

PART I
THE CURRENT SITUATION

1.1

From Primitive Selection to Genetic Modification, Ten Thousand Years of Plant Breeding

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Introduction

In the mid-1990s plant biotechnology burst onto the scene in world agriculture, beginning a second ‘green revolution’ and precipitating one of the great public debates of our time. Approximately a decade later, this book describes the impact of genetically modified (GM) crops on world agriculture, recent advances in the technology and the areas of research from which the next generation of GM crops is likely to emerge, as well as addresses the issues of safety and regulation that have dogged the technology, particularly in Europe.

This chapter defines exactly what GM crops are (in other words, what distinguishes them from other crops) and describes the GM crops that are currently in commercial use. It covers the traits of herbicide tolerance, insect resistance, virus resistance, increased shelf life and modified oil profile, as well as the genes used to impart them. It also chronicles the uptake of GM crop varieties around the world from their widespread introduction in 1996 to the present day, contrasting the situation in the Americas, Australia and Asia with that in Western Europe.

First, it is necessary to put the advent of plant genetic modification into the context of a long history of advances in plant breeding and genetics.

Early Plant Breeding

Arguably the most important event in human history occurred approximately 10 000 years ago when people in what is now called the Middle East began to domesticate crops and livestock, and adopt a sedentary way of life based on farming rather than a nomadic one based on hunting and gathering. Ultimately this led to the growth of villages, towns and cities, and provided the stability and time for people to think, experiment, invent and innovate. Technological advancement, which had barely progressed at all for half a million years, accelerated enormously (Figure 1.1.1). The great civilizations of ancient Mesopotamia (Assyria, Sumeria and Babylon) and Egypt arose within a few thousand years, laying the foundation of modern civilization.

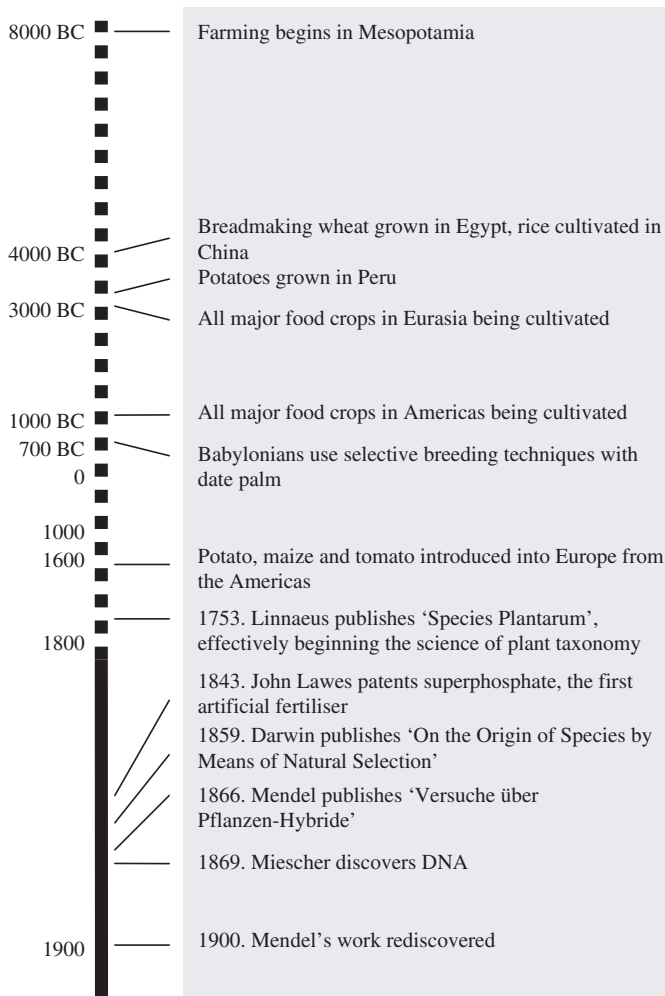


Figure 1.1.1 Timeline showing some of the major landmarks in the development of agriculture and plant breeding.

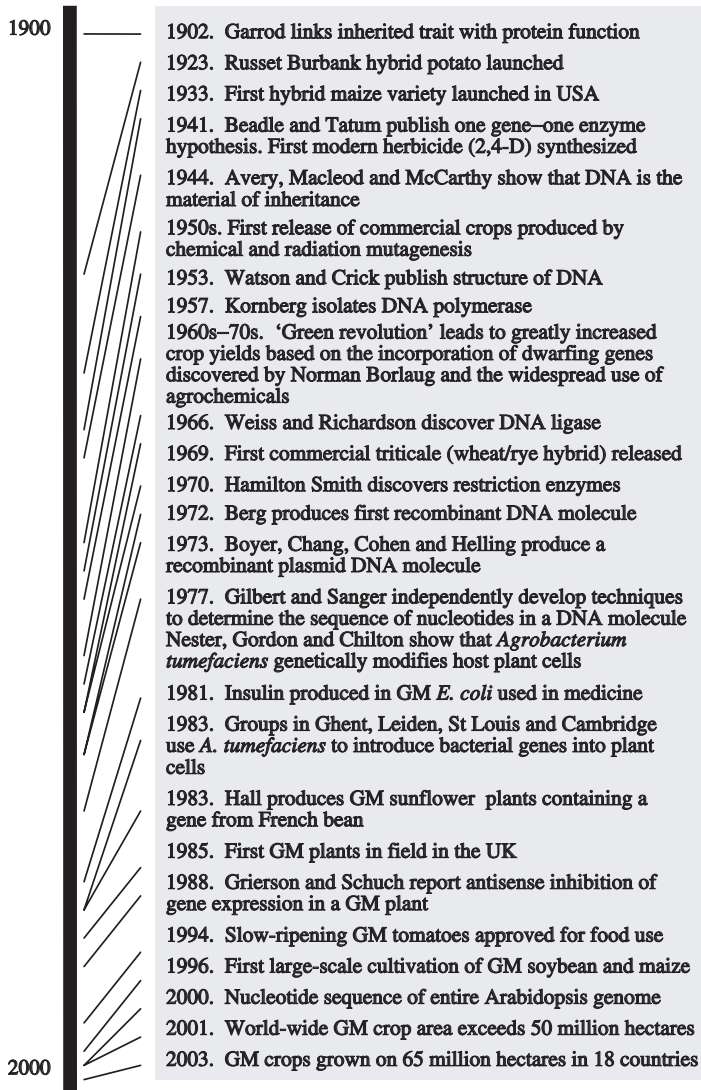


Figure 1.1.1 (Continued)

The crop species responsible for this change was probably wheat. Certainly by 6000 years ago, wheat was being baked into leavened bread in Egypt in much the same way as it is today. Farming was also developing in South America and China, with potato and rice, respectively, being the predominant cultivated crops.

It is probable that crop improvement began as soon as farming did. At first, such improvement may well have occurred unconsciously through the harvesting and growing of the most vigorous individuals from highly variable populations, but then became more systematic. For example, there is evidence that the Ancient Babylonians bred for

certain characteristics in palm trees by selecting male trees with which to pollinate female trees.

Over time such practices had dramatic effects on crop characteristics. For example, the wheat grain found in Ancient Egyptian tombs is much more similar to modern wheat than to its wild relatives. Indeed, breadmaking wheat arose through hybridization events between different wheat species that only occurred in agriculture; there is no wild equivalent. It first appeared within cultivation, probably in Mesopotamia between 10 000 and 6000 years ago, and its use spread westwards into Europe.

Another excellent example of the effects of simple selection is the cabbage family of vegetables, which includes kale, cabbage itself, cauliflower, broccoli and Brussels sprouts. The wild relative of the cabbage family was first domesticated in the Mediterranean region of Europe approximately 7000 years ago. Through selective breeding over many centuries, the plants became larger and leafier, until a plant very similar to modern kale was produced in the 5th century BC. By the 1st century AD, cabbage had appeared, characterized by a cluster of tender young leaves at the top of the plant. In the 15th century, cauliflower was produced in Southern Europe by selecting plants with large, edible flowering heads and broccoli was produced in a similar fashion in Italy about a century later. Finally, Brussels sprouts were bred in Belgium in the 18th century, with large buds along the stem. All of these very different vegetables are variants of the same species, *Brassica oleracea*.

The Founding of the Science of Genetics

The examples above show how crop plants were improved by farmers who for millennia knew nothing about the scientific basis of what they were doing. Modern, systematic plant breeding did not come about until the science of genetics was established as a result of the work of Charles Darwin and Gregor Mendel.

Darwin is regarded by many as the father of modern genetics but it was Mendel's work that showed how Darwin's theories on natural selection could work. Ironically the two men never met and Darwin died unaware of Mendel's findings. Darwin's seminal book, 'On the Origin of Species by Means of Natural Selection', was published in 1859. In it, Darwin described the theory of evolution based on the principle of natural selection. The theory was proposed independently at approximately the same time by Alfred Russell Wallace, but it was Darwin's meticulous accumulation of evidence collected over decades that gave weight to the hypothesis.

In simple terms, Darwin's theory of evolution proposed that the diversity of life on Earth had arisen through the adaptation of species to different and changing environments, leading to the extinction of some species and the appearance of others. Species that were similar had arisen from a recent common ancestor. This process was driven by natural selection, in which individuals competed with each other and those best fitted for their environment would be most likely to survive, reproduce and pass on their characteristics to the next generation. If the environment changed or a species colonized a new environment, different characteristics would be selected, leading to change and eventually to the evolution of a new species.

Natural selection (or artificial selection, for that matter) can only work because individuals within a species are not all the same; individuals differ or show variation. Darwin and his contemporaries believed that traits present in two parents would be mixed in the offspring so that they would always be intermediate between the two parents. This posed a problem for Darwin's theory of evolution because it would have the effect of reducing variation with every successive generation, leaving nothing for selection to work on.

The solution to the problem was provided by Gregor Mendel, a monk at the Augustinian monastery in Brno. In 1857, Mendel began experimenting with pea plants, noting different characteristics such as height, seed color and pod shape. He observed that offspring sometimes, but not always, showed the same characteristics as their parents. In his first experiments, he showed that short and tall plants bred true, the short having short offspring and the tall having tall offspring, but that when he crossed short and tall plants all of the offspring were tall. He crossed the offspring again and the short characteristic reappeared in about a quarter of the next generation.

Mendel concluded that characteristics were passed from one generation to the next in pairs, one from each parent, and that some characteristics were dominant over others. Crucially, this meant that variation was not lost from one generation to the next. Whether the offspring of two parents resembled one parent or were an intermediate between the two, they inherited a single unit of inheritance from each parent. These units were reshuffled in every generation and traits could reappear. Although Mendel did not use the term, units of inheritance subsequently became known as genes.

Mendel's findings were published by the Association for Natural Research in 1866, under the title 'Versuche über Pflanzen-Hybride', but were ignored until the beginning of the next century as the work of an amateur. Later they became known as the Mendelian Laws and the foundation of modern plant breeding.

The Elucidation of the Molecular Basis of Genetics

The pace of discovery accelerated greatly in the 20th century (Figure 1.1.1) and gradually the molecular bases for the laws of genetics were uncovered. In 1902, Sir Archibald Garrod found that sufferers of an inherited disease, alkaptonuria, lacked an enzyme that breaks down the reddening agent, alkapton, and therefore excreted dark red urine. This was the first time that a link had been made between a genetic trait and the activity of a protein. The significance of Garrod's work was only recognized decades later when George Beadle and Edward Tatum showed that a genetic mutation in the fungus, *Neurospora crassa*, affected the synthesis of a single enzyme required to make an essential nutrient. Beadle and Tatum published the one gene–one enzyme hypothesis in 1941 (Beadle and Tatum, 1941) and were subsequently awarded a Nobel Prize. The hypothesis was essentially correct, with the exception that some proteins are made up of more than one subunit and the subunits may be encoded by different genes.

Underpinning the laws of genetics and evolution, which have now been established, is the ability of organisms to pass on the instructions for growth and development to their

offsprings. The obvious question was in what substance was this information carried, in other words what was the genetic material. Deoxyribonucleic acid (DNA) was identified as this substance in 1944 by Oswald Avery, Colin MacLeod and Maclyn McCarty. Their conclusive experiment showed that the transfer of a DNA molecule from one strain of a bacterium, *Streptomyces pneumoniae*, to another changed its characteristics (Avery *et al.*, 1944).

DNA was first discovered in 1869 by Friedrich Miescher but its structure was not determined for another 84 years. The breakthrough was made by James Watson and Francis Crick in 1953 (Watson and Crick, 1953). They came up with their model after analyzing X-ray crystallographs produced by Rosalind Franklin and Maurice Wilkins, but it is fair to say that Watson and Crick made an intellectual leap that Franklin and Wilkins had failed to make. Watson, Crick and Wilkins were awarded a Nobel Prize; tragically, Franklin missed out because she died before the prize was awarded and it is not awarded posthumously.

The structure of DNA is so elegant that it has become iconic. The molecule consists of two strands (it is said to be double-stranded); each strand is made up of units of deoxyribose (a type of sugar) with an organic base attached, linked by phosphate groups. Each unit is called a nucleotide and there are four kinds, each with a different organic base: adenine, cytosine, guanine or thymine. These are often represented as A, C, G and T. The two strands run in opposite directions and are coiled into a double helix structure, the two strands linked together by hydrogen bonds between opposing bases. The separation distance of the two strands means that the bases on opposing strands occur in pairs (base pairs) that will fit: adenine on one strand always paired with thymine on the other, and cytosine always paired with guanine. This means that the sequence of bases on one strand determines the sequence on the other (they are said to be the reverse and complement of each other), an important factor when the molecule is being duplicated. If double-stranded DNA is unraveled to form two single strands, each strand can act as a template for the synthesis of a complementary chain and two replicas of the original double-stranded molecule are created. Information is encoded within DNA as the sequence of nucleotides in the chain, a four-letter language in which all the instructions for life on Earth are written.

Information encoded within the DNA molecule determines the structure of a protein through the process of gene expression. The first part of this process is called transcription, in which a molecule related to DNA called ribonucleic acid (RNA) is synthesized using the DNA molecule as a template. Like DNA, RNA consists of a sugar-phosphate backbone along which are attached organic bases, but the RNA molecule consists of a single strand, not two, and the base thymine is replaced with uracil (U). The sequence of nucleotides on the newly synthesized RNA molecule is determined by the sequence of bases on the DNA template.

The RNA molecule is processed and transported to protein complexes called ribosomes where protein synthesis occurs; this is called translation. Proteins consist of chains of amino acids and the amino acid sequence is specified by the sequence of nucleotides in the RNA molecule, each amino acid in the protein being represented by a triplet of nucleotides called a codon. It is the sequence of amino acids in the protein that determines its function and properties, and ultimately it is the protein structure and function that determines the characteristics of an organism.

This link from DNA to RNA to proteins explains the observations of Garrod and underpins the one gene—one enzyme hypothesis of Beadle and Tatum. Furthermore, the processes of evolution and the changes in plants and animals brought about by selective breeding can be seen to result from changes (mutations) in the DNA sequence that lead to variations between individuals and traits that are selected.

DNA molecules can be huge and in plants, animals and fungi they are wrapped around proteins to form structures called chromosomes. In humans, they are organized into 23 pairs of chromosomes, each chromosome containing a DNA molecule ranging from 50 to 250 million base pairs so that 23 individual chromosomes (one from each pair, making up the genome) comprise a total of approximately 3 billion base pairs. If this length of DNA were stretched out it would be several centimetres long, yet it has to be coiled and packaged to fit into a cell. In comparison, the rice genome contains only 466 million base pairs on 12 chromosomes, while that of *Arabidopsis*, a plant widely used as a model in plant genetics, contains approximately 126 million base pairs on five chromosomes. The maize genome contains 2.6 billion base pairs on 10 chromosomes, while that of wheat is estimated to contain more than 16 billion base pairs on seven chromosomes.

Distributed unevenly along these huge DNA molecules are genes, just below 30 000 in *Arabidopsis*, 30 000–40 000 in humans and 45 000–56 000 in rice. Genes can be over a million base pairs long but are usually much smaller, averaging about 3000 base pairs. In fact, they make up a small proportion of the total genome; the rest (often referred to as ‘junk DNA’) appears to have no function and its amount varies greatly between different species, hence the great disparity in genome size between quite closely related species such as rice and wheat.

There is no structure marking the beginning and end of a gene. Rather, the units of heredity described by Mendel can be defined simply as functional units within a DNA molecule. Perhaps the most readily recognizable part of the gene is that containing the information for the sequence of amino acids in the protein that the gene encodes. This part of the gene is called the coding region and at least it has a definite beginning and end, although it is usually split into sections called exons interspersed with non-coding regions called introns. A gene also contains information that determines when, where and in response to what the gene is active. This information is usually contained in regions of the DNA ‘upstream’ of the coding region in what is called the gene promoter, but it can be in regions downstream of the coding region or within introns. The region ‘downstream’ of the coding region also contains information for the correct processing of the RNA molecule that is transcribed from the gene and is called the gene terminator.

Genes that are active throughout an organism all the time are referred to as constitutive or house-keeping genes. Other genes are active only in certain organs, tissues or cell types, while some are active during specific developmental stages or become active in response to a particular stimulus. In the case of plants, genes respond to many stimuli, including light, temperature, frost, grazing, disease, shading and nutritional status.

The Manipulation of DNA and Genes

Once DNA had been identified as the genetic material and its structure described, studies on the properties of DNA itself and the enzymes present in cells, which work on it, began

in earnest. DNA polymerase, an enzyme that synthesizes DNA, was isolated by Arthur Kornberg in 1955 (Lehman *et al.*, 1958); DNA ligase, an enzyme that ‘glues’ two ends of DNA together, was isolated by Bernard Weiss and Charles Richardson in 1966 (Weiss and Richardson, 1967); a restriction endonuclease (also known as restriction enzyme), an enzyme that recognizes specific short sequences of base pairs in a DNA molecule and cuts the molecule at that point, was characterized by Hamilton Smith in 1970 (Smith and Wilcox, 1970). Both Kornberg and Smith received Nobel Prizes.

The molecular tools for repairing DNA, cutting it at specific places and sticking its pieces together in a test tube to make new molecules were now available. They were used by Paul Berg in 1972 to construct a DNA molecule by cutting viral and bacterial DNA sequences with restriction enzymes and then recombining them (Jackson *et al.*, 1972); he received a Nobel Prize in 1980. A year after Berg’s experiment, Stanley Cohen, Annie Chang, Herbert Boyer and Robert Helling demonstrated that DNA which had been cut with a restriction enzyme could be recombined with small, self-replicating DNA molecules from bacteria called plasmids (Cohen *et al.*, 1973). The new plasmid could then be reintroduced into bacterial cells and would replicate. If the bacterial cells were cultured, each cell carrying copies of the recombinant plasmid, large amounts of plasmid DNA with the new piece of DNA inserted in it could be isolated from the culture. This enabled a section of DNA from any species to be cloned and bulked up in bacteria to generate enough of it to work on. This process is often called gene cloning. The bacterium of choice for this purpose is usually *Escherichia coli* (*E. coli*). This is a human gut bacterium, although the strains used in the laboratory have been disabled so that they are not pathogenic.

The ability to clone genes underpinned the molecular analysis of gene structure and function. Some people regarded this as a new branch of science and called it molecular biology. Its commercial exploitation was termed biotechnology and the first example of this was in the pharmaceutical industry; insulin produced from a modified human gene in *E. coli* was approved by the Food and Drug Administration of the USA in 1981.

Two other advances are worthy of note: in 1977, Walter Gilbert and Fred Sanger separately developed methods for determining the sequence of nucleotides in a DNA molecule (Maxam and Gilbert, 1977; Sanger *et al.*, 1977), and in 1983, Kary Mullis invented a method called the polymerase chain reaction (PCR) by which short sections of DNA could be bulked up (amplified) without cloning in bacteria (Mullis and Faloona, 1987). All three received a Nobel Prize. The methods for determining the nucleotide sequence of a DNA molecule were developed and automated to such an extent by the early 1990s that projects were initiated to obtain the nucleotide sequence of entire genomes. A first draft of the nucleotide sequence of the human genome was published in 2001. The first plant genome sequence was that of Arabidopsis, which was published in 2000, and the first crop plant genome sequence to be published was that of rice in 2002.

Modern Plant Breeding

The practice of planting different variants of the same crop in adjacent plots to promote the production of hybrid seed is used widely today by farmers and plant breeders, and has probably been practiced for millennia. It is done to exploit hybrid vigor, the phenomenon

of a hybrid outperforming both of its parents. Hybrid vigor occurs because the ongoing process of genetic change by mutation leads to the existence of different forms of the same genes within a population. These different forms are called alleles, and the crossing of two parent lines with different characteristics results in a hybrid population with different combinations of alleles (genotypes) from the two parents. Some of these combinations are advantageous.

When Mendel's work on the inheritance of characteristics and the genetics of plant hybrids was rediscovered around 1900, plant breeding through the crossing of plants with different genotypes had a sound scientific basis. Plant breeders now understood what would happen to a genetic trait when it was crossed into a breeding line and how to produce a true-breeding line (a variety) in which that trait and other characteristics would be present in every individual in every generation. That is not to say that the process is simple; the fact that plants have several tens of thousands of genes which can be mixed in a myriad of combinations when a cross is made can make the outcome unpredictable. Furthermore, desirable traits may be linked with undesirable ones, usually as a result of being close together on the same chromosome.

Despite these difficulties, plant breeders have been incredibly successful at improving crop yield and it is just as well that they have. At the end of the 18th century, Reverend Thomas Malthus wrote in his 'Essay on the Principle of Population' that food supply could not keep up with rising population growth (Malthus, 1798). At that time, world population was approximately 1 billion. In 1999, the world population reached 6 billion, and yet famine remains relatively rare and localized and arises through extreme climate conditions combined with government incompetence and/or war, rather than inadequate crop plant performance.

An example of the dramatic increases in crop yield that have been achieved is that of wheat grown in the United Kingdom. It has increased approximately tenfold over the last 800 years, with more than half that increase coming since 1900. Similar increases have been achieved around the world with different crop species, the period of most rapid improvement being in the 1960s and 1970s when the incorporation of dwarfing genes into cereal crops together with increased mechanization and the widespread use of nitrogen fertilizers, herbicides and pesticides led to the so-called 'Green Revolution'. The dwarfing genes concerned actually affected the synthesis of a plant hormone, gibberellin, although it was not known at the time. Their incorporation reduced the amount of resources that cereal plants put into their inedible parts, making more available to go into the seed, and at the same time made the plants less susceptible to damage under damp and/or windy conditions. One of the pioneers of their use was Norman Borlaug, who not only used the technology himself in wheat breeding but also persuaded wheat breeders in Asia to do the same. Borlaug's actions are widely believed to have averted critical food shortages in Asia; indeed, it has been suggested that he is responsible for saving more lives than any other individual in history. No doubt Louis Pasteur and others would have supporters in a debate on that point, but Borlaug's success is something that all plant scientists can be proud of.

The seemingly inexorable rise in crop yield might be taken to indicate that the improvement will continue in perpetuity. However, improvement brought about by the recombination of existing genotypes is limited by the genetic variation that is present. Yield depends on many factors and is affected by many different genes, but eventually

the possible combinations of genotypes will be exhausted. Furthermore, some targets for plant breeders have been much less amenable to breeding. If a trait, whether it be for resistance to a disease, tolerance of a herbicide, the ability to survive and yield highly in a particular environment, or whatever the target might be, does not exist in any of the genotypes within a species then a breeder cannot simply invent it.

In the mid-20th century, plant breeders began to use two new methods to increase the genetic variation available in their breeding lines. The first was 'wide crossing', the creation of hybrids between crop plants and exotic relatives or even species with which they would not normally cross in nature. The second was to induce mutations by treatment with either ionizing radiation (neutrons, gamma rays, X-rays or UV radiation) or a chemical mutagen.

Wide crosses usually require rescue of the embryo to prevent abortion; the embryo is removed from a developing seed under sterile conditions and cultured in a nutrient medium until it germinates. If the cross is made between two different species then the hybrid is usually sterile. This is because the members of each pair of chromosomes have to come together at the beginning of the process of meiosis by which sperm and egg cells are formed. In a hybrid cell with one set of chromosomes from each parent species, either the chromosomes do not pair at all or they mispair; the result is that the sperm and egg cells that are formed have too many, too few or the wrong combination of chromosomes and are not viable. This can be overcome by inducing chromosome doubling, usually by treatment of anthers, immature inflorescences or cultured cells with a chemical called colchicine. The hybrid cells then have a pair of chromosomes originating from each parent and are said to be polyploid (having more than one genome).

The best known example of a crop plant produced in this way is triticale, a hybrid between wheat and rye. The hybrid is usually made between durum wheat, already a tetraploid (two genomes), and rye (a diploid) to produce a hexaploid triticale (three genomes), although it is also possible to cross hexaploid wheat with rye to produce an octoploid triticale (four genomes). The name triticale was first used in 1935 by Tschermak but it was not until 1969, after considerable improvement through breeding, that the first commercial varieties of triticale were released. Triticale is now grown on more than 2.4 million hectares worldwide, producing more than 6 million tonnes of grain per year. It combines the yield potential of wheat with the acid soil-, damp- and extreme temperature-tolerance of rye and is used mostly for animal feed.

Experiments with mutagenesis of crop plants began in the 1920s. The radiation or chemical treatment, usually of seeds, damages the DNA, resulting in changes in the DNA sequence and hence genetic variation. The process has the disadvantage of being entirely random, and therefore mutagenesis programs usually involve very large populations of at least 10 000 individuals to ensure that a useful mutant is produced. Nevertheless, it has proved successful; the first commercial varieties arising from mutation breeding programs were released in the 1950s and the technique was used widely in the 1960s and 1970s, and continues to be used today.

Mutagenesis played an important role in the improvement of oil quality of oilseed rape, the first variety produced in this way being Regina II which was released in 1953. Oilseed rape was first grown in the UK during World War II to provide oil for industrial uses, and some varieties are still grown for that purpose. Its oil was regarded as unfit for human consumption because it contained high levels of erucic acid and glucosinolates.

Both of these compounds are very poisonous and glucosinolates have a bitter flavor. Their levels were gradually reduced by breeders using mutagenesis and crossing, and oilseed rape was finally passed for human consumption and animal feed in the 1980s. The edible varieties were given the name canola in North America and this name is now used for all varieties in that part of the world. Mutagenesis has also played an important role in the improvement of pasta wheats, rice, white bean and barley.

Genetic Modification

In 1977, 4 years after the first recombinant plasmid DNA molecule had been produced, Nester, Gordon and Chilton showed that bacterial DNA was inserted into the DNA of host plant cells during infection by a bacterium called *Agrobacterium tumefaciens* (Chilton *et al.*, 1977). This bacterium causes crown gall disease, characterized by the formation of large swellings (galls) just above soil level. The piece of DNA that is inserted into the plant genome is called the transfer DNA (T-DNA) and is carried on a plasmid called the tumor-inducing or Ti plasmid. Besides causing the host cell to proliferate to form the gall, it also induces the production and secretion of unusual sugar and amino acid derivatives that are called opines, on which the *Agrobacterium* feeds. There are several types of opines, including nopaline and octopine, produced after infection with different strains of the bacterium.

The cells of the gall are not differentiated; in other words they do not develop into the specialized cells of a normal plant. They can be removed from the plant and cultured as long as they are supplied with light and nutrients and are protected from fungal and bacterial infection. A clump of these undifferentiated cells is called a callus and callus formation can be induced in the laboratory by infecting explants (e.g., leaf pieces, stem sections or tuber discs) with *A. tumefaciens*. All the cells in the callus contain the T-DNA that originated from the bacterium.

This discovery caused great excitement because it represented a means by which the genetic make-up of a plant cell could be transformed (the process is often referred to as transformation). In 1983, groups led by Schell and Van Montagu (Ghent), Schilperoort (Leiden), Chilton and Bevan (St. Louis and Cambridge) and Fraley, Rogers and Horsch (St. Louis) showed that bacterial antibiotic resistance genes could be inserted into the T-DNA carried on a Ti plasmid and transferred into plant cells (Bevan *et al.*, 1983; Fraley *et al.*, 1983; Herrera-Estrella *et al.*, 1983; Hoekema *et al.*, 1983). Michael Bevan in Cambridge developed the so-called binary vectors, plasmids that would replicate in both *E. coli*, in which it could be manipulated and bulked up, and *A. tumefaciens* (Bevan, 1984). Binary vectors contain the left and right T-DNA borders but none of the genes present in 'wild type' T-DNA. They are unable to induce transfer of the T-DNA into a plant cell on their own because they lack genes called virulence (*VIR*) genes that are required to do so. However, when present in *A. tumefaciens* together with another plasmid containing the *VIR* genes, the region of DNA between the T-DNA borders is transferred, carrying any genes that have been placed there in the laboratory.

Calli have to be kept under sterile conditions to prevent bacterial or fungal infection. They can be induced to form a shoot by treatment with a plant hormone; once a shoot

with a stem is formed, the hormone is withdrawn and hormones produced by the shoot itself then induce root formation and a complete plantlet is formed. The plantlet can be transferred to the soil and treated like any other plant. All the cells of the plant will contain the T-DNA integrated into its own DNA, and the T-DNA and all the genes in it will be inherited in the same way as the other genes of the plant. In 1983, Tim Hall used this method to produce a sunflower plant carrying a seed protein gene from French bean (Murai *et al.*, 1983). Not only was the gene present in every cell of the plant, but also it was inherited stably and was active. The era of plant transformation had begun.

Plants that have been altered genetically in this way are referred to as transformed, transgenic, genetically engineered (GE) or GM. The term transgenic is favored by scientists but GM has been adopted most widely by non-specialists. All plant breeding, of course, involves the alteration (or modification) of plant genes, whether it is through the selection of a naturally occurring mutant, the crossing of different varieties or even related species or the artificial induction of random mutations through chemical or radiation mutagenesis. Nevertheless, the term 'genetically modified' is now used specifically to describe plants produced by the artificial insertion of a single gene or small group of genes into its DNA. Genetic transformation mediated by *A. tumefaciens* is now not the only method available to scientists; other methods, including the latest advances, will be described in Chapter 2.1.

Genetic modification has been an extremely valuable tool in plant genetic research. It has been applied, amongst other things, to the analysis of gene promoter activity, the functional characterization of regulatory elements within gene promoters, the determination of gene function, studies on metabolic pathways, elucidation of the mechanisms by which plants respond to light, disease, grazing, drought, nutrition and other stimuli, and analyses of protein structure, function and regulation. However, this book is concerned with its use in crop plant breeding.

Out of the Laboratory and into the Field; Commercial GM Crops

Genetic modification has some advantages over other techniques used in plant breeding. It allows genes to be introduced into a crop plant from any source, so technically at least the genetic resources available are huge; it is relatively precise in that single or small numbers of genes can be transferred; the safety of genes and their products can be tested extensively in the laboratory before use in a breeding program; genes can be manipulated in the laboratory before insertion into a plant to change when and where they are active, or to change the properties of the proteins that they produce. These advantages have led to genetic modification becoming established as a new tool for plant breeders to add to (not replace) those already available.

Delayed Ripening/Increased Shelf Life

The first commercial GM plant varieties to be released were tomato varieties that had been modified to slow down the ripening process, giving them a longer shelf life, the first of which were approved for food use in the USA in 1994. A major problem in fruit

production is that consumers want to buy ripe fruit but ripening is often followed quite rapidly by deterioration and decay. Fruit ripening is a complex process that brings about the softening of cell walls, sweetening and the production of compounds that impart color, flavor and aroma. The process is induced by the production of a plant hormone, ethylene. Genetic modification has been used to slow ripening or to lengthen the shelf life of ripe fruit by interfering either with ethylene production or with the processes that respond to ethylene.

The development of these varieties went hand in hand with the invention of techniques that enabled scientists to use genetic modification to reduce the activity of (or silence) a specific plant gene. The first of these techniques was the so-called antisense method first described by Don Grierson in Nottingham (reviewed by Grierson, 1996). Antisense gene silencing involves the construction of a gene in which part of the gene to be silenced is spliced in the reverse orientation downstream of a promoter sequence. The promoter may derive from the same gene, but usually it is a more powerful one. When a GM plant is produced carrying this gene, it synthesizes RNA of the reverse and complementary sequence of that produced by the target gene. This antisense RNA interferes with the accumulation of RNA from the target gene, preventing it from acting as a template for protein synthesis. The second technique for silencing target genes in plants arose from the surprising observation that one or more additional copies of all or part of a gene even in the correct orientation sometimes had the same effect as antisense gene expression when introduced into a plant by genetic modification. This method of gene silencing is called co-suppression.

Gene silencing turned out to be a natural defense mechanism employed by plants against virus infection. It involves the production of small, antisense RNAs, 25 nucleotides in length, that interfere with the processing, transport and translation of RNA molecules produced by a target gene. The third method of gene silencing by genetic modification, called RNA interference (RNAi), involves inducing the plant to synthesize a double-stranded RNA molecule derived from the target gene. This has been done by splicing part of the gene sequentially in a head-to-tail formation downstream of a promoter. Introduction of such a gene into a plant causes the production of an RNA molecule that forms a hairpin loop, which is cleaved by enzymes naturally present in plant cells into short molecules, each 23 nucleotides long.

Antisense and co-suppression were used in the first GM tomato varieties to reduce the activity of a gene encoding polygalacturonase (PG), an enzyme that contributes to cell wall softening during ripening. Two competing groups developed these varieties at approximately the same time. Calgene in the USA used an antisense technique while Zeneca in collaboration with Grierson's group used co-suppression. The Calgene product was a fresh fruit variety called 'Flavr Savr'. It was first grown on a large scale in 1996 but was not a commercial success, and was withdrawn within a year.

Zeneca chose to introduce the trait into tomatoes used for processing and this proved to be much more successful. These tomatoes have a higher solid content than conventional varieties, reducing waste and processing costs in paste production and giving a paste of thicker consistency. This product went on the market in many countries and proved very popular in the UK from its introduction in 1996 until 1999 when most retailers withdrew it in response to anti-GM hostility.

Some GM tomato varieties with delayed ripening are still on the market in the USA. They have reduced activity of the enzyme aminocyclopropane-1-carboxylic acid (ACC) synthase, which is required for ethylene synthesis. ACC has also been targeted using a gene from a bacterium, *Pseudomonas chlororaphis*, that encodes an enzyme called ACC deaminase, which breaks down ACC. A similar strategy has been adopted to break down another of the precursors of ethylene, S-adenosyl methionine (SAM), using a gene encoding an enzyme called SAM hydrolase. Genetic modification to delay ripening and improve post-harvest shelf life is also being used in papaya, mango, pineapple and other fruits but there are no commercial varieties available yet.

Herbicide Tolerance

Tomato is an important fruit crop but its production is dwarfed by that of the major agricultural crops; and it was the release and success of GM varieties of two of these, soybean and maize (corn), that really established genetic modification as an important tool in plant breeding. These varieties were first grown on a large scale in the USA in 1996. The traits that they carried as a result of genetic modification were herbicide tolerance (soybean) and insect resistance (maize). These traits have now been introduced into other crops and combined (stacked) in some varieties.

Herbicide-tolerant GM crops were produced to simplify and cheapen weed control using herbicides. Of course, herbicides have been used since long before the advent of genetic modification, the first modern herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), was synthesized in 1941 and released in 1946. They are now an essential part of weed control for farmers in developed countries. However, besides the obvious considerations of equipment and labor costs, as well as the cost of the chemicals themselves, herbicides pose a number of problems for farmers. Most are selective in the types of plants they kill, and a farmer has to use a particular herbicide or combination of herbicides that is tolerated by the crop being grown but kill the problem weeds. Some of these herbicides have to be applied at different times during the season, including some that have to go into the ground before planting, some that pose a health risk to farm workers and some that are persistent in the soil, making crop rotation difficult.

The most successful herbicide tolerance trait to be introduced so far enables plants to grow in the presence of a broad-range herbicide, glyphosate. The soybean variety known as RoundUp Ready, marketed by Monsanto, was the first to carry this trait (Padgett *et al.*, 1995). Glyphosate is relatively safe to use, does not persist long in the soil because it is broken down by microorganisms and is taken up through the foliage of a plant, so it is effective after the weeds have established. It is also relatively cheap. Its target is an enzyme called 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS). EPSPS catalyzes the formation of 5-enolpyruvylshikimate 3-phosphate (EPSP) from phosphoenolpyruvate (PEP) and shikimate 3-phosphate (S3P). This reaction is the penultimate step in the shikimate pathway (Figure 1.1.2), which results in the formation of chorismate, which in turn is required for the synthesis of many aromatic plant metabolites including the amino acids phenylalanine, tyrosine and tryptophan. The shikimate pathway is not present in animals, which have to acquire phenylalanine, tyrosine and tryptophan (referred to as essential amino acids) in their diet; this is the reason for glyphosate's low toxicity in animals. The gene that confers tolerance of the herbicide is from the soil

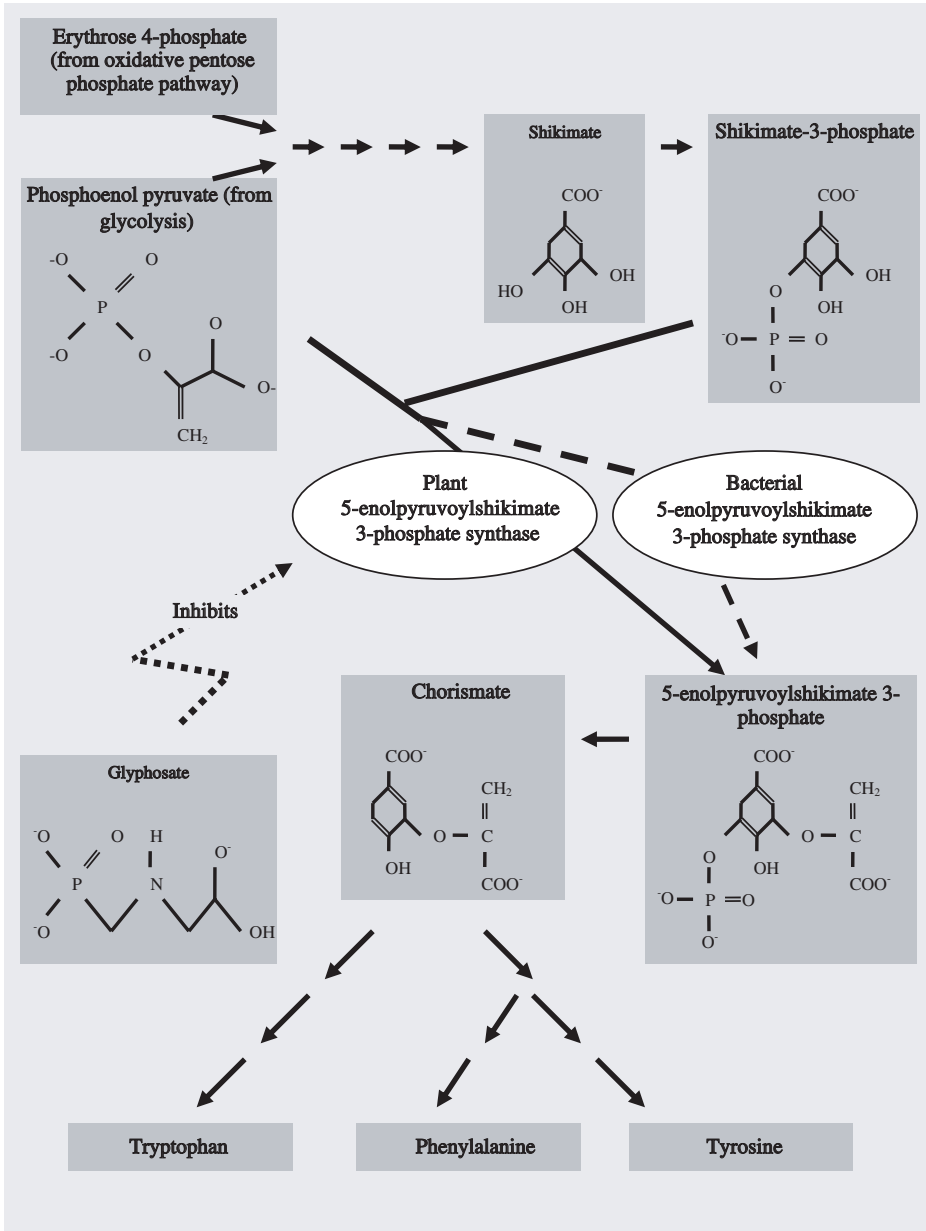


Figure 1.1.2 The action of glyphosate on the shikimate pathway.

bacterium *A. tumefaciens* and makes an EPSPS that is not affected by glyphosate. It has been introduced into commercial varieties of soybean, maize, cotton and oilseed rape, while glyphosate-tolerant varieties of many other crops, from wheat and sugar beet to onion, have been produced but not released yet.

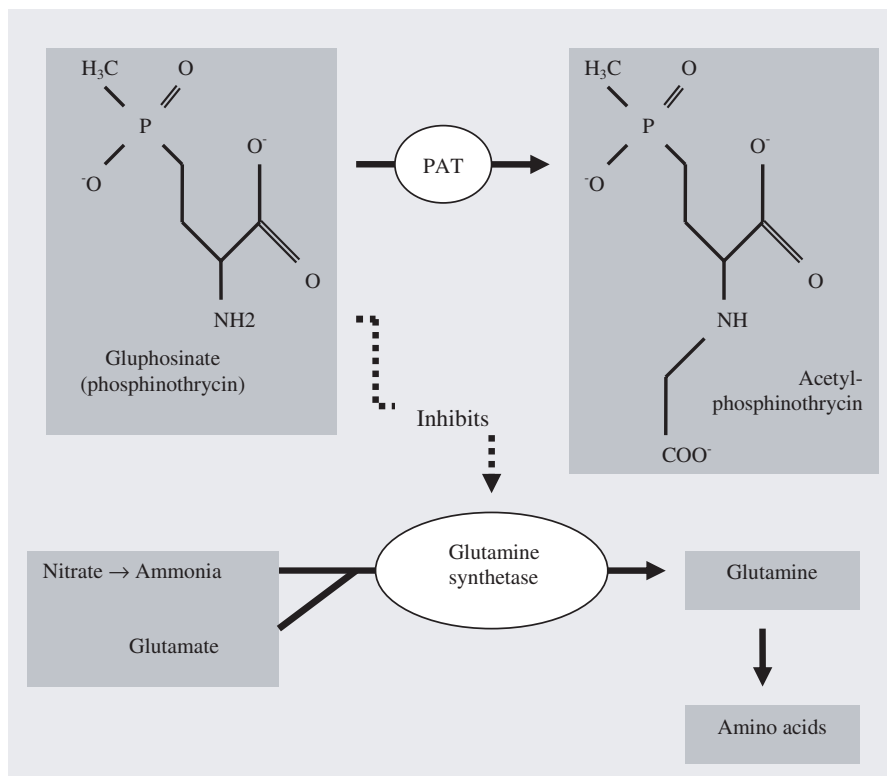


Figure 1.1.3 The action of glufosinate on amino acid synthesis and the detoxifying action of phosphinothricine acetyl transferase (PAT).

There are two other broad-range herbicide-tolerant GM systems in use, involving the herbicides glufosinate (or glufosinate) and bromoxynil, both marketed by Bayer. Glufosinate (Figure 1.1.3), the scientific name for which is phosphinothricin, is a competitive inhibitor of glutamine synthetase (GS), an enzyme required for the assimilation of nitrogen into the amino acid glutamine. The gene used to make plants resistant to glufosinate comes from the bacterium *Streptomyces hygroscopicus* and encodes phosphinothricine acetyl transferase (PAT), an enzyme that detoxifies the herbicide by converting phosphinothricin to acetylphosphinothricin (Figure 1.1.3) (Thompson *et al.*, 1987). Crop varieties carrying this trait include varieties of oilseed rape, maize, soybeans and cotton, and the trait has also been introduced into fodder beet and rice. The oilseed rape variety has been particularly successful in Canada.

The primary mode of action for bromoxynil (3,5-dibromo-4-hydroxybenzoxynitrile) is to inhibit photosynthesis by binding to the photosystem II complex of chloroplast membranes and blocking electron transport; tolerance is conferred by a gene isolated from the bacterium *Klebsiella pneumoniae ozanae*. This gene encodes for an enzyme called nitrilase, which converts bromoxynil into 3,5-dibromo-4-hydroxybenzoic acid, a

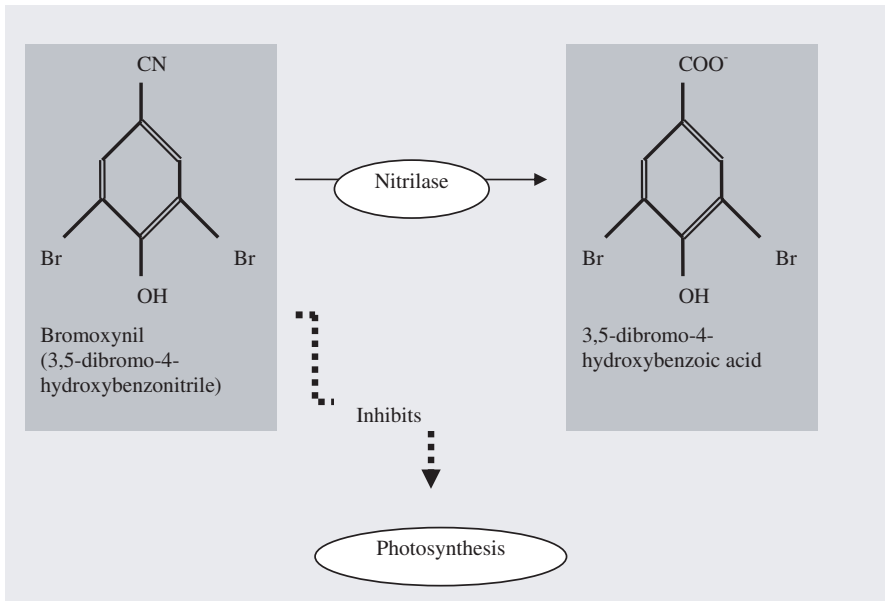


Figure 1.1.4 The detoxifying effect of nitrilase on bromoxynil.

non-toxic compound (Figure 1.1.4). So far this has only been used commercially in Canadian oilseed rape.

Interestingly there is a fourth broad-range herbicide tolerance trait available in commercial oilseed rape varieties in Canada. The herbicide in this case is imidazolinone and the varieties were produced by Pioneer Hi-Bred, now part of DuPont. However, the trait was produced by mutagenesis, not genetic modification.

Herbicide tolerance has now been engineered into many crop species and is undoubtedly the most successful GM trait to be used so far. In the USA in 2003, 81 % of the soybean crop, 59 % of the upland cotton and 15 % of the maize were herbicide tolerant (Benbrook, 2003). Herbicide-tolerant soybeans have been adopted even more enthusiastically in Argentina and now account for 95 % of the market, while herbicide-tolerant oilseed rape has taken 66 % of the market in Canada. This success is due to the factors such as simplified and safer weed control, reduced costs and more flexibility in crop rotation.

Insect Resistance

Organic and salad farmers have been using a pesticide based on a soil bacterium, *Bacillus thuringiensis*, for several decades. The bacterium produces a protein called the Cry (crystal) protein (often referred to now as the Bt protein); different strains of the bacterium produce different versions of the protein and these can be assigned to family groups, Cry1-40 (and counting), based on their similarity with each other. These families are further divided into subfamilies, Cry1A, B, C etc.

The Cry proteins are δ -endotoxins and they work by interacting with protein receptors in the membranes of cells in the insect gut. This interaction results in the cell membrane becoming leaky to cations, causing the cell to swell and burst. The interaction is very specific and different forms of the Cry protein affect different types of insects. Cry1 proteins, for example, are effective against the larvae of butterflies and moths, while Cry3 proteins are effective against beetles. The toxicity of all the Cry proteins to mammals, birds and fish is very low.

The fact that pesticides based on *B. thuringiensis* (Bt pesticides) had been used for a considerable length of time and had a good safety record, coupled with the fact that the insecticidal properties of the bacterium were imparted by a single protein, encoded by a single gene, made the Bt system an obvious target for adaptation for use in crop biotechnology. The first crop variety to carry the trait was a maize variety containing the *Cry1A* gene that was produced by Ciba-Geigy (now part of Syngenta) and first grown widely in 1996. Varieties of maize and cotton carrying the *Cry1* gene are also now marketed by Monsanto, Bayer, Mycogen and DeKalb. Aventis, subsequently acquired by Bayer, produced a maize variety called StarLink which carried the *Cry9C* variant, while Monsanto introduced the *Cry3A* variant into potato, marketing varieties carrying the trait as NewLeaf and NewLeaf Plus, the latter also carrying a gene for resistance to a virus (see below). Monsanto has also introduced the *Cry3B* variant into maize but this variety is not yet on the market. All these varieties are commonly referred to as Bt varieties.

The *Cry1A* and *Cry9C* proteins are effective against the European corn borer, a major pest of maize in some areas, while *Cry1A* is also effective against tobacco budworm, cotton bollworm and pink bollworm, three major pests of cotton. The *Cry3A* protein that was introduced into potato is effective against the Colorado beetle and the *Cry3B* protein against corn rootworm.

The benefits of using Bt varieties depend on many factors, most obviously the nature of the major insect pests in the area (not all are controlled by Bt) and the insect pressure in a given season. Bt varieties have been successful in many parts of the USA (in 2003, 29 % of the maize and 41 % of the upland cotton crop was Bt) and Bt cotton in particular is gaining ground in Australia, China, India and the Philippines. Farmers who use Bt varieties cite reduced insecticide use and/or increased yields as the major benefits (Gianessi *et al.*, 2002). A further, unexpected benefit of Bt maize varieties is that the Bt grain contains lower amounts of fungal toxins (mycotoxins) such as aflatoxin and fumicosin (Dowd, 2000).

Not all Bt varieties have been successful. NewLeaf and NewLeaf Plus potato were withdrawn in the USA due to reluctance to use them in the highly lucrative fast food industry. Farmers have adopted broad-range insecticides instead to combat the Colorado beetle. StarLink maize was an even more costly failure; it was not approved for human consumption because of doubts over the allergenicity of the *Cry9C* protein but, inexplicably given that maize is an outbreeding crop, the Environmental Protection Agency approved it for commercial cultivation for animal feed in 1998. Inevitably, cross-pollination occurred between StarLink and maize varieties destined for human consumption and StarLink had to be withdrawn.

Other approaches to engineering insect resistance into plants by genetic modification are being developed and tested but none have yet been used in a commercial crop variety. Many of the genes that are being used in these studies include those that encode

inhibitors of digestive enzymes, including trypsin, other proteases and α -amylase, and originate from a variety of plant sources. Although they occur naturally in many crop species, some are potentially toxic or allergenic to humans and their use in crop biotechnology may not be practical.

Similar reservations are held over another group of proteins that have insecticidal properties, the plant lectins. These proteins occur naturally in many kinds of beans, but most are toxic to animals, causing the clumping of erythrocytes, reduced growth, diarrhea, interference with nutrient absorption, pathological lesions and hemorrhages in the digestive tract, amongst other symptoms. However, not all lectins are toxic to animals and one such that retained its insecticidal properties would have potential in biotechnology.

Another group of proteins that are being investigated for their use in imparting insect resistance are the chitinases, enzymes that degrade chitin. Chitin is a polysaccharide present in fungal cell walls and chitinases are believed to have evolved as a defense against fungal attack. However, chitin is also present in the exoskeleton of insects, and although naturally occurring chitinases are not present in sufficient quantities to kill a grazing insect, it might be possible to increase their level by genetic modification to the point where they would cause lesions in the midgut membrane.

A concern with any strategy for engineering insect resistance into plants is the emergence of resistant insects. In the case of Bt this would not only nullify the advantage of using Bt crops but would also render spray-on Bt pesticides useless. Indeed, concern over resistance to Bt pre-dates the development of GM crops, but the rapid increase in the use of Bt corn and cotton in the USA from 1996 onwards necessitated action. The Environmental Protection Agency devised a solution in which farmers using Bt crops would have to plant a proportion of non-GM crop as well. This provides a refuge in which insects that have developed resistance to the effects of the Bt protein do not have a selective advantage over insects that have not (in fact they have a selective disadvantage). The proportion of non-GM crop that has to be grown varies according to what other insect-resistant GM crops are being grown in a particular area, and to prevent gene flow of the trait into wild species, Bt varieties cannot be grown where wild relatives occur (in the USA this affects cotton rather than maize). So far the refuge strategy appears to have been very successful in the USA but there is doubt as to whether every country that is growing or might grow Bt crops could enforce such a policy.

Another concern over the use of insect-resistant crops is their potential effect on non-target organisms. The obvious response to such concerns is that they are likely to have a beneficial effect by reducing the use of spray-on pesticides. There is plenty of anecdotal evidence, particularly from American cotton farmers, regarding this case, but it is difficult and expensive to undertake meaningful scientific experiments to confirm or contradict this. The largest field study on the effects of GM crops on biodiversity to be conducted so far was the United Kingdom's farm-scale evaluations program, but this concerned herbicide-tolerant not insect-resistant crops. Laboratory-based experiments are much less satisfactory and can give misleading results. One example of this was a study conducted by John Losey and his team at Cornell University and published in 'Nature' in 1999 (Losey *et al.*, 1999). Losey found that caterpillars of the monarch butterfly that were forced to eat large quantities of pollen from Bt maize suffered higher mortality levels than caterpillars that were not fed the pollen. In the wild, monarch

butterfly larvae eat milkweed, not maize pollen; in the experiment, pollen was spooned onto milkweed leaves so that the larvae had no choice but to eat it. Field-based studies subsequently showed that the larvae would never be exposed to such levels of maize pollen in the wild.

Similar laboratory-based experiments have shown that the survival rate of predator species such as lacewings and ladybirds can be reduced if they are fed exclusively on prey species that feed on GM insect-resistant plants. None of these results have been replicated in the field.

Virus Resistance

Virus resistance has been achieved using two methods; the first of these arose from studies on the phenomenon of cross protection, in which infection by a mild strain of a virus induces resistance to subsequent infection by a more virulent strain. Modifying a plant with a gene that encodes the viral coat protein has been found to mimic the phenomenon.

An example of the commercialization of this technology comes from the papaya industry in the Puna district of Hawaii (Ferreira *et al.*, 2002; Gonsalves, 1998). After an epidemic of papaya ringspot virus (PRSV) in the 1990s almost destroyed the industry, growers switched to a virus-resistant GM variety containing a gene that encodes a PRSV coat protein.

The second method used to impart virus resistance is to use antisense or co-suppression techniques to block the activity of viral genes when the virus infects a plant. The NewLeaf Plus potato variety discussed above, for example, carried a replicase gene from potato leaf roll virus (PLRV) in combination with the Bt insect-resistance trait. This technology is being applied to many other plant virus diseases and just one example of resistance being achieved, at least under trial conditions, is with potato tuber necrotic ringspot disease (Racman *et al.*, 2001). It has tremendous potential for developing countries where losses to viral diseases are the greatest and have the most severe consequences.

Modified Oil Content

The principle components of plant oils are fatty acids and the various properties of oils from different plants are determined by their differing fatty acid contents. Many hundreds of different fatty acids have been identified in plants, with diverse food and non-food uses. Lauric acid, for example, is used in cosmetics and detergents. Palmitic acid, stearic acid and oleic acid are used in foods, while γ -linolenic acid is used in health products. Erucic acid is poisonous but is used in the manufacture of plastics and lubricating oils.

GM crop varieties with modified oil content are already on the market in the USA. Calgene, subsequently taken over by Monsanto, genetically modified an oilseed rape variety to produce high levels of lauric acid in its oil. This variety was introduced onto the market in 1995. It contains a gene from the Californian Bay plant that encodes an enzyme that causes premature termination of growing fatty acid chains. The result is an accumulation of the 12-carbon chain lauric acid to approximately 40 % of the total oil content, compared with 0.1 % in unmodified oilseed rape. Lauric acid is a detergent traditionally derived from coconut or palm oil.

The other major crop that has been modified to increase the value of its oil is soybean. The GM variety was produced by PBI, a subsidiary of DuPont; it accumulates oleic acid, an 18-carbon chain fatty acid with a single unsaturated bond (a monounsaturate) to approximately 80 % of its total oil content, compared with approximately 20 % in non-GM varieties. In conventional soybean, relatively little oleic acid accumulates because it is converted to linoleic acid, an 18-carbon chain fatty acid with two double bonds (a polyunsaturate), by an enzyme called a Δ^{12} -desaturase. Some of the linoleic acid is further desaturated to linolenic acid, a polyunsaturate with three double bonds. In the GM variety, the activity of the gene producing this enzyme is reduced so that oleic acid levels are increased while linoleic and linolenic acid levels are decreased.

Oleic acid is very stable during frying and cooking, and is less prone to oxidation than polyunsaturated fats, making it less likely to form compounds that affect flavor. The traditional method of preventing polyunsaturated fat oxidation involves hydrogenation and this runs the risk of creating *trans*-fatty acids. *Trans*-fatty acids contain double bonds in a different orientation to the *cis*-fatty acids present in plant oils. They behave like saturated fat in raising blood cholesterol, contributing to blockage of arteries. The oil produced by high-oleic acid GM soybean requires less hydrogenation and there is less risk of *trans*-fatty acid formation.

Relatively small amounts of these GM oilseed rape and soybean varieties are grown on contract, but those farmers who can get into this business benefit from a premium price for their crop.

Current Status of GM Crops

Table 1.1.1 shows the global cultivation of GM varieties of the four major crops, soybean, maize, cotton and oilseed rape, for which GM varieties have been developed and commercialized. In 2003, the International Service for the Acquisition of Agri-biotech Applications (ISAAA) (www.isaaa.org) reported that GM crops were being grown commercially in 18 countries: Argentina, Australia, Brazil, Bulgaria, Canada, China, Colombia, Germany, Honduras, India, Indonesia, Mexico, Philippines, Romania, South Africa, Spain, Uruguay and the USA. Of these, Argentina, Brazil, Canada, China and the USA dominate in terms of total area (James, 2003).

Table 1.1.1 Global cultivation in 2003 of the four major crops for which GM varieties have been commercialized.

Crop	Global cultivation (million hectares)		Proportion of global crop GM (%)
	All varieties	GM varieties	
Soybean	76	41.40	54
Maize	140	15.50	11
Cotton	34	7.20	21
Oilseed rape	22	3.60	16
Total	275	67.70	25

Source: Food Standards Agency.

A remarkable feature of the global status of GM crops at present is the rapid and enthusiastic uptake of GM varieties in some countries and the lack of uptake of and resistance to GM crops in other countries, notably in Europe. The only significant use of GM crops in Europe at present is the cultivation of Bt maize in Spain. At the heart of the 'problem' for plant biotechnology in Europe is the hostile attitude of European consumers. This has led legislators at the European Union and national government level to introduce legislation to control the development and marketing of GM crops and foods, apparently in the hope that strict controls would reassure consumers. These controls are discussed in detail in Chapter 3.3. Briefly, any GM crop or food derived from it has to be approved for use within the European Union by the European Commission, and approval is extremely difficult to obtain. Furthermore, any food containing GM crop material above a threshold of 0.9 % has to be labeled, while novel foods produced in any other way need not. Unfortunately this legislation has undoubtedly deterred seed companies from developing GM crops for the European market but has so far failed to reassure consumers at all.

Exactly why European consumers have been so much more fearful of GM crops than other consumers is not clear. A recent poll showed that 66 % of consumers in China, Thailand and the Philippines believed that they would benefit personally from food biotechnology during the next 5 years. A different poll in the USA found that 71 % of US consumers would be likely to choose produce that had been enhanced through biotechnology to require fewer pesticide applications. Polls in the UK and Europe continue to show much less favorable attitudes amongst consumers.

Part of the answer lies in the reluctance of Europeans to trust their governments or scientific experts. GM foods were launched in Europe shortly after the epidemic of bovine spongiform encephalopathy (BSE) in the UK cattle herd had led to one of the biggest food scares in UK history. Rightly or wrongly, consumers felt that they had been given the wrong advice by scientists and government ministers on the safety of beef. However, food 'scares' are not unique to the UK and Europe.

Another reason for consumer antipathy towards GM crops in Europe is that the debate has been dominated by anti-GM pressure groups. European consumers have been bombarded with inaccurate information, half-truths and wild 'scare' stories. Even if they do not believe the more hysterical of these stories, why should they take the risk of buying GM food products?

The first imports of GM crop products into Europe began just before Christmas in 1996, with American soybean and maize, which at that time were approximately 2 % GM. American producers refused to segregate the GM from the non-GM and there was a flurry of media activity on the issue. This died down but Greenpeace, Friends of the Earth and other campaign groups had promoted the GM issue to the top of their list of campaign priorities (the title of a Greenpeace briefing pack in February 1997 was 'The end of the world as we know it') and it was only a matter of time before it returned to the top of the news agenda. It did, thanks to two scare stories originating from the legitimate scientific literature: the work of Dr Arpad Pusztai on feeding lectin-containing GM potatoes to rats (Ewen and Pusztai, 1999) and of John Losey on the effects of feeding monarch butterfly larvae on GM corn pollen (Losey *et al.*, 1999). Pusztai's paper was subsequently debunked by the Royal Society, while monarch butterfly larvae were found never to be exposed to the levels of maize pollen used in Losey's self-described

'preliminary' study. Incidentally, the monarch butterfly prospered after the introduction of GM insect-resistant corn and cotton into large areas of the USA in 1996, although it is now threatened by habitat destruction in its Mexican wintering sites. Despite this, I have been assured several times by different people in the UK that it is extinct as a result of the introduction of GM crops.

The GM crop debate has now become entangled with campaigns against capitalism, globalization and multinational companies, and spiraled out of the control of scientists to become a potent political issue. The only factor preventing the technology being lost to Europe now is the fact that GM crops are being used widely elsewhere in the world.

Conclusions

In this chapter genetic modification is described in context of a long history of plant breeding, which had become science-based long before genetic modification was invented. Genetic modification is now an established technique in plant breeding in many parts of the world. While not being a panacea, it does hold the promise of enabling plant breeders to improve crop plants in ways that they would not be able to through other methods. GM crops now represent approximately 6 % of world agriculture, and are being used in developed and developing countries. Farmers who use them report one or more of greater convenience, greater flexibility, simpler crop rotation, reduced spending on agrochemicals, greater yields or higher prices and increased profitability at the farm gate as the benefits.

The delay in allowing plant biotechnology to develop in Europe has already damaged the European plant biotechnology industry significantly and is putting European agriculture at an increasing competitive disadvantage. Europe desperately needs politicians and the food industry to show leadership on the issue, but there is little indication that they will. Powerful, multinational pressure groups continue to call the shots on GM crops and food in Europe, and these groups remain implacably opposed to the use of the technology. Despite this, it seems inconceivable that agricultural biotechnology will not continue and develop, at least outside Europe, given the success of GM crops and their popularity with farmers in those countries where farmers are allowed to use them.

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