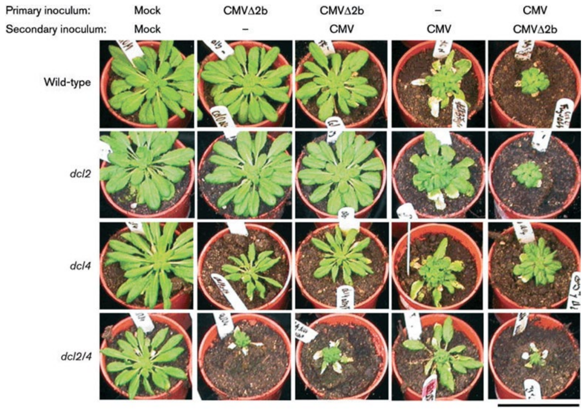
Mechanisms involved in cross-protection

RNA silencing mediated protection

- CMVΔ2b is a Cucumber mosaic virus (CMV) mutant not expressing the 2b silencing suppressor
- Use of CMVΔ2b as ”protective” strain (Ziebell et al., 2007):
    - Cross-protection against parental strain when challenged on the same leaf
    - Limited cross-protection against parental strain when challenged on upper leaves

Absence of the 2b silencing suppressor should induce a strong systemic silencing signal. However, strong systemic protection was not experimentally confirmed.

- Also: Cross-protection works in silencing-deficient *Arabidopsis* mutants



Ziebell and Carr (2009). JGV:90, 2288-2292

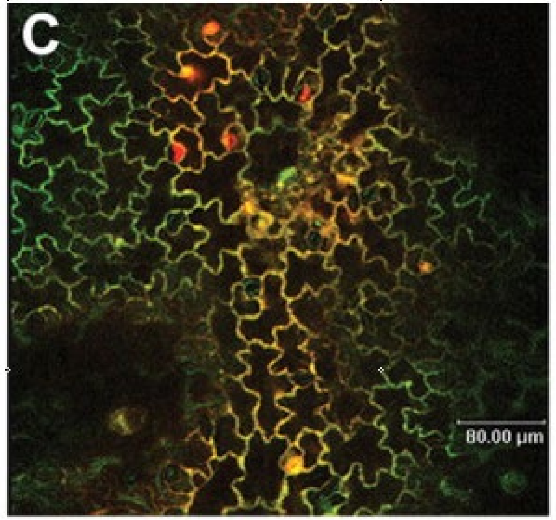
Conclusion: RNA silencing may play a crucial role in cross-protection but it is not the only mechanism involved

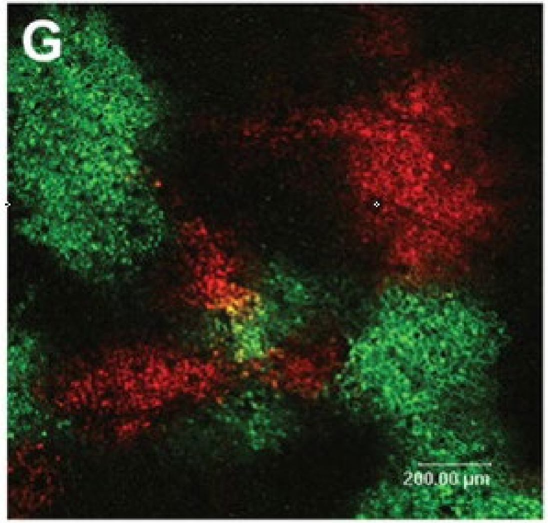
Mechanisms involved in cross-protection
Spatial separation

Observations: Different strains of the same virus exclude each other from infecting the same cell (spatial separation)

- Described for different virus species: Potex-/Potyviruses (Dietrich and Maiss, 2003), Apple latent spherical virus (Takahashi et al. 2007), CMV (Ziebell et al., 2007), AMV (Hull and Plaskitt, 1970), Tobamoviruses (Kunkel, 1934; González-Jara et al., 2009),...

Plum pox virus (green)
+ potato virus X (red)





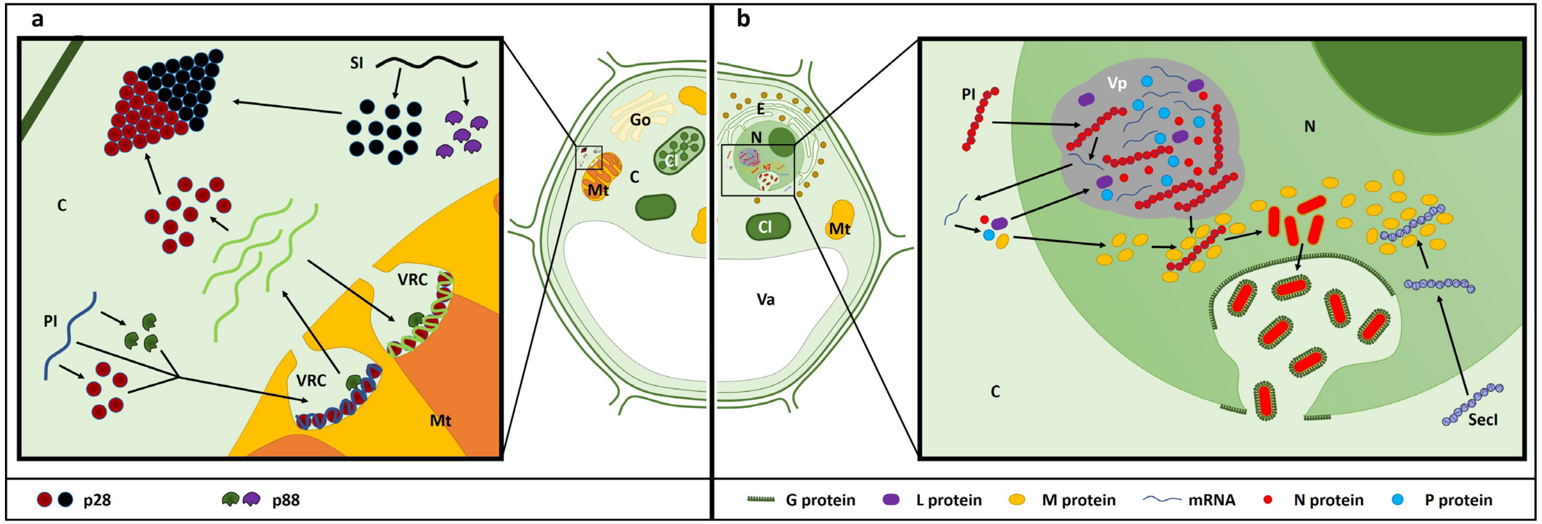
Plum pox virus (green)
+ plum pox virus (red)

Dietrich and Maiss (2003). J.Gen.Vir. 84: 2871-2876

- Clear spatial separation between the “protective” and “challenging” strain if closely related

Mechanisms involved in cross-protection
Superinfection exclusion

- Observed for taxonomically very different viruses (+ssRNA, -ssRNA; plant, human and animal viruses)
- Different players seem to be involved for each virus species
  - e.g. p28 (TCV), M-protein (SYNV), p33/5'-terminal region (CTV)



Final conclusion: For different viruses, different mechanisms of cellular exclusion may occur

Examples of applied cross-protection

- Zucchini yellow mosaic virus / cucurbits
- Papaya ringspot virus / papayas
- Citrus tristeza virus / citrus plants
- Pepino mosaic virus / tomatoes