Genetically Modified Crops

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When man evolved from a hunter gatherer they began to grow crops for food. They found that selection of crops improved the quality and yield of foods for food production. The selection of seeds led to the evolution of new crops that are more productive and nutritious crops.

The domestication of plants by humans to produce plants with more desirable traits than wild plants drove early man to become dependent on identification and propagation of plants with desirable attributes. Seed selection for desired traits began between 9000–11,000 years ago. Initially early farmers simply selected food plants with particular desirable characteristics, and employed these as progenitors for subsequent generations, resulting in an accumulation of valuable traits over time.

1856 and 1863 Gregor Mendel investigated of plant hybridization led to his laws of inheritance. This work became well known in the 1900s and formed the basis of the science of genetics. The laws he developed stimulated research by many plant scientists dedicated to improving crop production through plant breeding. He conducted hybridization experiments with garden peas (*Pisum sativum*) which led to two generalizations which later became known as *Mendel's Principles of Heredity* or *Mendelian inheritance*. Mendel discovered that, when he crossed purebred white flower and purple flower pea plants (the parental or P generation), the result was not a blend. Rather than being a mix of the two, the offspring (known as the F_1 generation) was purple-flowered. When Mendel self-fertilized the F_1 generation pea plants, he obtained a purple flower to white flower ratio in the F_2 generation of 3 to 1. The results of this cross are tabulated in the Punnett square in Fig. 14.1. Mendel hypothesized that allele pairs segregate, from each other during the production of gametes. Because allele pairs separate during gamete production, a sperm or egg carries only one allele for each inherited trait. When sperm and egg unite at fertilization, each contributes its allele, restoring the paired condition in the offspring. This is called the Law of Segregation. The presence of an allele does not mean that the trait will be expressed in the individual that possesses it. If the two alleles of an inherited pair differ (the heterozygous condition), one determines the organism's appearance and is called the dominant allele; the other has no noticeable effect on the organism's appearance and is called the recessive allele. Thus, in the example above dominant purple flower allele will hide the phenotypic effects of the recessive white flower allele. This is known as the Law of Dominance. These laws formed the basis of understanding inheritance and allowed more effective selection in breeding of plants.

Gregor Mendel's experiments with plant hybridization led to his establishing laws of inheritance. Once this work became well known, it formed the basis of the new science of genetics, which stimulated research by many plant

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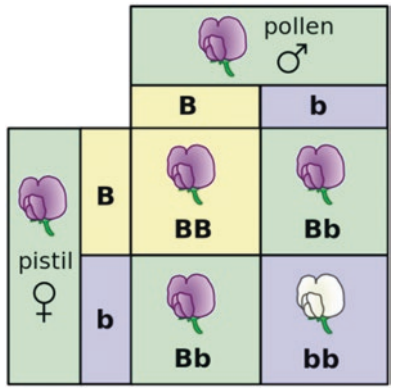

Fig. 14.1 Representation of the Punnett square for inherence of flower color in pea plants the https://commons.wikimedia.org/w/index.php?curid=2063426

scientists dedicated to improving crop production through plant breeding.

However, successful commercial plant breeding concerns began to be founded from the late nineteenth century. Gartons Agricultural Plant Breeders in England was established in the 1890s by John Garton, who was one of the first to cross-pollinate agricultural plants and commercialize the newly created varieties. He began experimenting with the artificial cross pollination first of cereal plants, then herbage species and root crops and developed far reaching techniques in plant breeding (Graften and Ridley 2006; Ford 1960).

In the late nineteenth and early twentieth centuries the better understanding of genetics provided plant breeders, with the tools to make modifications in plants with increasing precision. They changed the expression of traits in plants by crossing specific parent plants to produce new varieties with desired traits. They also developed more rapid methods to generate and detect genetic changes. The improved technology led to targeted and more efficient breeding of improved varieties (Mba2013). DNA mutation rare in nature (Ossowski et al. 2010), but scientists applied mutagenic chemicals or radiation to induce mutations in DNA at increased rates (Roychowdhury and Tah 2013) thus increasing the genetic variation in the species. Both natural and induced mutations are random so breeders must evaluate the progeny sorting for desired changes as well as undesired changes. In 1973, Cohen et al. described recombinant-DNA (rDNA) techniques that allowed scientists to cut gene sequences from the DNA of one organism and splice them into the DNA of another organism (Cohen et al. 1973), the path was paved for a new approach to increase genetic diversity for use in breeding organisms, including crops hence genetic engineering.

Many present-day crops currently under cultivation are traceable to plant domestication in ancient times. Nearly all of the domesticated plants used today for food and agriculture were domesticated in the various centers of origin around the world. In these centers there is still a great diversity of closely related wild plants. For example wheat in its early form came from the Fertile Crescent and corn as we know it came from Central America.

One major technique of plant breeding is selection, the process of selectively propagating plants with desirable characteristics and eliminating or "culling" those with less desirable characteristics.

Deliberate interbreeding of closely or distantly related individuals produces new crop varieties or lines with desirable properties. Plants are crossbred to introduce traits/genes from one variety or line into a new genetic background. One example is when a mildew-resistant pea is crossed with a high-yielding but susceptible pea the new cross bred peas with mildew resistance without losing the high-yield characteristics. Progeny from the first cross would then be crossed with the high-yielding parent to ensure that the progeny were most like the high-yielding parent in a step referred to as backcrossing. Plants may also be crossed with themselves to produce inbred varieties for breeding.

Classical breeding relies largely on homologous recombination between chromosomes to generate genetic diversity. Conventional plant breeders also make use *in vitro* techniques such as protoplast fusion, embryo rescue or mutagenesis to generate diversity and produce hybrid plants that would not exist in nature.

Breeders have attempted to incorporate the following traits into crops using conventional techniques:

1. Improved quality, such as increased nutrition, improved flavor, or greater beauty
2. Increased yield of the crop
3. Increased tolerance of environmental pressures (salinity, extreme temperature, drought)
4. Resistance to viruses, fungi and bacteria
5. Increased tolerance to insect pests
6. Increased tolerance of herbicides
7. Longer storage period for the harvested crop

The development of modern day corn serves as an example of the evolution of corn which began about 10,000 years ago. Starting with Teosinte we see the directed evolution of corn through selective plant breeding. Figure TSC1 illustrates the evolution of corn from teosinte to modern hybrid corn. The farmers selected corn seeds from crops that were easier to grind, tasted better or had larger kernels. The evolution from teosinte to modern corn illustrates man's direction of genetic selection to yield enhanced genotypes of a crop. It is important to realize that this is an early example of a genetically enhanced organism which resulted from selection and cross breeding. Figure 14.2 illustrates the evolution both the tassel (male) and ear (female) portion of corn from the parent teosinte to modern hybrid corn.

These breeding techniques resulted in large yield increase in the United States in the early twentieth century. After World War II, the Green Revolution increased crop production in the developing world in the 1960s. These breakthrough were based on three essential crops; maize, wheat and rice. The development of hybrid maize was followed by high-yielding and input-responsive "semi-dwarf wheat" (for which the CIMMYT breeder N.E. Borlaug received the Nobel prize for peace in 1970), and third came high-yielding "short statured rice" cultivars.

Dwarfing of wheat delivered a critical agronomic quality of dwarf plants with thick stems making them less prone to collapse under the

Fig. 14.2 Evolution of maze from teosinte to modern corn

weight of the extra grain — a trait called lodging. Borlaug worked wheat that had tall, thin stalks. The taller of wheat grasses better compete for sunlight, but tend to collapse under the weight of the extra grain resulting from rapid growth spurts from Nitrogen fertilizer. In 1953, he acquired a Japanese dwarf variety of wheat called Norin 10 developed by Orville Vogel, that had been crossed with a high-yielding American cultivar called Brevor Reitz (1970). Norin 10/Brevor is semi-dwarf (one-half to two-thirds the height of standard varieties) and produces more stalks and thus more heads of grain per plant. Borlaug crossbred the semi-dwarf Norin 10/Brevor cultivar with his disease-resistant cultivars to produce wheat cultivars that were adapted to tropical and sub-tropical climates (Hedden 2003).

In efforts to develop new varieties of grains seeds were submitted to mutation breeding. Mutation breeding is the process of exposing seeds to chemicals or radiation in order to generate mutants with searching for desirable traits to be bred with other cultivars. The mutated seeds were grown to screen for positive characteristics From 1930 to 2014 more than 3200 mutagenic plant varietals have been released (Schouten and Jacobsen (2007; FAO/IAEA 2014). The crops released are either direct mutants (70%) or their progeny (30%) (Maluszynsk et al. 2000), ot which food and agricultural crops account for 75% of released mutagenic species (Ahloowali 2004). The FAO/IAEA reported in 2014 over 1000 mutant varietals of major staple crops were being grown worldwide.

It is important to realize that mutation breeding produces completely random mutations which ultimately result in new or modified proteins in the new plant. The process is very tedious and it can take many years to identify a new and useful plant.

The accumulation of this previous work illustrates that plant scientists have been "engineering" plants for at least 10,000 years. In the early 1990s the next step in the evolution of the technology was to move genes from one species to another by direct gene insertion. The process is significantly faster and more precise than mutation breeding of crossing and back crossing. It also opened the possibility of plants with many new and useful attributes.

These directed changes in plants which includes direct transfer of a desired DNA from a microorganism or another plant into a trans-genic species that has the desired characteristics. Figure 14.3 illustrates the principal of gene insertion in plants.

Intensive research in molecular genetics has led to the development of recombinant DNA technology. Advancement in biotechnological techniques has opened many possibilities for breeding crops. Mendelian genetics allowed plant breeders to perform genetic transformations in a few crops, molecular genetics has provided the key to both the manipulation of the internal genetic structure, and the "crafting" of new cultivars with targeted attributes such as virus resistance, insect resistance and herbicide tolerance.

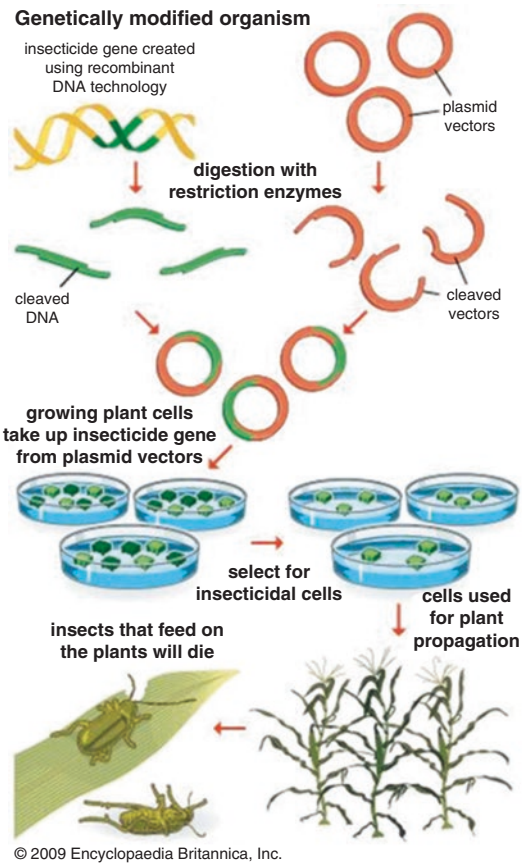

Fig. 14.3 The principal of gene insertion in DNA

Recombinant DNA technology is the joining together of DNA molecules from two different species which are inserted into a host organism to produce new genetic combinations. The recombinant organisms have value and promise in medicine, agriculture, and industry. Recombinant DNA technology allows the isolation of a segment of DNA or a gene for a specific protein. With that fragment the nucleotide sequence can be determined, the transcripts, mutate the sequence in highly specific ways if needed, and reinsert the modified sequence into a living organism. Both agriculture and medicine have benefited significantly from this technology. It should be pointed out that it is one more step in the timeline of plant science and improving our crops. Currently crops or foods modified by modern genic technology are referred to as Genetically Modified Organisms or GMOs. Simplistically GMO's have their genetic composition altered hence they can code for a new property. The gene needs a mechanism to turn it on. This on switch is called the promoter segment. One of the more widely used promoters is named 35S. When a new GMO has been developed with a new trait, the resulting gene construct is called an event with events being developed regularly. These events undergo various regulatory and safety reviews before being approved for use. One area that unfortunately is growing is the development of unapproved events.

Genetically modified (GM) foods were first approved for human consumption in the United States in 1994, and by 2014–2015 about 90% of the corn, cotton, and soybeans planted in the United States were GM. By the end of 2010, GM crops covered more than ten million square kilometres (3.86 million square miles) of land in 29 countries worldwide—one-tenth of the world's farmland. The majority of GM crops were grown in the Americas. In the agricultural arena, the technique was applied to soybeans in 1988, paving the way for one of the most successful crops, glyphosate tolerant soy. While of substantial importance to commercial agriculture, very few consumers were aware of this development. Likely, the introduction of the "Flavr Savr" tomato in 1994 was the first GMO crop many had seen. In the 1980s there was anecdotal information that the enzyme polygalacturonidase was a key since it dissolved cell wall pectin. A group from Celgene proposed to limit this enzyme by developing an antisense gene. The researchers hoped that this would retard ripening allowing it to remain firm longer. In 1987, Calgene identified and cloned the tomato fruit pg gene and in 1992 presented a petition to the FDA and in 1994 approved the addition of a kanamycin resistance gene construct needed to create the PG-antisense tomato. Work continued and in late 1994 the Flavr-Savr tomatoes was introduced. Demand was high and remained high but production costs were also high and the product was not profitable. While it may have been a technological success it was a commercial failure and did nothing for the cause of biotechnology so generally, the application of biotechnology and transgenic foods has become the purview of commercial agriculture. Currently there is a substantial amount of food grown using DNA recombinant technology with approx. 85% of the corn grown in the US being GMO and almost 90% of the soybeans. This is not all the GMO crops but encompass a substantial percentage. The GMO crops have various traits. Examples of two of the more common crops with their associated traits follow. Roundup Ready Soybeans contain a proteins that interferes most with the EPSPS pathway. Round Up known as glyphosate is a general purpose pesticide used not only in agriculture but in homes to eliminate weeds. While good to eliminate weeds, it also eliminates healthy crops such as flowers, crops and ornamentals. In the case of Roundup Ready Soy, the GMO trait allows the farmer the ability to use Round Up to eliminate weeds while not killing the soy. Furthermore, a farmer can be more productive eliminating tedious weeding. The second example is BT corn having been encoded with a gene that eliminates the corn borer allowing for more corn per acre. Based on data from the end of 2012 there were 170 million hectares in production that includes 312 events in 29 species with 3497 approvals in 59 countries. Table 14.1 provides a partial listing of some of the key proteins expressed by some GM crops. Figure 14.4 gives a simplistic view of the steps in genetic modification.

Table 14.1 Genetically engineered traits deregulated and approved for field release in the United States as of 2015

Crop	Scientific Name	Trait	Year approved	Developer
Alfalfa	*Medicago sativa*	Glyphosate HR[a,b]	2005, 2010	Monsanto/Forage Genetics
		Reduced Lignin	2015	Monsanto/Forage Genetics
Apple	*Malus domestica*	Non Browning	2015	Okanagan Specialty Fruits
Canola	*Brassica napus/ Brassica rapa*	Oil Profile Altered[c]	1994	Calgene
		Glufosinate HR	1995	Bayer
		Phytase	1998	BASF
		Glyphosate HR	1999	Monsanto
Maize, field	*Zea mays*	Glufosinate HR	1995	AgrEvo
		Bt IR	1995	Monsanto
		Glyphosate HR	1996	Monsanto
		Increased Lysine	2006	Monsanto
		Alpha-Amylase	2011	Syngenta
		Drought Tolerance	2011	Monsanto
		Male Sterility/Color	2011	DuPont
		ACCase[d] HR	2014	Dow
		2,4-D HR	2014	Dow
		Increased Ear Biomass	2015	Monsanto
Maize, sweet	*Zea mays*	Bt IR[e]	1998	Novartis
		Glyphosate HR	2011	Monsanto
Papaya	*Carica papaya*	Ring Spot Virus VR[f]	1996	Cornell University, University of Hawaii
				USDA Agricultural Research Service
Plum	*Prunus domestica*	Plum Pox VR[c]	2007	USDA Agricultural Research Service
Potato	*Solanum tuberosum*	*Bt* IR	1995	Monsanto
		Potato Virus Y VR[c]	1999	Monsanto
		Potato Leafroll VR[c]	2000	Monsanto
		Low Acrylamide	2015	Simplot Plant Sciences
		Nonbrowning	2015	Simplot Plant Sciences
		Resistance to Late	2015	Simplot Plant Sciences
		Blight Pathogen		
Rice	*Oryza sativa*	Gulfosinate HR	1999	AgrEvo
Squash	*Cucurbita pepo*	Zucchini Yellow VR	1994	Upjohn
		Watermelon Mosaic	1994	Upjohn
		VR	1996	
		Cucumber Mosaic VR		
Soybean	*Glycine max*	Glyphosate HR	1994	Monsanto
		Glufosinate HR	1996	Bayer
		Sulfonylurea HR	2007	DuPont
		Modified Oil	2009	DuPont
		High Oleic Oil	2010	DuPont
		Isoxaflutole HR[c]	2013	Syngenta
		Mesotrione HR[c]	2014	Syngenta
		Imidazolinone HR	2014	BASF
		2,4-D HR	2015	Dow
		Dicamba HR	2015	Monsanto
Sugar Beet	*Beta vulgaris*	Glyphosate HR[g]	2005	Monsanto
		Glufosinate HR	1998	AgrEvo
Tomato	*Solanum lycopersicum*	Fruit Ripening Altered[c]	1992	Calgene

[a]*HR* herbicide resistance
[b]Returned to regulated status in 2007; returned to deregulated status in 2011
[c]Not in production in 2015
[d]Acetyl CoA Carboxylase inhibitor herbicide
[e]*IR* insect resistance (different Bacillus thuringienis Cry genes inserted to encode proteins that kill specific species)
[f]*VR* virus resistance
[g]Returned to regulated status in 2010 because of litigation; Returned to deregulated status in 2011
DATA SOURCES: http://www.cera-gmc.org/GMCropDatabase; http://www.isaaa.org/gmapprovaldatabase/; http://www.aphis. usda.gov/biotechnology/petitions_table_pending.shtml.
Adapted from NAS, 2016

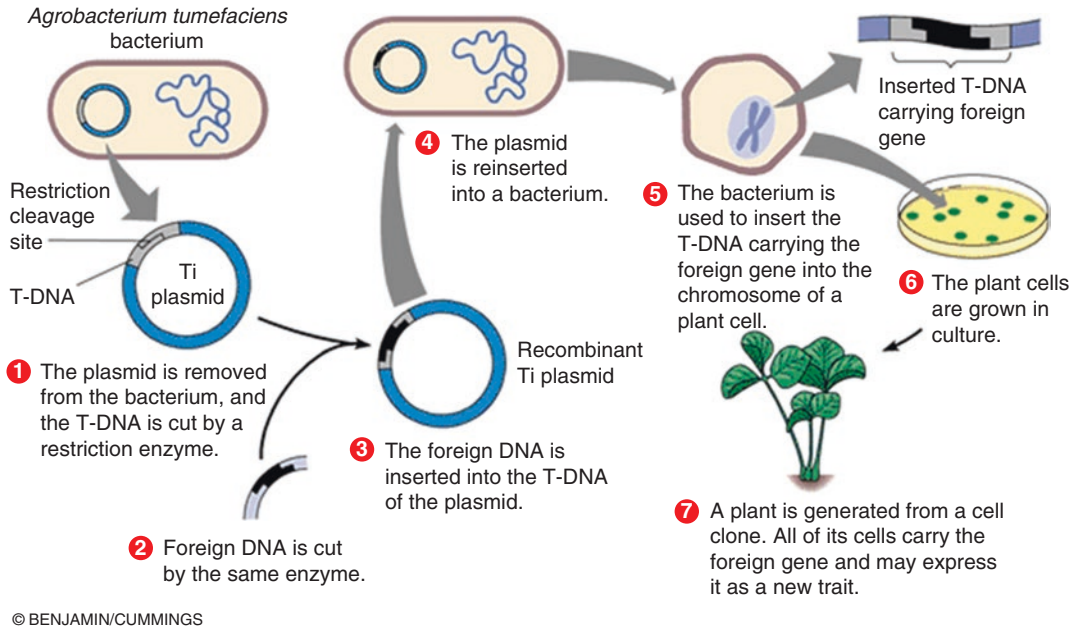

Fig. 14.4 Gene insertion in plants via Agrobacterium tranfection

One of the earlier techniques used to insert genes into plants was the use of the Agrobacterium as a vector to insert the new DNA into a plant. *Agrobacterium tumefaciens* causes a condition known as crown-gall disease in plants. Crown-gall is characterized by a tumor-like growth or gall on the infected plant. The tumors are initiated by the transfer of a DNA segment from the bacterial tumor-inducing plasmid. The plasmid T-DNA is integrated semi-randomly into the genome of the host cell where the tumor morphology genes on the T-DNA are expressed, causing the formation of a gall (Francis and Spiker 2004). The ability of *Agrobacterium* to transfer genes to plants and fungi is used in biotechnology, in particular, genetic engineering for plant improvement. A modified Ti or Ri plasmid can be used. The plasmid is 'disarmed' by deletion of the tumor inducing genes; the only essential parts of the T-DNA are its two small (25 base pair) border repeats, at least one of which is needed for plant transformation. Marc Van Montagu and Jozef Schell at the University of Ghent (Belgium) discovered the gene transfer mechanism between *Agrobacterium* and plants, which resulted in the development of methods to alter *Agrobacterium* into an efficient delivery system for gene engineering in plants (Schell and Van Montagu 1977; Joos et al. 1983). This work then laid the groundwork for the insertion of specific genes into a plant using the Agrobacterium. One can also argue that the gene transfer has been going on for a very long time and we have learned to use it effectively for specific crop improvements.

The genes to be introduced into the plant are cloned into a plant transformation vector that contains the T-DNA region of the bacterial plasmid, together with a selectable marker. Frequently an antibiotic marker gene was incorporated into the plasmid in conjunction with the other desired genes to enable selection for plants that have been successfully transformed. Plants are grown on media containing antibiotic following transformation, and those that do not have the T-DNA integrated into their genome will die. Transformation with *Agrobacterium* can be accomplished by incubating either protoplasts or leaf discs with the *Agrobacterium* to cause the plasmid insertion. From the callus that results, whole plants regenerated using plant tissue culture. The transformation with Agrobacterium is illustrated in Fig. 14.4.

Agrobacterium does not infect all plant species0 but other techniques have been applied for plant transformation one of which is the gene gun. A gene gun is biolistic particle delivery system, originally designed for plant transformation by injecting cells with genetic material. The plasmid DNA is coated on elemental particle of a heavy metal. The gene gun is able to transform almost any type of cell, including plants, and is not limited to genetic material of the nucleus: it can also transform organelles, including plastids.

Gene insertions intended to transform prokaryotic genomes generally have the gene or genes of interest, at least one promoter and terminator sequence, and a reporter gene; which is a gene used to ease detection or removal of those cells which didn't integrate the construct into their DNA.[5] These genes may each have their own promoter and terminator, or be grouped to produce multiple gene products from one transcript, in which case binding sites for translational machinery should be placed between each to ensure maximum translational efficiency. In any case the entire construct is flanked by regions called border sequences which are similar in sequence to locations within the genome, this allows the construct to target itself to a specific point in the existing genome (Slater et al. 2008). The target of a gene gun is often a callus of undifferentiated plant cells growing on gel medium in a Petri dish. After the gold particles have impacted the dish, the gel and callus are largely disrupted. However, some cells are not killed in the impact, and have incorporated enveloped a DNA coated gold particle, which eventually migrates to and integrates into a plant chromosome. Figure 14.5 illustrates the principal of the gene gum. The propellant can be compressed gas or 22 caliber blanks.

The modified cells from the callus are treated with a series of plant hormones, such as auxins and gibberellins, and each may divide and differentiate into the differentiated tissue cells of the plant. This capability of total re-generation is called totipotency. The new plant that originated from a successfully shot cell will express new genetic (heritable) traits as illustrated in Fig. 14.6. In the Figure the use of the gene gun is used to incorporate insect resistance into a tomato plant.

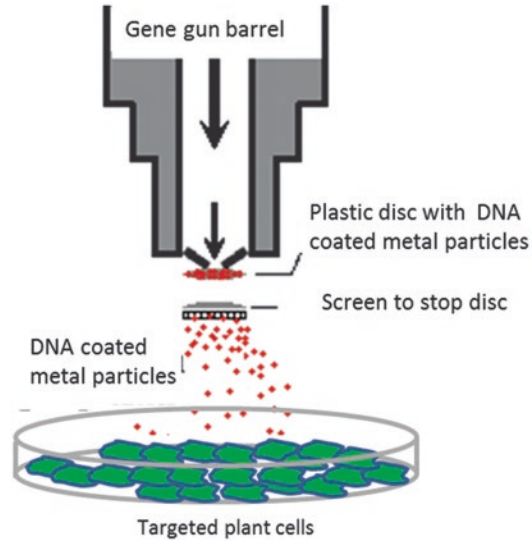

Fig. 14.5 Illustrates the principal of the Gene Gun shooting DNA coated particles into plant callus cells

The term genetic modification and so-called genetically modified organisms (GMOs) is frequently misused. All types (organic, conventional) of agriculture modify the genes of plants so that they will have desirable traits. Traditional forms of breeding change the plant's genetics indirectly by selecting plants with specific traits, while genetic engineering changes the traits by making changes directly to the DNA. In traditional breeding, crosses are made in a relatively uncontrolled manner. In conventional plant breeding, the breeder selects the parents to cross, the results are unpredictable because the DNA from the parents recombines randomly. In contrast, genetic engineering allows highly precise transfer of genes, quick and efficient tracking of genes in new varieties. This ultimately results in increased efficiency in developing new crop varieties with new and desirable traits (Popping 2010).

Applications of Genetically Modified Crops

The introduction of the "Flavr Savr" tomato in 1994 was the first GMO crop to be introduced into the market. In the 1980s there was anecdotal information that the enzyme polygalacturonidase

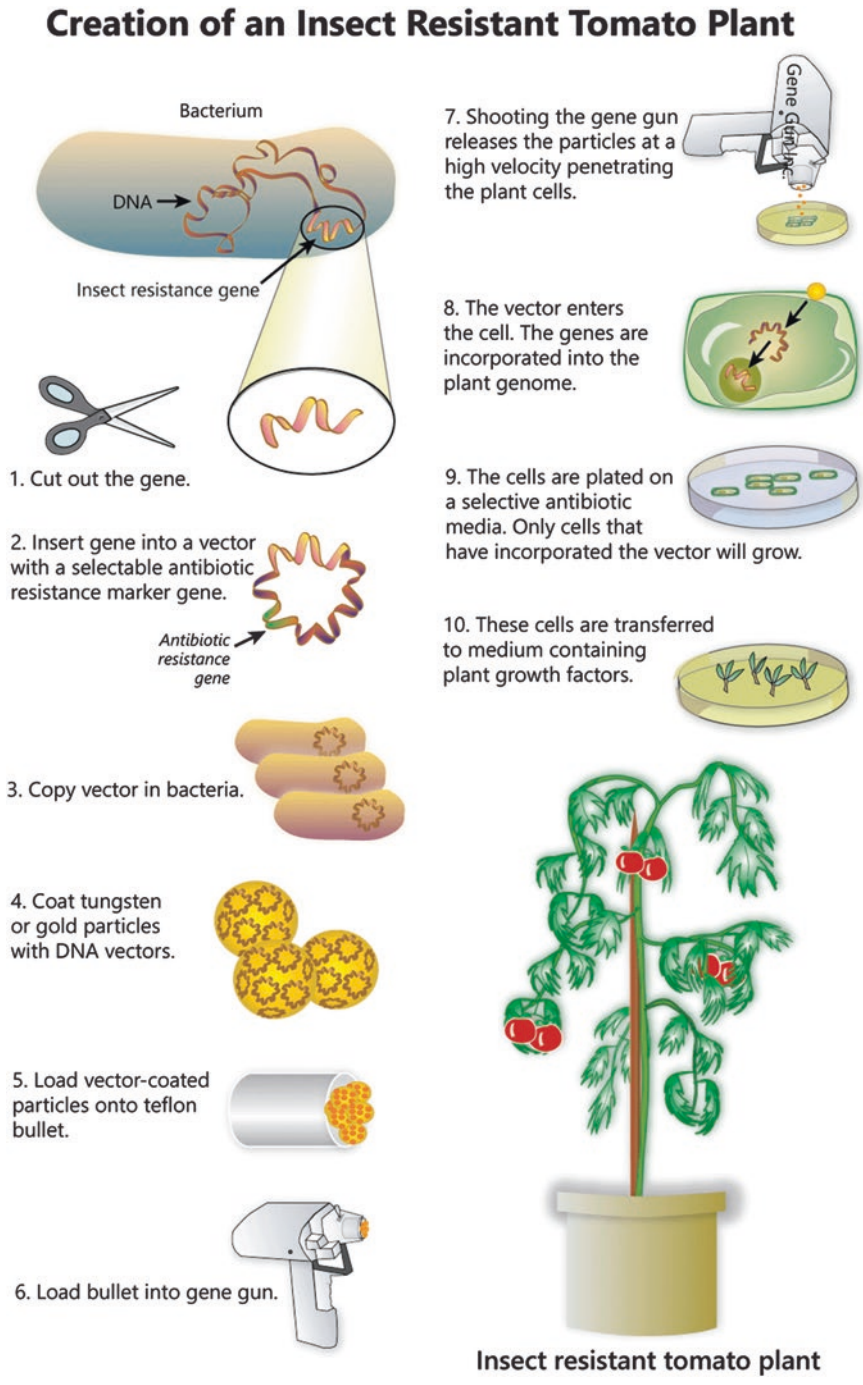

Fig. 14.6 Application of the Gene Gun to introduce insect resistance in a tomato plant

(PG) was key to softening of tomato fruit because it dissolved cell wall pectin. Calgene proposed to limit this enzyme by developing an antisense gene. The goal was to retard ripening allowing the tomatoes to remain firm longer. In 1987, Calegene identified and cloned the tomato fruit PG gene and in 1992 presented a petition to the FDA. In 1994 FDA approved the addition of a

kanamycin resistance gene construct needed to create the PG-antisense tomato. Work continued and in late 1994 the Flavr-Savr tomatoes was introduced. While it may have been a technological success it was a commercial failure and did nothing for the cause of biotechnology so generally, the application of biotechnology and transgenic foods has become a major point of discussion in agriculture. Currently there is a substantial amount of food grown using DNA recombinant technology with approx. 85% of the corn grown in the US being GMO and almost 90% of the soybeans. This is not all the GMO crops but encompass a substantial percentage. The GMO crops have various traits. Examples of two of the more common crops with their associated traits follow. Roundup Ready Soybeans contain a protein that interferes most with the EPSPS pathway. Round Up known as glyphosate is a general purpose pesticide used not only in agriculture but in homes to eliminate weeds. While good to eliminate weeds, it also eliminates healthy crops such as flowers, crops and ornamentals. In the case of Roundup Ready Soy, the GMO trait allows the farmer the ability to use Round Up to eliminate weeds while not killing the soy. Furthermore, a farmer can be more productive eliminating tedious weeding. The second example is BT corn having been encoded with a gene that eliminates the corn borer allowing for more corn per acre. Based on data from the end of 2012 there were 170 million hectares in production that includes 312 events in 29 species with 3497 approvals in 59 countries.

About 12 of global cropland was used to produce genetically modified crops in 2015 (FAO 2015; James 2015). Commercially available crops in production in 2015 included nine food crops, three non-food crops, and two types of flowers. Maize and soybean were the most widely grown genetically modified crops. Production of genetically modified genetically modified maize has increased substantially since its first commercial release in 1996 to include 53.7 million hectares by 2015. Genetically modified soybean also increased rapidly from their introduction in 1996 to over 92 million hectares in 2015 (James 2015).

The seven other food crops of which GE varieties were grown in 2015 were apple (*Malus domestica*), canola (*Brassica napus*), sugar beet (*Beta vulgaris*), papaya (*Carica papaya*), potato, squash (*Cucurbita pepo*), and eggplant (*Solanum melongena*) (James 2015). The contribution of GE varieties to the production of those crops was small, except for canola; GE varieties of canola constituted 24% of the 36 million hectares planted in 2015 (James 2015) rd of all land planted to maize worldwide that year (James 2006, 2015).

The most economically important crop modifications to date are herbicide resistance, Insect resistance and virus resistance.

Herbicide resistance introduces the ability of a crop to resist the application of certain herbicides that are used for weed control. Herbicide resistant traits have been developed for nine different herbicides and introduced into eight herbicide resistant traits for soybeans, six for cotton, three for canola, three for maize, two for sugar beet, and one for alfalfa. Some crop varieties that had stacked traits for resistance to two herbicides (for example, glyphosate and 2,4-D or glyphosate and dicamba). Since it was first introduced in for soybeans in 1996, glyphosate resistance has been introduced in alfalfa, canola, cotton, maize, and sugar beet by 2015.

Insect-resistant (IR) trait incorporates insecticidal properties produced internally by a plant itself. An example of insect resistance is the introduction of transfer of a gene coding for a crystalline (Cry) protein from the soil bacterium *Bacillus thermogenesis*. The Cry is toxic to the target insect when the insect feeds on the plant. The Cry proteins can control many insect pests—primarily moths, beetles, and flies (Höfte and Whiteley 1989). In 2015 insect resistant varieties of cotton, eggplant, maize, poplar, and soybean were in commercial production (NAS 2016).

Virus resistance prevents a plant from being susceptible to specific viral diseases. The virus resistance In crops target the coat-protein gene of the targeted virus. The transgene prevents the virus from replicating successfully in the host plant. Commercially grown virus resistant varieties of papaya were first introduced in the state of

Hawaii in 1998. Virus resistant squash ws also commercialized in the United States in the late 1990s NAS, 2016).

Testing

The ability to determine whether a crop has been genetically modified is important since consumers and regulators require that information. There are two basic types of testing that is performed on selected commodities; protein and DNA. In the development of the gene sequence for a crop, the new gene is sandwiched between two segments; a promoter and a terminator. There are a number of promoter and terminator segments which come from a novel source hence are readily identified. Two of the most common promoter segments are 34S and 35S which come from Cauliflower Mosaic Virus (CaMV) and the Figwort Mosaic Virus (FMV). A relatively common terminator marker is NOS from Nopaline Synthase. When testing for GM content there are two approaches. In the first approach, one can test for the expressed protein using an ELISA or an Immunochromatography method. ELISA tests for a large number of compounds have been in use for decades. While these are useful, the number of possible proteins to test for is limited and proteins are expressed at very low levels. An example of an immunochromatography based method, also called "dipstick" is seen in Fig. 14.7.

A second approach is to test for the fragments such as 34S, 35S and NOS using either PCR or RT-PCR with several commercial test protocols with kits available to test for the specific insert. Obviously before any of these techniques, samples need to be extracted and prepared for analysis using one of several techniques available. In qualitative PCR, the specifity of DNA polymerase is used to allow for amplification of target sequences. In standard PCD, two pairs of primers are used with one being a sense sequence and the other being antisense. These sequences are amplified numbers of times approaching a million. After the amplification, these segments can be separated by agarose gel electrophoresis but other techniques such as HPLC have been used. The approach that is an alternative to the qualitative PCR is Quantitative Real time PCR in which the separation of fragments is performed automatically. Should an organization not choose to perform testing, there are several contract labs that can perform this assay. (Ahmed 2002).

While there is interest in the various technology involved in GMO testing, in recent years a new phenomenon has come into being which is GMO verification services with the most visible

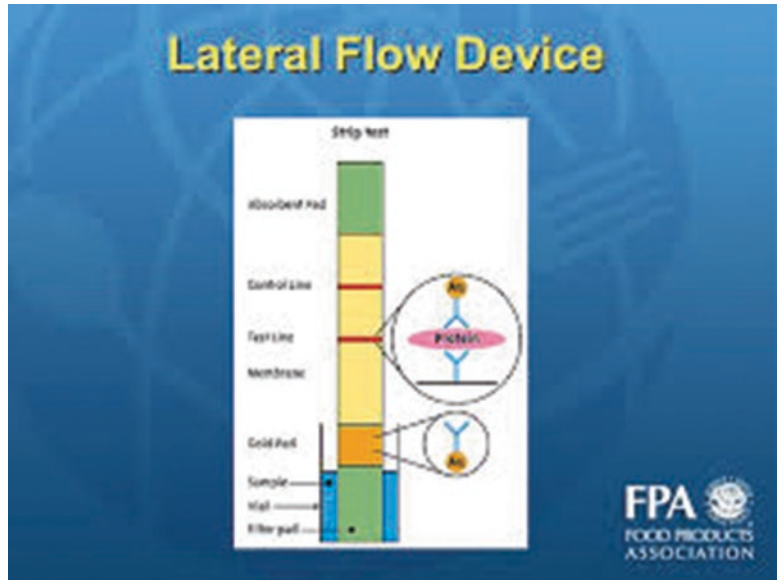

Fig. 14.7 Sample lateral flow device

Fig. 14.8 Non-GMO project verified symbol

Fig. 14.9 USDA PVP symbol

being the non-GMO Verification Project. The project has numerous requirements with the final result being that a manufacturer then can add the symbol to their product (Fig. 14.8).

Additionally, in late 2015, the USDA announced a Process Verified Program (PVP) which allows a supplier manufacturer to place another type of symbol on their project (Fig. 14.9).

Regulation

At the time this chapter is being written, the regulatory landscape is unclear since countries and now individual states in the US are developing action levels that could trigger a requirement for labeling a product as containing GMO. In 1997 the EC developed the novel food regulation 1997/258/EC. For GMO containing foods it required evidence that foods were safe for human consumption and required labeling if foods were not substantially equivalent. It was interesting that this did not trigger labeling. Regulation 1998/1139/EC which regulated Round Up Ready soy and BT137 maize and 1997/1813/EC required labeling of biotech corn and soy based on the presence of transgenic protein or DNA. These regulations created additional confusion as no thresholds were established and 2000/49/EC and 2000/50/EC introduced a 1% labeling threshold for adventitious material which is defined as material that is contained in the food even after all attempts to exclude it. Over the next 3 years there was an evolution of the regulations with 2003/1829/EC/and 2003/1830/EC which added additional biotech crops past Round Up Ready Soy and BT maize and added feed in addition to food. The current level that triggers labeling is 0.9% GMO content but there is still confusion on this topic as there can be different interpretations as what this means. Obviously unapproved events are not allowed. In contrast to Europe, Japan has focused on the GMO content of final food product rather than ingredients with a 5% level (European Union 2011).

The US does not have universal labeling requirements but two bills have been introduced in the US congress that would have standardized labeling with one of them recently defeated. In a parallel fashion a number of states have passed laws requiring GMO labeling with Vermont being the farthest along with food manufacturers preparing for its implementation in 2017 (Figs. 14.10 and 14.11).

Future Challenges

As this topic area evolves, there are going to be challenges on a number of fronts. With the total number of approved events in the hundreds, a category of unapproved events continues to grow. A paper published in the International Journal of Food Contamination on the GMO Contamination Register between the years of 1997 and 2013 indicated that it had recorded 396 incidents across 63 countries (Price and Cotter 2014). An in-depth analysis revealed that rice had the highest number accounting for almost 1/3 of the incidents even though there are is no commercial

Fig. 14.10 Pro-GMO Labeling Ad

Fig. 14.11 Example of a GMO label

growing of gm rice anywhere in the world. The majority of the rice incidents occurred with LLRICE in the US and BT63 Rice from China with the conclusion that the detection of these unapproved events being dependent on both routine and targeted monitoring. As a corollary to these developments, a review of test kits whether it be for expressed protein or genetic markers is limited indicating the need for additional emphasis in the area. A final concern is the need to feed an ever increasing world population making the need for more efficient production of food. In a recent article in **Nature** titled "India needs Home Grown GM Food to Stop Starvation", the author of this commentary stresses the need for India to develop "homegrown" GM IP focusing on commodities that are critical to the country.

The area of transgenic crops is going to continue to evolve with not only development in food for human consumption but also the continuing developments in Pharm animals where modifications are made providing for the development of selected pharmaceuticals. Finally this chapter presents a snapshot of this topic area in early 2016 with changes occurring on a regular basis.

GMO Dictionary

0.9%: The level used in EU countries to determine labeling thresholds.

35S: Promoter DNA fragment from CaMV used as marker to indicate GM content. Also used as a marker when testing samples.

34S: Promoter DNA fragment from FMV used as marker to indicate GM content. Also used as a marker when testing samples.

17,025: A number indicating a lab has met certain quality requirements. Many customers require that 17,025 accredited labs perform the analysis of their samples

Adventitious Contamination The presence of GMOs in traditional crops is difficult to avoid. Minute traces in food products are tolerated if their presence is accidental or the result of technically unavoidable contamination during growing, harvesting, transport or processing.

Base Building blocks of the nucleic acids DNA and RNA**.** Four bases are present in DNA: adenine (A), cytosine (C), guanine (G) and thymine (T). In RNA, thymine is replaced by uracil (U). These four bases encode the genetic information; thus, the four letters A, C, G and T are sometimes called "the alphabet of life".

Bt A protein that is toxic to chewing insects and is produced by the soil bacterium ***Bacillus thuringiensis*** and has long been used as a biological pesticide.

Chromosome: The self-replicating genetic structure of cells, containing genes, which determines inheritance of traits. Chemically, each chromosome is composed of proteins and a long molecule of DNA.

Dipstick; Defined as immunochromatography and used on commodities in the field to determine GM content. Not suitable for processed foods. Uses same technique as home pregnancy test kits.

Cross-pollination: Fertilization of a plant with pollen from another plant. Pollen may be transferred by wind, insects, other organisms, or humans.

DNA (deoxyribonucleic acid): The chemical substance from which genes are made. DNA is a long, double-stranded helical molecule made up of nucleotides which are themselves composed of sugars, phosphates, and derivatives of the four bases adenine (A), guanine (G), cytosine (C), and thymine (T). The sequence order of the four bases in the DNA strands determines the genetic information contained.

EU Labeling: see 0.9%.

Event: A set of trait in a plant giving it unique properties such as herbicide resistance with the provider of the traits having IP.

GMO Analysis: A series of steps including extraction, isolation, analysis and interpretation of data.

Genetic engineering: Manipulation of an organism's genes by introducing, eliminating or rearranging specific genes using the methods of modern molecular biology, particularly those techniques referred to as recombinant DNA techniques.

Genetically engineered organism (GEO): An organism produced through genetic engineering.

Genetic modification: The production of heritable improvements in plants or animals for specific uses, via either genetic engineering or other more traditional methods. Some countries other than the United States use this term to refer specifically to genetic engineering.

Genetics: The study of the patterns of inheritance of specific traits.

Genome: All the genetic material in all the chromosomes of a particular organism.

Herbicide-tolerant crops: Crops that have been developed to survive application(s) of particular herbicides by the incorporation of certain gene(s) either through genetic engineering or traditional breeding methods. The genes allow the herbicides to be applied to the crop to provide effective weed control without damaging the crop itself.

Identity preservation: The segregation of one crop type from another at every stage from production and processing to distribution. This process is usually performed through audits and site visits and provides independent third-party verification of the segregation.

Insecticide resistance: The development or selection of heritable traits (genes) in an insect population that allow individuals expressing the trait to survive in the presence of levels of an insecticide (biological or chemical control agent) that would otherwise debilitate or kill this species of insect. The presence of such resistant insects makes the insecticide less useful for managing pest populations.

Insect-resistance management: A strategy for delaying the development of pesticide resistance by maintaining a portion of the pest population in a refuge that is free from contact with the insecticide. For Bt crops this allows the insects feeding on the Bt toxin to mate with insects not exposed to the toxin produced in the plants.

Insect-resistant crops: Plants with the ability to withstand, deter or repel insects and thereby prevent them from feeding on the plant. The traits (genes) determining resistance may be selected by plant breeders through cross-pollination with other varieties of this crop or through the introduction of novel genes such as Bt genes through genetic engineering.

Intellectual property rights: The legal protection for inventions, including new technologies or new organisms (such as new plant varieties). The owner of these rights can control their use and earn the rewards for their use. This encourages further innovation and creativity for the benefit of us all. Intellectual property rights protection includes various types of patents, trademarks, and copyrights.

Molecular biology: The study of the structure and function of proteins and nucleic acids in biological systems.

Nucleotide: A subunit of DNA or RNA consisting of a nitrogenous base (adenine, guanine,

thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA), a phosphate molecule, and a sugar molecule (deoxyribose in DNA and ribose in RNA). Many of nucleotides are linked to form a DNA or RNA molecule.

Plant-incorporated protectants (PIPs): Pesticidal substances introduced into plants by genetic engineering that are produced and used by the plant to protect it from pests. The protein toxins of Bt are often used as PIPs in the formation of Bt crops.

Polymerase chain reaction (PCR): A technique used to create a large number of copies of a target DNA sequence of interest. One use of PCR is in the detection of DNA sequences that indicate the presence of a particular genetically engineered organism (Vollenhofer et al. (1999)).

Promoter: A region of DNA that regulates the level of function of other genes. Also see 35S and 34S.

Protein: A molecule composed of one or more chains of amino acids in a specific order. Proteins are required for the structure, function, and regulation of the body's cells, tissues, and organs, and each protein has a unique function.

Recombinant DNA (rDNA): A molecule of DNA formed by joining different DNA segments using recombinant DNA technology.

Recombinant DNA technology: Procedures used to join together DNA segments in a cell-free system (e.g. in a test tube outside living cells or organisms). Under appropriate conditions, a recombinant DNA molecule can be introduced into a cell and copy itself (replicate), either as an independent entity (autonomously) or as an integral part of a cellular chromosome.

Ribonucleic Acid (RNA): A chemical substance made up of nucleotides compound of sugars, phosphates, and derivatives of the four bases adenine (A), guanine (G), cytosine (C), and uracil (U). RNAs function in cells as messengers of information from DNA that are translated into protein or as molecules that have certain structural or catalytic functions in the synthesis of proteins. RNA is also the carrier of genetic information for certain viruses. RNAs may be single or double stranded.

Terminator A segment of DNA indicating the end of a particular gene sequence. See NOS

Non-GMO: An internal term indicating a product or ingredient does not require llabeling of GM content

Non-GE: See Non-GMO

Test Free: Test results indicating the sample was Test negative or had detectable but no quantifiable GM content

Test Negative: Test results indicating no detectable GM content

Quantitative Result: A test result indicating the presence or absence of GM content. A result indication the presence of Gm content would like trigger the need for a quantitative test

Qualitative Result; A test result resulting in how much GM content in a sample

RTPCR; The final step in GMO analysis when isolate DNA are amplified 2,000,000 times and are able to be detected

NOS: Terminator DNA fragment from Nopaline Synthase ad used as marker to indicate GM content. Also used as a marker when testing samples

Real Time PCR: See RTPCR

Round up: A type of herbicide used to eliminate weeds. Chemically known as glyphosate

Roundup Ready: A particular type of plant resistant to Roundup allowing for increased productivity

Ingredients

Flour

There are currently no approved non-GMO wheat events. Additionally, based on analytical data and discussions with laboratories and vendors, it is unlikely that one will be able to finds a test negative source of flour. There are vendors that sell a non-GMO flour that tests positive. What this means is the samples likely have been tested with result indicating a detectable but not quantifiable result. One of the reasons for this phenomena is a concept called adventitious contamination. In the case of flour, this concept mean that in each load of wheat there can be several percent GMO corn or soy resulting in a detectable result for the flour and products made with it. A follow on quantitative determination will not be successful as the sample contains not quantifiable DNA. The final result is

that almost 100% of flour will test positive. This should be taken into account as we move forward.

Sunflower Lecithin: With increasing pressure on IP soy lecithin supplies, manufacturers have looked for alternatives such as sunflower lecithin. There currently is no GMO sunflower.

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