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Genetically modified organism

- An organism that has been genetically modified in a laboratory

Types

- Transgenic
- Across species
- Bacteria -> corn
- Cisgenic
- Same species
- Editing the genetic material to make an apple rot slower
- Intragenic
- Within same species
- One tomato has a better gene, we put that gene in the other tomatoes

History

- Selective breeding
- Used in agriculture for 12000 years
- Artificial selection
- Humans identify an appealing natural trait variation
- Organisms with that characteristic are bred to produce offspring with the same trait
- 1972: scientists discovered how to put genes inside bacteria
- Beginning of genetic engineering
- Genetic engineering: artificial manipulation of DNA in a lab to benefit humans
- Paul berg
- Combined the genes of two virus
- He produced a unique combination of genes
- Referred to as recombinant DNA or rDNA
- First GMO
- Made by Boyer and Cohen in 1972
- Inserted kanamycin in antibiotic resistance gene into a plasmid
- Came from a different strain of bacteria
- Plasmid was inserted into E. coli
- Result: E. coli became resistant to kanamycin

- First transgenic GMO
- Boyer and Cohen inserted frog ribosomal RNA into plasmid
- Plasmid was inserted into E. coli
- First GMO approved by FDA
- Flavr-Savr made by Calgene in 1994
- Problem: food waste due to fruit over-ripening
- Gene that dissolves pectin in the skin of the tomato was disabled
- Was removed from the market due to public expressed opposition that made the product unprofitable

Why GMOS?

- Climate change
- Human-induced changes to the climate of our planet
- Results
- Changes in precipitation
- Ecosystems altered
- Solutions
- Wheat with increased photosynthesis in CO_2-rich environments
- Rice modified to survive in high-salt environments
- Drought-resistant corn and soybeans
- Pest management
- Pesticides naturally select for resistant insects
- Some pesticides have been used to cause adverse in humans
- Solutions
- Naturally occurring endotoxin gene from the bacteria Bacillus thuringiensis (Bt) inserted into several varieties of corn, soybean, and cotton
- Viral resistant papaya
- Growing population
- Solutions
- Modify plants so that they offer more nutrition
- Modify plants so that they increase their yield per acre

Examples

- Bacteria
- Why GM bacteria?
- Small genome with <100 genes
- Contain plasmids

- Rapid generation time of 15 minutes
- Can be grown in mass to produce large quantities of a desired product in a short time frame
- Uses
- Makes biotechnology products
- Medicine (insulin, etc)
- Vaccine for Hep B
- Clotting factor for hemophilia
- Hormones (human growth hormone)
- Treat and prevent tooth decay
- Treat inflammatory diseases of large intestine like Chron’s disease
- Treat phenylketonuria by breaking down phenylalanine
- Protection of plants
- Produce proteins to protect plants from freezing
- Bt gene protects against insect predation
- Bioremediation
- Bacteria can be used to break down toxic chemicals in the environment
- Bacterial enzymes just need to be enhanced
- Synthesis of organic chemicals
- Can be used to produce biofuels used in manufacturing
- Animals
- Models for human disease
- Mice with sickle cell disease
- Biotechnology products
- Pharmaceutical compounds in milk, meat, or eggs
- Increased nutritional value
- Reduce susceptibility to disease
- Increase rate of growth
- Xenotransplantation
- Pigs are being investigated for the production of human organs
- Pest control
- Mosquitos modified to become sterile
- Plants
- Reduce impact of pests like insects
- Increase nutritional value or reduce spoilage
- Resist environmental challenges due to climate change
- Produce biotechnology products
- Herbicide-resistant crops

- Problem: weeds are also plants, difficult to produce a chemical that targets weeds but leaves the crops to survive
- Solution: make crops resistant to glyphosate
- Glyphosate (RoundUp): prevents plants from making a specific amino acid

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Basic plant characteristics

- Multicellular
- All plants are composed of cells arranged into more complex tissues and organ systems
- Eukaryotes
- Plant cells contain a nucleus and membrane-bound organelles
- Photosynthetic
- Plants are autotrophs
- Autotrophs have the ability to make their own food
- Plants use sunlight as their source of energy and CO_2 as their carbon source

Evolution of plants

- Charophytes = green freshwater algae
- Once plants left the aquatic environment they had to adapt
- Prevent drying out
- Develop a way to transport water and nutrients to all of their cells
- Change how they disperse their gametes in the absence of water
- Protect embryos from the temperature
- Mosses
- Primitive, no vascular system, restricted to moist environments
- First to have embryo protection
- Lycophytes
- First plants
- Ferns
- Large leaves (megaphylls) increase surface area for photosynthesis
- Gymnosperms
- First plants to develop seeds
- Angiosperms
- Developed an alternative reproductive structure, the flower

Phylogeny

- Shows all major adaptive events

Life cycle of a plant

- Both the sporophyte and gametophyte stages are present in an individual plant
- Sporophyte stage is responsible for photosynthesis
- Meiosis reduces the chromosome number
- But mitosis produces the gametes in plants

Life cycle of an angiosperm

- Flower = sporophyte reproductive structure
- Pollen grains and embryo sacs = gametophyte reproductive cells
- Anther produces pollen grains
- Select cells in the ovary become the egg and embryo sac
- Pollen grain reaches the ovule with the help of a pollinator
- Germinates to release haploid sperm
- Pollen grain contain two sperm
- One unites with the egg to produce a diploid embryo
- Second unites with a diploid cell in the ovary to produce the endosperm (3n)
- Diploid embryo is enclosed in a seed
- Endosperm is used as a food source for the developing embryo

Photosynthesis

- Series of chemical metabolic reactions used by plants to produce their own food
- Majority of photosynthesis occurs in the leaves
- Mesophyll cells house the cellular machinery for photosynthesis
- Chloroplasts: organelle responsible for chemical reactions
- Thylakoids: disc-like structures containing chlorophyll (a pigment)
- Grana: stacks of thylakoids
- Stroma: space between the grana stacks of thylakoids
- Two stages
- Light-dependent reactions
- Captures energy from the sun and transfers it to ATP and NADPH
- NADPH carries high-energy electrons
- Light-independent reactions
- Synthesize carbohydrates
- Occurs within the stroma of the chloroplast

- Uses energy from light-dependent reactions to convert CO_2 into carbohydrates
- Also called the Calvin cycle
- CO_2 is added to a 5-carbon molecule (RuBP)
- 6 carbon molecule produced is unstable
- Split into 3-phosphoglycerate
- Converted into 2 molecules of glyceraldehyde 3-phosphate
- Uses ATP and NADPH
- Some glyceraldehyde 3-phosphate molecules are combined to form glucose
- Others reused in the Calvin cycle
- Several turns of the cycle are needed to produce glucose
- Products
- Oxygen
- Carbohydrates
- Fruits, vegetables, grains
- Oils
- Energy source
- Protection against fungi and bacteria
- Fiber
- Structural component of plants
- Used for wood, paper, dietary fiber
- Chemical compounds
- Tannins, alkaloids, phenols
- Table 2.1

Why should we genetically modify plants?

- Solution to food waste
- Improves fruit ripening
- Fresh produce ripens and spoils quickly
- Once they are spoiled we throw them away
- Historical solution: crops picked before they are ripe and then exposed to a plant hormone (ethylene) when at destination
- Browning is caused by polyphenols
- Polyphenol oxidase catalyzes a reaction between polyphenols and oxygen causing the fruit to brown
- Gene that produces the enzyme in fruit can be turned off
- Example: arctic apple
- RNA interference

- Small pieces of RNA are introduced into the cells
- RNA finds a complementary strand on the mRNA
- Double-stranded RNA is targeted for destruction
- Translation does not occur
- Protein is not mad
- Solution to growing population
- Farmland is finite
- Need to increase the amount of food produced per acre
- Increases crop yield
- Yield is generally increased by preventing loss
- Predation from insects decreased
- Insertion of Bacillus thurignesis (Bt) endotoxin gene
- Competition from weeds
- RoundUp (glyphosate) inhibits the enzyme EPSPS
- EPSPS produces amino acids and vitamins for plant growth
- Plants can become resistant to glyphosate via insertion of genes from bacteria or by the insertion of genes that produce an enzyme that breaks down glyphosate
- Increase yield per acre by up to 25%
- Increase health outcomes
- Plants can also be modified to produce more of a compound
- Canole plants produce more specific fatty acids in their oils
- Solution to nutrient deficiencies
- Produces more nutritious foods
- 600,000 children die each year due to vitamin A deficiency
- Solution = golden rice
- Beta-carotene is a precursor of vitamin A
- Not naturally found in rice
- Two genes (daffodil and soil bacterium gene) inserted into rice

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How do we genetically modify a plant?

1. Identify the gene to modify
2. Find the gene in the genome
3. Make several copies of the gene
4. Modify the gene
5. Insert it into the plant

Proteins

- Responsible for all cell activities
- Processing energy and materials
- Maintaining an internal environment
- Responding to stimuli from the environment
- Reproducing
- Adapting to changing environmental conditions

DNA structure

- Polymer made of nucleotide monomers
- Nucleotides consist of a phosphate, deoxyribose sugar, nitrogenous base
- Two complementary antiparallel strands
- Adenosine pairs with thymine
- Cytosine pairs with guanine
- Nitrogenous bases held together by weak hydrogen bonds

Antiparallel DNA structure

- Two strands oriented in opposite directions
- At the bottom of one strand is a hydroxyl group (3 prime end)
- A phosphate group is at the bottom of the other end (5 prime end)

Converting PPO gene into a protein

- Transcription
- DNA transcribed into mRNA
- mRNA transported from the nucleus to the cytoplasm
- Translation
- The genetic information is interpreted into the structure of a protein

Enzyme

- Enzymes are proteins with a distinct 3D shape
- They are catalysts
- Increase reaction rate

DNA sequencing

- Identifies the order of nucleotides in a sequence
- Technique uses fluorescent dyes

- A different dye used for each nucleotide
- A chemical reaction removes one nucleotide at a time
- A laser identifies the dye at each location

1. DNA polymerase and a primer are added to the DNA sample
2. Fluorescent-labeled nucleotides are added
3. When added the replication reaction is halted
4. Yields different sizes of DNA fragments complementary to the original sequence
5. Laser and photodetector are used to read the colors of the fluorescently labeled nucleotides

DNA probe

- Uses single strand of DNA attached to a fluorescent label
- Strand is complement to the gene you are looking for
- Limit: you have to have the sequence for a similar gene in another plant

DNA microarray with DNA probes

- DNA probe can be used to identify the gene in the genome
- Genome is inserted in pieces on a gene chip
- Plastic or silicon slide containing segments of DNA from the organism of interest
- Brighter regions indication locations that closely match your gene

Gene cloning

1. PPO gene extracted from a cell
2. PPO gene is inserted into a bacterial plasmid
3. Circular plasmid DNA is cut open with a restriction enzyme
4. PPO gene is inserted with the help of DNA ligase
5. Recombinant DNA is inserted into a bacteria host cell
6. Recombinant DNA: DNA not found naturally in the environment
7. The cell produces multiple copies of the gene product
8. Copies are referred to as clones

Restriction enzymes

- Proteins that cut DNA
- Recognize a sequence 6-12 nucleotides long
- Cuts both strands typically leaving overhanging DNA

Polymerase Chain reaction

- Denaturation (95 C)
- Double-strand DNA is separated into two single strands of DNA
- Annealing (50-60 C)
- Primer binds to a specific DNA segment
- Primer = small synthetic complementary DNA piece
- Extension (72 C)
- Optimal temp for DNA polymerase to add complementary nucleotides to single strands
- DNA polymerase comes from the bacterium Thermus aquaticus found in hot springs

Mutagenesis

- Mutation: natural product of DNA replication
- DNA polymerase occasionally makes error during DNA replication process
- Can be manipulated to make purposeful changes in the DNA
- To understand gene function
- To understand gene product (protein) function
- Site-directed mutagenesis
- Add change into the synthetically produced PCR primers

CRISPR/Cas9 technology

- First discovered in bacteria as a type of immune system against bacteriophages
- PAM sequence prevents Cas9 from cutting up bacterial DNA
- Consists of two components
- Cas9 (molecular scissor)
- Guide RNA
- Used in genome editing
- Used to understand the role of a gene
- Inactivate a gene and observe effects

Gene delivery

- Agrobacterium tumefaciens
- Plant bacterial pathogen
- Plasmid can be inserted inside pathogen
- Contains new gene
- New gene added with restriction enzyme and ligase
- Added to plant cells
- Cells used to make new plants

- Problem pathogen doesn't infect every plant
- Particle bombardment
- Small particles of tungsten or gold are coated with modified DNA
- Fired at plant tissue using gene gun
- RNA interference
- Small RNA pieces introduced into the cells
- RNA finds a complementary strand on the mRNA
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- Protein is not made

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First FDA-approved GMO

- Flavr-savr made by Calgene in 1994
- Gene that dissolves pectin in tomato skin disabled
- Solution to food waste
- Remove from market due to public opposition (made it unprofitable)

GMO concerns

- Horizontal gene transfer: transfer of genes between species
- Common natural occurrence
- Bacteria routinely share genes between species
- Viruses routinely insert their genes into our genomes
- What is glyphosate-resistant gene in the crop is transferred to the weeds
- Insecticide-resistant weeds have been found
- Genetic analysis shows that this is mostly due to natural selection as a result of the widespread use of glyphosate and not gene transfer
- Adverse human reactions
- Starlink corn
- GMO corn with gene for insecticide resistance and pesticide resistance
- Pesticide resistance due to Bacillus thuringiensis
- Approved for use in animal feed in May 1998
- Was in the process of proving that Cry9C was not allergenic in order to get approval for human consumption
- One would have to already be allergic to the product being inserted
- It is virtually impossible to have a food allergy to a protein that is new to the human diet

- Impact on non-pest species
- Meta-analyses on non-target species generally do not appear to be impacted

Why do we need GM plants?

- Climate change
- Wheat with increased photosynthesis in CO2-rich environments
- Rice modified to survive high salt environments
- Drought-resistant corn and soybean
- Goal: grow more food on less land using less water and fewer chemical insecticides
- Pest management
- Growing population

Future GM plants

- Edible vaccines
- Easier to deliver
- Zmapp developed from GM tobacco plants to treat Ebola patients
- Elimination of harmful chemicals
- Potatoes that can not produce acrylamide when fried
- Removal of carcinogens from tobacco plant
- Yeast modified to accelerate the brewing process
- Cattle that produce less methane
- Edible vaccines
- Produced in chicken to prevent human disease
- Mouse models for human disease
- pork/beef with more beneficial fats
- AquaAdvantage Salmon
- Contains two genes from two other fish
- Result: salmon grow faster

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