Cisgenese rond aardappel en appel

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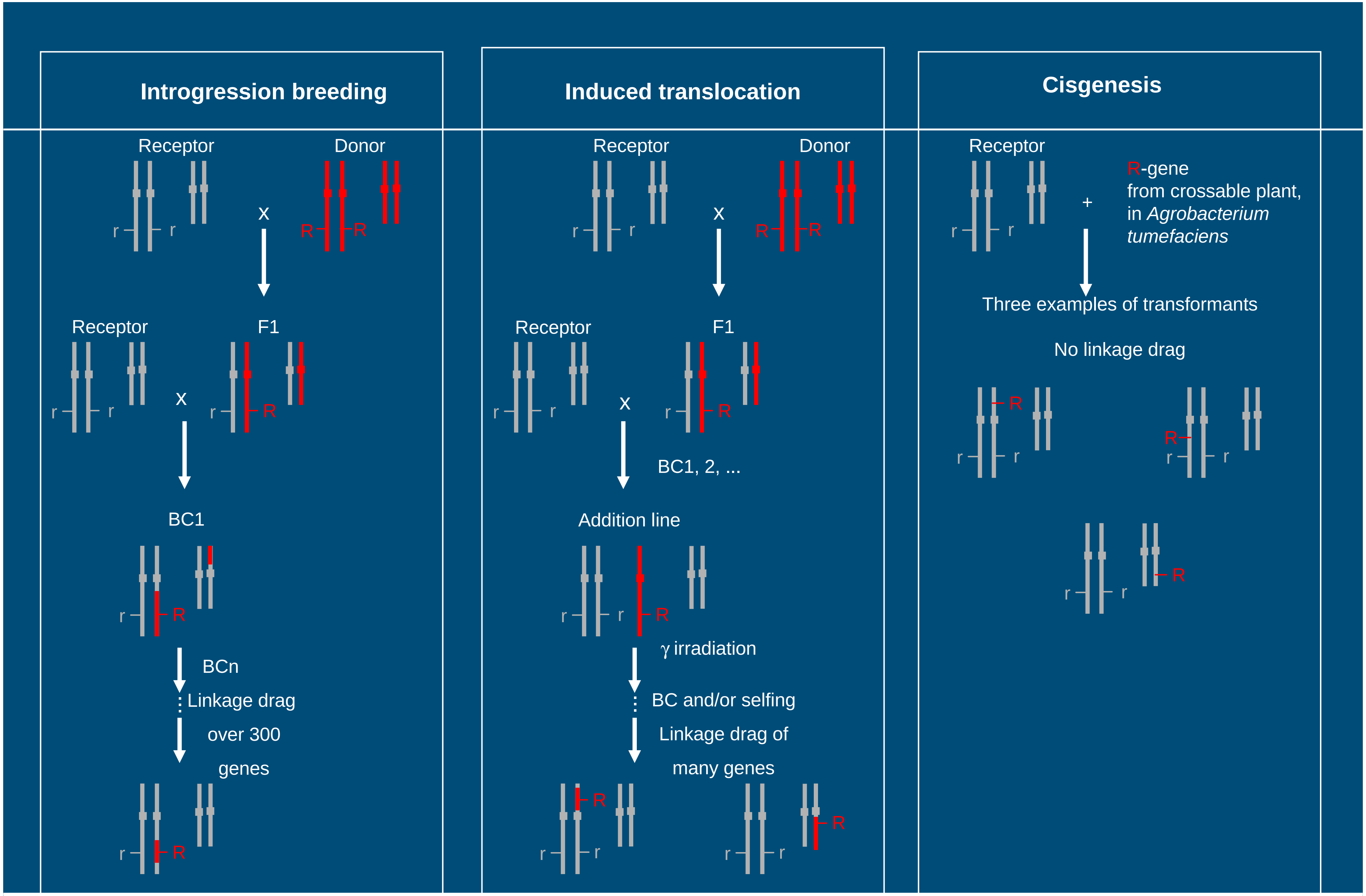
Today's problem of classical breeding

- Too many seedlings (10^5 – 10^6) needed for a new variety
- Improvement of more and more agricultural and quality traits
- More wild species needed for improving genetic variation
- Through linkage drag, introgression breeding of, for example, several resistances (viruses, fungi, nematodes, etc..) is complicated; more pre-breeding needed
- Wild species can introduce new types/high content of glyco-alkaloids
- A popular (free) variety with good practice can be improved by mutations (restricted) and GMO (not accepted)



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Principle of Introgression breeding for a single trait

Species A ***Species B (donor)***

rr x *RR*

↓

Rr x rr (species A)

↓

rr Rr x rr

↓

rr Rr

↓

Rr + **linkage-drag**

- recombination around *R*-gene restricted
– marker assisted breeding speeds up
- pre-breeding for breeding parents needed

slow, multiple step, genetic domestication of a resistance gene with linkage drag

Interspecific/bridge crosses in introgression breeding

37 years ago – **Bridge crosses** for *Phytophthora* resistance

S. acaule 4x × ***S. bulbocastanum*** 2x (*R genes*)

↓

AB hybrid 3x

↓ colchicine doubling

AB hybrid 6x × *S. phureja* 2x

↓

ABP hybrid 4x × *S. tuberosum* 2x

↓

ABPT material 4x *R*-gene + linkage-drag

First resistant varieties come out, all with **only 1 *R*-gene?**

Stacking of R-genes for **sustainable** resistance in this way is difficult and always accompanied with a lot of **linkage-drag**

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Biotechnology and introgression breeding

Biotechnology is assisting **introgression breeding:**

1. **In vitro techniques**: embryo rescue, prpl fusion, propagation, (**transformation**)
2. **Genomics**: genetic mapping, marker assisted breeding, genome sequencing and (**gene isolation**)

First Generation of Transgenic-Food Plants with Agronomical Traits

- Improved disease resistance
  (viruses, fungi)
- Improved pest resistance
  (lepidoptera, beetles)
- Tolerance for herbicide
  (glyphosate, glufosinate)
- Slow ripening

Causes of problems in acceptance of GMO-crops in Europe

- Dependence of chemical industry: Herbicide resistance
- Regulations developed for **transgenes**
- Genetic make-up of plants has been made inflexible
- Antibiotic resistances have been made a successful political issue
- NGO lobby underestimated
- A **no risk** approach and not a **risk-benefit** approach
- GMO-traits not differentiated

Today: New chances

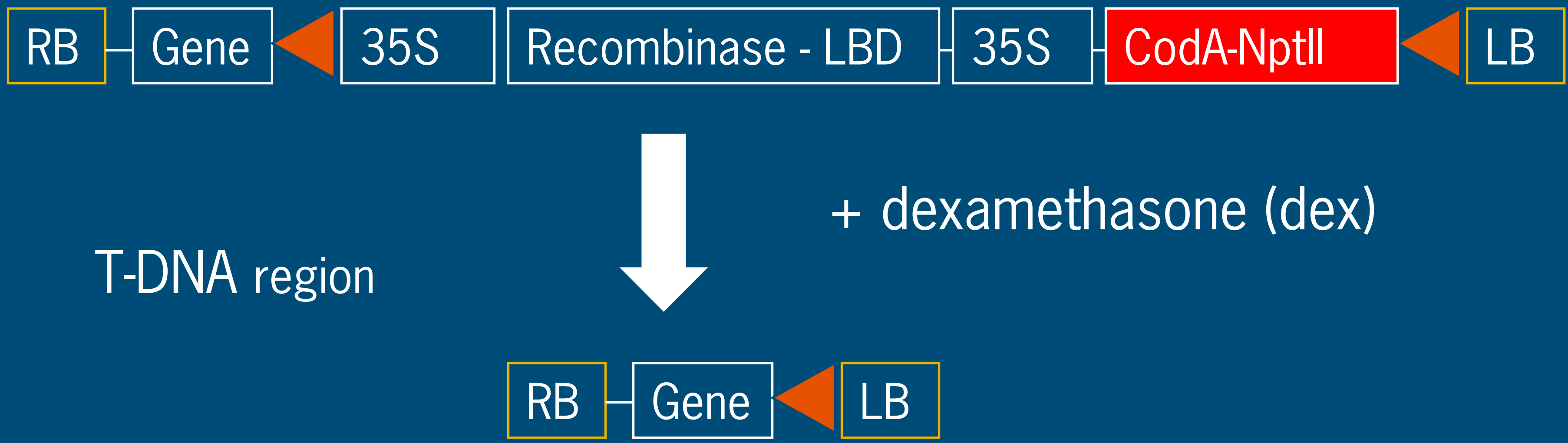
- Cisgenic resistances with plant-own genes are more often an option
- Intragenic traits only with functional parts of plant-own genes
- Marker-assisted breeding on field resistance more effective
- Combination of field resistance and cisgenic resistance will improve sustainability
- In parallel with Bt-resistance, new resistance strategies can be developed and tested
- In complex crops, like potato, existing varieties with a long safe use, can be improved with cisgenic traits (resistances, quality, ..)

Transgenes versus Intragenes and Cisgenes

- A transgene is a natural gene from a non-crossable species or it is a synthetic gene. It represents the new gene pool
- An intragene is coding for a trait with functional parts of genes from the crop plants itself or crossable species. It represents the new gene pool using functional parts of only the breeders gene pool
- A cisgene is a natural gene, coding for a trait, from the crop plant itself or from a crossable species which is normally used in conventional breeding. It represents the breeders gene pool

Clean vector systems

A. The Standard Vector, pMF1000



B. *A.tumefaciens* without selection genes

C. Mixed inoculation followed by genetic segregation

Transgenesis versus **cisgenesis**

- Directive 2001/18/EC is based on **domestication** of transgenes, representing a new gene pool, which is comparable with horizontal gene transfer
- Cisgenesis is based on **domestication** of cisgenes using clean vector transformation. It is comparable with translocation or introgression of the breeders gene pool traits

Introgression versus cisgenic resistance breeding

- **Modern introgression breeding**:
  - **MAS** can speed up multiple step **domestication** and the **selection** process during backcrosses (including translocation) and reduce **linkage-drag** but not completely
  - Example: MAS of **insect resistance in lettuce**
- Cisgenic resistance breeding:
  - Linkage **drag-free**
  - Single step **insertion** and **domestication** of a *R*-gene

GMO-legislation

- Actual releases of only GM-varieties with **foreign transgenes**
- No example developed with **cisgenic** GM-varieties
- ‘clean’,antibioticum resistance gene-free, GM-plants possible
- GM-plants with **only** **cisgenes** will more frequently replace **introgression** breeding in the near future
- Cisgenic approach enables **single step domestication** of **natural** genes from crossable species without linkage-drag.
- **Cisgenic** GM-plants are more **comparable with introgression** resistance breeding, using **the same gene pool**, without undesired linkage-drag than transgenes from **other gene pools**

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Proposed cis-, intra- and transgenic GM-plant classification to facilitate the notification procedure for the information required in directive 2001/18/EC

Category	type of genes	notification
1	**new transgenes**	**full**
2	**New events** in existing gene-crop combination and **intragenes**	**partial**
3	**cisgenes**	**exempted**

Definition GMO, including proposed change

EU 2001/18/EC

Definition of GMO: Organisms of which the genetic material has been changed in a non-natural way, by

- Recombinant DNA-techniques
- Micro-injection with DNA
- Fusion of cells of non-crossable organisms

Techniques not leading to a GMO (Annex 1A):

- Polyploidisation
- In vitro-fertilisation
- ....

Techniques leading to a GMO, but which is exempted from the GMO-legislation (Annex 1B):

- Mutagenesis
- Fusion of cells of crossable plants
- IN THE FUTURE : **SELF-CLONING, including CISGENESIS**


Picture stolen from G. Flachowsky, Braunschweig

Two examples for cisgenesis

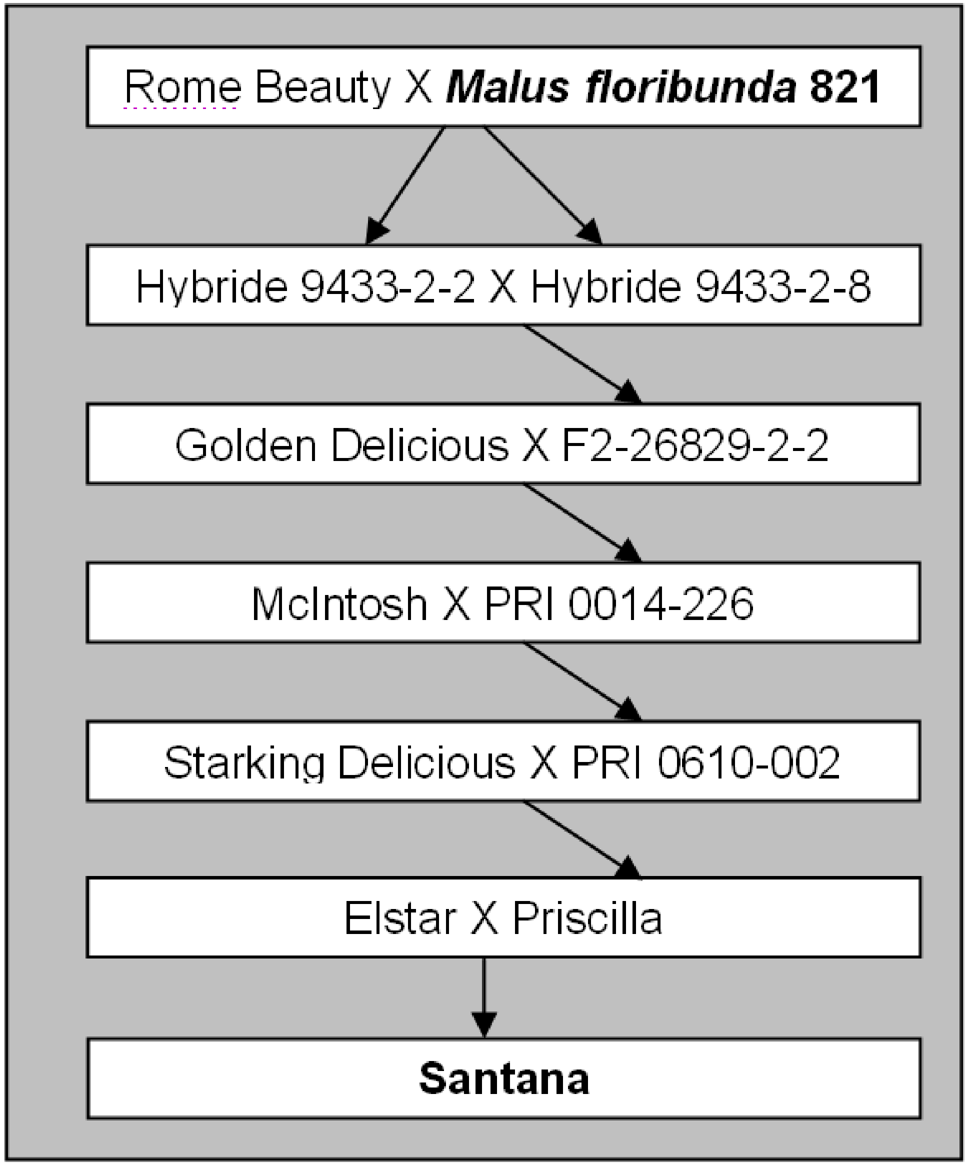
- 1. Apple with the problem of scab and human health keeping colored fruit flesh

- 2. Potato with the problem of late blight

Apple Scab Resistance by Introgression Breeding

- Example: Vf-varieties
- 1953: Vf-resistance detected in the ornamental apple *Malus floribunda* 821 in USA.

- Worldwide heavily used
- After about **50 years** of crossing and selection Vf-varieties obtained with good fruit quality



Introgression breeding of apple scab resistance or by cisgenesis

- **However:**
  - Vf-resistance is not sustainable. Is already broken. Effort of 50 year was within 10 years cancelled!
- **Needed:**
  - Stacking more than one resistance gene and development of a resistance strategy
- **Problems:**
  - Lasting(!) process
  - Testing for presence of multiple R-genes in one plant not easy.
- **Solutions:**
  - Marker assisted breeding (longterm)
  - **Cisgenic GM-plants with R-gene stacking (Vf1 +Vf2+ V25 genes)**

Rood vruchtvlees als gezongheidseigenschap via introductie van het MIP10 gen

The new concept: HEALTHY POTATO

a cisgenic GM strategy for durable resistance based on *R* and *Avr* genes

- Phytophthora resistance in potato is easily broken. A better strategy is needed for **sustainable** resistance
- **GM** potato is the **only solution** in the short run
- Many *R*-genes are available in crossable wild species, enabling the development of a **resistance strategy**
- More useful molecular knowledge is coming rapidly available from the pathogen such as ***Avr*** genes
- **Proof of principle** will be developed in the field
- Communication
- **Legislation** Directive 2001/18/EC has to be adapted for cisgenes

Cisgenic-resistance breeding

- Selection of *R*-gene containing transformants with sufficient biological expression of the resistance trait
- Insertion (TDNA) random in the genome. Selection out of more transformants helps to prevent undesired side-effects
- Random insertion comparable with:
  1. Translocation breeding in wheat
  2. Fixed and active transposons
  3. Existing GMO-crops
- Breeding for compensation of negative side-effects not needed
- Ideal approach for adding strong resistance gene(s) to **existing** (field resistant) varieties

Host resistance - New sources of resistance

- Source: accessions of wild species
- Screening for resistance:
  - In vitro inoculation
  - Detached leaf essay
  - Field trial
- Genetics: mapping, cloning
- *P.infestans*: complex isolate 90128

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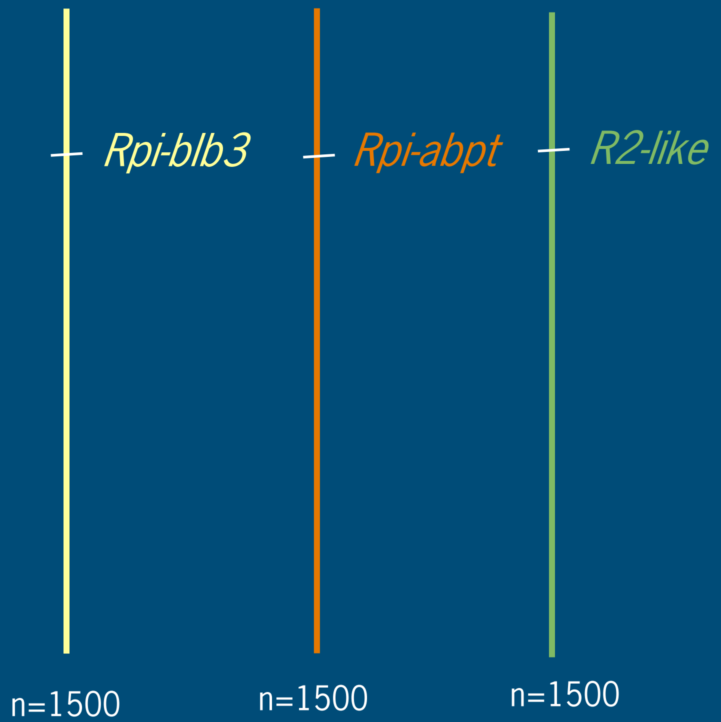
Host resistance – Cloning of *R* genes

- Map based cloning of 3 genes on chr. 4
- Cosegregating markers



- BAC sequencing
- Subcloning candidate genes
- Complementation
- Cross reacting *Avr* gene

Cloning of *Rpi-blb3*: complementation analysis

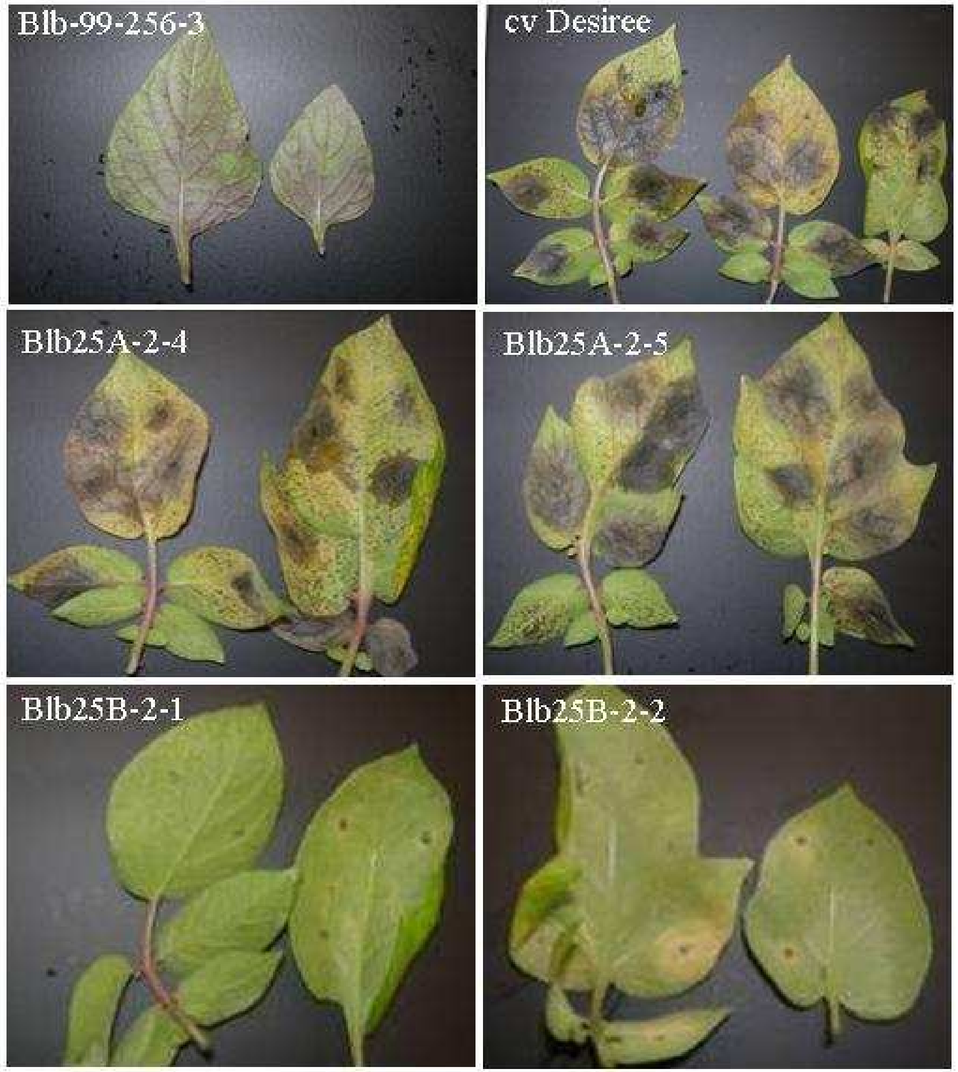
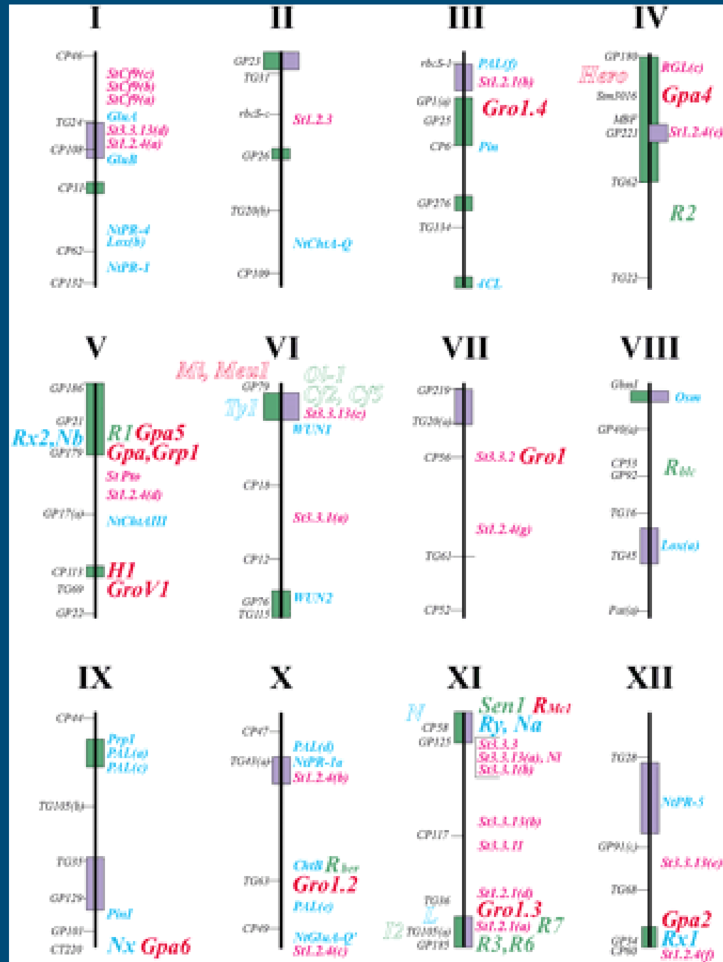


Figure . Genetic complementation for late blight susceptibility.
Typical disease phenotype 8 days after inoculation with a sporangiospore suspension of Phytophtora infestans isolates 90128.
Blb-99-256-3: resistant parental clone; cv. Desiree: potato cultivar used for transformation; Blb25A-2-4 and Blb25A-2-5: primary transformants harboring RGH-Blb25A; Blb25B-2-1 and Blb25B-2-2: primary transformants harboring RGH-Blb25B (*Rpi-blb3*).

Host resistance - *R* genes in *Solanum* spp

- *S. demissum* — *R1* (V)
  - *R2* , *R2 like* (IV)
  - *R3a, R3b, R6, R7* (XI)
- *S. bulbocastanum* — *RB, Rpi-blb1* (VIII)
  - *Rpi-blb2* (VI)
  - *Rpi-blb3* (IV)
  - *Rpi-abpt* (IV)
- *S. berthaultii* — *Rpi-ber1* (X)
- *S. microdontum* — *Rpi-mcd1* (IV)
- *S. pinnatisectum* — *Rpi-pnt1 (Rpi1)* (VII)
- *S. mochiquense* — *Rpi-mcq1* (IX)
- *S. neorossii* — *Rpi-neo1* (IV/VII)
- *S. okade* — *Rpi-oka1* (IX)
  - *Rpi-oka2* (IV)
- *S. stoloniferum* — *Rpi-sto1*
- *S. papita* — *Rpi-pta1*



Gebhardt & Valkonen (2001) Annu. Rev. Phytopathol 39: 79-102

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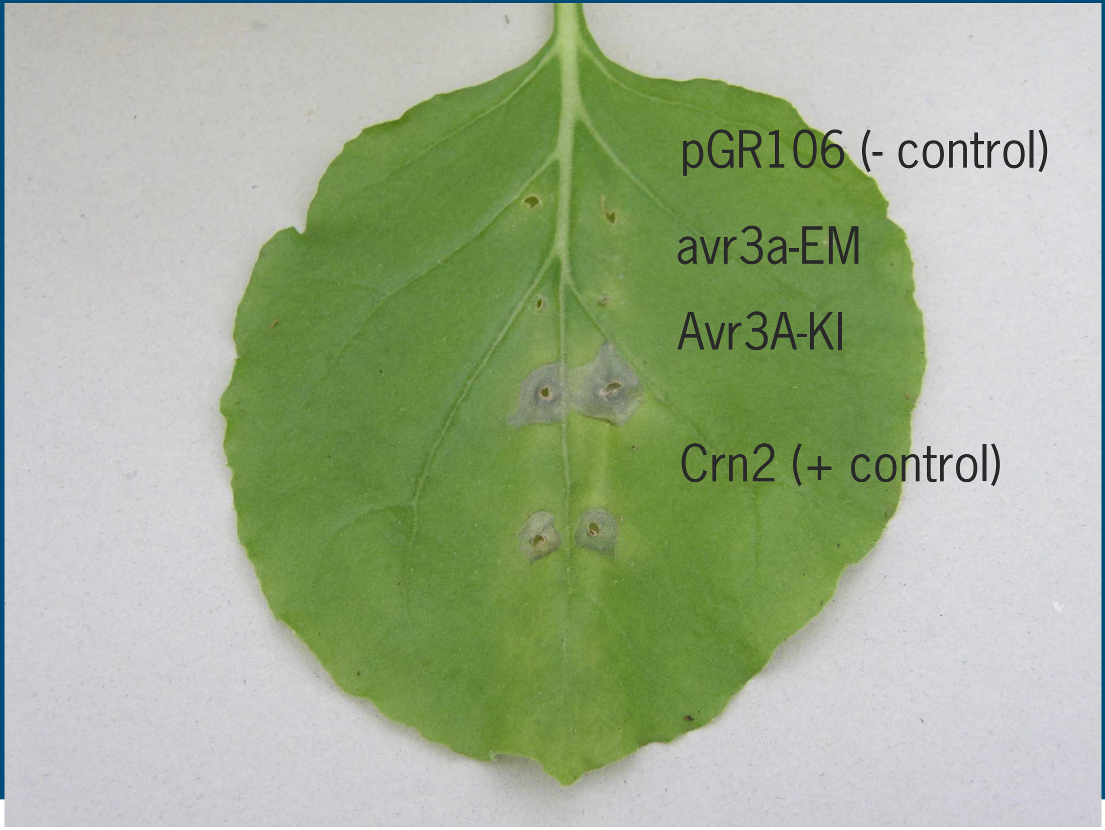
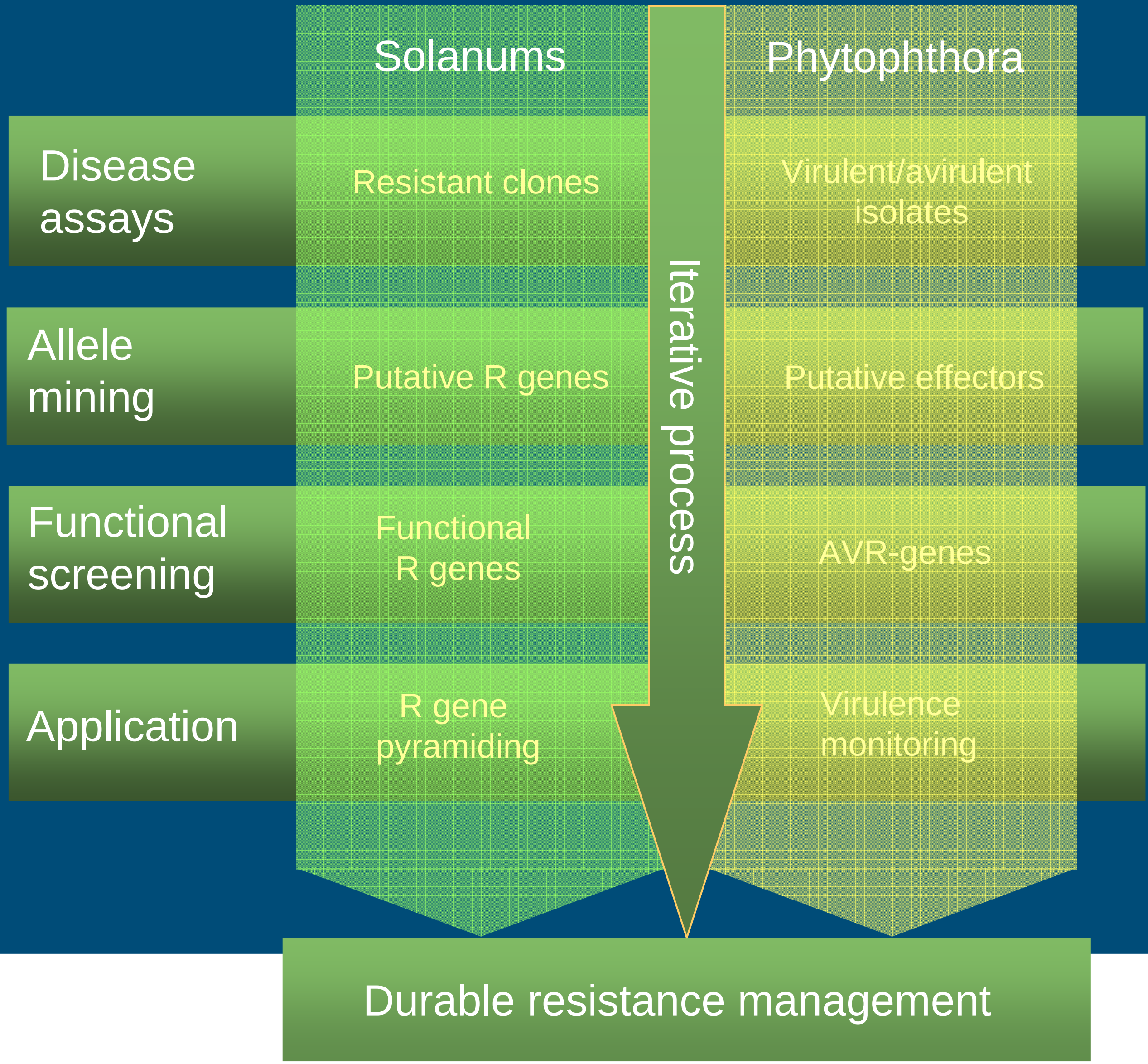
Functional screens with candidate effectors

- *R3a-Avr3a* interaction in transgenic *R3a N. benthamiana*
- 5 *Avr* genes (*R3a, R4, Rpi-blb1, Rpi-blb2, Rpi-blb3*) isolated sometimes reacting in different species



Functional allele mining strategy



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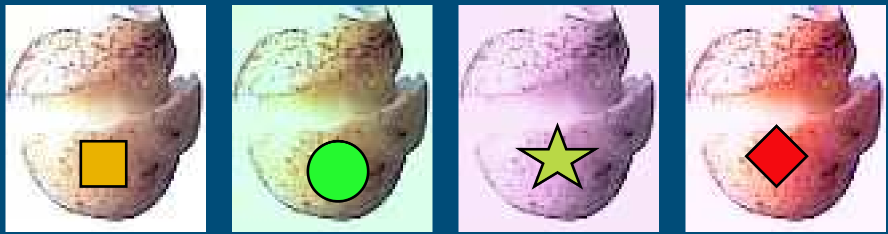
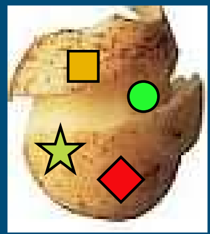
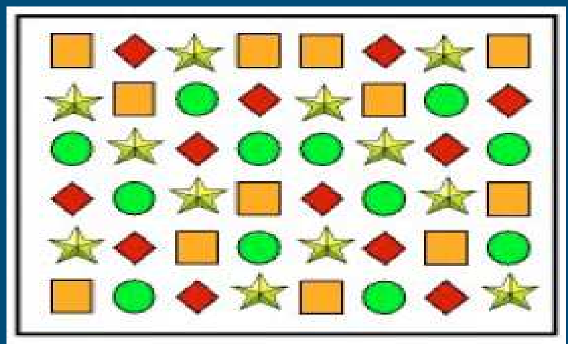
Three paradigms in deployment of *R* genes based on diagnostic *Avr* research of isolates



→ Monoculture-boom-bust



→ Pyramiding by **stacking**



→ Polyculture

Conclusions

- **Cisgenesis has to be exempted of EU 2001/18/EC**
- Cisgenesis is important for **improving existing** varieties of complex crops like apple and potato
- Cisgenesis brings new possibilities for resistance strategy
- Stacking of *R* genes is much more easy to handle
- *Avr* genes are helping (traditional) breeding to chose the same class of *R* genes in different species: *S. stoloniferum* instead of *S. bulbocastanum*
- *Avr* genes will help to develop, locally the most durable resistance strategy
- Cisgenesis is also very attractive for SME's and developing countries
- Cisgenic resistance breeding using wild species is more safe

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Toekomst verwachting

- Als cisgenese niet wereldwijd geaccepteerd wordt, zal al onze voedselproductie van een paar multinationals gaan afhangen met veel minder biodiversiteit per gewas en extra risico's
- Dit gaat ten koste van MKB en ontwikkelingslanden
- Op dit moment lopen tegengestelde belangen van NGO's (**one liners**) en multinationals paralel. Dit dreigt voorlopig zo te blijven
- Regelgeving werkt als een patent voor multinationals en maakt voedsel onnodig duur