The Environmental Impact of Biotechnology:

current status and new technologies

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"La Biotecnología Moderna y sus Impactos en la Agricultura"

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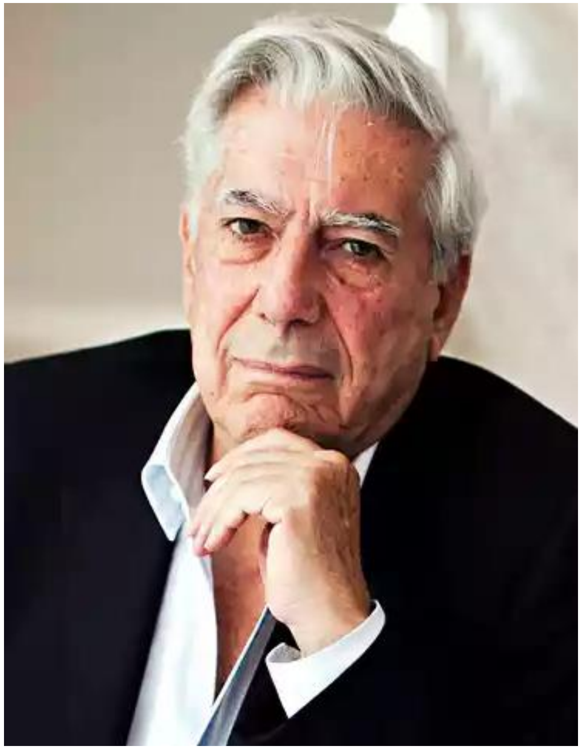
Overview

- It is all in the DNA:
  Domestication, Breeding and Genetic Engineering
- What traits have been and can be engineered?
- What is the potential of new biotechnology?
- What can go wrong?
- What can we do about it?
- What is the potential for Peru?

What makes the difference?



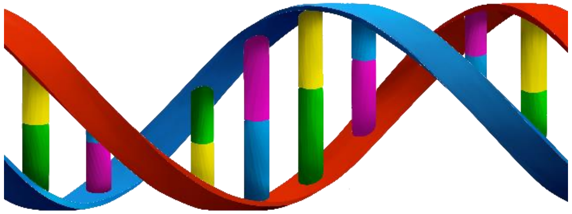
Mario Vargas Llosa
Nobel Price for Literature

Genetic similarity human

human	99.9%
chimp	96%
cat	90%
mouse	85%
fly	61%
banana	60%

Ramsey and Lee, 2016

- A-T-G-C –



-T-A-C-G -

Carlos Bustamante
NAS, Scientist

Both have about 3.2 Gbp (Giga base pairs)
= pairs of A-T or G-C.

Giga = 10^9 or 1.000.000.000

What kind of mutations can occur?

Single Nucleotide Polymorphisms (SNPs)

Substitution

```
A-T-T-G-A-C-A-A-T
A-T-T-G-G-C-A-A-T
```

Insertion

```
A-T-T-G-A - C-A-A-T
A-T-T-G-A-T-C-A-A-T
```

Deletions

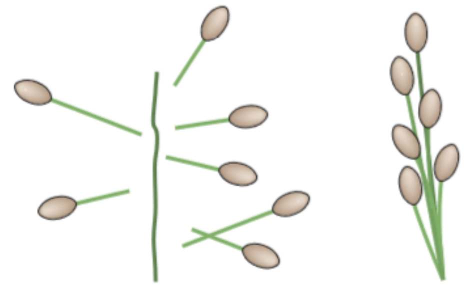
```
A-T-T-G-A-C-A-A-T
A-T-T-G - C-A-A-T
```

SNPs occur normally once in every 300 nucleotides on average, which means there are roughly **10 million SNPs in the human genome**.

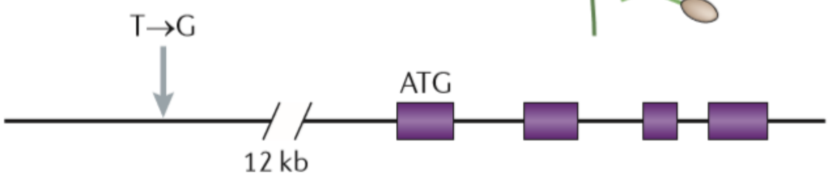
Does a SNP make a difference?

Most of the time, they do not, but sometimes they make all the difference:

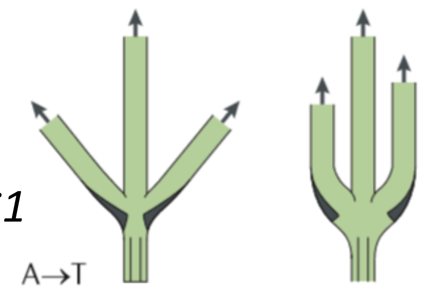
Oryza sativa qSH1



non-shattering phenotype in rice and wheat



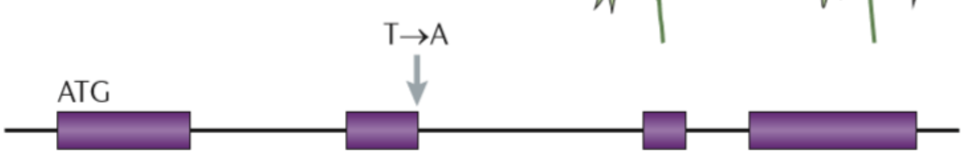
Oryza sativa PROG1



erect growth in domesticated Asian rice varieties

Phaseolus vulgaris TFL1

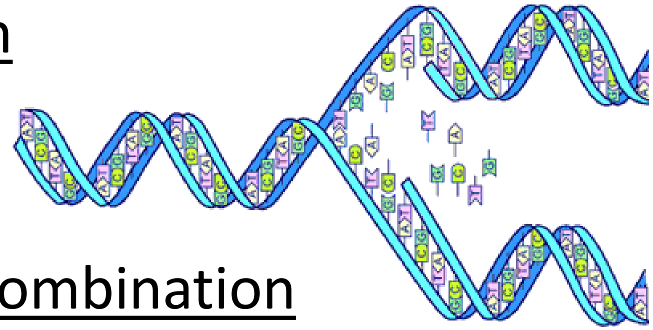
determinate inflorescences in bean



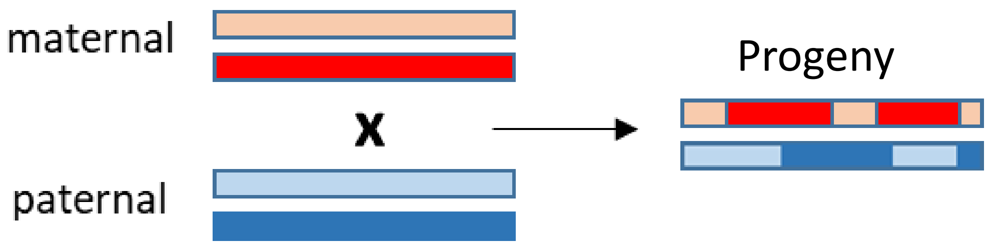
How does the DNA “naturally” change?

Errors during replication

Cells duplicate DNA
before they divide

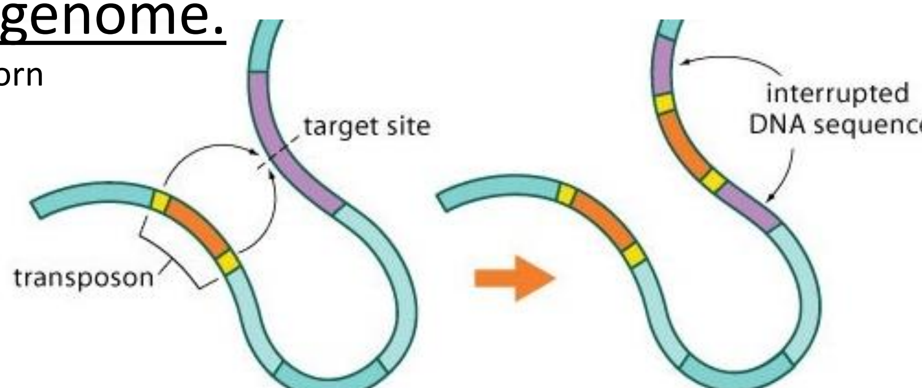


Errors during repair/recombination

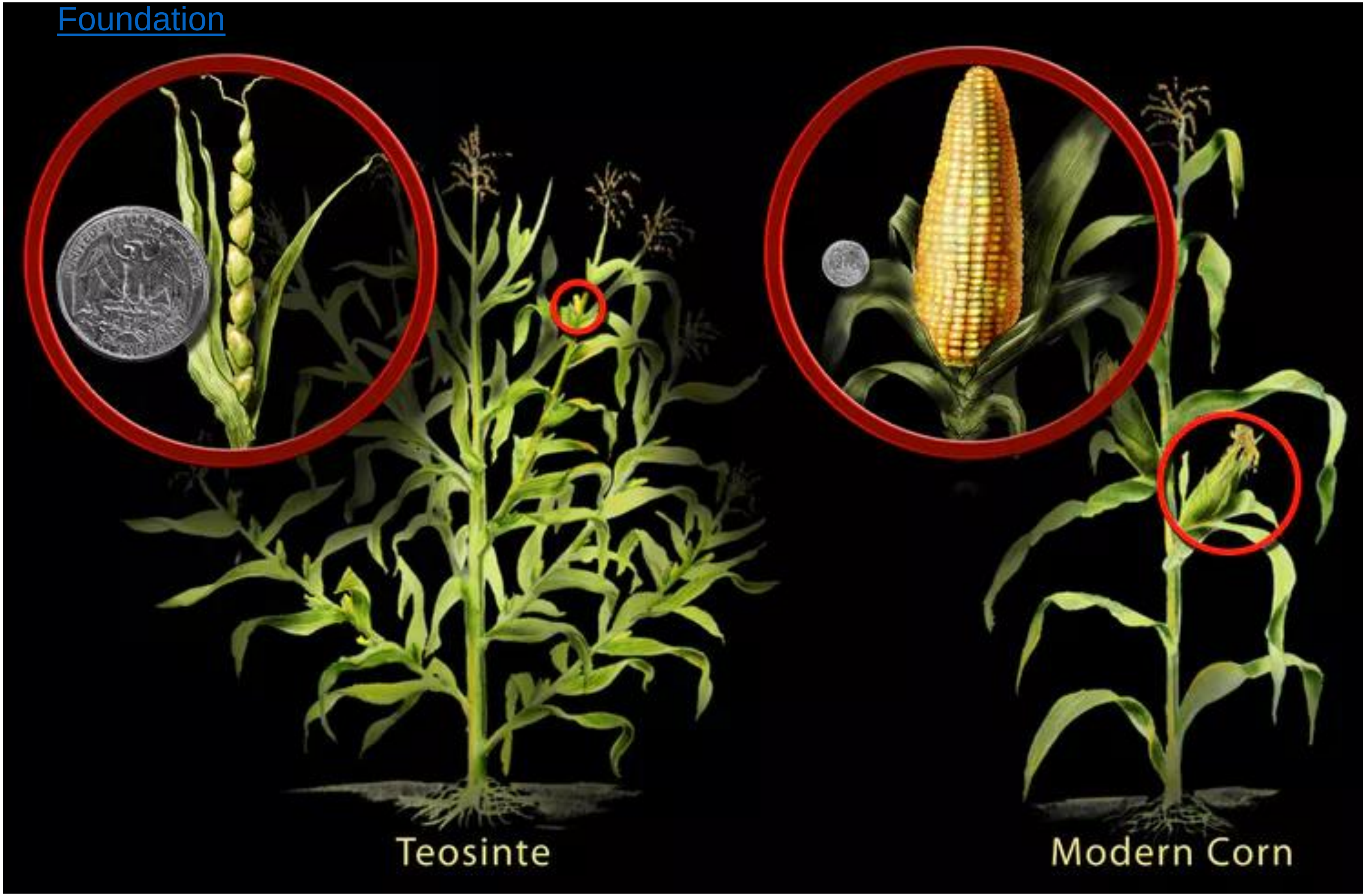


Transposons are DNA segments that jump around in the genome.

30,000 transposons in corn



Modern cultivated corn was domesticated from teosinte, an ancient grass, over more than 6,000 years through conventional breeding. Nicole Rager Fuller, National Science Foundation

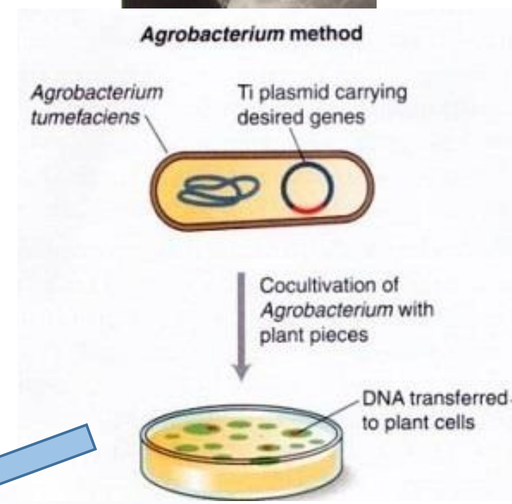


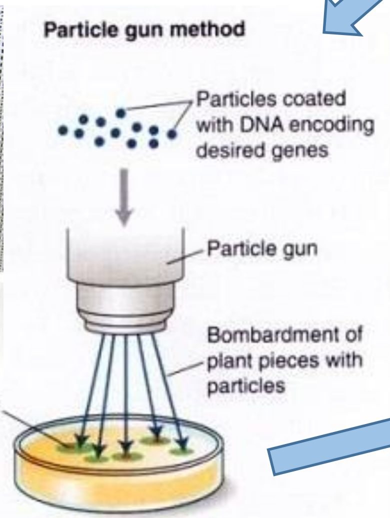
Selection and breeding resulted in a loss of about 30% of genetic variation

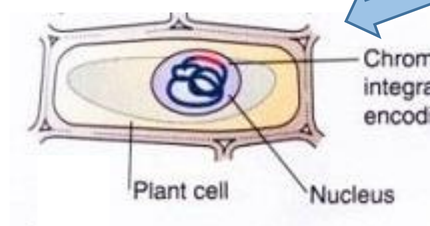
Biotechnology – How do we transfer genes?

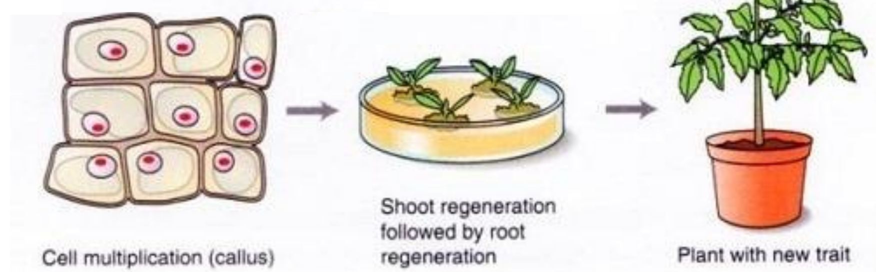
All cells in an organism contain the same DNA

Specific DNA pieces can be cut and pasted into other DNA



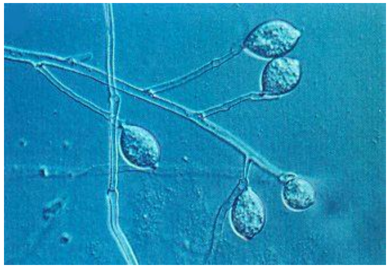






Biotechnology – generating genetic changes in a laboratory

Cisgenesis: transferring a gene within the same species



Phytophthora infestans

infection

Conventional treatment: spray fungicide

Tomato blight

potato blight

Late blight is the major threat to potato production, responsible for yield losses of around 16 % of the global crop and representing an annual financial loss of approximately € 6 billion (Haverkort *et al.* 2016).

Wild potato

Resistance gene

Rpi2

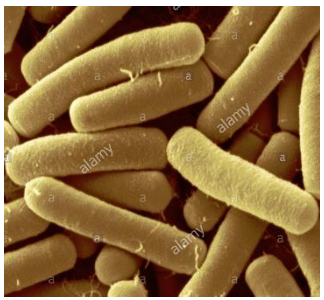
Single dominant resistance gene, *Rpi2*, from the wild species *Solanum pinnatisectum* confers broad spectrum resistance to late blight.

Resistant varieties remained healthy with an average 80% to 90% reduction of the fungicide input.

Biotechnology – generating genetic changes in a laboratory

Transgenesis: transferring a gene between different species or introducing synthetic genes

Lepidoptera larvae

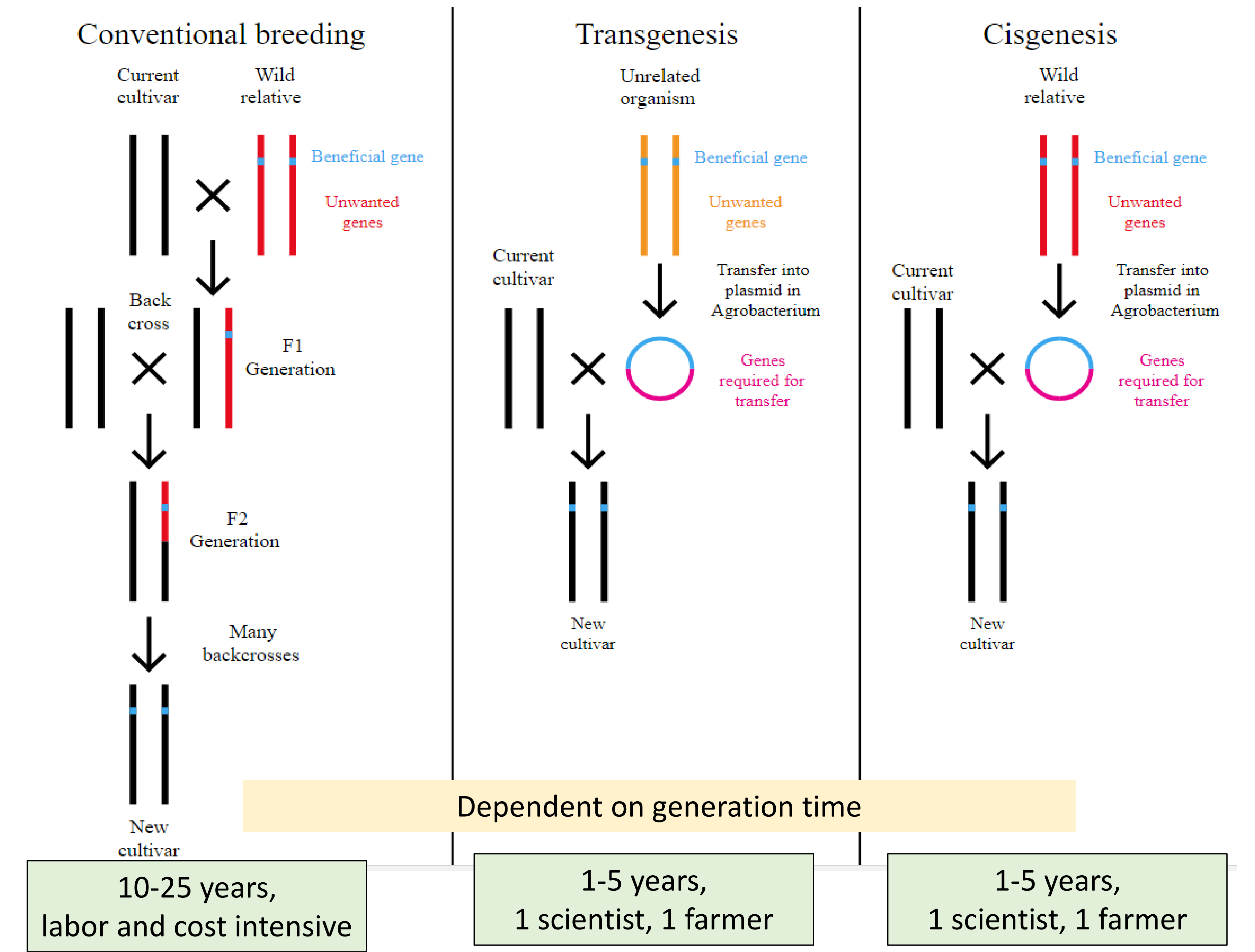


Bacillus thuringiensis

Bt endotoxin gene

Bt corn

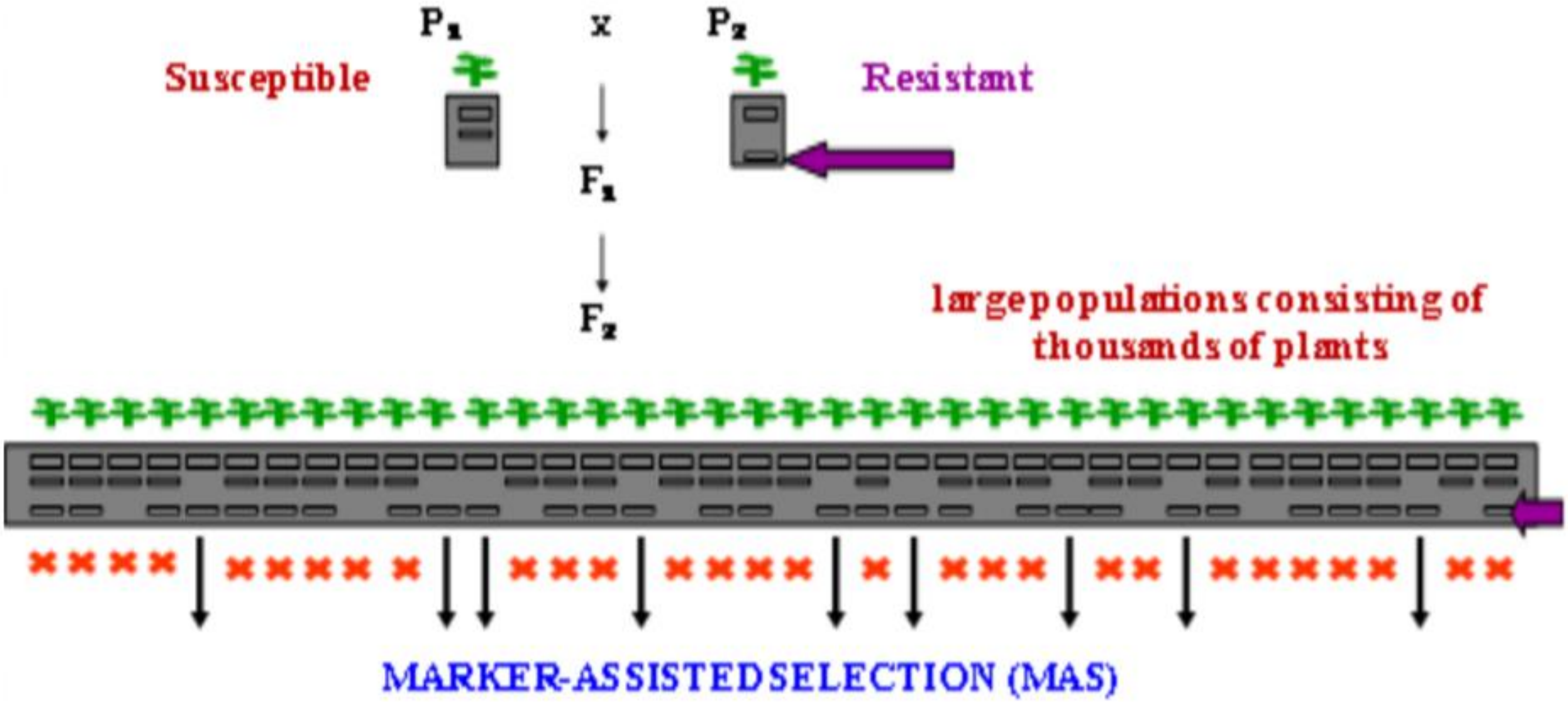
Bacillus thuringiensis gene of interest produces a protein called delta endotoxin that kills Lepidoptera larvae, in particular, European corn borer.



Conventional breeding
Current cultivar
Wild relative
Beneficial gene
Unwanted genes
Back cross
F1 Generation
F2 Generation
Many backcrosses
New cultivar
Transgenesis
Unrelated organism
Beneficial gene
Unwanted genes
Current cultivar
Transfer into plasmid in Agrobacterium
Genes required for transfer
New cultivar
Cisgenesis
Wild relative
Beneficial gene
Unwanted genes
Current cultivar
Transfer into plasmid in Agrobacterium
Genes required for transfer
New cultivar
Dependent on generation time
10-25 years, labor and cost intensive
1-5 years, 1 scientist, 1 farmer
1-5 years, 1 scientist, 1 farmer

New Molecular Breeding Technologies: **M**arker **A**ssisted **S**election

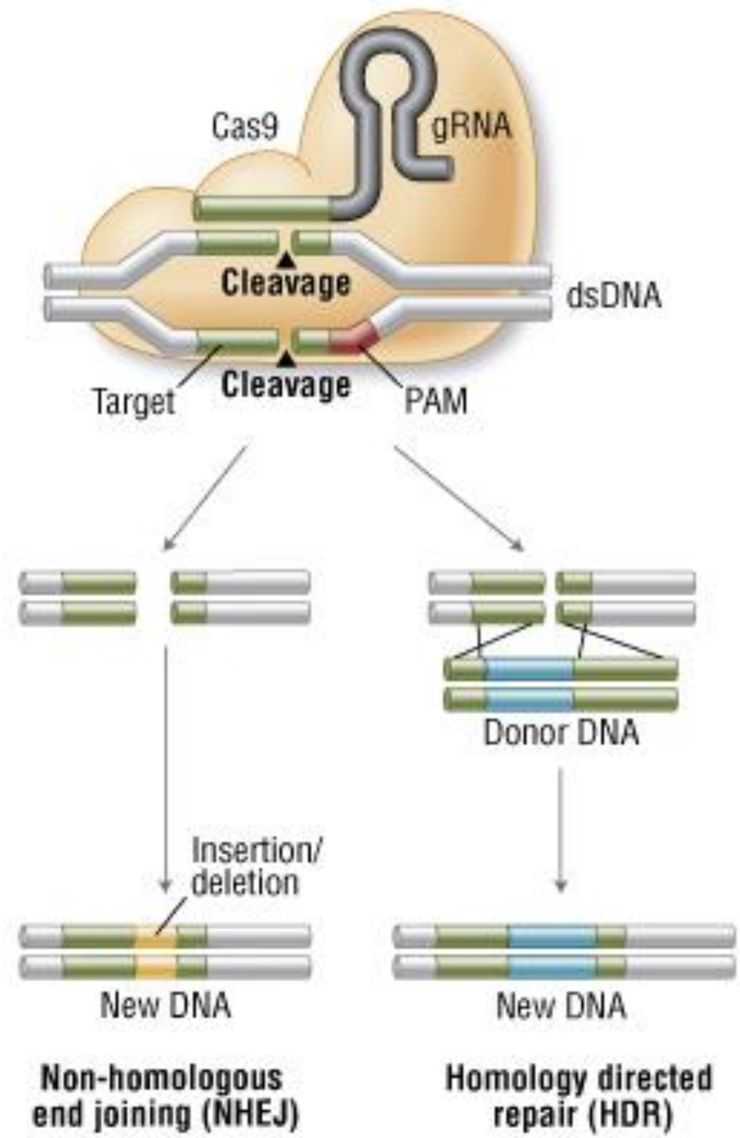
MAS allows the identification and elimination of an individual plant from a population on the basis of its genetic composition and, as a consequence, reduces the costs associated with both continued propagation and downstream phenotyping ([Ru et al., 2015](#)).



http://www.ijabpt.com/pdf/56042-P.Jhansi%20Rani.pdf

New BioTechnologies – Gene Editing CRISPR/Cas

Originated from a bacterial "immune system" to fight off bacterial viruses.



www.neb.com

Two genes are inserted into a plant:

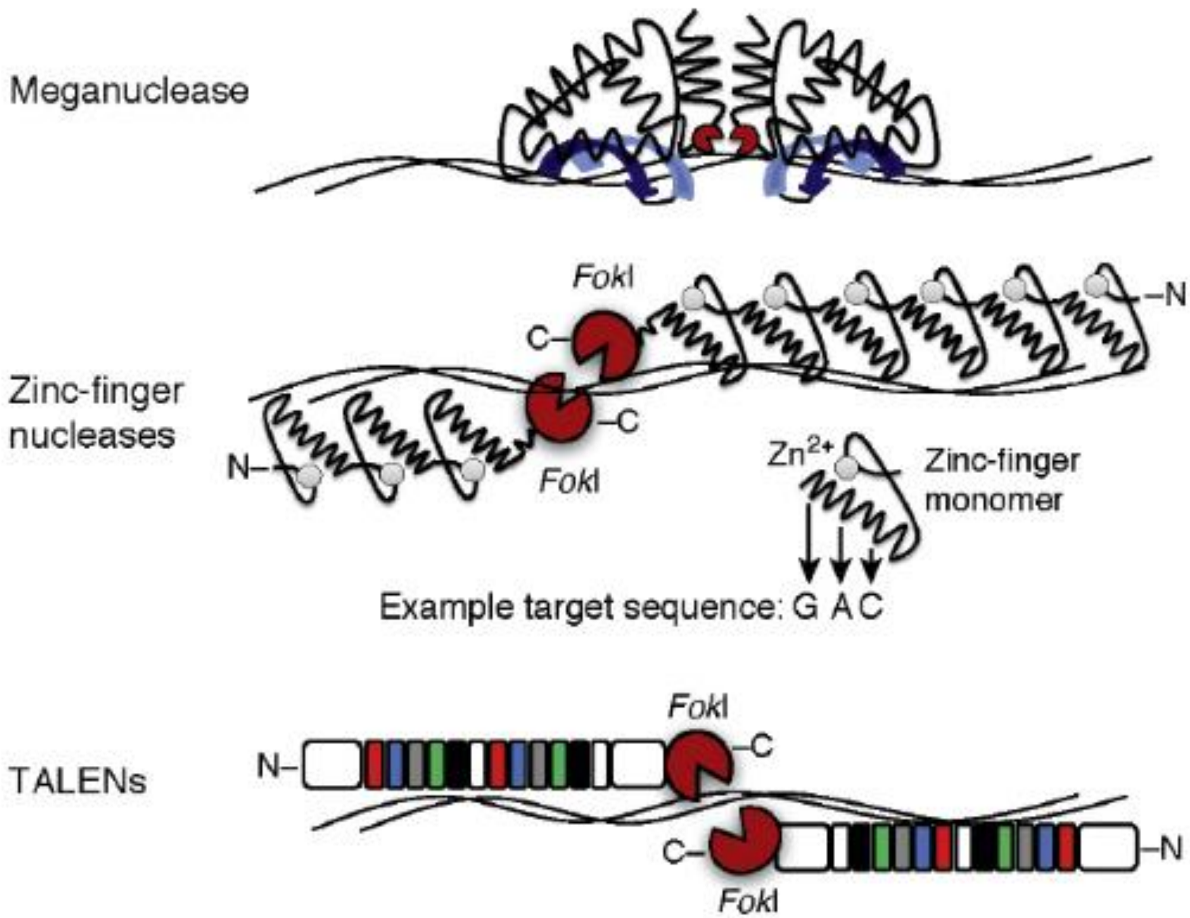
1. **Cas9**, an enzyme that cuts DNA
2. **gRNA**, a gene that produces RNA to guide Cas9 to the desired target site in the DNA

Cas9 and gRNA generate a cut at the target site. The cells repair mechanism (NHEJ) repairs the cut but makes mistakes.

Plants are grown and selected for the desired phenotype (and sequenced for the modification).

Plants are crossed back to removed Cas9 and gRNA but retain the edit.

Other Gene Editing Technologies with Similar Outcome to CRISPR

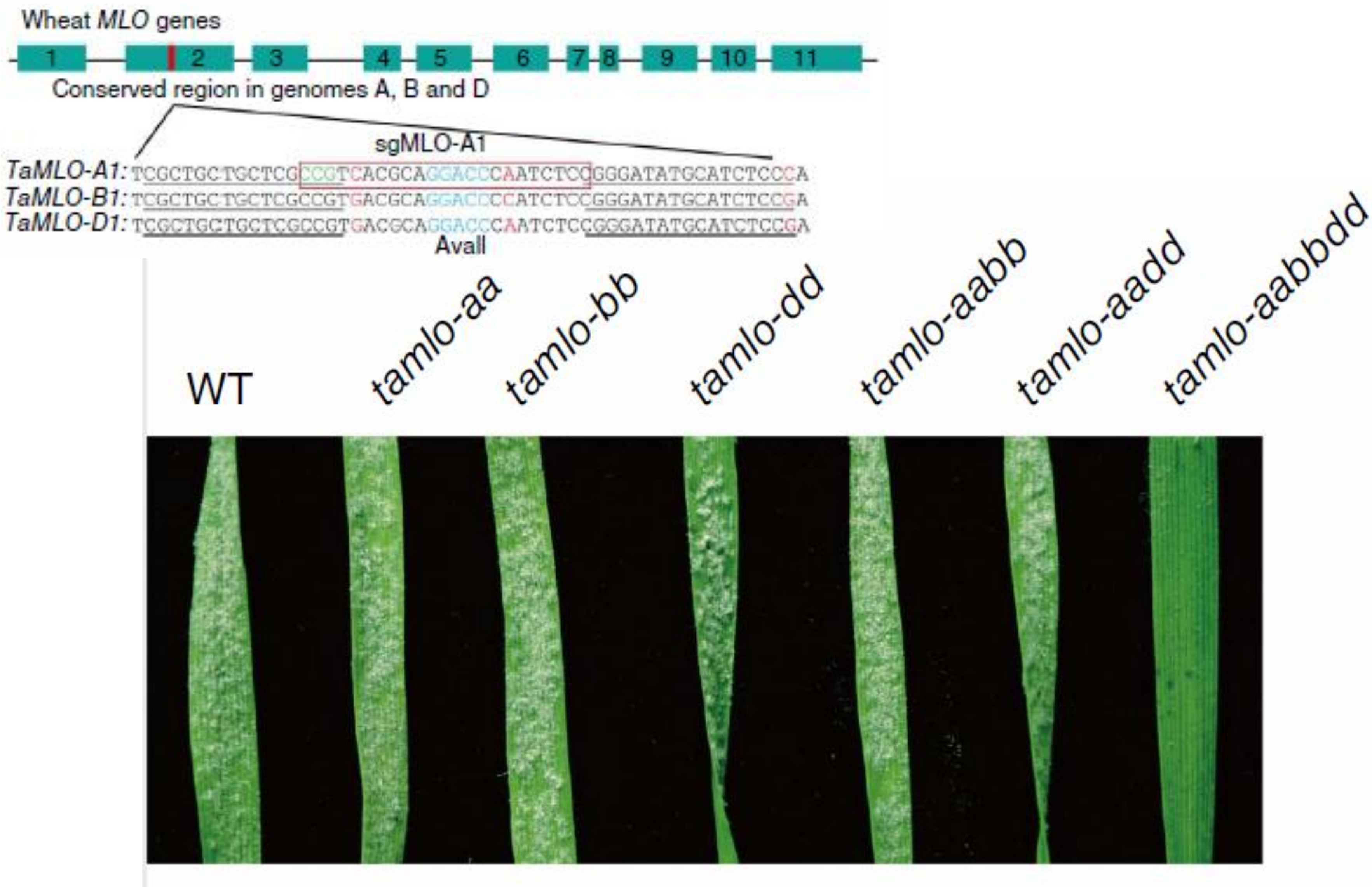


SOURCE: Baltes and Voytas (2014).

New BioTechnologies – Gene Editing CRISPR/Cas

nature biotechnology

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C. and Qiu, J.L**., 2014**. **Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew**. *Nature biotechnology*, *32*(9), p.947.



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Citrus canker, caused by Xanthomonas citri subsp. Citri (Xcc), is severely damaging to the global citrus industry.

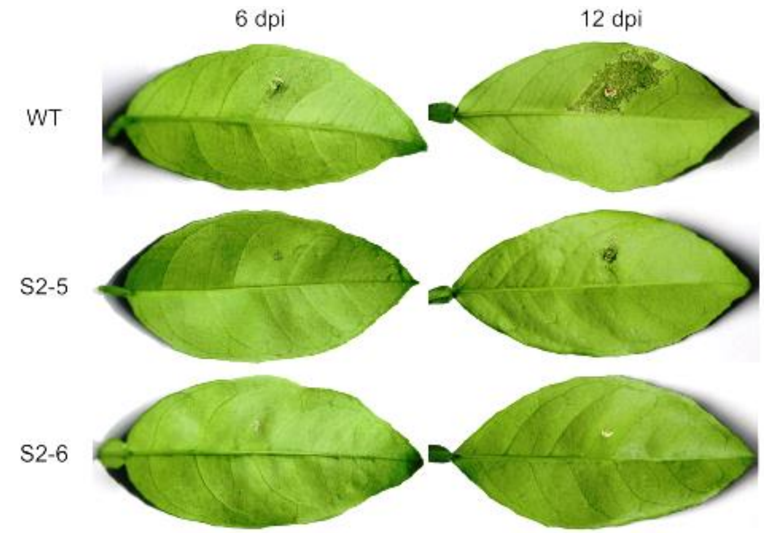
Plant Biotechnology Journal

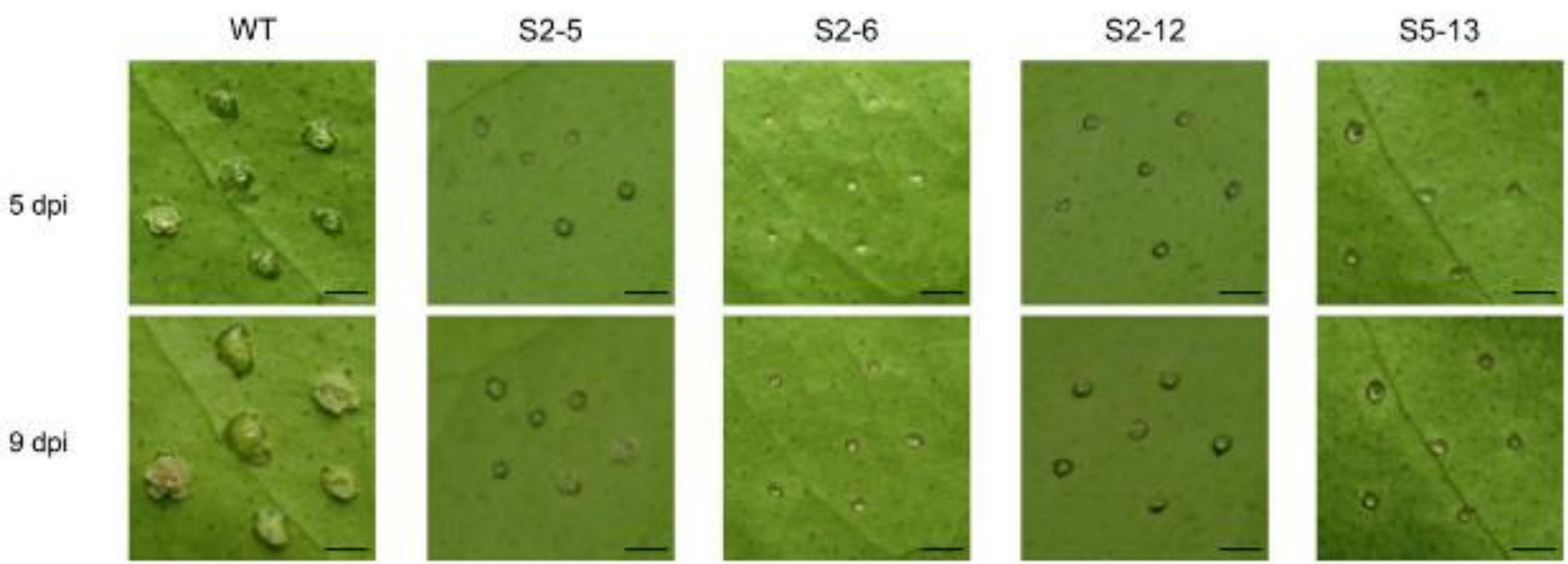
Plant Biotechnology Journal (2017) **15**, pp. 1509–1519 doi: 10.1111/pbi.1273

Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene *CsLOB1* promoter in citrus

Aihong Peng[1,2,†], Shanchun Chen[1,2,†], Tiangang Lei[1,2], Lanzhen Xu[1,2], Yongrui He[1,2], Liu Wu[2], Lixiao Yao[1,2] and Xiuping Zou[1,2,*]

[1]Citrus Research Institute, Chinese Academy of Agricultural Sciences and National Center for Citrus Variety Improvement, Chongqing, China



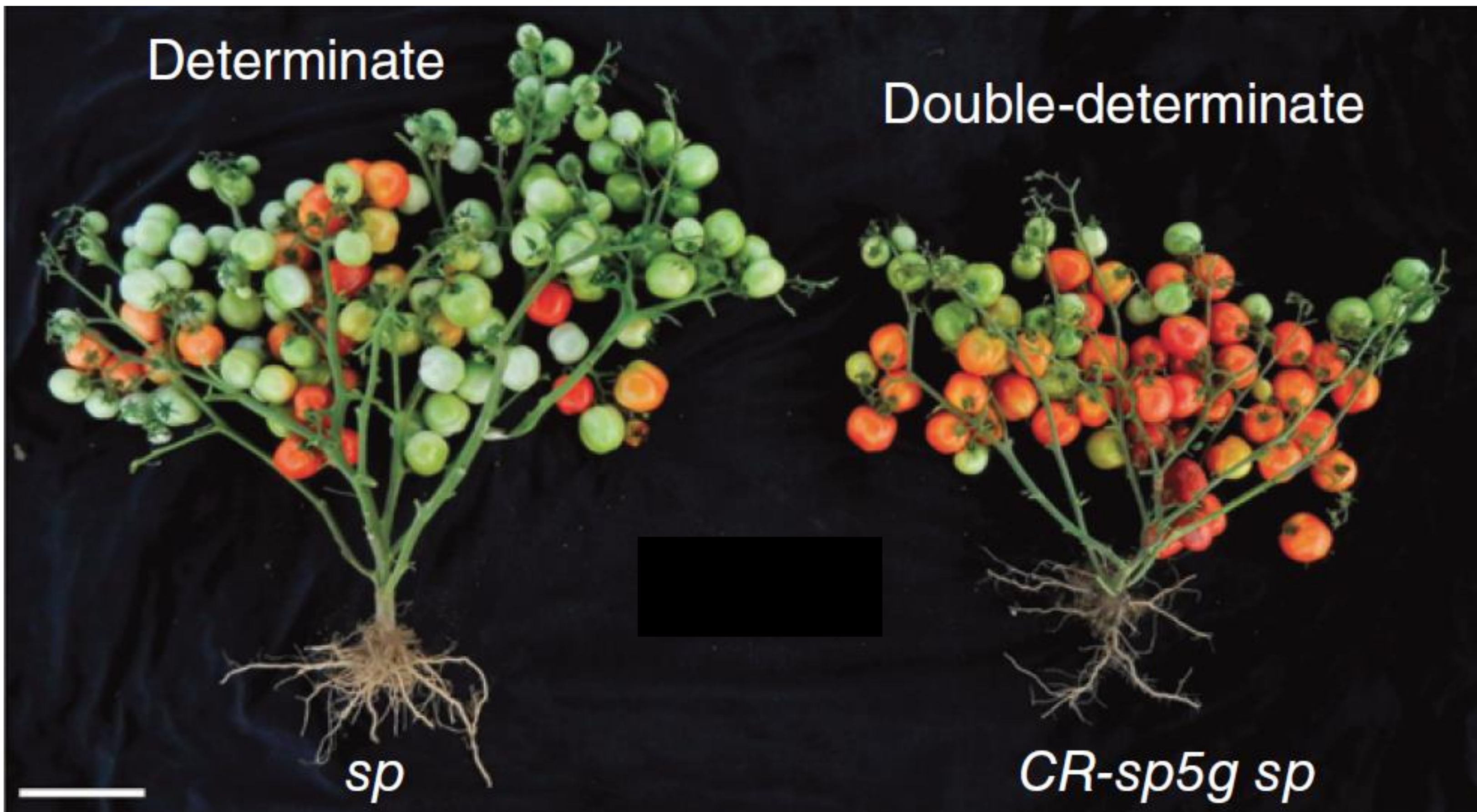


nature genetics

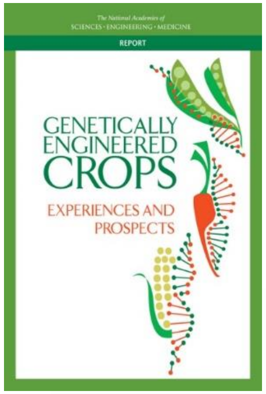
Variation in the flowering gene *SELF PRUNING 5G* promotes day-neutrality and early yield in tomato

Sebastian Soyk[1], Niels A Müller[2,7], Soon Ju Park[3], Inga Schmalenbach[2], Ke Jiang[1,7], Ryosuke Hayama[4,7], Lei Zhang[2], Joyce Van Eck[5], José M Jiménez-Gómez[2,6] & Zachary B Lippman[1]

CRISPR/Cas9-engineered mutations in *SP5G* cause rapid flowering and enhance the compact determinate growth habit of field tomatoes, resulting in a quick burst of flower production that translates to an early yield. This work suggest that pre-existing variation in *SP5G* facilitated the expansion of cultivated tomato beyond its origin near the equator in South America.



Environmental Impact of Crop Biotechnology



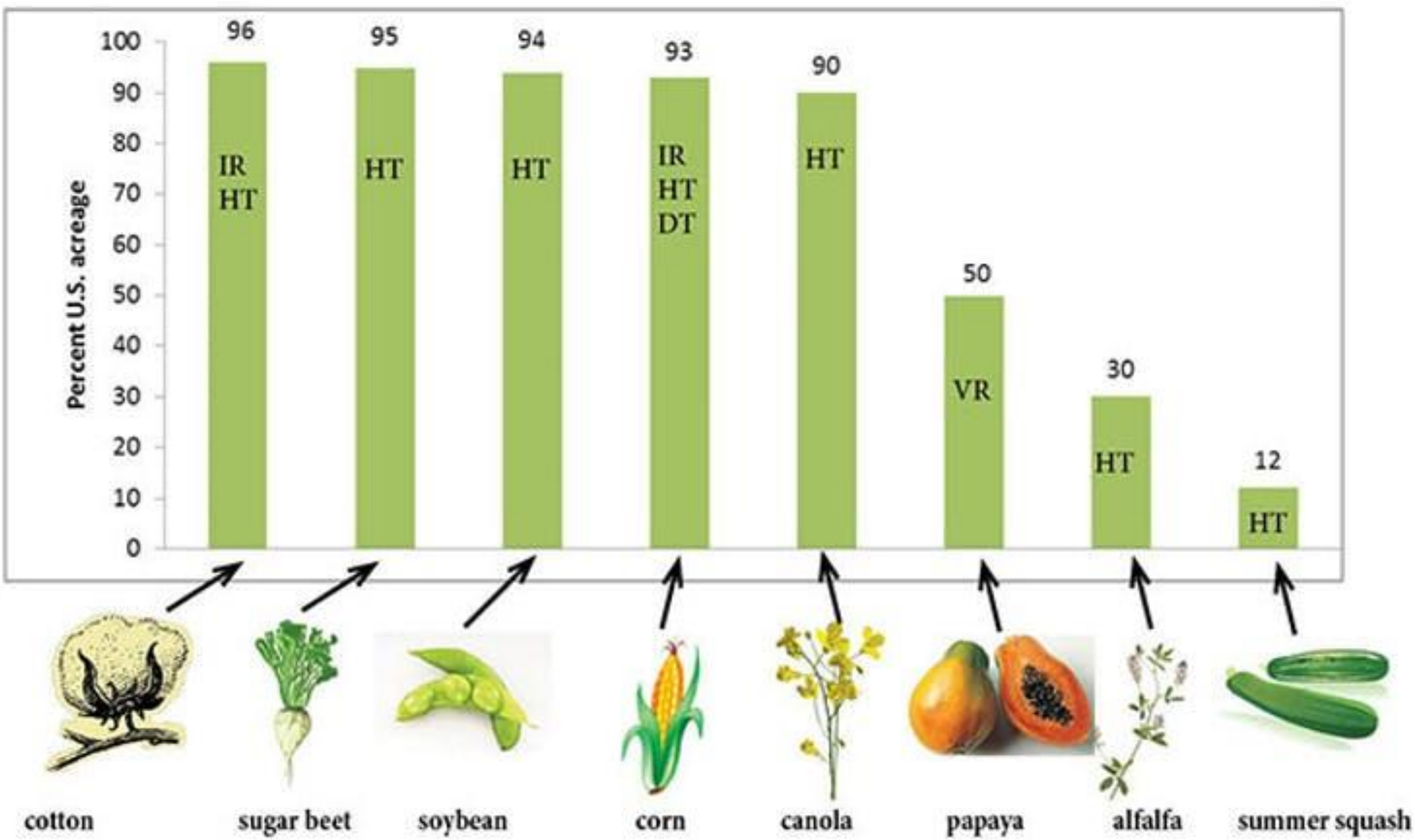
Which crops are grown and what impact did they have?

(US National Academy of Sciences Study 2017)

FIGURE S-1 Type and location of commercially grown genetically engineered (GE) crops in 2015.[1] NOTE: In 2015, almost 180 million hectares of GE crops were planted globally. Over 70 million hectares were planted in the United States. GE crops produced in Brazil, Argentina, India, and Canada accounted for over 90 million hectares. The remaining hectares of GE crops were spread among 23 countries.

Current GM crops

Most released GM crops have Herbicide Resistance (HR) or Insect Resistance (IR) or Drought Tolerance (DT) – traits that benefit the farmer by increasing yield or reducing use of pesticide and herbicide.



Potential and real benefits and adverse effects/risks:

1. Yield increases ? Crops containing Virus Resistance:
   Crops containing Herbicide Resistance:
   Crops containing Insect Resistance (Bt)

2. Reduction in herbicides and pesticides ?

3. Other changes in/to the environment
   – biodiversity and invasiveness ?

4. Evolution of Resistance to herbicides and pesticides ?

Higher yields per hectare reduces pressure on farmland efficiency and allows more conservation of native reserves.

Average % yield gains GM IR cotton and maize 1996-2016

Country	IR Maize Corn boring	Cotton IR
USA	**7.0**	**9.9**
China	N/a	**10.0**
South Africa	**11.1**	**24.0**
Argentina	**6.0**	**30.0**
Colombia	**21.8**	**18.0**
India	N/a	**30.0**
Canada	**7.0**	N/a
Brazil	**11.8**	**1.3**
Australia	N/a	**0**
Honduras	**23.8**	N/a
Mexico	N/a	**11.0**

Brookes and Barfoot (2018).

Average Gross Farm income change from Herbicide tolerant (HT) Crops

Country	HT corn	HT cotton	HT canola
	Gross farm income benefit after deduction of technology cost **$/hectare**		
USA	**28**	**20**	**49**
Australia	**-**	**28**	**45**
South Africa	**5**	**33**	**-**
Argentina	**108**	**43**	**-**
Colombia	**15**	**95**	**-**
Canada	**15**	**-**	**57**
Brazil	**38**	**62**	**-**
Philippines	**31**	**-**	**-**
Paraguay	**3**	**-**	**-**
Vietnam	**37**	**-**	**-**

Brookes and Barfoot (2018) Farm Income and production impacts of using GM crop technology 1996-2016.

Eggplant farmers in Bangladesh suffered significant yield losses at 51-73% annually due to insect damage.

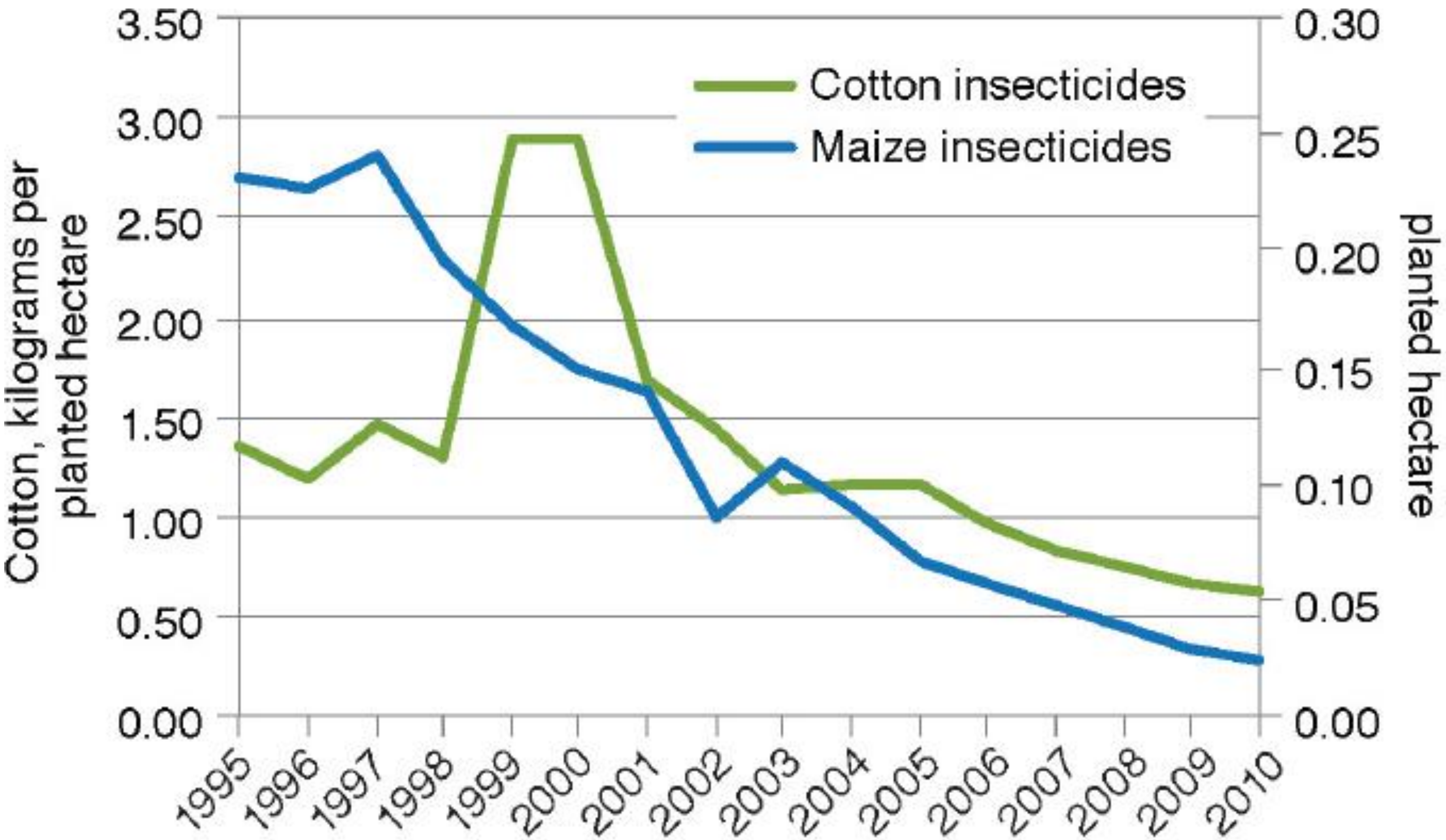
***Bt* eggplant** was grown commercially only in Bangladesh. It was first commercialized in spring 2014, when 20 farmers in four regions planted one of the four *Bt* varieties of eggplant (brinjal) on a total of 2 hectares They found **yield of uninfected fruit to be 117 percent greater in *Bt* eggplant hybrids than in insecticide-treated isogenic non-*Bt* hybrids** (Choudhary et al., 2014). .

Papaya ringspot virus arrived in Hawaii in 1992.

From 1992 to 1998, papaya production was over **30% reduced by the virus** from 33,065 kg/hectare to 21,072 kg/hectare.

Fruit production in field trials of **VR papaya** planted in 1995 **was 3 times greater than the average production in 1988–1992**, before the papaya ringspot virus affected production. VR papaya was introduced in 1998; as of 2009, it accounted for over 75 percent of papaya hectares in Hawaii (USDA–NASS, 2009).

Do GM crops reduce herbicide and pesticide use ?



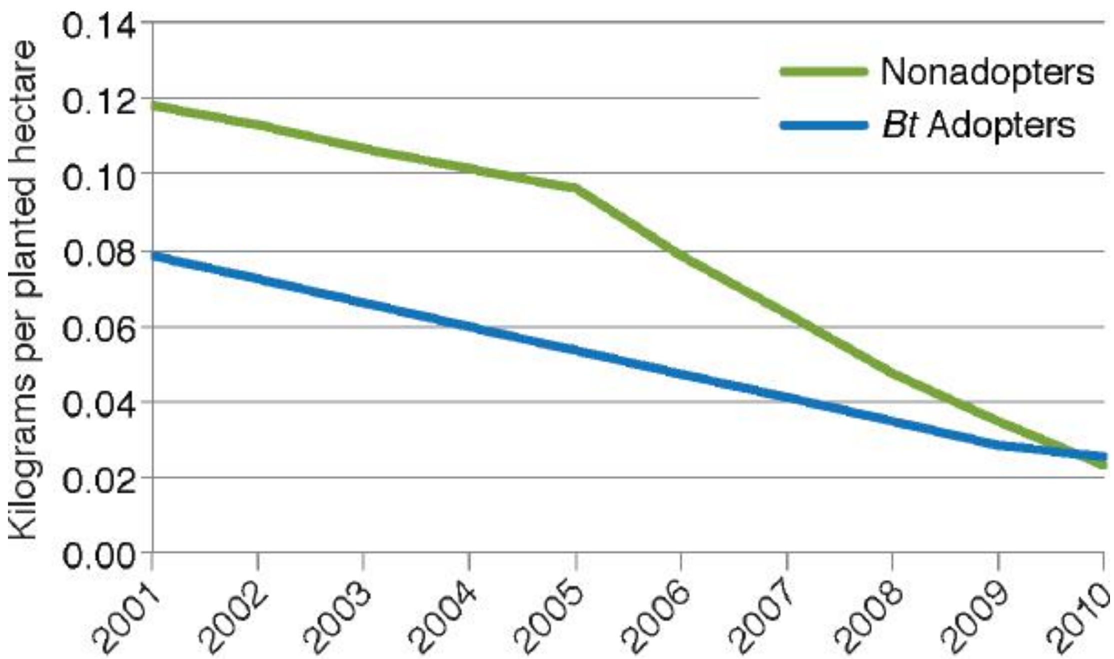
Rates of insecticide application for maize and cotton in the United States from 1995 to 2010. [Fernandez-Cornejo et al. (2014)](#).

Science AAAS

Areawide Suppression of European Corn Borer with Bt Maize Reaps Savings to Non-Bt Maize Growers

W. D. Hutchison,[1*] E. C. Burkness,[1] P. D. Mitchell,[2] R. D. Moon,[1] T. W. Leslie,[3] S. J. Fleischer,[4] M. Abrahamson,[5] K. L. Hamilton,[6] K. L. Steffey,[7†] M. E. Gray,[7] R. L. Hellmich,[8] L. V. Kaster,[9] T. E. Hunt,[10] R. J. Wright,[11] K. Pecinovsky,[12] T. L. Rabaey,[13] B. R. Flood,[14] E. S. Raun[15‡]

We found that **areawide suppression of the European corn borer** is associated with Bt maize use. Cumulative benefits over 14 years are an estimated $3.2 billion for maize growers in Illinois, Minnesota, and Wisconsin, with more than $2.4 billion of this total accruing to non-Bt maize growers.



Rates of insecticide application for maize and cotton in the United States from 1995 to 2010 for BT adopters and non-adopters.
[Fernandez-Cornejo et al. (2014)](#).

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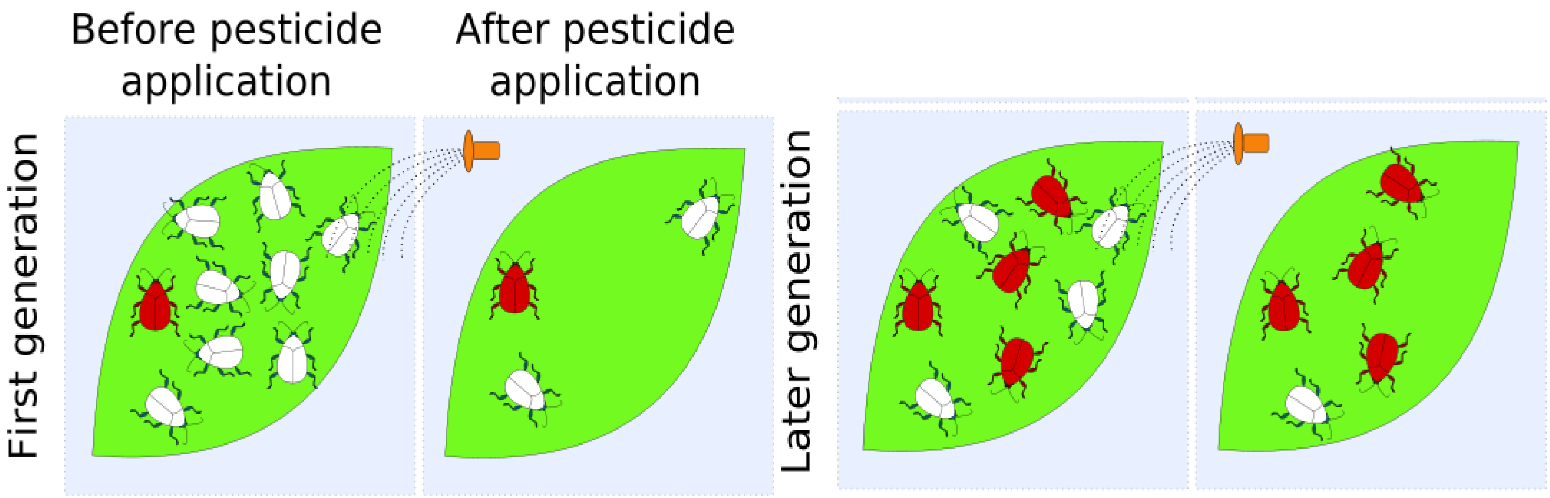
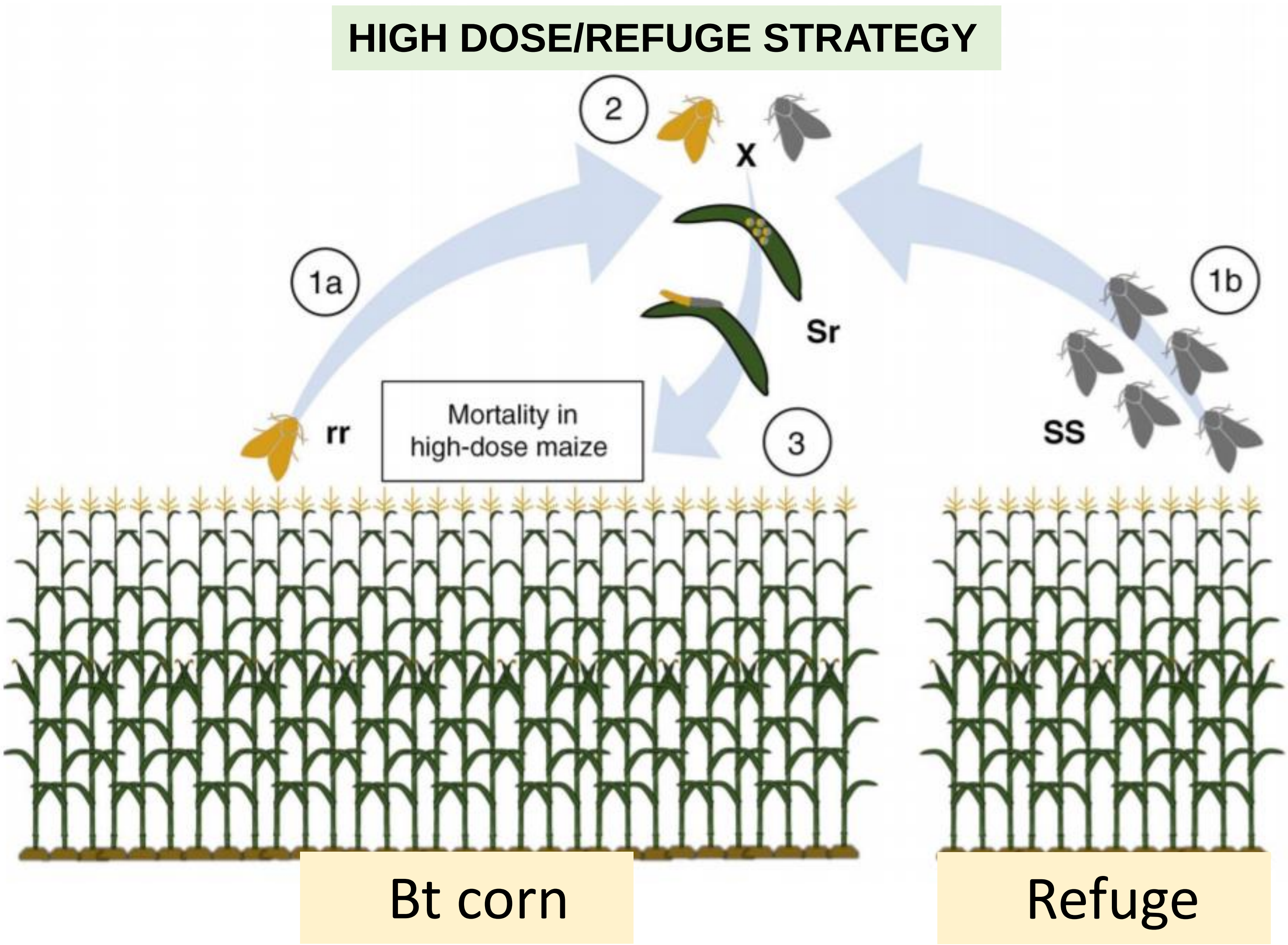
Resistance Evolution and Resistance Management in *Bt* Crops

The evolution of target insects with resistance to *Bt* toxins has resulted in substantial economic losses for farmers of *Bt* crops.



Resistance occurs by natural mutations. Resistant organisms have an advantage under "selection pressure" = insecticide/herbicide use.

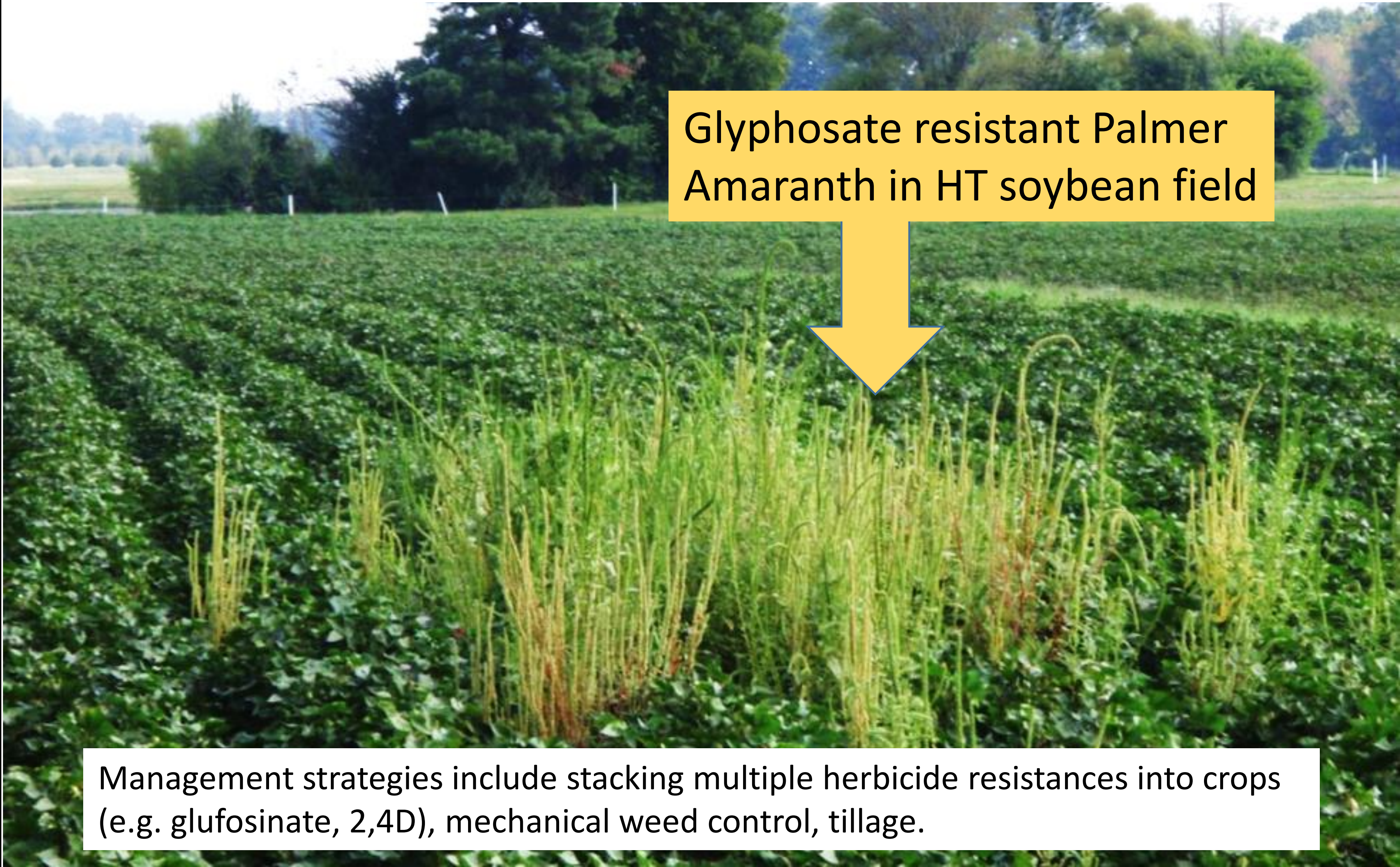
Resistance Evolution and Resistance Management in *Bt* Crops



Vélez, Ana M., et al *Current opinion in insect science* 15 (2016): 116-124.

Herbicide resistant weeds

Single herbicide (glyphosate) resistance has evolved in many weeds.
Management is successful by using other herbicides (glufosinate).



Management strategies include stacking multiple herbicide resistances into crops (e.g. glufosinate, 2,4D), mechanical weed control, tillage.

Can transgenic plants become invasive – or transfer their genes?

Can GM crops transfer their genes to other species?

– no, only to those they are sexually compatible to. There is no evidence of “horizontal” transfer of transgenes between different crop or plant species, plant : animal transfer or plant” bacteria transfer.

Can GM crops become invasive?

nature

News

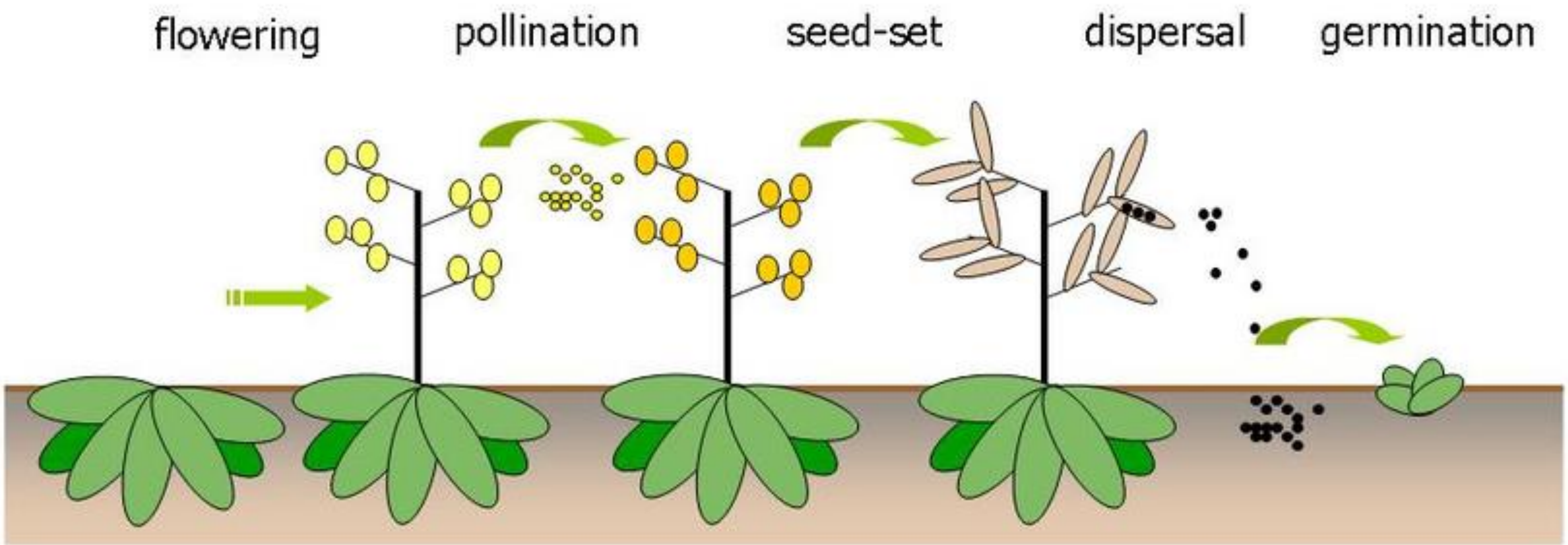
GM crop escapes into the American wild

Transgenic canola found growing freely in North Dakota.

Natasha Gilbert 2010

Researchers found two varieties of transgenic canola in the wild — one modified to be resistant to Monsanto's Roundup herbicide (glyphosate), and one resistant to Bayer Crop Science's Liberty herbicide (gluphosinate)..

Transgene containment



1.) Physical and biological containment strategies

2.) make the second generation of seeds sterile or dependent on a chemical for fertility.

3.) GMO plants can only fertilize other GMO plant with same trait.

4.) include synthetic amino acids that are not present in the wild.

5.) Expression in plastids produces transgene-free pollen.

Plastids are only maternally inherited.

FOR THE FARMER

Input traits

Stacked herbicide tolerance

Biotic stress resistance

Microbial resistance

- Major resistance genes
- Phytoalexin engineering[a]
- Novel resistance mechanisms[a]
- Viral RNA interference or coat protein[a]

Insect resistance

- Stacked insecticidal genes[a]
- RNA interference[a]

Abiotic stress tolerance

- Drought tolerance
- Water-use efficiency
- Cold tolerance
- Heat tolerance
- Salt tolerance

Nutrient-uptake and nutrient-use efficiency

Nitrogen fixation (in cereals)[a]

Phosphorus-use efficiency

Carbon fixation

- Improved Rubisco[a]
- C4 photosynthesis in C3 grasses[a]
- CAM in C4 plants[a]

Post harvest improvements

- Microbial resistance
- Increased shelf-life[a]
- Reduced bruising[a]
- Silage stability[a]
- Standardized quality

FOR THE CONSUMER

Output traits

Enhanced nutritional content
- Micronutrients
- Amino acids
- Vitamins
- Fatty acid profiles
- Flavonoids and nutraceuticals

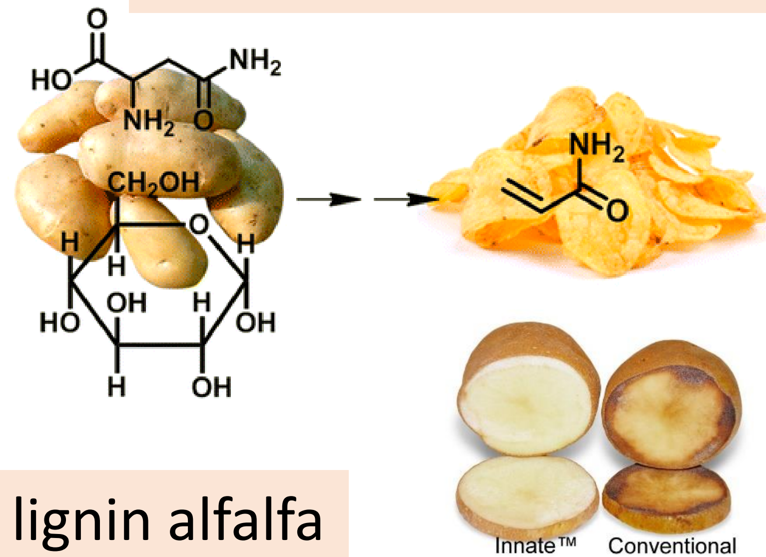
Food safety
- Reduced acrylamide formation[a]
- Reduced aflatoxin concentrations[a]

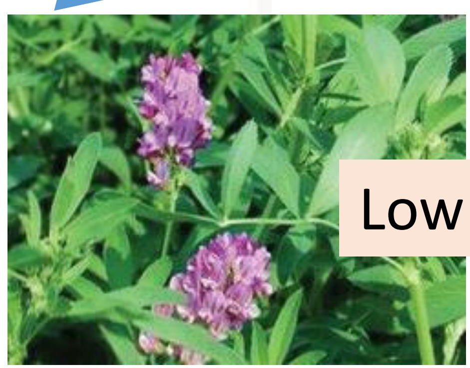
Forage quality
- Digestibility
- Nitrogen protection

Biofuels and industrial byproducts

Golden Rice (Vitamin A)

Low acrylamide potato





Low lignin alfalfa

A new trait for Peruvian cacao?

Cacao beans contain high levels of cadmium.

The cadmium comes from contaminated soil. It is taken up through the root, transported up into the shoot and flower/seed and accumulates in the beans.

Selection and breeding of “low cd” varieties is underway but will take years to market.

Cadmium (Cd) transporter genes have been identified in several plants (rice, mulberry).

Sequence the Theobroma cacao genome, Identify transporter and edit it out = limit Cd transport to fruit.

The worlds largest resources of resistance (R-) genes are the rain forests.

Resistance to pathogens (bacteria, viruses, fungi) evolve in environments where the plants (animals) are exposed to those pathogens. Plants in the rainforests have survived pathogens for a long time and there should be a large amount of different Resistance genes.

An untapped resource for Peru (and the world).

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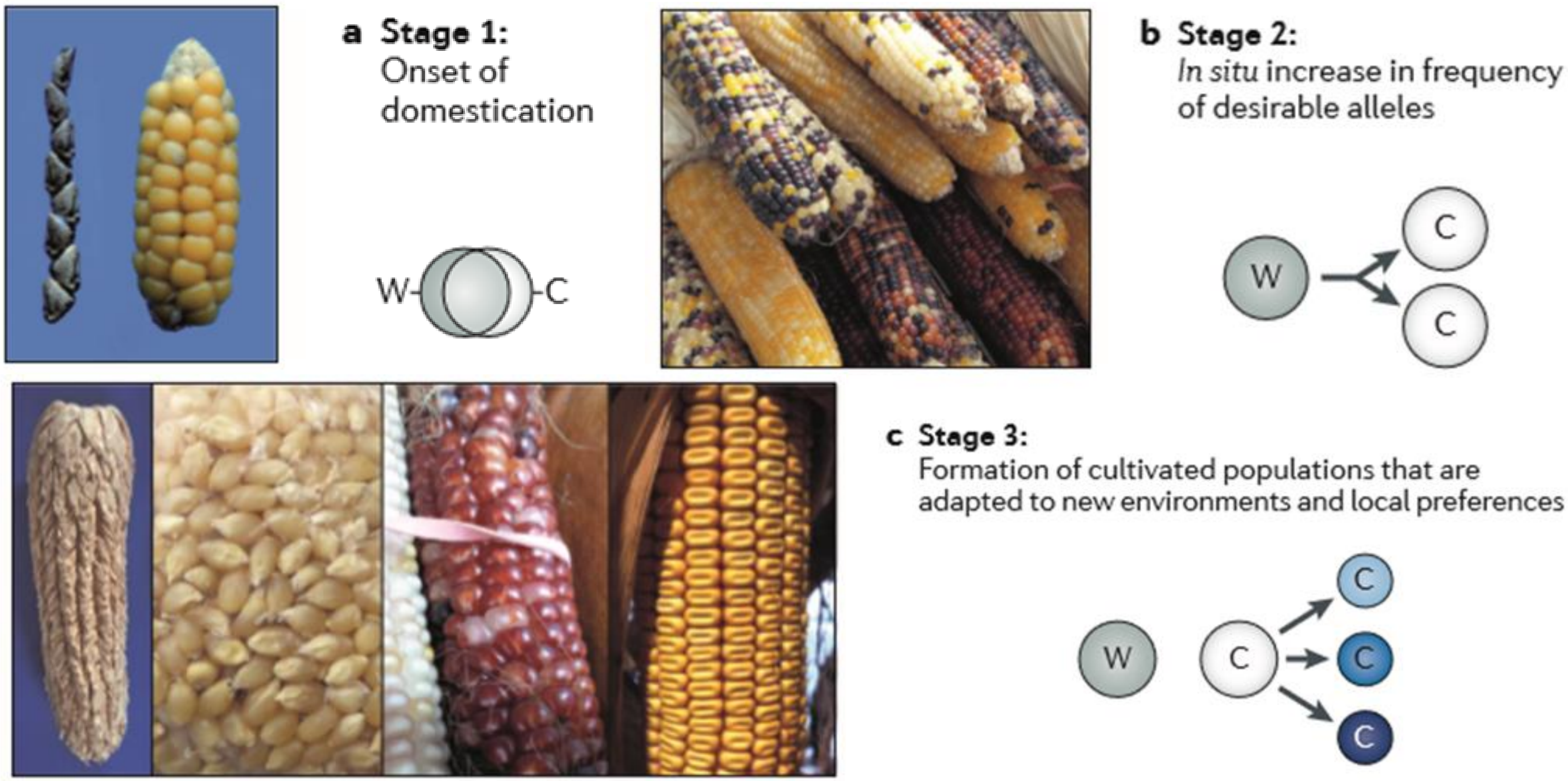
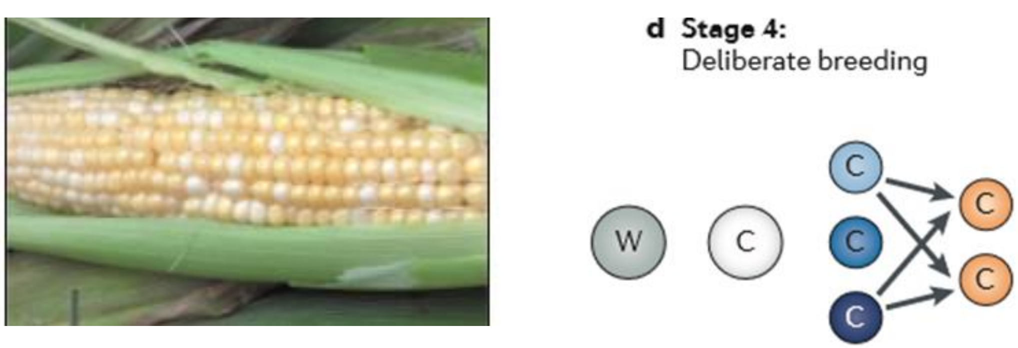
Genetic Basis for Domestication and Crop Improvement





Selection and breeding resulted in a loss of about 30% of genetic variation in grasses (corn, wheat, rice, sorghum)

Meyer, R.S.; Purugganan, M.D. (2013). "Evolution of crop species: Genetics of domestication and diversification". *Nature Rev Genetics*. **14** (12): 840–52.

Changes in secondary insect pests due to Bt crops

The control of targeted species by *Bt* toxins sometimes provides an opportunity for populations of "secondary" insect species to increase. The secondary insect-pest populations increase because they are not susceptible to or have reduced susceptibility to the specific *Bt* trait in the crop.

In a 10-year study in China conducted from 1997 (when *Bt* cotton was introduced) through 2008, populations of a mirid bug, which is not affected by the *Bt* toxin in the cotton, steadily increased because broad spectrum insecticide was no longer applied (Lu et al., 2010).

A summary assessment of the effects of secondary pests on *Bt* cotton in China (Qiao, 2015) concluded that the **effects were minor in comparison with the decreases in major insect pests and insecticide use**.