The evolution of breeding approaches: from crosses to genome editing

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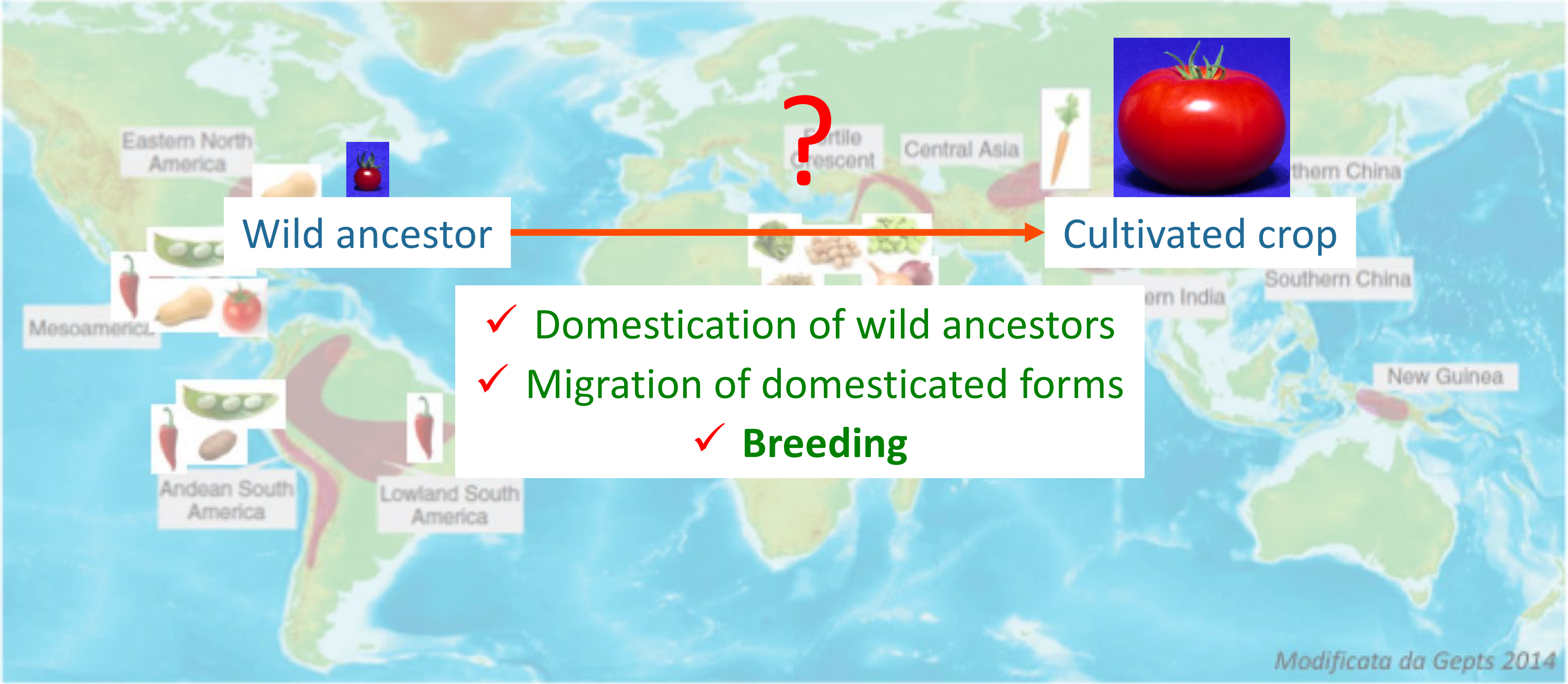
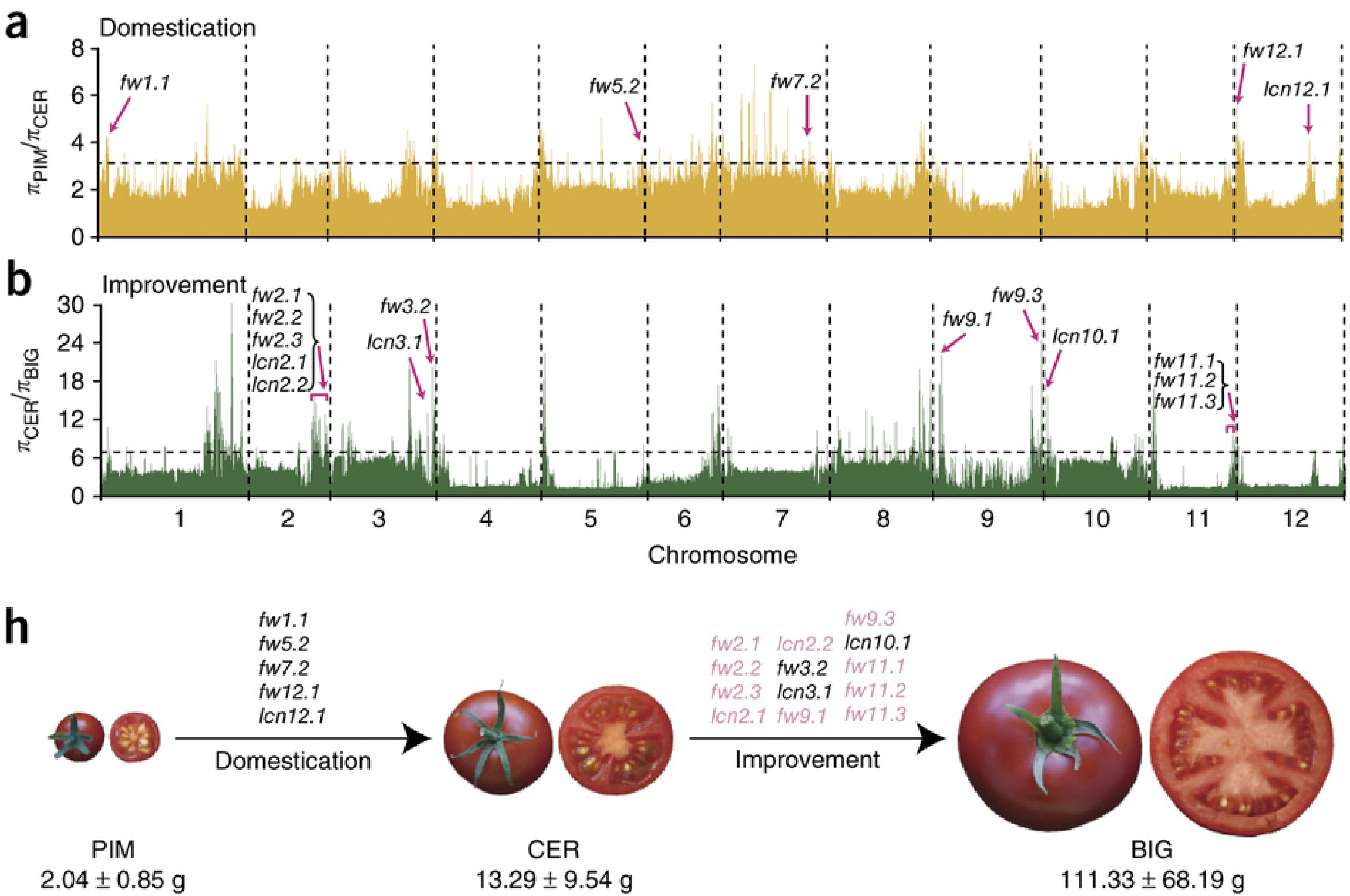
The Neolithic revolution



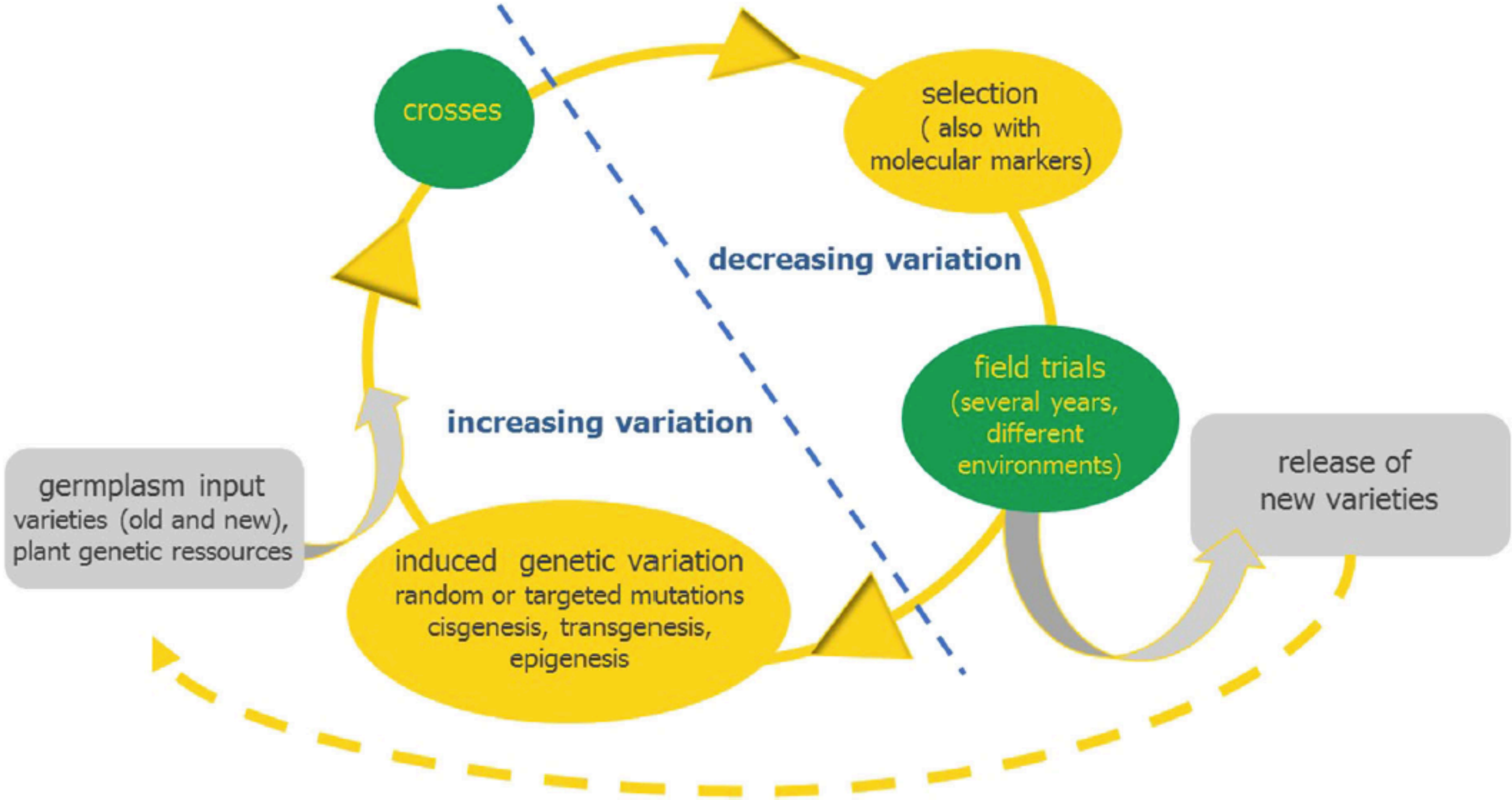
Domestication and breeding



Solanum pimpinellifolium | *S. lycopersicum var. cerasiforme* | *S. lycopersicum var. lycopersicum*

Lin et al., 2014

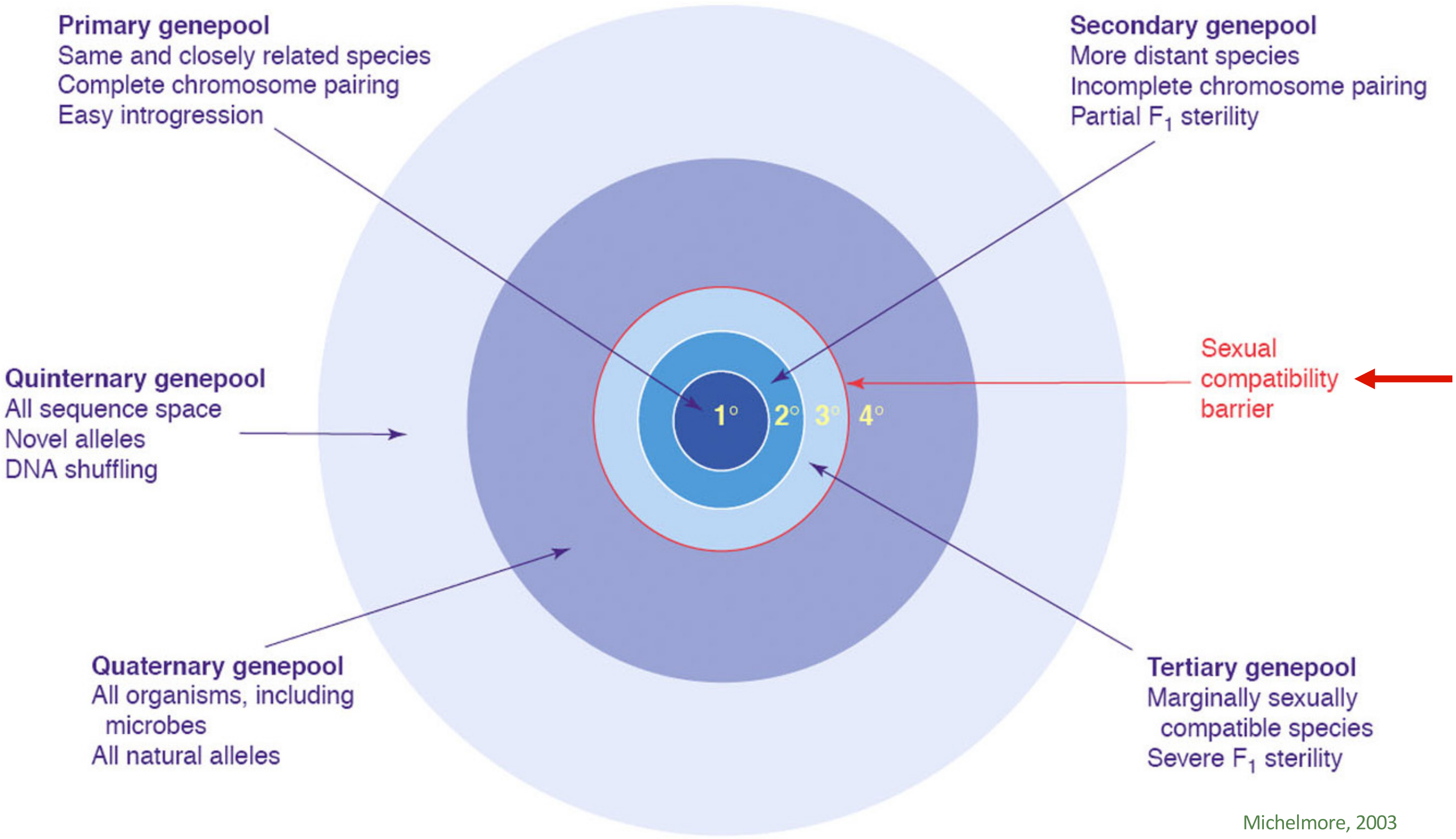
Plant Breeding



Jorasch 2019

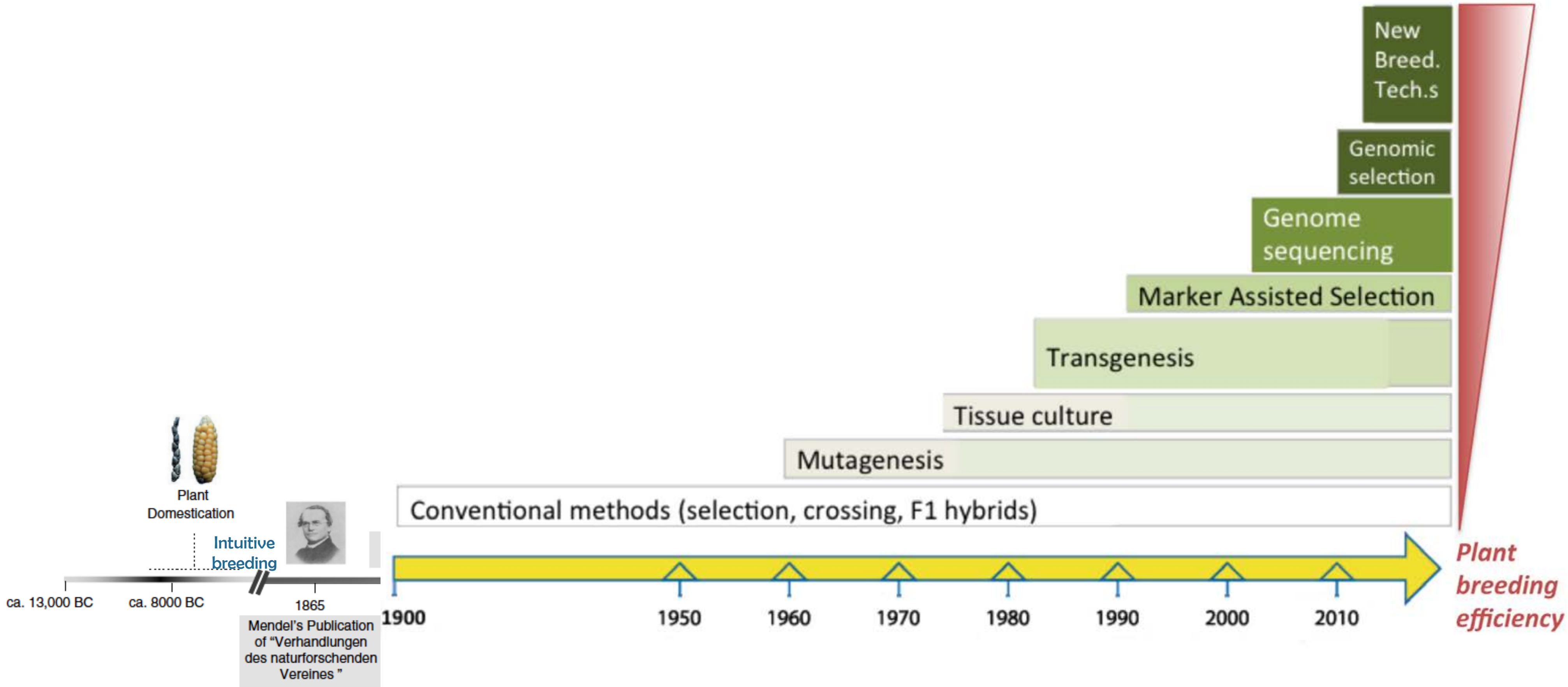
Fig. 3 The Plant Breeding Innovation Cycle

"Gene-pool" and breeding



Michelmore, 2003

Plant breeding



Francis et al. 2017
Mod. from Fricano et al. 2016

New Techniques in
Agricultural Biotechnology

High Level Group of Scientific Advisors
Explanatory Note 02

Brussels, 28 April 2017

Plant breeding

Conventional Breeding Techniques

- Selection in natural populations
- Intra-specific crossing and selection
- Mutagenesis
- Wide hybridization (Inter-specific hybridization)
- Tissue culture techniques (included somatic hybridization between sexually compatible species)

Established Techniques of Genetic Modification

- Somatic hybridization between sexually incompatible species
- Transgenesis

New Breeding Techniques

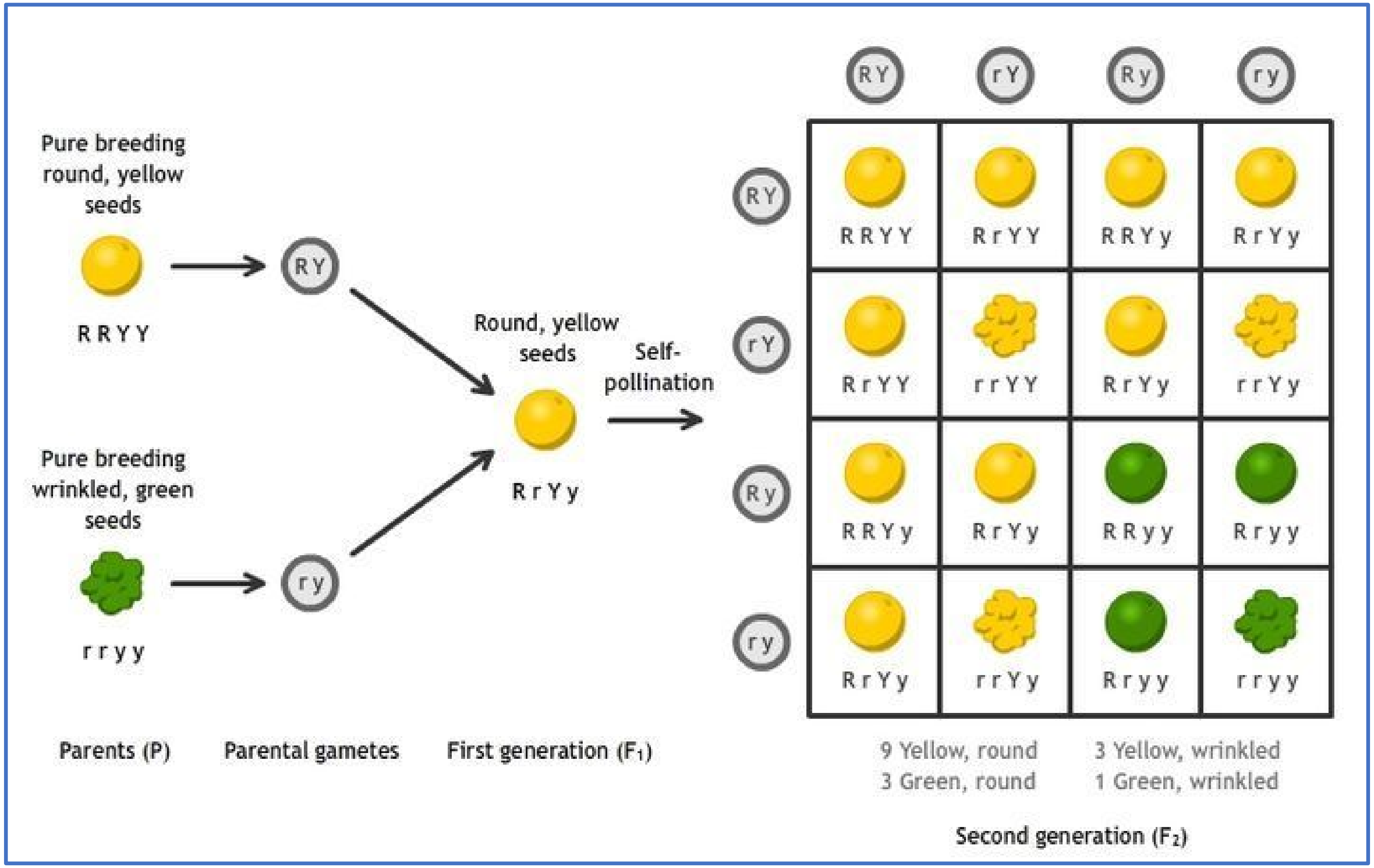
- Oligonucleotide Directed Mutagenesis (ODM)
- Nuclease-based Genome Editing
- Cisgenesis and Intragenesis
- Grafting (on GM rootstocks)
- RNA-dependent DNA methylation (RdDM)
- Reverse breeding
- Agro-infiltration
- Synthetic genomics
- ……………………………..?
- ……………………………..?

Hybridization

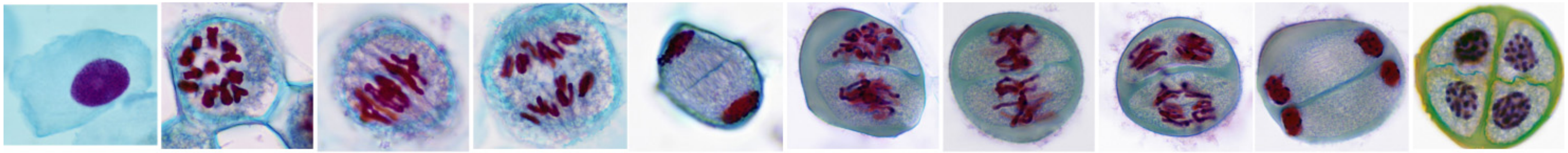
Mutations generate new traits. Crossing allows to combine them determining the appearance of new phenotypes

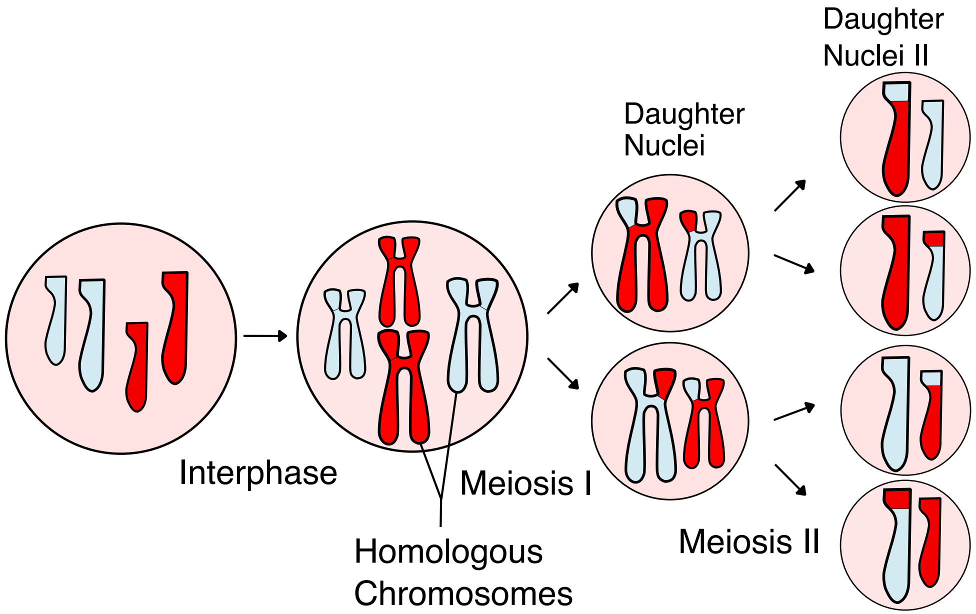
- **After crossing yellow and round peas with green and wrinkled peas, Mendel obtained new phenotypes: green and round or yellow and wrinkled peas.**

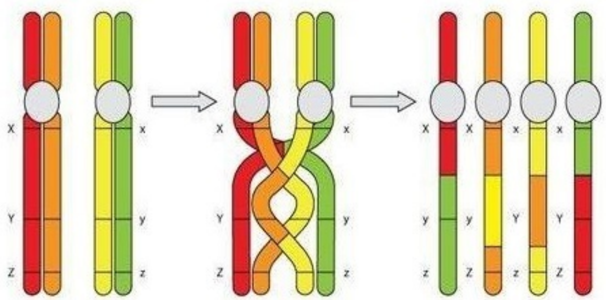
1865 (1900)



Meiosis and Genetic variability







During meiosis:

- Parental chromosome sets reassort
- Parental chromosomes recombine

⬇

Novel genetic variability!

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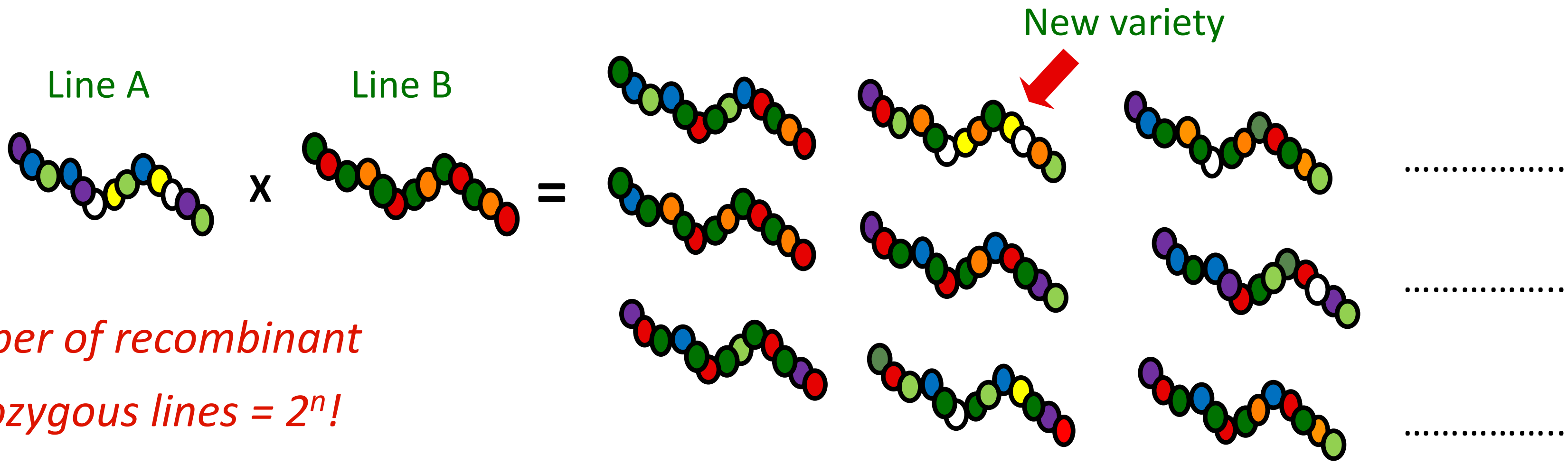
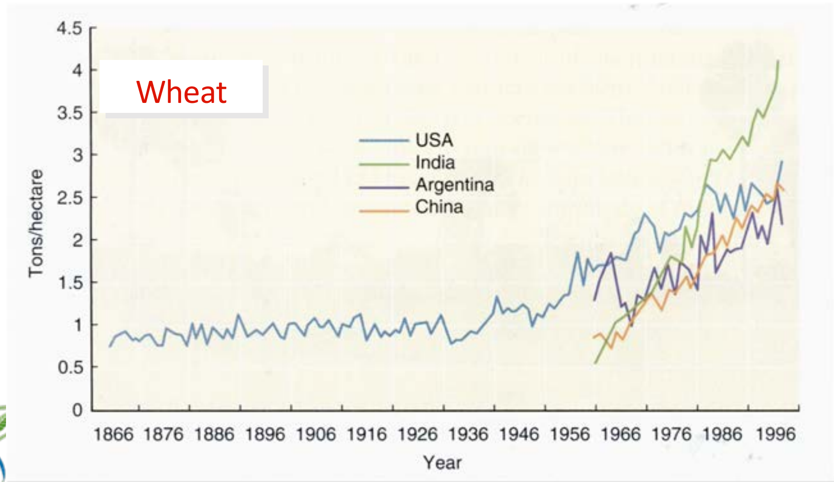
Hybridization

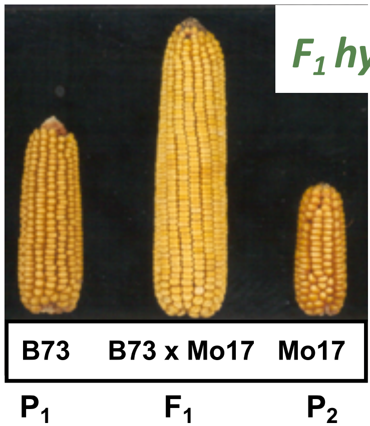


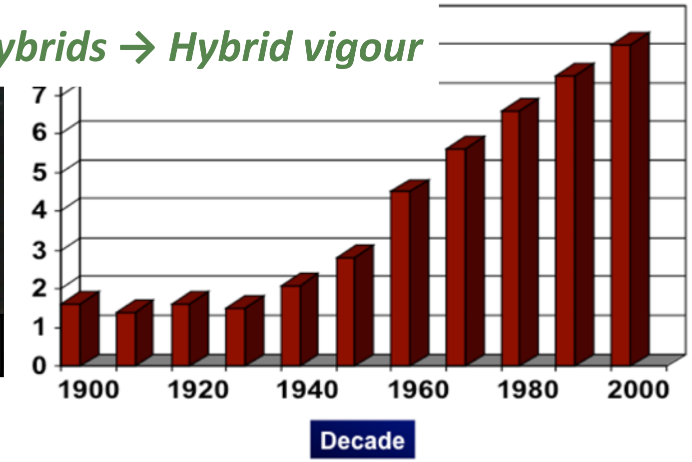
Number of recombinant homozygous lines = 2^n!

- Random, long and tedious process. The selection of desired products is not an easy task due to the high number of possible combinations and the confusing effects of gene interactions and the environment
- Unwanted genes are transferred along with those of interest
- Only genes within sexually compatible gene-pools can be transferred.



F_1 hybrids → Hybrid vigour





1960-1980

Mutagenesis

Induction of novel genetic variation by the generation of transmissible mutations in an otherwise good genetic background

Classical approaches

- Mutagenesis with physical or chemical mutagens ➡ Induced mutagenesis
- Treatment of seeds and pollen in sexually propagated plants or vegetative organs in vegetatively propagated plants
- Phenotypic selection in derived progenies

Technological advances

- Integration of tissue culture techniques ⇨
  - Exploitation of somaclonal variation
  - *In vitro* dissociation of chimeras
  - Mutagenesis of explants *in vitro*
  - *In vitro* selection of mutants
  - Micropropagation of selected mutants
- Integration of molecular techniques ⇨
  - Molecular markers
  - Tilling, Eco-tilling, DEco-tilling
  - Sequencing
- Transposon / T-DNA -based tagging ⇨
  - Insertion mutagenesis (➔ Arabidopsis, rice, ...)
- Use of NBT (ODM, SDN) ⇨
  - Site-directed mutagenesis

1960-1980

Mutagenesis

Induction of novel genetic variation by the generation of transmissible mutations in an otherwise good genetic background

- **> 3200 varieties released wordwide**
- **Direct / Indirect use in research and breeding**
- **Important tool for discovery of genes underpinning agronomic traits**
- **But several drawbacks…**

Classic

- Muta chem
- Treat in sex or ve vegetatively propagated plants
- Phenotypic selection in derived progenies

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s

- Integration of tissue culture techniques ⇨
  - Mutagenesis of explants *in vitro*
  - *In vitro* selection of mutants
  - Micropropagation of selected mutants
- Integration of molecular techniques ⇨
  - Molecular markers
  - Tilling, Eco-tilling, DEco-tilling
  - Sequencing
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  - Insertion mutagenesis (➔ Arabidopsis, rice, ...)
- Use of NBT (ODM, SDN) ⇨
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Wide hybridization

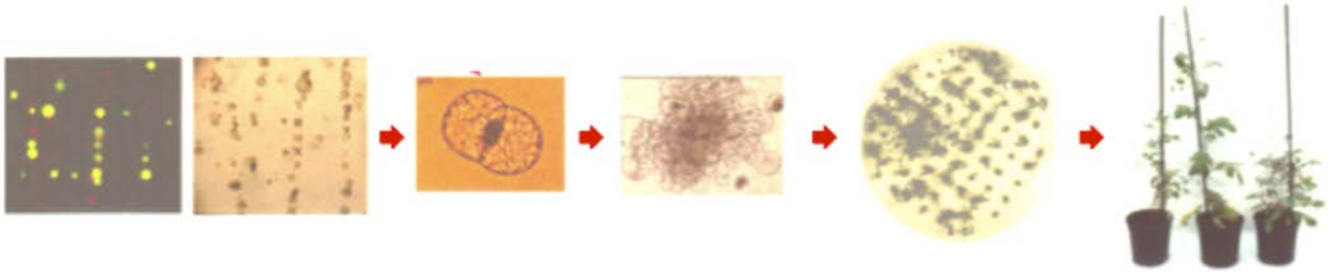
Pre- and post-fertilization barriers can hinder the access to genetic variability in secondary and tertiary gene pools

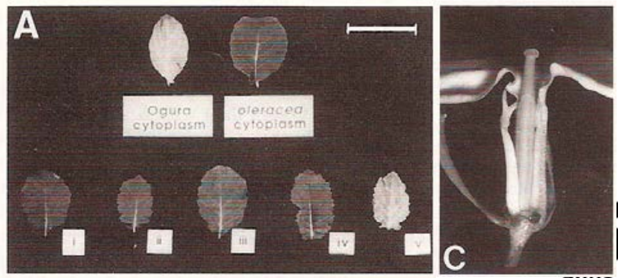
1950 - 1960…

Embryo rescue ➔
- based on *in vitro* culture of the immature hybrid embryo (before seed abortion occurs)
- useful to overcome post-zygotic barriers
- In various crops (e.g. *Solanum lycopersicum* and *S. peruvianum*) used to transfer disease resistance genes from wild relatives
- In cereals helped produce new crops from intergeneric crosses (e.g. triticale)

1970 - 2000

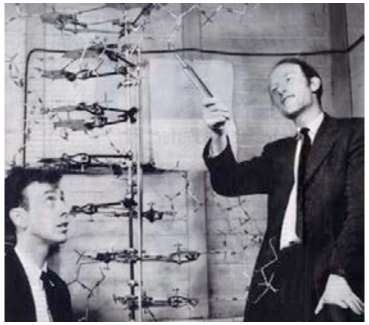
Somatic hybridization ➔
- protoplasts derived from somatic cells are fused *in vitro* using either chemical or physical methods
- useful to overcome both pre- and post-zygotic barriers
- in various crops (e.g. potato, Citrus, Brassica) allowed to transfer genes for tolerance/resistance to abiotic/biotic stresses, produce seedless hybrids, male sterile genotypes, etc.
- cybridization (cytoplasmic hybridization) allows independent transmission of mitochondria and chloroplasts (e.g. Ogura CMS cold tolerant *Brassica spp.*)

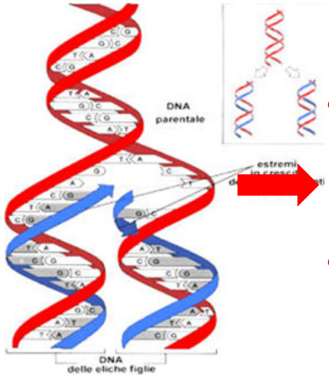




Genetic engineering and plant transformation

1953

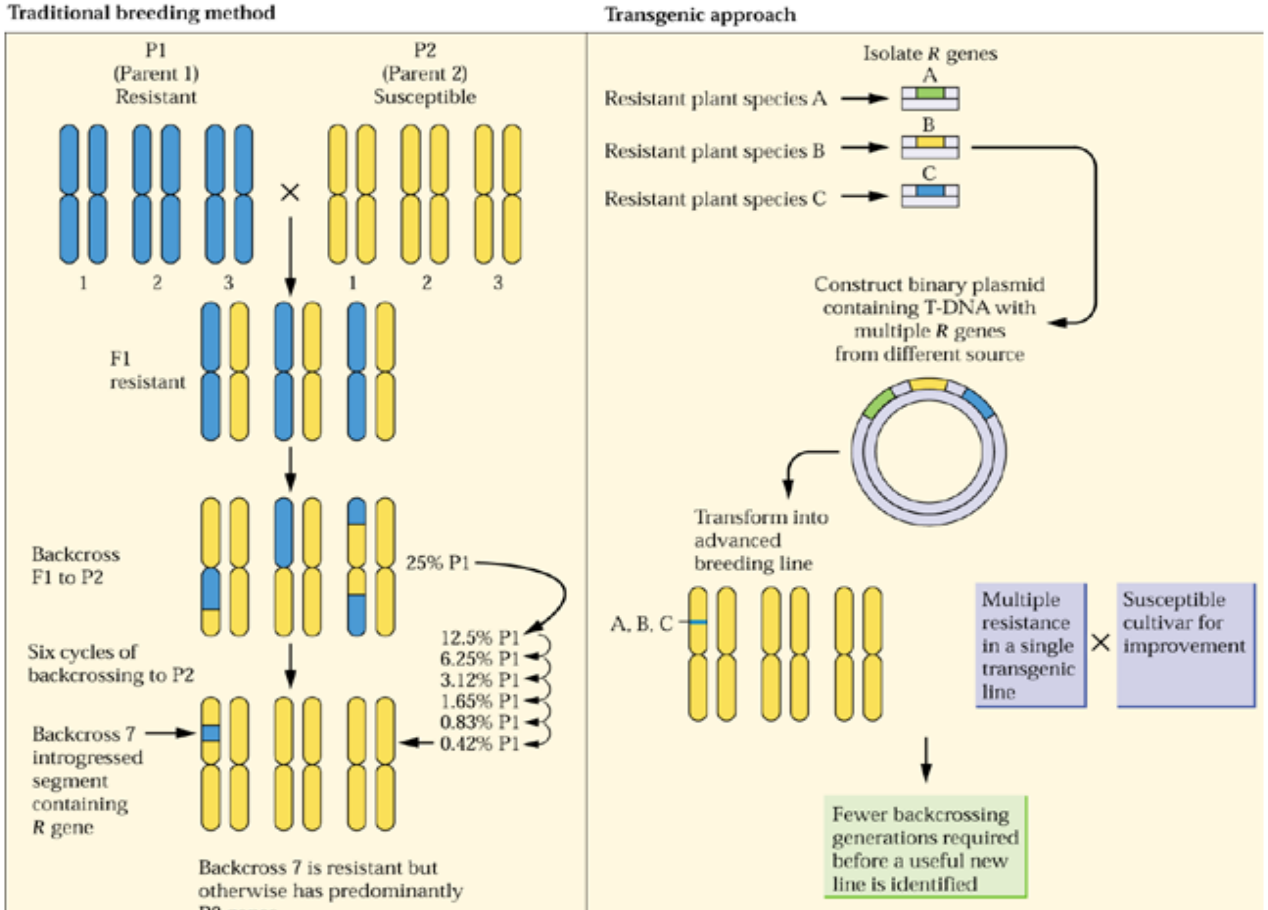




- Development of molecular biology tools
- Improvement of tissue culture protocols

Transgenesis: 1983…

A gene of interest can be isolated from any organism, engineered with appropriate regulatory sequences and inserted in plant cells, usually using *in vitro* systems

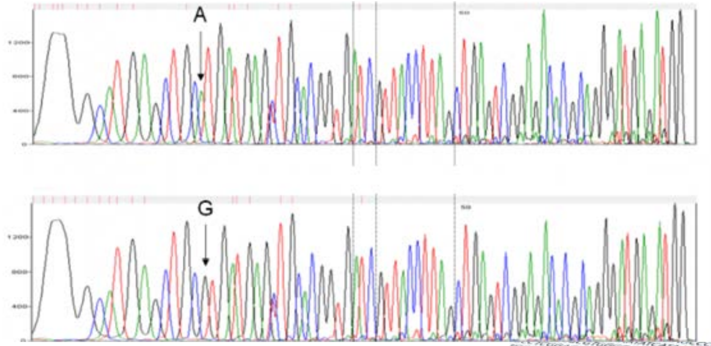


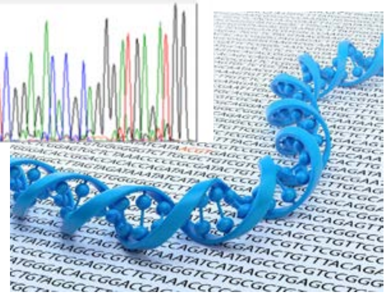
Hammond-Kosack and Jones 2000

Genomics

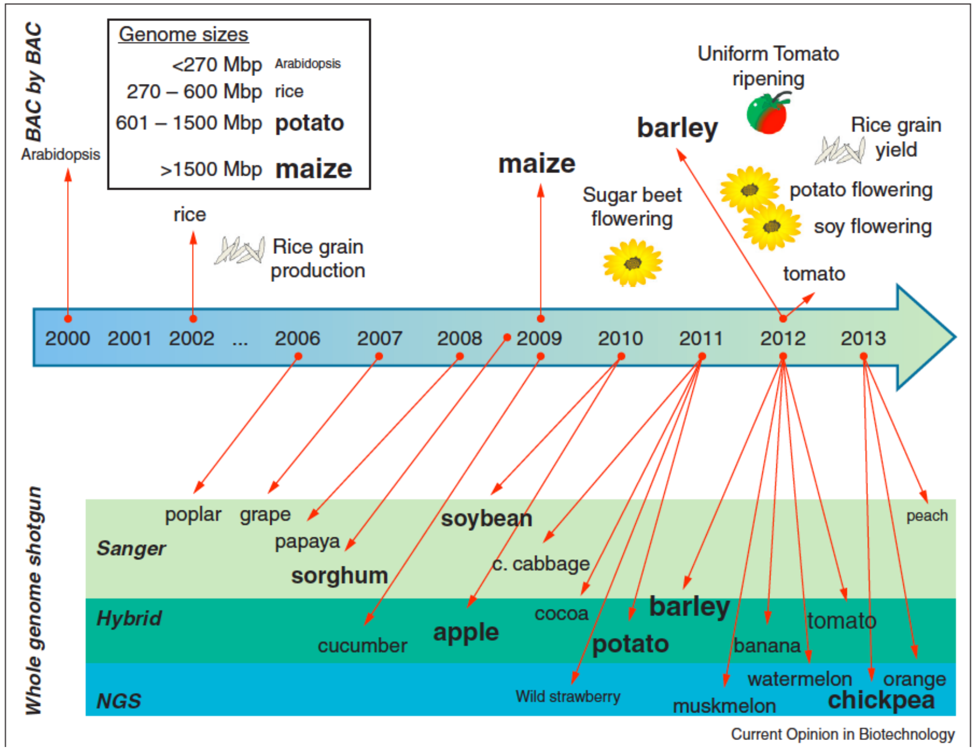
2000…

High efficiency technologies for DNA sequencing





- ***High perfomance computers***
- ***Bioinformatics***



- ✓ Acquisition of structural and functional information of whole plant genomes and single genes
- ✓ Essential tool to:
  - understand molecular bases of natural variation and "mine" novel alleles
  - isolate, modify and transfer genes with the original regulatory sequences from related species
  - act in a targeted manner only on the gene responsible for the character to improve
  - develop genome-wide markers and more efficient selection approaches

Review

Opportunities for Products of New Plant Breeding Techniques

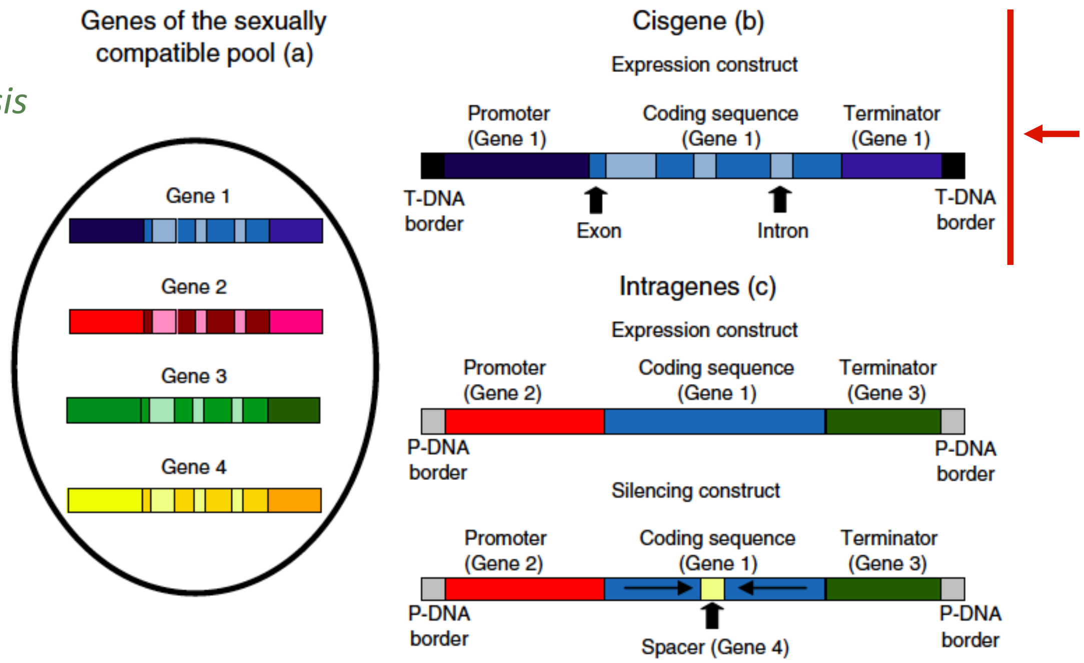
Jan G. Schaart,[1,*] Clemens C.M. van de Wiel,[1] Lambertus A.P. Lotz,[2] and Marinus J.M. Smulders[1]

2010…

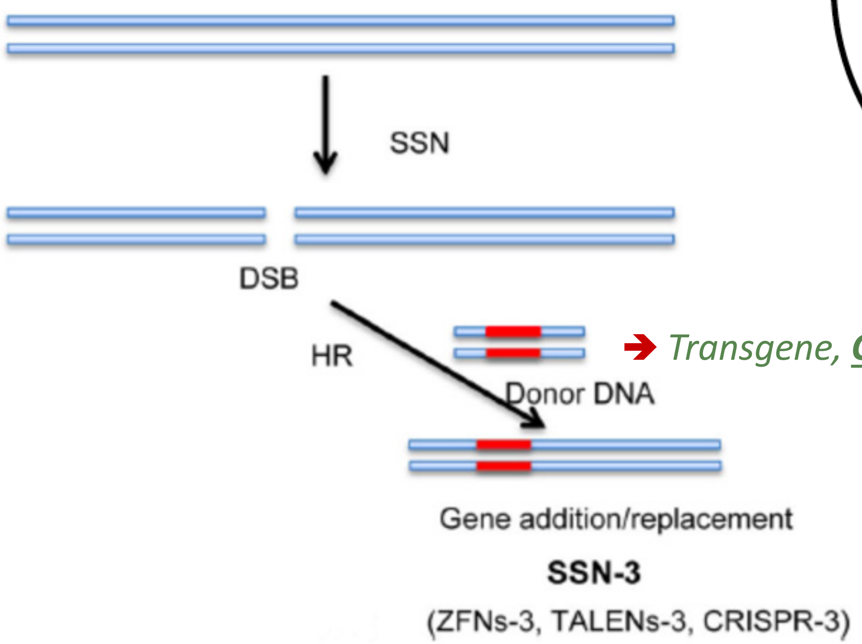
New (Plant) Breeding Techniques

(i) The plant (final product) contains a new DNA fragment (*e.g.*, a new gene)

- ***Cisgenesis***
- *Intragenesis*



- ***SDN-3 (SSN-3)***



➔ *Transgene, __**Cisgene**__, Intragene*

Holme et al. 2013
Chen and Gao 2014

New (Plant) Breeding Techniques

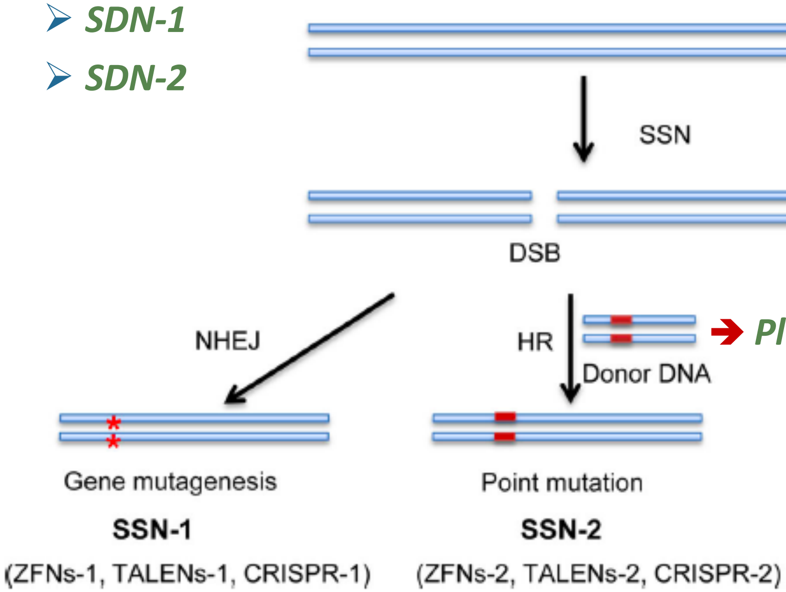
(ii) The plant (final product) does not contain a new DNA fragment, but contains (small) modifications of its own DNA (indels or point mutations)

Review

Opportunities for Products of New Plant Breeding Techniques

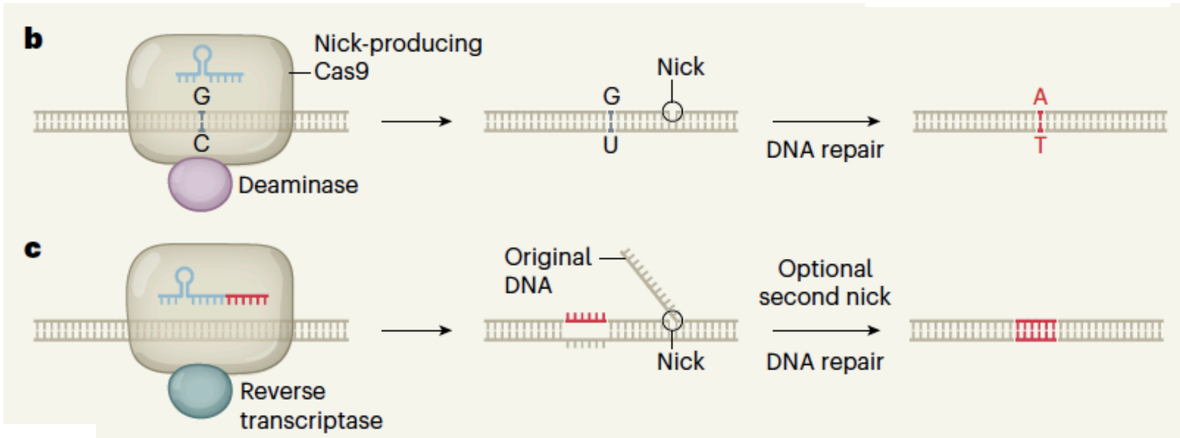
Jan G. Schaart,[1,*] Clemens C.M. van de Wiel,[1] Lambertus A.P. Lotz,[2] and Marinus J.M. Smulders[1]

- *SDN-1*
- *SDN-2*

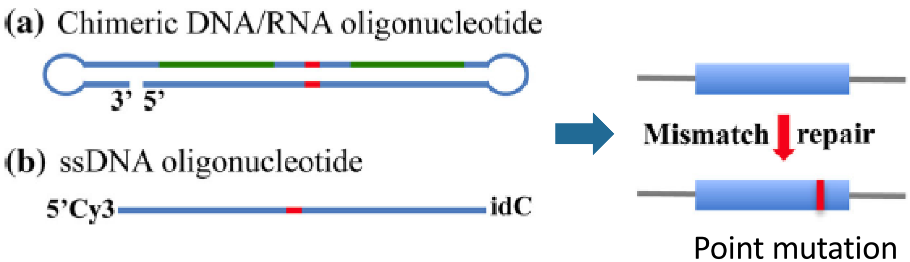


➔ *Plasmid, dsOligo, ssOligo*

- *Base editing*
- *Prime editing*



- *ODM*



Chen and Gao 2014
Platt 2019
Cardi and Stewart 2016

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Improvement of selection efficiency

➢ Doubled haploids (DH) to obtain homozygous lines

TABLE 9.1 Percentage of Homozygosity and Number of Self Pollinations Needed to Obtain Inbred Lines in a Traditional Breeding Program of Autogamous Species

Traditional Method				
Seasons	Generation Planting	Harvesting	% Homozygosity	⊗[1]
1	Crossing block	F1	0%	
2	F1	F2	50%	1
3	F2	F3	75%	2
4	F3	F4	87.50%	3
5	F4	F5	93.75%	4
6	F5	F6	96.88%	5
7	F6	F7	98.44%	6
8	F7	F8	99.22%	7

[1] *Number of self pollinations.*

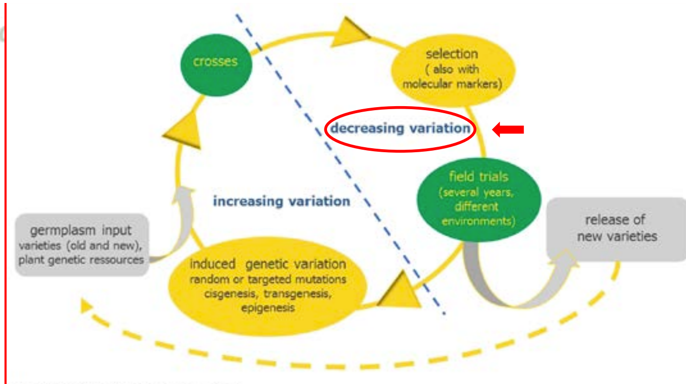
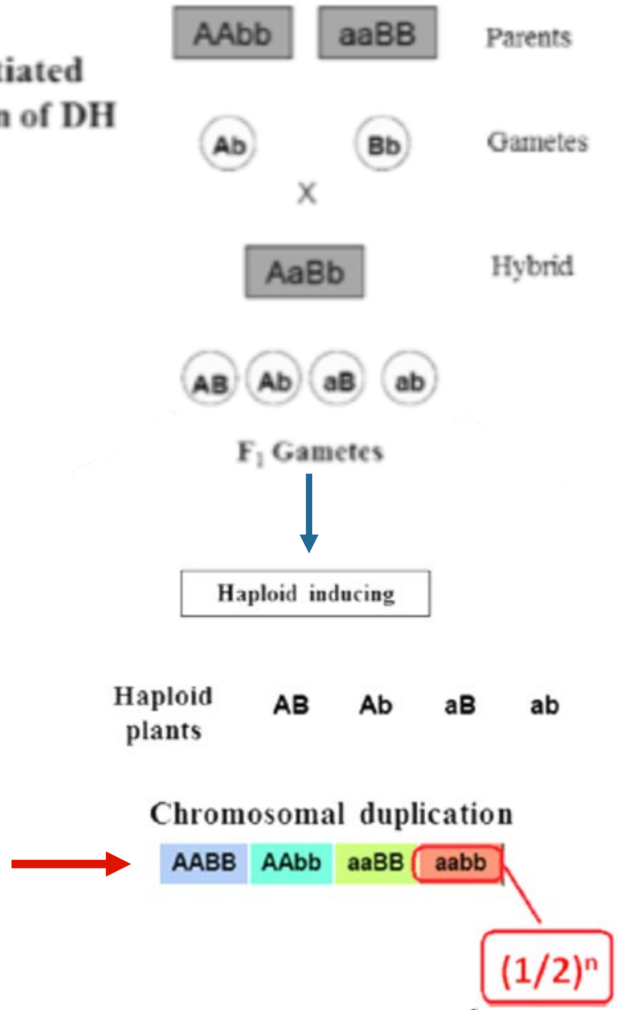


Fig. 3 The Plant Breeding Innovation Cycle

Differentiated segregation of DH



Fritsche-Neto et al. 2014
Sood and Dwivedi 2015

Improvement of selection efficiency

➢ ***Doubled haploids (DH) to obtain homozygous lines***

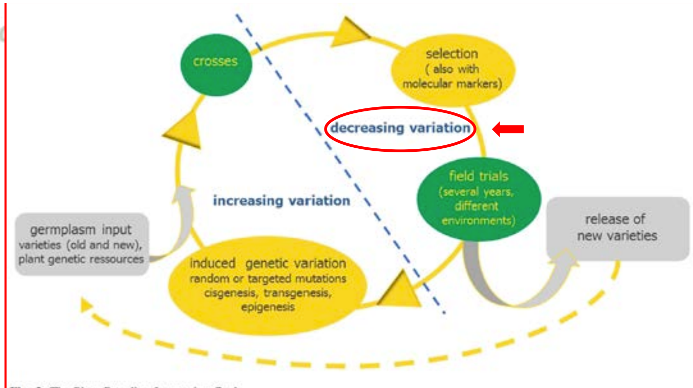


Fig. 3 The Plant Breeding Innovation Cycle

TABLE 9.1 Percentage of Homozygosity and Number of Self Pollinations Needed to Obtain Inbred Lines in a Traditional Breeding Program of Autogamous Species

- ✓ >> 300 new varieties developed worldwide using DH technology
- ✓ Most DH varieties in barley, rapeseed, wheat, pepper, rice, tobacco, eggplant, melon, triticale and asparagus
- ✓ In vegetable crops, DHs used primarily as parents for F_1 hybrid seed production

4	F3	F4	87.50%	3
5	F4	F5	93.75%	4
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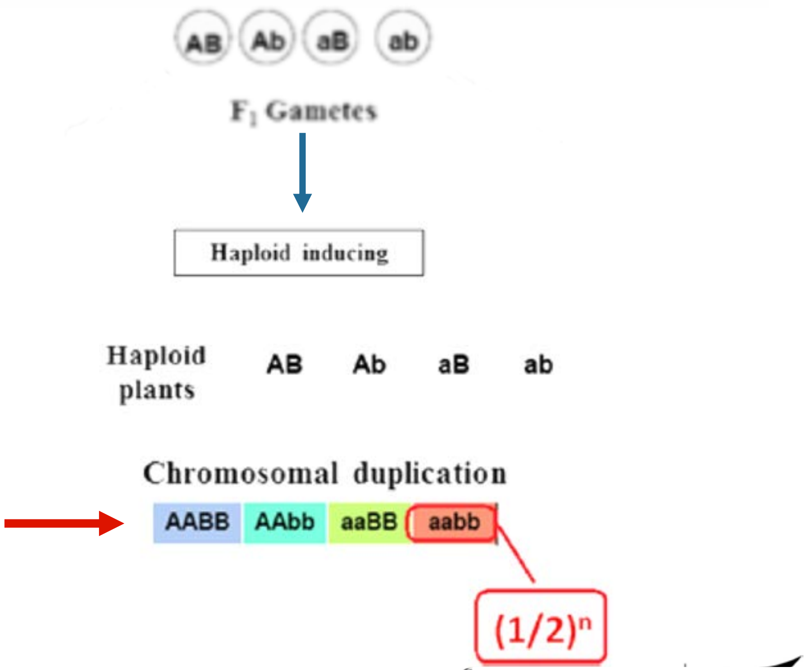
[1] Number of self pollinations.



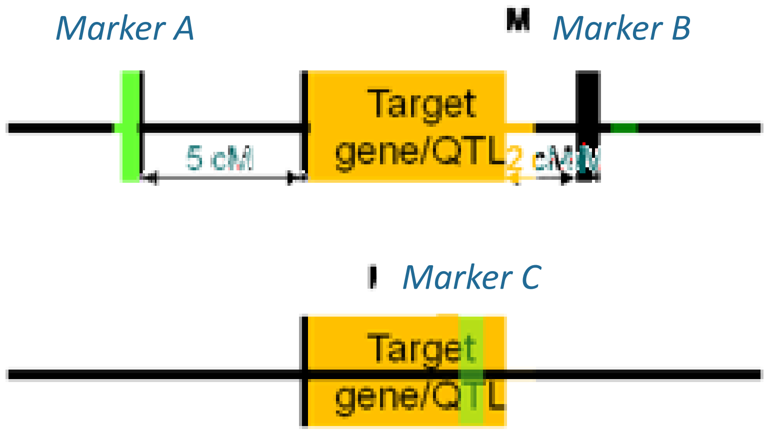
Fritsche-Neto et al. 2014
Sood and Dwivedi 2015

Improvement of selection efficiency

1980…

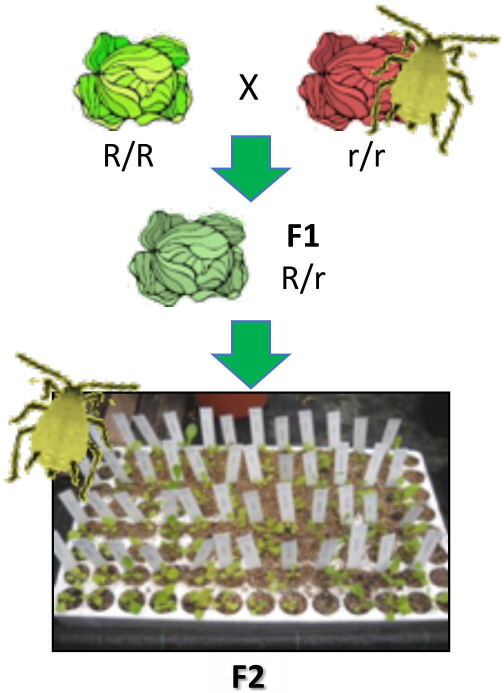
Molecular markers

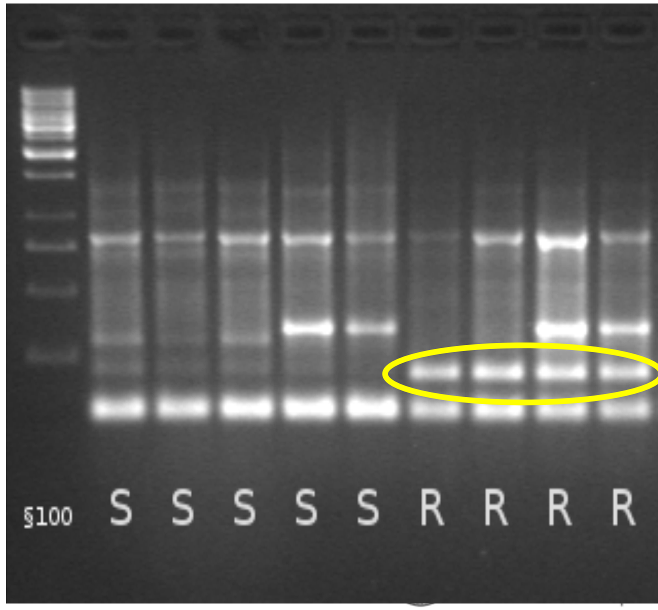
- Molecular markers show DNA polymorphisms distributed on the genome
- They can be detected in different ways
- Their presence / absence, and detection, does not depend on the environment or the stage of development
- Most useful markers for marker assisted selection are those closely associated with genes and characters of interest



Marker Assisted Selection (MAS)

Molecular marker associated to the aphid *Nasonovia ribisnigri* in *Lactuca sativa*

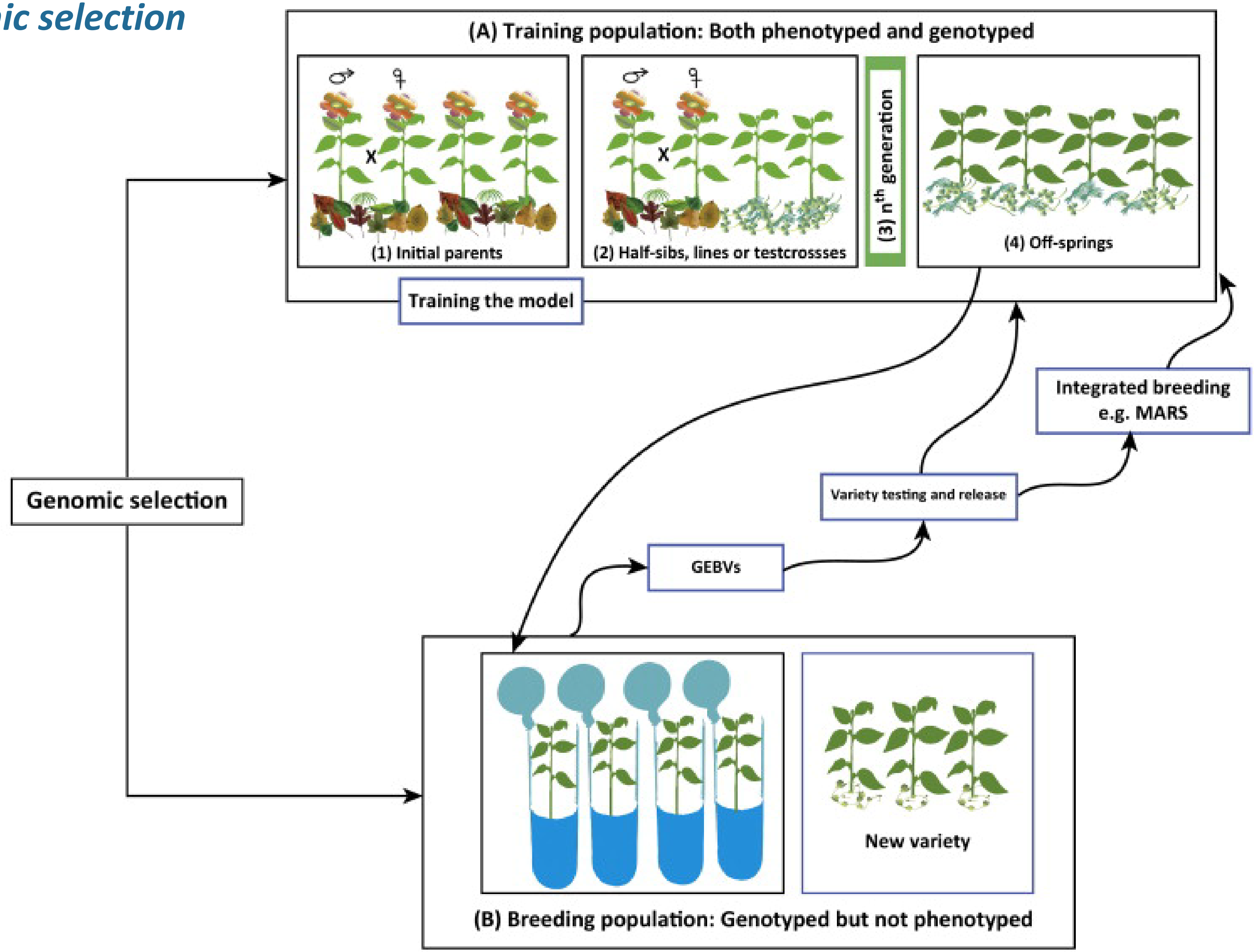




Next generation breeding

2010…

Genomic selection



Abera Desta and Ortiz, 2014

Conclusions and perspectives

- Since the rediscovery of Mendelian laws at the beginning of the last century, plant breeding has being played a major role in increasing crop productivity, food security and safety, quality and diversity of agricultural products, sustainability of agriculture
- Breeding approaches based on hybridization and phenotypic selection have been an important component of this process. Nevertheless, they pose some limits and, in some cases, show a low efficiency
- During the years, new technologies have been gradually integrated in plant breeding approaches increasing their efficiency and efficacy
- It can be envisaged that knowledge of plant genomes and new biotechnologies, wisely integrated in breeding programmes, will play a major role in the future to face up to old and new challenges

Thank you for your attention

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