Herramientas moleculares para el mejoramiento genético de plantas

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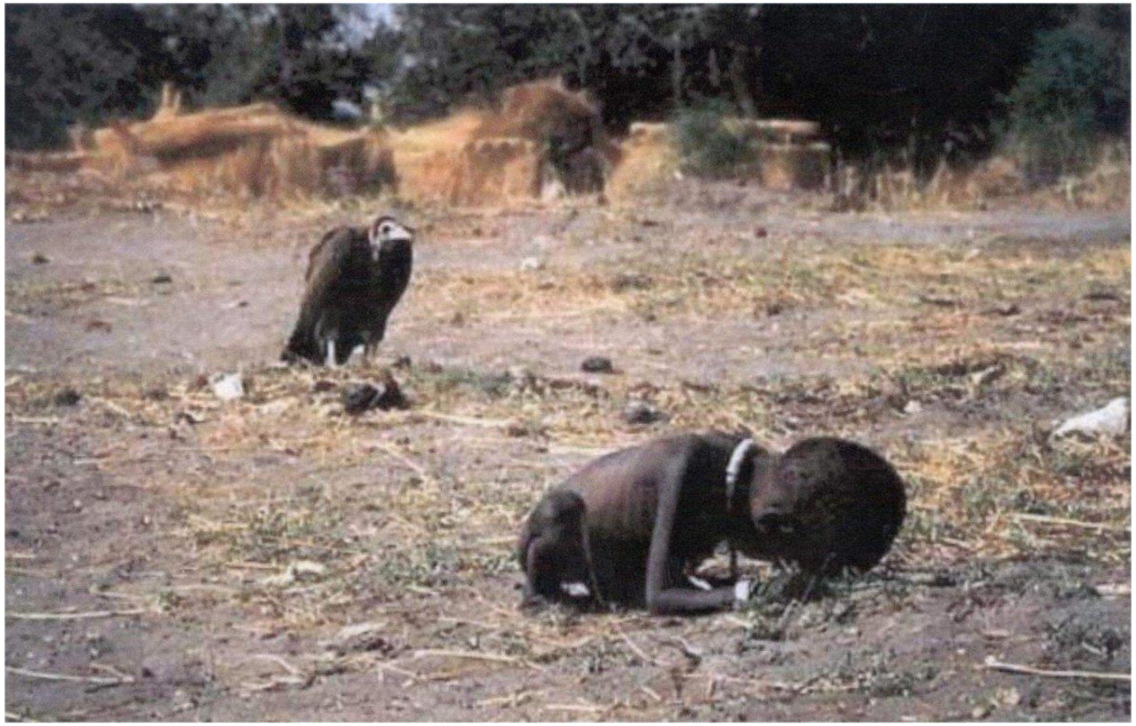
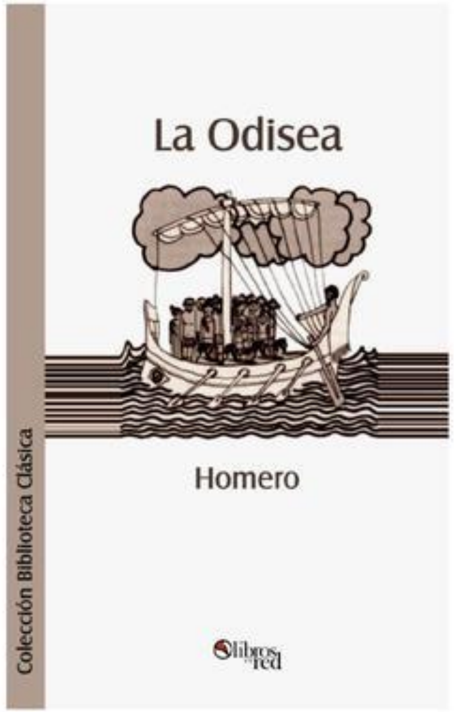
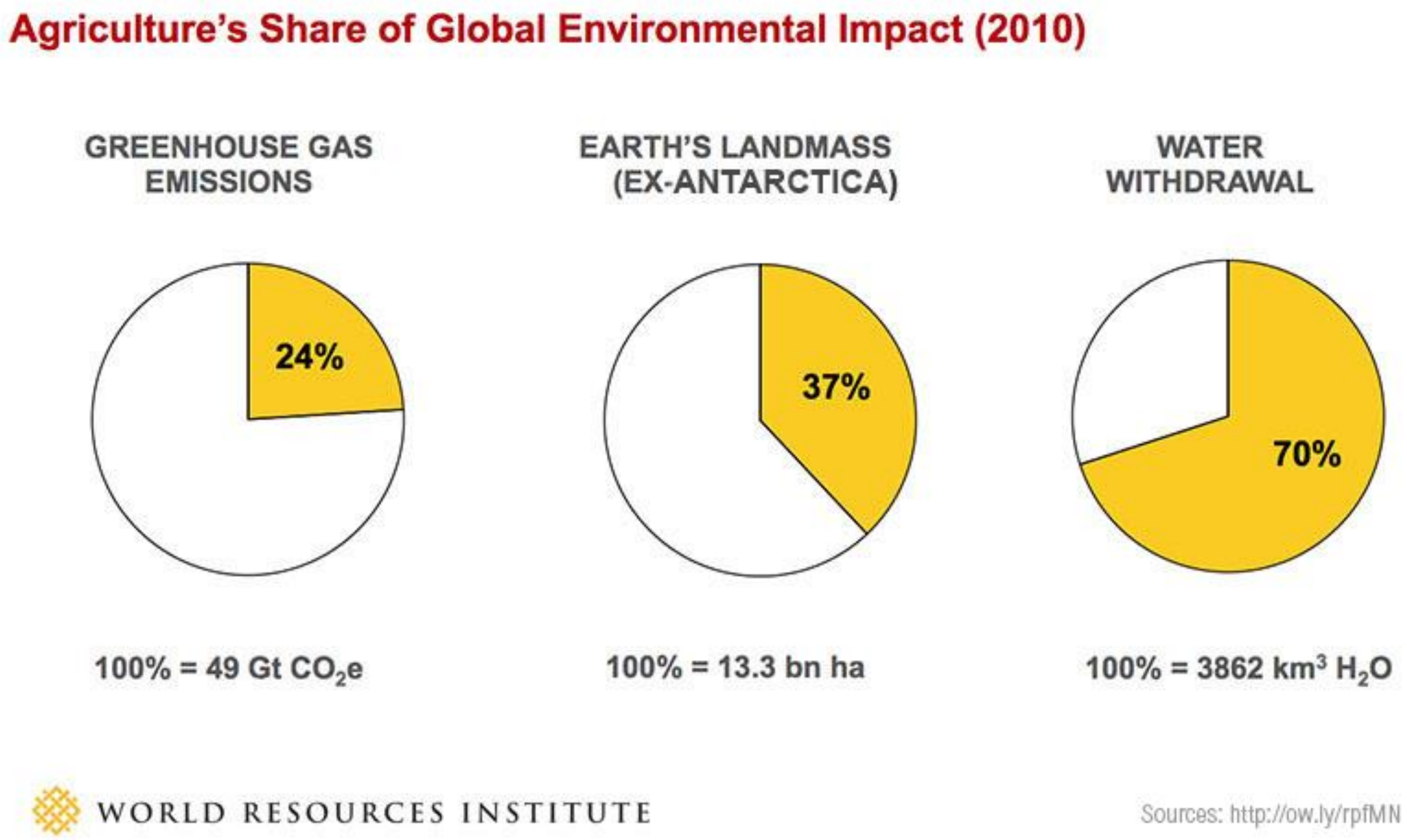
“Morir de hombre es el más amargo de los destinos”

Homero, La Odisea.



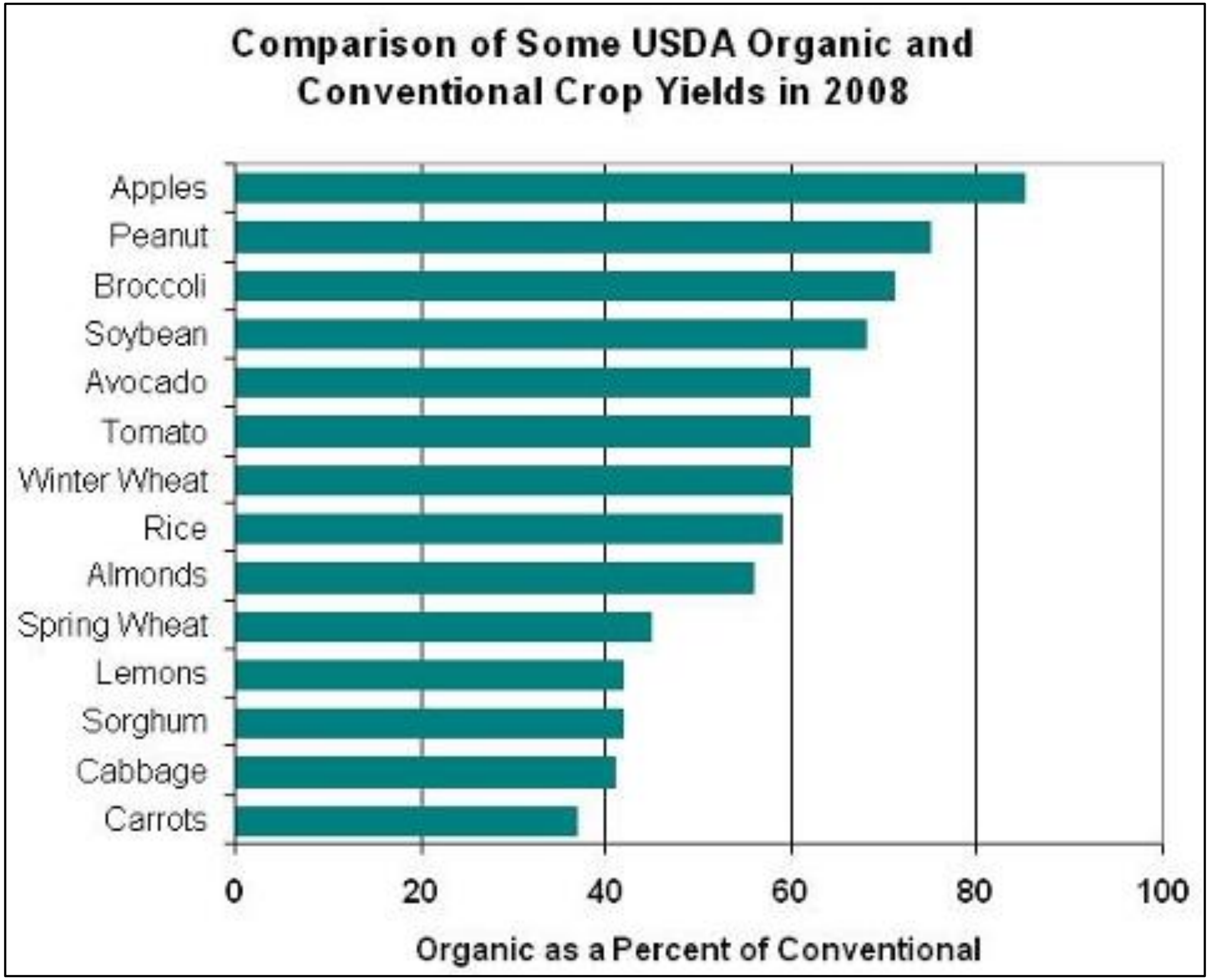


La agricultura tiene impactos . . .



El Mito de “La Agricultura es Natural”

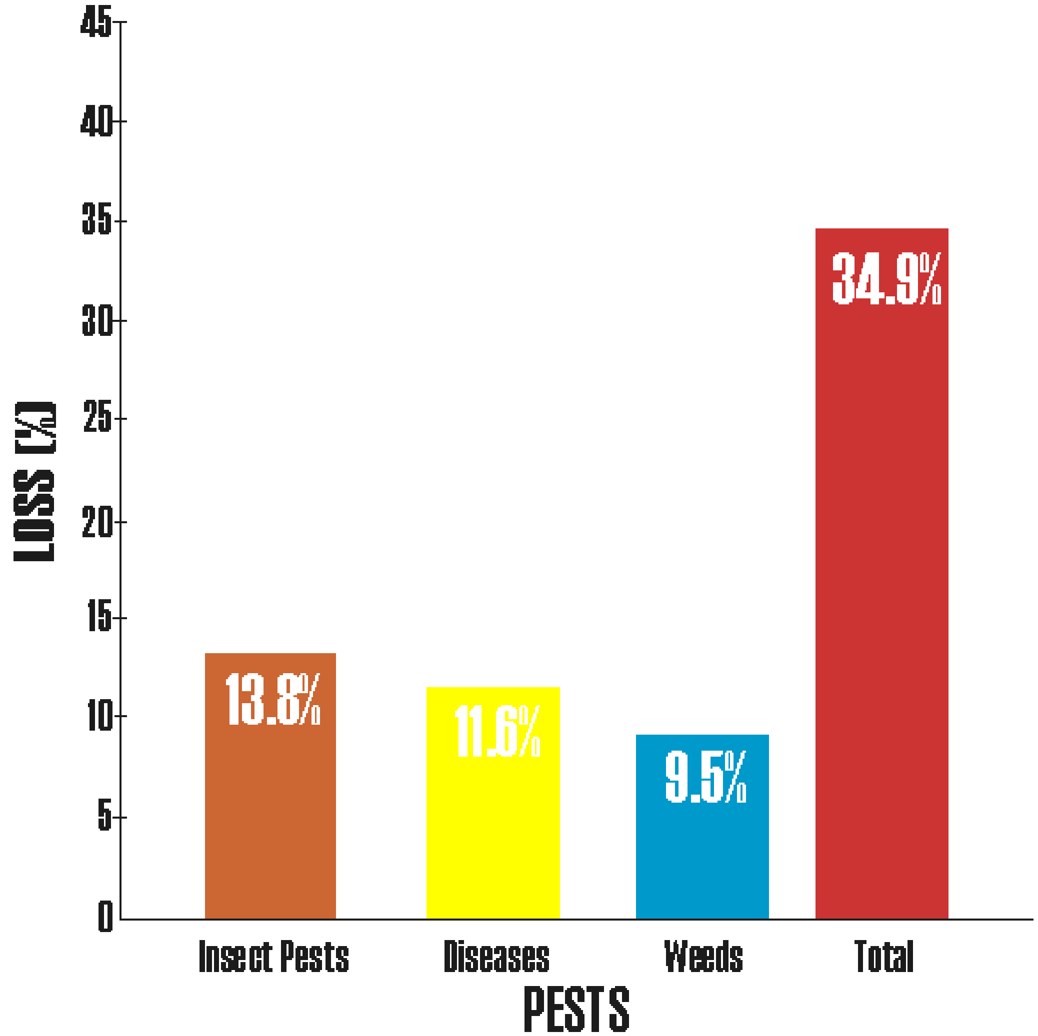
Agricultura Orgánica rinde menos



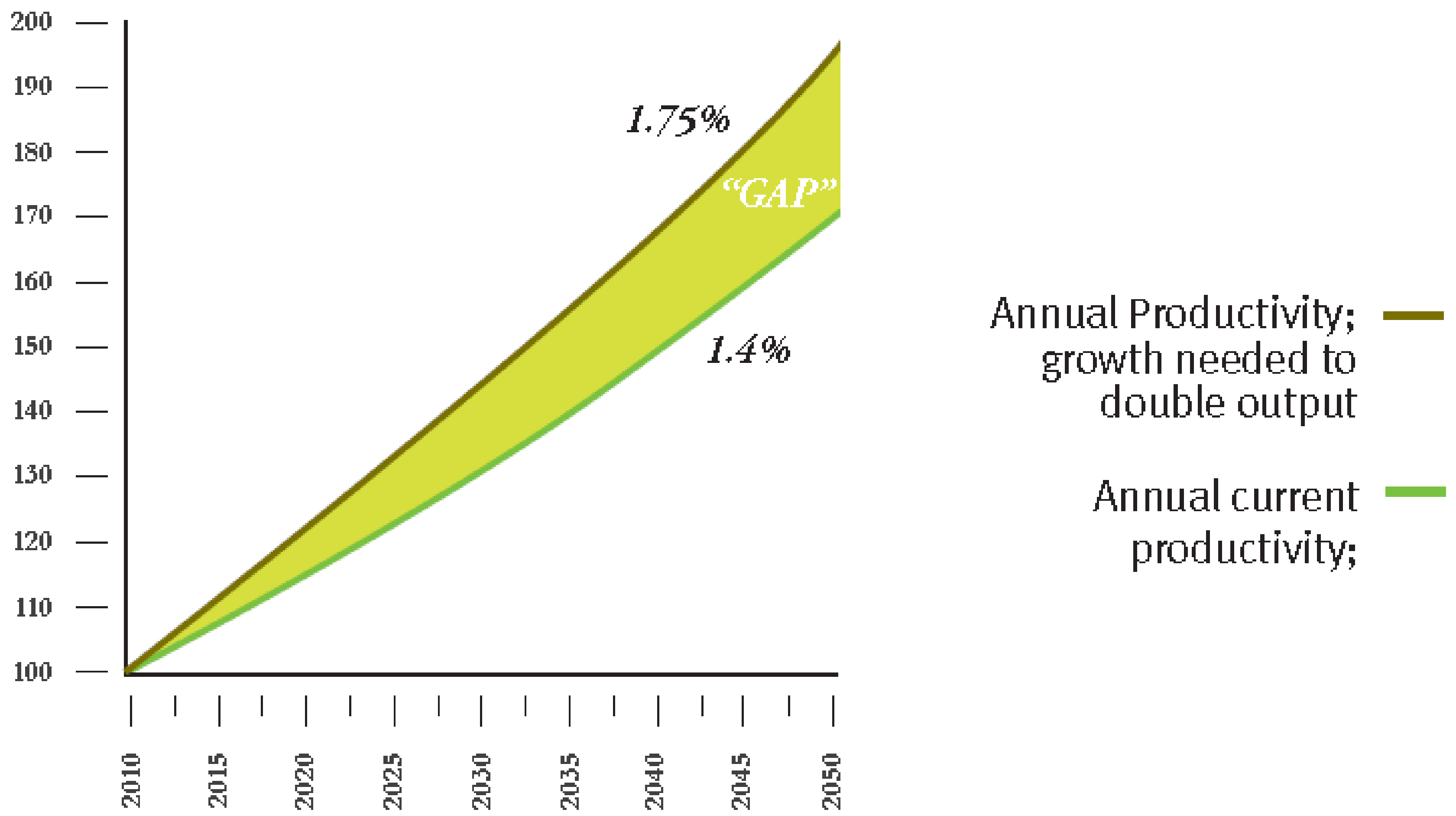
Rendimientos Promedios y Récords de algunos cultivos importantes (Bray *et. al.*, 2000)

Cultivo	Rendimiento Récord (Kg/Há)	Rendimiento Promedio (Kg/Há)	Rendimiento Promedio (% del récord)	Pérdida Promedio por factor Biótico (% del récord)	Pérdida Promedio por factor Abiótico (% del récord)
Trigo	14,500	1,880	13.0	5.0	82.1
Cebada	11,400	2,050	18.0	6.7	75.4
Soya	7,390	1,610	21.8	9.0	69.3
Maíz	19,300	4,600	23.8	10.1	65.8
Papa	94,100	28,300	30.1	18.9	54.1
Remolacha Azucarera	121,000	42,600	35.2	14.1	50.7

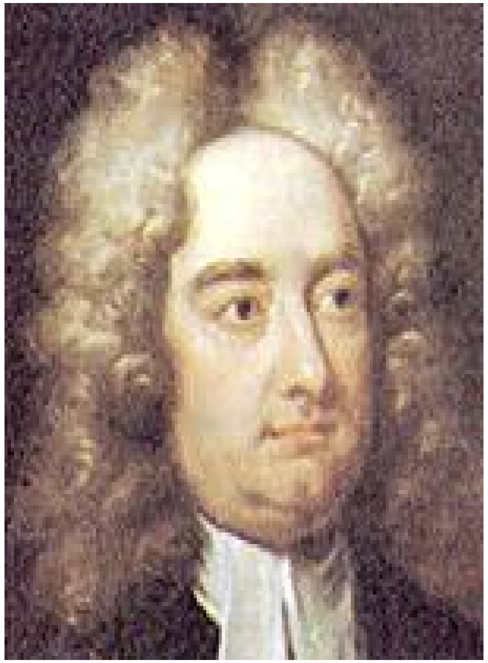
Factores que causan pérdidas en agricultura



La brecha global de Productividad Agrícola: 2010-2050



Jonathan Swift



“And he gave it for his opinion, "that whoever could make two ears of corn, or two blades of grass, to grow upon a spot of ground where only one grew before, would deserve better of mankind, and do more essential service to his country, than the whole race of politicians put together.”

– Jonathan Swift, Gulliver's Travels

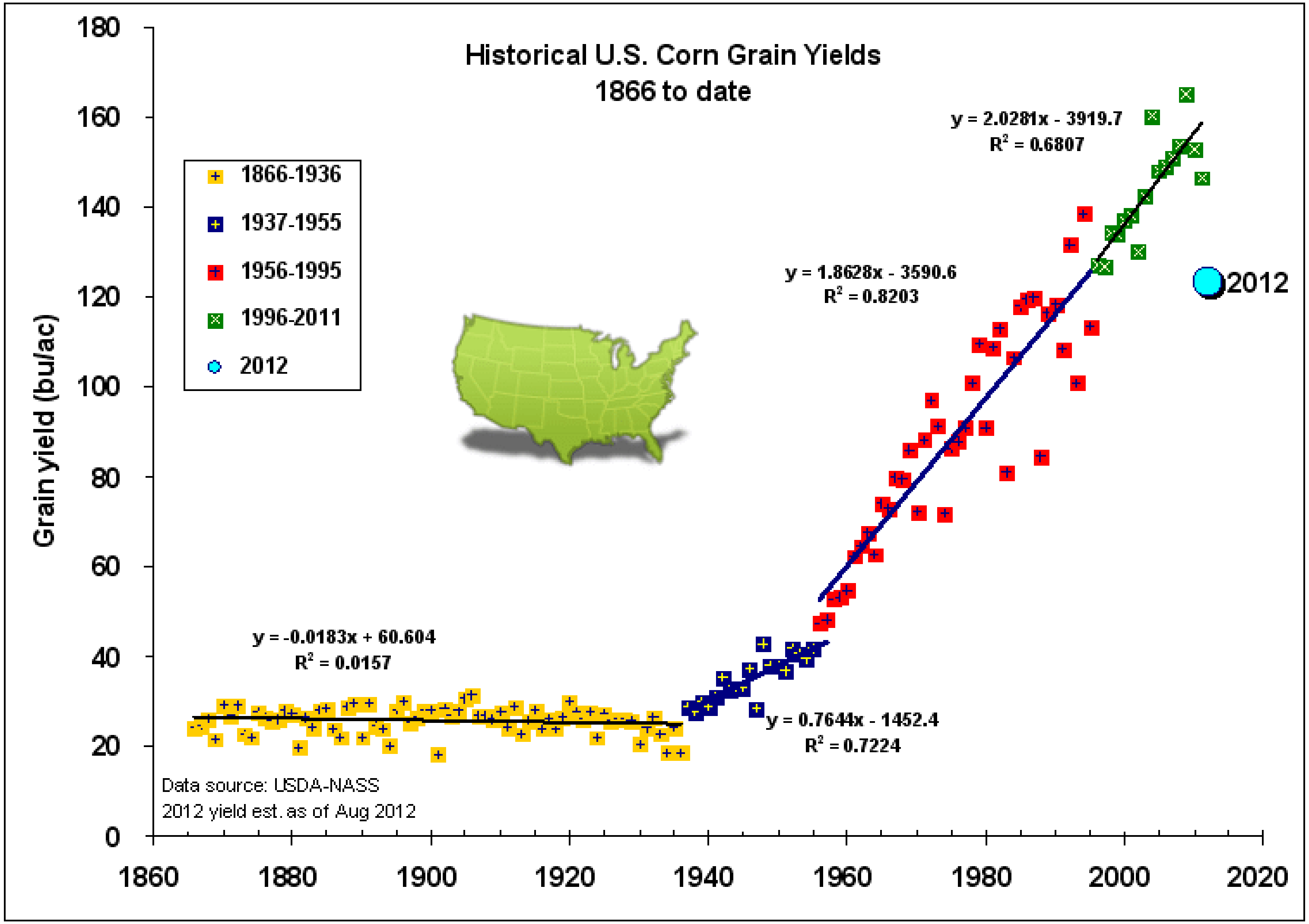
"Y él lo dio por su opinión", que cualquiera que pudiera hacer crecer dos mazorcas de maíz, o dos hojas de pasto, sobre un terreno donde solo creció uno antes, se merecería más de la humanidad, y haría un servicio más esencial para su país, que toda la raza de políticos juntos ".

Mejoramiento Genético de Plantas

Bajo relieve asirio de 750 AC polinización artificial de una palmera datilera

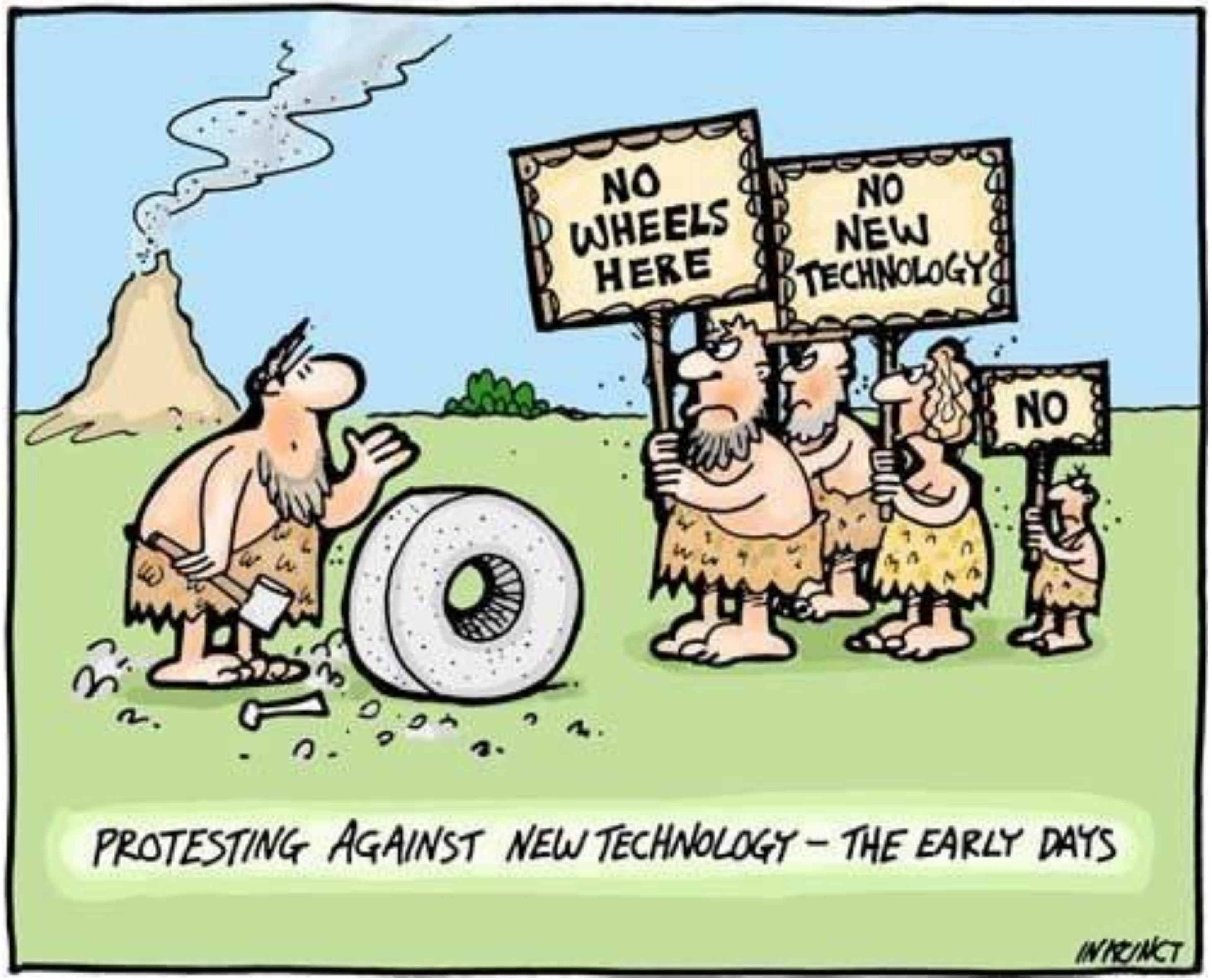
Mejorador de maíz contemporaneo

Rendimientos Históricos del Maíz (1866-2014)



Resistencia al cambio tecnológico (I)

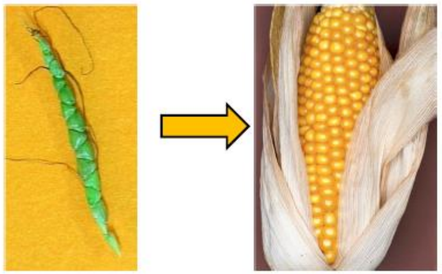
Resistencia al cambio tecnológico (II)

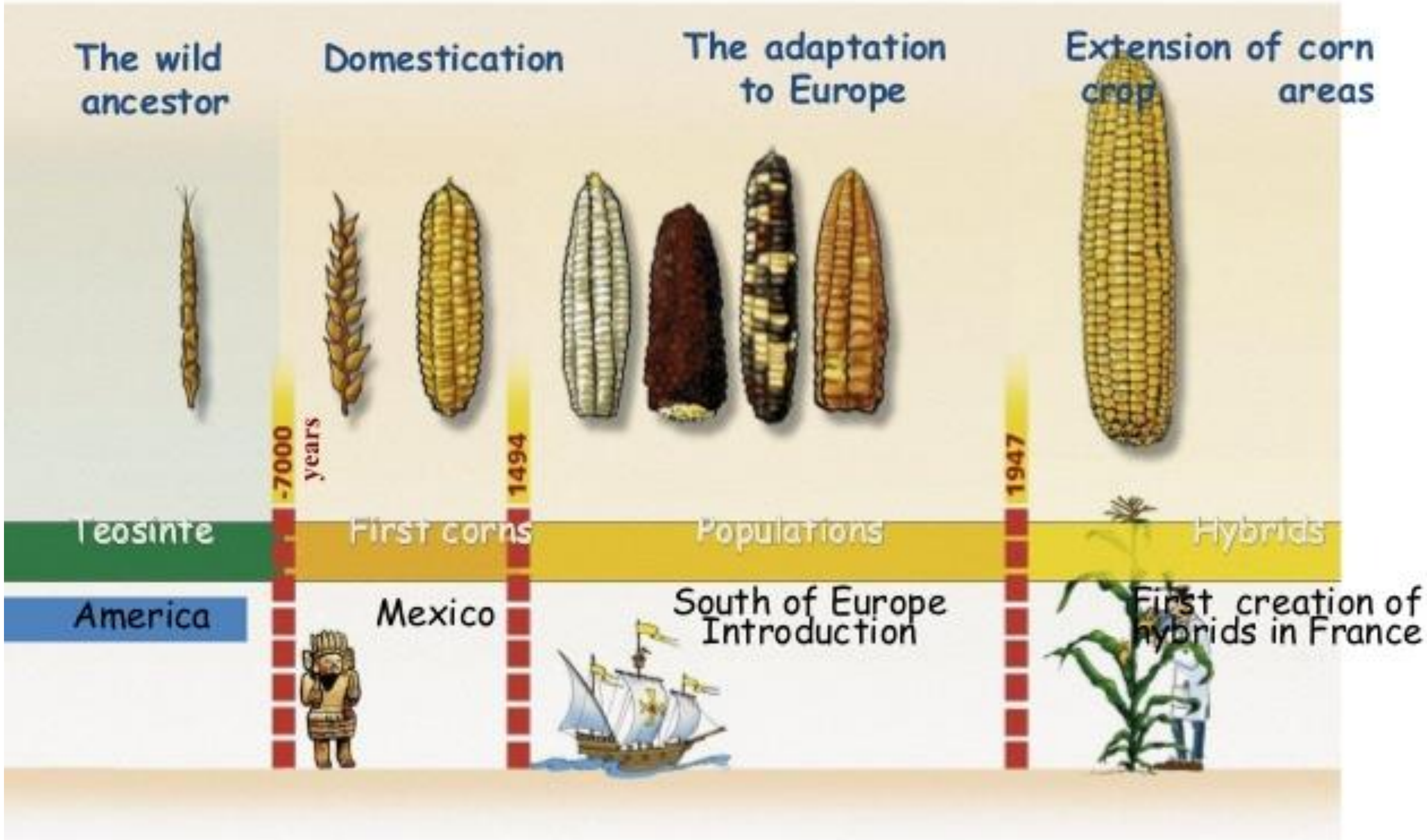


Domesticación de Cultivos

Centers of origin of selected crops

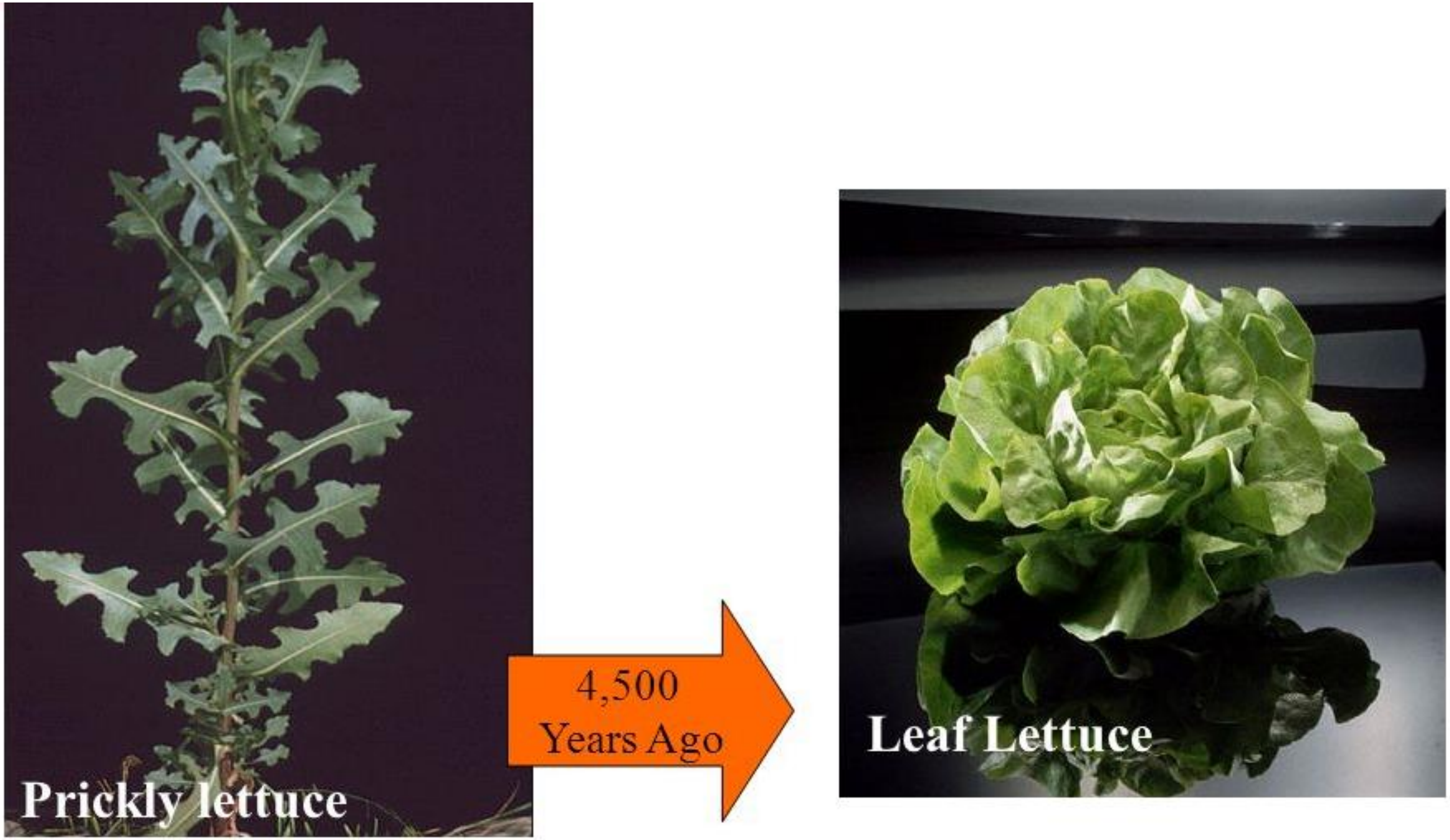
La “Creación” del Maíz





Evolución del Maíz

Domesticación de la Lechuga

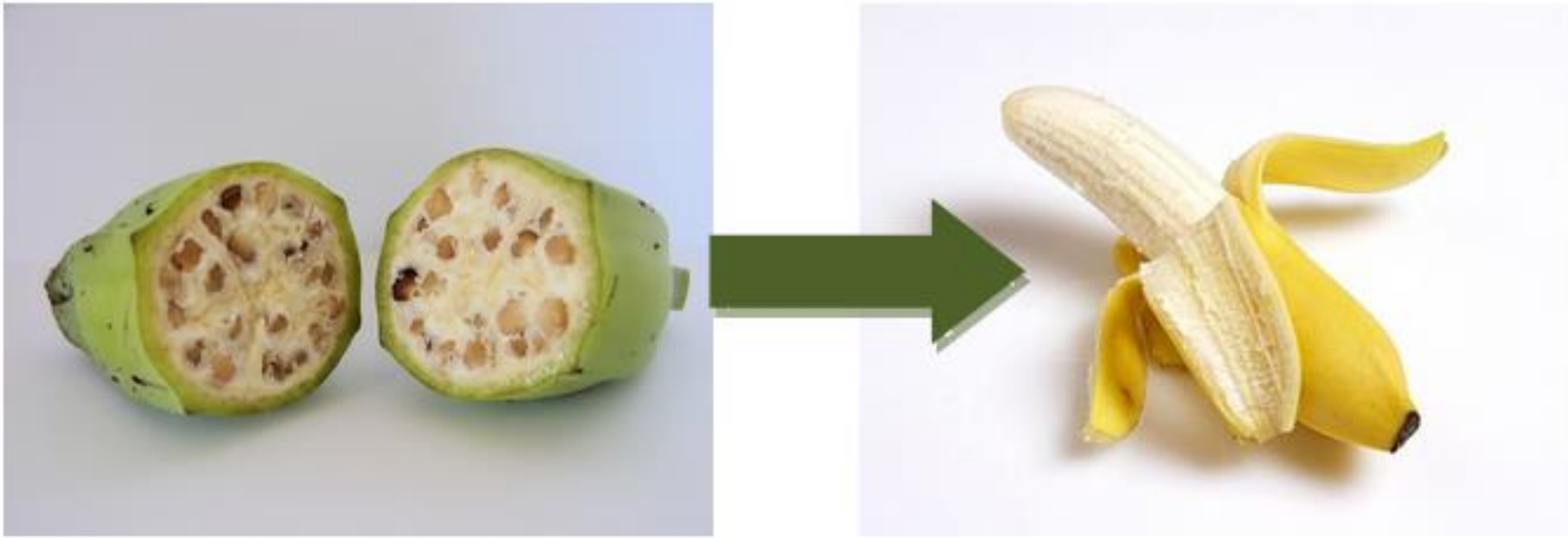


Lots Of
Lettuce
purple kale
Boston bibb
romaine
nappa cabbage
green leaf
cabbage
frisee
spinach
mache
treviso
rainbow chard
arugula
little gem
mesclun
kale
belgian endive
watercrest
collards
redicchio
red leaf
iceberg
dandilion greens
butter lettuce

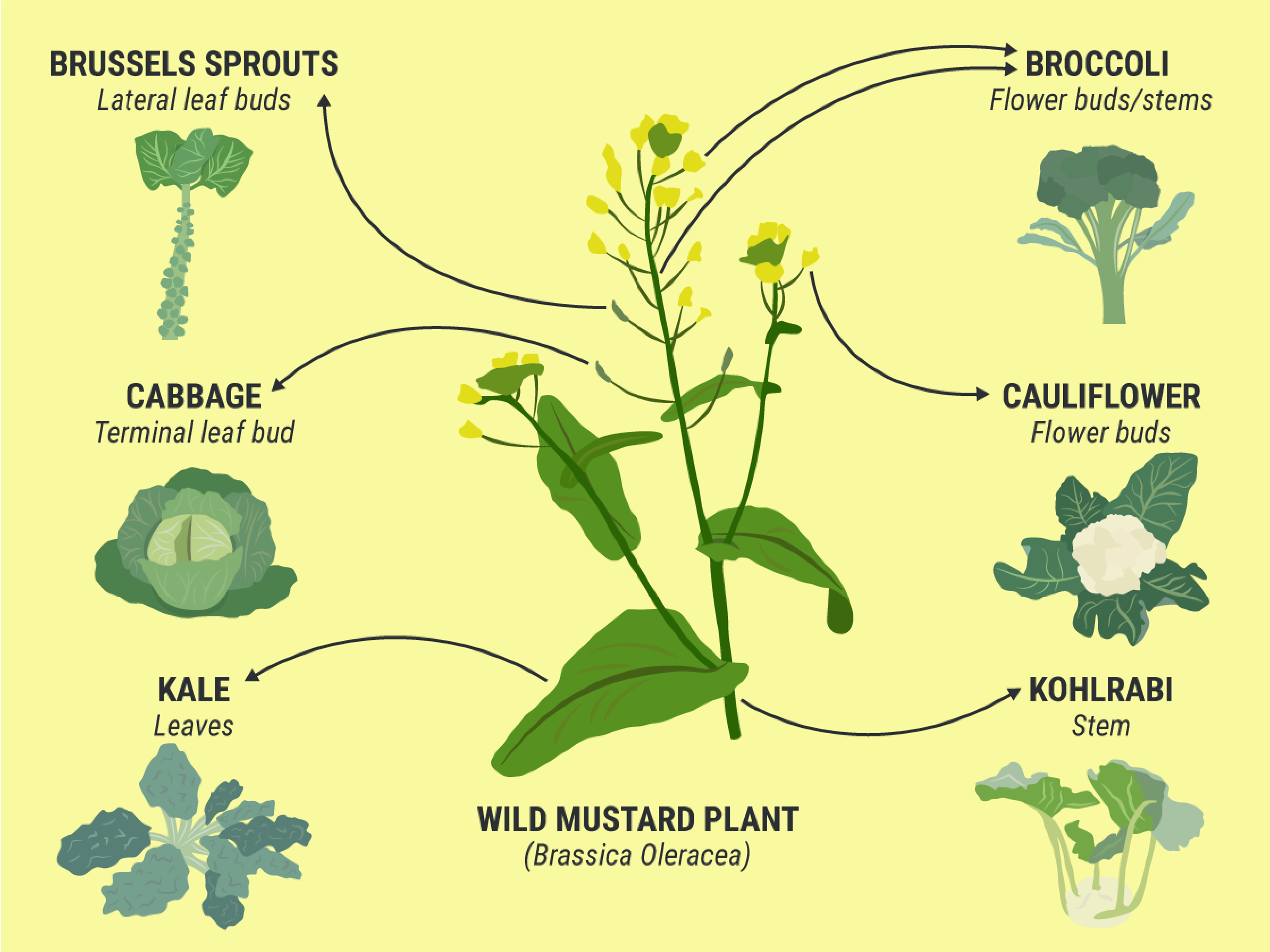
Domesticación de la Zanahoria


Mantis
Expect Big Things
www.mantis.com

Domesticación del Banano



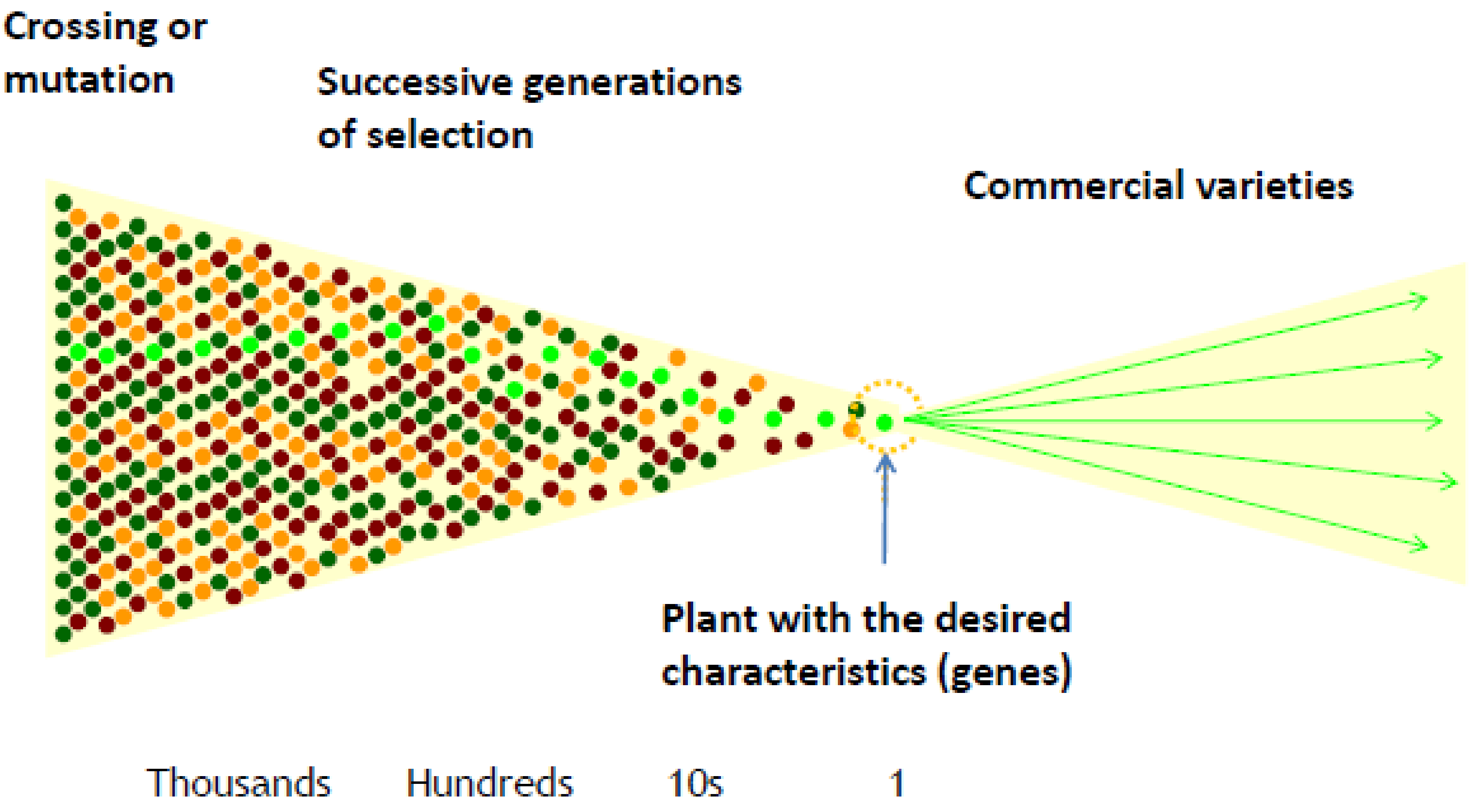
Diversas Variedades del Banano


BRUSSELS SPROUTS
Lateral leaf buds
BROCCOLI
Flower buds/stems
CABBAGE
Terminal leaf bud
CAULIFLOWER
Flower buds
KALE
Leaves
KOHLRABI
Stem
WILD MUSTARD PLANT
(Brassica Oleracea)

Principales Objetivos de los Mejoradores

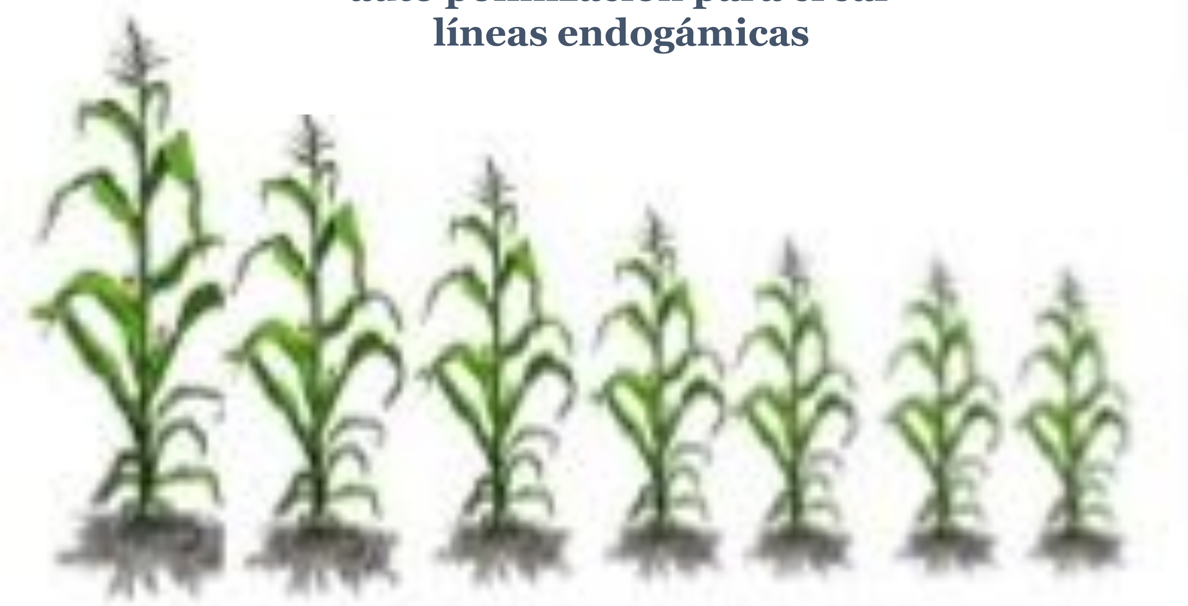
- Tolerancia múltiple a herbicidas
- Resistencia a enfermedades
- Producción de híbridos
- Uso eficiente de nutrientes
- Mejor calidad de aceite, almidón y aa´s esenciales
- Tolerancia al frío y a las heladas
- Tolerancia a la salinidad
- Resistencia a insectos
- Calidad nutritiva

Mejoramiento Tradicional de Plantas



Producción de Híbridos a partir de Líneas Endogámicas

Se necesitan 7 generaciones de auto polinización para crear líneas endogámicas



Depresión endogámica

B73 Mo17

¿Cómo se diferencia el MGP tradicional del actual?

- Aceleración vía marcadores moleculares
- Genético antes que fenotípico
- Identificación de los genes que contribuyen a la característica
- Eficiencia a través del uso de herramientas moleculares y robótica
- Toma menos generaciones
- Adición de genes específicos
- Cambios específicos por genes específicos
- Mayor comprensión de la estructura del genoma

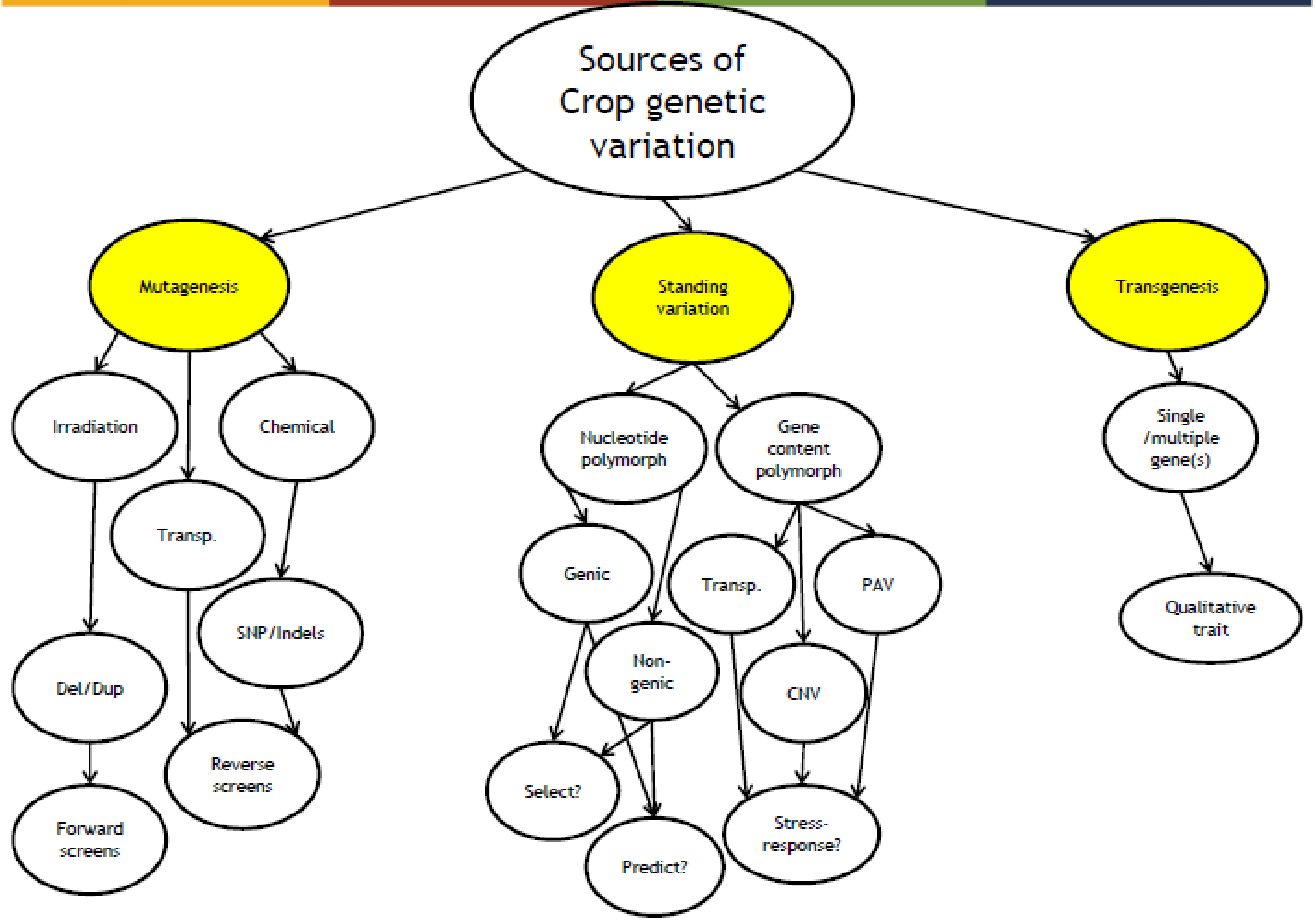

Sources of Crop genetic variation
Mutagenesis
Standing variation
Transgenesis
Irradiation
Chemical
Transp.
Del/Dup
SNP/Indels
Forward screens
Reverse screens
Nucleotide polymorph
Gene content polymorph
Genic
Non-genic
Select?
Predict?
Transp.
PAV
CNV
Stress-response?
Single /multiple gene(s)
Qualitative trait

Table 1 Ten leading institutions developing new plant breeding technologies ranked according to absolute number of publications and number of covered techniques

Institution	Location	Number of publications	Techniques[a]
Wageningen University	Wageningen, The Netherlands	21	C,R,G,B,A
University of California, Riverside	Riverside, CA, USA	11	O,R,G,A
John Innes Centre	Norwich, UK	9	C,R,G,A
J.R. Simplot[b]	Boise, Idaho, USA	9	C
Austrian Academy of Sciences	Salzburg, Austria	9	R
University of Amsterdam	Amsterdam	6	Z,O,C,R
Iowa State University	Ames, Iowa, USA	6	Z
Max-Planck Institute	Koln, Germany	4	O,R,G
University of Michigan	Ann Arbor, Michigan, USA	4	C,Z
Institute of Plant Genetics and Crop Plant Research (IPK)	Gatersleben, Germany	4	O,G

[a]Each technique is represented by a letter. Z, ZFN; O, ODM; C, cisgenesis/intragenesis; R, RdDM; G, grafting; B, reverse breeding; A, agro-infiltration. [b]Private institution.

Nuevas Tecnologías de Mejoramiento Genético de Plantas

- Tecnología de las Nucleasas de los Dedos de Zinc
- CRISPR/Cas9
- Mutagénesis de Oligonucleótido Dirigida
- Metilación del ADN dependiente del ARN
- Cisgénesis e Intragénesis
- Grafting
- Reverse Breeding
- Agroinfiltración

NPBT: New Plant Breeding Techniques

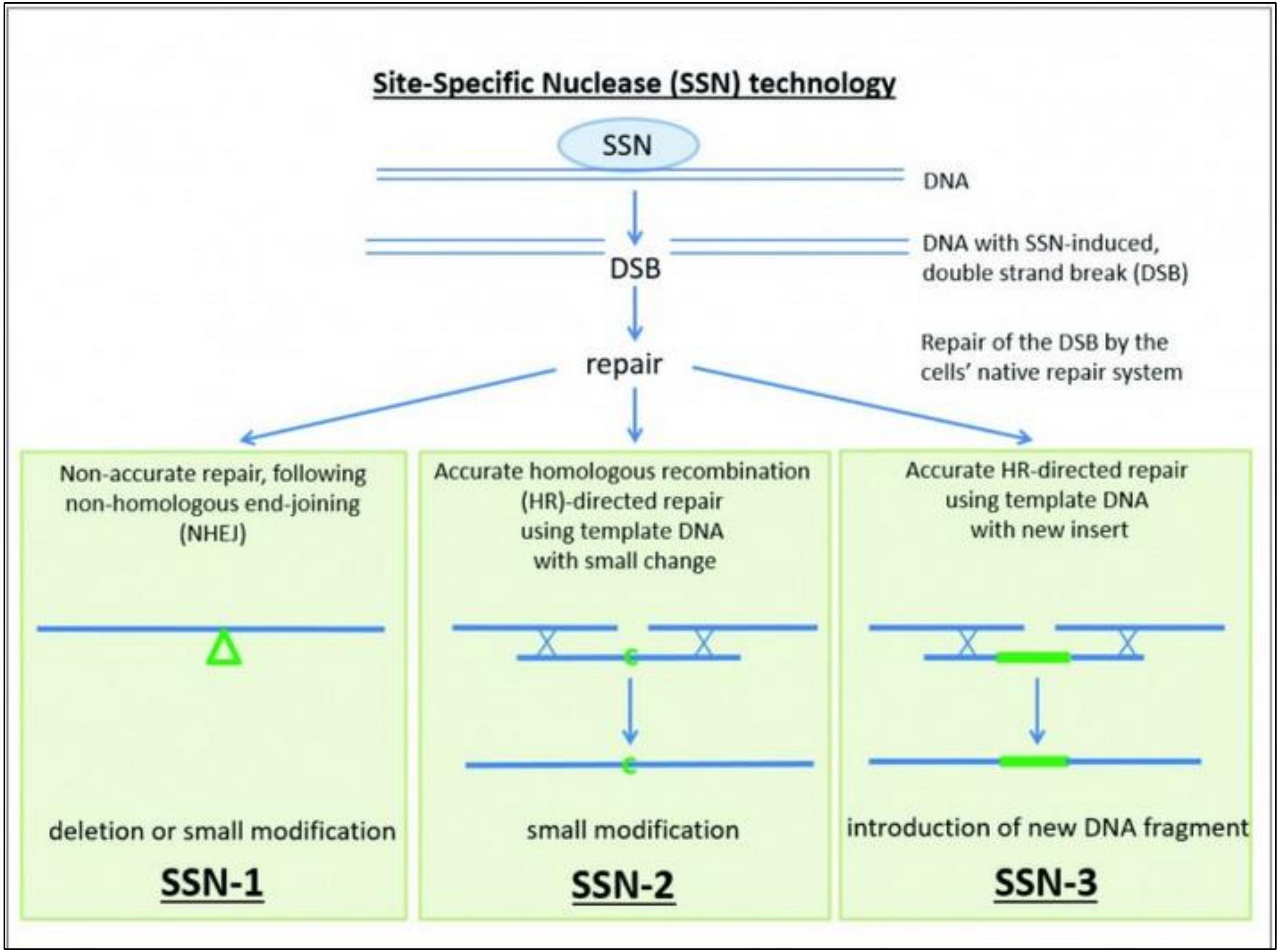
BOX 2

Groups of NPBTs

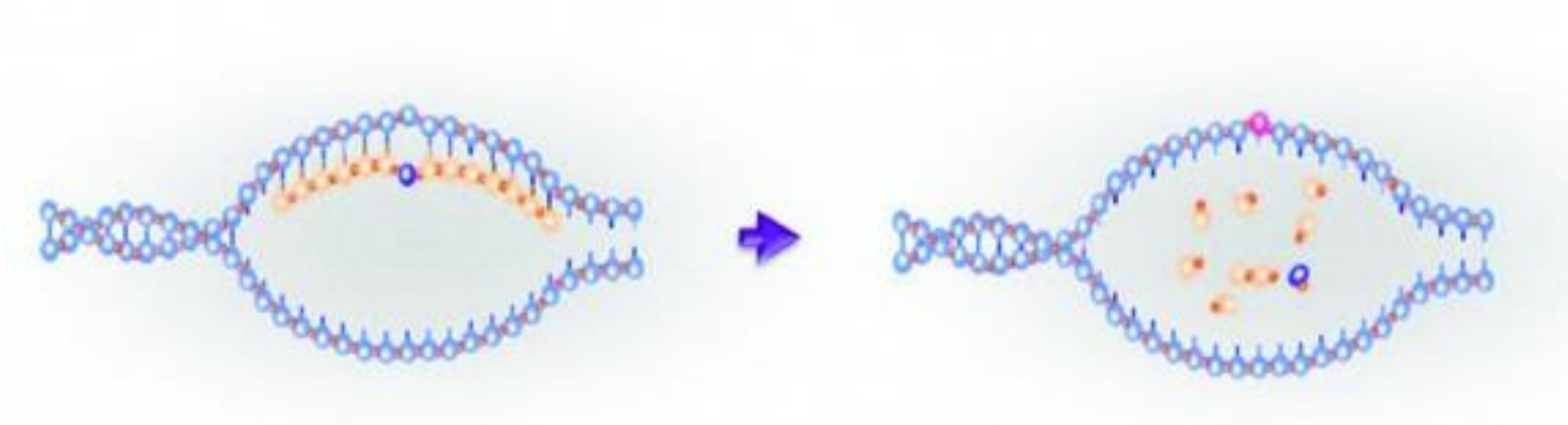
Group 1:	**Site specific mutagenesis**	Zinc Finger Nuclease (ZFN) technique Meganuclease (MN) technique Transcription Activator-Like Effector Nuclease (TALEN) technique Oligonucleotide-Directed Mutagenesis (ODM)
Group 2:	**Cisgenesis and Intragenesis**	Cisgenesis Intragenesis
Group 3:	**Breeding with transgenic inducer line**	RNA-dependent DNA methylation (RdDM*) Reverse breeding Accelerated breeding following early flowering
Group 4:	**Grafting techniques**	Grafting on GM rootstock
Group 5:	**Agro-infiltration techniques**	Agro-infiltration 'sensu stricto' Agro-infection Floral dip

* RdDM leads to changes in the methylation status of the genome (epigenetic effect) but not to changes in the DNA sequence. Our view of a genome change is confined to changes in the DNA sequence.

Mutagénesis de Sitio Dirigido

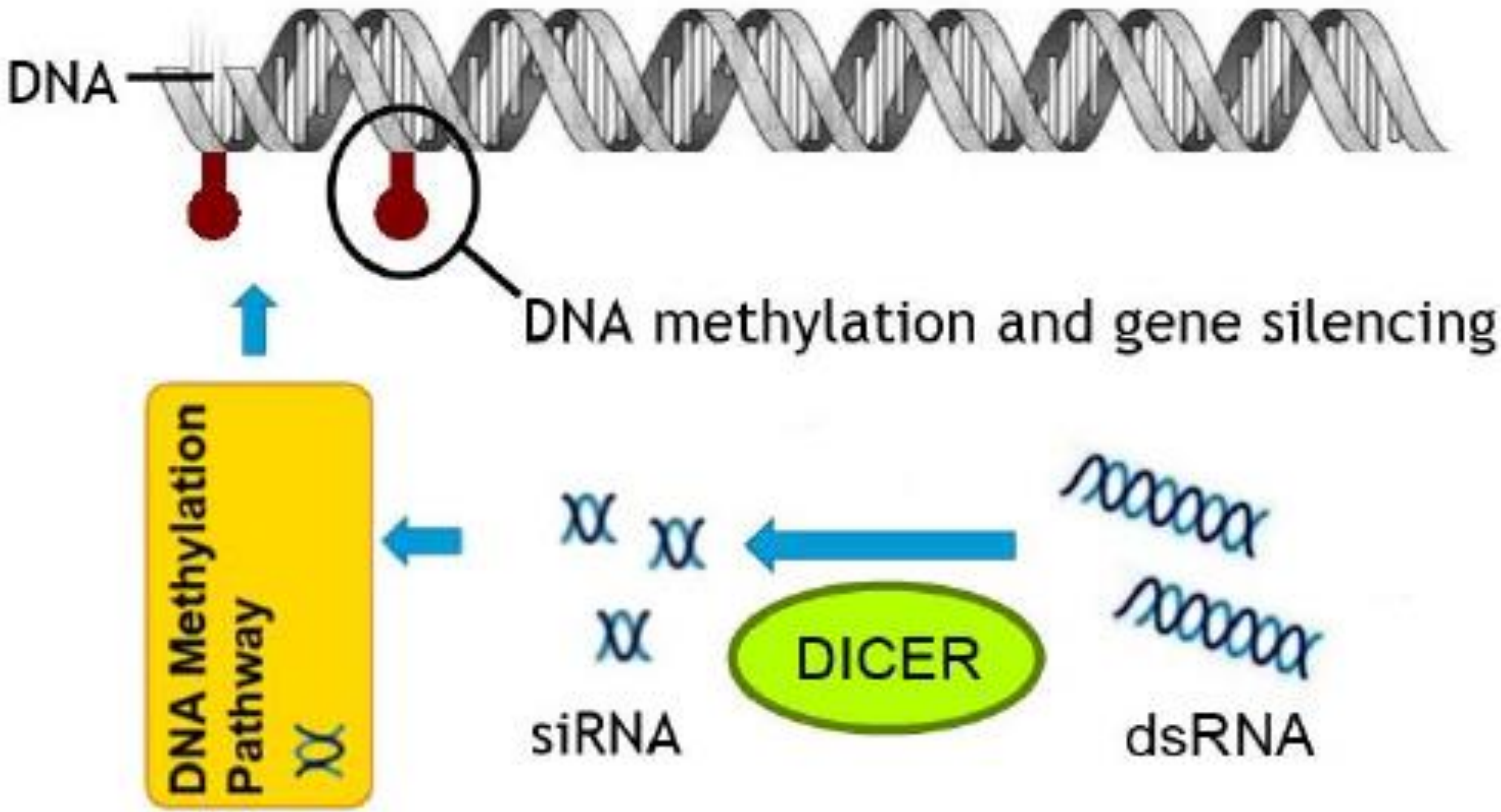


Mutagénesis Dirigida por Oligonucléotido (ODM)

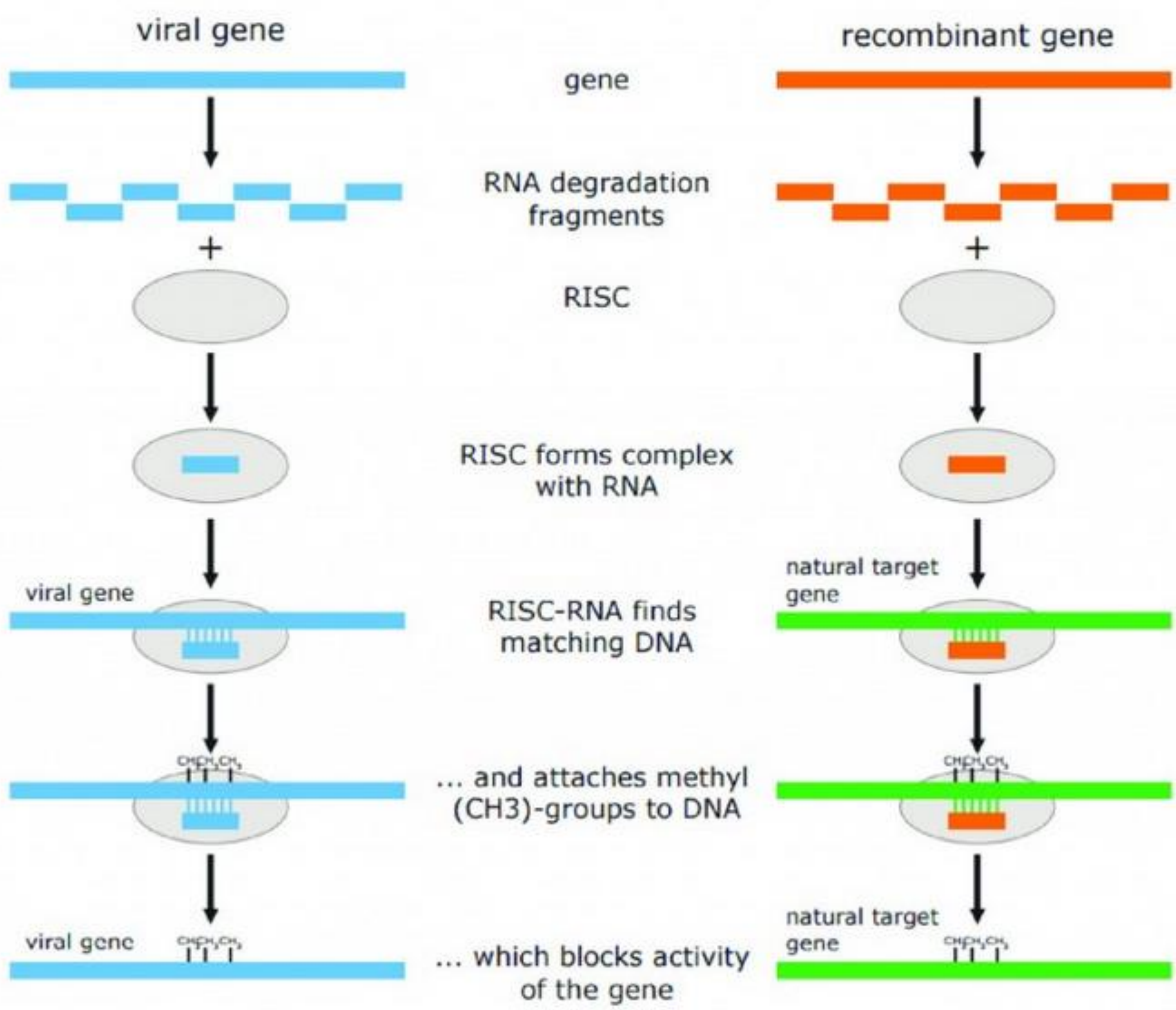


Solo funciona con plantas que pueden ser regeneradas a partir de protoplastos

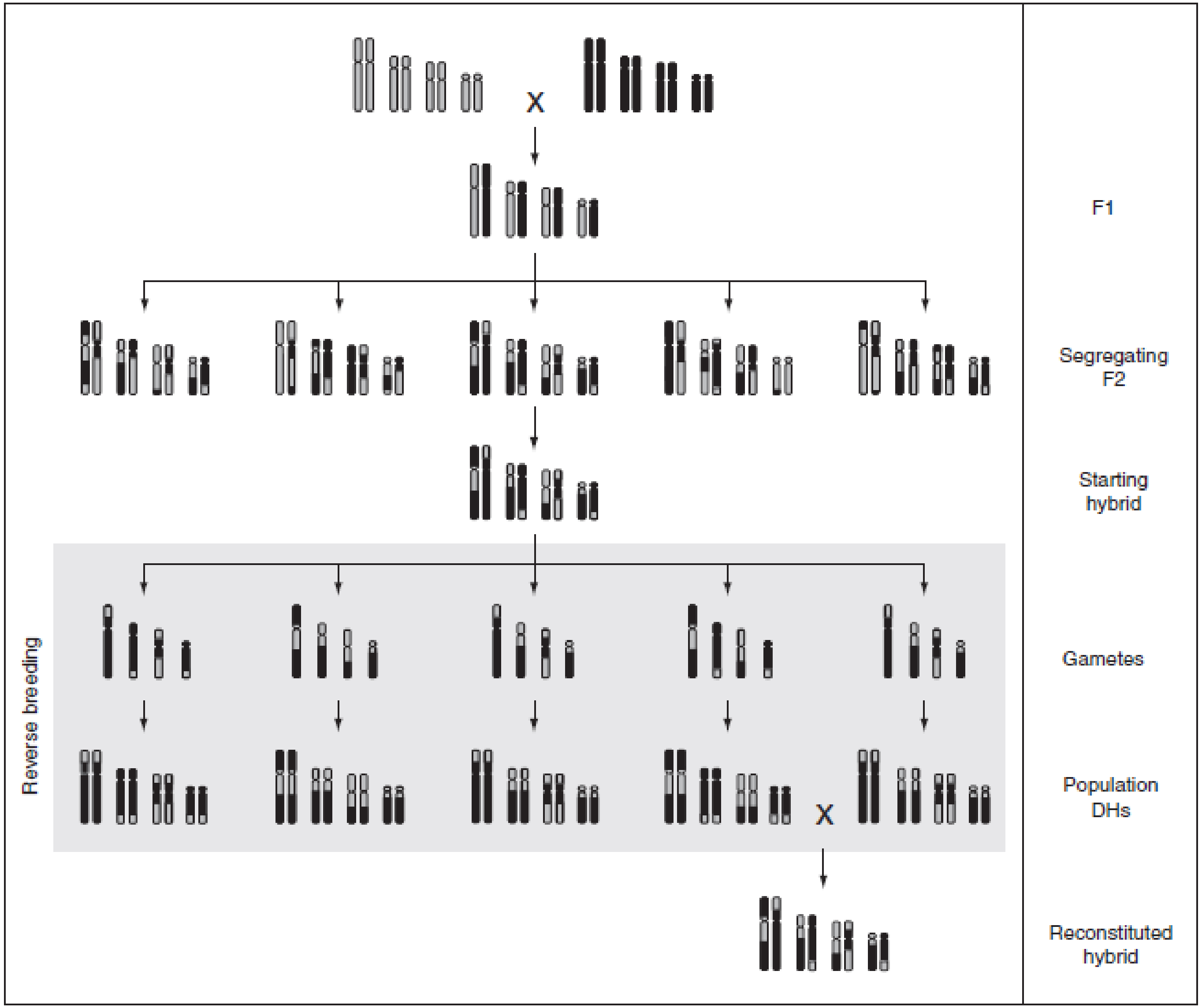
RNA-dependent DNA Methylation



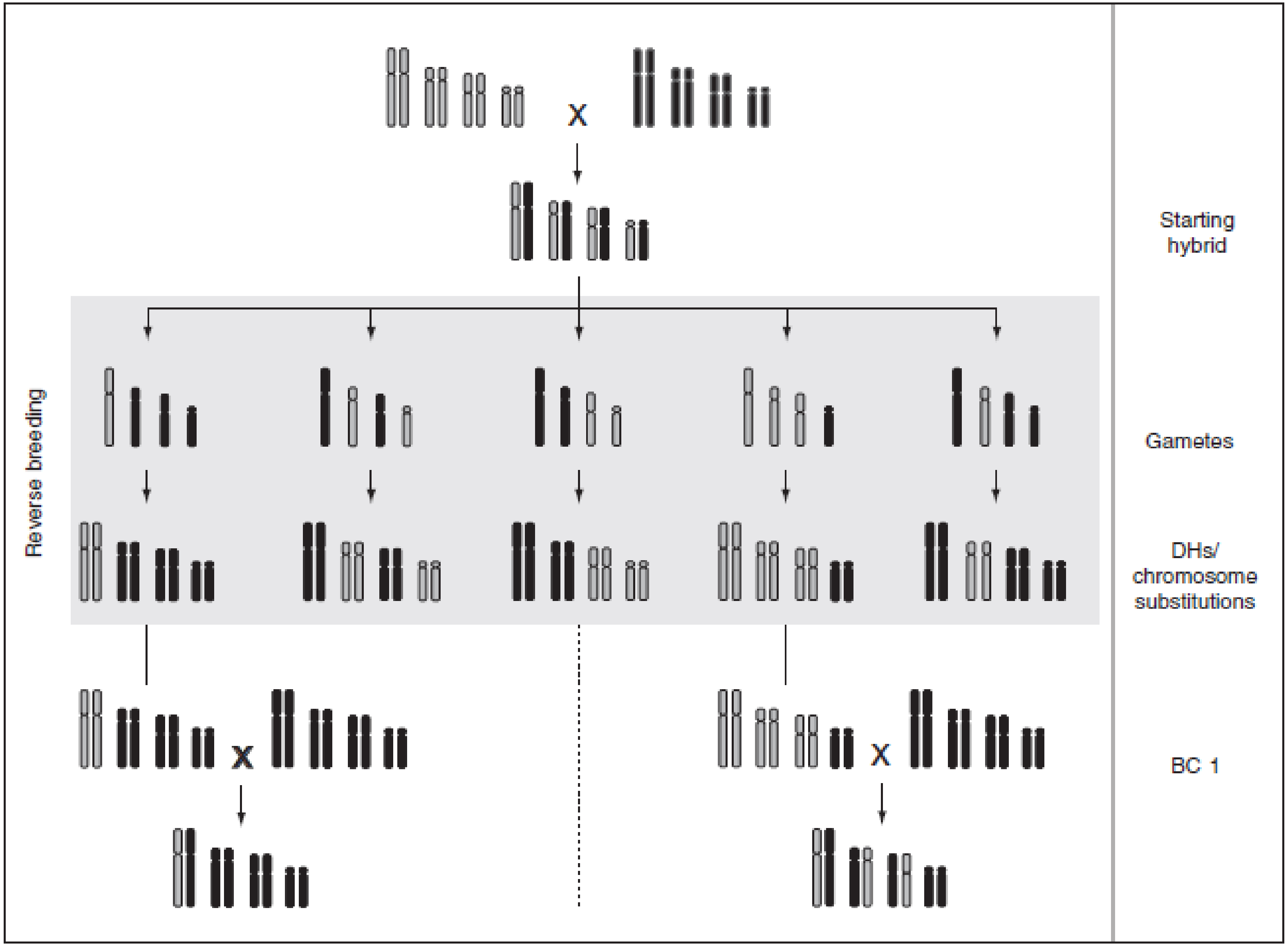
RdDM en detalle



Reverse Breeding (I)



Reverse Breeding (II)

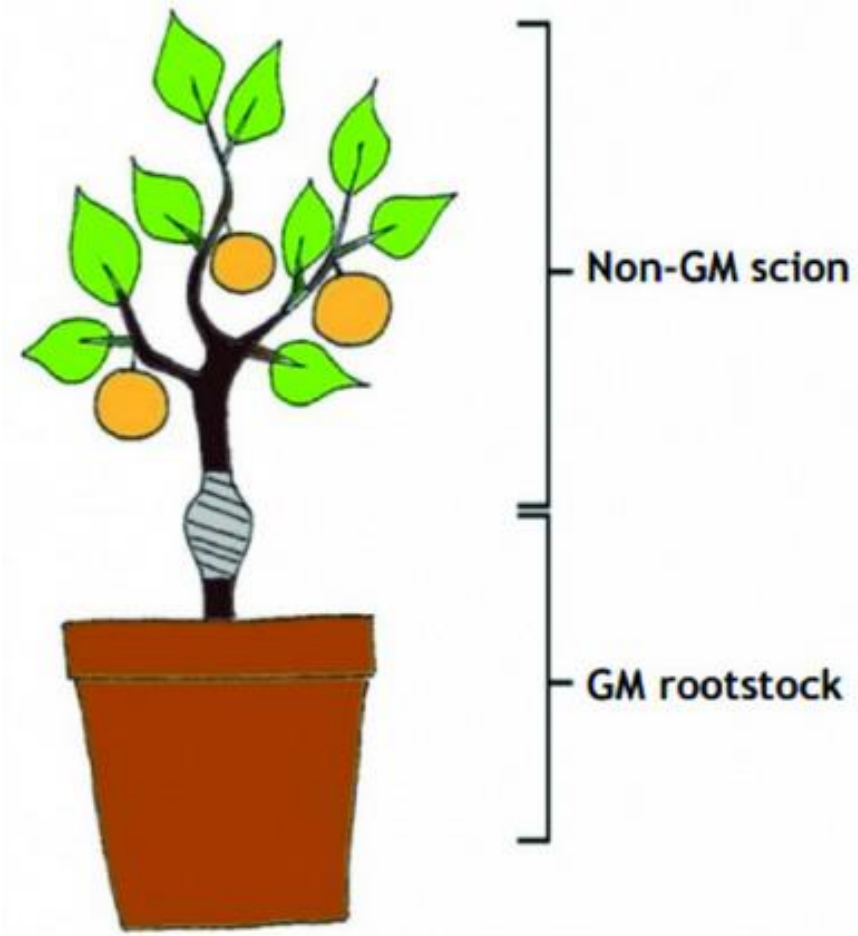


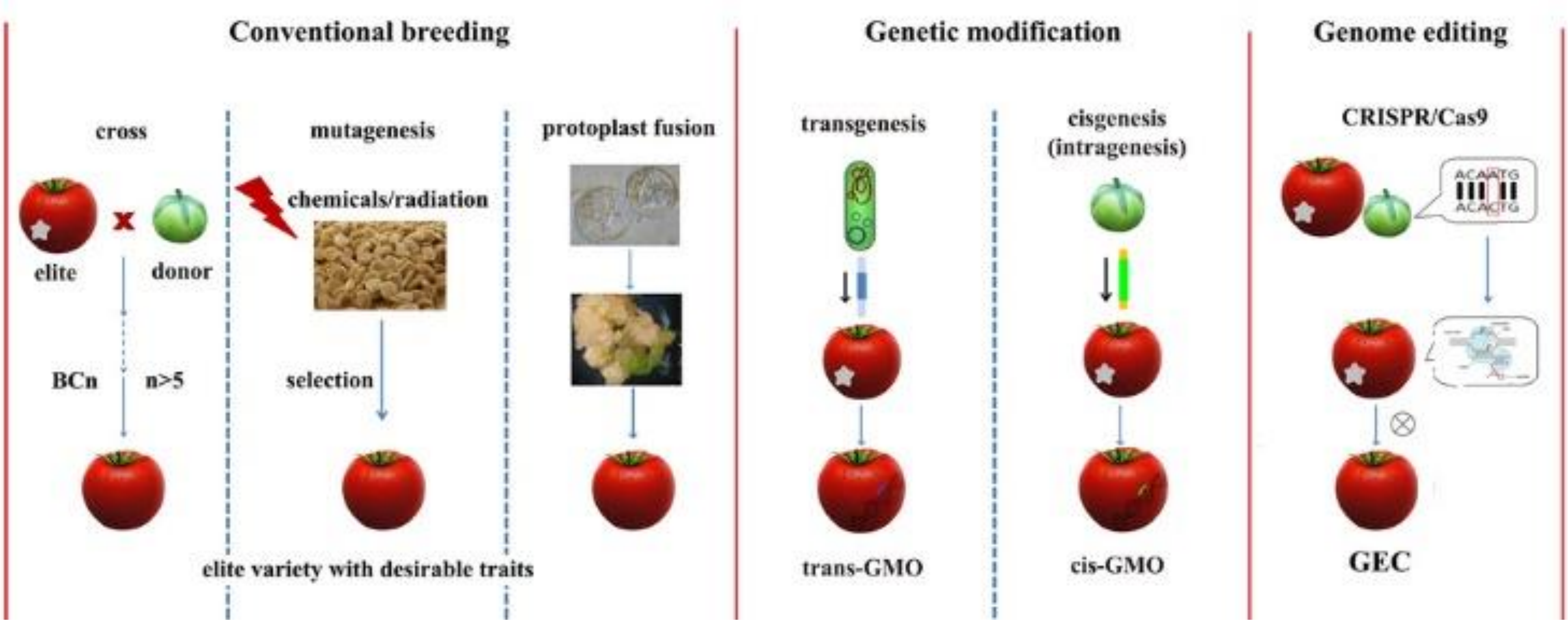
Reverse Breeding (III)

Table 1 Number of non-recombinant DHs required for reconstructing the original starting plant at different probability levels in various species

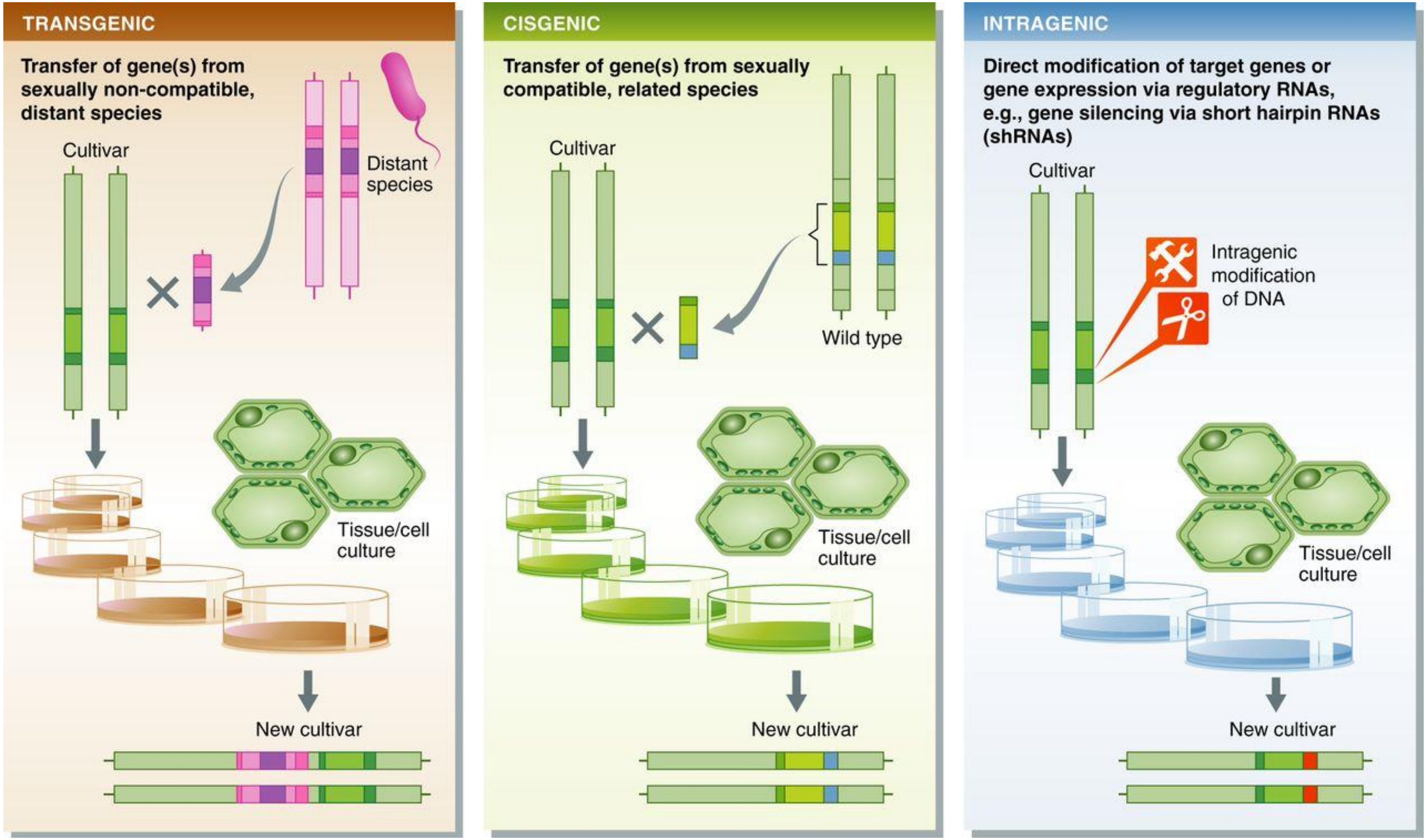
Haploid chromosome number (x)	Probability				Model species/crop
	0.90	0.95	0.99	1.00	
5	13	14	18	47	*Arabidopsis*
6	18	20	25	67	Spinach, corn salad
7	25	28	35	94	Cucumber
8	35	40	49	133	Onion
9	49	56	69	188	Barley, carrot, sugarbeet, most vegetable Brassicas, lettuce
10	69	79	98	266	Maize, sorgum, asparagus, cocoa
11	98	111	138	377	Banana, watermelon, celery, fennel, common bean
12	138	157	195	532	Tomato, pepper, melon, rice, egg plant

Injerto de Patrón OGM (Grafting)





Conventional breeding
Genetic modification
Genome editing
cross
mutagenesis
protoplast fusion
transgenesis
cisgenesis
(intragenesis)
CRISPR/Cas9
elite
donor
chemicals/radiation
ACAATG
ACACTG
BCn
n>5
selection
elite variety with desirable traits
trans-GMO
cis-GMO
GEC



TRANSGENIC
Transfer of gene(s) from sexually non-compatible, distant species
Cultivar
Distant species
Tissue/cell culture
New cultivar
CISGENIC
Transfer of gene(s) from sexually compatible, related species
Cultivar
Wild type
Tissue/cell culture
New cultivar
INTRAGENIC
Direct modification of target genes or gene expression via regulatory RNAs, e.g., gene silencing via short hairpin RNAs (shRNAs)
Cultivar
Intragenic modification of DNA
Tissue/cell culture
New cultivar

Aceleración de la Domesticación de Plantas Silvestres

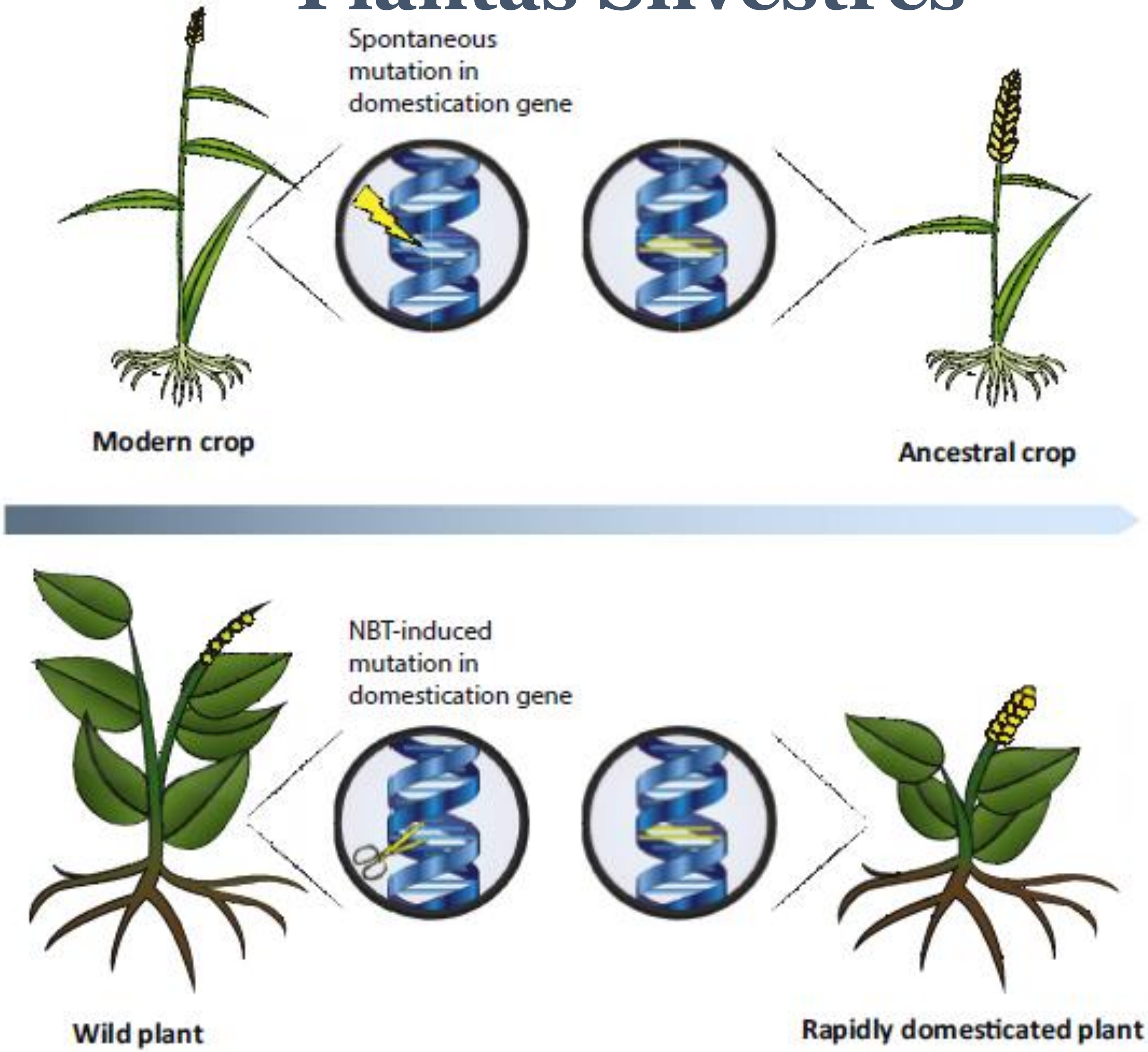
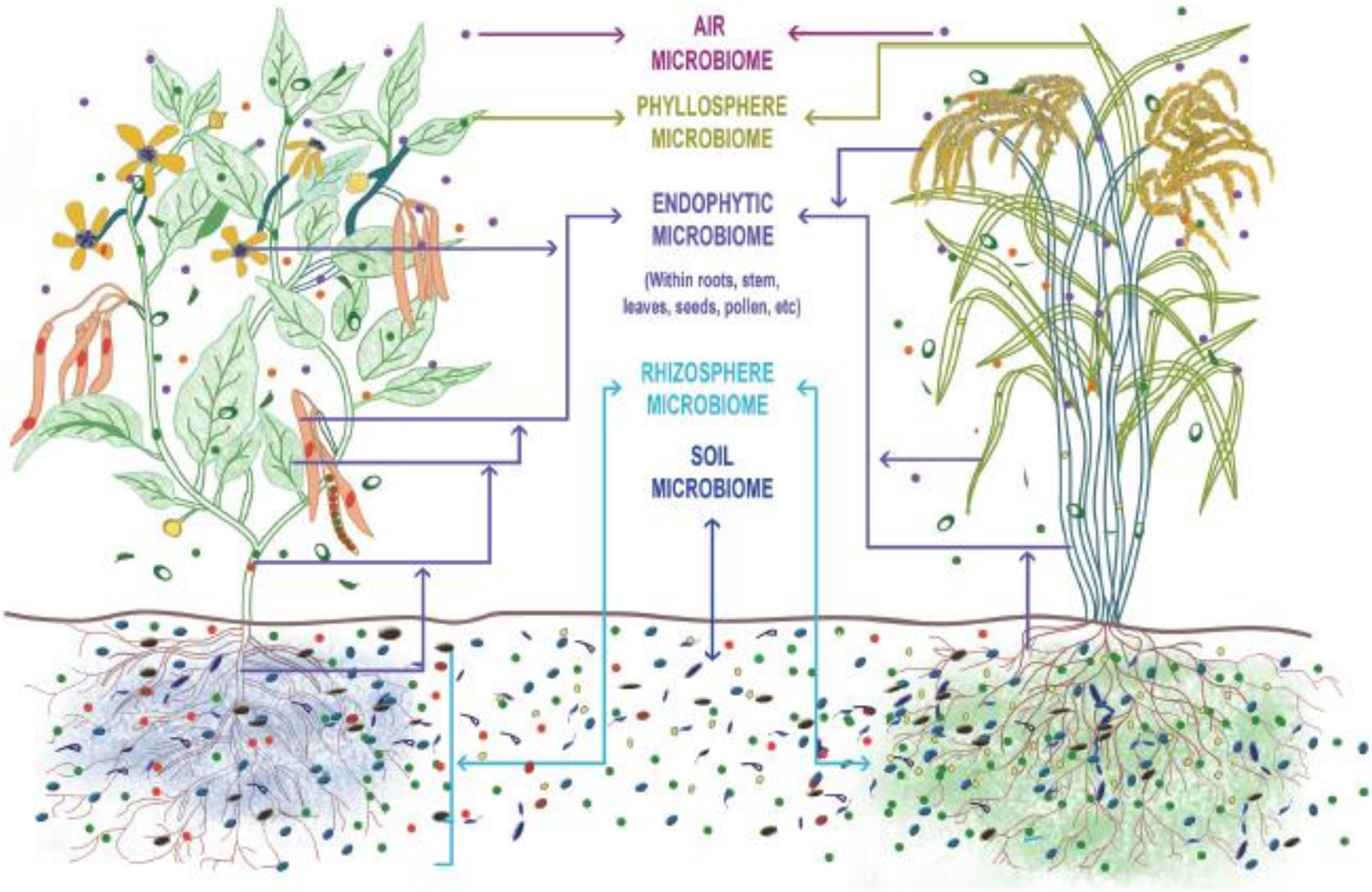


Table 1. Examples of Genes Important for Crop Domestication Where Mutations Creating Similar Loss of Function Could Be Achieved By Genome Editing

Crop	Gene	Function	Trait	Mutation	Refs
Barley (*Hordeum vulgare*)	*Btr1/2*	Unknown	Brittle rachis	Shortened transcript[a]	[83]
	Vrs1	Transcription factor, HD-ZIP	Inflorescence structure	Shortened transcript	[84]
Maize (*Zea mays*)	*Tga1*	Transcription factor, SBP	Inflorescence structure	Amino acid change[b]	[85]
	ZmSh1-1/1-5	Transcription factor, YABBY-like	Seed shattering	Structural change/ regulatory change[c,d]	[86]
Rice (*Oryza sativa*)	*LABA1*	Cytokinin-activating enzyme secreted peptide	Inflorescence structure	Shortened transcript	[26]
	RAE2	EPF/EPFL family	Inflorescence structure	Shortened transcript	[27]
	sh1	Transcription factor, YABBY-like	Seed shattering	Regulatory change[d]	[86]
	sh4	Transcription factor, Myb3	Seed shattering	Amino acid change[d]	[87]
	qSH1	Transcription factor, BEL1-type	Seed shattering	Regulatory change	[88]
	PROG1	Transcription factor, C2H2-type	Plant structure	Amino acid change	[89,90]
Sorghum (*Sorghum bicolor*)	*SbSh1*	Transcription factor, YABBY-like	Seed shattering	Shortened transcript/ regulatory change[d]	[86]
Tomato (*Solanum lycopersicum*)	Style2.1	Transcription factor, HLH	Inflorescence structure	Regulatory change	[91]

El Microbioma de la Planta



Gracias, ¿preguntas?