



Bioteknologi Tanaman

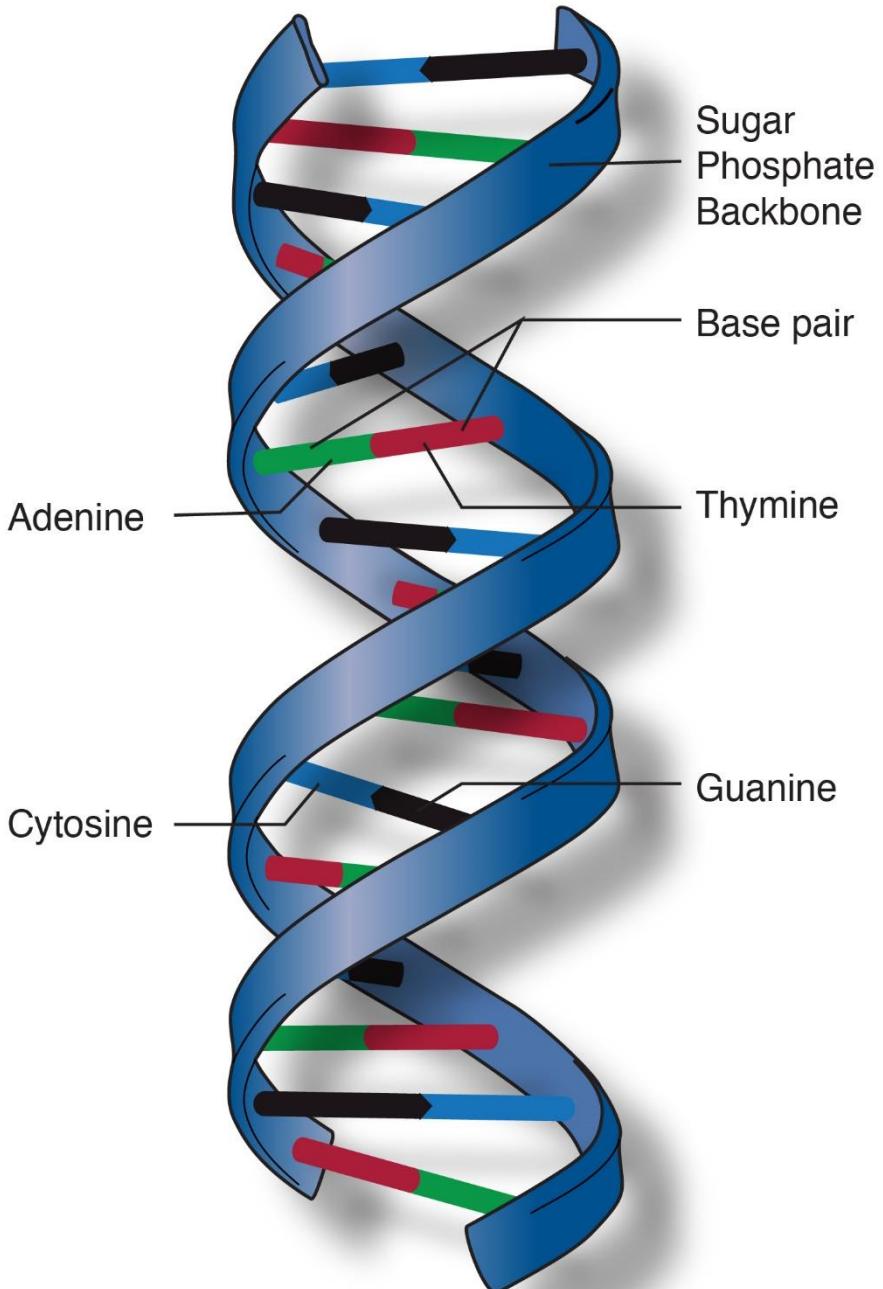
REKAYASA GENETIKA TANAMAN

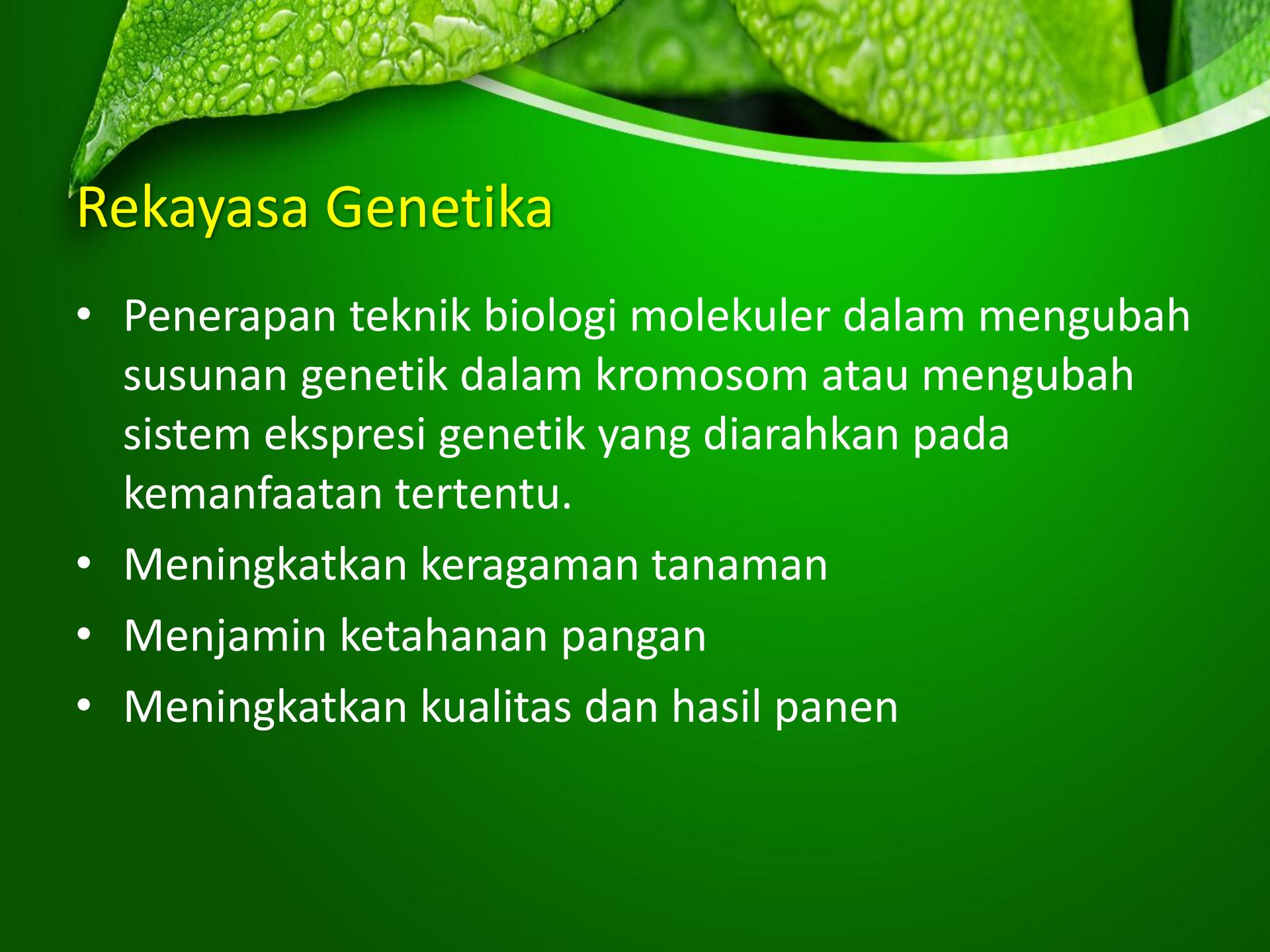
Gabriel Tirtawijaya





Apa itu rekayasa genetika?





Rekayasa Genetika

- Penerapan teknik biologi molekuler dalam mengubah susunan genetik dalam kromosom atau mengubah sistem ekspresi genetik yang diarahkan pada kemanfaatan tertentu.
- Meningkatkan keragaman tanaman
- Menjamin ketahanan pangan
- Meningkatkan kualitas dan hasil panen

Tujuan Rekayasa Genetika Tanaman

Memberikan karakter baru pada tanaman melalui pengintegrasian gen-gen yang berasal dari organisme lain untuk perbaikan sifat tanaman itu sendiri.

- ✓ to produce needed chemicals
- ✓ to carry out useful processes
- ✓ to give an organism desired characteristics

A close-up photograph of a vibrant green leaf. The leaf's surface is covered in numerous small, clear water droplets, which catch the light and appear to glisten. The leaf has a slightly serrated edge and a prominent central vein. The background is dark and out of focus, making the green of the leaf stand out.

Bioteknologi Tanaman

Traditional Biotechnology

- Growing plants
- Plant breeding (selective & interspecies crosses)

Gene Manipulation & Introduction

- Identify gene from another species which controls a trait of interest or modify an existing gene (create a new allele)
- Introduces that gene into an organism
→ transformation (forms transgenic organisms)

THE EVOLUTION OF MAIZE

The wild ancestor



Teosinte

Domestication



±5000 BC

First corns

The adaption to Europe



1494

Populations

Extension of corn crop areas



1947

Hybrids

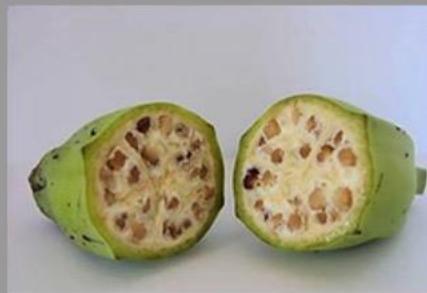
GET TO KNOW GMO BASICS



watermelon



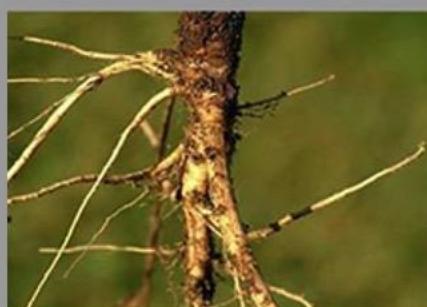
corn



banana



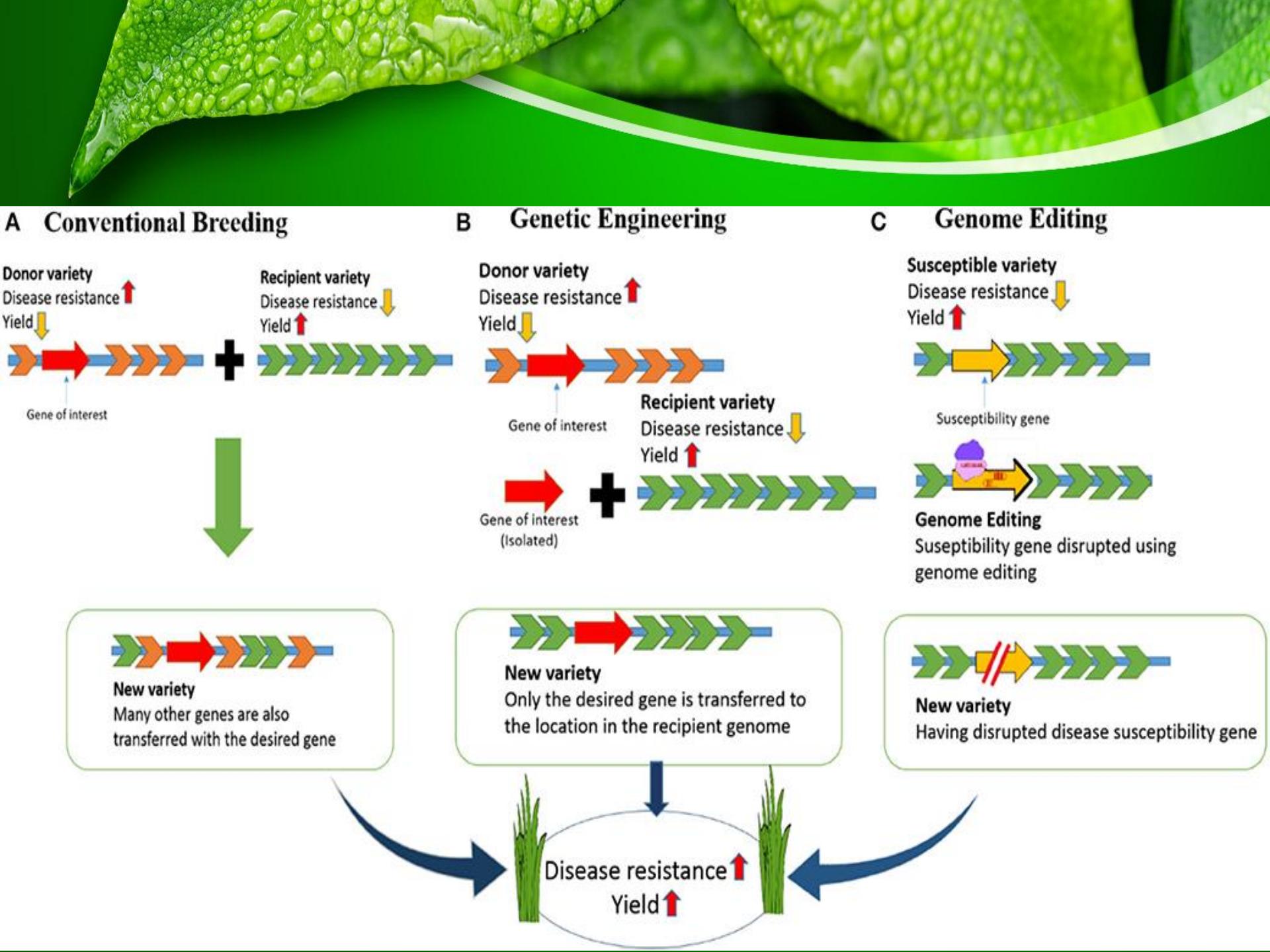
aubergine / eggplant



carrot



cabbage, kale, broccoli, etc.



Transformasi

- Memperkenalkan DNA ke dalam host sel lain sedemikian rupa, sehingga gen tersebut dapat diekspresikan
- Gen pada DNA yang ditransformasikan harus mampu:
 - a. Transkripsi dan translasi untuk membentuk molekul protein
 - b. Replikasi dan dapat diturunkan ke generasi berikutnya



Transformasi

- Untuk mengontrol replikasi dan ekspresi DNA asing yang telah disisipkan kedalamnya dapat digunakan Vektor ,plasmid maupun virus
- Transformasi dapat didefinisikan: Perubahan keadaan/sifat satu strain oleh DNA asing
- Dapat dilakukan dengan memasukan DNA donor ke dalam sel inang
- DNA donor bisa utuh ataupun rekombinan



Sejarah Organisme Rekayasa Genetika (GMO)

- 1982 1st transgenic plant produced (antibiotic resistance tobacco)
- 1984 1st transgenic plant produced using Caulimovirus vector (CaMV)
- 1994 1st GMO approved for sale in US (Flavr-Savr tomato)
The EU approved GMO herbicide-resistance tobacco → 1st commercially marketed in Europe
- 1995 US-EPA approved Bt-potato (insecticide-producing potato)

Sejarah GMO

- 1996 1st GM flower introduced (Moondust-bluish colored carnation)



- 2000 Rice containing β -carotene (Golden rice)



Syarat Dasar Transformasi Genetik

1. Gen target
2. Vector pembawa gen
3. Modifikasi DNA asing untuk meningkatkan tingkat ekspresi gen
4. Metode pengiriman plasmid DNA ke dalam sel
5. Metode identifikasi *transformed cell*
6. Kuljar untuk memulihkan tanaman yang layak dari sel yang diubah



Prinsip Dasar Rekayasa Genetika

1. Identify required gene and isolate it.
2. Make multiple copies of the gene.
3. Insert the gene into the cells of the host organism.
4. Identify the cells that have the gene and clone it.

Genetic engineer's toolkit:

- a. Enzymes
- b. Vectors
- c. Marker genes



Microorganism



Plant



Animal

Functional
genomics



Genes of interest

Recombination



Recombinant DNA

Transformation



Transformed
Plant cell

Regeneration



New variety

Breeding

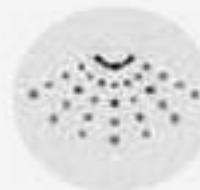


Safety assessment

Safety
assessment



Transgenic plant



Particle gun



Aplikasi Rekayasa Genetika Tanaman

- Toleran terhadap zat kimia tertentu (misal herbisida)
- Tahan terhadap hama dan penyakit tertentu
- Memiliki sifat khusus, misalnya longer-lasting tomato dan padi yang mengandung β -carroten & vitamin A.
- Dapat mengambil nitrogen langsung dari udara.
- Tahan terhadap lingkungan ekstrim, misalnya kekeringan, cuaca dingin, dan tanah bergaram tinggi.



Keilmuan yang Mendasari Rekayasa Genetika

- Restriction Enzymes & Recombinant DNA
- Gene Discovery, Isolation and Cloning
- Move Foreign Gene or Transgene from Any Organism to Any Other Organism



Metode Rekayasa Genetika Tanaman

Mediasi Vektor

Biologi

- Mediasi bakteri *Agrobacterium tumefaciens* & *A. rhizogenes*
- Mediasi virus

Tanpa Mediasi Vektor

Fisik

- Microinjection
- Pressure
- Biolistic
- Electroporation
- Silica carbide fibers

Kimia

- PEG
- DEAE-dextran
- Calcium phosphate
- Artificial lipids
- Proteins

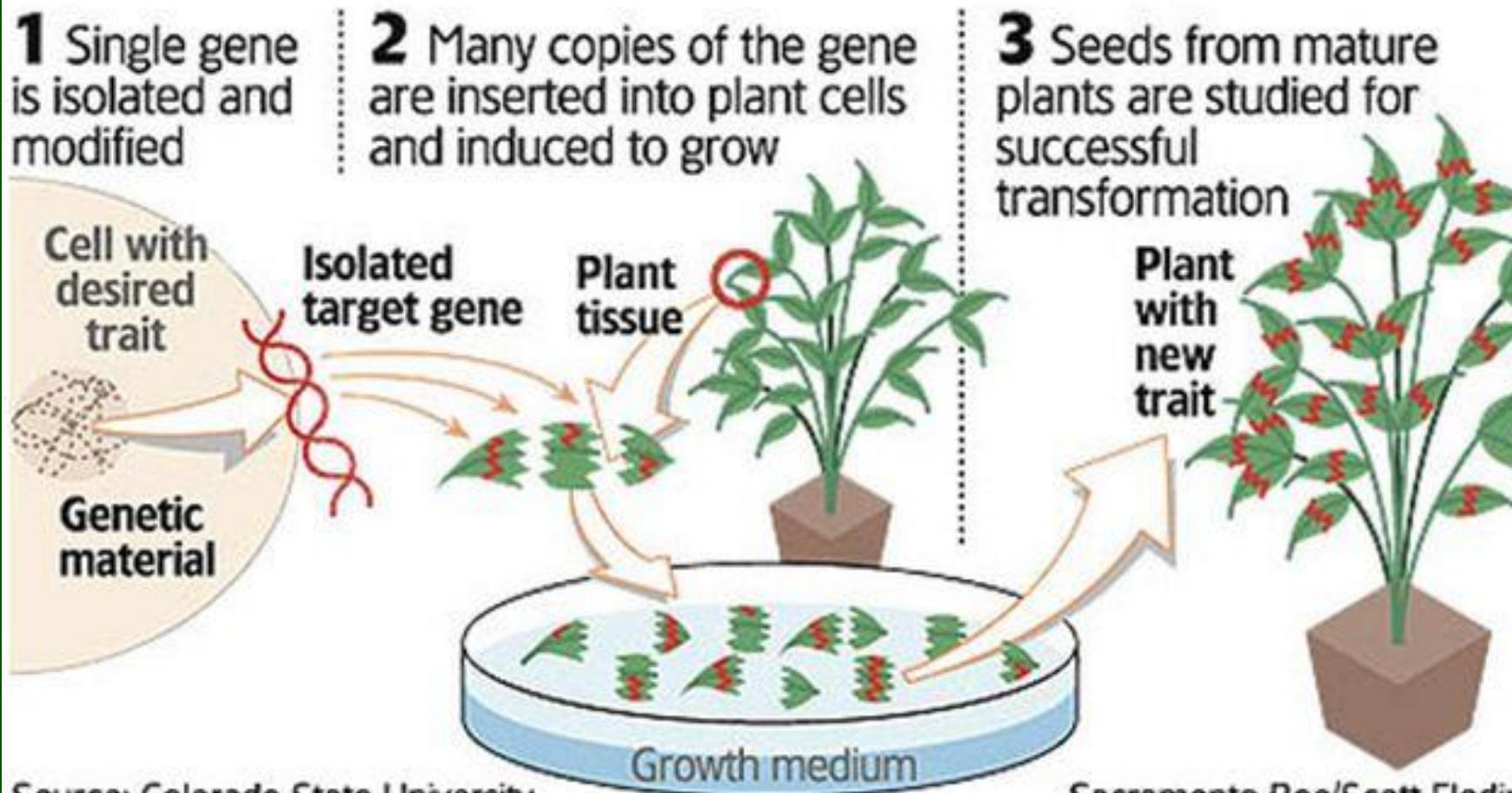
Metode Rekayasa Genetika Tanaman

TABLE 49.1 Gene transfer (DNA delivery) methods in plants

<i>Method</i>	<i>Salient features</i>
I. Vector-mediated gene transfer	
Agrobacterium (Ti plasmid)-mediated gene transfer	Very efficient, but limited to a selected group of plants
Plant viral vectors	Ineffective method, hence not widely used
II. Direct or vectorless DNA transfer	
<i>(A) Physical methods</i>	
Electroporation	Mostly confined to protoplasts that can be regenerated to viable plants. Many cereal crops developed.
Microprojectile (particle bombardment)	Very successful method used for a wide range of plants/tissues. Risk of gene rearrangement high.
Microinjection	Limited use since only one cell can be microinjected at a time. Technical personnel should be highly skilled.
Liposome fusion	Confined to protoplasts that can be regenerated into viable whole plants.
Silicon carbide fibres	Requires regenerable cell suspensions. The fibres, however, require careful handling.
<i>(B) Chemical methods</i>	
Polyethylene glycol (PEG)-mediated	Confined to protoplasts. Regeneration of fertile plants is frequently problematical.
Diethylaminoethyl (DEAE) dextran-mediated	Does not result in stable transformants.

Genetic engineering

Researchers isolate a gene from an organism that has the trait they want to impart to a plant.

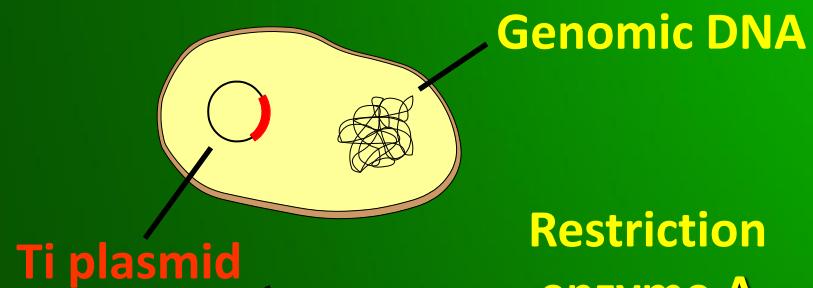


Source: Colorado State University

Sacramento Bee/Scott Flodin

Agrobacterium tumefaciens

Agrobacterium



Ti plasmid
Genomic DNA

Restriction enzyme A

Empty plasmid

Gene of interest

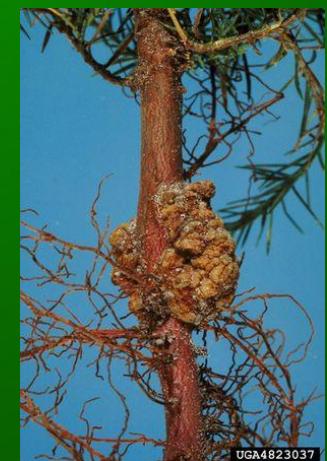
Ti plasmid with the gene of interest

Plant cell

Genomic DNA (carries the gene of interest)

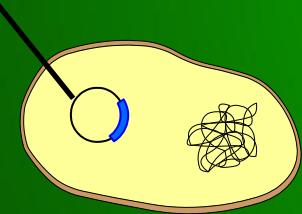
Restriction enzyme A

Gene of interest

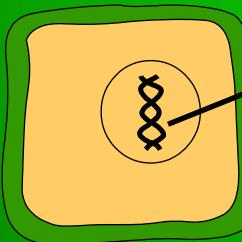


Agrobacterium tumefaciens

Ti plasmid with **the new gene**

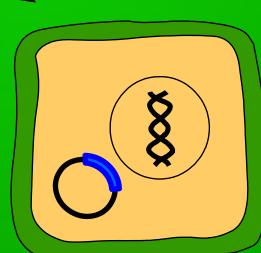


Agrobacterium

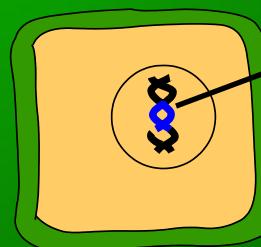


Plant cell

cell's
DNA



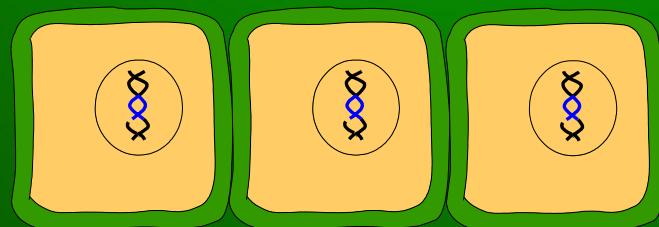
Transformation



**The new
gene**



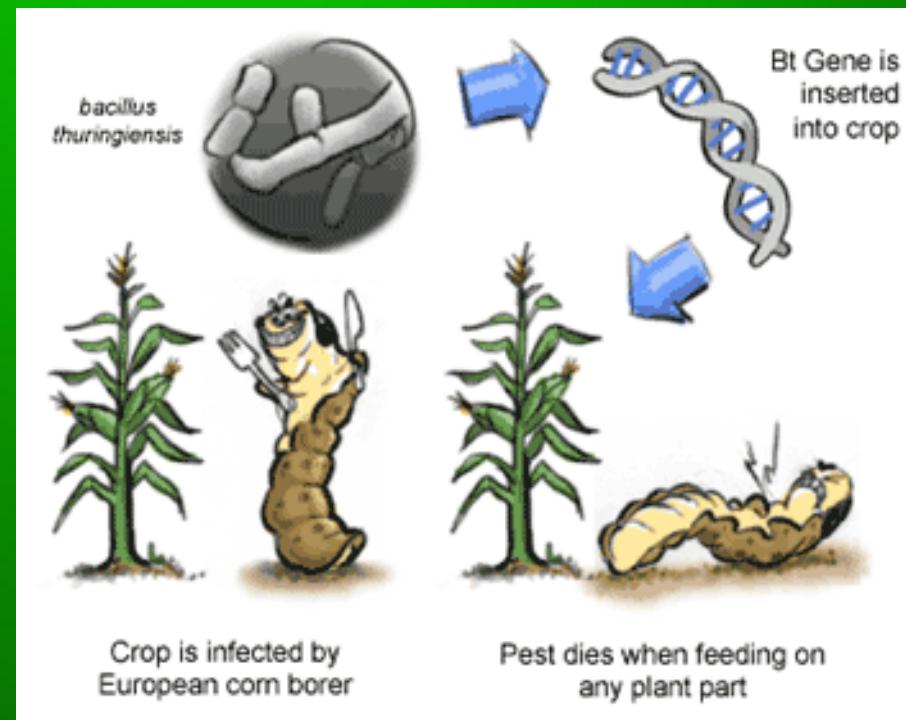
Transgenic plant



Cell division

Gen yang disisipkan pada tanaman GM

- Endo-toxin dari Bt
- Protease inhibitors
- Alpha amylase inhibitors
- Lectins
- Enzymes
- Pyramiding genes



Gen yang disisipkan pada tanaman GM

- Protease inhibitors (PI)

Menghambat protease pada pencernaan serangga.

Contoh: Gen trypsin inhibitor (CpTi) dari Cowpea disisipkan pada tembakau untuk mencegah serangan

Helicoverpa





Gen yang disisipkan pada tanaman GM

- Alpha Amylase Inhibitors (AAI)

Menghambat digesti karbohidrat pada serangga.

Contoh: Tomat & kentang dengan gen AAI resisten terhadap serangan hama Lepidopteran

- Lectin genes

Lectin mengikat karbohidrat, termasuk kitin pada saluran cerna serangga. Sehingga mengganggu penyerapan nutrisi

Contoh: Gen pea lectin (P-Lec) disisipkan pada tembakau untuk mencegah serangan *Helicoverpa*



Gen yang disisipkan pada tanaman GM

- Enzymes

Gen chitinase, cholesterol oxidase, dan lipo-oxygenase disisipkan pada tanaman untuk memunculkan sifat insektisida
Contoh: Gen bean chitinase (BCH) disisipkan ke tembakau untuk mecegah serangan Aphid

- Pyramiding Genes

Penyisipan > 1 gen untuk memperoleh banyak mekanisme pertahanan

Contoh: CpTi + P-Lec disisipkan pada tembakau

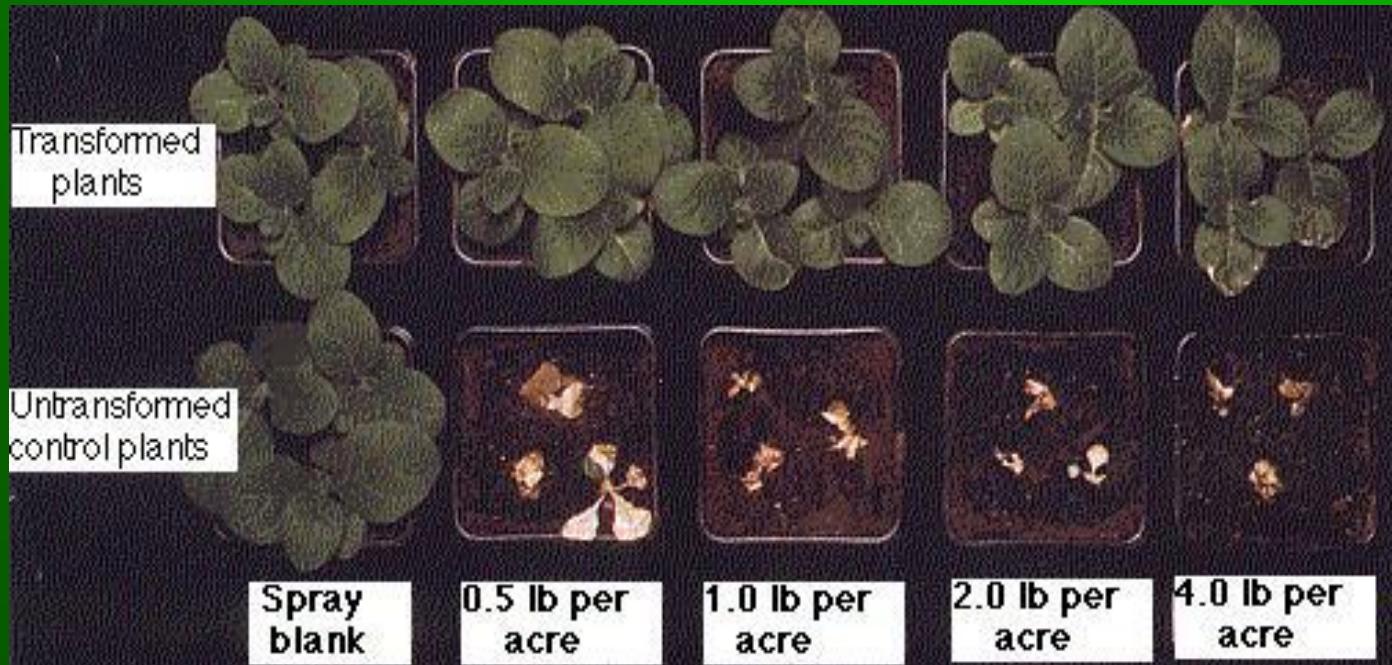
C5 Plum pox resistant plums



Plums that have been genetically engineered to be resistant to the plum pox virus



Pesticide resistance

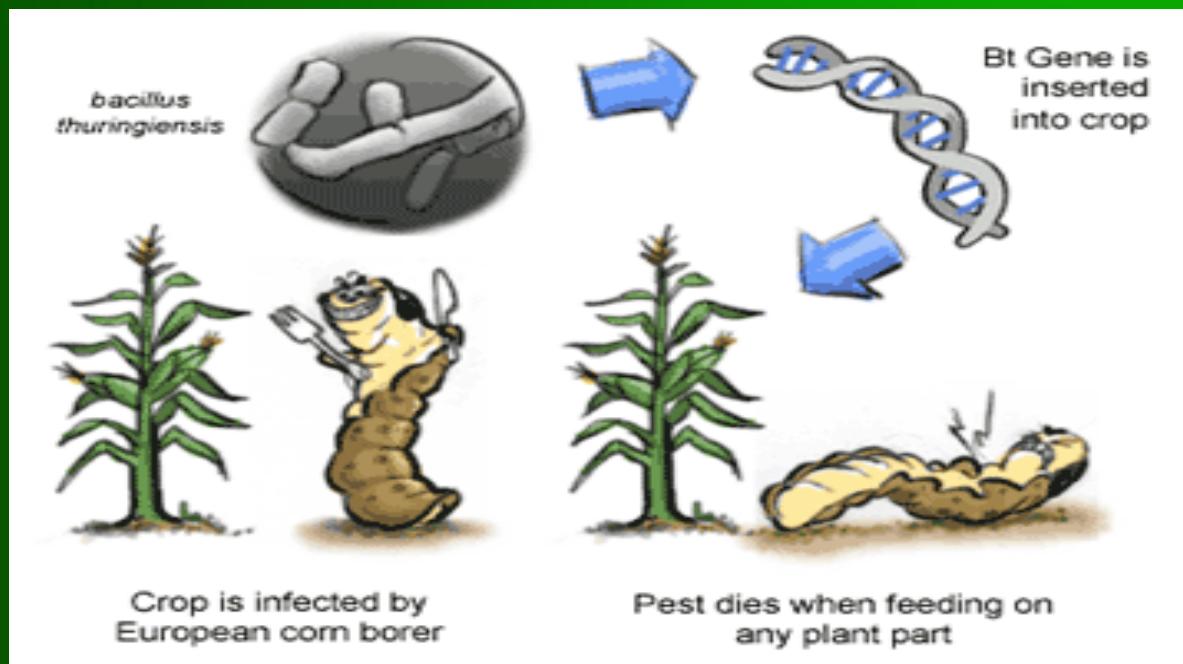


Effect of the herbicide bromoxynil on tobacco plants transformed with a bacterial gene of which a product breaks down bromoxynil (top row) and control plants (bottom row). "Spray blank" plants were treated with the same spray mixture as the others except the bromoxynil was left out.

(Courtesy of Calgene, Davis, CA.)

Bt Corn

Bt corn is a variant of maize, genetically altered to express the bacterial Bt toxin, which is poisonous to insect pests. The pest is the European Corn Borer.



Bt Cotton



Insect Control Ledger for 2000: Conventional Insecticides versus Bollgard® Cotton on Five Million Acres

Conventional Cotton	Manufacturing	Distribution	Application	Financial Benefit	Stewardship	Consumer Benefit
						
Bollgard® Cotton						

Conventional Cotton

- Saves 3.46 M lb raw material
- Conserves 1.48 Mgal fuel oil
- Eliminates 2.16 M lb industrial waste

Bollgard® Cotton

- Transports and stores 416,000 gal insecticide less
- Conserves 604k gal fuel oil

Financial Benefit

- Fewer insecticide use and storage
- Saves workdays for insecticide application
- Conserves 2.41 Mgal fuel and 93.7 Mgal water

Stewardship

- Reduces pesticide exposure risk
- Preserves beneficial insect populations
- Creates wildlife benefits
- Gives cotton producers more time for family and community activities
- Gives cotton producers peace of mind

Consumer Benefit

Produces fiber equivalent to that found in all consumer products derived from cotton

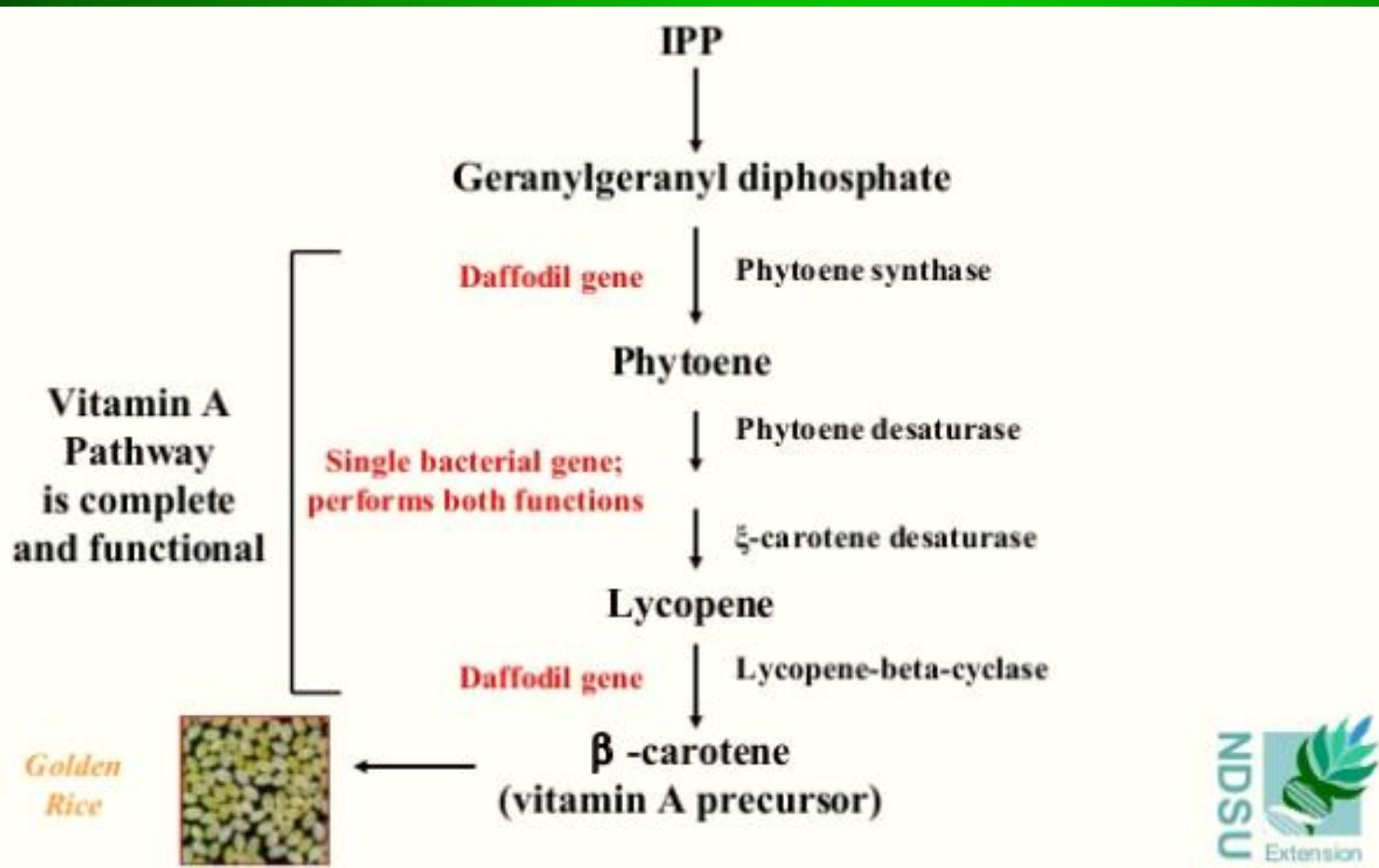
Golden rice



Production of transgenic cereals for developing countries

- Introduction of genes that produce β -carotene in the rice grain.
- Beta-carotene is present in the leaves of the rice plant, but conventional plant breeding has been unable to put it into the grain.
- Dr. Ingo Potrykus of the Swiss Institute of Plant Sciences in Zurich, with Rockefeller funding, transferred one bacterial and two daffodil genes.
- The transgenic rice grain has a light golden-yellow color and contains sufficient beta-carotene to meet human vitamin A requirements from rice alone.
- Potrykus has also added a gene from the French bean to rice that increases its iron content over threefold.

β -Carotene Pathway in Golden Rice



New Ways to Protect Drought-Stricken Plants

Anne Simon Moffat. Science 296:1226-1229, May 17 2002.

With drought an ever-present threat, researchers are identifying genes that can help plants tolerate arid conditions in hopes of using them to produce hardier crops.



Tomato plants carrying a foreign gene that protects their cells from salt-induced dehydration thrive in a 200-mM salt solution, whereas unaltered plants wither.

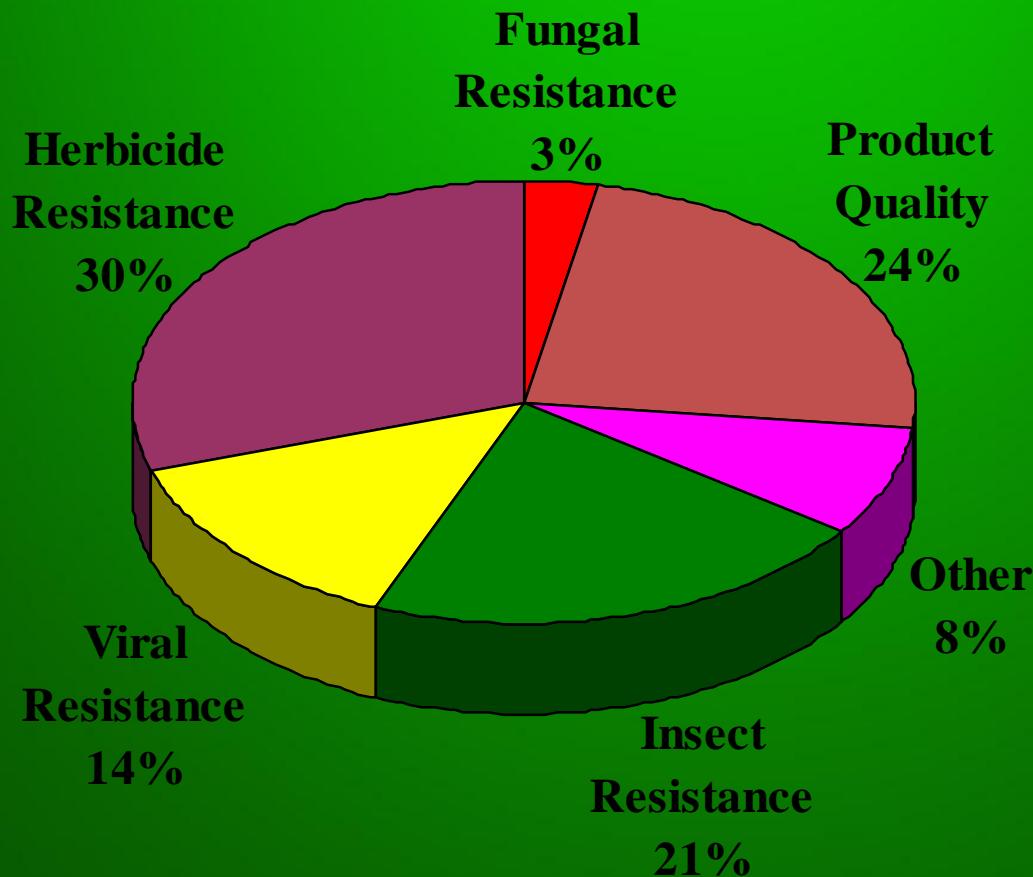


Improving the drought tolerance of corn could make dried-out crops like this one a thing of the past.

Credit: Richard Hamilton Smith/Corbiss

What's Being Tested

(US field releases, cumulative since 1987-2015)



Genetic traits expressed in GMOs in the US

FIELD CORN

Genetic Traits

Insect Resistance

Herbicide Tolerance

Uses

- Livestock and poultry feed
- Fuel ethanol
- High-fructose corn syrup and other sweeteners
- Corn oil
- Starch
- Cereal and other food ingredients
- Alcohol
- Industrial uses



SOYBEAN

Genetic Traits

Insect Resistance Herbicide Tolerance

Uses

- Livestock and poultry feed
- Aquaculture
- Soybean oil (vegetable oil)
- High oleic acid (monounsaturated fatty acid)
- Biodiesel fuel
- Soymilk, soy sauce, tofu, other food uses
- Lecithin
- Pet food
- Adhesives and building materials
- Printing ink
- Other industrial uses



CANOLA

Genetic Traits

Herbicide Tolerance

Uses

- Cooking oil
- Animal feed



ALFALFA

Genetic Traits

Herbicide Tolerance

Uses

- Animal feed



RAINBOW PAPAYA

Genetic Traits

Disease resistance

Uses

- Table fruit



COTTON

Genetic Traits

Insect Resistance

Herbicide Tolerance

Uses: Fiber, Animal feed, Cottonseed oil



SUGAR BEETS

Genetic Traits

Herbicide Tolerance

Uses: Sugar, Animal feed



SWEET CORN

Genetic Traits

Insect Resistance

Herbicide Tolerance

Uses: Food



SUMMER SQUASH

Genetic Traits

Disease resistance

Uses: Food



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.



Open to Your Questions
About How Our Food Is Grown

GET TO KNOW GMO BASICS

Why GMO? SEED IMPROVEMENT

SEED IMPROVEMENT TECHNIQUE	SELECTIVE BREEDING	INTERSPECIES CROSSES	MUTAGENESIS	TRANSGENESIS (GMOs)
What is it?	10,000 years ago to today	Late 1800s to today	1930s to today	1990s to today
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 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.



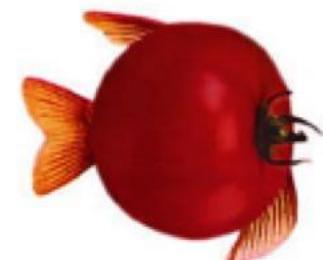
#2. GMOs aren't safe and they're only tested by the companies making them.

#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.



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



safe to grow



safe for the environment



safe to eat

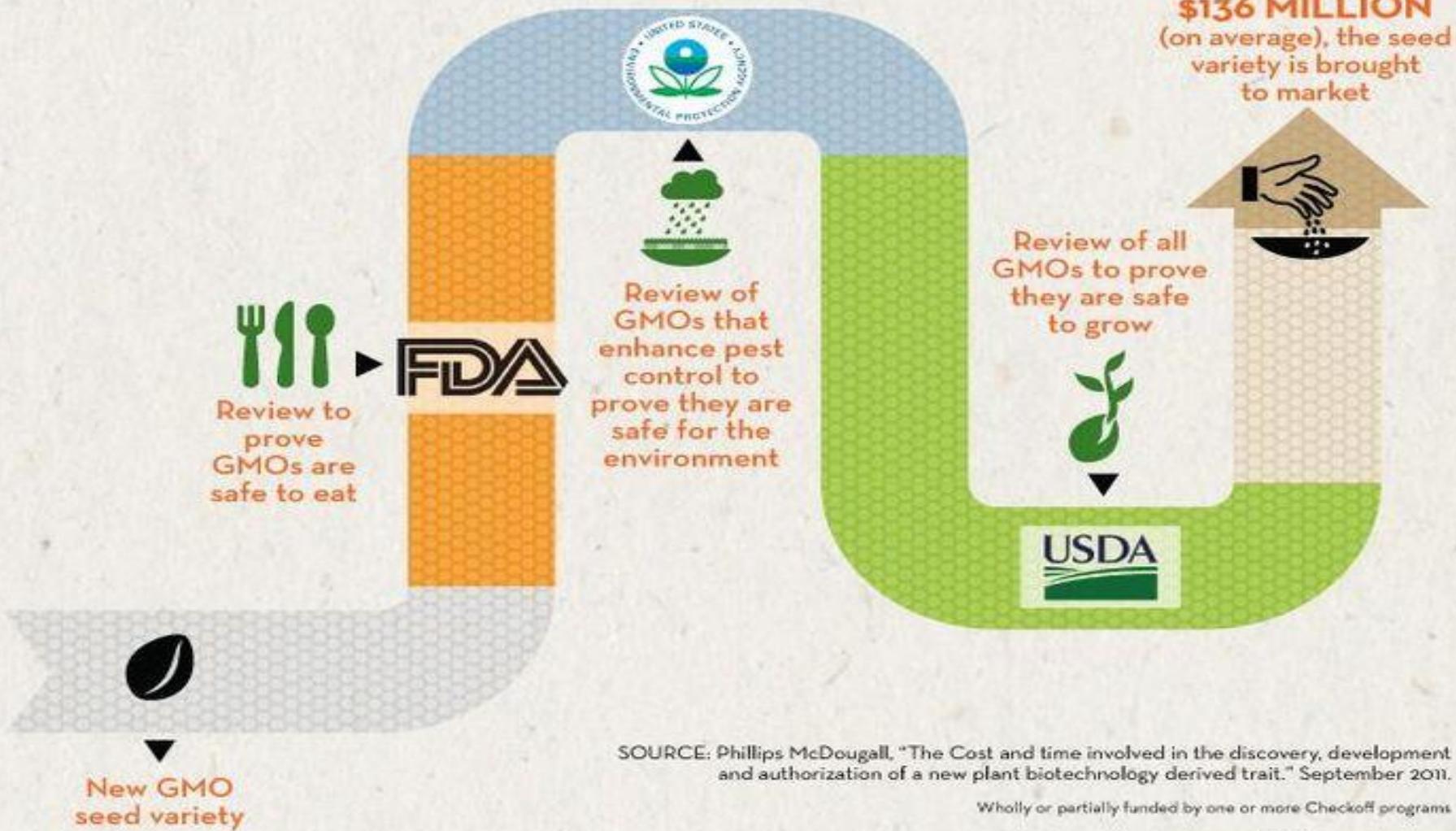


Open to Your Questions
About How Our Food Is Grown

HOW A GM SEED GETS TO MARKET

No other type of new seed that comes to market from other breeding methods goes through regulatory approval, including the thousands of conventional and organic seeds developed from mutagenesis*. Only GMOs are required to be reviewed. Even before the new seed goes through the review process, years of testing and research take place.

*Deliberately engineered DNA mutations

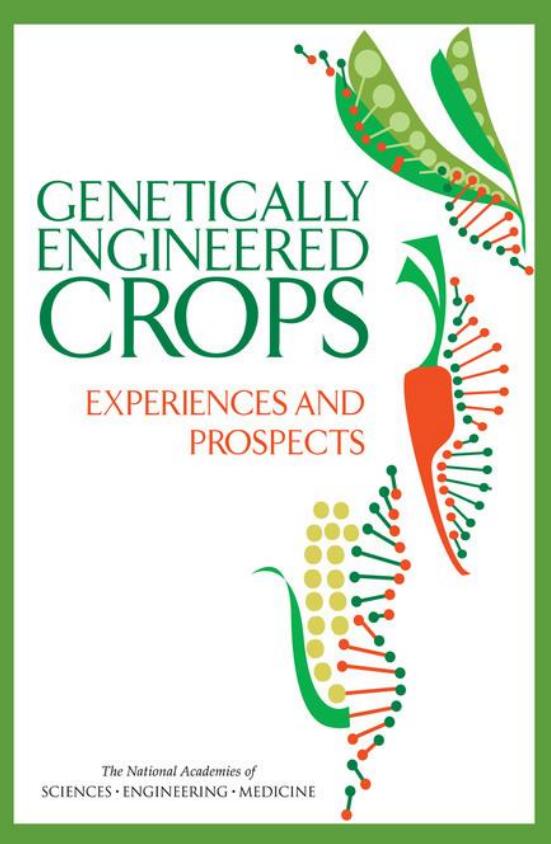


Januari 2022

Label pada produk yang
mengandung GMOs



National Academy of Science Report on GE Crops - May 2016



Tidak menemukan bukti nyata tentang perbedaan risiko terhadap kesehatan manusia antara tanaman rekayasa genetika (RG) yang saat ini dikomersialkan dan tanaman yang dibiakkan secara konvensional, juga tidak menemukan bukti sebab-akibat yang meyakinkan tentang masalah lingkungan dari tanaman RG.

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