Sweetpotato breeding course
24 August 2012

Genetic modification ≠ breeding?

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Contents

- Introduction DNA & breeding
- Phytophthora-problem & resistance
- Genetic engineering of plants
- A Belgian field trial with GM potatoes
- Some final remarks on HGT and cisgenesis

DNA= identical code for all

DNA = genetic information encoded in
4 letters

GAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCCGCGGGATTGA
CTGCGTACAAGCTCGGCCTCCAGTACATTGAATGGCTTGCCTGTCTTCAC
AAAAGCTTCAGCACACTTGCGGTTACGACCGCCAAAGCACGTAGTTATGA
GATCAGCCACACCAGCACTTGTTTCGGTGAAAGTTTCAGGGCGCACATCT
TTGAAGAACTCGAGCGCGAAACGTCGCATCTCCACCAAACCGATACGCAT
GATGGCGGCCTTCGCATTACCGCCCCAACCAAGACCATCAACGAAGCCGG
CACCCACAGCCACGATATTCTTCAATGCACCACACAGACTCACACCCGCC
ACGTCTTCAATCATGCCCACGCGGAACTTATGCGTGTCAAAGAGCTTGAC
ATAATACTCAGCCAACGCGCGTTGGTGTGGACGATATCCGACAGTTGTCT
CCGAAAAGAGACCAGACGCTACTTCATTCGCAATGTTTGCGCCAATTACT
CAGGACTCATCGTCAATCACTAGTGCGGCCGCCTGCAGGTCGACCATATG
GGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGT

Some genes are very conserved in evolution

16/18S rRNA-gen

human
GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCAGTTA

yeast
GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTA

Corn
GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTA

E.coli
GTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA

Breeding: what happens to the DNA?

Crossing a tomato with a wild relative

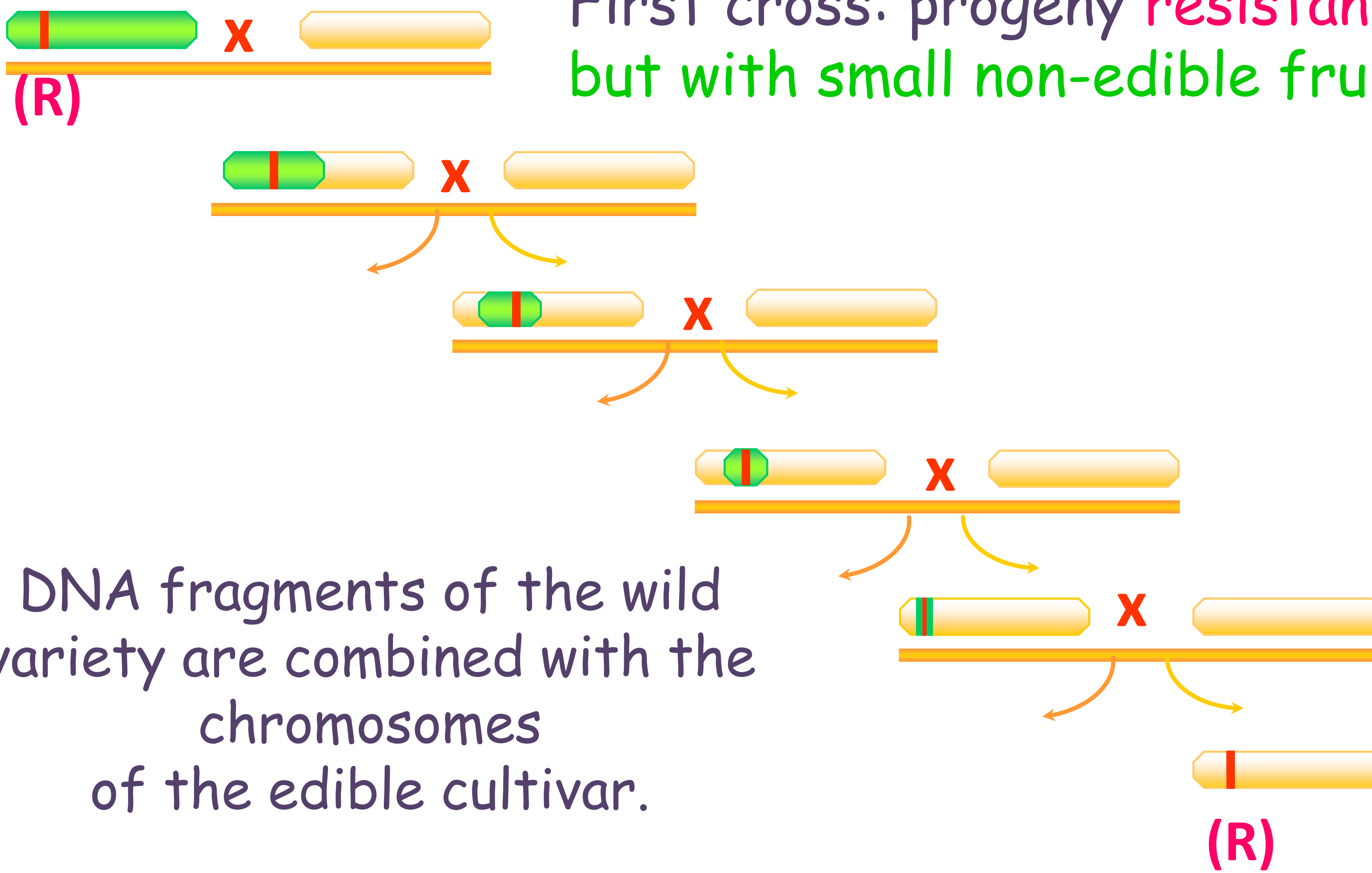
Backcrossing with the cultivated tomato plant to retrieve all the good characteristics

EDIBLE

X

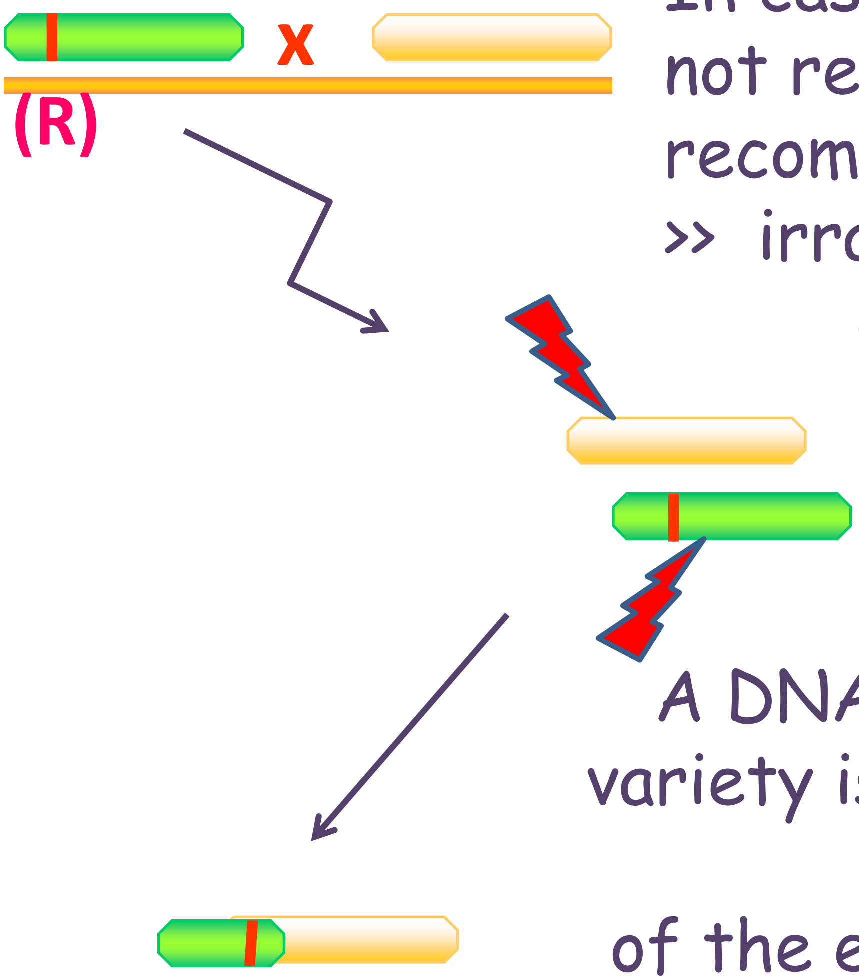
Breeding: what happens to the DNA?

First cross: progeny resistant but with small non-edible fruits



DNA fragments of the wild variety are combined with the chromosomes of the edible cultivar.

Breeding: what happens to the DNA?



In case the wild relative is not related enough, no natural recombination can occur,

>> irradiation is used to break chromosomes.

A DNA fragment of the wild variety is attached to one of the chromosomes of the edible cultivar, example: current wheat varieties.

Table 1. Examples of gene transfer by random insertion using induced translocation breeding by irradiation

Crop	Source	Trait	Ref
Wheat	*Aegilops umbellulata*	Brown rust	[3]
Wheat	*Secale cereale*	Mildew and brown rust	[3]
Wheat	*Agropyron elongatum*	Brown and black rust	[3]
Wheat	*Agropyron intermedium*	Brown and yellow rust	[3]
Wheat	*Aegilops speltoides*	Brown rust	[3]
Oats	*Avena barbata*	Mildew	[3,25]
Beet	*Beta patellaris*	Nematodes	[26]
	Beta procumbens		
Tobacco	*Nicotiana glutinosa*	Tobacco mosaic virus	[27]
Radish	*Brassica rapa*	Spread leaf type	[28]

Jacobsen & Schouten, 2007

Genetic engineering is often seen as unnatural in contrast to breeding

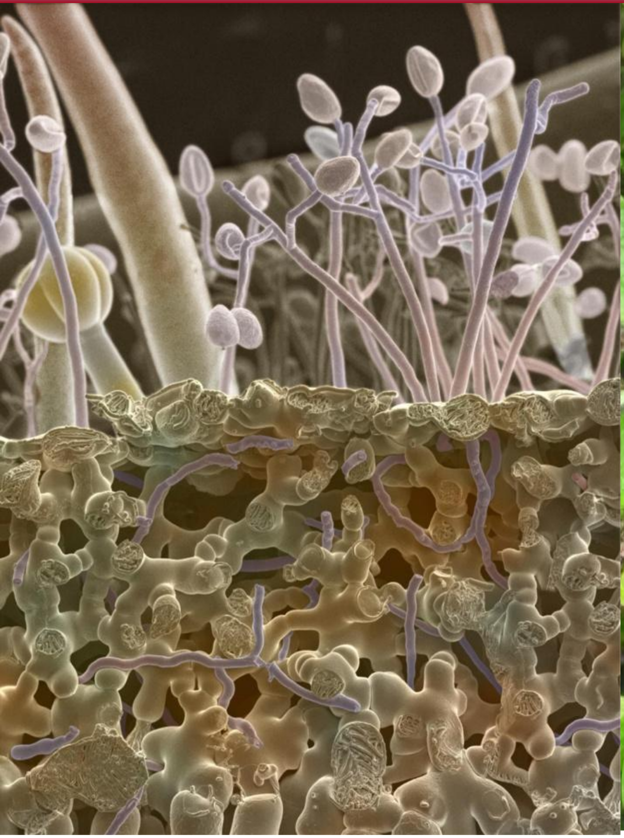
Breeding is often seen as something that spontaneously happens in nature, but it is a man driven process.

Breeding is seen as a natural process, but…

- It is not only done within species but also between species (interspecific) and even between genera (intergeneric) such as wheat (*Triticum*) resistance breeding with grasses (*Agropyron & Aegilops*).
- Irradiation can be used to break the chromosomes.
- Colchicine is a chemical that blocks chromosome separation during meiosis to induce higher ploidies.
- Progeny are often two weak to survive without help

  >> in vitro embryo rescue.

The late blight problem in potato



Phytophthora is the biggest threat for potato cultivation

Phytophthora costs in Belgium: >1000 tons of fungicides and 10 -15 times spraying / season ≈ 55 million euro / year

Estimation for Europe > 1 billion euro costs / year

Phytophthora is a world wide problem on potato

Food security issue

21 million ha globally, losses about 10 billion €…

Resistant varieties are the best solution

- Resistance genes available in wild relatives

  (*S. stoloniferum, S. venturi, S. bulbocastanum* and others,

  > 20 genes in total)

- Introduction into potato through, either:
  - Conventional breeding
  - Genetic modification

Resistant varieties obtained by breeding

Sarpo Mira
(Danespo)

Bionica
(C.Meijer)

Toluca
(Agrico)

Bionica &Toluca contain blb2

Conventional breeding is very slow and in case of interspecific crosses involves in vitro techniques (embryo rescue, colchicine*).

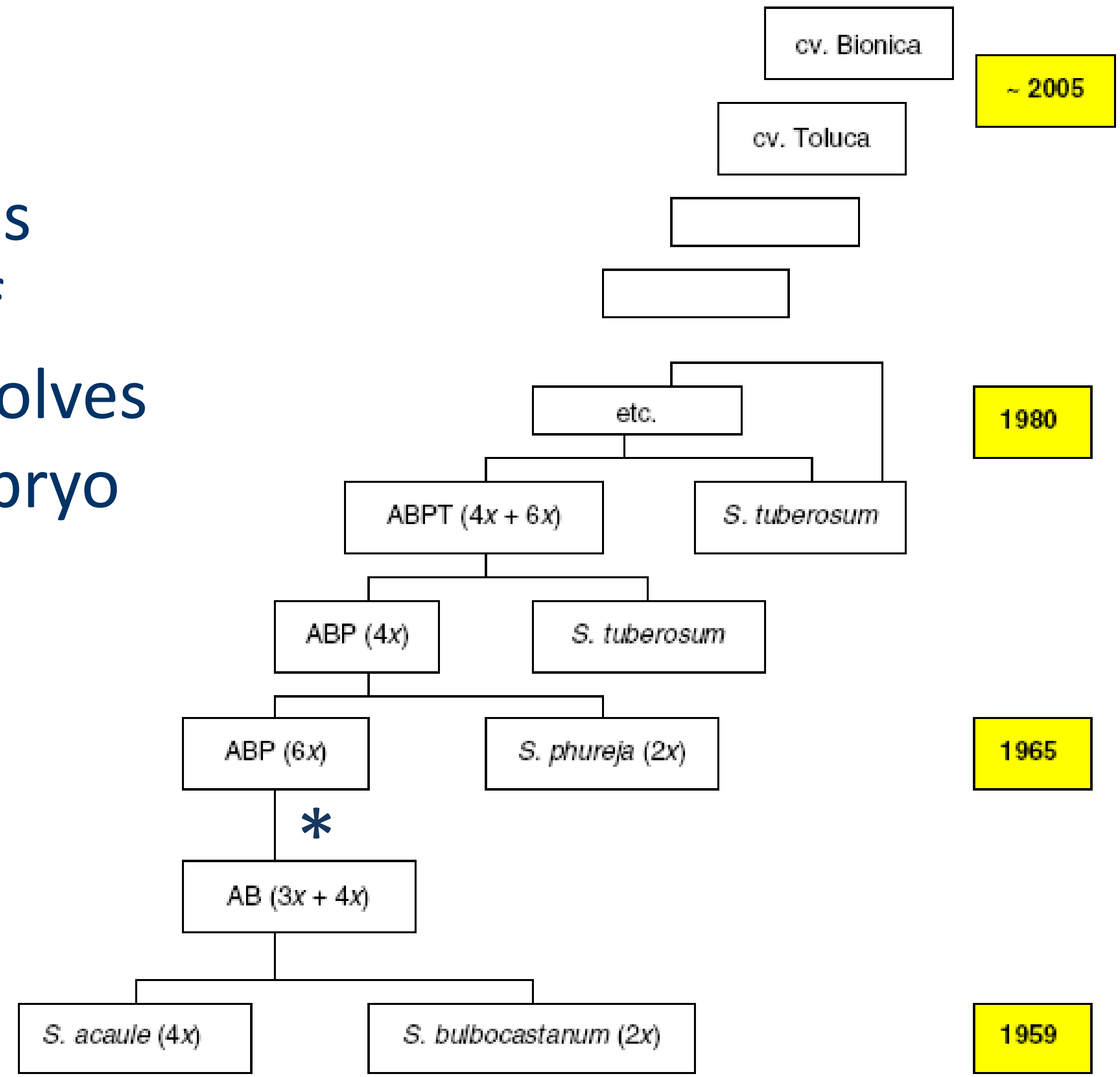


Fig. 3 Scheme of interspecific bridge cross breeding activities with late blight resistant *S. bulbocastanum* at Wageningen University and Research Centre and private breeding companies in the Netherlands. After 46 years the first resistant varieties Bionica and Toluca appeared, containing the single broad spectrum resistance gene *Rpi-blb2*. Note that stacking of *R* genes through this approach would even be more complicated and slow

Resistant varieties obtained by breeding: results from the field trial in 2011, Belgium

Sarpo Mira
(Danespo)
Several R genes

Toluca
(Agrico)
Blb2

Bionica
(C.Meijer)
Blb2

Disadvantages of breeding

- Sarpo Mira has several resistance genes (Rietman et al., 2012, MPMI), but the eating and processing qualities are low (only suitable for french fries).

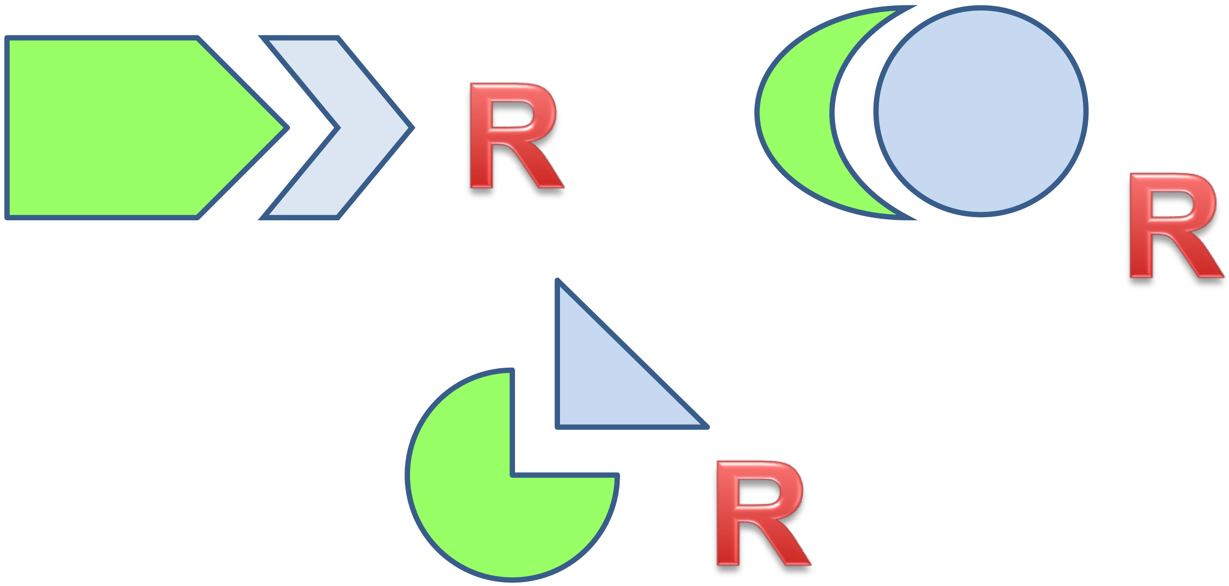
- Bionica and Toluca are more palatable but not good for processing and they contain only one resistance gene > virulent Phytophthora strains develop very fast > resistance is not functional anymore.

Monogenic resistance

- Very strong defense response.
- Very specific (= not toxic).
- Based on recognition of a protein from the pathogen by a plant protein ("immune response").

- Mutation of the gene for the pathogen protein = no recognition anymore by the plant.

Pyramiding resistances = durable resistance

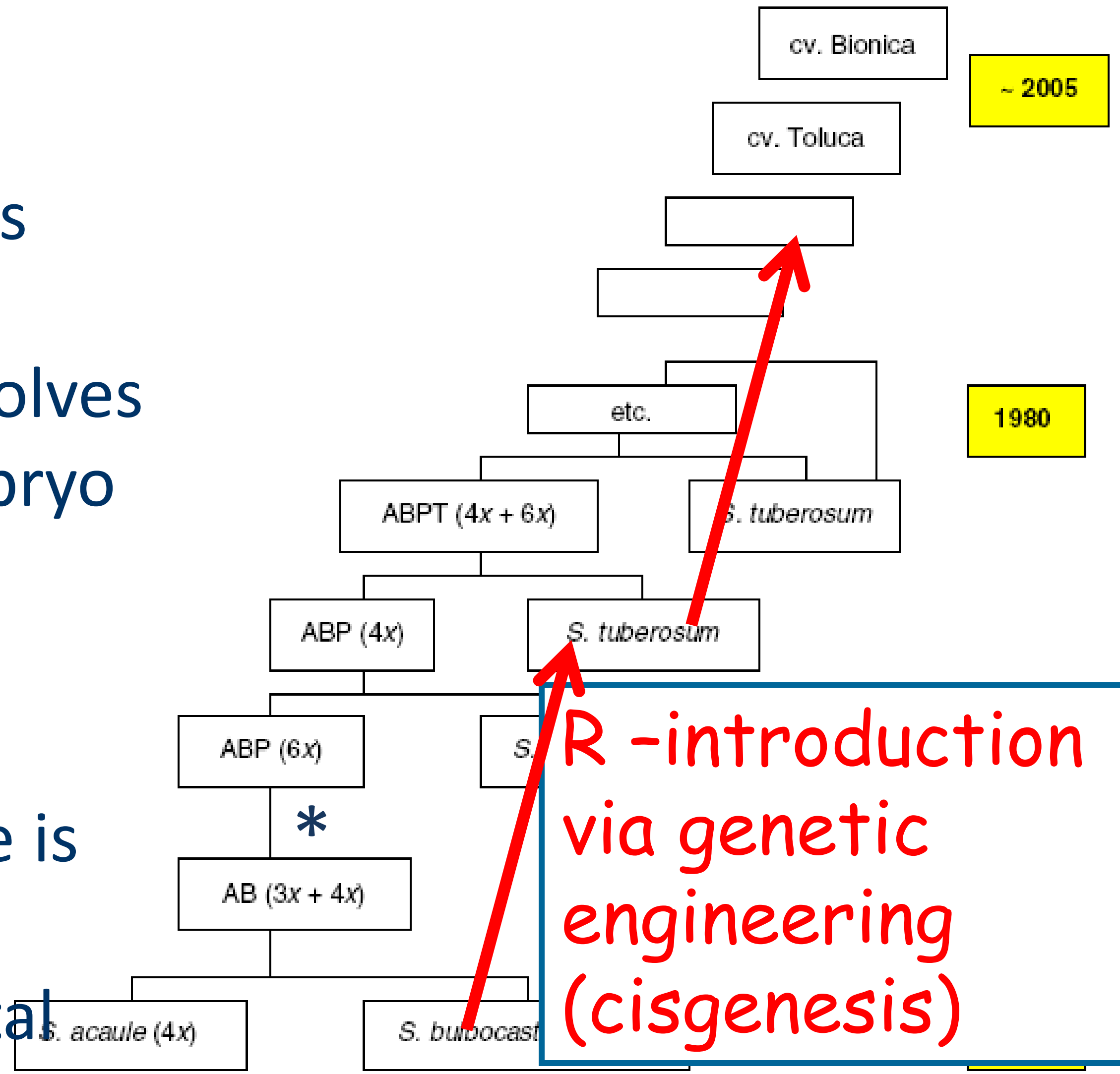


Phytophthora easily overcomes a single resistance e.g. 1/1000
Double resistance is much more durable 1/1000 x 1/1000
Triple resistance is even more durable 1/1000000000

Bionica & Toluca contain blb2

Conventional breeding is very slow and in case of interspecific crosses involves in vitro techniques (embryo rescue, colchicine*).

GM is fast but the authorisation procedure is time consuming and expensive. Environmental and food safety tests required (animal testing).



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Genetic engineering of potato is fast and efficient

Differences with breeding:

- Resistance in one step through isolation of one gene out of 20-40,000 and introducing it into a good variety.

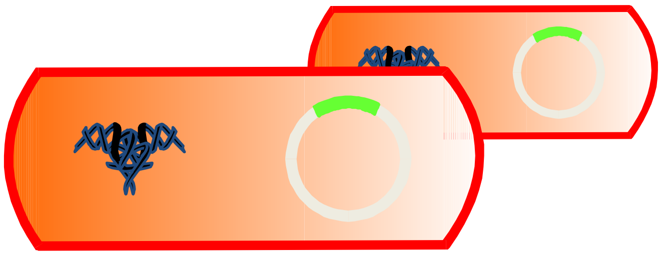
- Variety characteristics remain.
- Possible to introduce multiple resistance (R) genes at the same time: potato lines in the field trials have R genes from *Solanum bulbocastanum, S. venturi & S. stoloniferum.*

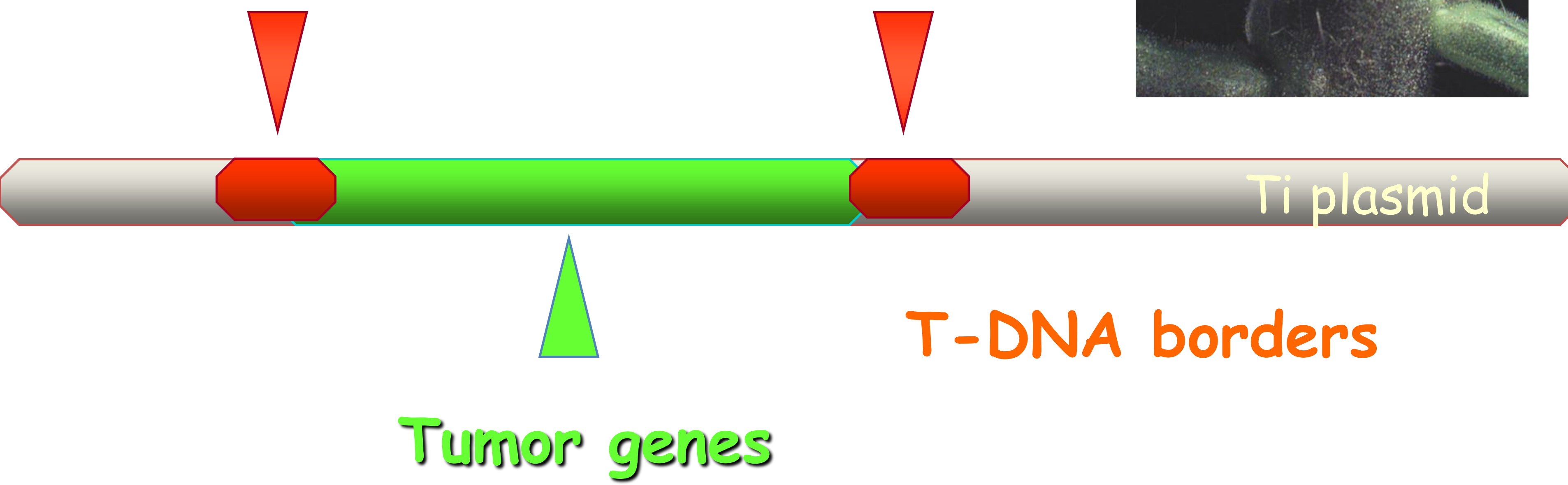
The essence of plant genetic engineering

- A specific piece of DNA is introduced into the plant cell.
- Plant transformation methods use Agrobacterium or physical means (microparticles) to introduce the DNA.
- DNA integration into the plant genome has been studied very well.

- The DNA is inserted in one of the chromosomes of the target plant by natural DNA repair enzymes (>> **event**).
- Very precise technology: one gene can be isolated from one organism and introduced in “another”, this new gene is stably integrated and inherited as any other gene, location unknown at forehand but characterised > known.

Agrobacterium tumefaciens is a natural genetic engineer: T-DNA transfer into the plant cell





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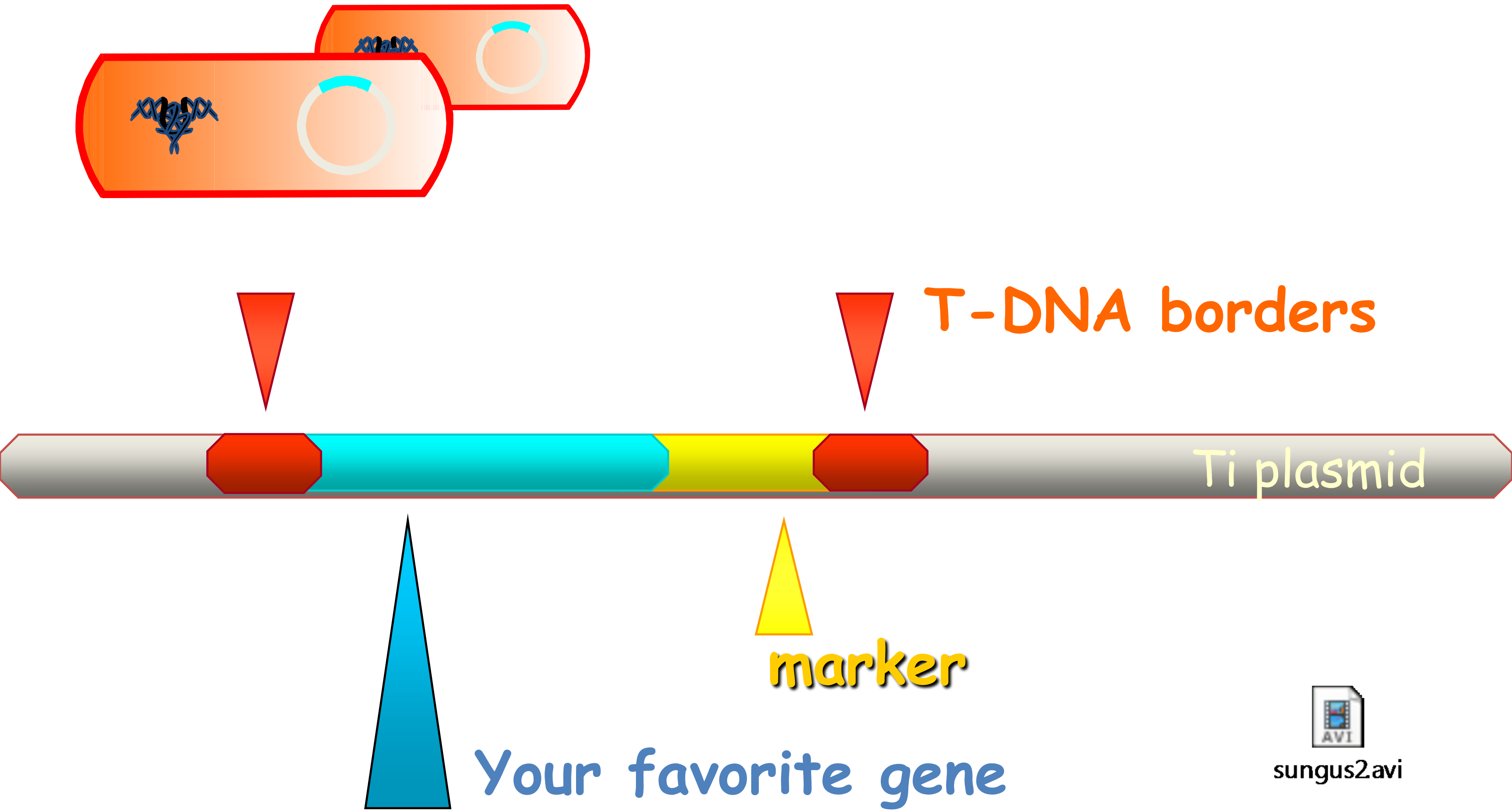

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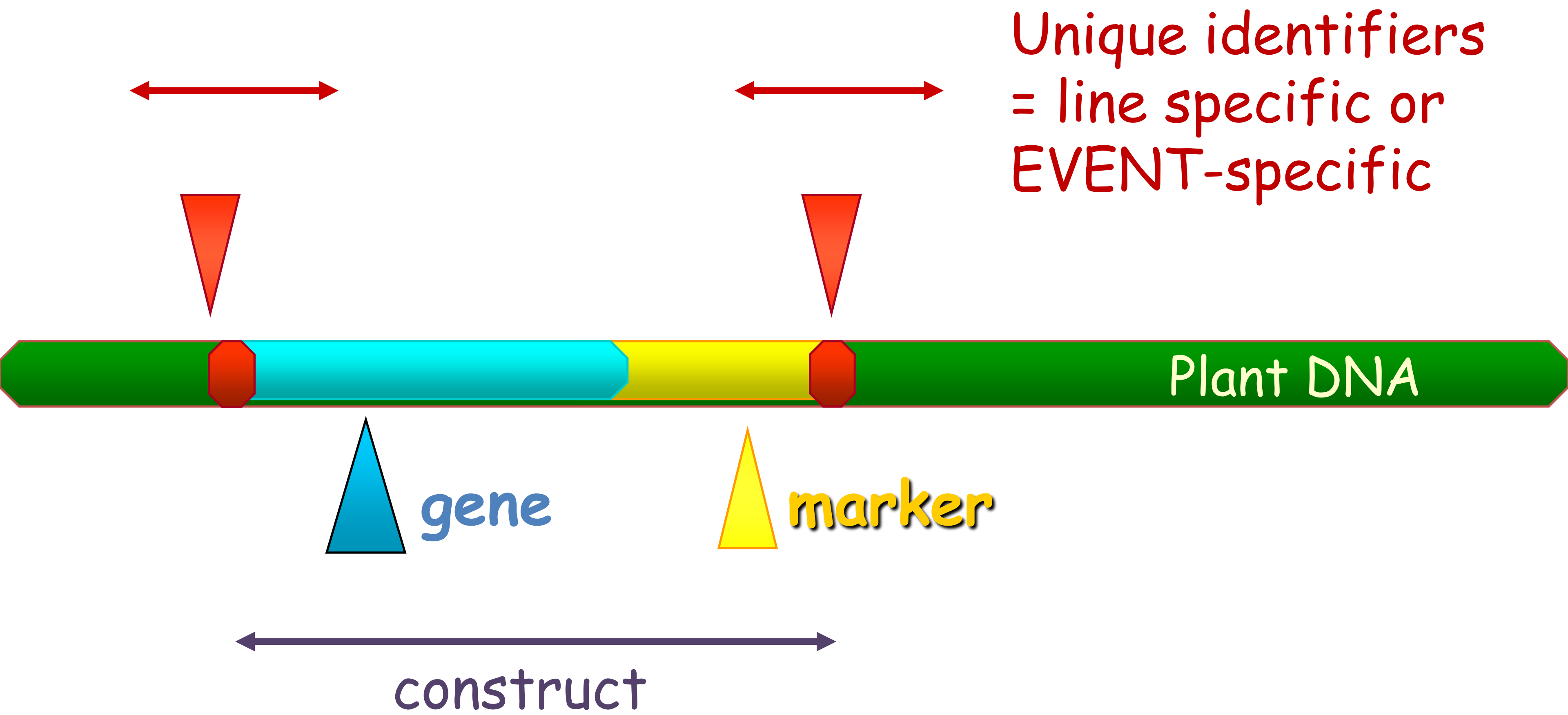
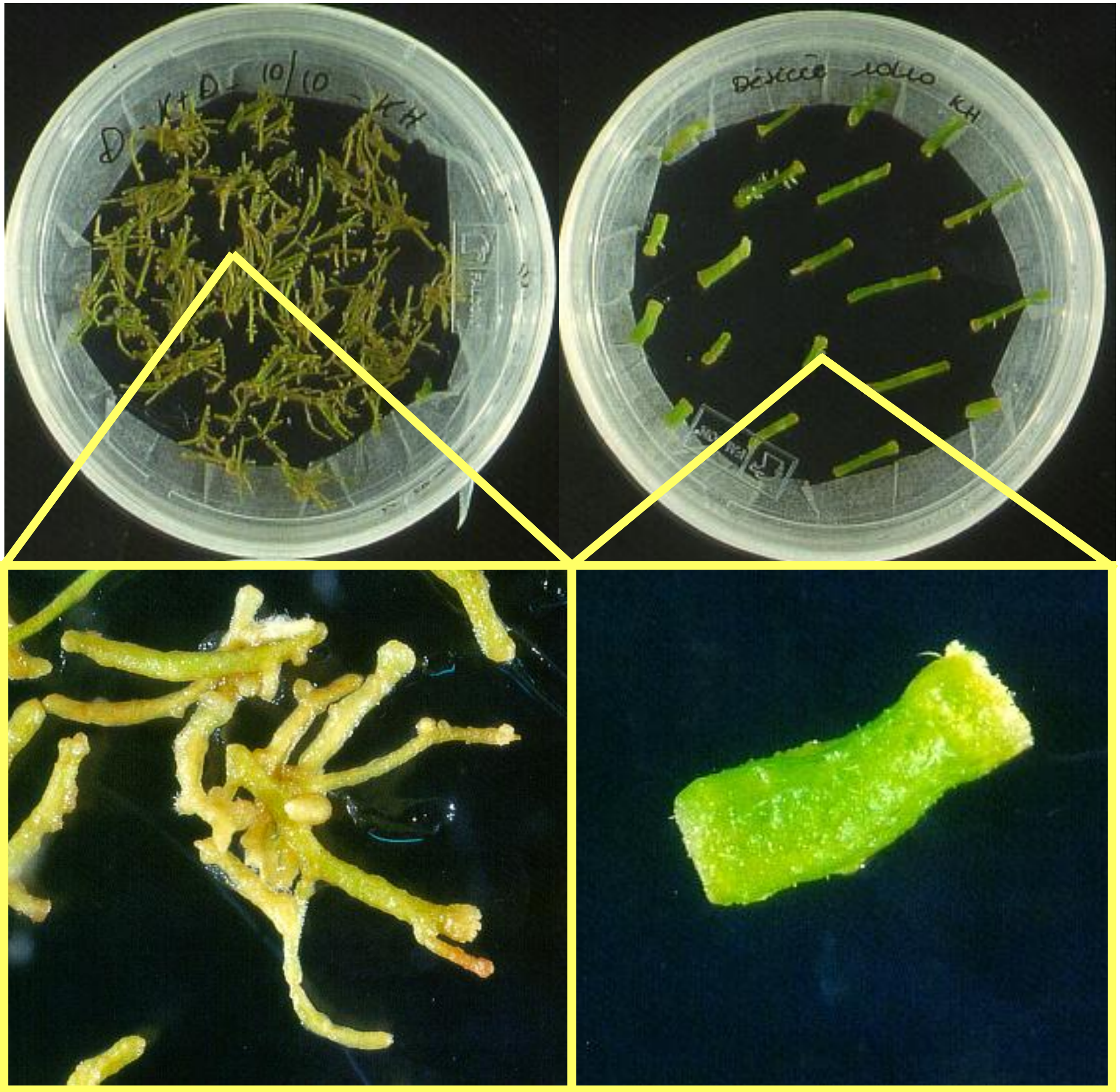


Selection

- Transformation is not 100% efficient
- Regeneration of only the transgenic cells can be selected on the basis of an introduced gene eg. antibiotic- or herbicide resistance (put plant tissue on selective medium) or a screening can be done (PCR)

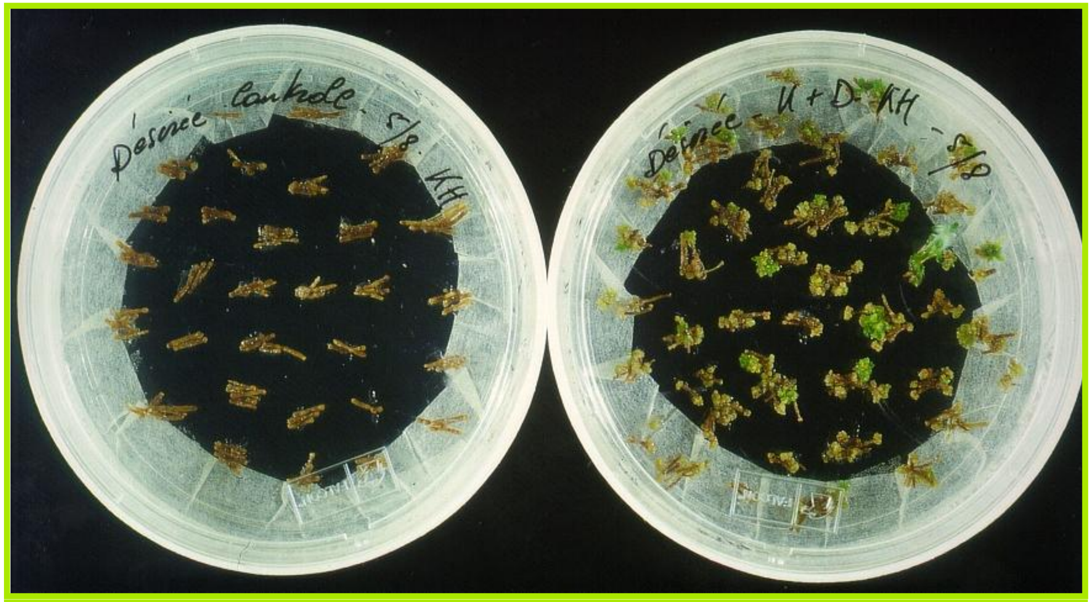
Cocultivation of potato with Agrobacterium



Start of transformation

A. Depicker

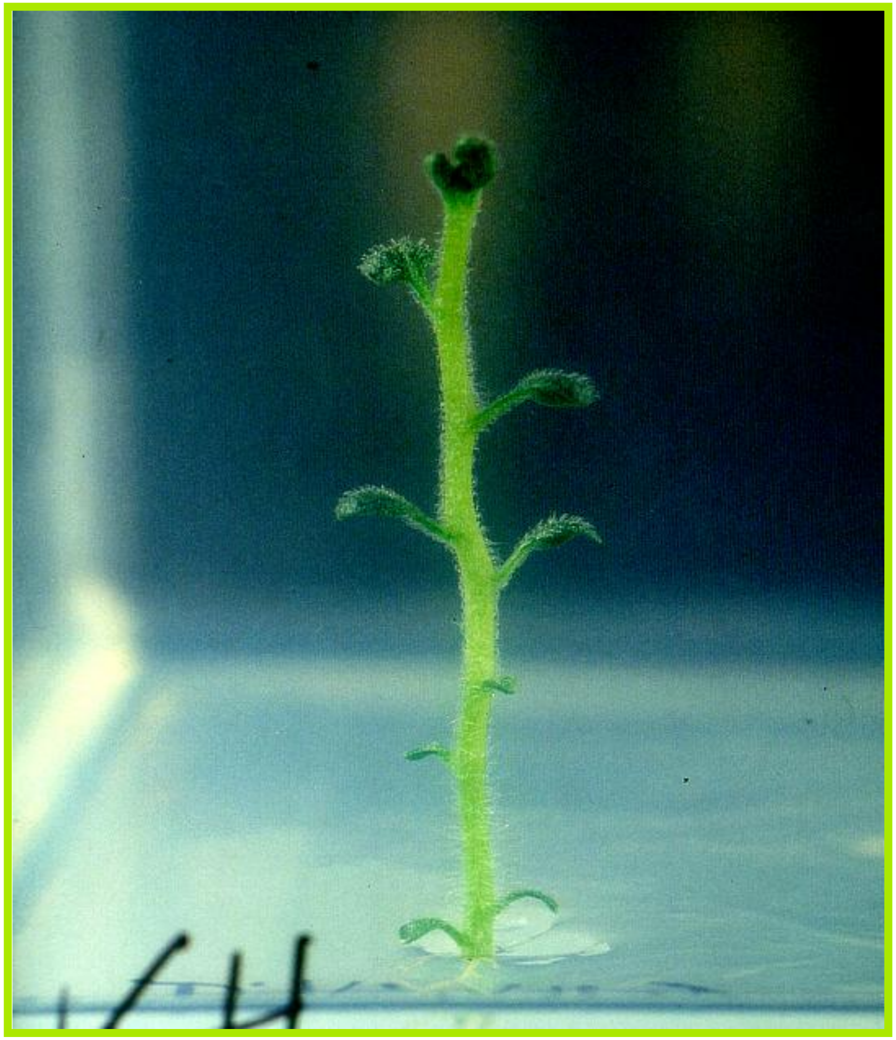
Callus and shoot formation



3 months

A. Depicker

Transfer of shoots



4 months

A. Depicker

Rooting, amplification in vitro

5 months

A. Depicker

Transfer of plantlets to soil

6 months

A. Depicker

Growth of plants in greenhouse

9 months

A. Depicker

Transgenic potato tubers

A. Depicker

From construct to tuber production:

Min. 9 months

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ILVO

VIB

HOGESCHOOL GENT
LID VAN DE ASSOCIATIE UNIVERSITEIT GENT

WAGENINGEN UNIVERSITY
WAGENINGEN UR

A Belgian field trial with GM late blight resistant potatoes

Belgian field trial with GM late blight resistant potatoes

- Wageningen University: DuRPh potatoes
- University of Ghent: coordination
- Institute for Agriculture and Fisheries Research: practical execution
- Flanders Institute for Biotechnology: regulatory issues
- University College of Ghent: late blight expertise

The GM potatoes in the Belgian trial in 2012

From Wageningen UR (DuRPh project):

- 7 lines with *sto1* resistance gene + *nptII* marker
- 8 lines with *vnt1.1* resistance gene
- 10 lines with *sto1*, *vnt1.1.* and *blb3* resistance genes + nptII marker

All in Désirée

From several sources: resistant and susceptible reference lines.

UNIVERSITEIT GENT ILVO VIB HOGESCHOOL GENT LID VAN DE ASSOCIATIE UNIVERSITEIT GENT

Lab and greenhouse tests

Resistance tests in the lab and greenhouse to identify the best resistance genes and the lines with best performance.

Désirée

Désirée + Rpi-chc1

The results

7th august 2012

Catherine Blancquaert

Results of the field trial

Resistent GMO lines: no spraying is needed for late blight protection

Susceptible reference: destroyed by late blight if not sprayed

Sustainability

- Economy
  - Late blight costs Belgian farmers about 55 M€ / year.
- Ecology
  - Belgian farmers spray up to 20 times / year.
- Social aspects
  - Farmers do not need to constantly check the crop and be alert for potential infections.
- Sustainable long lasting resistance through multiple gene approach.
- Changing farmers' livelihoods: less costs and work, better yields, easier management.

Common arguments against GMO’s

- GMO’s are no solution for real problems, farmers do not want this.
- GMO’s increase pesticide use.
- GMO’s are risky for health or environment.
- GMO’s are being developed and commercialised by multinational companies to increase their profits.
- GMO’s are not useful/needed in Europe.
- GMO’s are unnatural.

However: “ the“ GMO does not exist!

Some genes have been transferred from one organism to another in evolution by HGT (horizontal gene transfer)

Adzuki Bean Bee

ana tabacum

Elysia chlorotica

Risk assessment / major public concerns?

- Bioafety Issues:
- Human and animal health
- ✓ Natural resistance genes also introduced by breeding, cultivars commercially grown. Genes are not toxic but work as an immune response.

- Environment
- ✓ Less fungicide spraying, ecological effects can be lower than those of traditional agriculture
- ✓ Specific recognition, no non-target effects expected
- ✓ Gene flow? can also happen with genes introduced by breeding, and berries are not used for propagation.

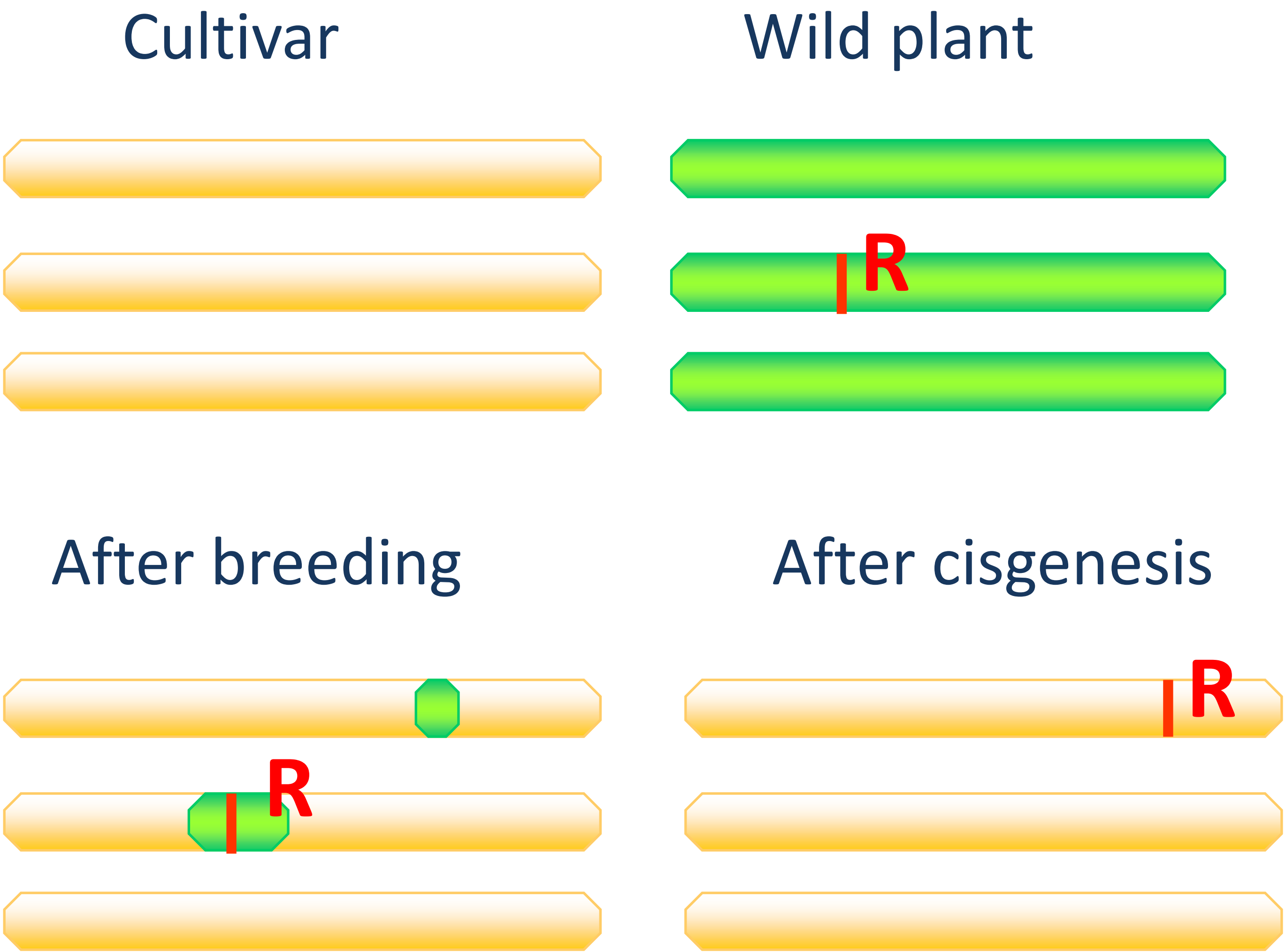
Transgenesis & cisgenesis

- Cisgenic plants are produced by the same transformation techniques as transgenic plants, both are GMO's.
- A genetically modified organism (GM) that has obtained DNA from another organism = transgenic.
- A genetically modified organism (GM) that has obtained DNA (native non-modified genes) from an organism that belongs to the same or a crossable species = cisgenic. This DNA could also be introduced through breeding.

Applications of cisgenesis

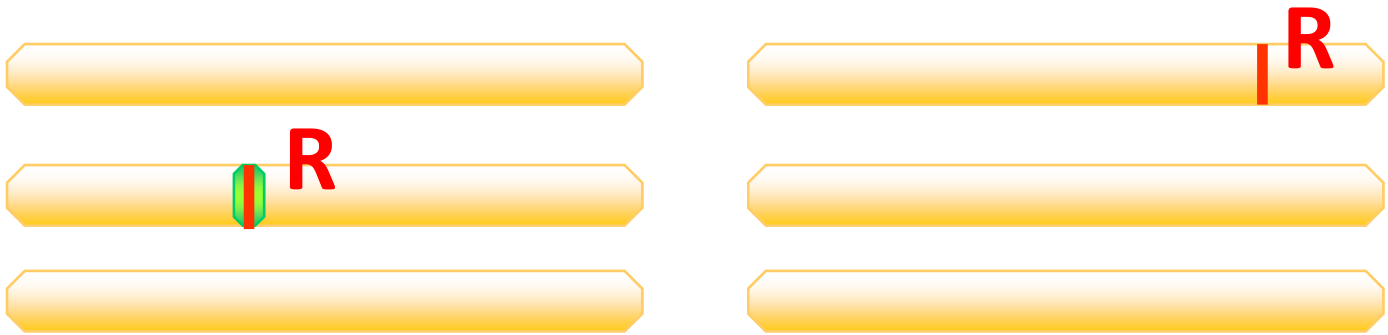
- Recurrent back crossing is not needed: cisgenesis allows the fast introduction of resistance gene(s) by maintaining the agricultural value: only adding resistance trait(s).
- Recurrent back crossing is often not feasible:
  - Crops multiplied vegetatively (e.g. apple, grape, strawberry, banana, …)
  - Long life cycles e.g. trees
  - When resistance gene has to be introgressed into heterozygous material (e.g. apple variety Elstar, grape variety Merlot, …)
  - Self-incompatibilities

Breeding versus cisgenesis



Conclusion

Classical breeding | Cisgenesis



- EFSA Scientific Opinion, 2012:

  The Panel concludes that similar hazards can be associated with cisgenic and conventionally bred plants.

- EU Working Group New Breeding Techniques, 2012:

  Cisgenesis …. could be considered to be excluded from the EC Directive on GMO's.