Genetic Engineering of Agronomic Crops

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Outline

- Background Information
- Why I Chose A Module
- Module
- Quiz Questions
- Questions

Background Information

- Raised in Dodge City, Kansas
- Started working on a farm in Ensign, Kansas at the age of 14
  - Picking sweet corn
  - General farm hand
    - Life Lessons

Ensign, Kansas grain elevator. Photo courtesy of PrideAg

Education

- **Sterling College**
  - **General Education**
- **Kansas State University**
  - **B.S. in Agronomy**
    - **Crop Consulting Option**
- **Iowa State University**
  - **Working towards M.S. in Agronomy**

Internships

- **Flat Lands Ag Consulting**
  - **Pierceville, Kansas**
  - **2002, 2003**
- **Servi-Tech Incorporated**
  - **2004**

- **Corn, Soybean, Alfalfa,**
- **Cotton, Sunflower, Sorghum,**
- **Wheat**

Professional Experience

Monsanto Company

- Temporary seed laborer (Wichita, KS)
- Seed/Field Technician (Wichita, KS)
- Research Associate (Wichita, KS/Garden City, KS)
- Site Responsible Researcher (Garden City, KS)
- Field Preparation and Planting Manager (Maui, HI)
- Site Lead (Lebanon, IN)

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Why I Chose A Module

- **Started working on a research project prior to a relocation**
  - **Drought in corn relating to maturities versus water input and return**
- **Land is a premium in Hawaii, which made it difficult to conduct a research project**

Why I Chose A Module

- **In 2014, a ballot initiative was proposed in Maui county**
  - **The initiative proposed a moratorium on planting genetically modified crops**
  - **During campaign efforts, I noticed strong emotions and a lack of science in terms of thinking**

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Introduction

Perceptions of Genetically Modified Organisms

Genetically modified organisms (GMO) are currently a 'hot button' issue across the county.

Advocates of GMO's believe that GMO:

- Help to meet the demand of the market
- Help to increase yields
- Are reducing the pesticides that are needed to raise a crop
- Reducing the amount of inputs required to produce a crop
- Some GMO products have increased the nutrition of the commodity

The belief is the GE products are safe because of the strenuous testing that occurs by world governments, universities, and independent firms.

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Introduction

Perceptions of Genetically Modified Organisms

Genetically modified organisms (GMO) are currently a 'hot button' issue across the county..

Opponents of genetically modified organisms:

- Have a general belief that they are harming either the environment, people or animals.
- More testing needs to be performed prior to the release of any GMO product to determine the potential impact on the environment or consumers.
- Due to increased tolerance of products to pesticides, application rates have increased causing more harmful chemicals to be put into the environment.
- In addition to these ills, organic and traditional farmers are being harmed because they cannot deliver a pure product to market due to pollen drift or contamination.
- Consumers have a right to know what they are eating, so they can make healthy decisions. If a product contains any organism that has been modified, it should be disclosed on a food label.
- There is current research that shows the harm that genetically modified organisms can have. The research ranges from an increase in autism and cancer to a decreased population of insects or decline of the environment.

Introduction

Perceptions of Genetically Modified Organisms

Objectives:

- Understand what a genetically modified organism is, and how it is created.
- Understand how a GMO is developed versus other methods used to alter an organisms genetics.
- Understand why GMO's are used.

Ears of corn ready for harvest.

What is a GMO/GE Organism

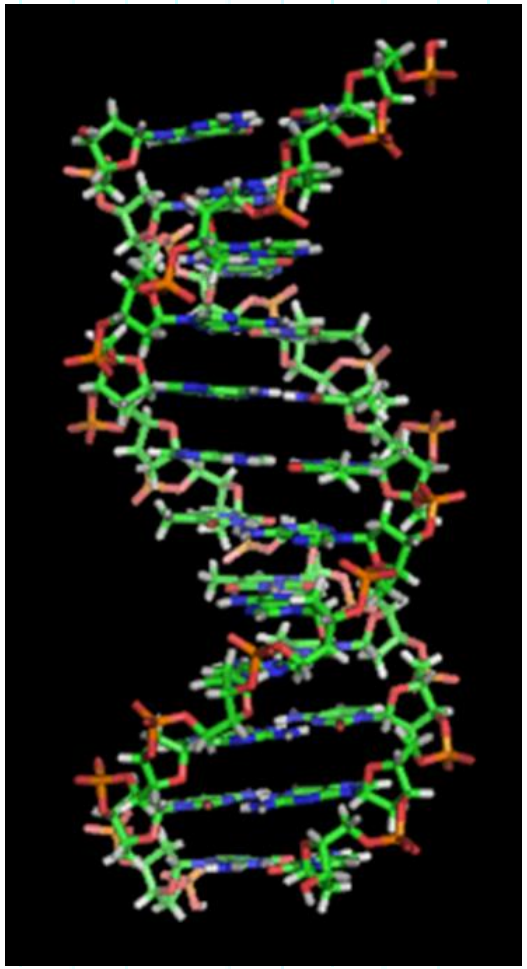


Illustration of a nucleic acid double helix. (Wikipedia, 2015)

A **genetically modified organism** (GMO) or **genetically engineered** (GE) organism is an organism whose genetic characteristics have been altered (Biology Online, 2009). This alteration in the DNA of the organism can occur by using a gene from another organism or by genetic engineering. Genetic engineering is altering the structure of genetic material in a living organism.

DNA: Deoxyribonucleic acid - the hereditary material in humans and almost all other organisms (Genetics Home Reference, 2015).

Gene: The heredity transmitted from generation to generation (GMO Answers, 2015).

What is a GMO/GE Organism

A **transgene** introduces the desired trait to the organism. The transgene must have additional genetic material to fill the space of the gene. Often the additional material will consist of a **marker gene**, a **promoter**, and a **termination sequence**.

The **marker** is used to identify the cells or tissues that have been transformed. The marker is important to determine the expression of the transgene since the adoption rate is typically low.

The **promoter** controls when and where the transgene will be expressed.

The **termination** sequence is used to signal the cells that the gene sequence has been completed (Fenwick, 2004).

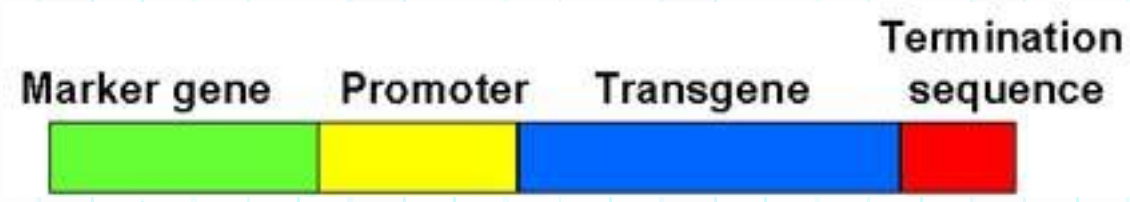


Illustration showing the different components of a transgene. (Fenwick, 2004)

What is a GMO/GE Organism

To add a non-native trait to a crop, a transgene needs to be added among additional genetic material (Federation of American Scientists, 2011). This process is called **transformation** (Fenwick, 2004).

A gene is commonly added to DNA using two different methods.

- ***Agrobacterium tumefaciens***
- **Gene gun** (Federation of American Scientists, 2011)

Agrobacterium tumefaciens is the preferred method because there are more single site insertions of the transgene compared to gene gun insertion (Fenwick, 2004).

Older gene gun used for bombardment. (Wikipedia, 2016)

Helios gene gun. (University California San Diego)

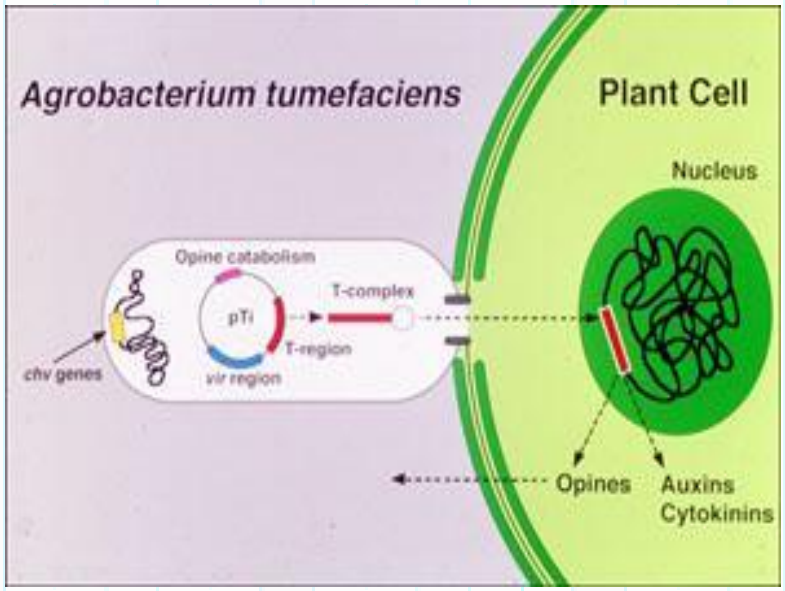
What is a GMO/GE Organism

Agrobacterium tumefaciens

Agrobacterium tumefaciens is a soil dwelling bacteria that can infect plant cells with its DNA. The DNA of *Agrobacterium tumefaciens* is in the chromosome as well at tumor-inducing plasmids. This DNA is termed **T-DNA**.

During the transformation process, a plant is wounded, which allows tumor-inducing plasmid to transfer the T-DNA. Virulence genes attach T-DNA to the host plant (Fenwick, 2004). Bacterial proteins attach to the T-DNA allowing it to cross the transport channel and be inserted into the host cell (Nguyen, 2015).

This method of introducing transgenes has typically been used for dicot crops, but has recently been adopted to monocot crops.



***Agrobacterium tumefaciens* infection process in a plant cell. (Look For Diagnosis, 2014)**

What is a GMO/GE Organism

From A Tissue Culture to a Plant

The cells that are present in the media used for growth are not differentiated. In the initial stages, growth hormones signaling the cells to **differentiate**. Without the growth hormones, a mass of cells, termed a **callus**, develops on the media. When growth hormones are added to the media, it will trigger the cells to start producing specific tissue, such as roots or shoots.

There are many cells growing in the petri dishes, and the chances are that only a minority of them obtained the transgene. To separate the transgenic from non-transgenic cells, the marker that was attached to the transgene can be used to determine if the cells are transgenic. A common marker is an herbicide resistance to either glyphosate or glufosinate. The herbicide can be applied to the media which leaves only the resistant and transformed cells living (Hain et al., 2015).

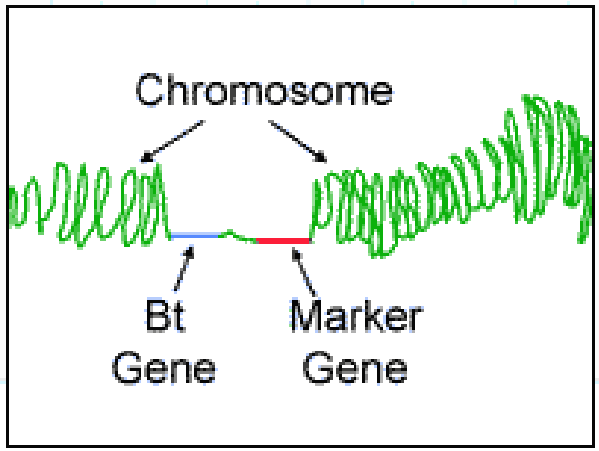


Illustration shows a transgene, in this case a *Bt* gene, with a marker that would be used for selection. (Hain et al., 2015)

What is a GMO/GE Organism

From A Tissue Culture to a Plant

The cells that were selected by their markers are then grown in a sterile environment. Once the growth hormones have been added, and enough growth has occurred, the tissues can be transplanted into soil. The tissue will continue to grow and ultimately become a full grown plant. The formation of a plant happens due to a process called **totipotency** (Mineo, 1990). This process is not unique to genetic engineering as it is also a method used in traditional propagation.

Plant callus cells in a petri dish. Photo Courtesy of Wikipedia, 2015.

Cultured tissue growing in a medium. Hormones have already been added to stimulate root and shoot development. (American Phytopathological Society, 2015)

Why are GMO's used?

Changing the genetic structure of an organism can create a desired trait or characteristic. The desired traits can help the organisms growth or development. For crops specifically, these traits can:

- **defend against pests**
- **reduce inputs**
- **increase nutritional value**

GMO's are used in a range of various industries. The primary focus of this module will be related to agronomic crops, but other examples will be presented, such as the medical field.

Corn plants representing *Bt* corn.

Picture of soybean plant representing glyphosate resistant soybeans. (Ciha)

Syringe representing medical uses derived from GMO's. Photo OJ medical, 2015

Why are GMO's used?

In agriculture, GMO traits largely relate to the management of pest problems. The most notable are herbicide and insect management.

Corn roots with healthy corn roots on the left and roots damaged by western corn rootworm beetle (*Diabrotica vigifera vigifera)* larvae on the right. Courtesy of Cornell University - http://web.entomology.cornell.edu/shelton/veg-insects-ne/images2/wcrw-damage2b.jpg

European corn borer (*Ostrinia nubilalis)* larvae in a corn stalk. Courtesy of Purdue University - http://extension.entm.purdue.edu/radicalbugs/images/pests/larva/europeanCornBorerLarva.jpg

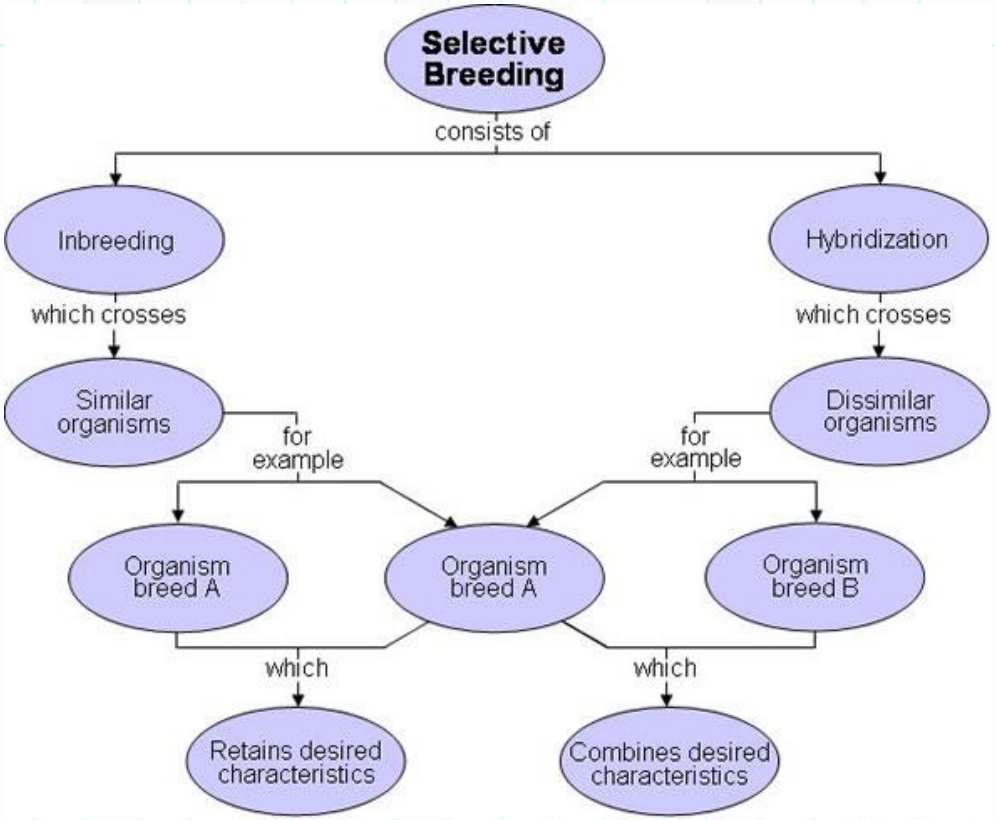
Corn earworm (*Helicoverpa zea)* eating the top rows of corn. (Reisig, 2015)

Why are GMO's used?

Many of the same traits that genetic engineering of crops seek may be accomplished through **traditional breeding**. Traditional breeding is seeking the same goal of producing a plant with desired characteristics.

The advantage of genetic engineering is that this can be accomplished in a shorter time frame.

Inserting a known gene onto a known loci can accomplish what traditional breeding hopes to achieve through the random luck of pollination.



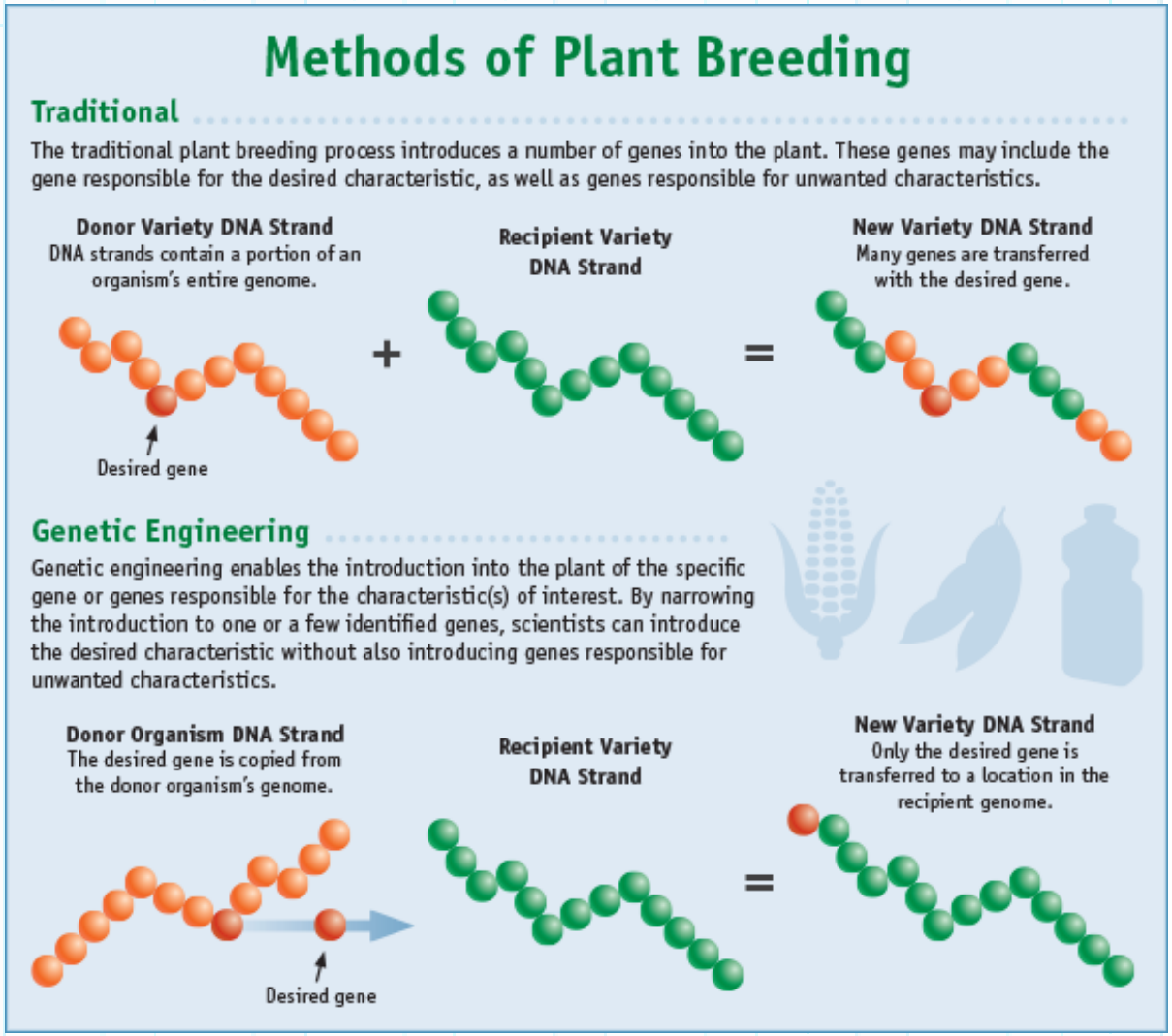
A simple diagram showing inbred breeding to make a hybrid. Courtesy of http://corncorps.com/2014/10/08/four-reasons-your-gmo-fears-are-unfounded/

Why are GMO's used?

The illustration shows why some believe genetic engineering to be a quicker process compared to traditional plant breeding.

In a traditional cross to obtain a particular trait, there is the potential for many traits to be included from the donor organism.

With genetic engineering, only the desired trait is introduced.



U.S. Food and Drug Administration, 2015.
http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm352067.htm

Why are GMO's used?

Cheese Production

Rennet is a key ingredient used to make hard cheese. This product was obtained from the 4th stomach lining of **unweaned** calves. During the 1960's, the demand for hard cheeses outpaced the availability of rennet, so another source was sought. It was discovered that enzymes from some plants and microbes could produce enzymes that act to coagulate milk, like rennet, but there were also undesired side reactions. In the 1980's, it was discovered that a bovine gene could be transformed into microbes, which could be cultivated and used in the production of cheese. This process is used in ninety percent of the cheese in the United States (Etine, 2015).

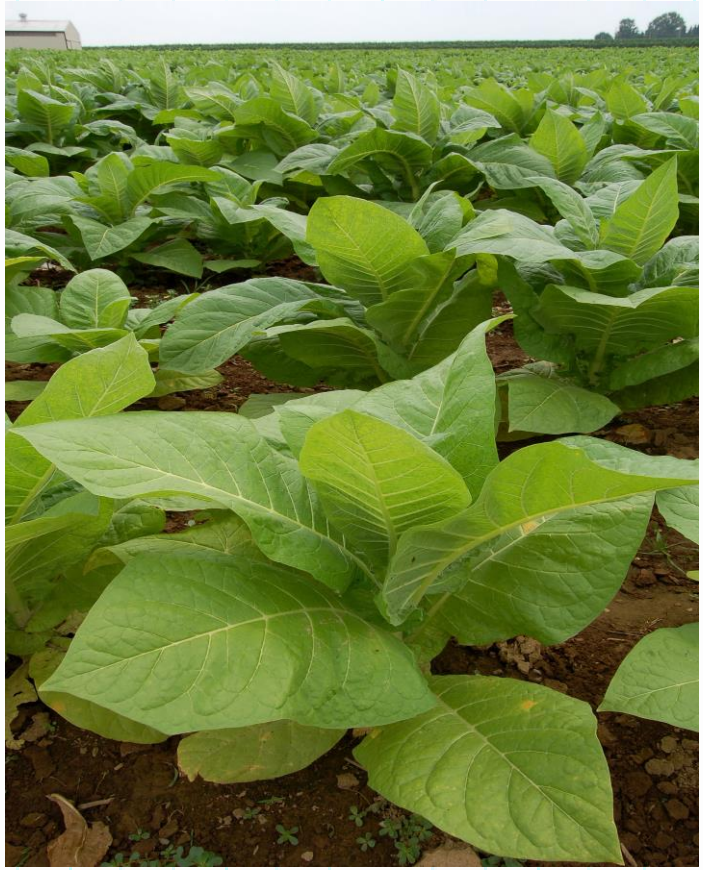
Picture representing hard cheeses made with GMO's.
http://www.geneticliteracyproject.org/2015/05/15/cheese-gmo-food-die-hard-gmo-opponents-love-and-oppose-a-label-for/

Why are GMO's used?

Medical Use of GMO's

Genetically modified organisms are often thought of in terms of crops. However, they are also used to deliver many key medical products.

- **Insulin for diabetes**
- **Cancer treatments**
- **Human Growth Hormone**
- **Ebola**
- **Vaccines**
  - Hepatitis A
  - Hepatitis B
  - Diphtheria
  - Tetanus
  - Whooping cough
  - Polio



Tobacco plant. Courtesy of Wikimedia
https://upload.wikimedia.org/wikipedia/commons/a/ae/Nicotiana_Tobacco_Plants_1909px.jpg

Are GE Crops Safe?

Who Regulates GMO's?

There are three different regulatory agencies that regulate genetically modified crops.

- **EPA** -The **Environmental Protection Agency** regulates biopesticides. This would include the widely used *Bt* toxin that is derived from soil bacteria. Product developers must show environmental and food safety data to the EPA .

- **FDA** -The **Food and Drug Administration** regulates the crops from a food safety aspect. Testing must be done to ensure the nutritional and potential allergenic properties of the crops are equivalent or better than a non-genetically engineered crop.

- **USDA** -The **United States Department of Agriculture** regulates GE crops under the Plant Protection Act of 2000. They are responsible for regulating "plant pests" which also include the agrobacterium that is used to transform many GE crops. Environmental assessments or environmental impact statements are required prior to the USDA approval to test a crop (Federation of American Scientists, 2011).

Are GE Crops Safe?

Are GMO Foods Safe to Eat?

Several food safety tests are performed on food that results from genetic engineering. These tests are conducted by the United States Food and Drug Administration as well as the World Health Organization (WHO). The tests consist of:

- Direct health effects (toxicity)
- Potential for allergic reaction
- Components thought to have nutritional or toxic properties
- Stability of the inserted gene
- Nutritional effects
- Unintended effects from the gene insertions.

(Wikipedia, 2015)

These tests are performed to ensure that safety of the food for both human and animal consumption. The following article lists 1,783 studies that have been conducted affirming the safety of genetically engineered foods: http://www.geneticliteracyproject.org/2013/10/08/with-2000-global-studies-confirming-safety-gm-foods-among-most-analyzed-subject-in-science/. Click on the hyper link “1783 studies” to open the spreadsheet (Wendel, 2013).

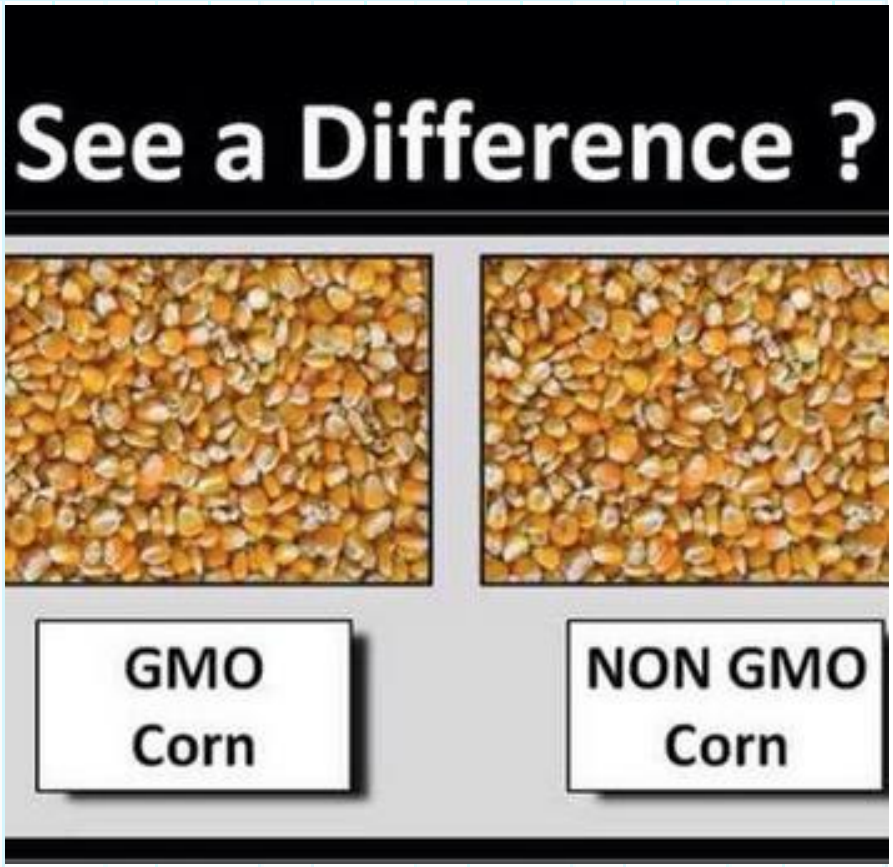
Are GE Crops Safe?

GMO Foods or Feeds

Common foods that have been genetically modified:

- Alfalfa
- Rapeseed
- Cotton
- Rice
- Soybean
- Sugar cane
- Tomatoes
- Corn (sweet and field)
- Canola
- Potatoes
- Flax
- Papaya
- Squash
- Red-hearted chicory
- Cotton seed oil
- Meat
- Peas
- Sugarbeets
- Dairy products

(Langtree, 2009)



Two pictures of corn. The picture on the left is GMO, and the picture on the right is non GMO. Is there a visual difference? (Genetic Literacy Project, 2014)

Goals of GE crops

Creating pest tolerance

Creating a tolerance within the plant to allow for pest control:

- Herbicide resistance
- Insect resistance
- Drought tolerance
- Nutrient deficiency tolerance
- Disease resistance

European corn borer (*Ostrinia nubilalis)* feeding inside of a corn plant. (Obermeyer, 2009).

Comparison of standard rice versus white rice. (Wikipedia, 2011)

Drought tolerant corn on the left. (Bio)

In Hawaii, papaya has been genetically engineered to resist ringspot virus: infected plants on (left), virus-resistant (right).

Photo courtesy of http://ucce.ucdavis.edu/files/repository/calag/img6602p68.jpg

Soybean plants tolerant to dicamba herbicide on the right to help manage glyphosate-resistant Palmer Amaranth (left). (Orso, 2014)

Goals of GE crops

Creating pest tolerance – Drought Tolerance

Drought tolerance is a product that is fairly new to the market in corn products. The intent of the product is to slow the plant's respiration to help it survive during small periods of drought (Stecker, 2012). An example of how these products are reaching a broader audience is the Water Efficient Maize for Africa Project (WEMA). Maize is a staple product for Africa. Some small farmers are reliant on the crop which is often affected by drought. Early results of the products are showing a doubling of yield compared to the national average (Werehire, 2014).

The picture to the left shows Dr. Sylvester Oikeh standing between rows of corn. The row on the left is a hybrid from WEMA while the row on the right in a traditional variety. (The Guardian, 2015)

Goals of GE crops

Creating pest tolerance – Nutrient Deficiency Tolerance

Nitrogen is a primary micronutrient needed for plant growth and development. Legumes are capable of fixing their own nitrogen, but all other crops may need additional nitrogen added to the soil. The problem with adding nitrogen is that crop use is not always efficient. It is estimated that only 30-50% of the nitrogen applied is absorbed by the plants. While some of the nitrogen is bound in organic matter, some of the nitrogen is leached into groundwater. Although there is not currently a GE product commercially available for nitrogen-use efficiency, there are studies being conducted with corn, wheat, rice, canola, sugarbeet, and sugarcane. The goal of the studies is find the transgenic varieties that are able to utilize more of the nitrogen that is in the soil. This would result in lower nitrogen needs during fertilization and decrease the potential nitrogen loss from the soil (ISAAA, 2016).

The rice pictured to the left is a product of NUE rice trials. Early results of the trials show a 50% reduction in nitrogen applied with yield gains of up to 30%. (ISAAA, 2016)

Goals of GE crops

Adding value to the crop - Increasing Nutrient Quality

Golden rice is a genetically engineered crop that was created to help developing countries. In some developing countries, rice accounts for up to 60% of calories consumed. The problem with consuming rice as a primary source of food is that it is poor in vitamins (International Rice Research Institute). One of the vitamins that is lacking is vitamin A. The golden rice project sought to develop rice that contains beta carotene. The beta carotene is converted by the human body in vitamin A. It is estimated that this change in rice could save the lives of 2.7 million children under the age of five. Vitamin A deficiencies can lead to:

- Blindness
- Exposure to infections
- Reduced immune response
- Impaired production of blood cells and platelets
- Reduced skeletal growth

(Golden Rice Project, 2015)

The picture shows non-modified rice on the left versus rice modified to have beta carotene on the right. (Golden Rice, 2004)

Goals of GE crops

Adding Value to the Crop

This lesson has focused mainly on benefits of genetically modified crops to the grower. There are also benefits to the consumer that need to be examined. There are two main areas of products that have been developed:

- Increasing produce shelf life
- Increasing nutritional quality

Peaches at a grocery store. The quantity in stock shows the importance of shelf life. (Bartel, 2016)

Various fresh produce at a grocery store, each providing different nutrients to the consumer (Bartel, 2016)

Proper Management of GE Crops

Like other crop protection products, proper management of genetically engineered crops must occur to lower the risk of resistance. The three main principles are:

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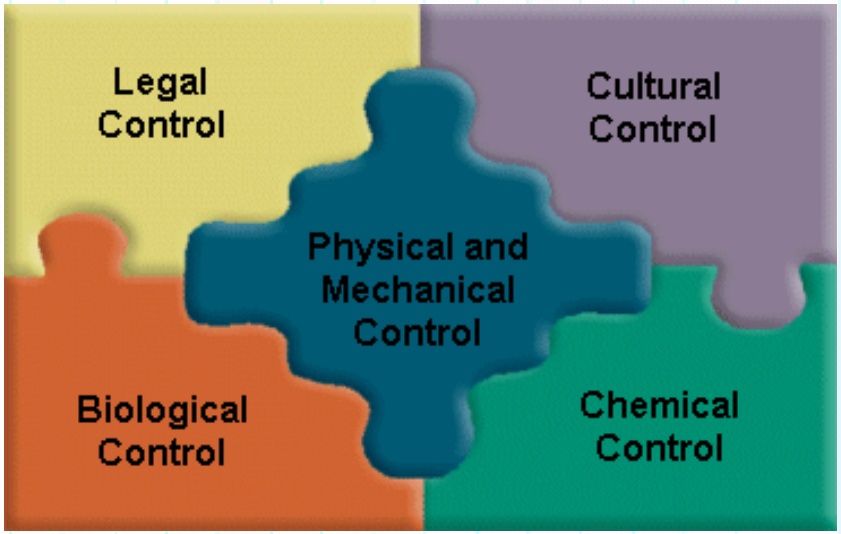
- **Crop Rotation**
- **Refuge Areas**
- Rotation of Chemistries



This figure represents the different steps to controlling pests with a healthy IPM program.

While each of these management factors aids in warding off resistance, and combination of the practices will provide a more robust approach. These techniques would follow some key steps of an integrated pest management program (IPM).

Proper Management of GE Crops

Crop Rotations

Crop rotations are an important tool for managing various agronomic aspects. When planting transgenic crops, rotation is important to prevent resistance.

By constantly changing the crops in the fields, the suitable environment for weeds or insects also changes. Different crops may require different pesticides to be applied. Changing pesticides prevents insects and weeds from being exposed to the same chemicals year after year.

For the best results, a diverse rotation is encouraged. Rotating between two crops provides a benefit, and adding more diversity to the rotation increases the benefits (Nickel, 2014).

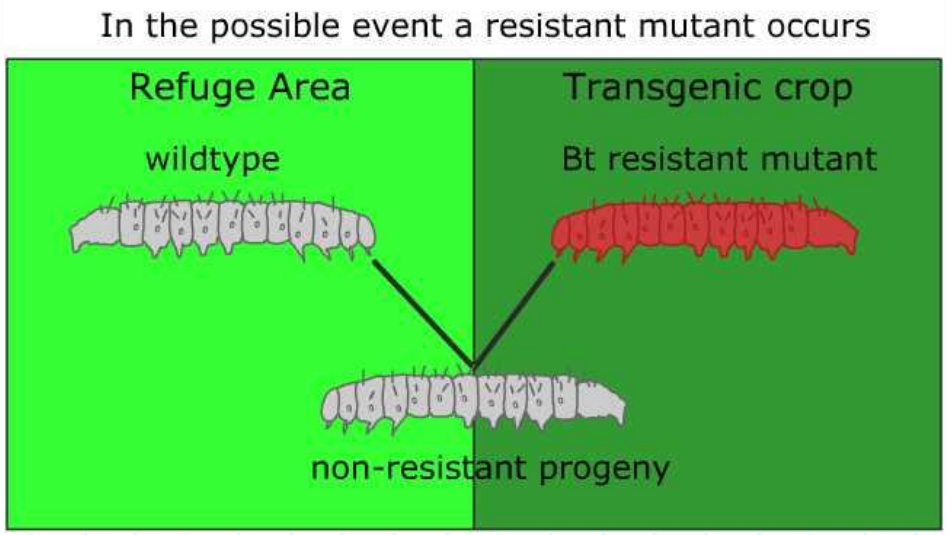
The illustration shows a common crop rotation between corn and soybeans. (Monsanto, 2015)

Proper Management of GE Crops

Refuge Areas

Refuge areas are established to provide a habitat for insects outside the transgenic section of the field.

The theory behind refuge areas is that should insect resistance develop, the resistant insect can reproduce with a non-resistant insect (susceptible) that was feeding in the refuge area. The progeny of this reproduction would not be Resistant (University of California San Diego).



The above illustration shows the principle of how planting refuge Areas prevent the buildup of resistant insect populations. Illustration courtesy of University of California San Diego.

The amount of refuge needed depends on the product. Products with multiple modes of action will typically need less refuge than products with a single mode of action. In order to simplify management and increase compliance with refuge policies, some products come pre-mixed with the appropriate amount of refuge, otherwise known as refuge in a bag (RIB).

Proper Management of GE Crops

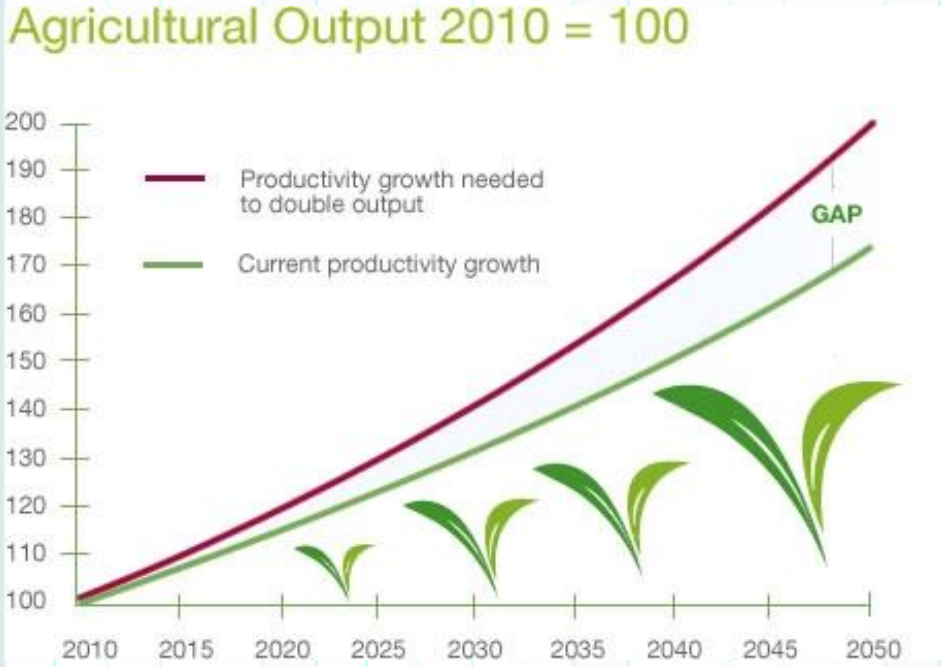
Rotation of Chemistries

Effective pesticides can lull producers into reducing their control strategy. This is evident in the use of herbicide-resistant crops. The reliance of glyphosate for weed control has led to an increase in the number of glyphosate-resistant weeds (Mortensen et al., 2012). This can be a problem whether a producer is planting a GMO crop or a traditional crop.

Biotypes exist within a population that may have resistance to a particular class of herbicides. When the population of susceptible weeds declines, the biotype population begins to increase which highlights the problem of resistance (Weed Science Society of America, 2016).

The same principle will hold true for genetically modified crops used to control insects or disease. The same trait used for control should not be used continuously as at it may allow biotype populations to increase. Rotating control methods will keep the resistant populations in check since other control methods may be effective.

Where do GE crops fit within Agriculture?



The graph shows that in 2010, producers were able to match production with demand. As the population continues to grow, productivity will need to increase in order to meet demand. (Stutman, 2015)

Genetically modified crops are not the answer to all problems in agriculture.

- A wide ranging approach is still needed in agriculture:
  - Traditional breeding techniques
  - Integrated pest management
  - Agronomic practices.
- Modified crops should be used in a targeted environment to help solve a problem.
- As the world continues to change, GE crops have the potential to help meet the demand for food.

Summary

Genetically modified crops, or genetically engineered crops are an additional tool that can be used to increase production agriculture. These crops provide producers with targeted technology to help control some potential problems that they may encounter. These crops should not be used as standalone technology to solve problems, but rather as part of a full system of management.

With a changing world the demand for food will increase while agronomic problems shift. The world population is increasing, diets are changing, and agricultural land is decreasing Genetic engineering of crops has the potential to help agriculture shift with these changes by quickly delivering targeted traits that can be combined with new germplasm.

Safety and regulation are top concerns. This is a concern that will need to be addressed by government agencies as crop are evaluated for commercial release. To date, extensive studies have been performed on GMO crops with excellent safety results. The results of genetic engineering extend beyond food to other products, such as medicine.

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Example Quiz Questions

What is the correct sequence of genetic material during transformation?

1. *Marker gene, promoter, transgene, termination sequence
2. Termination sequence, transgene, marker gene
3. Marker gene, transgene, marker gene, promoter
4. Promoter, transgene, termination sequence

Traditional breeding differs from genetic engineering in what way?

1. The gene of interest can be placed in a specific loci
2. No genetic material foreign to the plant can be introduced during traditional breeding.
3. Traditional breeding is a quicker process
4. Traditional breeding will only insert one trait

Genetically engineered crops targeted toward insect management work in which of the following ways:

1. Target specific insects when the insects feed on the plant
2. Produce broad spectrum insecticides to defend the plant from all potential insect pests
3. Exude pesticides to prevent insect feeding
4. Adjust physiological characteristics of the plant to that it is no longer a target

Questions