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Innovative potato breeding to reduce the dependency on plant protection products

16 June 2017

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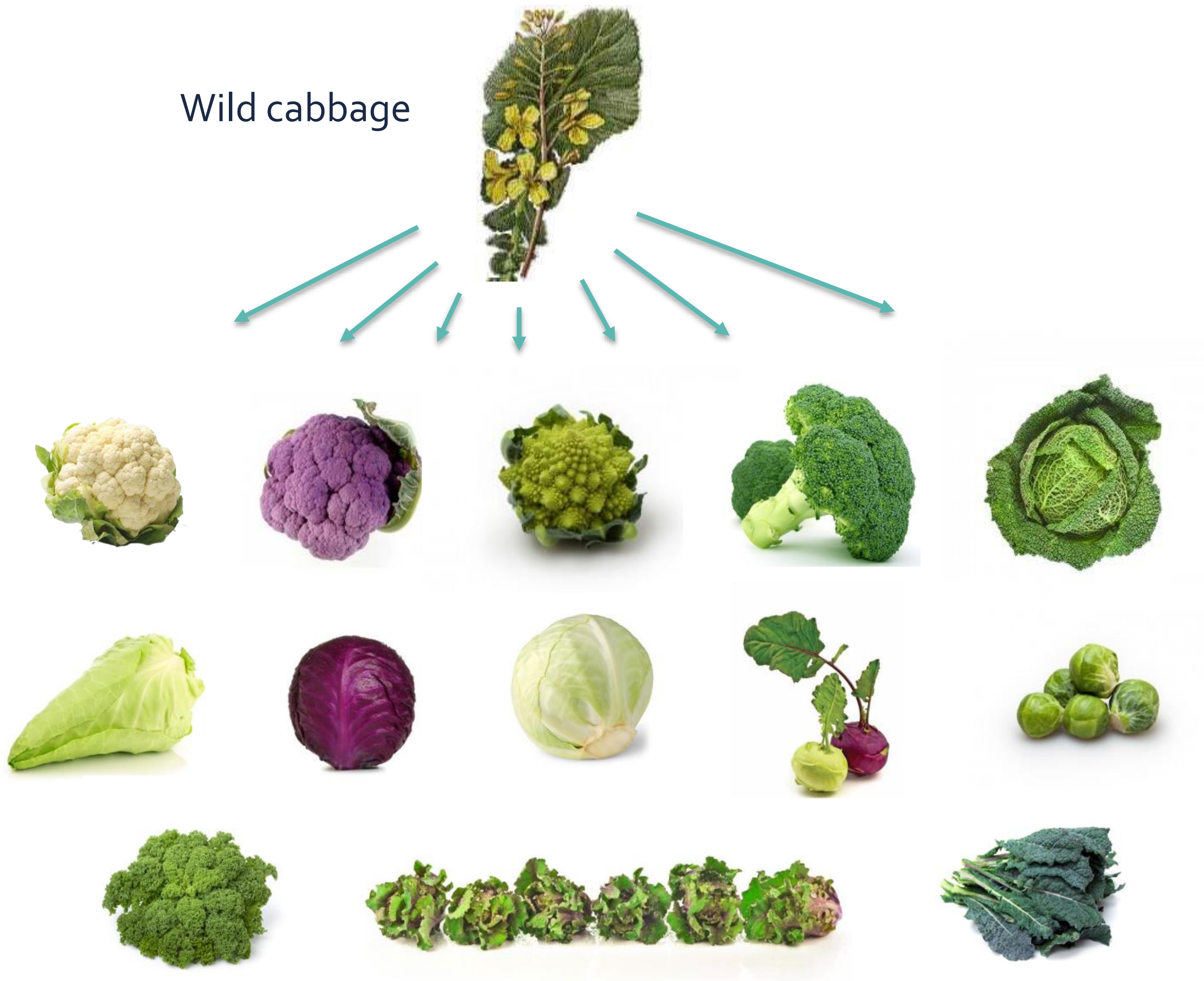
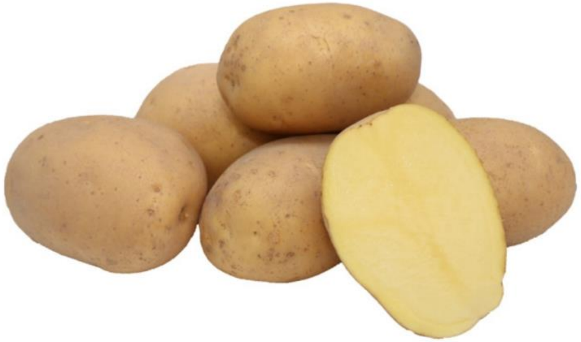
Plant Breeding

= Exploitation of genetic variation

- Additional crop variation
- Introduce beneficial properties for the farmer, processor, consumer
- Combat major challenges
  - Stress caused by disease, climate, marginal soils
  - Environmental pollution
  - Growing world population
  - Health


Wild cabbage



Disease in potato

1. Late blight (*Phytophthora infestans*)
2. Early blight (*Alternaria* ssp)
3. Potato cyst nematode
4. Insects (potato beetle, aphids)
5. Stem canker (*Rhizoctonia solani*)
6. Dry rot (*Fusarium* spp.)
7. Potato virus X, potato virus Y
8. Potato leaf roll virus
9. …

Fighting disease in potato using plant protection products (PPP)

1. Fungicides (*Phytophthora, Alternaria*)
2. Insecticides (potato beetle, aphids, potato leaf roll virus)

Additional use of PPP:

3. [[Herbicides (full crop or between row; before crop closes)]]

Use of PPP in potato is high

Crop	Region	Chemical load per hectare of crop grown per farm kg ai/ha		
		Average	Range	
Wheat	Hannover (D)	4.5	0.08	8.5
	E Anglia (UK)	4.6	0	10.1
	N Central France	3.8	0.7	13.7
	Piemonte (I)	2.1	0.02	7.3
Potatoes	Lüneburg (D)	9.8	2.7	22.3
	Flevoland (NL)	12.6	1.6	34.6
	E Anglia (UK)	13.1*	2.0	26.7
	N E France	32.0	9.0	73.7
Apples	S E France	41.4	1.7	146.7
	Trentino (1)	33.7	0.6	83.4
	Lerida (E)	27.4	1.4	109.6
Vines	Bordeaux (F)	46.0	7.9	87.3
	Rioja (E)	16.8 (42) **	2.9	146.9
	Verona (I)	33.6 (43) **	0.8	142.4

Source: LEI

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Breeding to increase disease resistance and lower PPP use

1. Increase general resilience
2. Introduce specific disease resistances

Sources:

1. Potato gene pool
2. Solanum gene pool
3. Non-related sources

Continuous innovation in plant breeding

Cross-breeding and selection

F1 Hybrids (1+1 = 3)

Embryo rescue (crossing the not-crossable)

Induced mutagenesis

recDNA technology (foreign genetic material)

Marker assisted breeding

Cisgenesis

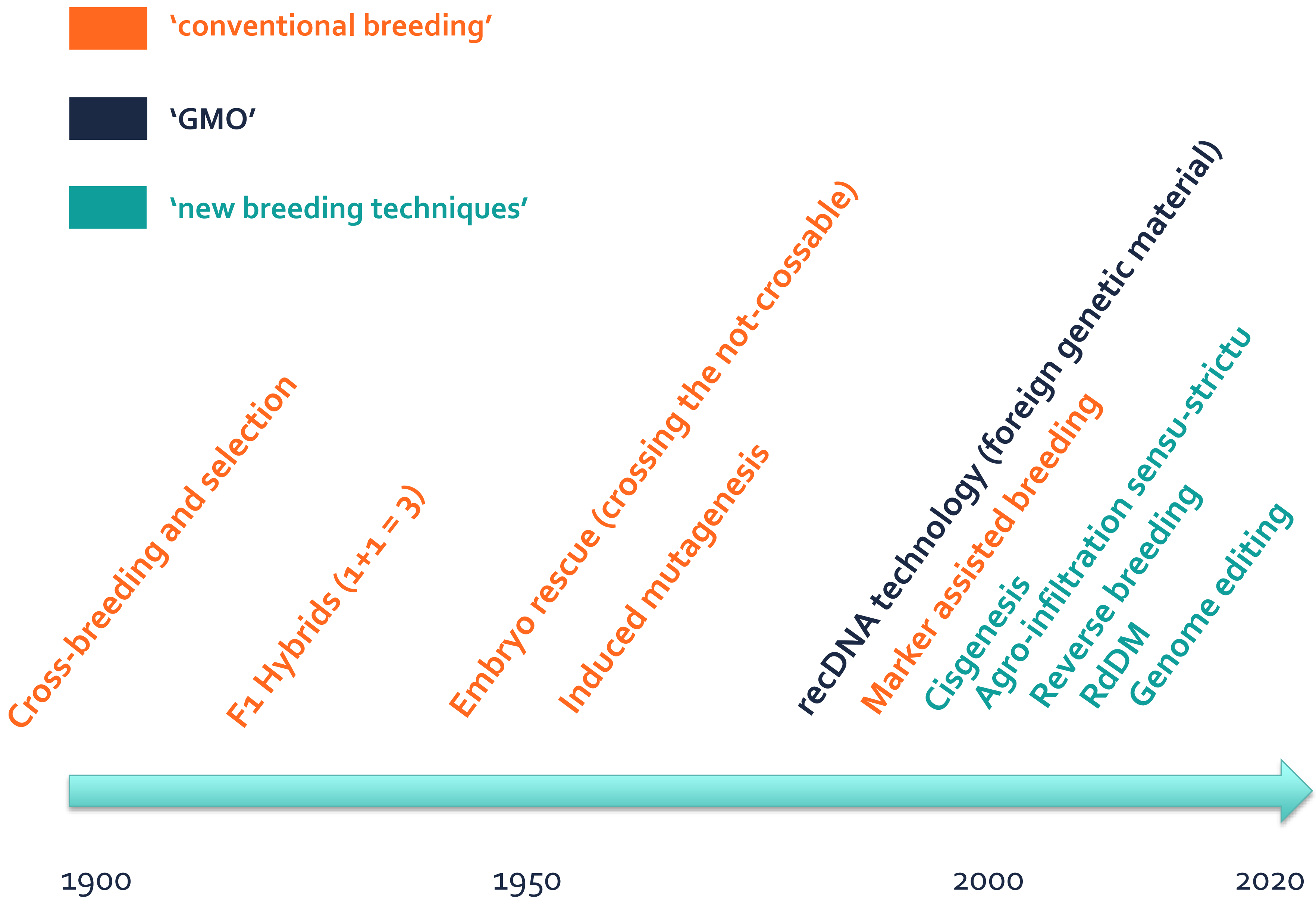
Agro-infiltration sensu-strictu

Reverse breeding

RdDM

Genome editing

1900 1950 2000 2020



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Genetic variation that can and cannot occur in nature

Organisms with genetic variation within natural boundaries

Organisms with genetic variation beyond what is possible through mating and natural recombination

Likely or possible to occur in nature

Cannot occur in nature

Known natural phenomena that contribute to genetic variation:

- Mutations (point mutations, INDELS, frameshift mutations)
- Recombination
- Duplications, inversions
- Insertial mutagenesis (transposons, ...)

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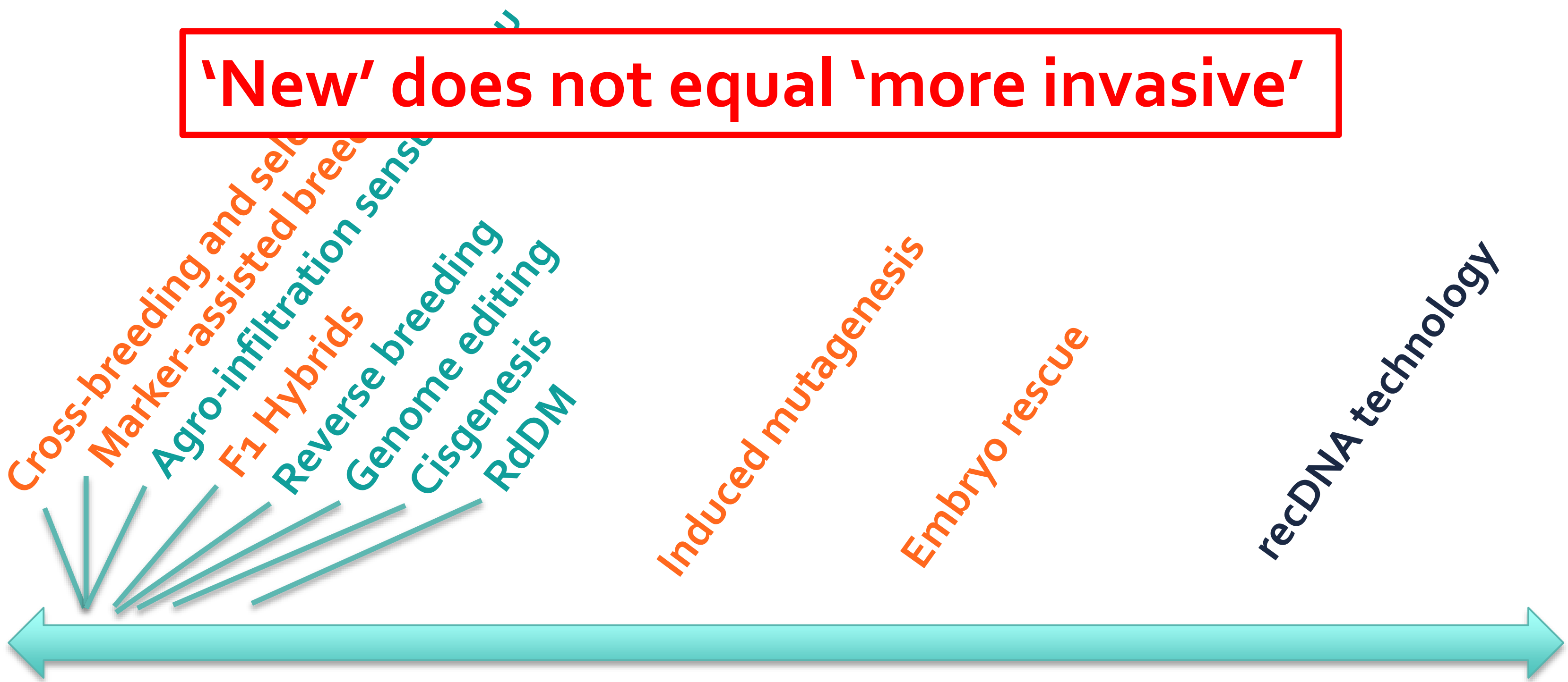
'conventional breeding' 'GMO' 'new breeding techniques'

'New' does not equal 'more invasive'



Genetic variation within natural boundaries

Ability to create novel genotypes beyond what is possible through mating and natural recombination

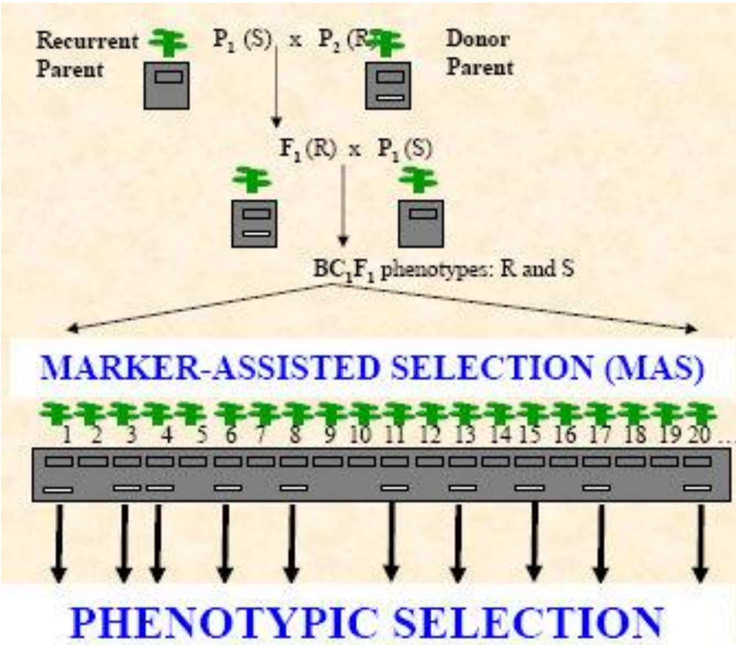
Innovative breeding in potato

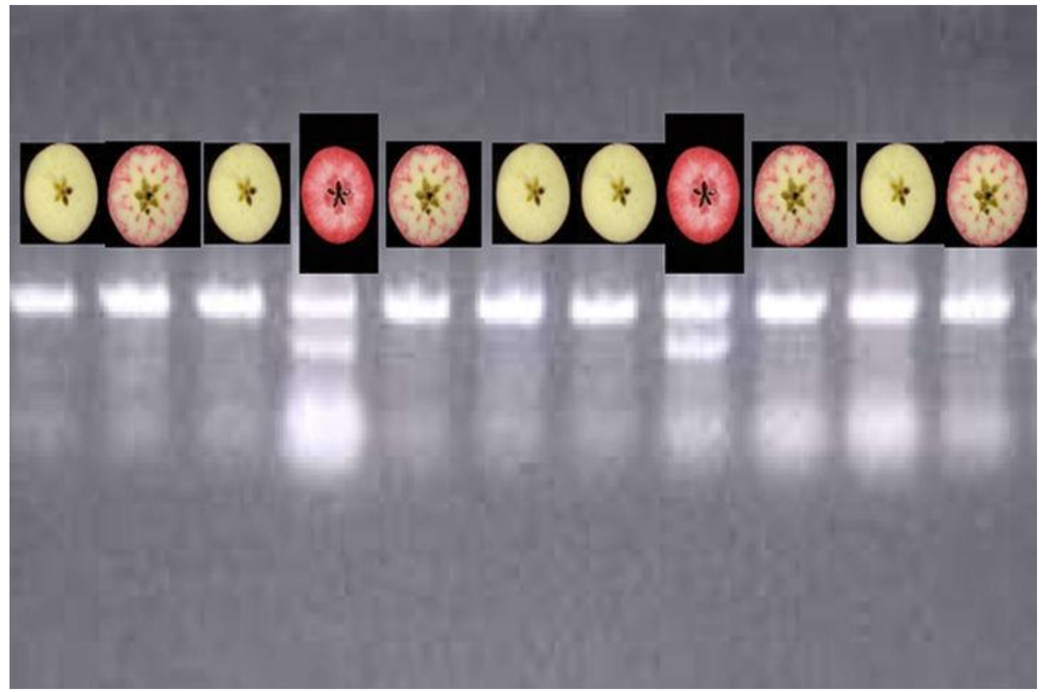
1. Marker-assisted selection
2. Transgenesis
3. Cisgenesis
4. F1 hybrids
5. Gene/genome editing

Marker-assisted selection

- Potato genome knowledge
- Used for selecting dominant traits that are available in the potato gene pool
    - Late blight resistance

→ Speeds up the conventional breeding process, but is still slow

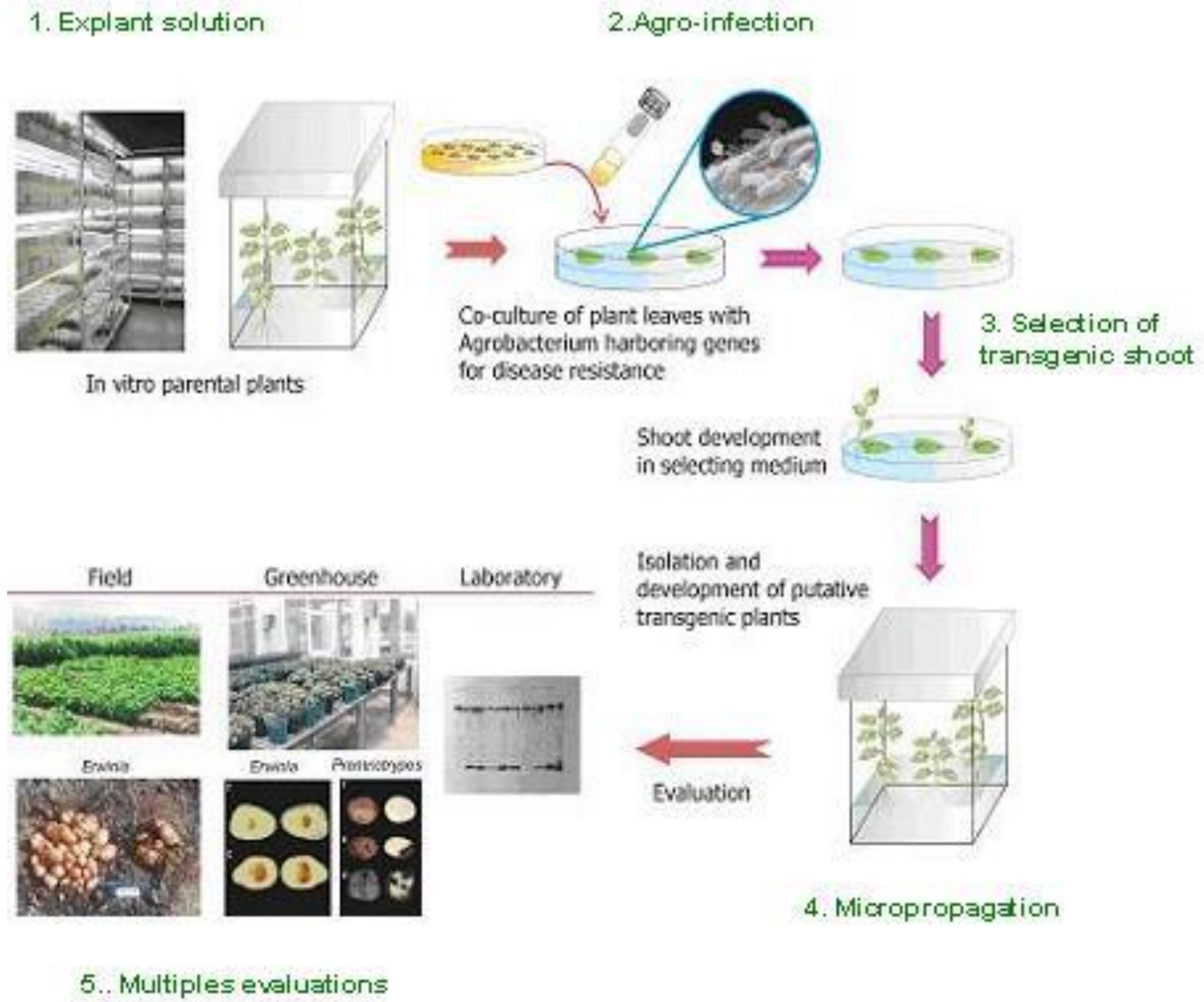

Recurrent Parent
P1 (S) x P2 (R)
Donor Parent
F1 (R) x P1 (S)
BC1F1 phenotypes: R and S
MARKER-ASSISTED SELECTION (MAS)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 …
PHENOTYPIC SELECTION



Transgenesis (the introduction of 'foreign' genes)

- *Agrobacterium tumefaciens* mediated transformation
- Used for traits/genes that are not available in the potato gene pool
  - Cyst nematode resistance
  - Resistance against virus
  - Resistance against insects (potato beetle)
  - Bacterial diseases

→ Ideal for rapid stacking of traits
→ Costly, heavily regulated, debated and politicized

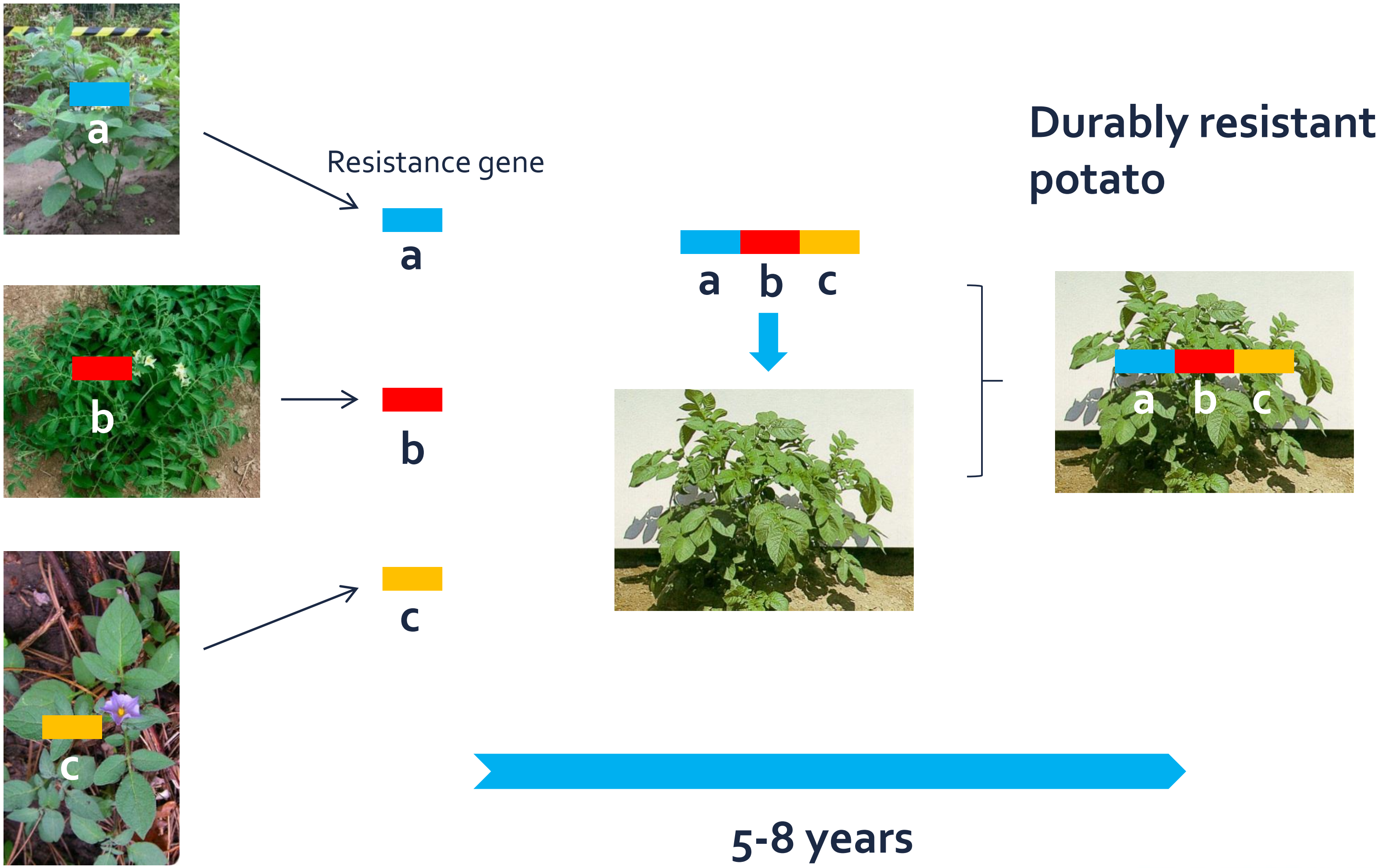

1. Explant solution
2.Agro-infection
In vitro parental plants
Co-culture of plant leaves with Agrobacterium harboring genes for disease resistance
3. Selection of transgenic shoot
Shoot development in selecting medium
Isolation and development of putative transgenic plants
Field
Greenhouse
Laboratory
Evaluation
4. Micropropagation
5.. Multiples evaluations

Cisgenesis (the introduction of naturally occurring potato genes)

- *Agrobacterium tumefaciens* mediated transformation
- Used for traits/genes that are naturally occurring in the potato gene pool
  - Late blight resistance

→ Ideal for rapid stacking of naturally occurring traits
→ Regulated as GMOs?

Cisgenesis



a
b
c
Resistance gene
a
b
c
a b c
Durably resistant potato
a b c
5-8 years

F1 hybrids (old technology!, but new in potato)

- Diploid potato (instead of tetraploid)
- Multiplication via True Potato Seeds (and not tubers)
- Previously deemed impossible due to the severe inbreeding depression and self-incompatibility
- Used for selecting both dominant and recessive traits, and making homozygous F0 parents
    - Late blight resistance
    - …

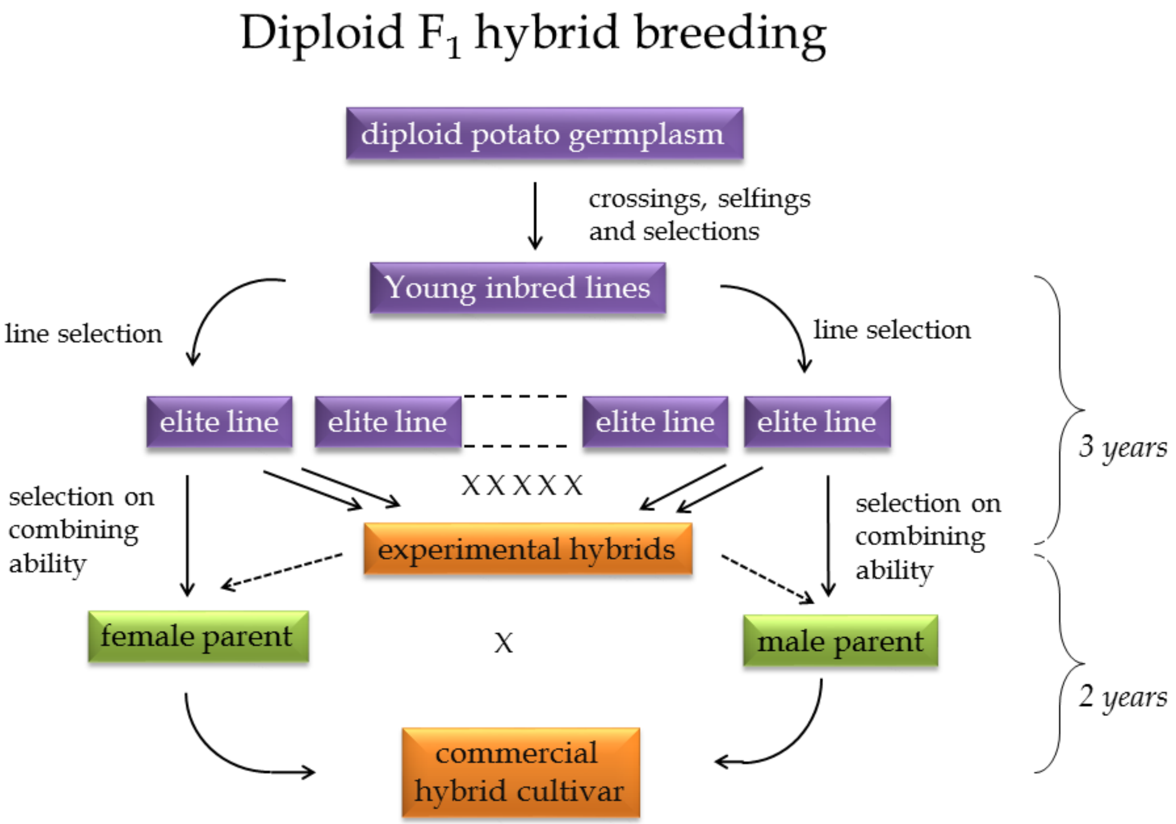
→ **High acceleration of the conventional breeding process**

→ **Overcomes the problems with high heterogeneity of tetraploid potato**

→ **Very fast multiplication via true seeds**

→ **Limited to traits/genes available in the potato gene pool**

True Potato Seed



Source: www.solynta.com

Gene/genome editing

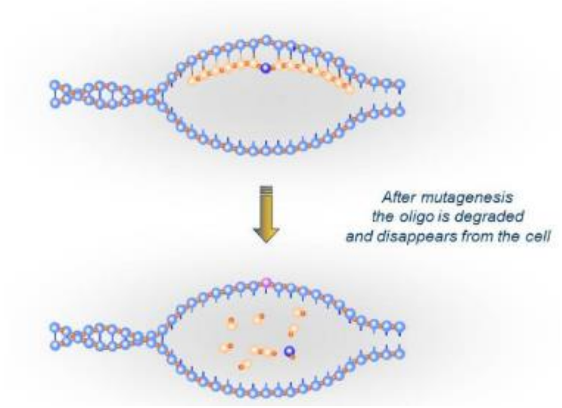
- Revolutionary CRISPR/Cas9 technology!!
- Precise, targeted, knowledge-based introduction of small alterations to the genome
- Used for altering disease sensitivity genes (S-genes)
  - Late blight resistance
  - …

→ **High acceleration of the conventional breeding process**

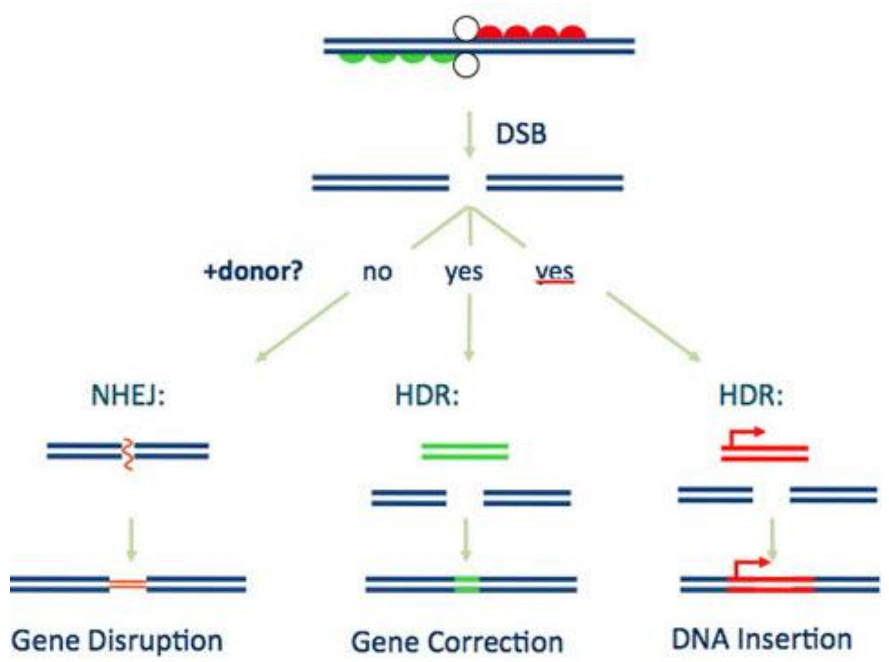
→ **Low cost, easy to apply**

→ ***Regulatory uncertainty!***

Gene editing technology



ODM



SDN-1 SDN-2 SDN-3

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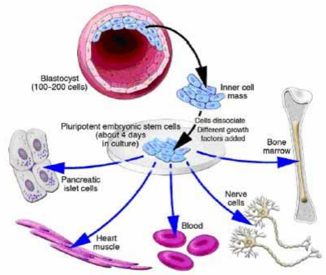
Gene editing examples

Type of edit	Example	Edit technology	Mechanism
Single nucleotide alteration	…ATA… → …ACA…	Oligo-Directed Mutagenesis	DNA mismatch repair
Single nucleotide alteration	…ATA… → …ACA…	Type 2 site-directed nuclease technology (SDN-2)	Double strand break repair via homology directed repair (HDR)
Single nucleotide deletion	…AATAGC… → …AAAGC…	Type 1 site-directed nuclease technology (SDN-1)	Double strand break repair via non-homologous end-joining (NHEJ)
Multiple nucleotide deletion	…AATAGC… → …AC…	SDN-1	NHEJ
Complete gene deletion	….Gene-X…. → ……..	SDN-1 using a double cut	NHEJ
Single nucleotide addition (frameshift)	…AATAGC… → …AATTAGC…	SDN-1	NHEJ
Multiple nucleotide addition	…AATAGC… → …AATTGTAGC…	SDN-1	NHEJ
Complete allele replacement	…Allele-1… → …Allele-2…	SDN-2 using a double cut	HDR

Genome editing is everywhere

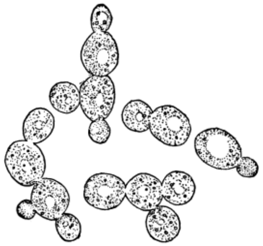
Cell culture



Human

Insects

Plants



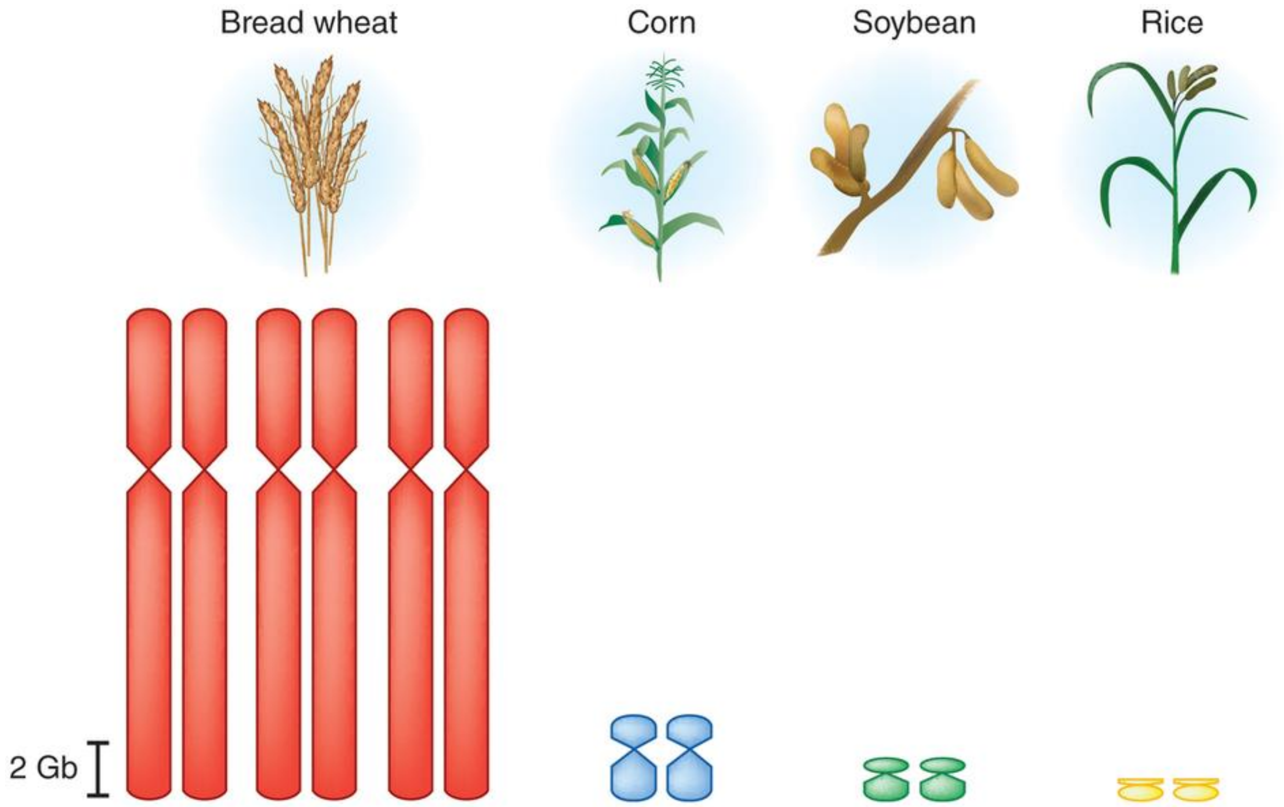
Micro-organisms

Animals

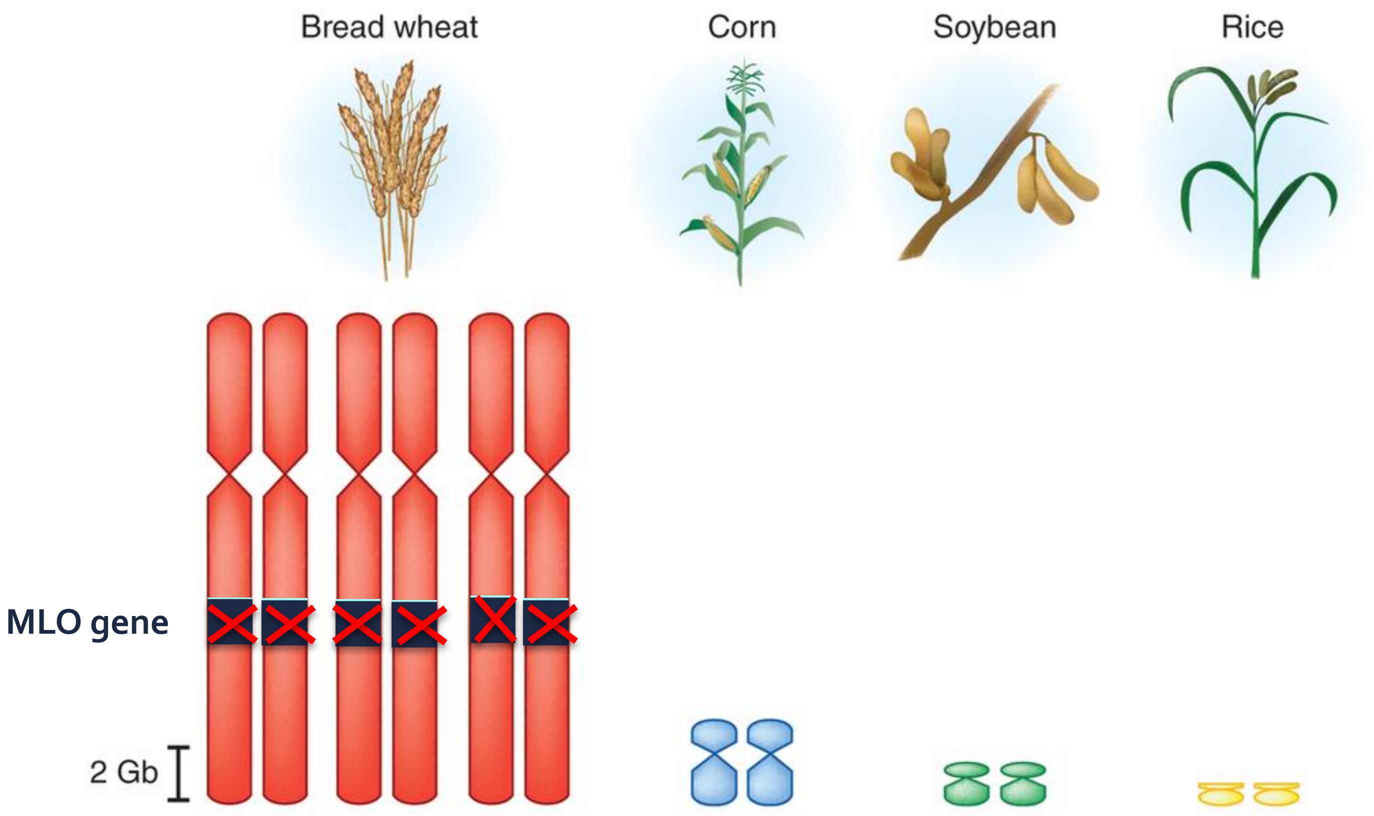
Fish

amphibians

Mildew resistant wheat through editing



Mildew resistant wheat through editing



Innovative breeding for potato disease resistances

Late blight; *Phytophthora infestans*

Rpi-genes, S-genes, other resistance

Durable resistance against late blight can reduce fungicide use in potato with 80%

1. Conventional (ma… …g

   New var… …onica, Carolus, Avito

2. Cisgenesis

   Wageninge… UR (NL), Bintje[PLUS] consortium (BE), Simplot (US), Sainsbury lab (UK), CIP (Peru)

3. Genome editing

   Calyxt (US), …

Deployment of transgenesis for disease resistance in potato

1. Potato cyst nematode

cystatin genes from rice and maize

Sainsbury lab (UK), University of Leeds (UK)

2. Resistance against potato beetle

Cry genes from Bacillus thuringiensis

Different labs in the world.

2. Resistance against potato viruses

RNAi approaches

Different labs in the world.

Bintje^{PLUS} project

Project consortium: **UGent, ILVO, VIB**

UNIVERSITEIT GENT

INSTITUUT VOOR LANDBOUW-, VISSERIJ- EN VOEDINGSONDERZOEK

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Contribute to sustainability in potato cultivation by developing cisgenic, durably resistant Bintje potatoes

- Stacking of four Rpi-genes

TSL Potato Partnering Project

Project partners

TheSainsburyLaboratory TSL

UNIVERSITY OF LEEDS

Simplot

BioP

Project funders

BBSRC bioscience for the future

NERC SCIENCE OF THE ENVIRONMENT

Maris Piper potato with:
(1) Late blight resistance, (2) potato cyst nematode resistance, (3) lower levels of reducing sugars and asparagine, (4) less prone to bruise damage.

Simplot Innate® potatoes

Innate® Generation 2

- Contains 1 Rpi-gene (Rpi-vnt1)
- Reduces fungicide application with ≈ 50%
- Closed, contact based system

Innate® Generation 3

- In development
- Will contain improved late blight resistance

Innovative potato breeding and effects on PPP use

In the next 5 to 15 years innovative potato breeding is expected to reduce especially fungicide use by at least one third.

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Are the products of innovative potato breeding regulated *(as GMOs)* ?

Products from:	Under GMO safely legislation	No safety legislation
Marker assisted selection		X
Transgenesis	X	
Cisgenesis	← ?	→
F1 Hybrid technology		X
Gene/genome editing	← ?	→

a GMO is …

Does this refer to the technique used, the end product, or both?

'… an organism, with the exception of human beings, in which the genetic material ***has been altered in a way that does not occur naturally*** by mating and/or natural recombination;'

Article 2(2) of EU Directive 2001/18/EC

Other clues

the technique

the end product

Techniques of genetic modification listed in annex IA part 1:

(1) recombinant nucleic acid techniques involving the formation of ***new combinations of genetic material*** by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation ***into a host organism in which they do not naturally occur*** but in which they are capable of continued propagation;

the technique

the end product

(3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with ***new combinations of heritable genetic material*** are formed through the fusion of two or more cells by means of methods that do not occur naturally

As a consequence

1. Recombinant nucleic acid technology and cell fusion ***only*** result in genetic modification when a ***'new combination of heritable genetic material'*** is formed

2. To be internally consistent, the phrase ***'has been altered in a way that does not occur naturally'*** has to ***refer to both the method of alteration AND the end product***

Cartagena Protocol on Biosafety

the end product

LMO:

Any living organism that possesses a ***novel combination of genetic material*** developed through ***modern biotechnology***

the technique

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The EU GMO legislation is *NOT purely process based*

- The use of modern techniques is a first regulatory trigger
- The resulting organism has to fulfill certain criteria

Are genome edited potatoes subject to the EU GMO legislation?

NO, when the genome editing has led to a combination of genetic material that can occur naturally through mating and/or natural recombination

→ It's not a GMO in the first place

YES, when the genome editing has led to a combination of genetic material beyond what does occur naturally through mating and/or natural recombination

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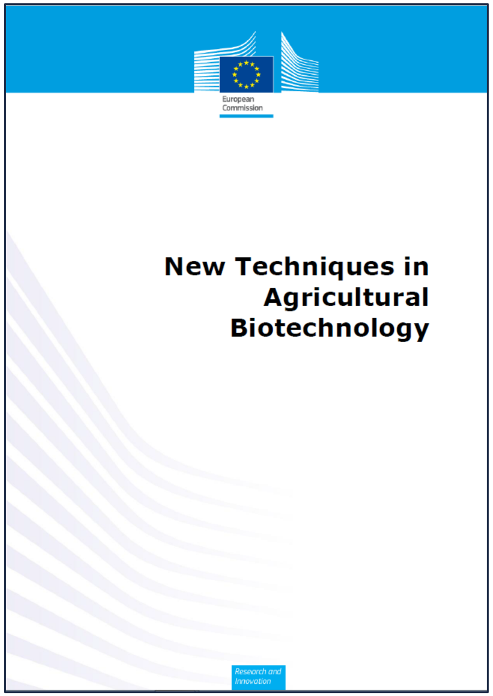
Are cisgenic potatoes subject to the EU GMO legislation?

NO, when you consider the location of the cisgene to be irrelevant

SAM Explanatory Note


New Techniques in Agricultural Biotechnology

- Key characteristics of the NBTs
- Comparison with conventional breeding and established GM techniques
  - Differences in safety
  - Possibilities for detection
  - Speed and cost to achieve expected results

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European Commission

SAVE THE DATE

EUROPEAN COMMISSION HIGH LEVEL CONFERENCE ON

"MODERN BIOTECHNOLOGIES IN AGRICULTURE – PAVING THE WAY FOR RESPONSIBLE INNOVATION"

28 SEPTEMBER 2017, 09.30-18.00

BRUSSELS, BELGIUM

To conclude

1. Innovative potato breeding is contributing seriously to achieving disease resistant potatoes, especially in late blight.
2. Especially fungicide use is expected to go down as the result of the introduction of more and more LBR potato varieties.
3. Cisgenesis and gene editing are becoming serious accelerators in the development of disease resistant potatoes.
4. Cisgenesis and gene editing will only become true contributors in practice if they are not heavily regulated like GMOs.