Chapter 2

Genetically Engineered Plants

2.1 Terminology

The term biotechnology has been used broadly to describe all genetic modifications, particularly in the field of agriculture (Shandro, 2000). Genetic engineering is an application of biotechnology involving the transfer of a gene between species in order to encourage the desired trait. Plant genetic research in the last decade has focused on a large number of global agricultural challenges (Ghatnekar and Ghatnekar, 2000). Genetic transformation is the heritable change in a cell or an organism brought about by the insertion of foreign DNA into its genome (Agbios, 2001). With the help of gene technology scientists aim to introduce, enhance or delete particular characteristics of a living thing depending on whether the characteristics are considered desirable or undesirable (CSIRO Australia, 2000).

Plants, in which new pieces of DNA are introduced by means (or methods) other than sexual crossing, are referred to as genetically modified (GM), genetically enhanced or transgenic. The broadest definition of plant genetic engineering is changing the genetic make-up of plants to provide plants, plant products and processes for our needs. In this sense, plant genetic engineering has been around for a very long time (Boulter, 1997). A plant contains transgenes that have been artificially inserted instead of acquiring them through pollination is referred to as a transgenic plant. A transgene refers to a gene or group of foreign genes, from one organism that has been inserted into the genome of a different unrelated organism via biotechnology techniques (agbios, 2001).

Proponents argue that advances in this new technology can produce food with yields to feed the growing world population in the 21st century. Critics are concerned that this technology produces uncertainties about the long-term impact on the environment. A criterion by which the importance of this new technology might be evaluated is its contribution to solving or avoiding deleterious consequences of agricultural production practices. Soil erosion and agricultural residues in soil and water are two such problems, where a solution may in part be made possible with the addition of crop varieties designed to be genetically comparable with broad spectrum, high potency, environmentally safe herbicide (Goodman, 1987). Further, traditional corporations, which are among the main proponents of plant biotechnology, view transgenic crops as a way to reduce dependence on inputs such as pesticides and fertilizers (Altieri, 1999).

2.2 Plant engineering

2.2.1 Genetic engineering vs. traditional breeding

Genetic engineering clearly differs from traditional breeding in a number of ways. Traditional breeding relies primarily on selection, using a natural process of sexual and asexual reproduction. Genetic engineering utilizes a process of insertion of genetic material. This can be via the application of a bacterium as a gene vehicle, a gene gun or other direct gene introduction methods (Hansen, 2000). Traditional breeding has typically only recombined genetic traits within species and between related ones. In contrast, genetic engineering can move fully functional gene traits between completely different sorts of organisms (Regal, 1994). Because of the wide variety of genes available for transfer as transgene and the types of alterations that are possible by molecular techniques, gene technology is inherently different to traditional breeding methods (Rogers and Parks, 1995).

Further, one of the disadvantages of traditional breeding is that this process is extremely time consuming. It can take 10 to 12 years to bring new varieties to the market (Scandizzo, 2002). Also, breeders frequently face a situation in which a resistant gene is closely linked to a gene that adversely affects the quality of a crop that is where the two traits are always inherited together. For example, insect resistance in lettuce plants might be inherited along with a tendency for the lettuce to taste bitter (Designer seeds, 1998). Despite these disadvantages, traditional plant breeding will, however, continue to be a vital tool for improvement of plant crops complementing the strategies of genetic engineering (Keshun, 1999).

2.2.2 Genetic engineering techniques

The process of insertions of foreign genetic material via genetic engineering into the host plant genome and the expression of such material are called plant transformation (Hansen, 2000). This provides plants an opportunity for improving their usefulness (Murray, 1997).

For the last two decades there is much development in plant transformation. There are two main methods of transforming a plant cell or plant tissue. The direct gene transfer is obtained by electroporation, biolistics or micro-injection. The method of electroporation is based on the fact that electric pulses can open the cell membrane and allow penetration of alien DNA. Heat shock, in combination with electroporation, has been resulting in a higher efficiency of the transformation. The advantage of electroporation is that it can be applied to protoplasts in angiosperms. The chemical compound polyethyleneglycol changes the pore size of the cell membrane, which enhances the probability of an alien DNA molecule penetrating into the cell. This method has been used for transfer of DNA to monocots as well as dicots, including transfer of DNA to protoplasts. The biolistic approach is using the principle to shoot particles coated with DNA into selected tissues or cells by a particle gun. The particles may consist of either tungsten or gold carrying the DNA and the size may vary. This method is useful for both monocots and dicots (Simonson and Jorgensen, 1997).

DNA transfer can also be obtained by a biological vehicle such as *Agrobacterium spp.* This technique was the first one and has been used for almost 20 years to produce transgenic plants. Many plants are susceptible to this bacterium (Simonsen and Jorgensen, 1997). Within the last few years this Agrobacterium infection was also amended to allow DNA uptake by cereal tissue. *Agrobacterium tumefaciens* is a naturally occurring pathogenic bacterium in the soil that has the ability to transfer part of its tumor-inducing DNA (T-DNA) located on the *Ti* (tumor-inducing) plasmid into a plant genome, which is required for its survival (Figure 2.1). It can only infect a plant and transfer genes through wounds and causes a characteristic growth called crown gall tumor (Figure 2.2). Researchers have, however, disarmed this tumor-inducing part of DNA and have replaced this DNA with genes coding for useful characteristics (Designer seeds, 1998). During transformation the bacterium binds to a wounded plant cell at specific site. Phenolic compounds exuded by the plant cell activate the virulence genes that control the excision and export of the T-DNA segment from the bacteria to the plant cell. (Galun and Breiman, 1997). The most widely transferred traits so far by this technique are genes coding for resistance to herbicides, pests and pathogens (Dunwell, 1998).

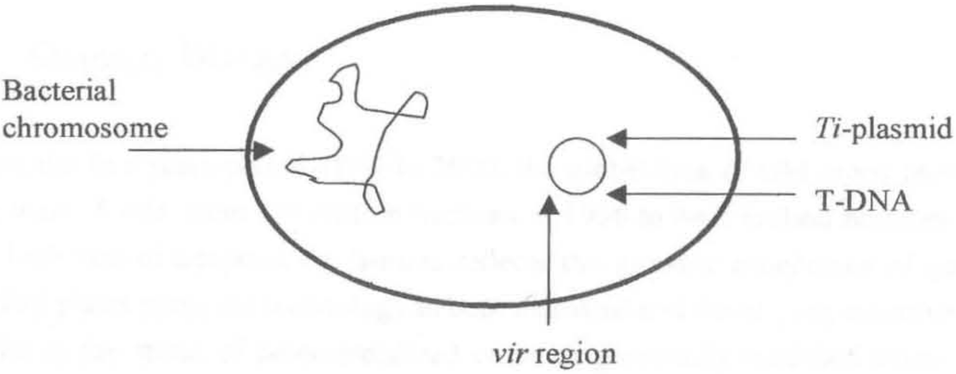

Figure 2.1: *Agrobacterium tumefaciens* cell with *Ti*-plasmid containing T (transfer)-DNA, which is transferred into the plant genome and *vir* (virulence) region, which is required for T-DNA transfer and genome integration.

Figure 2.2: Crown gall tumor on plant. Crown gall disease, which is caused by *Agrobacterium tumefaciens*, produces a tumor-like growth on stems of susceptible plants such as *Kalanchoe* (Source: Designer seeds, 1998).

2.3 Growth of GM crops

During the five years period 1996 to 2000, the global area of GM crops increased by more than 25-fold, from 1.7 million hectares in 1996 to 44.2 million hectares in 2000. This high rate of adoption by farmers reflects the growing acceptance of genetically modified plants using the technology in both industrial and developing countries. Figure 2.3 shows the status of commercialized crops of genetically modified plants in 2000 (James, 2000) and the total acreage has been estimated having increased by 11% when compared to 1999.

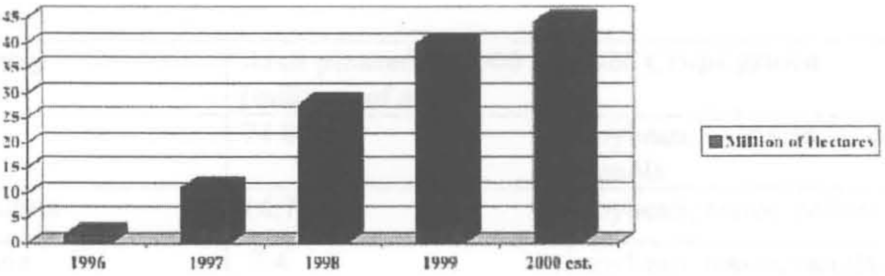

Figure 2.3: The worldwide areas of GM crops over a five-year period. The estimated global area of genetically modified crops for 2000 was 44.2 million hectares.

In 2000, 13 countries grew GM crops (Table 2.1) but four countries had 99% of the global GM crop area. These were the US, Canada, Argentina and China. Among these, the US had the largest GM crop area with 30.3 million hectares followed by Argentina with 10 million hectares, Canada with 3 million and China with 0.5 million hectares. In 2000, herbicide-resistant GM soya occupied 58% (25.8 million hectares) of the global area of GM crops. GM maize followed with 10.3 million hectares, GM cotton covered 5.3 million hectares and GM canola covered 2.8 million hectares. Figure 2.4 shows that out of total worldwide planting, GM soya accounts for 36%, GM cotton for 16%, GM canola for 11% and GM maize accounts for 7% of the total respective crop planting.

Table 2.1: GM crops grown and production area in 2000 (James, 2000).

Country	**Area planted in 2000 (millions of acres)**	**GM Crops grown**
USA	74.8	soybean, maize, cotton, canola
Argentina	14.7	soybean, maize, cotton
Canada	7.4	soybean, maize, canola
China	1.2	cotton
South Africa	**0.5**	**maize, cotton**
Australia	0.4	cotton
Mexico	minor	cotton
Bulgaria	minor	maize
Romania	minor	soybean, potato
Spain	minor	maize
Germany	minor	maize
France	minor	maize
Uruguay	minor	soybean

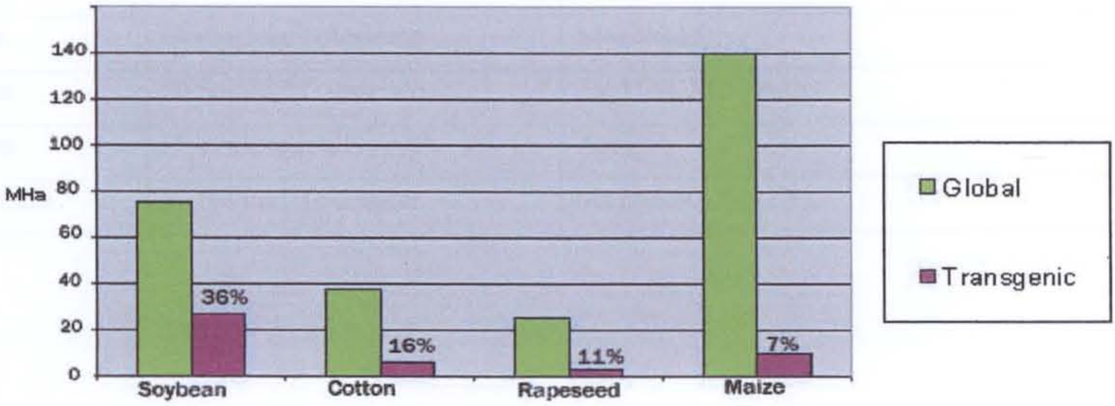

Figure 2.4: Growth of GM crops (Transgenic) in million hectares (M ha) in comparison to total worldwide growth (M ha) of four important crops - soybean, cotton, rapeseed (canola) and maize.

2.4 Traits of GM crops

Plant biotechnology has the capacity to create a greater variety of commercial plants. Genes from sexually incompatible plants or from animals, bacteria or insects can now be introduced into plants. The first transgenic plant, a tobacco plant resistant to an antibiotic, was created in 1983 (BBC News, 1999). Others that followed include Calgene's *Flavr Savr* tomato, a variety of virus- and herbicide resistant crop plants and insect resistant cotton and maize expressing a Bt (*Bacillus thuringiensis*) toxin (Table 2.2). Currently, the major GM crops are soya, canola, cotton and maize (Keshun, 1999).

Table 2.2: Commercially available GM crops.

Crop	**Trait**	**Developed by:**
Soya	Herbicide Tolerance	Monsanto, AgrEvo
Soya	Modified Oils	Monsanto, DuPont
Maize	Herbicide Tolerance	Monsanto, AgrEvo
Maize	Hybridization System	AgrEvo
Maize	Insect Resistance	Monsanto, Novartis, AgrEvo
Potato	Insect and Virus Resistance	Monsanto
Cotton	Insect Resistance	Monsanto, AgrEvo, Dow AgroSciences
Cotton	Herbicide Tolerance	Monsanto
Canola	Herbicide Tolerance	AgrEvo, Monsanto
Canola	Hybridization System	AgrEvo
Sugarbeet	Herbicide Tolerance	Monsanto, AgrEvo

Based on the major outcomes of the traits under modification, GM crops fall into two major categories: those with improved agronomic traits and those with altered quality traits (Table 2.3). Herbicide-tolerant crops and Bt-based insect protected crops are best examples of improved agronomic traits (Keshun, 1999). Wilkinson (1997) indicated that about one third of GM crops approved or under review by regulatory agencies for commercialization are with improved agronomic traits including those with tolerance to a broad-spectrum of herbicides. Farmers are usually increasing their acreage of herbicide-resistant crops, because the use of those plants reduces the need to plough, decreases the amount of herbicides and can deliver a cleaner and higher grade of grain and product (Vogt and Parish, 1999).

Table 2.3: Possible benefits of crops with improved agronomic and quality traits.

Crops with improved agronomic traits	**Crops with improved quality traits**
Often reduced use of agrochemicals	Offering new ways to produce raw materials or ingredients
Offering better pest-control tools	Providing healthier food
Achieving high yield potential	Offering superior functionality
Improving crop production efficiency	By-pass processing
Promoting conservation tillage techniques	Facilitate processing
Creating crops which are less harmful to the environment	Offering products with better flavour, colour and taste
Contributing to sustainability of global agriculture	Offering products with longer shelf life
Increasing the world food supply	Improved end-use value

2.4.1 GM crops and herbicide resistance

For an economically superior weed control with higher labour and energy efficiency than the manual or mechanical cultivation methods, herbicides are an indispensable tool of modern agriculture and they are also the targets for GM crops to increase their efficiency. Herbicides are substances that kill plants especially weeds by inducing numerous changes in the growth of plants and their structure. Herbicides reduce manual and mechanical weeding and can also prevent soil erosion. In dry land agriculture, effective herbicidal control ensures higher water availability to the crops and less crop failure due to drought

(Syngenta, 2002). Herbicides may affect the entire plant or only alter particular organs. Presently, different types of herbicides are available. This includes herbicides with either a broad spectrum of action killing crops and weeds indiscriminately (Simonsen and Jorgensen, 1997) or being selective having no or only minor effects on crops. Herbicides might be applied before a crop is planted or when the crop is starting to grow, some herbicides work through the root system of the plants, others through its leaves, some kill the plant on contact, others translocate within the plant.

Various reasons motivated scientists to develop herbicide-resistant GM crops (Le Baron, 1987). This includes:

- Farmers can use the presently available herbicides on additional crops.
- There is a decline in the number of herbicides developed commercially.
- It is time consuming and expensive to develop new herbicides.
- Crops resistant or tolerant to herbicides having long soil residual could be rotated without fear of carry-over injury from the herbicides.
- Weed scientists and plant physiologists have determined the mechanism of action for most herbicides, which is essential before genetic engineering for herbicide selectivity is possible.

The availability of herbicide-resistant GM crops might help farmers to control weeds to a greater extent. But it requires a great deal of administration on the part of farmers (Marshall, 1997). Most common possible risks involved in the introduction of herbicide-resistant GM crops are:

- The potential for over-use of herbicides.
- The risk that herbicide resistance might spread from a transgenic crop to weed species
- The potential for long-term impact on the environment including herbicide residues in soil and water.
- Increased selection for herbicide-resistant weeds by greater reliance on a single herbicide.

- Opportunities for multi-national companies to monopolize the sale of herbicide-resistant GM crop seeds and herbicides through international patents.
- The safety of food harvested from GM crops.

To improve the selectivity of herbicides genes have been identified that express proteins detoxifying herbicides to give crops a selective advantage in the field upon spraying (Stalker, 1989; Andel *et al.*, 1997). Much effort has been put into developing GM crops with tolerance to the non-selective herbicide glyphosate (Figure 2.5). The herbicide glyphosate (phosphonomethyl glycine), which is commonly known as 'Roundup', is one of the most widely used weed control agents today in both developed and developing countries. The herbicide is non-selective and is used in a wide range of applications for total weed control (Ray, 1989). Target of the herbicide is the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), which is naturally found in all plants, fungi and bacteria but is absent in animals. The enzyme is an important catalyst in the biochemical pathway for synthesis of the aromatic amino acids phenylalanine, tryptophan and tyrosine (Felsot, 2000). Glyphosate kills plant cells due to the inhibition of the enzyme EPSPS. In GM crops, EPSPS is greatly overproduced due to the insertion of an extra exogenous EPSPS gene into the crop plant allowing the modified plant to survive the herbicide action. However, Glyphosate have no detrimental effect on bacteria and fungi (Carpenter *et al.*, 2002; Busse *et al.*, 2001).

As the metabolic paths of plants differ from animals, glyphosate can be considered as rather safe to humans. Tests have also shown that glyphosate when used according to label directions has no weed killing activity once in contact with the soil. Glyphosate will not move in or on the soil to affect non-target vegetation and it does not move through the soil to enter other non-target plants by the root system. Glyphosate is only effective when it comes into contact with the green growing parts (Annual Report of Monsanto, 2001).

Figure 2.5: Action of the herbicide glyphosate. GM glyphosate-resistant canola surrounded by weeds (left). After spraying of glyphosate only GM canola survives (right; photo courtesy of Monsanto Co.; Source: Virginia Cooperative Extension, 2001).

2.4.2 GM crops and insect resistance

Bt crops are resistant to certain insect pests due to the transfer of a gene to plants from a natural soil bacterium. Bacillus thuringiensis (Bt), which is a common soil bacterium, was first isolated in the Thuringia region of Germany (Cropbiotechnet, 2002). This bacterium has the ability to produce a crystal like protein (delta endotoxin), which can selectively kill Lepidopteran insects. Once the cry protein is eaten, the digestive system of insects activates the toxic form of the protein that can kill the insect within a few days. (University of Minnesota, 1997).

Bt toxins are currently the most widely used naturally occurring agricultural pesticide. These organic insecticides are safer and more benign than the chemical pesticide they are replacing and are used extensively both by organic farmers and in transgenic crops to provide safe protection against insect pests (Aroian, 2001). Also due to its target specificity and as it is not synthetic, the organic industry relies heavily on Bt.

Environmental Protection Agency (EPA) in USA classifies Bt pesticides as toxicity III-slightly toxic the same rating as glyphosate (Nelson and Pinto, 2001).

There might be several advantages of using a Bt crop, which includes:

- Improved pest management.
- Reduction in insecticide use.
- Greater yield.
- Improved conditions for non-target organisms.

In 2001, an estimated 12 millions ha of land were planted with crops containing the Bt gene. Table 2.4 shows countries that have commercialized Bt cotton and/or Bt maize.

Table 2.4 Countries that have commercialised Bt cotton and or Bt maize (Source: CropBiotechNet, 2002).

CROP	COUNTRY
Cotton	Argentina Australia China India Indonesia Mexico South Africa United States
Maize	Argentina Canada European Union South Africa United States

Many non-agrochemical organization scientists and farmers around the world are opposed to the use of Bt crops for a variety of reasons including:

- Bt-spray may lose its value as an effective pest management tool if Bt crops are planted extensively. Organic farmers used Bt spray as an effective pesticide control measure for a long time. It has the advantage of Bt toxin breaks down rapidly in the environment and also does not persist in water. Now, because of widespread use of Bt crops in which the Bt does not break down and there is increased exposure to the toxin, insect resistance could develop and this valuable pest management tool could be lost.
- Bt crops may pose serious long-term risks to monarch butterflies.
- Bt crops can contaminate organic and conventional crops and results in loss of bio-diversity
- Bt crops may cause allergic reactions (Pesticide action network update, 2001).

Usage of Bt crops identifies two types of potential risks.

- The development of insect resistance
- The risk to non-target insects (OSTP, 2001)

The lifespan of the Bt product would be significantly shortened if long-term exposure to such insecticide crops were to intensify selection pressure for resistant insects. Insect resistance might also reduce the future utility of naturally occurring microbial Bt. (Levidow *et al.*, 1999).

In order to prevent or mitigate adverse effects, these risk scenarios require:

- Proper monitoring of insect resistance
- Implementation of resistance management plans and
- Examination of potential non-target influence on insects inhabiting the area of Bt maize planting (OSTP, 2001).

There are a variety of insect resistant management (IRM) practices designed to reduce the potential for insect pests to become resistant to a pesticide. The threat that insect resistance presents to the future use of Bt plant pesticide and Bt technology as a whole is a major reason Bt IRM is of great importance. Specific IRM strategies such as the high-dose/ structural refuge strategy will diminish insect resistance to specific Bt proteins produced in maize, cotton and potatoes (Biopesticides registration action document, 2000).

The potential for toxicity to non-target organisms is another area where adverse effects could occur. A Study by Losey *et al.* (1999) shows that Bt-maize pollen can be toxic to monarch larvae when present in significant amounts. Further studies have confirmed that the risk to monarch butterflies from Bt-maize pollen is extremely low (Hansen and Obrycki, 2000).