

27.-28. April 2009, FIAP, Paris, France



Genetic modification hidden in modern plant breeding techniques

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Novel breeding techniques:

- The introduction of Cytoplasmic Male Sterility (**CMS**) by **cell fusion** techniques
- **DNA Marker** assisted selection
- **Induced mutagenesis**
- **Dihaploid** plants from tissue culture and their application in “**reverse breeding**”
- Gene transfer from related species: **cisgenesis**

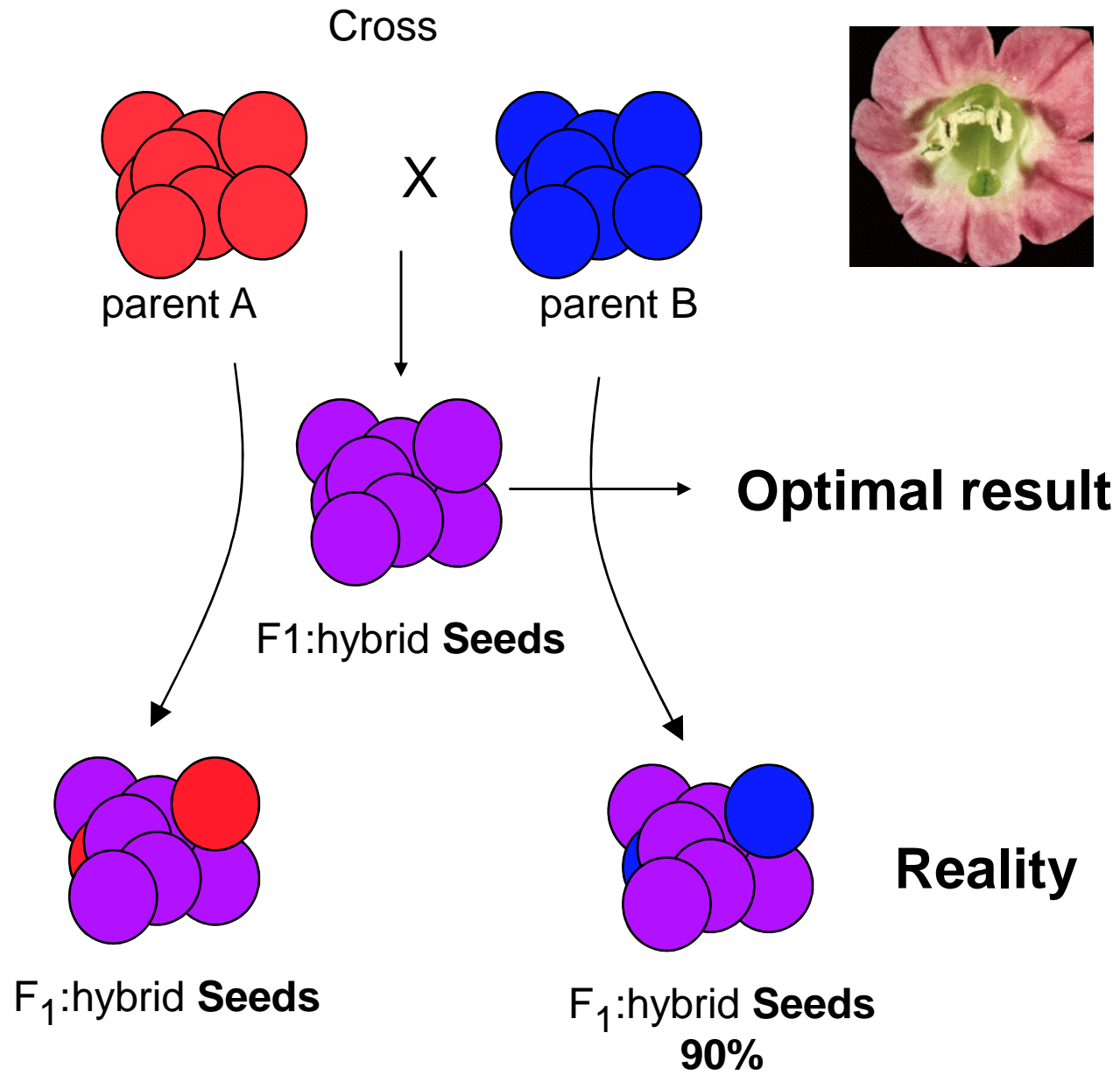
*Modern plant breeding:
Selecting inbred lines for
 F_1 -hybrid seed production*



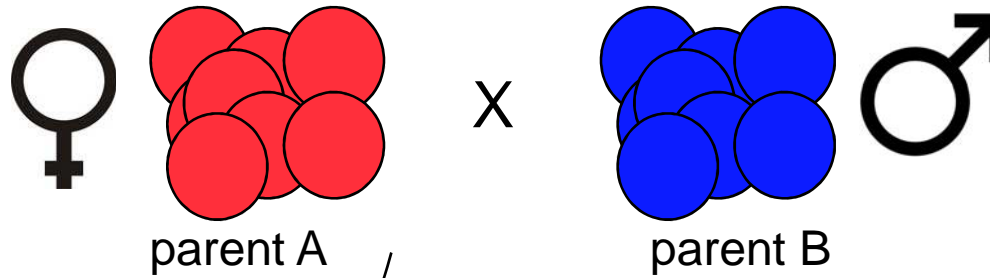
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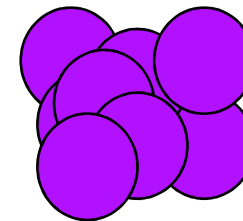




Cross

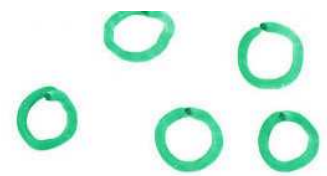


CMS: cytoplasmic male sterility



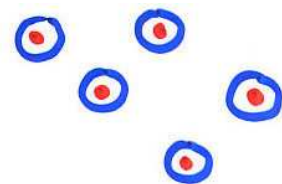
Optimal

**F₁: hybrid Seeds
100%**

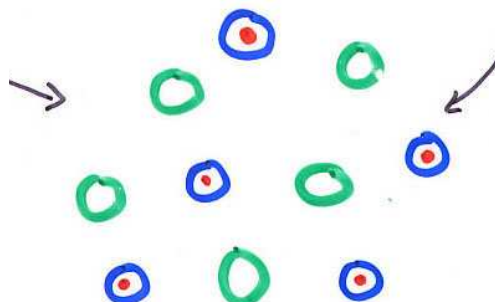


CMS cells
without
nucleus

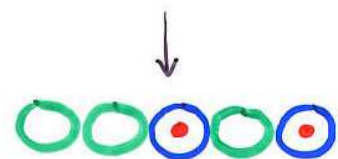
Cytoplasm
fusion



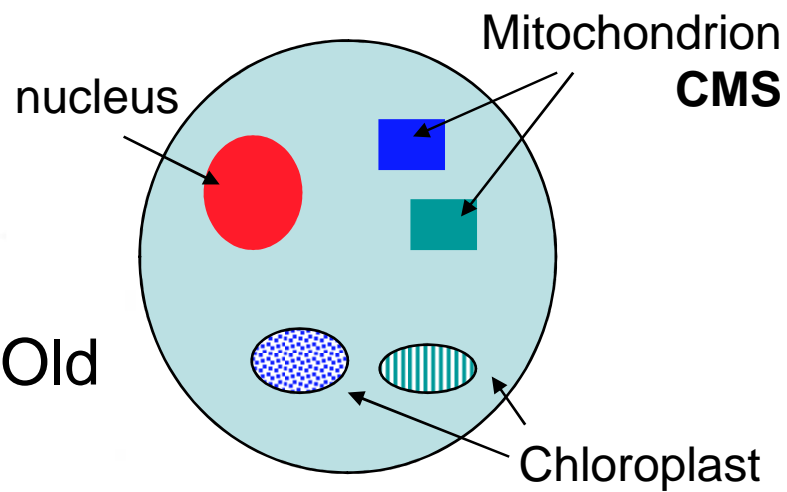
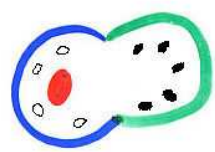
protoplasts



Cytoplasm Fusion:
only Mixing of
Cytoplasmic traits



Cell fusion



Old

New, CMS!
Only transfer of mitochondria

Sources of Cytoplasmic Male Sterility

Radish: Ogura CMS

Now in cauliflower

Brassica: polima CMS

Petunia CMS

Maize: Texas CMS

Sunflower: petiolaris CMS

Now in chicory

Bean CMS

Sugarbeet: Beta vulgaris CMS

Theor Appl Genet (1997) 94: 213–220

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M. A. Sigareva · E. D. Earle

Direct transfer of a cold-tolerant Ogura male-sterile cytoplasm into cabbage (*Brassica oleracea* ssp. *capitata*) via protoplast fusion



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United States Patent 6,803,497

Delesalle, et al. October 12, 2004

Methods of making *cytoplasmic male sterile* chicory plants comprising the ORF 522 of *helianthus annuus*

Abstract

The invention is directed to methods of making *cytoplasmic male sterile* chicory plants by identifying a **diagnostic 347 bp fragment** of the orf 522 of *Helianthus annuus*.

Inventors: **Delesalle; Louis** (Cappelle en Pevelle, FR); **Dhellemmes; Charles** (Cappelle en Pevelle, FR); **Desprez; Michel** (Cappelle en Pevelle, FR)

Assignee: **Florimond Desprez Veuve et Fils** (Cappelle en Pevelle, FR)

Appl. No.: **194598**

Filed: **November 30, 1998**

PCT Filed: **May 30, 1997**

PCT NO: **PCT/FR97/00944**

PCT PUB.NO.: **WO97/45548**

PCT PUB. Date: **December 4, 1997**

Foreign Application Priority Data

May 31, 1996[FR] 96 06725

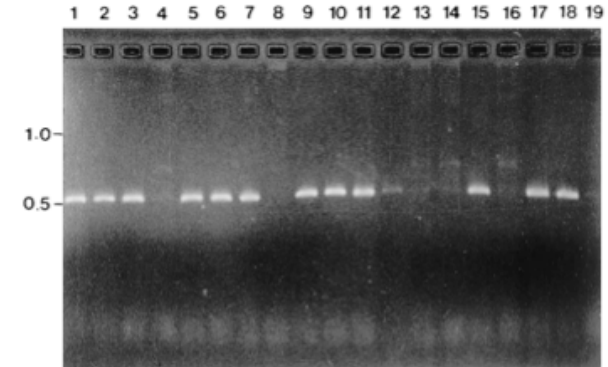


Fig. 4 PCR analysis with CMS-specific primers. Lanes 1-3 CMS broccoli lines AB-2, AB-3, and AB-4 used as parents showing the characteristic 0.5-kb band; lane 4 fertile cabbage A-5; lanes 5-7, 9-12, 15, 17, 18 CMS cybrids; lanes 8, 13, 14, 16, 19 fertile cybrids

Example of DNA fingerprint to identify CMS in breeding lines

Novel breeding techniques:

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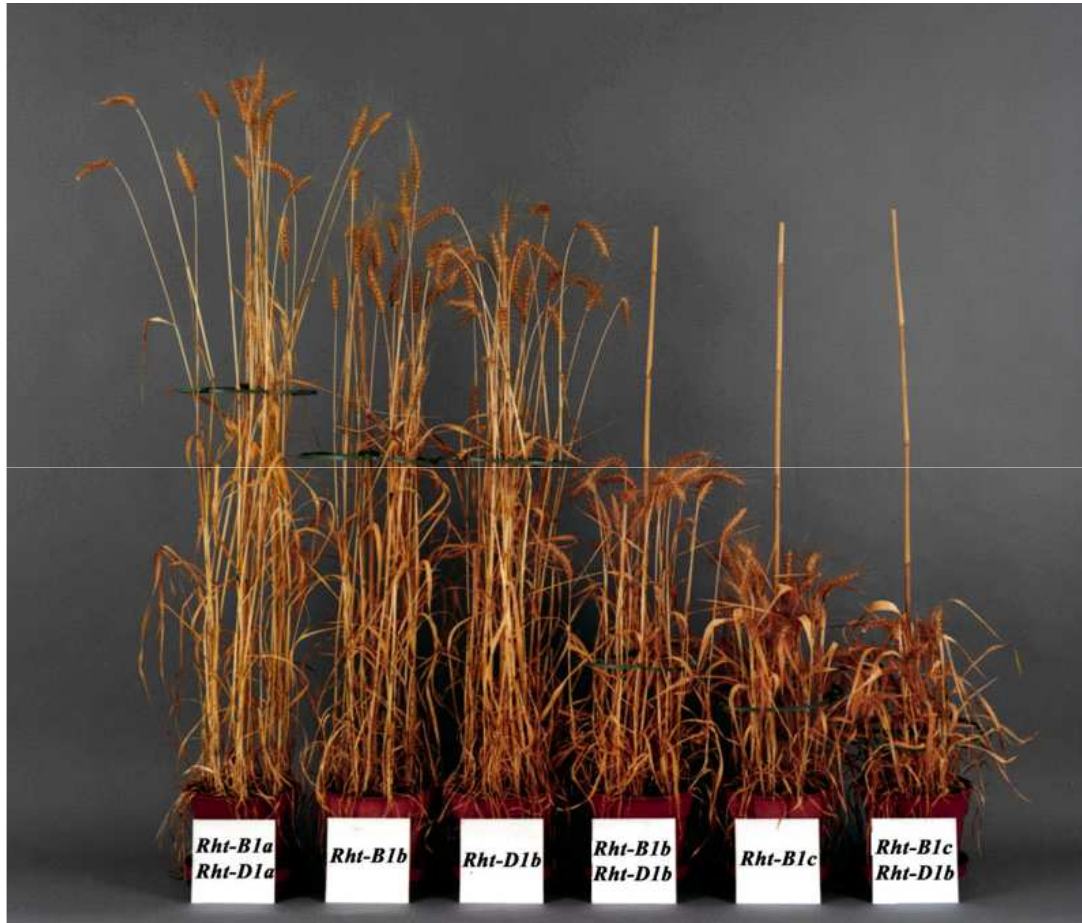
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Mutagenese in de plantenveredeling



Using **chemicals or ionizing radiation** *random mutations* have been induced in plant genomes resulting in new phenotypes: these have been introduced in breeding programs.

The “green revolution”
Dwarf-varieties of wheat



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Directed mutagenesis in plant breeding

570 Val Val Gln **Trp** Glu Asp Arg
GTG GTT CAA **TGG** GAG GAT CGG

Original gene

+

ChALS-1719

TGCGCG---ac cac caa guT AAC Cuc cua gcc aa T
T T
TCGCGC TG GTG GTT CAA **TIG** GAG GAT CGG TT T
3' 5'

**Synthetic
DNA/RNA
hybrid**



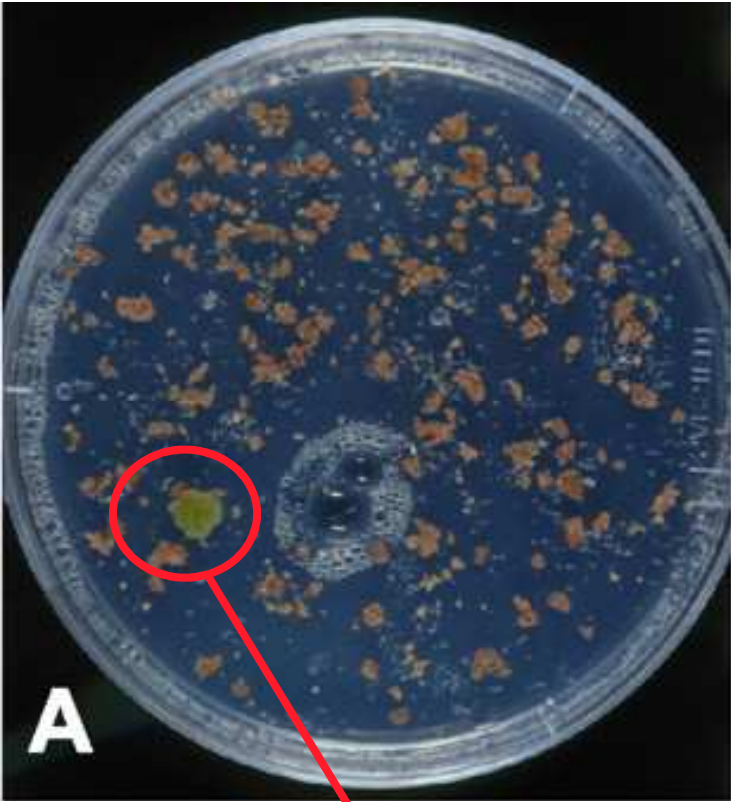
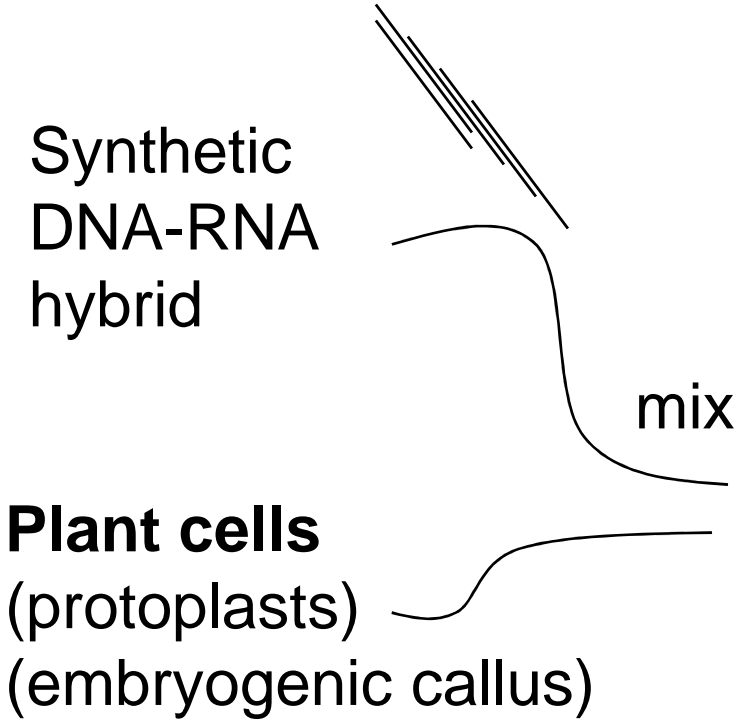
570 Val Val Gln **Leu** Glu Asp Arg
GTG GTT CAA **TIG** GAG GAT CGG

“New” gene

Result: herbicide tolerant plant

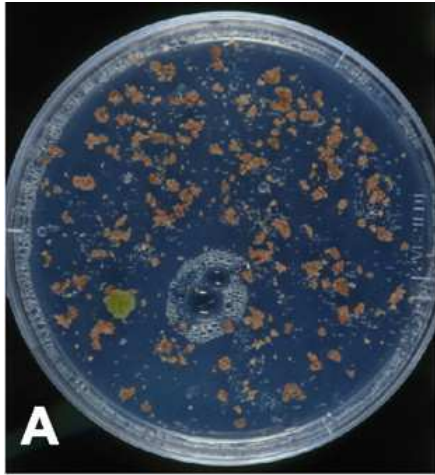


Directed mutagenesis



Grow plantlet

Select for resistance against herbicide



A



B

R

WT

84 μ M

140 μ M



C

R

WT

280 μ M



D

R

WT

R

WT

R

WT

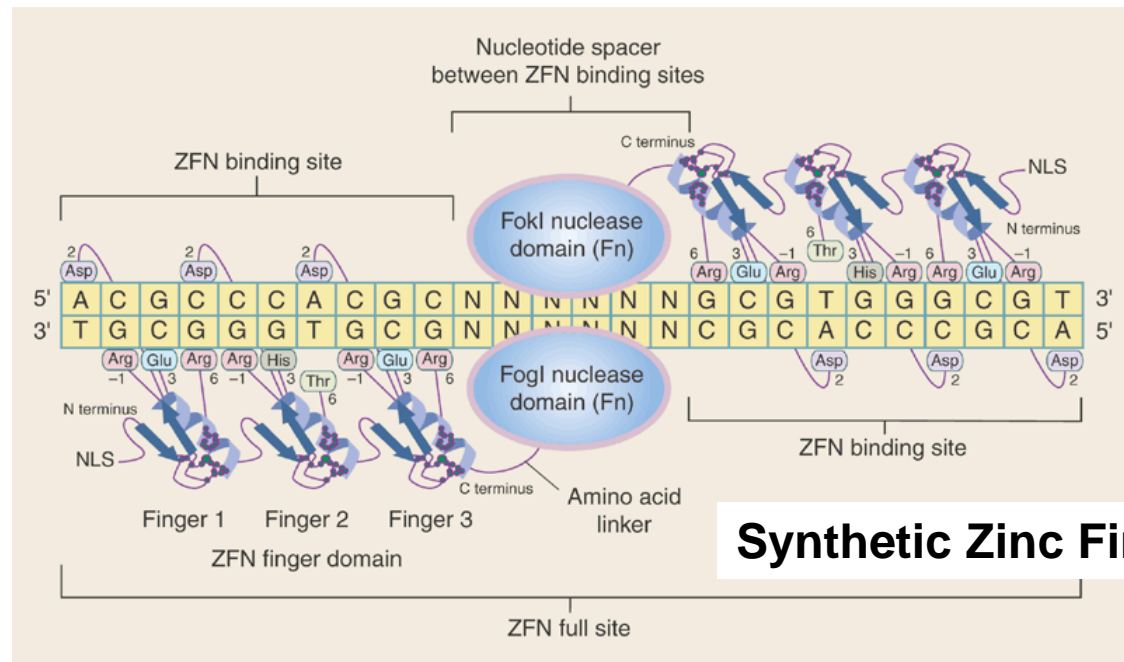


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Result: chlorosulfuron tolerant plant

Directed mutagenesis in plant breeding:
Still very inefficient: works only for traits that can be selected for (herbicide tolerance)

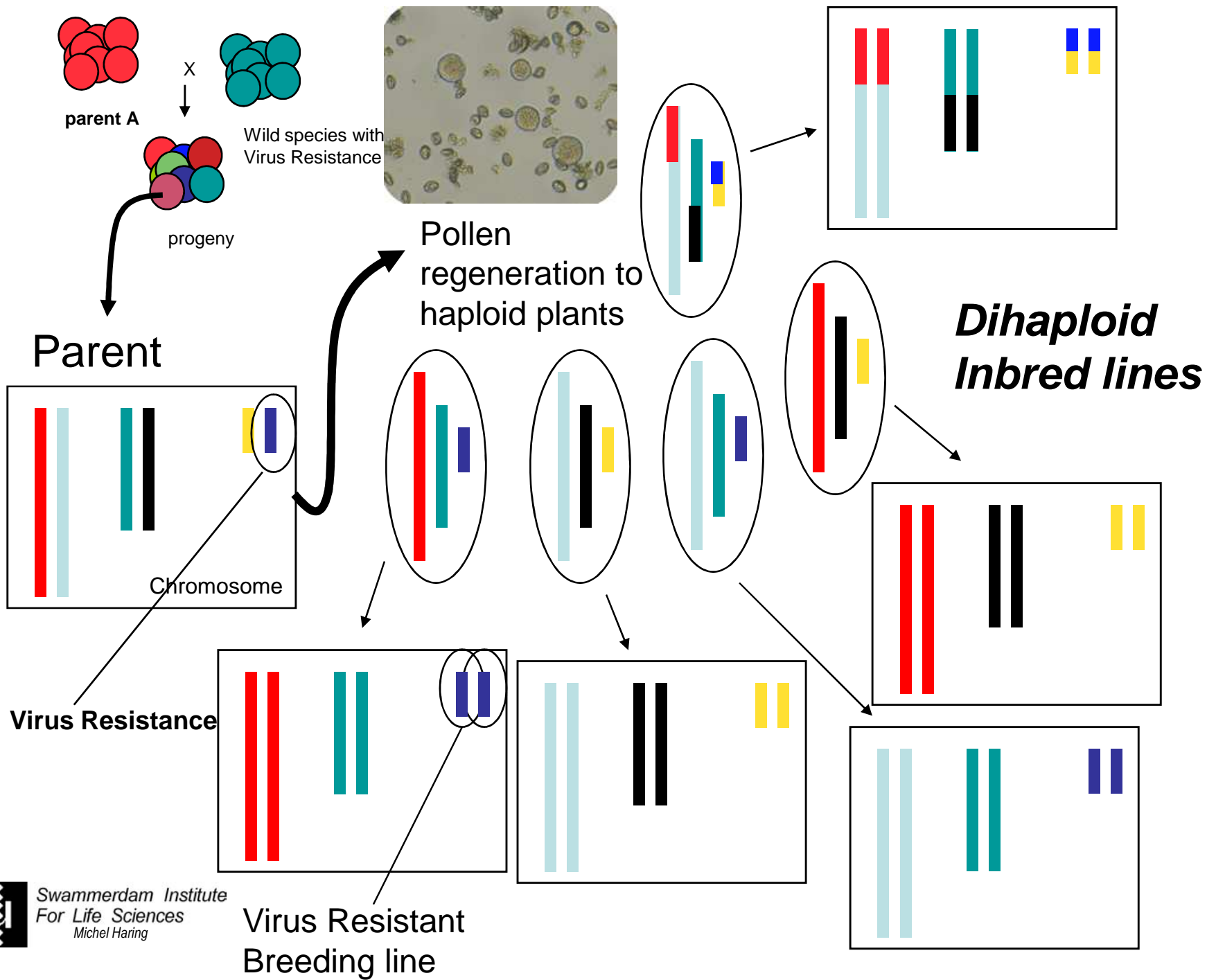
In combination with a GM strategy:
Introduce a specific “Zinc Finger” gene to induce the mutation in the desired gene. **Still in development!**



Novel breeding techniques:

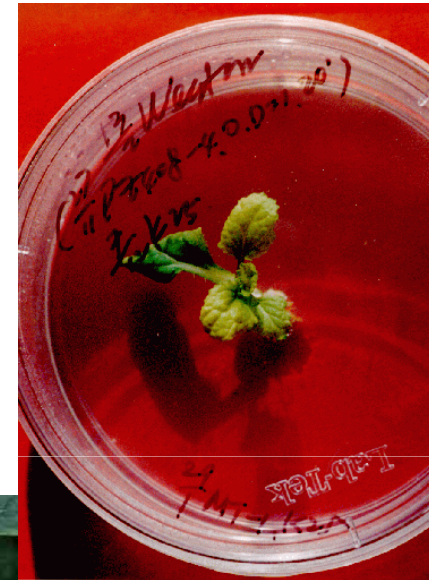
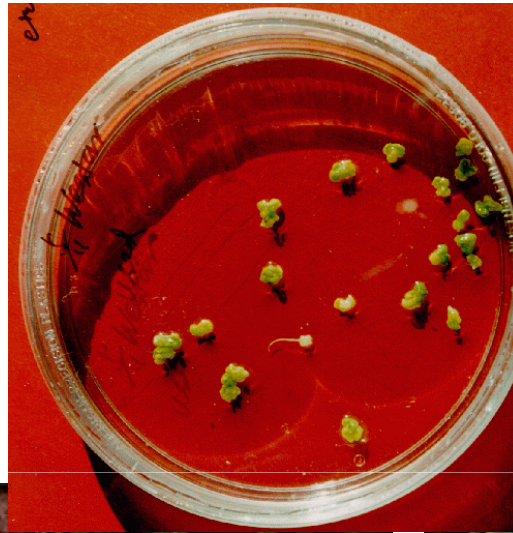
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Laboratory technique

Example:



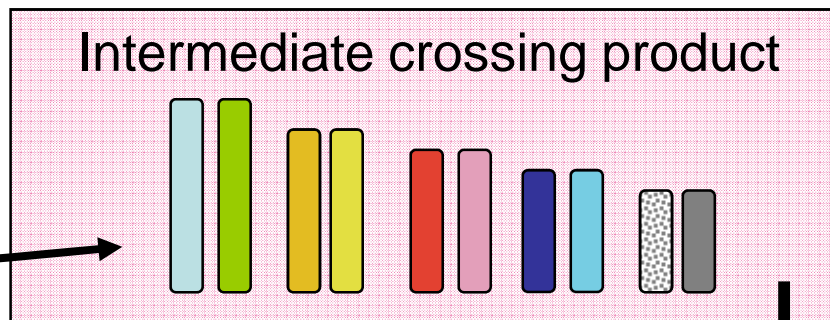
Dihaploid Cucumber

Haploid Cucumber

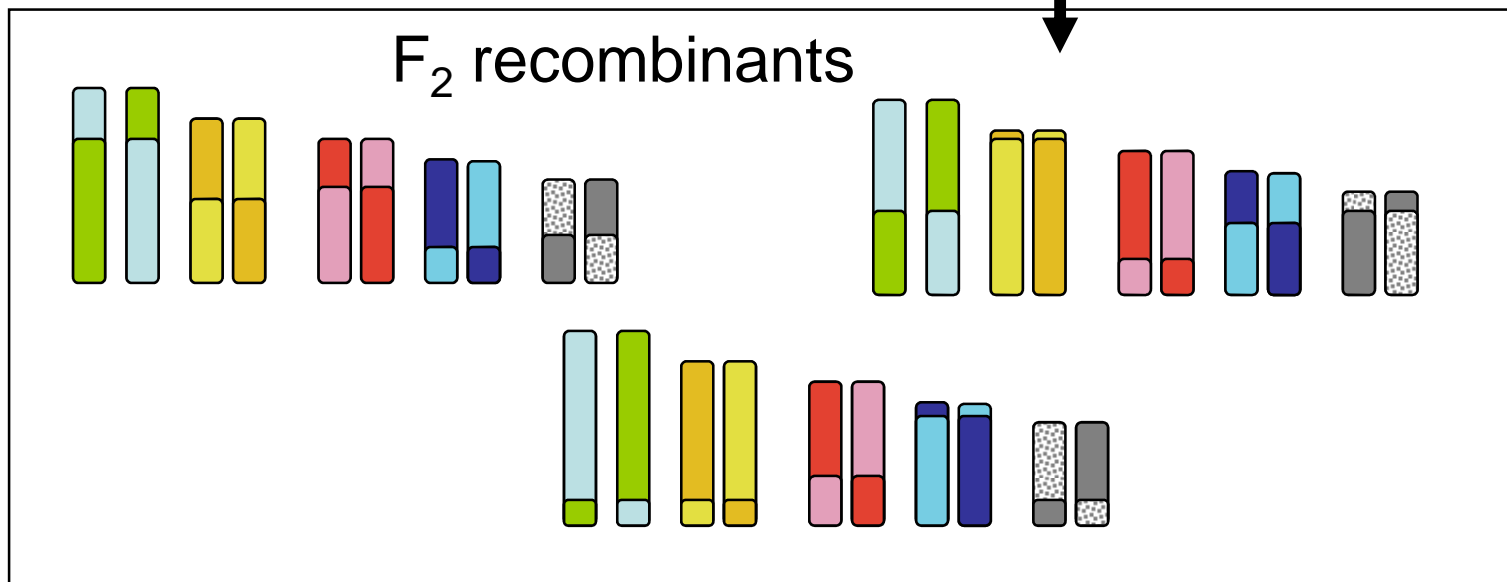
?



Interesting properties

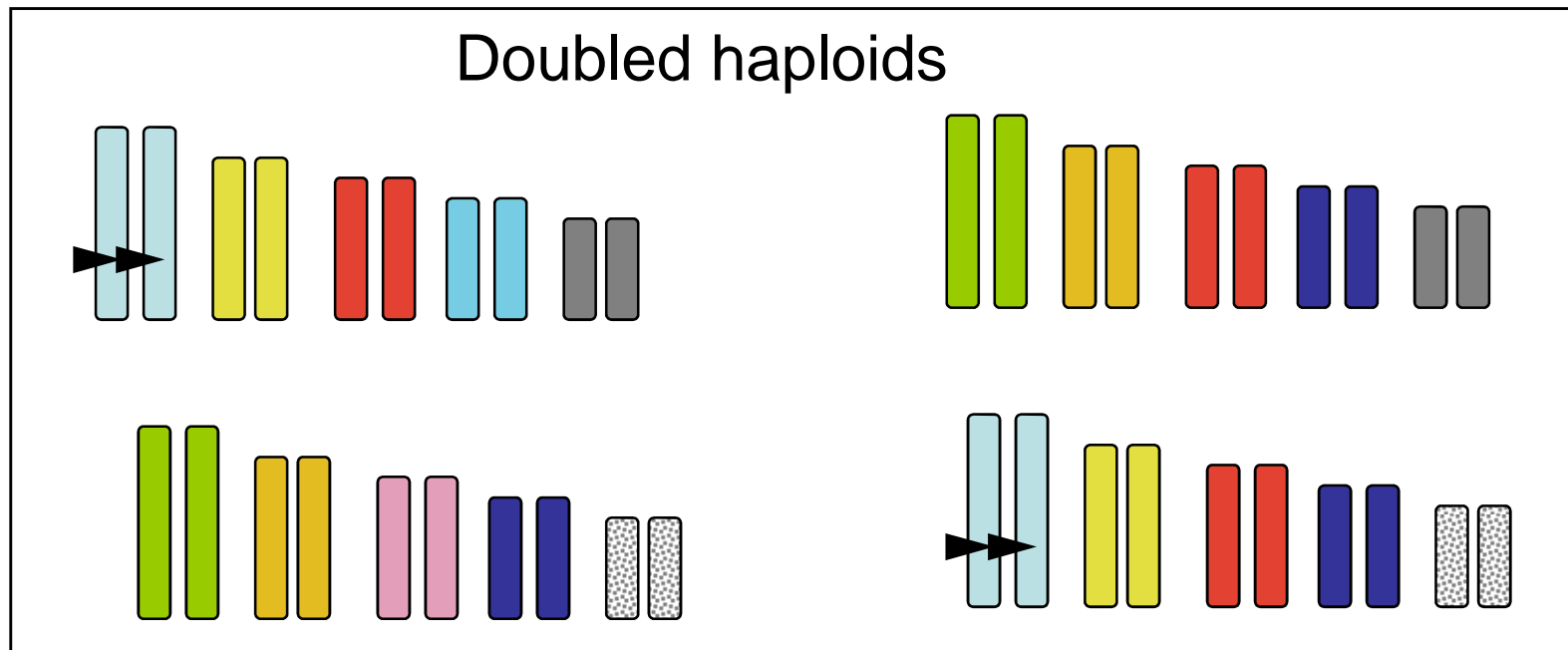
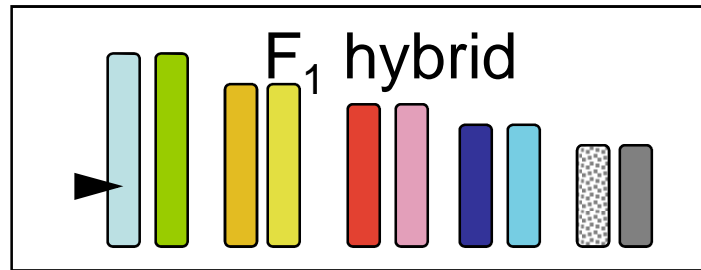


How to reconstitute parents for a desired product ?



Create transgenic line
of interesting F₁ hybrid:
Meiotic recombination is blocked

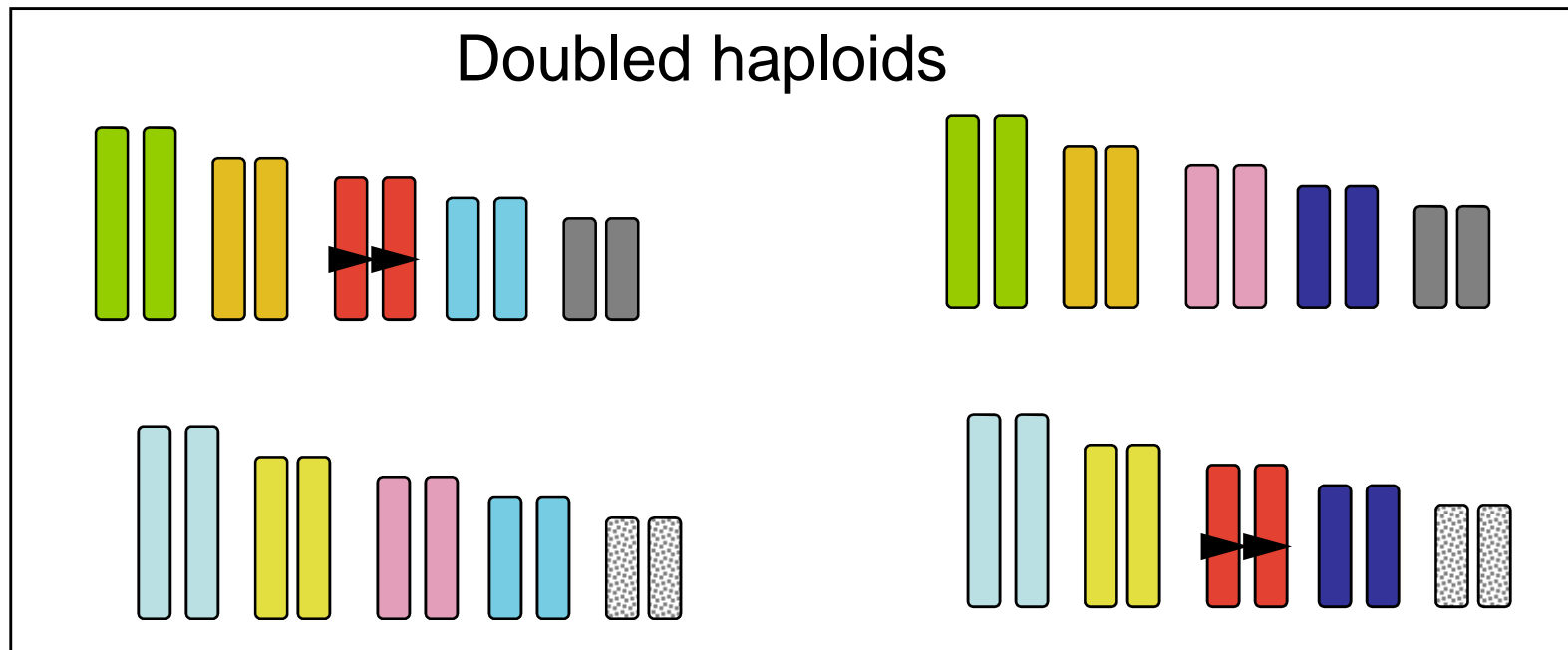
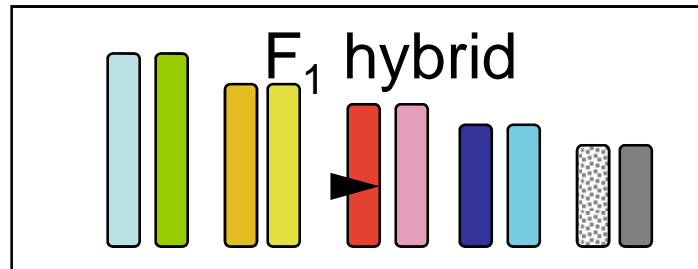
▶
transgene



DNA marker analysis

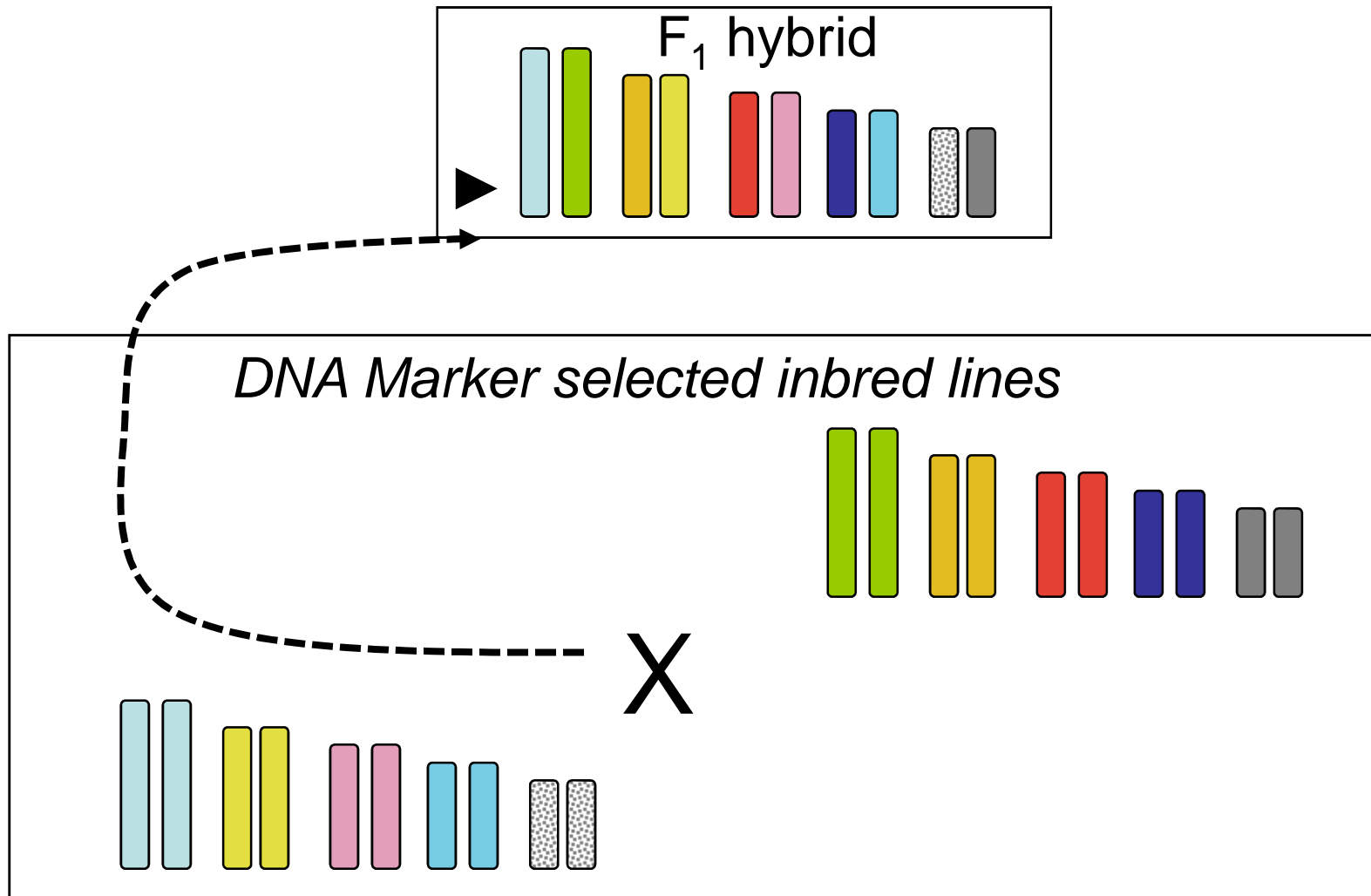
Create 2nd transgenic line
of interesting F₁ hybrid:
Meiotic recombination is blocked

▶
transgene



DNA marker analysis

Re-constituted F₁ hybrid **without transgene** “REVERSE BREEDING”

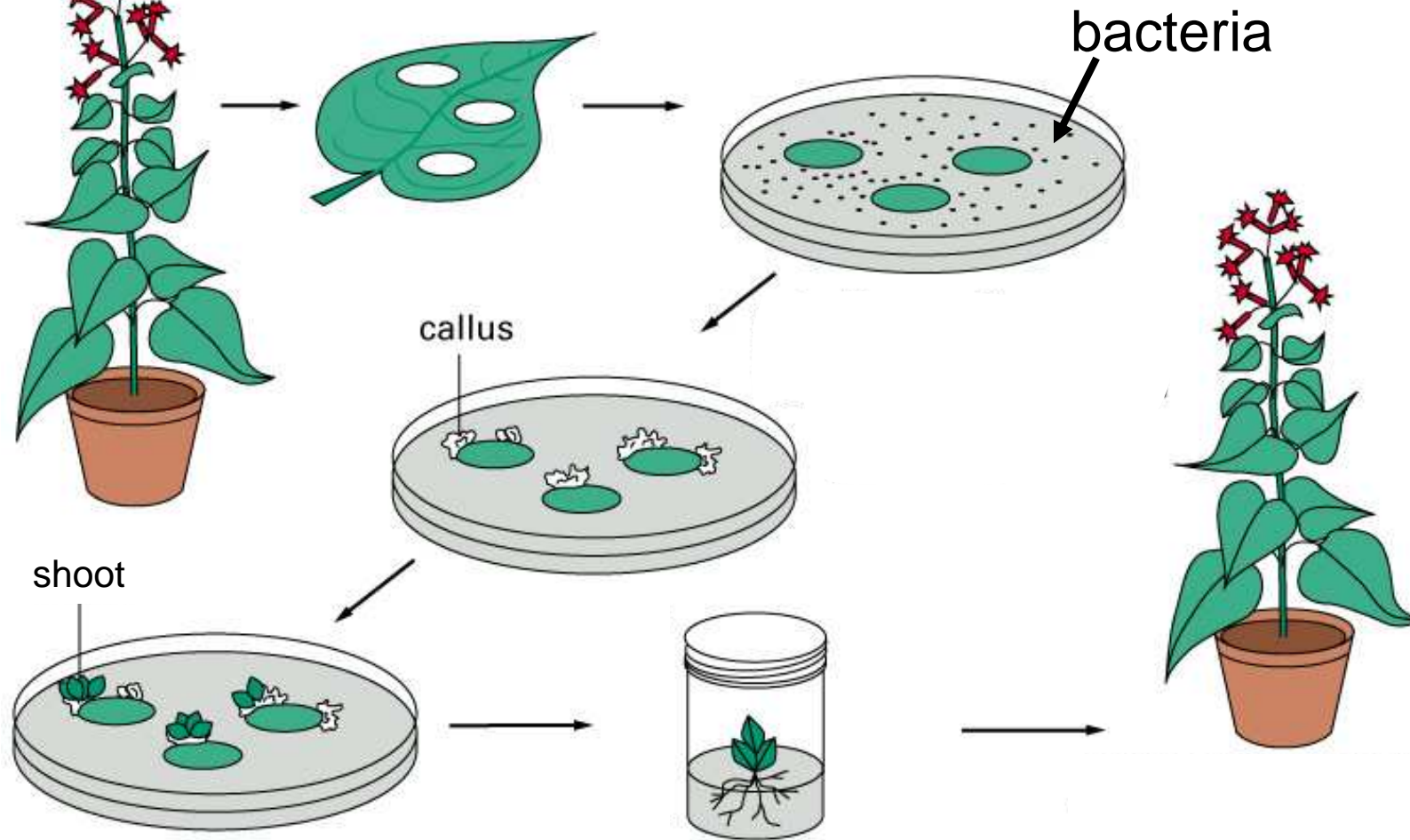


Novel breeding techniques:

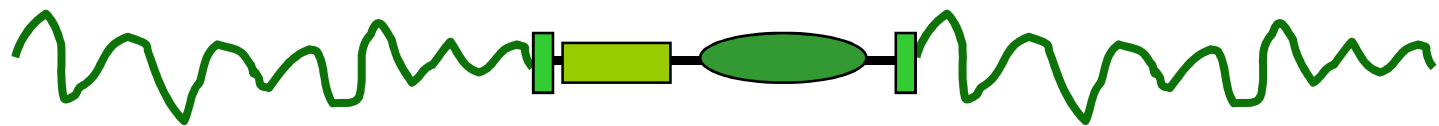
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Gene transfer: insert DNA in the plant genome



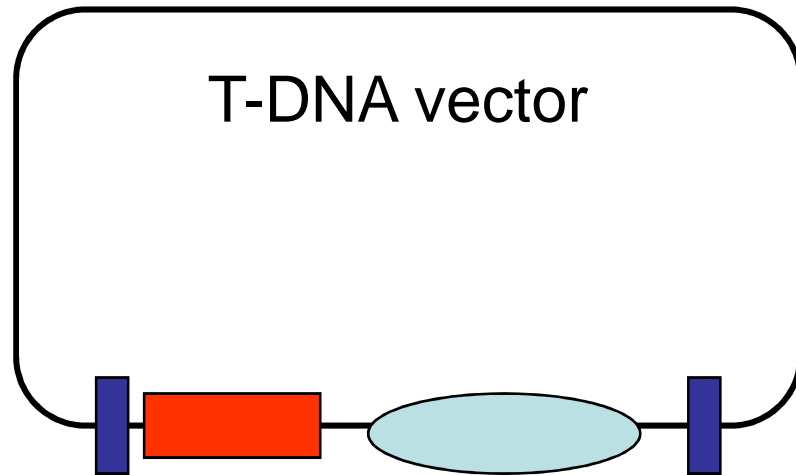
DNA
Plant



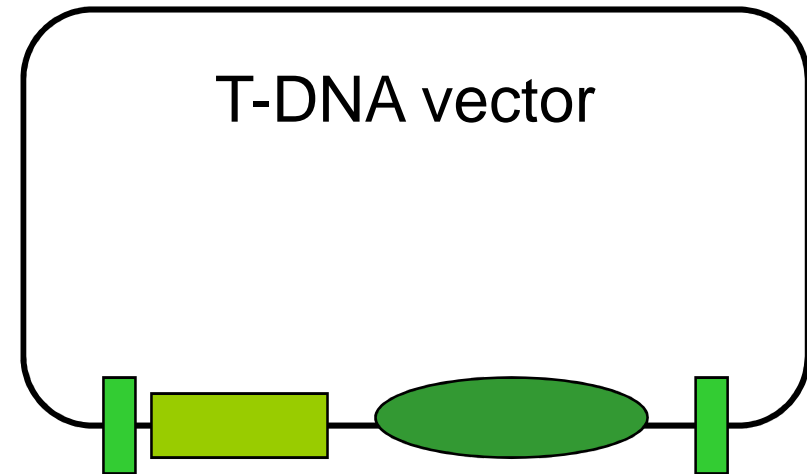
"extra gene"



Trans-gene Vector



Cis-gene Vector



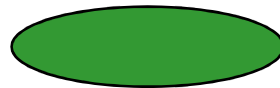
Foreign DNA elements:
Bacteria
Virus
Unrelated plant species



Plant T-DNA transfer Fragments



Plant selection marker



“Cis” plant gene

APPLICATION:

Phytophthora resistance in Potato

Scab resistance in Apple

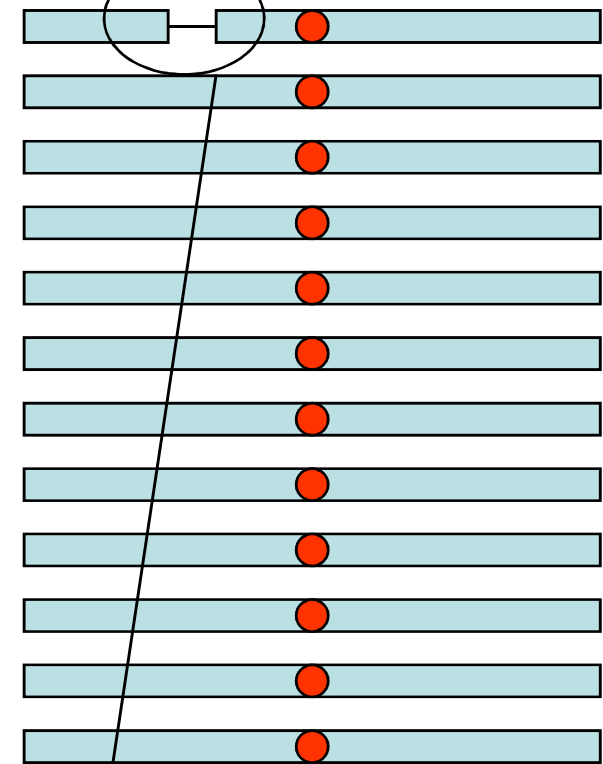
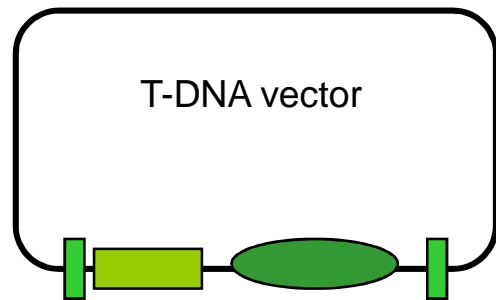
Botrytis resistance in Strawberry



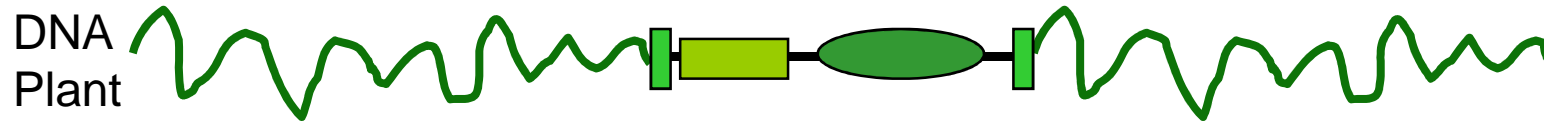
Is cisgenesis a different GM technique ??

Transformation

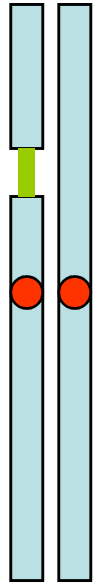
Integration



Random Integration in a Chromosome

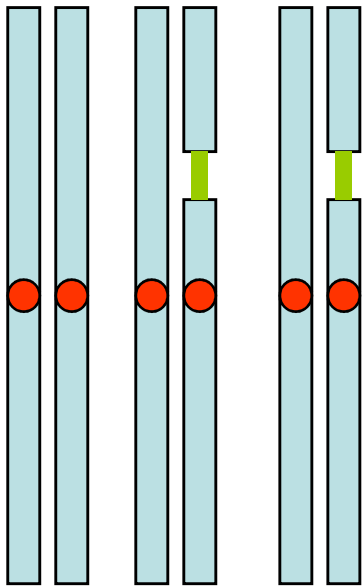


Structure of a "cis" gene in the plant genome

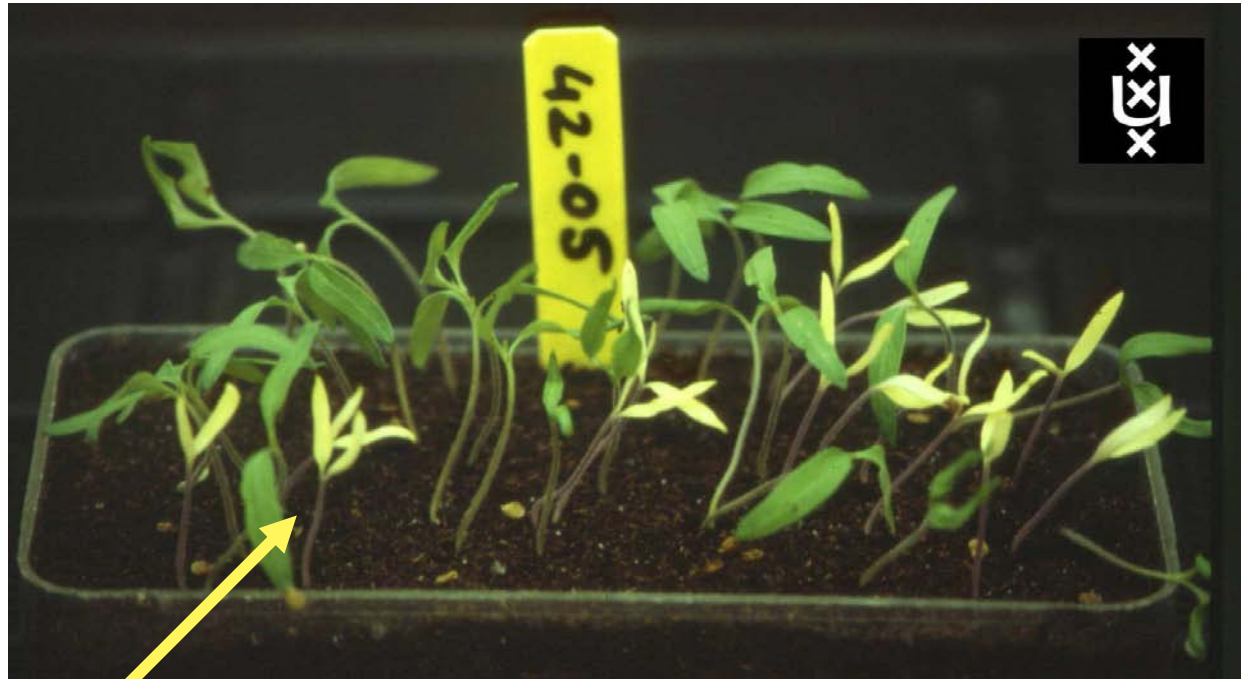
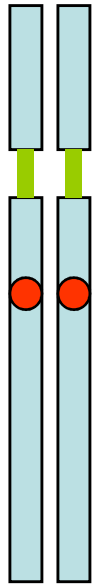


Primary Transformant: heterozygous for inserted cis-gene

selfing



progeny



Also for cisgenic plants.....

Potential effect of the insertion:

Mutation of *unknown* plant gene!



Novel breeding techniques:

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2 Directed Mutagenesis

3 Dihaploid plants from tissue culture and their application in “**reverse breeding**”

4 Gene transfer from related species: **cisgenesis**

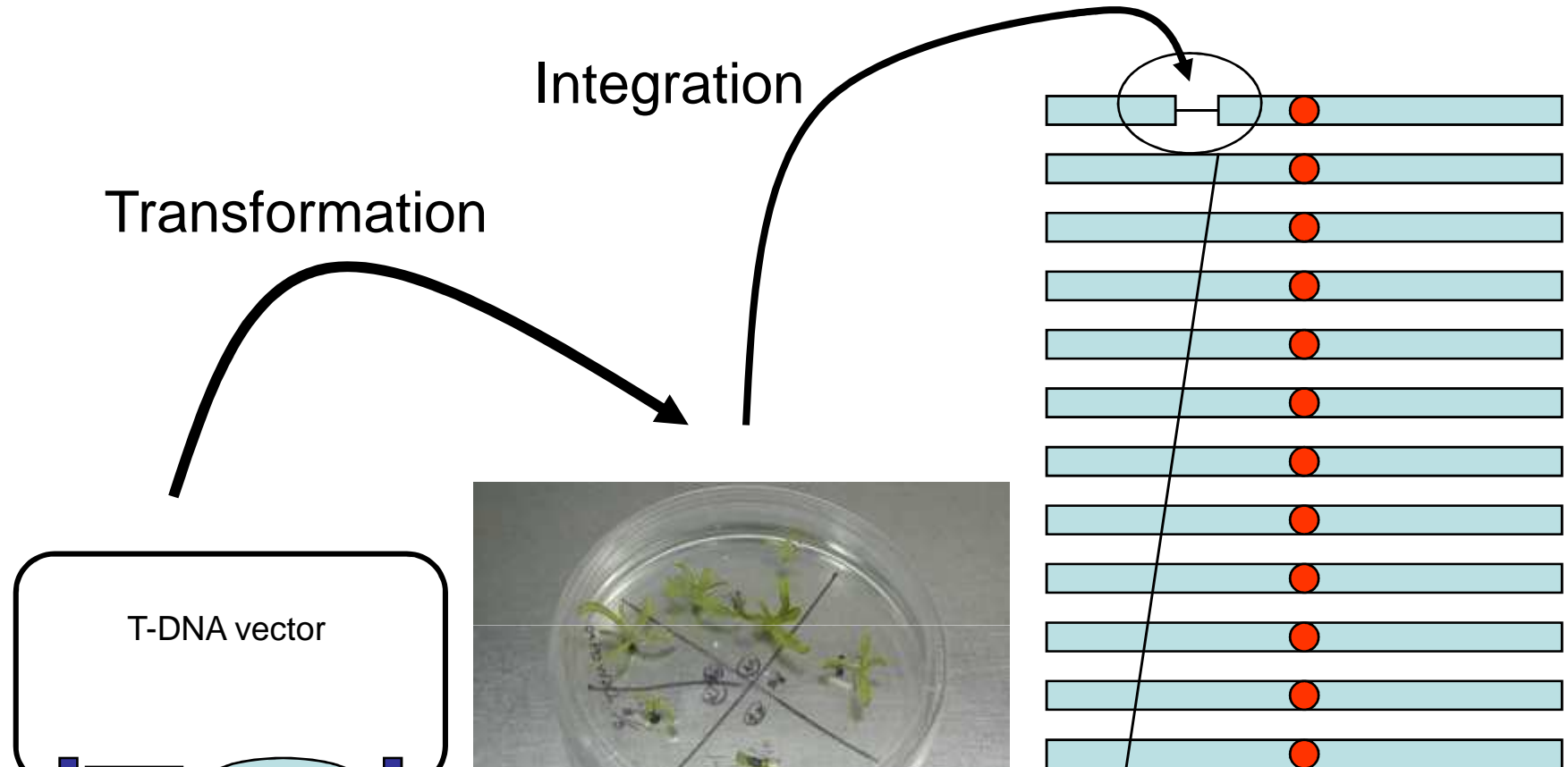
Assessment with regard to GM:

*In the **process** GM applied (1, 2, 3, 4)*

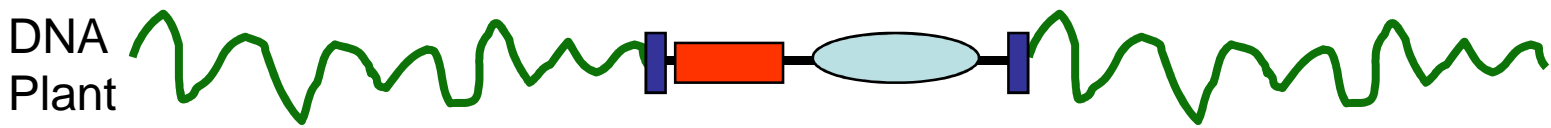
*GM cannot be recognized in the **product** (2, 3)*

*Cisgenesis **is** GM (4)*

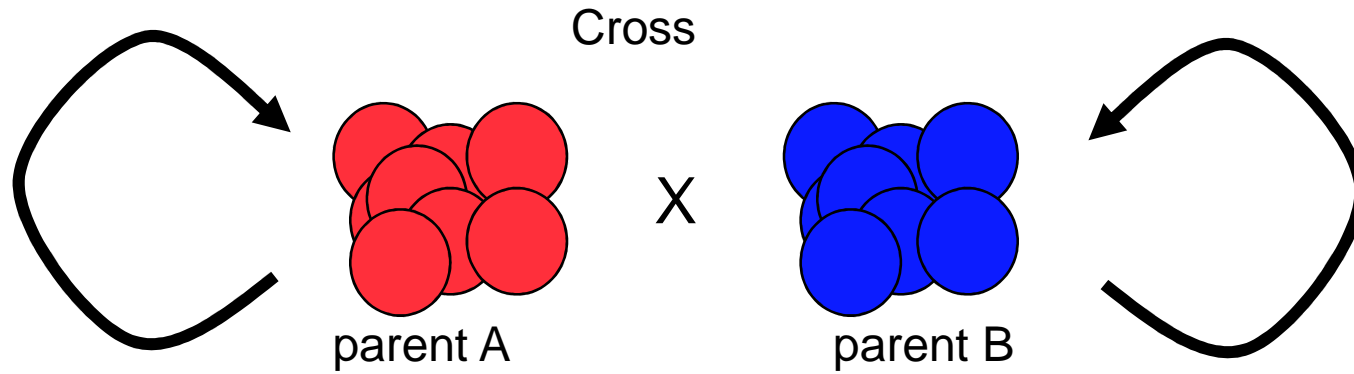
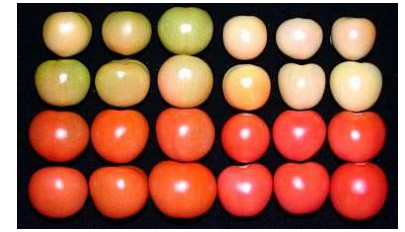




Random
Integration in a
Chromosome

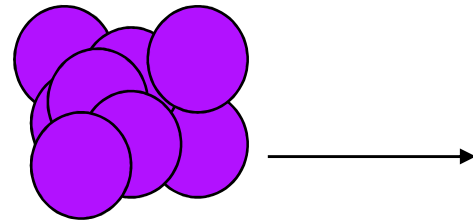


Structure of a "trans" gene in the plant genome



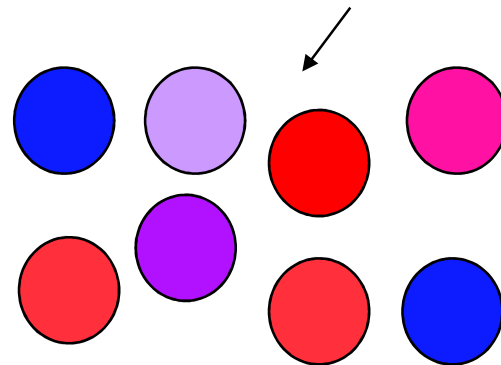
Inbred

Inbred

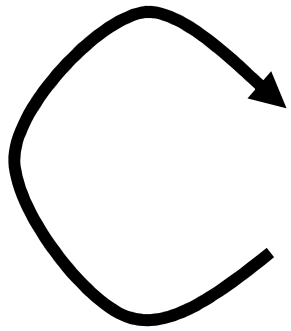


product

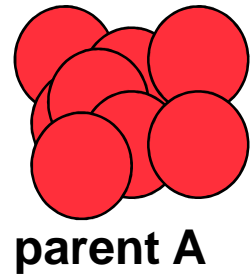
F₁ - hybrid Seeds



*Modern plant breeding:
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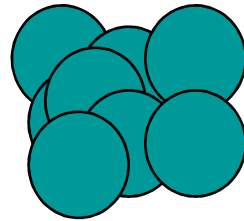


Inbred

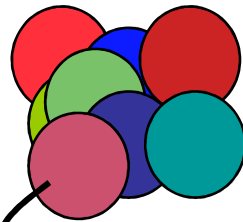


parent A

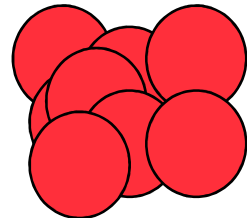
X



Wild species with
Virus Resistance

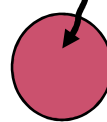


progeny

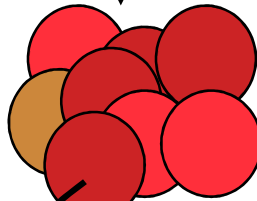


parent A

X



Hybrid with
Virus Resistance



progeny

Repeat
6-10x

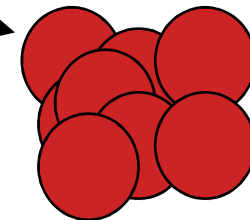


Hybrid with
Virus Resistance



Breed new variety

5-10 years



parent C with
Virus Resistance



Targeted breeding

Parents

AA bb x aaBB



Progeny (F₁)

AaBb x AaBb



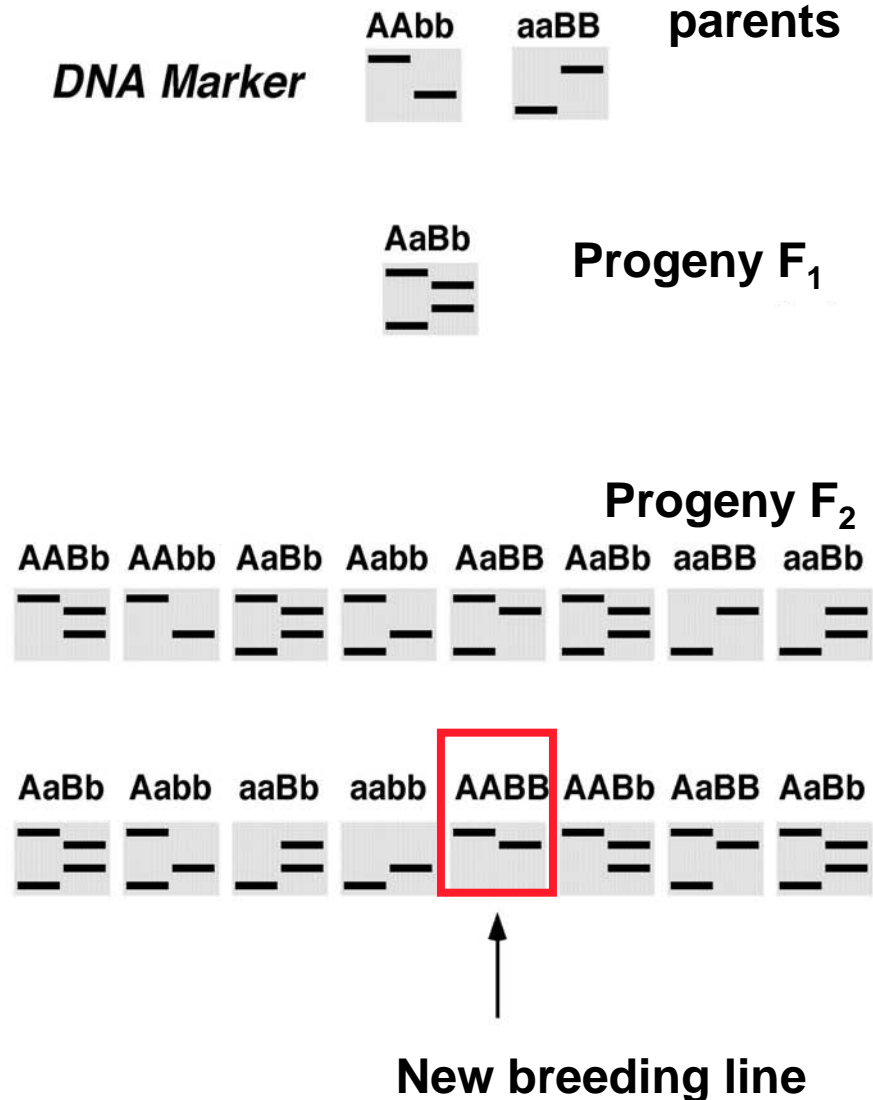
Progeny (F₂)



	AB	Ab	aB	ab	
1 : 16	AB	AABB	AABb	AaBB	AaBb
	Ab	AABb	AAbb	AaBb	Aabb
	aB	AaBB	AaBb	aaBB	aaBb
	ab	AaBb	Aabb	aaBb	aabb



Marker assisted selection



DNA diagnostics

Extract DNA from parents and progeny:

Desired combination of DNA markers determines which plant lines are used in breeding program

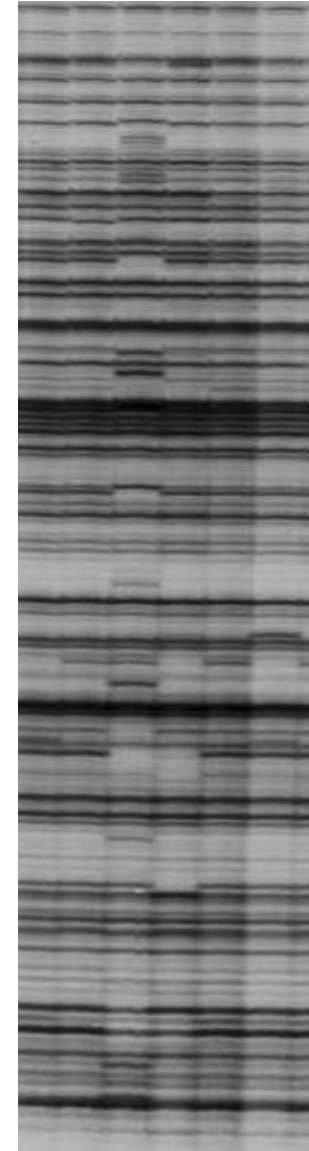
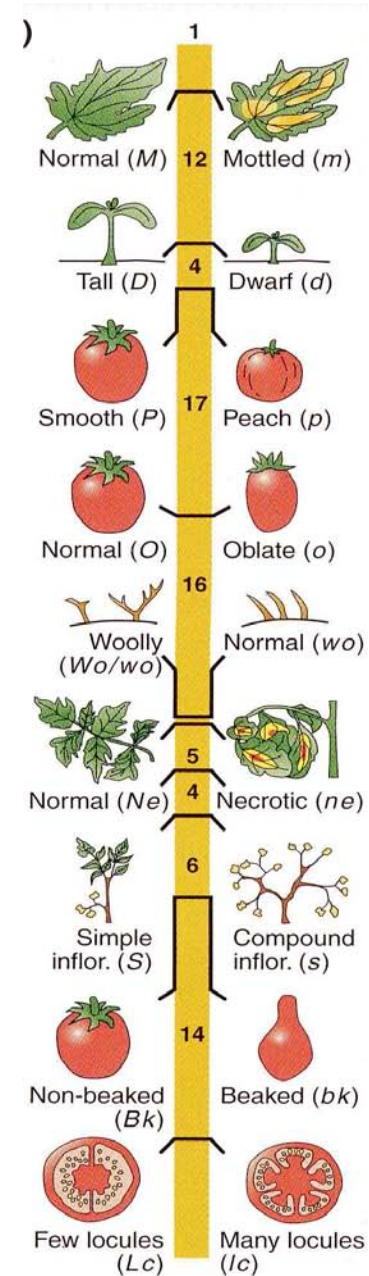


Mapping traits to the genome:
chromosome maps

Translate traits to **DNA fragments**

Select seedlings based on DNA pattern

**Application ONLY to known traits:
no novel traits can be identified**



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