# Transgenic resistance to plant pathogens

Gonsalves et al. (2000) Plant Health Progress

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Literature: Collinge DB, Jørgensen HJL, Lund OS, Lyngkjaer MF (2010) Engineering pathogen resistance in crop plants: Current trends and future prospects. Annual Review of Phytopathology 48: 269-291

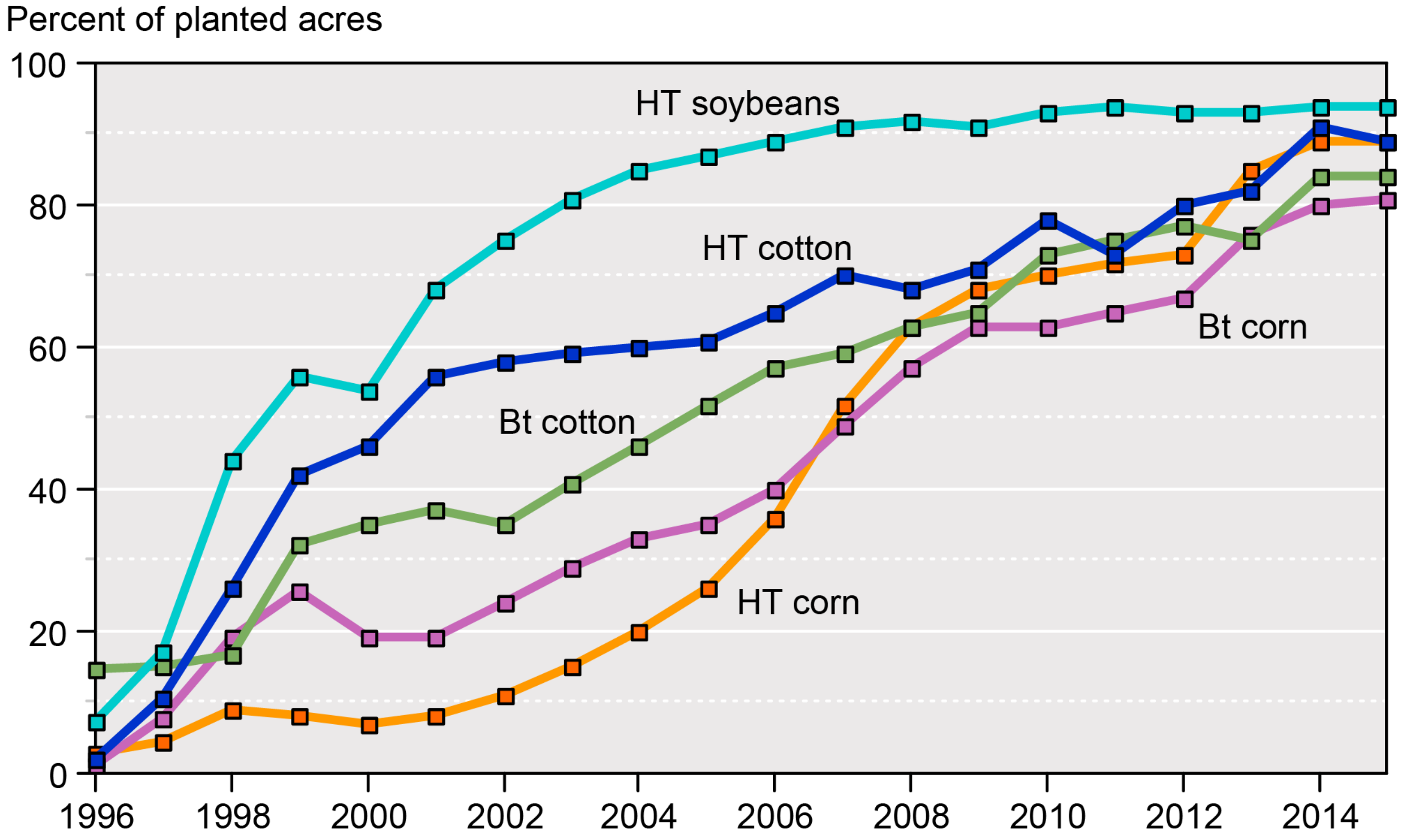
Study question: Discuss why viruses have been a major target for transgenic resistance against pathogens in plants.

# GMO traits in crop plants

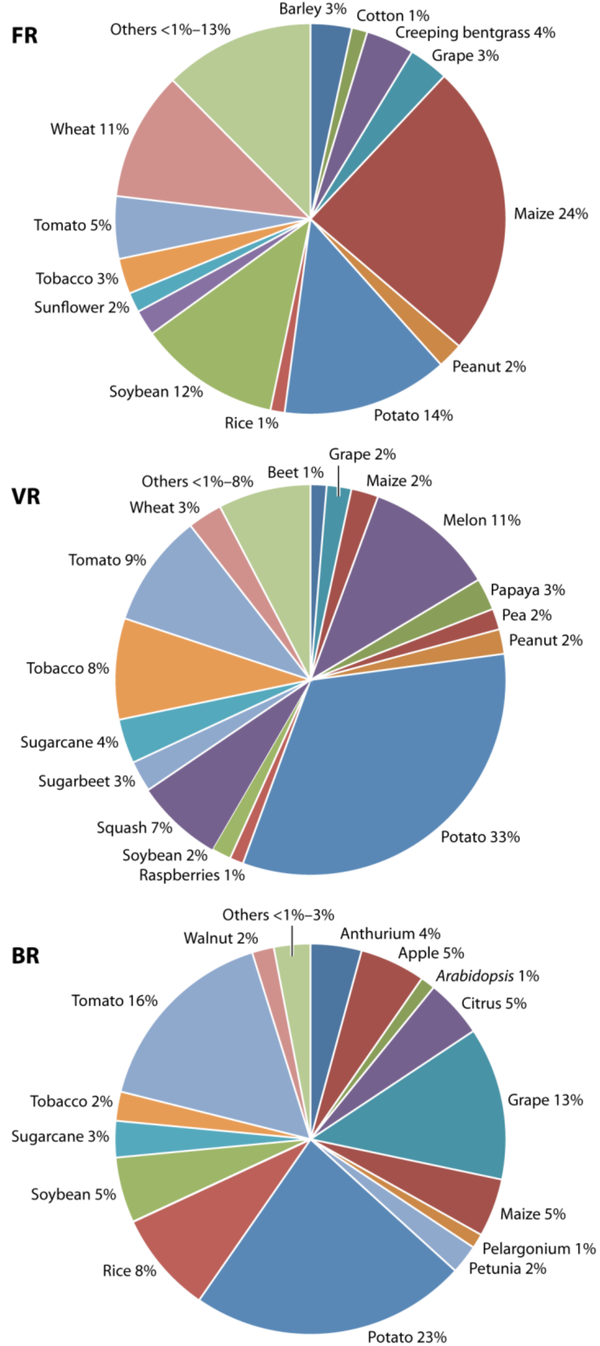
- Herbicide tolerance
  (glyphosate, glufosinate)
- Insect resistance (Bt)
- Pathogen resistance
- Added value: Golden rice (high vitamin A), changed starch, pharmaceuticals, flower colour etc.

# Frequent use of GMO crops in the U.S.

**Adoption of genetically engineered crops in the United States, 1996-2015**




Data for each crop category include varieties with both HT and Bt (stacked) traits.
Sources: USDA, Economic Research Service using data from Fernandez-Cornejo and McBride (2002) for the years 1996-99 and USDA, National Agricultural Statistics Service, *June Agricultural Survey* for the years 2000-15.

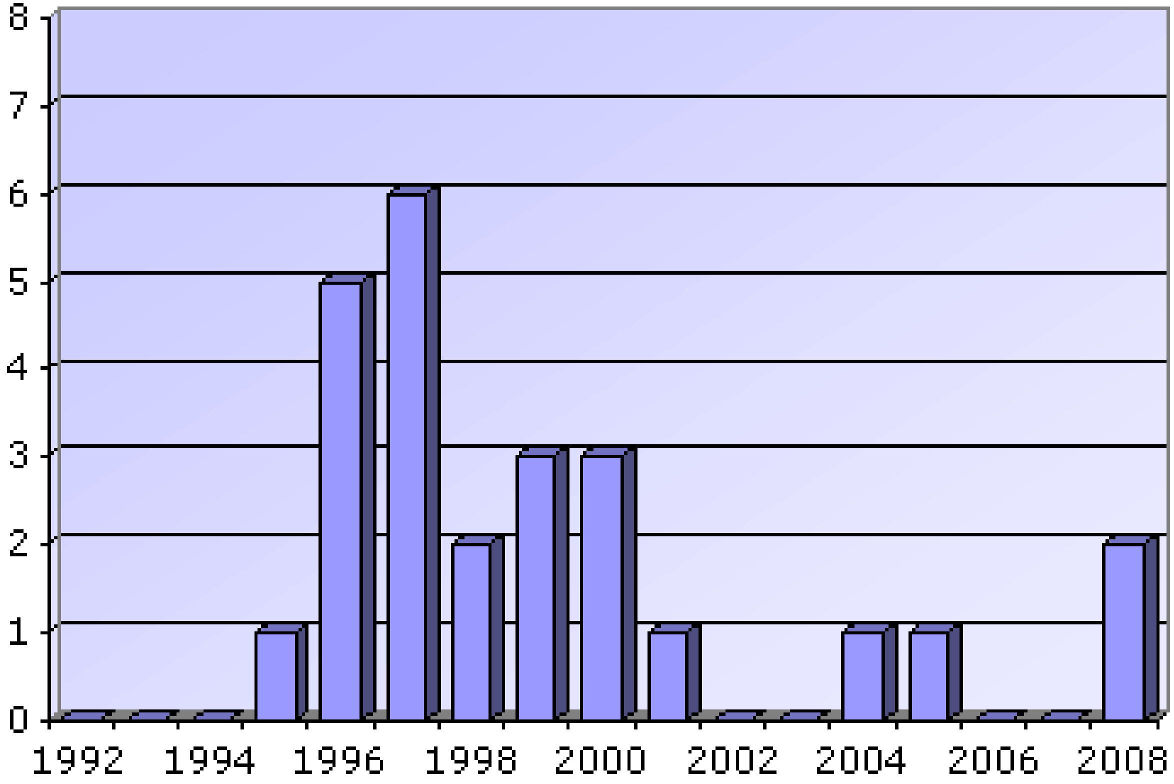



Collinge DB, et al. 2010.
Annu. Rev. Phytopathol. 48:269–91

**Distribution of transgenic crops with fungal (FR), viral (VR), and bacteria (BR) disease resistance in the U.S. field trials application from 1987 to 2009.**

**In total, there have been 15,850 field test release applications for transgenic crops in the United States. Out of these, 2003 occurrences deal with disease resistance: 853 for fungal, 983 for virus, and 167 for bacterial resistance.**

# Finland: Deliberate releases of GMOs into the environment for field trials (1992-2008)

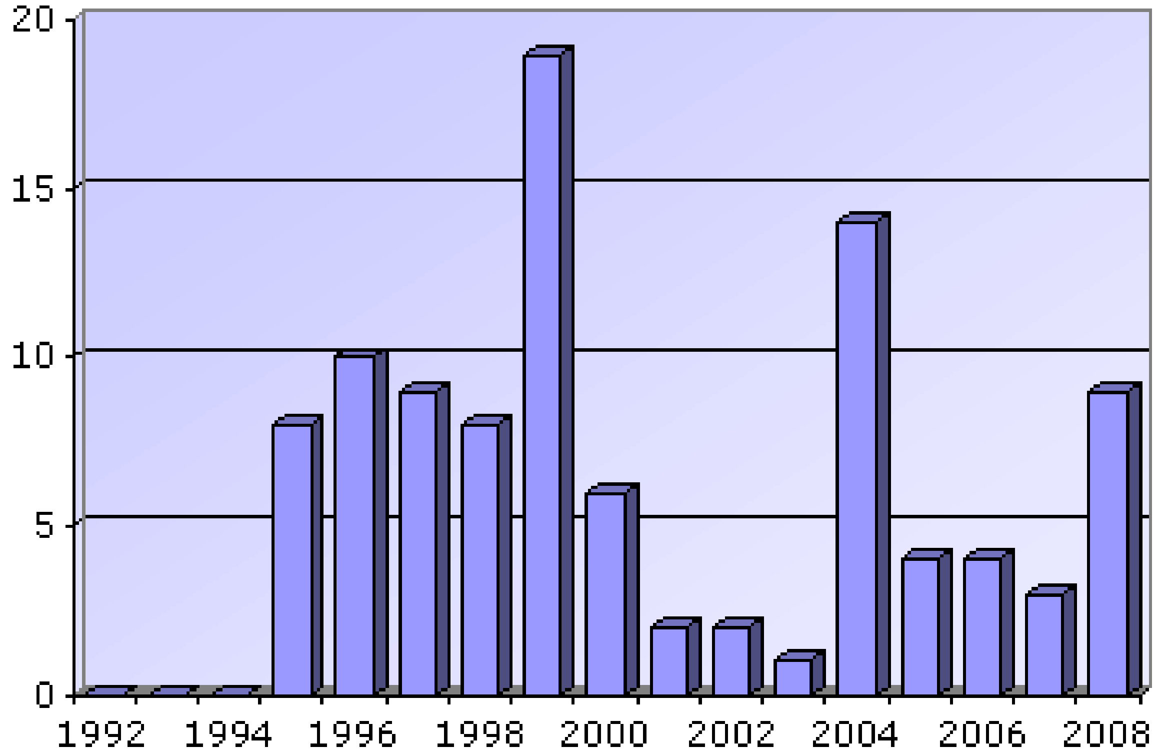



Plants: Trees, beet, potato, cabbage, oilseed rape, barley, cauliflower, turnip rape

Traits: herbicide resistance, modified composition, resistance (virus, fungi, insects)

Data from GMO Compass

# Sweden: Deliberate releases of GMOs into the environment for field trials (1992-2008)




<u>Plants:</u> Oilseed rape, potato, beet, thale cress

<u>Traits:</u> herbicide resistance, modified composition, resistance (virus), frost tolerance

Data from GMO Compass

# Resistance will protect plants from pathogen infection without the need of chemicals



Gene-for-gene resistance against *Tobacco mosaic virus* in tobacco based on the N gene

# Why produce transgenic resistance?

- Economically important crop
- Natural resistance is lacking or not sufficient
- Difficult to introduce resistance by breeding
  - genetic incompatibility
  - linkage of undesired traits
  - polyploid crops
  - takes long time

# Strategies for transgenic pathogen resistance in plants

- Synthesis of antimicrobial agents:
  proteins and metabolites
- Induction of plant resistance
  e.g., pathogen-derived resistance, resistance genes
- Knock-out of susceptibility genes

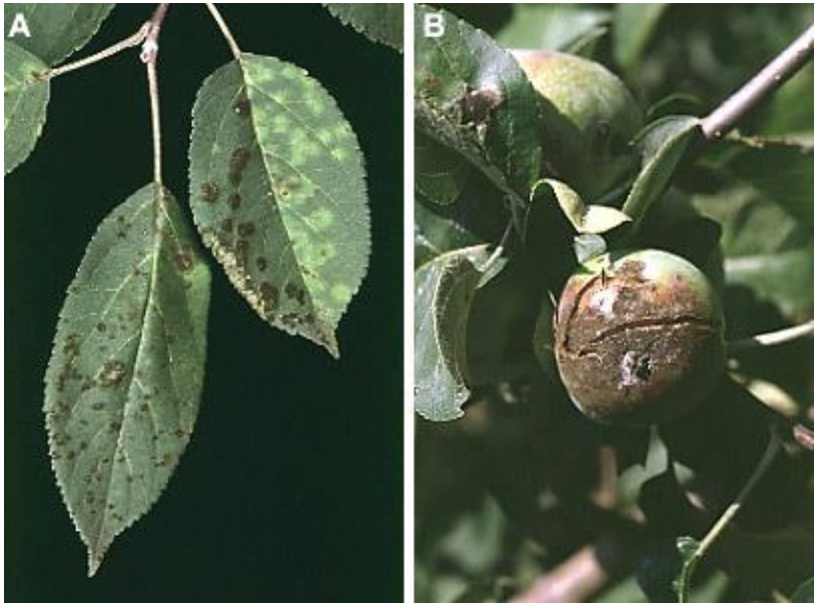
# Antimicrobial proteins

- Pathogenesis-related proteins (PR proteins)
- Chitinase: degradation of fungal cell walls
- Lysozyme: degradation of bacterial cell wall
- Polygalacturonase-inhibiting protein prevents degradation of plant cell wall

# Antimicrobial proteins

- Gives most often only partial resistance
- Gene source may be other plants, fungi, insects, animals

# Transgenic resistance against *Venturia inaequalis*




Scab-infected apple leaves (A) and fruit (B). Trees can be defoliated by the leaf infections and scabby fruits are rejected by consumers and have poor processing and storage qualities.


Transgenic McIntosh apple expressing endo- and/or exo-chitinase genes have increased resistance.

http://www.apsnet.org/publications/apsnetfeatures/Pages/AntimicrobialProteins.aspx

# Antimicrobial metabolites

- Production of antimicrobial stilbenes can be obtained by transfer of a single gene: stilbene synthase
- Transfer of complete biosynthetic pathway: aim to produce benzyl-glucosinolate from *Arabidopsis thaliana* in potato for resistance against potato late blight

# Pathogen-derived resistance (PDR)

- Sanford & Johnston (1985): ”The concept of parasite-derived resistance - Deriving resistance genes from the parasite´s own genome”. J Theor Biol 113: 395-405

- First application: expression of TMV coat protein in tobacco (Powell-Abel et al. (1986) Science 232: 738-743)

# Virus resistance I

Pathogen-derived resistance (PDR):

Transfer of virus gene to crop plant

↓

Plant produces virus RNA

↓

Plant virus defence is activated (RNA silencing)

↓

Virus RNA is degraded by plant enzymes

# Virus resistance II

<u>Pathogen-derived resistance (PDR):</u>

Transfer of virus gene to crop plant

↓

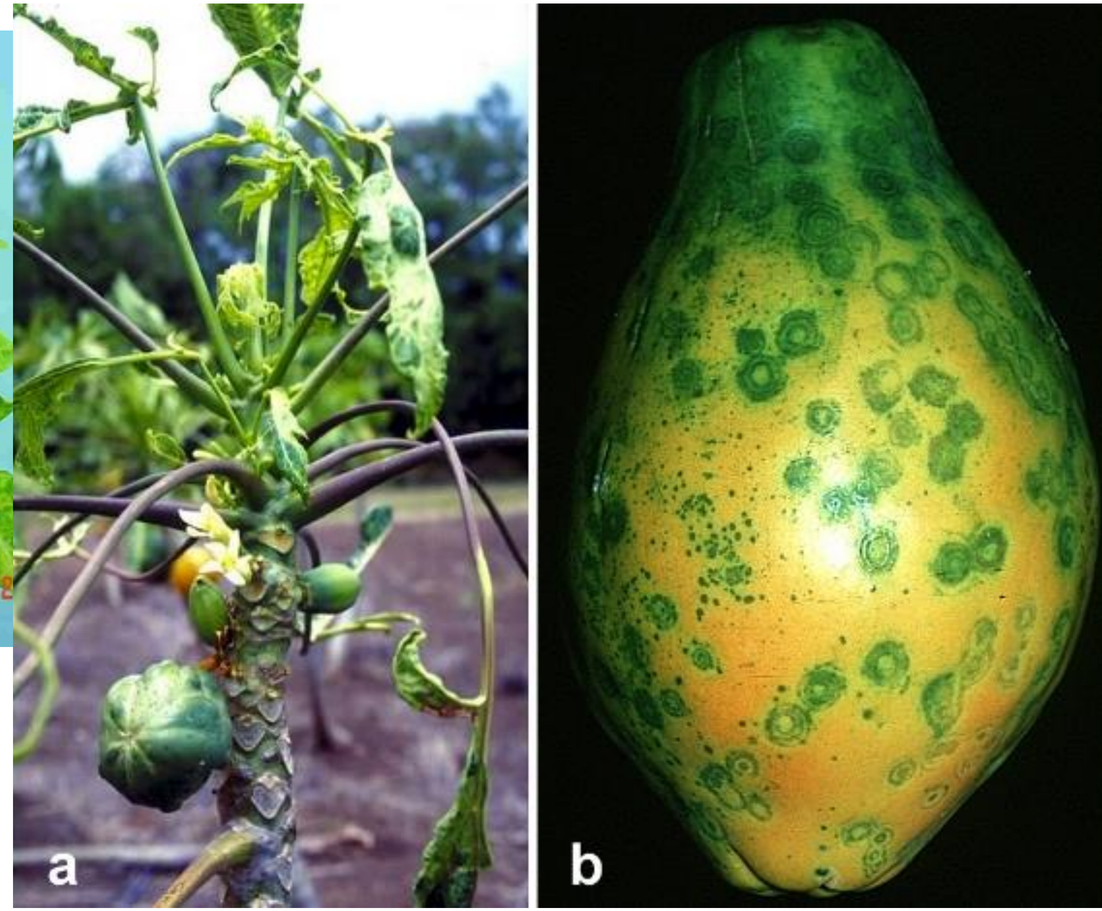
Plant produces virus mRNA and protein (often coat protein)

↓

Virus life cycle is blocked

# Papaya ringspot virus: Symptoms

BSPP




http://www.apsnet.org/

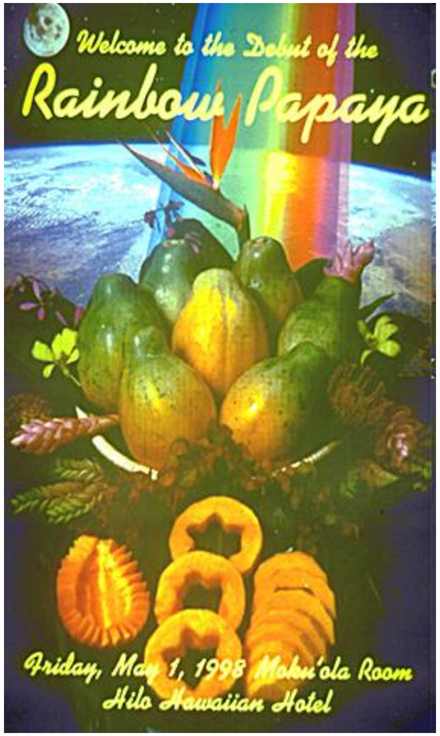
# Papaya ringspot in Hawaii 1994

PRSV-infected papaya

Healthy papaya

Gonsalves et al. APSnet 2010

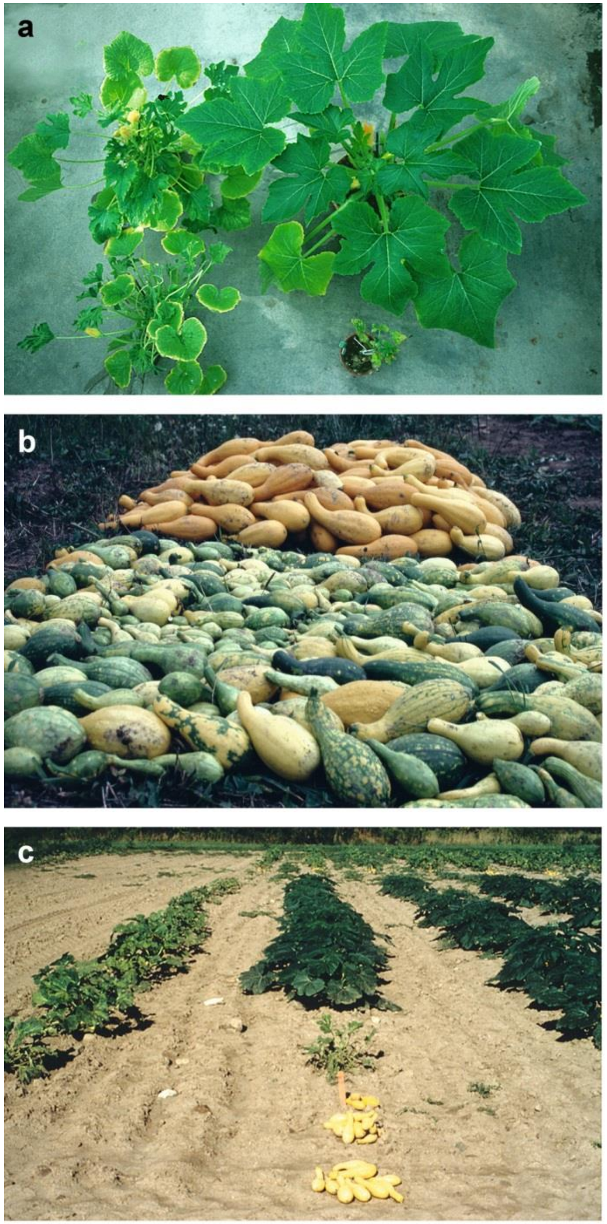
# PRSV-resistant transgenic variety Rainbow




http://www.apsnet.org/

Susceptible Resistant

http://www.hawaiipapaya.com/#gmo-papayas

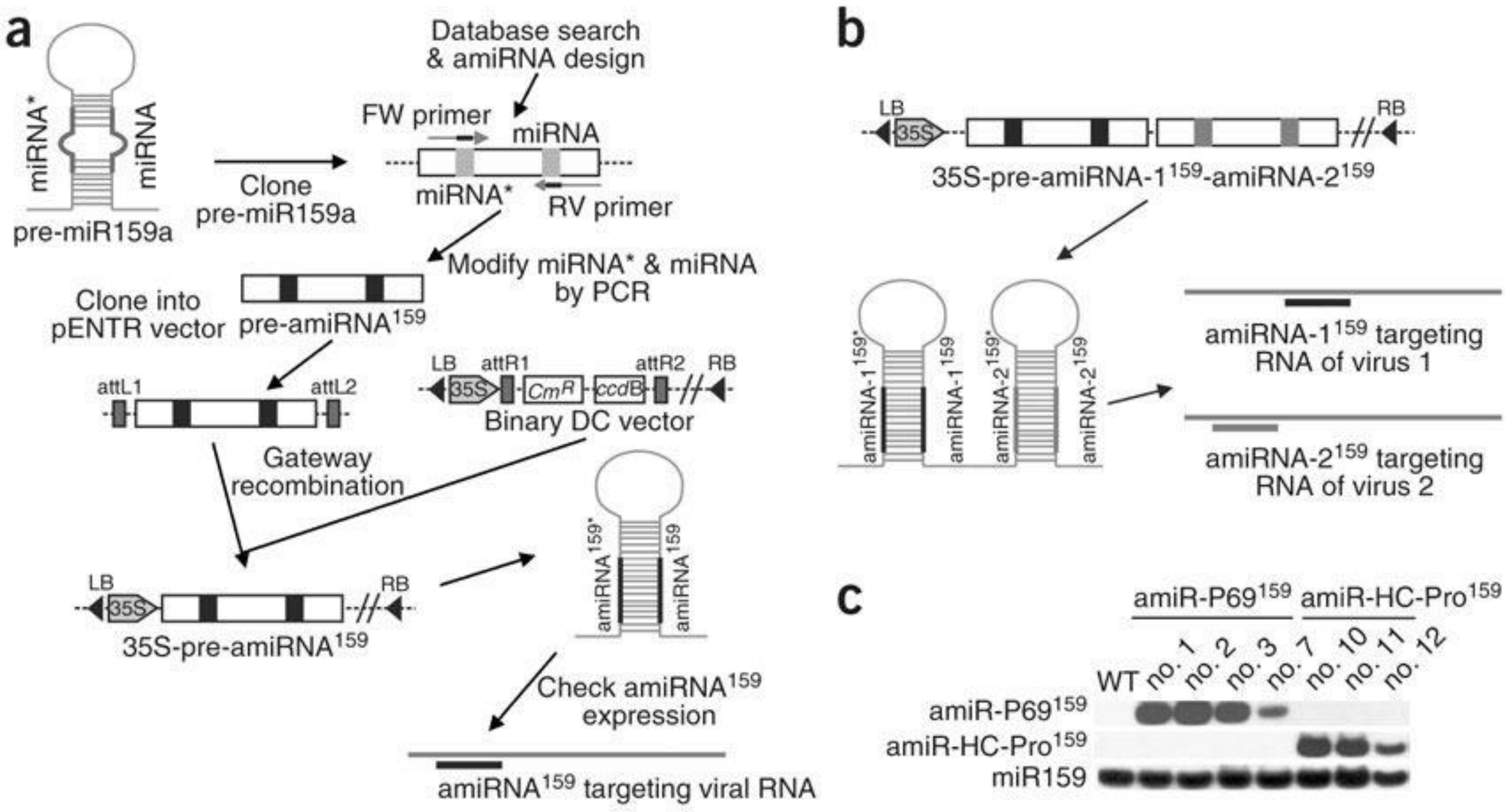



Fuchs M, Gonsalves D. 2007.
Annu. Rev. Phytopathol. 45:173–202

# Multiple virus resistance

Squash expressing coat protein gene of *Zucchini yellows mosaic virus* (ZYMV) *Cucumber mosaic virus* (CMV) and *Watermelon mosaic virus* (WMV) is highly virus resistant

# Expression of artificial microRNAs in transgenic *Arabidopsis thaliana* confers virus resistance

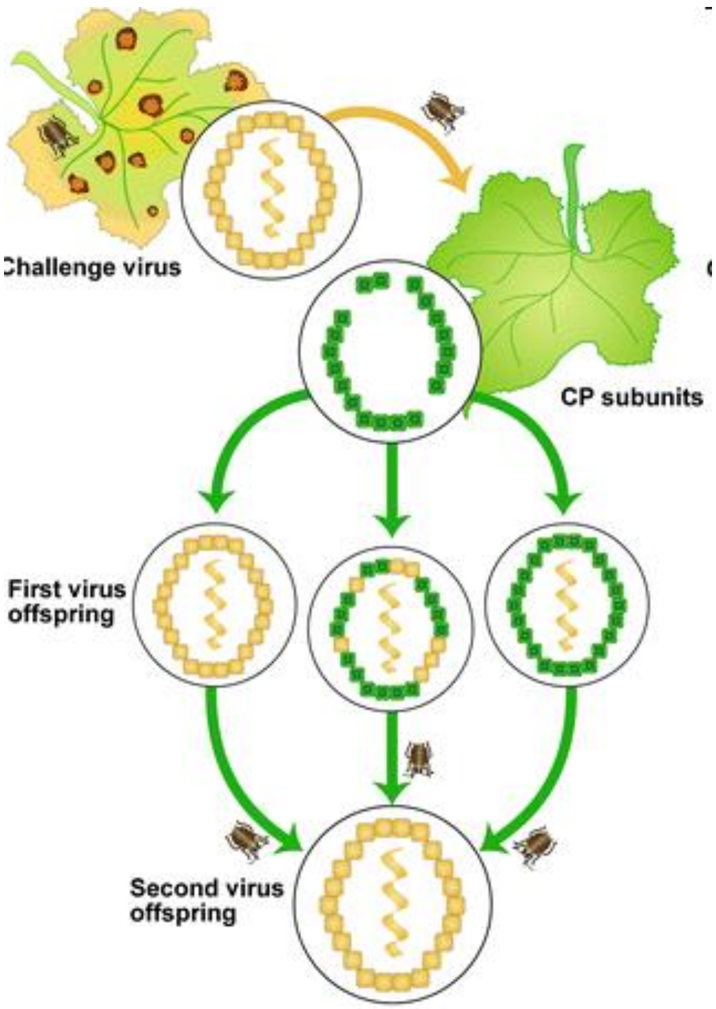



Niu et al. (2006) Nature Biotechnology 24: 1420-1428

# Transgenic resistance – potential safety issues

- Food safety (human health)
  - allergens
  - toxins
- Environment
  - gene flow (transgene escape)
  - loss of biodiversity
  - contamination of organic crops
  - heteroencapsidation, recombination (virus resistance)

# Heteroencapsidation

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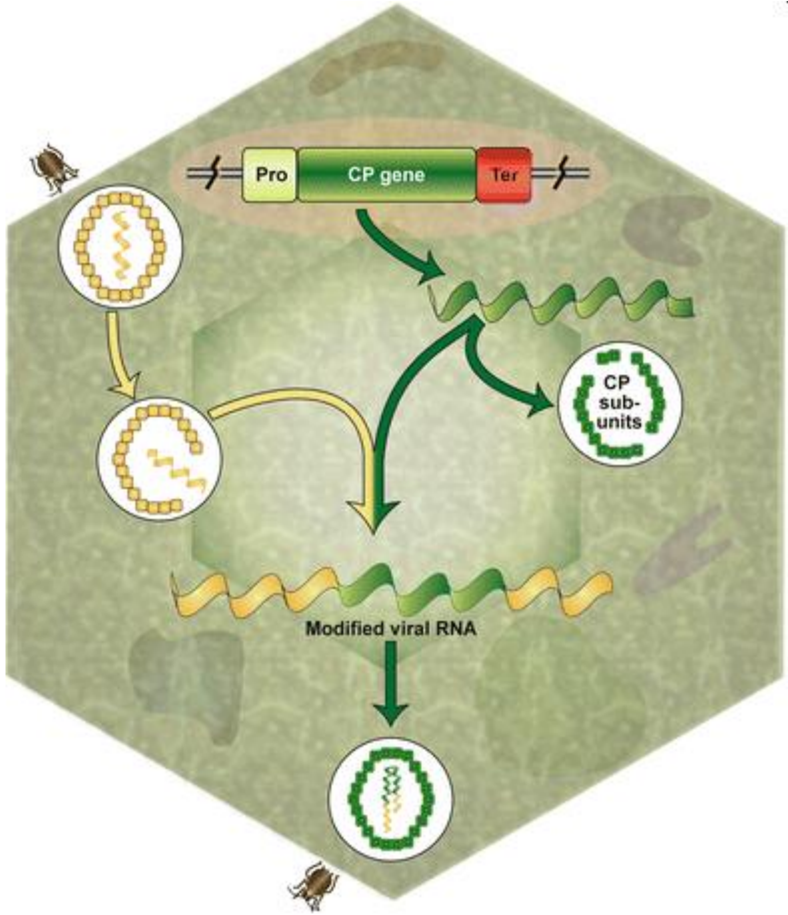
Infecting virus takes the coat protein produced by the transgenic plant

# Recombination

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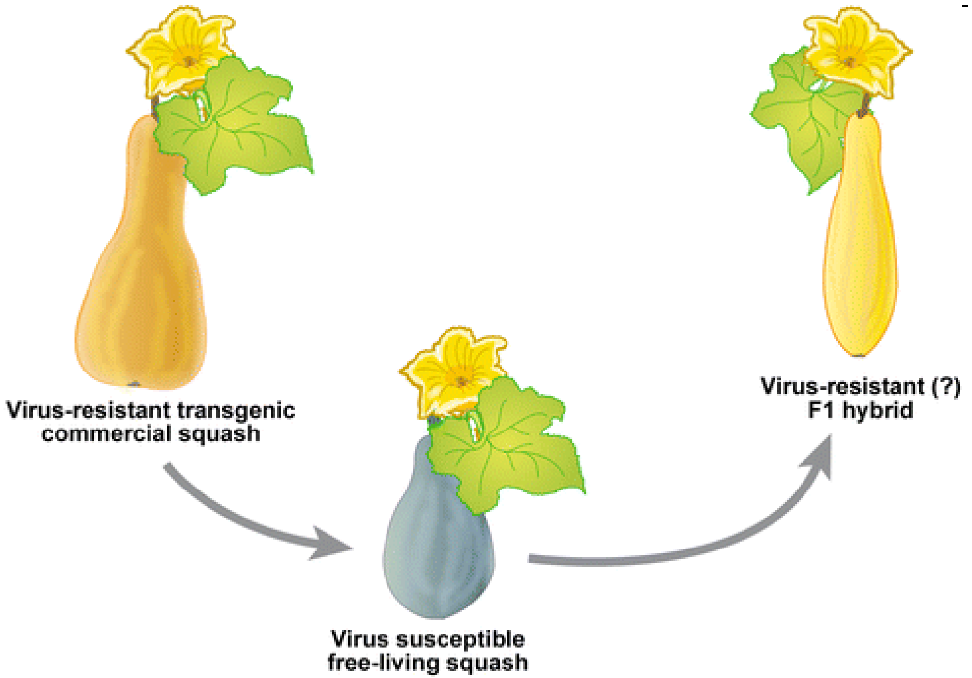
Virus recombines with viral gene mRNA produced by transgenic plant

# Transgene escape

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Gene flow from transgenic plant to wild relatives or conventional crops

# Plants with transgenic resistance grown in Sweden in field trials

- Sugar beet with resistance to *Beet necrotic yellow vein virus* (Syngenta)
- Potato with resistance to *Phytophthora infestans* (BASF Plant Science)

# Rhizomania

***Beet necrotic yellow vein virus***

# ***Beet necrotic yellow vein virus***

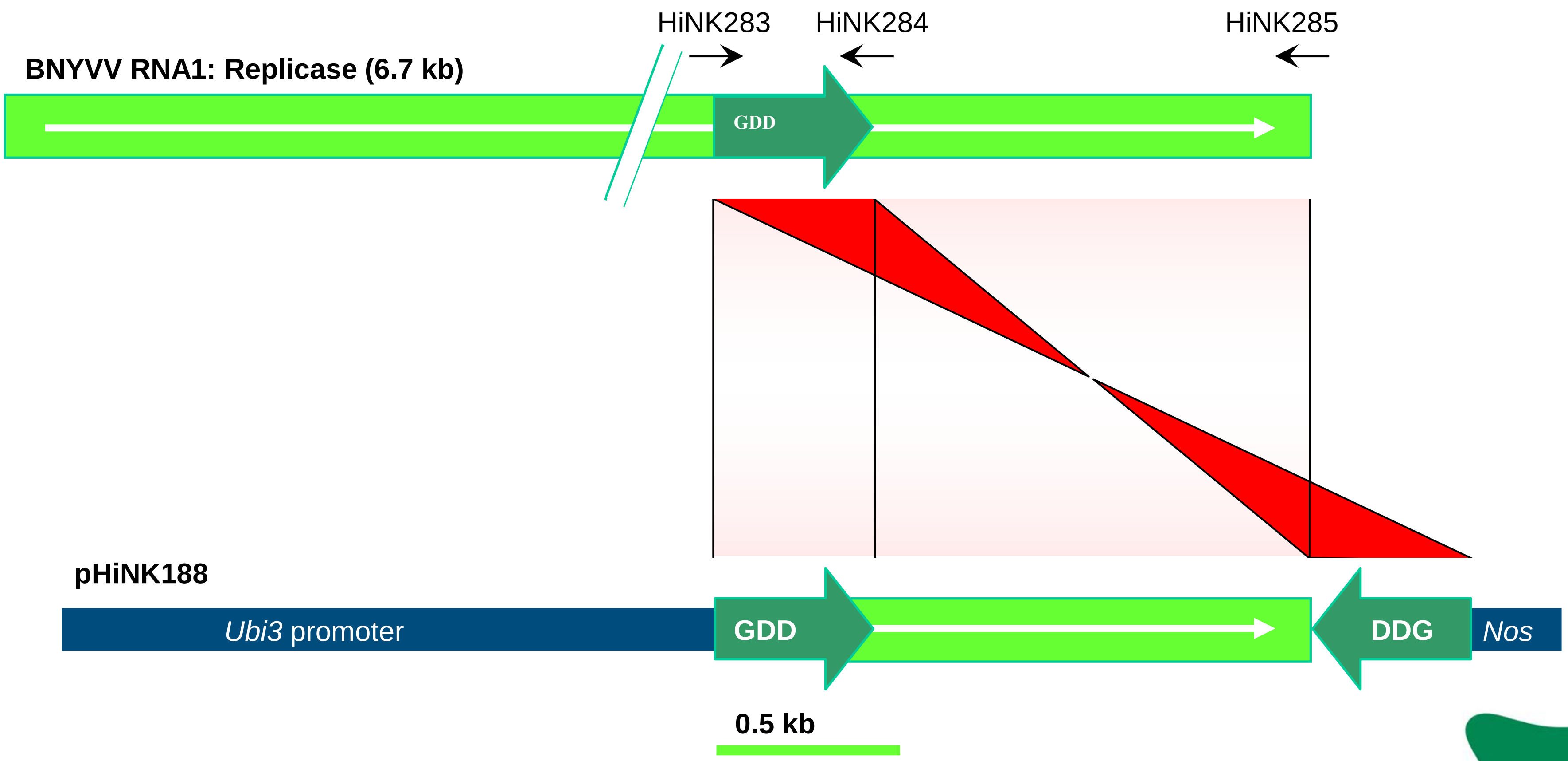
- Causes one of the most important diseases of sugar beet worldwide
- Genus *Benyvirus*
- Genome of single-stranded (+)RNA with 4 RNA molecules
- Soil-borne by the plasmodiophoromycete *Polymyxa betae*

# There is conventional resistance to rhizomania (*Rz1* gene), but there are resistance-breaking virus genotypes

Sugar beet plants grown in the same field with rhizomania. The plant to the left is resistant and the plant to the right is susceptible.

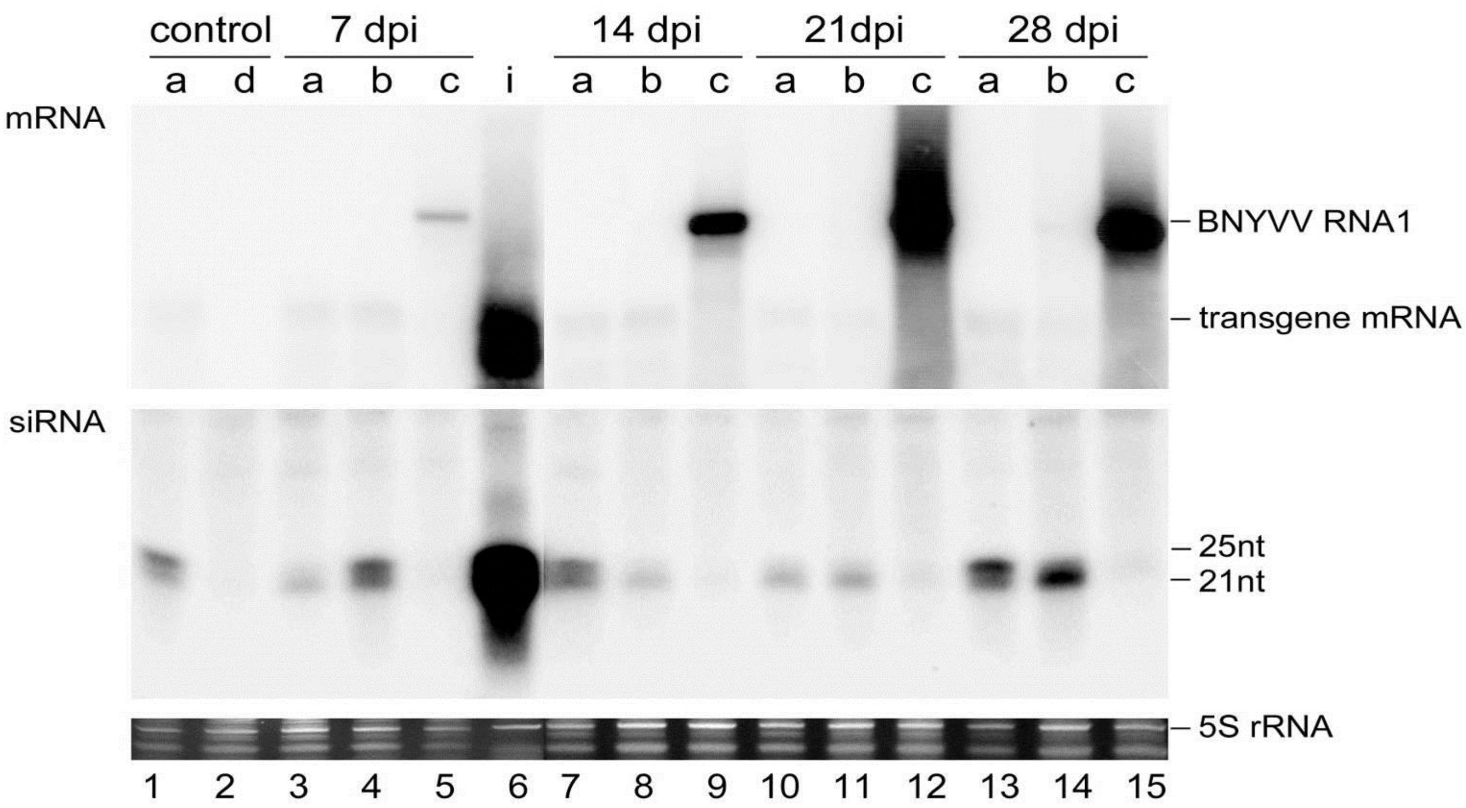
PhD thesis Britt-Louise Lennefors, SLU

# Inverted repeat construct for BNYVV replicase gene




Lennefors et al. (2006) Mol Breeding 18: 313-325

# Northern blot analysis of the accumulation of mRNA and siRNA resulting from the transgenic expression of the BNYVV gene-derived inverted repeat replicase




a) Transgenic plant grown in sterile sand b) in infested soil.
c) Non-transgenic plant grown in infested soil d) in sterile sand.

Lennefors et al. (2006) Mol Breeding 18: 313-325

# Resistance to BNYVV in transgenic sugar beet

Transgenic resistant genotype

Susceptible genotype

Lennefors et al. (2006)
Mol Breeding 18: 313-325

# Transgenic resistance to rhizomania in sugarbeet

- Promoter: *Arabidopsis thaliana* promoter used to drive constitutive expression
- RZM: From BNYVV. Resistance to BNYVV
- Terminator: *Agrobacterium tumefaciens* polyadenylation sites used to terminate transcription
- Hsp80: Hsp promotor from *Brassica sp.* used to drive constitutive expression of the PMI gene (Brunke and Wilson, 1993)
- PMI: Phosphomannose isomerase gene derived from *E. coli* (Joersbo et al., 1998) used as selectable marker
- 35S: 35S Terminator from Cauliflower Mosaic Virus (Odell et al., 1985)

Information from EU

# **Transgenic resistance to *Phytophtora infestans* in potato**

- New resistance genes against *P. infestans* found in the wild potato relative *Solanum bulbocastanum* (ornamental nightshade): *Rpi-blb1*, *Rp1-blb2*, *Rpi-blb3*
- Confer broad resistance against different *P. infestans* genotypes
- Difficult to cross into potato

# Transgenic resistance to *P. infestans* in potato (Fortuna)

- T-DNA borders, pTiT37, from *Arabidopsis thaliana* for stable incorporation into plant chromosome.
- acetohydroxyacid synthase gene (ahas, EC 2.2.1.6, 2013 base pairs, mutation S653N) from Arabidopsis thaliana, conferring imidazolinone tolerance in plant material.
- Promoter and terminator from nopaline synthase gene, *Agrobacterium tumefaciens*, gene regulation.
- Resistance genes *Rpi-blb1* and *Rpi-blb2* from *Solanum bulbocastanum*, with endogenous promoters and terminators for improved resistance to *Phytophthora infestans*.

Information from EU

# For discussion

What do you think are the possible benefits and drawbacks with these two transgenic cultivars for sugar beet and potato?

# Useful links

- GMOinfo: gmoinfo.jrc.ec.europa.eu
- GMO compass: www.gmo-compass.org
- Swedish Board of Agriculture: www.sjv.se
- The Swedish Gene Technology Advisory Board: www.genteknik.se
- Information Systems for Biotechnology/USDA: http://www.isb.vt.edu/