RAMA UNIVERSITY

www.ramauniversity.ac.in

FACULTY OF ENGINEERING & TECHNOLOGY

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Genetic Engineering and Biotechnology

- What is Biotechnology?
- How Genetic Engineering Is Accomplished
- Success Stories in Plants
- Issues

What is Biotechnology?

"Biotechnology" means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Definition from Convention on Biological Diversity
https://www.cbd.int/

Biotechnology Over Time

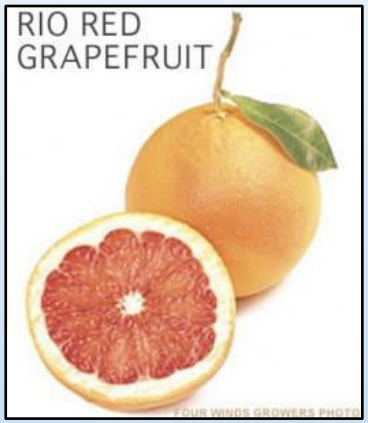
- **Traditional Biotechnology**
  - Growing plants
  - Raising animals
  - Plant and animal breeding
  - Fermentation (bread, beer, wine, fish sauce)

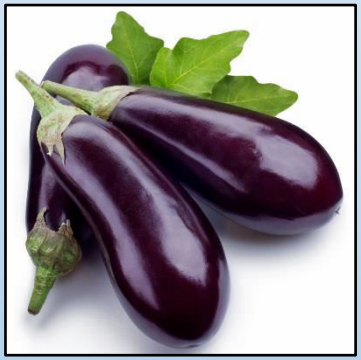
- **Genetic Engineering** - Recombinant DNA and tissue-culture-based biotechnology
  - Genome Editing – Precision breeding

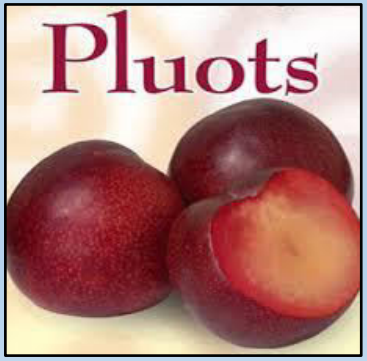
Current Uses of Biotechnology

- **Agriculture**
  - Transgenic Plants [disease resistance, drought tolerance, nutrient use efficiency, plant-based products such as vaccines]
  - Transgenic Animals
  - Transgenic Microbes
- **Pharmaceutical**
  - Insulin
  - Antibiotics
  - Cancer therapy
- **Others**
  - Mining
  - Petroleum spill clean-up with microbes

Food Quiz: Pick the GMO

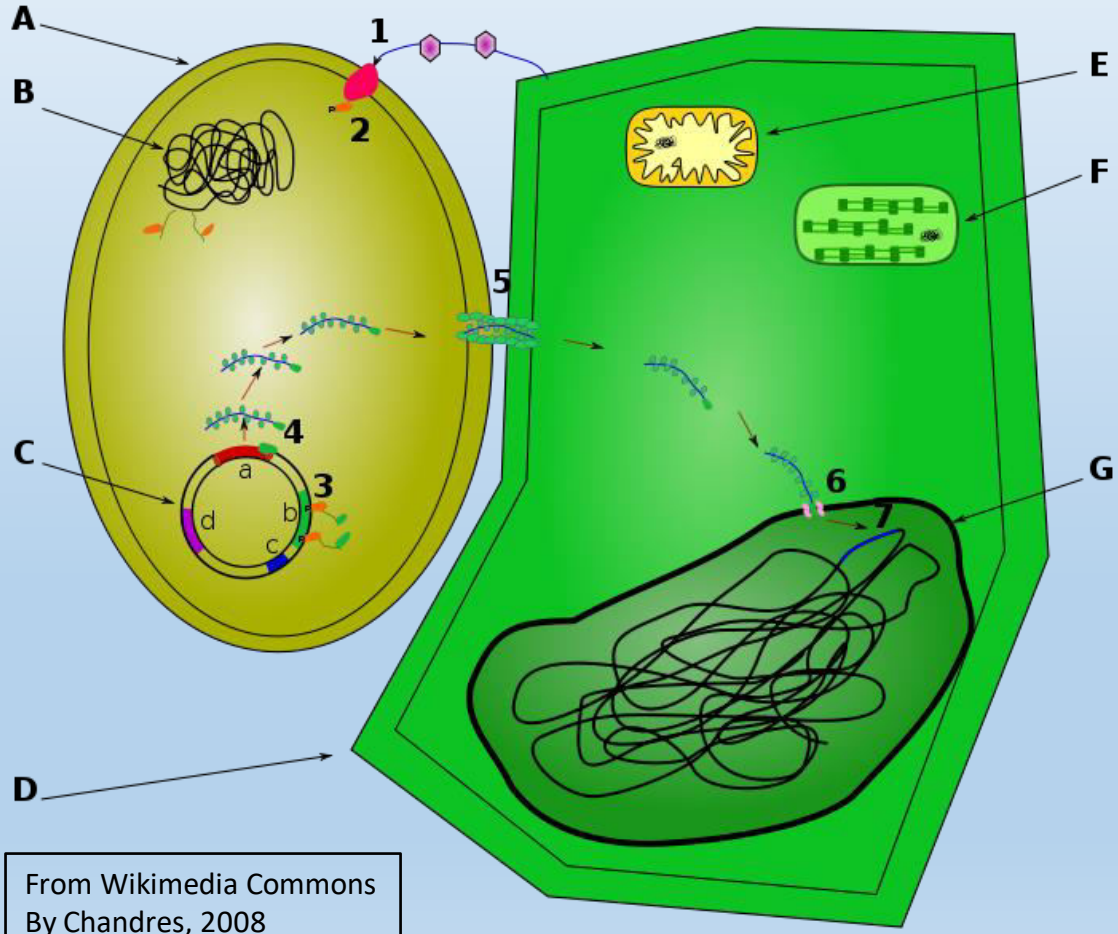






Plant Genetic Engineering History

Agrobacterium tumefaciens has been naturally genetically engineering plants for centuries!



From Wikimedia Commons
By Chandres, 2008

A: *Agrobacterium tumefaciens*

B: Agrobacterium genome

C: Ti Plasmid :
- a: T-DNA
- b: *Vir* genes
- c: Replication origin
- d: Opines catabolism genes

D: Plant cell

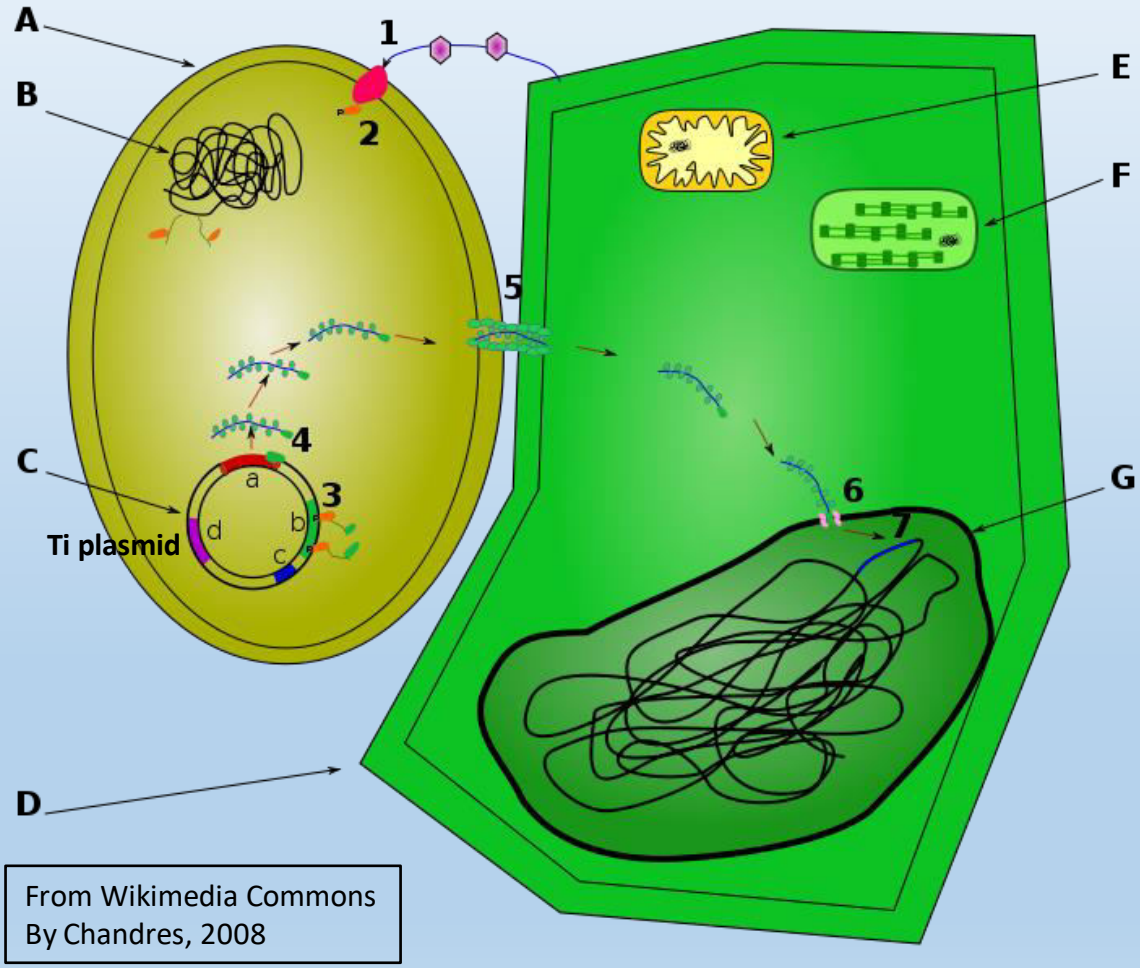
E: Mitochondria

F: Chloroplast

G: Nucleus

Plant Genetic Engineering History

Agrobacterium tumefaciens infects plant cell



From Wikimedia Commons
By Chandres, 2008

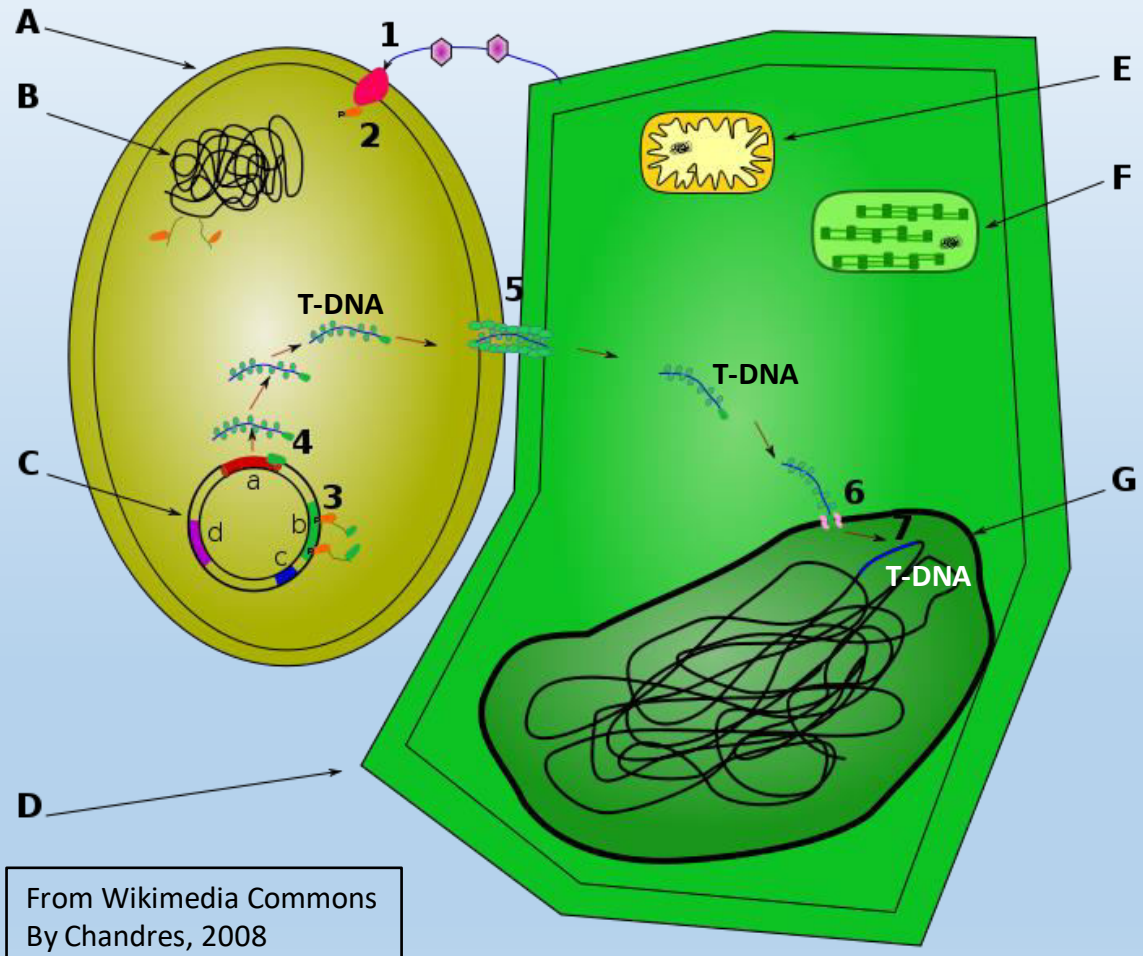
Steps 1 & 2: Bacterial cell weakly attaches itself to the plant cell. It then produces cellulose fibrils to anchor it to the plant cell (infection).

Step 3: When the bacterium detects certain compounds produced by the plant in response to bacterial infection, *vir* (virulence) genes [located on b of the Ti plasmid (C)] start producing various compounds.

Step 4: One *vir* gene complex cuts the T-DNA (a) from the Ti plasmid (C).

Plant Genetic Engineering History

Agrobacterium tumefaciens infects plant cell



From Wikimedia Commons
By Chandres, 2008

Step 5: In the meantime, other *vir* genes produce compounds that coat the T-DNA to help export it into the recipient plant cell

Step 6: Other *vir* genes make the nucleus of the plant cell receiving the T-DNA more receptive.

Step 7: T-DNA is integrated into the host genome.

T-DNA contains genes that will force the plant to produce special amino acids called **opines**, which the bacteria can metabolize as its food source!

Plant Genetic Engineering History
Agrobacterium tumefaciens

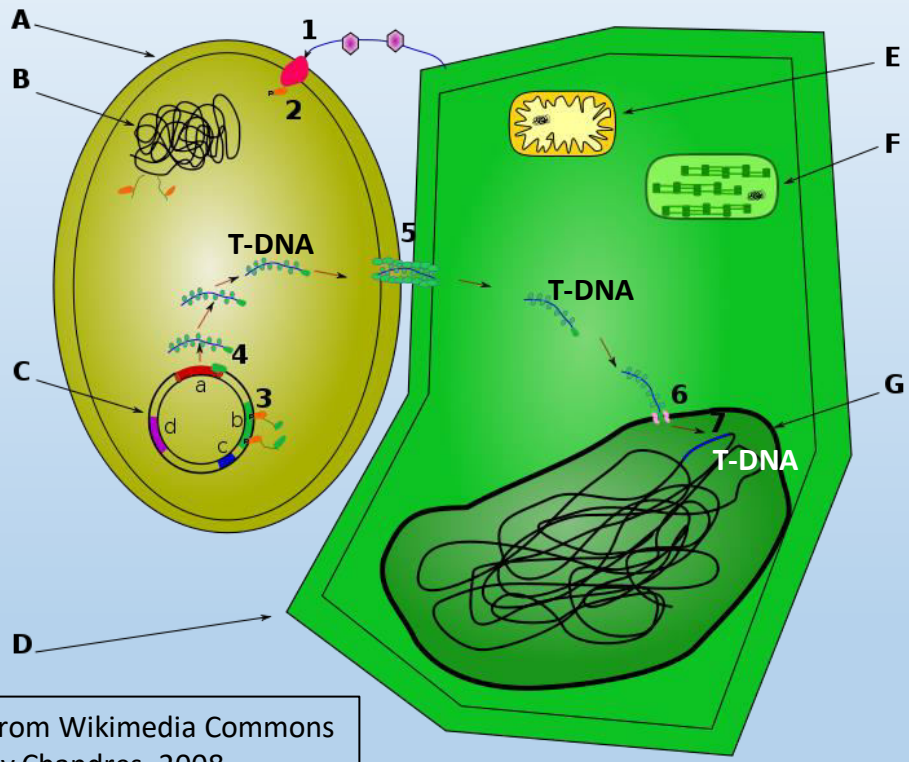
To cause gall formation, the T-DNA encodes genes for the production of auxin or indole-3-acetic acid via a pathway not normal for plants, so the plant can't regulate its production . . . only the pathogen! Other T-DNA genes code for production of cytokinins. Together, cell proliferation and gall formation occur.

Plant Genetic Engineering

- Agrobacterium-mediated transformation
- Gene Gun
- Protoplast transformation and fusion
- Genome editing

Plant Genetic Engineering History

Agrobacterium tumefaciens infects plant cell



From Wikimedia Commons
By Chandres, 2008

- To transform plant cells, the desired gene sequence is cloned into the T-DNA (a).
- The T-DNA is the delivery vehicle of the desired gene sequence into the plant cell.

Plant Genetic Engineering
Using *Agrobacterium tumefaciens*

"Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants"
Ow et al. Science (Nov. 1986)

When tobacco plant was sprayed with the chemical substrate luciferin, the plant glowed temporarily.

Gene Gun - Biolistic

Hawaiian Rainbow Papaya

Inserted papaya ring spot virus coat protein using high speed particle bombardment method (relies on pressure)

https://www.youtube.com/watch?v=2G-yUuiqIZ0
(5 ½ minutes)

http://www.apsnet.org/publications/apsnetfeatures/Pages/papayaringspot.aspx

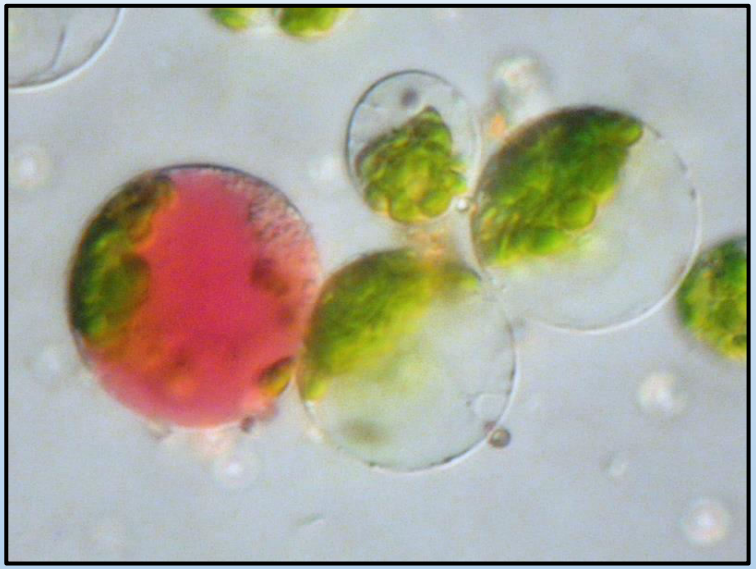
Gene Silencing

RNA interference, or RNAi, a molecular mechanism that defends plants, fungi, and animals against viruses made of RNA, a chemical relative of DNA. When a RNA virus takes over a host cell, it needs to copy itself and the copying process creates double strands of RNA. The RNAi defense mechanism recognizes these double-stranded RNAs as foreign and degrades them plus any single-stranded RNAs that it "recognizes".

Proteins are made on single-stranded RNA templates, so a gene targeted by RNAi can't produce the protein that it usually makes. The gene has not been changed, but it no longer can be used to make proteins or duplicate the viral RNAs. We speak of a RNAi-targeted gene as being "knocked down" or "silenced." This natural gene silencing mechanism is why genetically modified (GM) papaya that contains a coat protein gene from Papaya Ringspot Virus is able to resist the virus

Biotech in Focus, April 2016, University of Hawaii Cooperative Extension Service
http://www.ctahr.hawaii.edu/biotechinfocus/backissues.html

Protoplast Fusion
Somatic Fusion



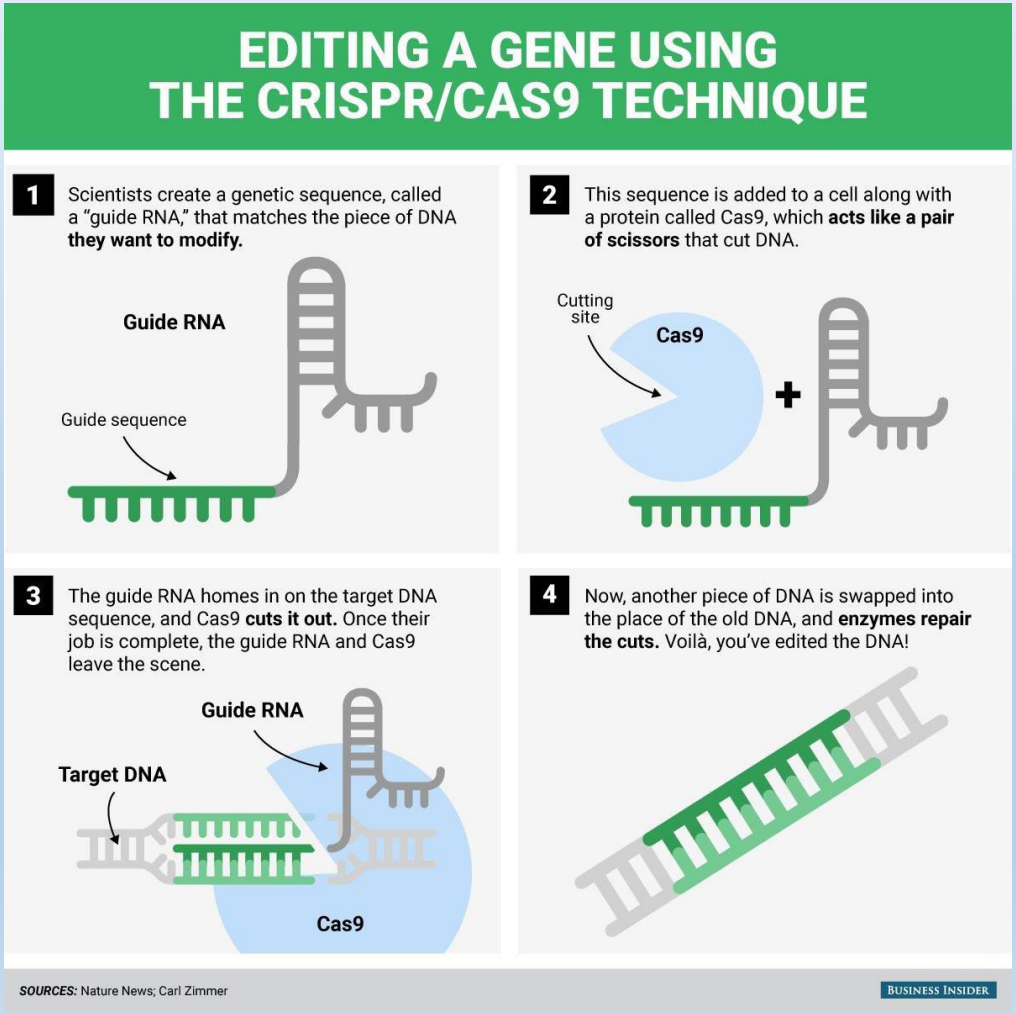
From Wikimedia Commons, Mnolf, 2009

Protoplasts of two distinct species of plants are fused together to form a new hybrid plant with the characteristics of both

Example for plant disease resistance: Plant Cell Reports, August 2013, Volume 32, pp. 1231-1241 Development of somatic hybrids Solanum × michoacanum Bitter. (Rydb.) (+) S. tuberosum L. and autofused 4x S. × michoacanum plants as potential sources of late blight resistance for potato breeding by P. Smyda et al.

Genome Editing Using CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats



How CRISPR/Cas9 technology works:

- The "guide RNA" is attached to Cas9, a bacterial enzyme that will cut the DNA sequence at the desired site in the genome.
- Once the genome is broken, the guide RNA/Cas9 disappear, and the cell will try to repair the cut, which can disable or knock out a particular gene.
- Or, scientists can insert a new segment of DNA into the cut, essentially pasting a gene into the desired location and changing the genome.

Genome Editing Using CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats

- The CRISPR system was first discovered in bacteria and functions as a defense against foreign DNA, either viral or plasmid.
- Used for gene knock-out, repression or activation
- CRISPR does have limits! But, because of its precision and simplicity, it is "the" genetic tool of the moment.

[addgene (non-profit): https://www.addgene.org]

GMO Answers is funded by the members of The Council for Biotechnology Information, which includes BASF, Bayer, Dow AgroSciences, DuPont, Monsanto Company and Syngenta.

The independent experts who answer consumer questions are not paid by GMO Answers to answer questions. Experts donate their time to answer questions in their area of expertise for the website. They do so because they are passionate about helping the public better understand GMOs and how our food is grown.

GMOAnswers.com

Supporting partners are organizations, companies and others who are committed to the five core principles of GMO Answers and have added their support to this initiative. To date those partners include The American Council on Science and Health, The American Farm Bureau Federation, American Seed Trade Association, American Soybean Association, The American Sugarbeet Growers Association, Minnesota Crop Production Retailers, National Association of Wheat Growers, National Corn Growers Association, National Cotton Council, Ohio AgriBusiness Association, South Dakota Agri-Business Association, The U.S. Beet Sugar Association, Western Sugar

GET TO KNOW

GMO BASICS

How We Got Here

THE HISTORY OF GENETIC MODIFICATION IN CROPS

10,000 years ago

Humans begin crop domestication using selective breeding.

1700s

Farmers and scientists begin cross-breeding plants within a species.

1940s and 1950s

Breeders and researchers seek out additional means to introduce genetic variation into the gene pool of plants.

1980s

Researchers develop the more precise and controllable methods of genetic engineering to create plants with desirable traits.

1990s

The first GMOs are introduced to the marketplace.

gmo answers Open to Your Questions About How Our Food Is Grown

GET TO KNOW GMO BASICS

watermelon

corn

banana

aubergine / eggplant

carrot

cabbage, kale, broccoli, etc.

gmo answers Open to Your Questions About How Our Food Is Grown

GET TO KNOW GMO BASICS

Why GMO? SEED IMPROVEMENT

SEED IMPROVEMENT TECHNIQUE	SELECTIVE BREEDING 10,000 years ago to today	INTERSPECIES CROSSES late 1800s to today	MUTAGENESIS 1930s to today	TRANSGENESIS (GMOs) 1990s to today
What is it?	Combining traits from similar and dissimilar plants by crossing into one genetic background with improved traits	Breeding and tissue culture techniques that permit genetic exchange between plants not crossing naturally	Using chemicals or radiation on seeds to change DNA and occasionally induce a favorable trait	Adding a specific, well-characterized gene to a new seed to transfer a specific trait
Examples	 Almost everything we eat	 Pluots, tangelos, some apples, rice and wheat	 Many plants and fruits including pears, apples, rice, yams, mint, some bananas	 Alfalfa, canola, corn (field and sweet), cotton, papaya, potatoes, soybeans, squash, sugar beets. Apples approved and coming to market soon.
Improved by breeding?	YES	YES	YES	YES
How many genes are affected?	10,000 to 300,000+	10,000 to 300,000	Random and unknown, likely thousands	1 to 3
Do we know which genes in the seed are affected?	NO	NO	NO	YES
Research and development time?	5 to 30 years	5 to 30 years	5+ years	5 to 10 years
Tested by regulatory agencies to ensure safety for people, animals and the environment?	NO	NO	NO	YES
Can the seeds be patented?	YES	YES	YES	YES
Approved for non-GMO and organic farming?	YES	YES	YES	NO
Are people asking for labeling?	NO	NO	NO	YES

THIS CHART COMPARES AND CONTRASTS MODERN METHODS OF SEED IMPROVEMENT.

How do we create new and improved varieties of plants? It starts with the seed. Plant breeders and scientists work together to create new varieties to address evolving challenges to farming and changing consumer preferences. Humans have been central in seed improvement for over 10,000 years, and in the last 100 years our understanding of genetics has accelerated and enabled new seed improvement techniques. Compared to earlier methods, breeders can now make improvements to seeds by moving more precisely one or a few genes into a seed.

gmo answers Open to Your Questions About How Our Food Is Grown

Sourced by Dr. Kevin M. Folta, Professor and Chairman, Horticulture Sciences Department, University of Florida, GMOAnswers.com and Scitable by Nature Education.
Visit GMOAnswers.com for more information.

GMO MISCONCEPTIONS

The top five misconceptions see across social media are:

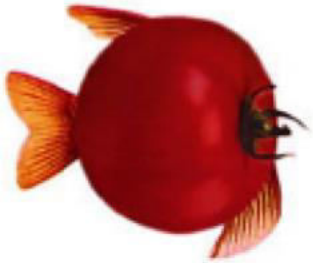
#1. If it's extra-large, seedless, looks weird, tastes bad and feels squishy – it must be a GMO.

#2. GMOs aren't safe and they're only tested by the companies making them.

#3. There is animal DNA in GMOs.

#4. GMOs have pesticides injected into them.

#5. GMO companies force farmers to grow their crops, or sue farmers if GMO seeds or pollen blow into their fields.



gmo answers Open to Your Questions About How Our Food Is Grown

GET TO KNOW GMO BASICS

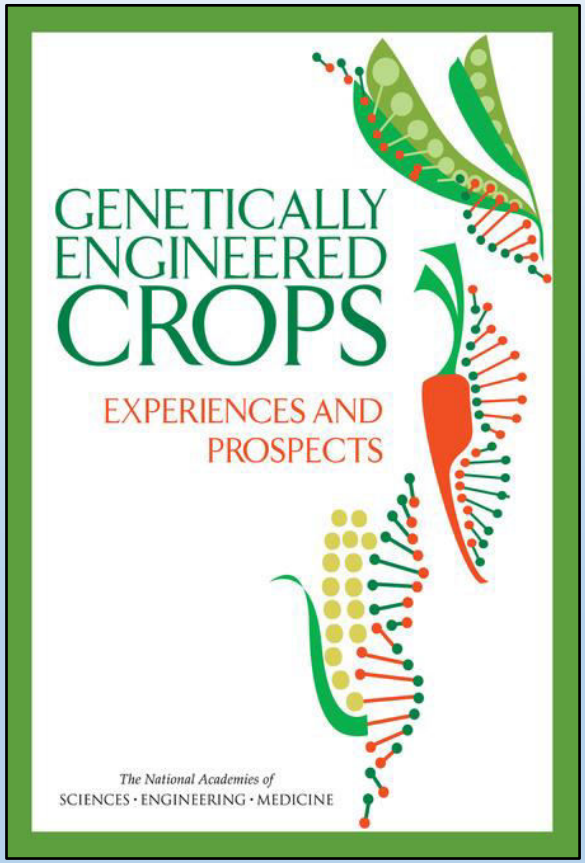
How do we ensure that GMOs are safe for use and consumption?

- GMO crops are studied extensively to make sure they are safe for people, animals and the environment
- GM seeds take an average of $136 million and 13 years to bring to market because of research, testing and regulatory approvals conducted by government agencies in the United States and around the world.[1]

gmo answers Open to Your Questions About How Our Food Is Grown

1 McDougall,P. (2011). The Cost and Time Involved in the Discovery, Development and Authorization of a New Plant Biotechnology Derived Trait

National Academy of Science Report on GE Crops - May 2016



While recognizing the inherent difficulty of detecting subtle or long-term effects in health or the environment, the study committee found no substantiated evidence of a difference in risks to human health between currently commercialized genetically engineered (GE) crops and conventionally bred crops, nor did it find conclusive cause-and-effect evidence of environmental problems from the GE crops.

http://nas-sites.org/ge-crops/ FREE: full report; short report; slides from news release

National Academy of Science Report on GE Crops - May 2016

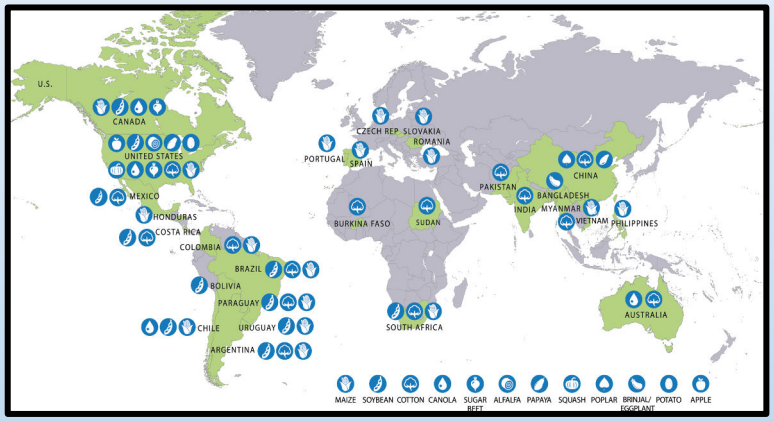
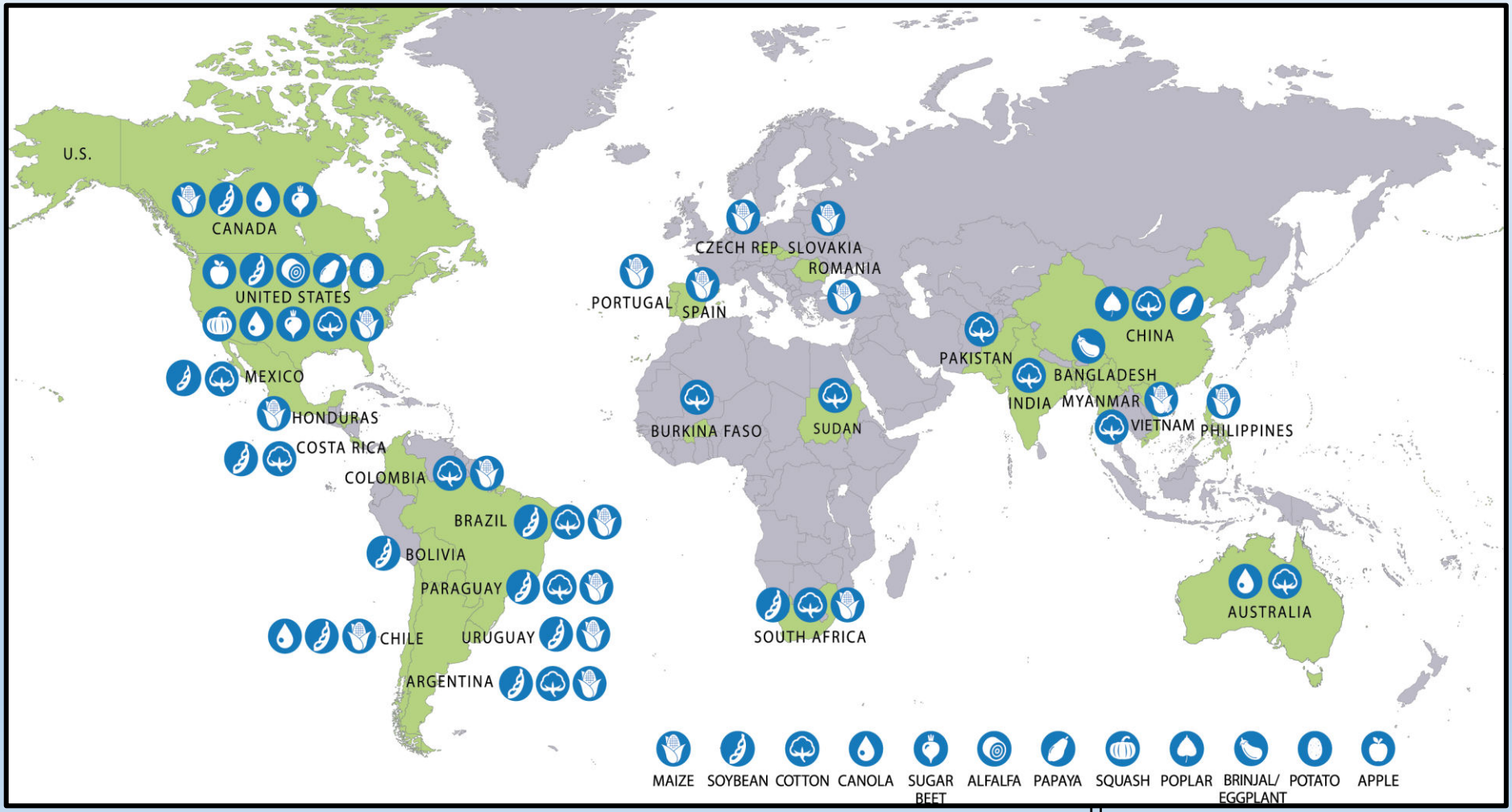


Figure 1. Commercially Grown Genetically Engineered Crops Worldwide.

In 2015, almost 180 million hectares of GE crops were planted globally, which was about 12% of the world's planted cropland that year. There were herbicide-resistant varieties of maize (corn), soybean, cotton, canola, sugar beet, and alfalfa, and insect-resistant varieties of maize, cotton, poplar and egglplant.

http://nas-sites.org/ge-crops/ full report; short report; slides from news release

National Academy of Science Report on GE Crops - May 2016



Genetically Modified Crops currently planted in the U.S.

CROP	TRAITS
Maize (corn)	Herbicide tolerance, insect resistance
Soybean	Herbicide tolerance, insect resistance, high oleic acid content
Cotton	Herbicide tolerance, insect resistance
Canola	Herbicide tolerance, high oleic acid content
Sugar beet	Herbicide tolerance
Alfalfa	Herbicide tolerance
Papaya	Disease (virus) resistance
Squash	Disease (virus) resistance
Potato	Resists bruising; reduced asparagine content
Apple	Delayed browning

Genetically Modified Crops

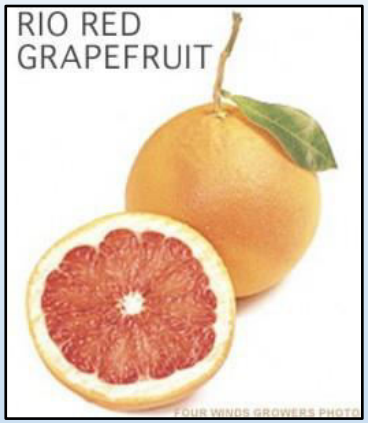
- Intelligence Squared U.S., December 3, 2014 Debate
  http://intelligencesquaredus.org/debates/past-debates/item/1161-genetically-modify-food (about 20 minutes)
  - This page is good for obtaining information for both sides of the debate. Spoiler alert: GM Foods win!

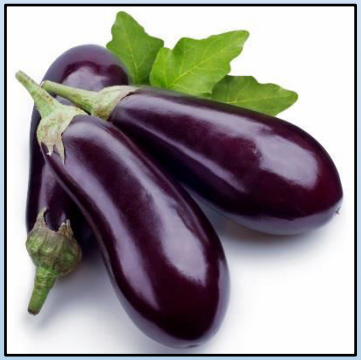
- Genetic Literacy Project https://www.geneticliteracyproject.org/

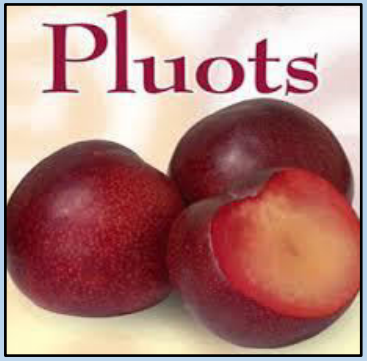
- GMOanswers https://gmoanswers.com
  - can ask any question; PowerPoint; posters, brochures, etc. - all free and free to use

- Florida Tomatoes – GM tomatoes have been developed to resist a devastating bacterial disease, but . . .
  - The Good, the Bad, and the Ugly: What the Future Could Hold for Bs2 Tomatoes http://edis.ifas.ufl.edu/hs1259

Food Quiz: Pick the GMO







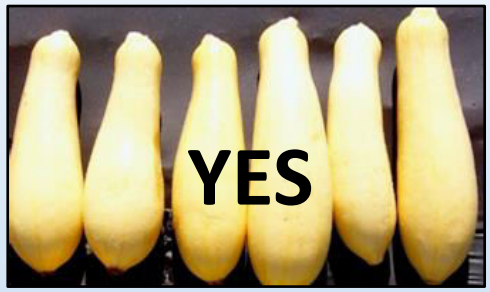
Food Quiz: Pick the GMO

No, derived by mutagenesis

No

interspecies cross

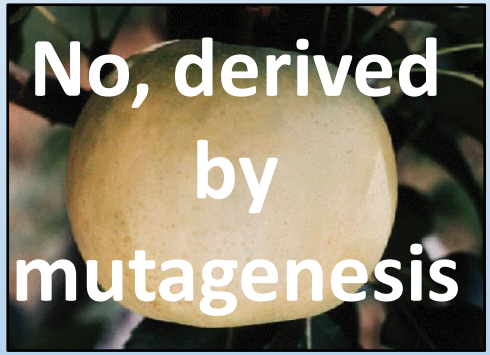
grapefruit x tangerine



YES

No

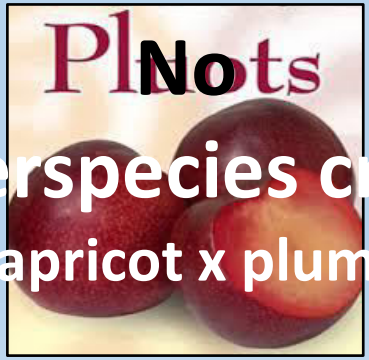
MAYBE



No, derived by mutagenesis

No

YES



No

interspecies cross

apricot x plum