



Chapter 4

Risk of GM Crops

4.1 Types of risk

The benefits and risks of genetically engineered plants vary with the specific crop and trait. Even for a specific GM crop, any assessment could vary dramatically from one geographic area to another. Any risk by genetic engineering must be further evaluated in the context of the risk involved in current agricultural practices. Further, rigorous scientific studies are also conducted and reviewed by three agencies of the federal government (Environmental Protection Agency, Food and Drug Administration and Department of Agriculture) before products are commercialized in the US (James and Krattiger, 1996). These testing procedures meet or exceed global standards developed by an international panel of experts formed by the World Health Organization.

4.1.1 Bt crops

Laboratory studies have shown that expression of the Bt gene can affect caterpillars of the monarch butterflies (Losey *et al.*, 1999) when they consume large quantities of Bt corn pollen blown onto milkweeds around maize fields on which the insect is feeding. However, the chance of high amounts of Bt corn pollen settling on milkweeds is rather small (CSIRO Australia, 2000). Another concern is about the development of pest resistance to Bt toxin. The widespread planting of transgenic crops containing the Bt gene will accelerate the development of Bt resistance in pest populations (Gould, 1994). During a study in 1997 in Arizona, scientists projected an increase in resistance to Bt cotton in pink bollworm. In this study, the frequency of a resistant gene in the pink bollworm was about 1 in 10, which was roughly 100-times higher than estimated when compared to other pests of Bt crops. Subsequent studies proved that the estimated

frequency of resistance did not increase from 1997 to 1999 (McGinley, 2000). A first strategy to manage insect's resistance includes the planting of 'refuge' or non- Bt crops near Bt cotton, to provide a source of non-resistant target species (in this case *Helicoverpa*) to prevent domination by a non-resistant population. The second strategy is 'pyramiding' which involves creating genetically modified plants with genes of two different toxins. This avoids the risk of loss of single gene resistance (Skerritt, 2000).

A Cornell University study further showed that pollen from Bt maize (altered to contain an insect-killing protein from *Bacillus thuringiensis*) could kill monarch butterfly larvae (Losey *et al.*, 1999). This simple laboratory study would be significant - if monarch larvae eat maize pollen, which they don't. Even dusting of their only food source, milkweed, is unlikely because:

- Larvae may not be present when maize is pollinating.
- Few milkweeds are in and near maize fields.

Bt, which is specific to target pests and harmless to birds, mammals and most other insects, is generally far less risky to monarchs and other beneficial insects than alternative chemical control measures.

A Swiss study also showed that Bt maize could harm beneficial insects including lacewings (Hilbeck *et al.*, 1998a). In this laboratory study, lacewing larvae were fed nothing else but European corn borer larvae, which are killed when they eat Bt maize. For about 21 days, one group of lacewings ate corn borer larvae. However, a large percentage of the lacewings that ate corn borers not subjected to Bt maize also died. They were possibly sick from eating only corn borers, as corn borer larvae are only a minor part of lacewings diet. Interestingly, field studies show that lacewings and other beneficial insects thrive in Bt fields, much better than in fields sprayed with insecticides (Milloy, 1999).

4.1.2 Herbicide-resistant crops

Typical risks for engineered herbicide-resistant crops are shown in Figure 4.1 (Altieri, 1999; Wolfenbarger and Phifer, 2000; Marshall, 1997)

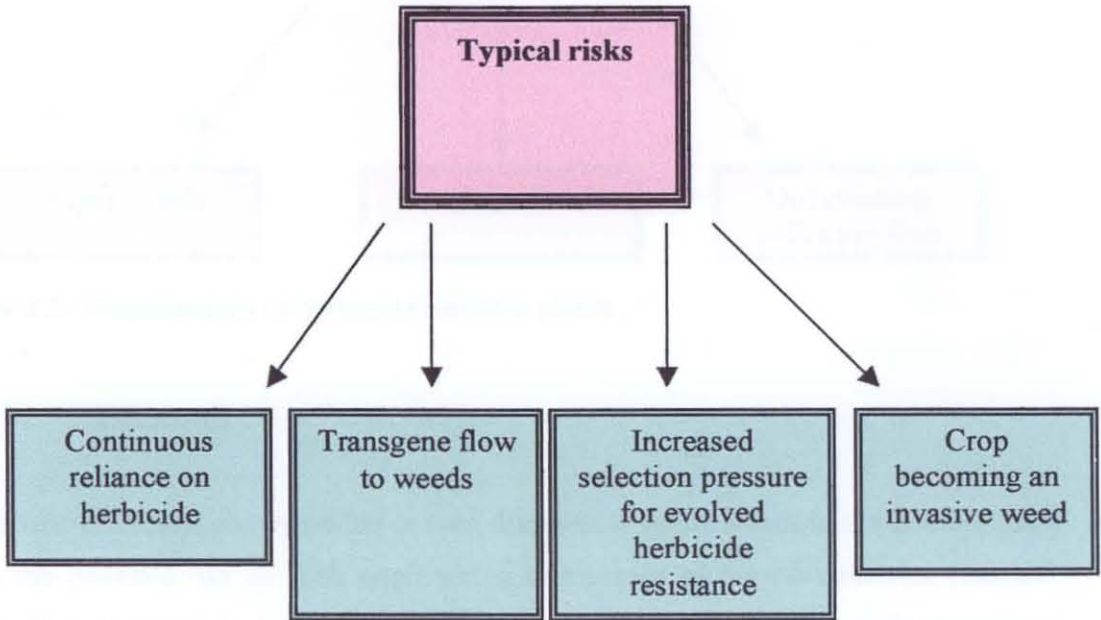


Figure 4.1: Typical risks of herbicide resistant crops. These risks have the potential to offset the prolonged benefits associated with a particular genetically modified crop.

Without doubt, the greatest risk of GM crops is, however, the transgene flow to weedy relatives. The likelihood that a transgene, which increases fitness of a plant, would be accidentally transferred to sexually compatible weeds depends on the degree to which out-crossing can occur in the crop species, and whether there are weedy relatives in the vicinity (FAO conference, 2002). Engineered *Brassica* species, as typical out-crossers, would pose a greater risk than for example engineered soybean, which is almost entirely self-pollinated, or engineered maize, which is grown near a wild relative only in Mexico (Stewart *et al.*, 2002). Possible risks for herbicide-resistant plants are outlined in Figure 4.2.

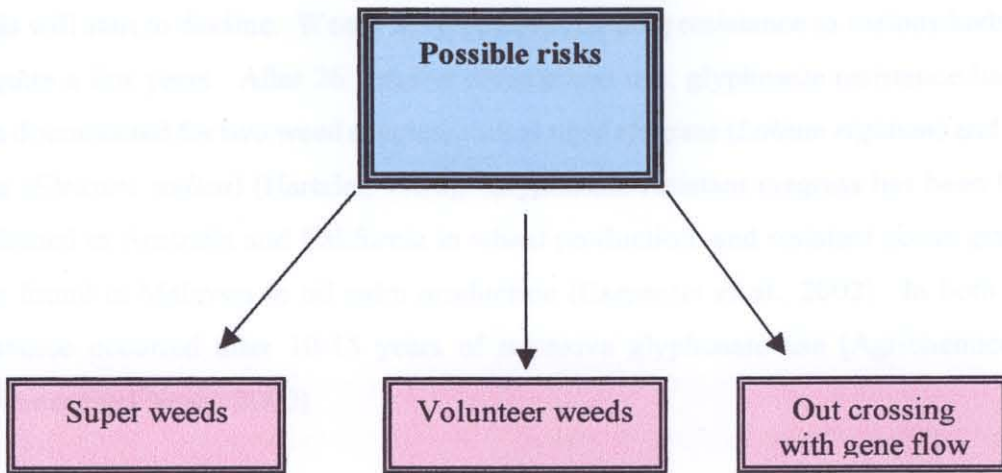


Figure 4.2: Possible risks of herbicide resistant plants.

4.1.2.1 Super-weeds

The escape of a transgene by pollen or seed dispersal creating weeds has raised concerns about the possible risk of such engineering technology to the environment (Daniell, 1999). The major risk is that large-scale release of GM crops may promote the transfer of a transgene from crops to wild species by sexual hybridization and the hybrid may become a “super weed” (Raybould and Gray, 1994). When a single herbicide is used repeatedly on a crop, the chances of herbicide resistance developing in a weed population greatly increases. The increased herbicide use may lead to reduction in crop yield as two kinds of plant competition occur with the increased use (Altieri, 1999). The genetic engineering of crops that are resistant to herbicides might enable the widespread use of these herbicides without concern for the stage of the crop growing cycle. But these herbicides, although considered to be environmentally safe, would no longer be effective against weeds that had captured a transgene for herbicide resistance, leading to the use of more dangerous chemicals. These herbicide resistant crops could cause the creation of “super-weeds” (Ellstrand and Hoffman, 1990). Other crops would thus be forced to compete with these new super-weeds. For example, populations of annual ryegrass (*Lolium rigidum*) have been found in Australia, which are resistant to herbicides from ten



different chemical classes (Rogers and Parkes, 1995). As crops are forced to compete with new super-weeds that no longer succumb to the usual herbicide treatment their yields will start to decline. Weeds have been developing resistance to various herbicides for quite a few years. After 26 years of commercial use, glyphosate resistance has only been documented for two weed species, annual rigid ryegrass (*Lolium rigidum*) and goose grass (*Eleusine indica*) (Hartzler, 1998). Glyphosate-resistant ryegrass has been further confirmed in Australia and California in wheat production, and resistant goose grass has been found in Malaysia in oil palm production (Carpenter *et al.*, 2002). In both cases, resistance occurred after 10-15 years of intensive glyphosate use (Agrichemical and Environmental News, 2000)

It has been found that genetically modified herbicide-tolerant oilseed rape pose a potential risk of spreading herbicide-tolerant genes to other rape crops or to weedy relatives (Squire *et al.*, 1997). Oilseed rape belongs to the *cruciferae* family, is indigenous to Western Europe and is one of the most problematic crops concerning gene flow from the crop to weedy relatives (Kapteijns, 1993). For example, of the 160 species of *Brassicaceae* present in Australia, several species are important weeds of the Southern Australian cropping zone with overlapping flowering time. Among these weeds are *B. rapa*, *B. juncea*, *B. tournefortii*, *diplotaxis tenuifolia* (Lincoln weed) and *Raphanus raphanistrum* (wild radish) (Virtue, 1996). *B. rapus* is derived from the cross between *B. rapa* and *B. oleracea*. *B. juncea*, is a hybrid between *B. rapa* and *B. nigra* and is generally thought to have originated in the Middle East (Reiger *et al.*, 1999). Inadvertent hybridization could generate persistent herbicide-tolerant weeds that would limit the efficiency of the herbicide in rape. Such scenarios could adversely affect overall herbicide usage or could preclude options that are environmentally preferable. Transgene flow can very likely occur in rape, because viable pollen can travel up to 2 km (Timmons *et al.*, 1996). Volunteer rape has, for example, a constant flux with feral rape outside the field, via a flow of pollen and of seeds (Squire *et al.*, 1997). When interspecies hybridization was tested in field experiments studying the initial hybrid and then back crosses with the weed, fertile, weed-like plants were found after just two generations of crosses between *Brassica napus* and *Brassica campestris/rapa* (Jorgensen *et al.*, 1996;

Mikkelsen *et al.*, 1996). When GM oilseed rape with tolerance to three different herbicides was cultivated in close proximity, some progeny had multiple herbicide tolerance (Reboud *et al.*, 1998).

4.1.2.2 Volunteer-weeds

The seeds remaining in the soil from the previous season's herbicide-resistant GM crop might germinate the following year in rotational crops. If these "volunteer-weeds" are resistant to herbicides, which are used on the new crop, competition may become a critical yield-limiting factor, because crop yield is dependent on the plants not growing too close together (Rautenberg, 1998). Volunteer crops are already considered to cause significant problems in weed control. Careful considerations must be given to the herbicide/crop combinations sold in a particular country. For example, volunteer potatoes are particularly troublesome in the UK, for which the herbicide glyphosate is a valuable herbicide (Marshall, 1997). However, if glyphosate-tolerant engineered potatoes would be introduced to the UK, their volunteers would undoubtedly become a serious weed problem, given that there are no satisfactory alternative herbicides for their control (Marshall, 1997). The widespread use of herbicide resistant crops is also likely to increase herbicide use, as they have to control the engineered super and volunteer-weeds.

4.1.2.3 Out-crossing

4.1.2.3.1 Gene flow

As gene flow is a natural process, it is important to improve our understanding of this phenomenon, and modern biotechnology is helping make research into gene flow more accurate and informative. It is the movement of gene mediated by pollen flow and seed dispersal (Rieger *et al.*, 1999) and this is a major process determining the genetic structure of a plant population. Pollen will be the most important vehicle for the escape of transgenes (Rongnli *et al.*, 2000). The difference between gene flow either by pollen or seeds is that seeds usually only transfer cytoplasmic inherited genes (Raamsdonk *et al.*,



1997). The gene transfer is further divided into vertical gene transfer and horizontal gene transfer. Vertical gene transfer is the sexual transfer of genes between two genetic different entities e.g.: two distinct populations of two species. Hybridization action through out-crossing is needed for a successful gene transfer. Out-crossing is thus a prerequisite for gene transfer. Horizontal gene transfer refers to non-sexual gene-transfer among organisms, which may belong to unrelated systematic groups e.g.: gene transfer between higher plants and microbes (Kjellsson *et al.*, 1997). Gene flow within and between populations has an important role in maintaining population genetic structure, enabling adaptation to changing environmental circumstances and reducing vulnerability to evolutionary hazards, such as inbreeding depression and genetic drift (Rieger *et al.*, 1999).

There are various methods used to measure pollen flow. These methods can be classified under two main groups, which are “Indirect” and “Direct” (Real, 1983). Due to the fact that pollen reaches a target from any direction, determining the movements of pollen grains from specific sources is difficult. In the first indirect method, dyes or powders are placed on the anthers and then after a period of pollination activity, surrounding plants are searched for the marker (Real, 1983). Further, indirect methods are secondly where chemicals are introduced into or within the pollen grains. Then after the pollen disperses, surrounding plants are searched for the labeled grains. The movement of all-potential pollinators is observed in a third method so that their behavior and movement patterns can be observed when in contact with pollen (Real, 1983).

As direct methods artificial samplers have been used to understand pollen flight dynamics for forest trees and for wind pollinated crop and weed stands (Ogden *et al.*, 1974). The accuracy and efficiency of these samplers depends on the mechanical design especially on the speed of the air as it passes through the collector and the size and shape of the collecting surface (Ogden *et al.*, 1974). A second method includes the use of stigmas, where counting of pollen grains eliminates the possible sources of error that the mechanical devices create (Real, 1983).



Gene flow is not reserved strictly for GM crops as gene flow has occurred since crop domestication. Gene flow will occur if there are wild relatives in the vicinity of the crop plant (Carpenter *et al.*, 2002). Sorghum and sunflowers have a greater likelihood for cross-pollination with weedy relatives, when these weeds exist where the crop is grown. Should a sorghum or sunflower plant derive through application of genetic engineering, the regulatory review process would require consideration of the potential impact on weediness of wild or weedy relatives (Council for biotechnology information, 2001). The potential for gene flow between weedy species and a related GM crop is a key component of the risk assessment required by regulatory agencies for every new plant variety developed through biotechnology.

In the US and Canada, crops, such as corn and soybeans, do not have wild relatives nearby. Gene flow is therefore limited to neighboring cultivated plants within the same field or to nearby fields. The likelihood of gene flow diminishes the further apart plants are located, even if they are in the same field or region. For some crops, such as soybeans, pollination characteristics limit gene exchange even between neighboring plants in the same field. For others, such as sorghum and sunflowers, more attention must be given to cross-pollination with weedy relatives.

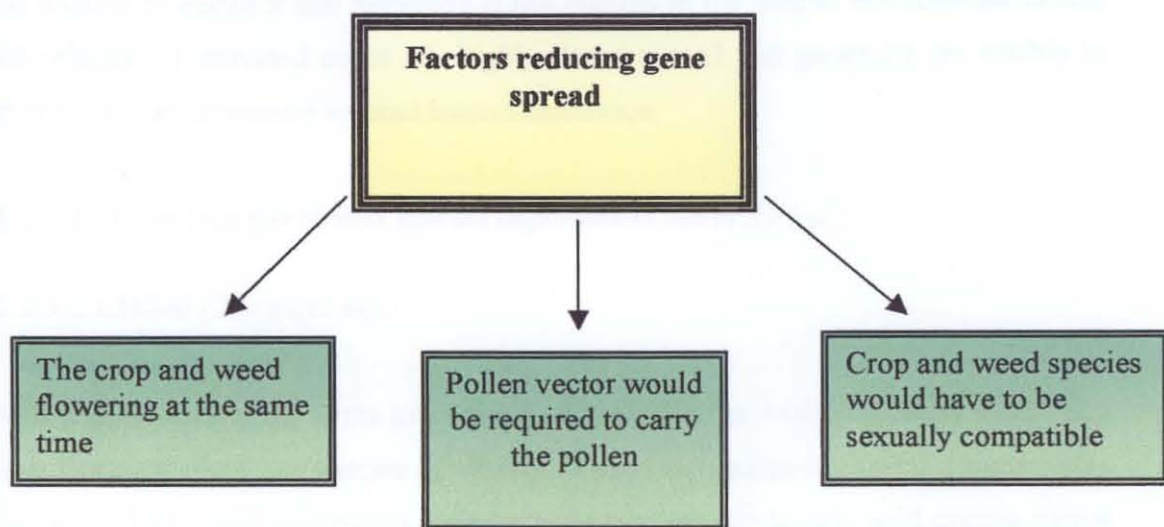


Figure 4.3: Factors reducing gene spread.

The chance of a gene spread is greatly reduced by some factors (Figure 4.3). This includes that (1) the crop and weed would have to flower at the same time, (2) a pollen vector, such as insects or wind, would be required to carry the pollen and (3) the crop and weed species would need to be sexually compatible and capable of producing fertile progeny weed plants. If there is no sexual compatibility between plants, there can be no gene flow - just as a dog cannot successfully mate with a cat. The pollinating characteristics of the particular plant species are important as well.

Some crops, such as maize, are cross-pollinators and can exchange genes relatively easily with other maize plants or with wild relatives under appropriate conditions. Gene flow in self-pollinators, such as wheat and soybeans, occurs infrequently. In addition, the crop must be grown in an area where a wild relative is native. For example, there are no wild relatives of maize or soybean, two of the most widely planted crops, in the US. Gene flow from these crops into wild populations, therefore, does not occur (Rissler and Mellon, 1993). Also, there must be a benefit associated with the gene of interest in order for it to persist. Genetic modifications must increase a plant's ability to survive and reproduce in order for any gene to be actively selected and preserved over generations.

There is little selective advantage to having a trait that confers herbicide-tolerance in a wild relative of maize if that herbicide is not present in the natural environment of that wild relative. Cultivated crops are highly domesticated and generally are unable to survive in the environment without human assistance.

4.1.2.3.2 *Gene transfer to wild species important to South Africa*

4.1.2.3.2.1 *Maize (Zea mays ssp.)*

Zea is a genus belonging to the grass family *Poaceae* in the *Andropogoneae* tribe. The genus *Zea* consists of four species of which *Zea mays* ssp (maize or corn) is economically important. The other *Zea* species, referred to as teosinte, are largely wild grasses native to Mexico and Central America (Doebley, 1990). Teosinte species show little tendency to spread beyond their natural range and distribution is restricted to North, Central and South America. The nearest teosinte relative to *Z. mays* ssp is *Z. mays* ssp. *Mexicana* Iltis (previously classified as *Euchlaena mexicana*, *Zea mexicana*) ($2n = 20$) has limited use as a forage and green fodder crop, but can be problematic due to weedy tendencies (Doebley, 1990). This central Mexican annual teosinte is large flowered; mostly weedy with a broad distribution across the central highlands of Mexico.

Gene exchange between cultivated and genetically engineered maize occurs naturally at the present time (Agbios, 2001). Cross-fertilization normally occurs when a transgene-laden pollen is carried by bees or blown with the wind from one field to another. The resulting contamination from GM maize can ruin any normal or organically grown maize crop by rendering traditional hybrid maize worthless for export to countries where consumers are wary of the gene transfer technology (Agbios, 2001).

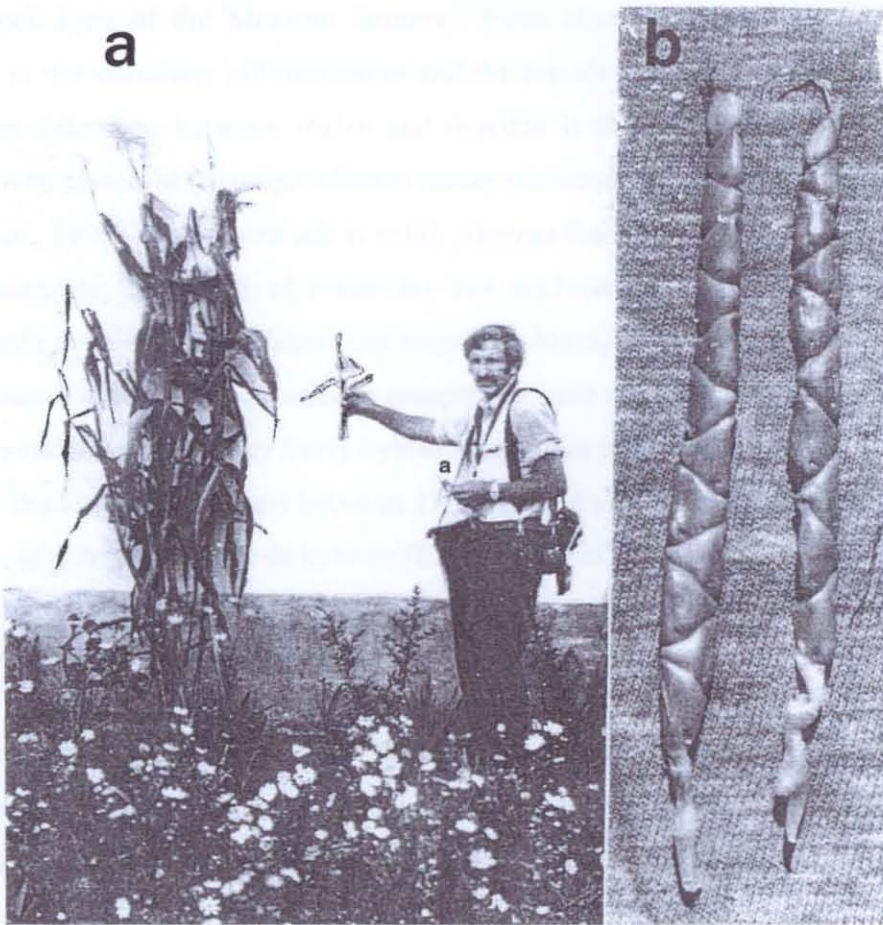


Figure 4.4: The teosinte, *Zea mays* spp. *Mexicana*, in the Valley of Mexico. (a) A robust teosinte plant taken from a maize field and a smaller teosinte plant found growing along the edge of the highway. (b) The ears or female inflorescences of the teosinte *Zea mays* ssp. *Mexicana* which differ remarkably from ears of maize in appearance and structure despite the plants being members of the same biological species (source: Dobley, 1990).

A recent genetic experiment suggests maize (corn) to be more related to annual teosinte than to any of its other relatives (Galiant, 1984). Both have the same chromosome number and they hybridize readily. The fertility of the hybrids is high because their chromosome pairing is regular and virtually complete. Morphologically, teosinte is

similar to maize; and indeed when both grow together in the maize fields of the valley of Mexico, distinguishing one from the other before they blossom is not an easy task, even for the keen eyes of the Mexican farmers. Even after flowering there is a marked similarity in the staminate inflorescences and the tassels (Soriano and Klevezas, 2000). The major difference between maize and teosinte is that teosinte typically has long branches with tassels at their tips whereas maize possesses short branches tipped by ears (Wang *et al.*, 1999). The maize cob is solid, whereas the teosinte cob is brittle and come apart at maturity; the seeds of maize are not enclosed, while those of teosinte are encapsulated in fruit cases. Maize (*Zea mays ssp. Mays*) and annual teosinte (*Zea mays ssp. Mexicana*) is, however, genetically compatible, both are wind pollinated, and in areas of Mexico and Guatemala they freely hybridize when in proximity to each other (Agbios, 2001). In the US a cross occurs between *Tripsacum* (a genus closely related to *Zea*) and *Zea mays*, which produce sterile hybrids (Carpenter *et al.*, 2002).

4.1.2.3.2.2 Cotton (*Gossypium Hirsutum L.*)

The cotton genus (*Gossypium L Malvaceae*) consists of approximately 50 species of shrubs and small trees found worldwide in both tropical and subtropical areas (Wendel *et al.*, 1992). It is subdivided into four sub-genera and these further subdivided into sections and subsections. *Gossypium L.* includes four species of cultivated cotton (*G. arboreum L.*, *G. barbadense L.*, *G. herbaceum L.*, *G. hirsutum L.*) (Biotech basics, 2001). *Gossypium hirsutum* in its wild and commercial form grows in the drier areas of Middle America, northern South America, the West Indies, the southern tip of Florida and through introduction in Northern Africa and Southern Asia. The wild population is rare and widely dispersed (Lee, 1984).

At least seven genomes (chromosome sets with distinctive gene groupings) designated A, B, C, D, E, F and G are found in the genus. Diploid species ($2n=26$) are found on all continents. The A genome is restricted to diploids of two species (*G. arboreum* and *G. herbaceum*). The D genome is restricted in diploids of some species, such as *G. thuberi*. *G. hirsutum* and *G. barbadense* are both allo-tetraploids (plants with four sets of

chromosomes derived by doubling of chromosomes from a hybrid plant) (Biotech basics, 2001). *G. tomentosum*, *G. hirsutum*, and *G. barbadense* have compatible genotypes and can be crossed to produce viable offspring, although crosses with *G. tomentosum* are only known with certainty from artificial crosses in breeding programs (Biotech basics, 2001). The bulk of the world's cotton is supplied by modern cultivars of *G. hirsutum* (Fryxell *et al.*, 1991). These four species of *Gossypium* are/have been widely cultivated in Africa (Vollesen, 1986). Because *G. hirsutum* and *G. barbadense* are sympatric in the Caribbean and co-occur to a limited extent in Central America, it was expected that inter-specific introgression would be most frequently detected (Wendel *et al.*, 1992). *G. barbadense* is also an alien plant in South Africa coming from North Africa. Scientific study proves that cross between *G. hirsutum* and *G. barbadense* can take place successfully (Schendiman *et al.*, 1974). There is hybrid vigour present in F1 hybrid progeny with good productivity, length and strength of the fiber (Ano *et al.*, 1983). This cross occurs mainly because cotton is partially an insect pollinator. Secondly both are tetraploid species and they can cross successfully and give a vigorous F1 hybrid (Hutchinson, 1940). The evidence shows that a cross between *G. barbadense* and Bt cotton can take place in South Africa as they are both tetraploid species and can be pollinated by insects.

The inter-specific crossing between the same genome groups of cotton can produce fertile F1 progeny, but it will segregate and lose its vigor (Harland, 1936; Hutchinson, 1940). Backcrossing with cultivated species can bring back the desirable characteristics (Munro, 1987). In inter-specific crossing the cross may be between a diploid and a diploid plant, or between a diploid and tetraploid plant, giving respectively a diploid or a triploid hybrid. Doubling the chromosome number, giving a tetraploid or a hexaploid plant, can restore fertility. It is usual to try to synthesize such a tetraploid from diploids with the A and D genome, so that it will produce fertile seed when crossed with one of the cultivated tetraploid species (AD) (Munro, 1987). The fertility may not be complete, and may require several backcrosses to the natural tetraploid before full fertility is restored (Munro, 1987).

Under controlled conditions hybridization between plants of *G. hirsutum* and wild *G.*



thuberi would likely result in a triploid ($3X=39$) sterile plant, because *G. hirsutum* is an allo-tetraploid ($4X = 52$) and *G. thuberi* is a diploid ($2X = 26$) plant (LaSota, 1996). *G. herbaceum africanum*, which is a wild form of cotton indigenous to South Africa, is a bushy perennial shrub and is considered to be the most primitive cotton (Munro, 1987). LaSota (1996) showed that a cross between the diploid ($2X=26$) *G. herbaceum* and allo-tetraploid cultivated cotton could occur, because they are insect pollinated plant, but the cross produces sterile plants.