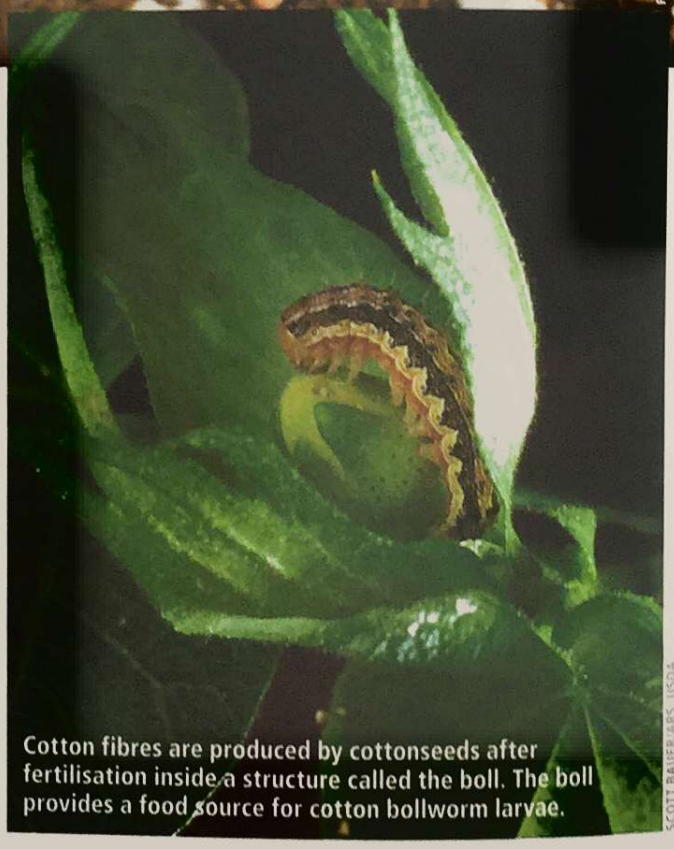
Insecticidal cotton

A GM success story

The first **transgenic** crop was developed in 1996. Ever since, genetically modified (GM) crops have been controversial. Opponents argue that the use of herbicide-resistant crops may lead to an increase in the amount of weedkiller applied to farmland. Fears have been expressed that foreign genes from transgenic plants may enter wild plants through cross-pollination, and others are worried that food from transgenic crops may contain new allergens or toxins. Here we consider the impact of transgenic cotton on farmers, the economy and the environment.

Despite public concerns, transgenic crops are now grown in more than 25 countries worldwide. More than 45% of the world's cotton production comes from GM seed. Box 1 describes how cotton fibres form. There are concerns that powerful global biotechnology companies may exploit the agricultural industry, which is becoming increasingly reliant on supplies of transgenic seed in order to maintain high yields. However, the development and widespread use of strains of GM cotton in India has demonstrated that such crops can have a beneficial environmental and economic impact.



Cotton fibres are produced by cottonseeds after fertilisation inside a structure called the boll. The boll provides a food source for cotton bollworm larvae.

KEY WORDS

Insecticide
Bioaccumulation
Transgenic
Genetic engineering
Bollworm

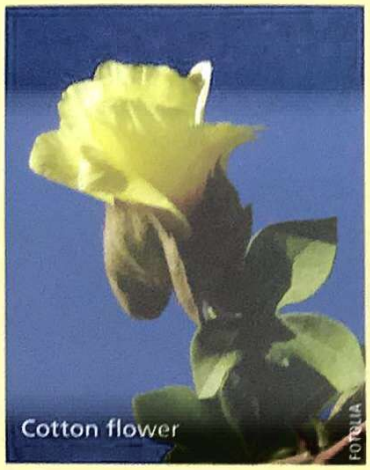
What is Bt cotton?

Bt cotton is a transgenic strain of cotton containing a gene from the bacterium *Bacillus thuringiensis*. This gene has been introduced to cotton plants to enable the plant to produce a toxin that kills insect pests, including the cotton bollworm. The adult bollworm is a moth that damages cotton plants by boring holes in the flowers and pods, and the caterpillars feed on young leaves and buds. Plants damaged by the action of bollworms are often unable to produce much cotton fibre, and in some cases the damage is so bad that the flowers fall off and no seed or cotton is produced at all. Yields of cotton may be reduced by up to one-third due to the effects of the cotton bollworm.

BOX 1

What is cotton?

The cotton plant (*Gossypium hirsutum*) is cultivated in tropical and sub-tropical climates throughout the world. Cotton is grown and harvested annually, taking approximately 25 weeks to grow to maturity ready for harvest. Cotton plants produce a small flower bud called a square, which develops into an attractive flower. Pollination usually occurs within 3 days: cotton flowers can self-pollinate but often pollination is aided by bees or other insects. A boll then forms when the flower falls off. Each boll contains around 30 seeds from which the cotton fibres will grow. The fibres grow quickly at first then start filling with cellulose. Once this process is complete, the bolls burst open and the cotton dries out and becomes fluffy, ready for harvest. Seeds for planting the following season can be removed from the fibres by machines called strippers, but many farmers buy commercial cottonseed from horticultural suppliers.



The cotton boll matures, the cotton fibres puff up and the cotton is ready for harvesting.

Why use a gene from *B. thuringiensis*?

Bacillus thuringiensis (Bt) is an insect pathogen found in many habitats including soil. Bt produces a range of proteins called **endotoxins**, which can cause disease or death to various invertebrates. Crystal protein (Cry) endotoxins are one example — they cause damage to the mucosal **epithelium**. This is the layer of cells lining the insect gut.

Bt makes an inactive form of Cry endotoxin, and this must be activated in order to cause damage. Activation occurs due to the effect of digestive enzymes in the insect gut, and can only take place in alkaline pH conditions. This helps to ensure that the toxin only harms certain groups of invertebrates as not all groups have the necessary combination of conditions and enzymes in their gut to allow activation of the toxin.

Once activated, the endotoxin enters the plasma membrane of the cells lining the insect gut wall, creating pores in the membrane. This leads to changes in membrane permeability, which results in the influx of water and ions. The cells of the insect swell and eventually burst. This is known as cell lysis (see Figure 1). The affected insect suffers gut paralysis and stops feeding. Breakdown of the gut wall also allows spores and gut bacteria to enter the body cavity of the insect, leading to septicaemia (infection of the blood) and death.

Selective toxicity

There are a number of Cry endotoxins, each with its own spectrum of activity. The toxic effects are apparent in only a few classes of invertebrates. The Cry1Ac toxin has been shown to be particularly toxic to moths and butterflies. It does not have significant effects on other invertebrates due to the lack of specific gut conditions necessary to activate the

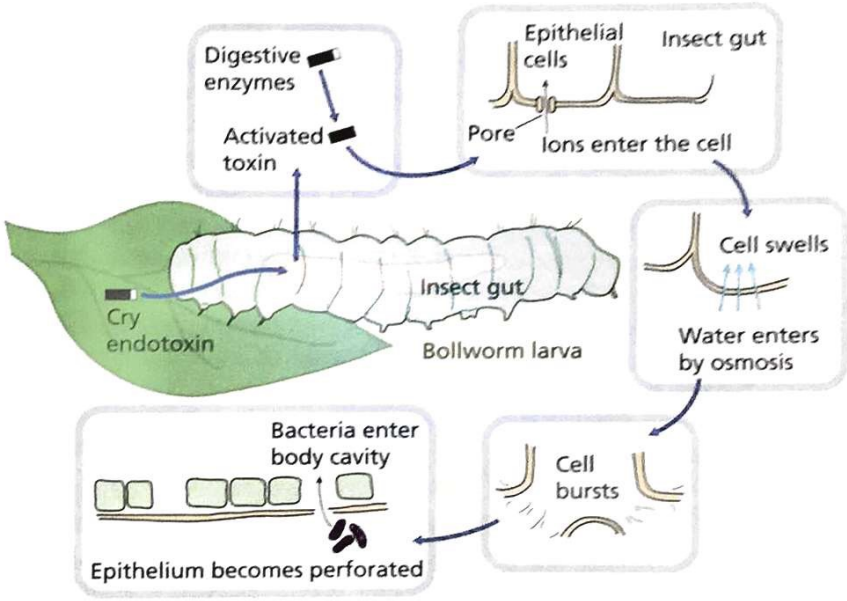


Figure 1 Cry endotoxins cause damage to the lining of the insect gut.

TERMS EXPLAINED

Bioaccumulation The build-up of toxic chemicals in living organisms.

Endotoxin A toxin produced inside certain bacteria, which is released when the bacterial cell is destroyed.

Epithelium Thin tissue forming the outermost layer of cells of the skin and mucous membranes.

Explants Samples containing a small number of living cells from an organism that can be grown into an adult plant on a nutrient medium.

Plasmid A small circular piece of DNA found in bacteria and some other organisms.

Transgenic An organism that has been modified by the insertion of a gene from a different species.

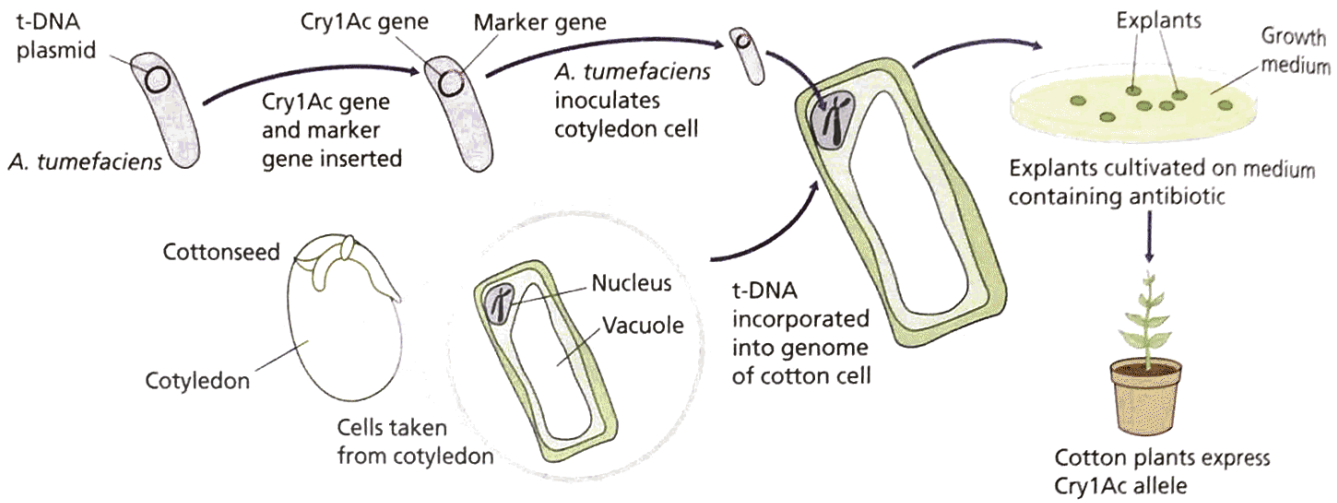


Figure 2 Transgenic cotton containing the Cry1Ac gene is created using the bacterium *A. tumefaciens*.

toxin. The Cry1Ac gene was therefore selected for use in the development of Bt cotton, in order to create an effective toxin directed against the target species without adversely affecting the population of other insects that may be exposed to the plant.

How is Bt cotton made?

The Cry1Ac gene is inserted into cotton plant cells using the bacterium *Agrobacterium tumefaciens*, which normally causes the growth of tumours in plants. *A. tumefaciens* attaches to plant cells and inserts a small section of DNA known as transfer DNA (t-DNA) into the cell. This t-DNA is then incorporated into the plant genome at a random location (see Figure 2).

The first stage in the process is the isolation and copying of the Cry1Ac gene. The gene is then inserted into the t-DNA **plasmid** of *A. tumefaciens*. In addition to the Cry1Ac gene, a marker gene is inserted into the plasmid to allow plants in which the gene transfer has been successful to be identified. In most cases, the marker gene used confers resistance to an antibiotic.

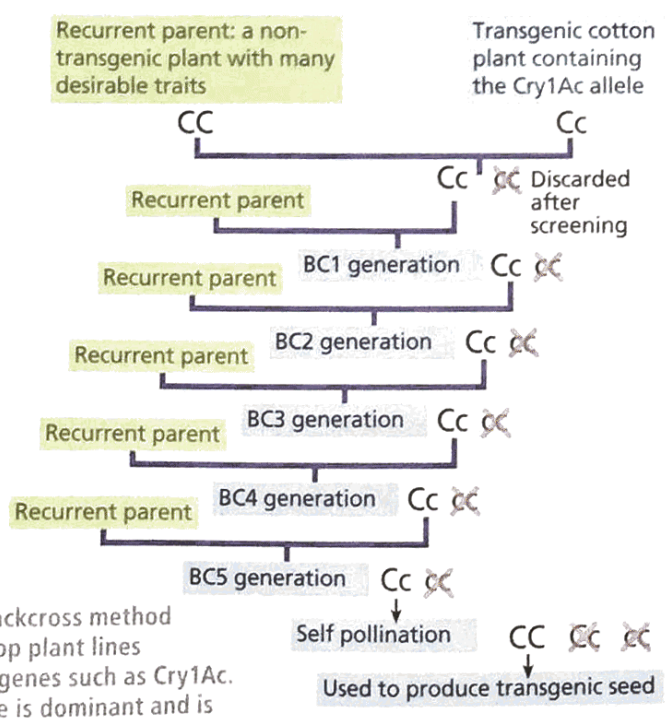


Figure 3 The backcross method is used to develop plant lines containing new genes such as Cry1Ac. The Cry1Ac allele is dominant and is represented by the letter C.

The cotyledon is part of the embryo in the seed of the plant, which will eventually give rise to the first leaves. Cells are taken from the cotyledons of cottonseeds and transferred to a growth medium. *A. tumefaciens* is used to inoculate these **explants** with the plasmid containing the Cry1Ac gene and the marker gene conferring antibiotic resistance. The explants are then cultivated on a growth medium containing the antibiotic. Any explants in which the gene transfer has been unsuccessful will fail to grow. This allows the selection of explants containing the Cry1Ac gene.

The transgenic plantlets are grown to adulthood so that biochemical tests such as an enzyme-linked immunosorbent assay (ELISA) can be carried out. This test will determine whether sufficient endotoxin is produced. Proteins are first extracted from the seed by crushing the seeds and breaking open the cells. The extracted material is attached to a solid surface, then tested for the presence of the Cry1Ac protein by adding an antibody specific for the protein. This antibody is in turn linked to an enzyme. If the toxin is present, the antibody–enzyme complex sticks to the immobilised sample; if not then no antibody–enzyme complex will attach. Detection is achieved by adding a substrate for the enzyme, which results in a colour change if the enzyme is present. Suitable transgenic plants can then be selected to use in the production of seed for sale to farmers.

How are transgenic seeds produced?

Having cultivated individual transgenic plants expressing the Cry1Ac gene, a plant breeding programme must be undertaken to produce seed for commercial sale. This is necessary because the first generation transgenic plants may not display all the characteristics needed to make a successful cotton plant for agricultural use. A backcross method is used to incorporate the Cry1Ac gene into a cotton plant with other desirable traits, such as high yield and drought tolerance.

In backcross breeding (see Figure 3), a non-transgenic parent plant is selected that displays numerous desirable traits. This plant is known as the recurrent parent. The recurrent parent is crossed with a transgenic plant containing the Cry1Ac gene by using pollen from one plant to fertilise the flowers of the other. The plants resulting from this cross are then bred with the recurrent parent. The plants in this generation are known as the BC1 (backcross 1st)

generation. Individuals from the BC1 generation are screened and those expressing the Cry1Ac allele are selected to be crossed with the recurrent parent again to produce the BC2 generation. This process is repeated several times, and the final backcross generation is self-pollinated to produce individuals homozygous for the Cry1Ac allele. These individuals are then selected and used in the production of seed for commercial sale.

The backcross method ensures the final seed will produce plants as similar as possible to the recurrent parent, displaying all the desirable traits but which are guaranteed to express the Cry1Ac gene.

Bt cotton in India

Strains of Bt cotton are grown in many countries around the world, but farmers in developing countries such as India have experienced the most dramatic benefits from using the transgenic seed. India first developed its own strains of Bt cotton in 2002 under licence from the multinational biotechnology company Monsanto. Additional strains have been developed since, including some unlicensed products, which are produced and sold on the black market. Government incentives and the high availability of licensed and unlicensed strains has resulted in widespread use of the transgenic crop (see Table 1), with estimates suggesting 80% of India's cotton originated from Bt strains in 2008.

Impacts of Bt cotton use

Conventional control of cotton bollworm usually involves the use of chemical insecticides, together with land management techniques such as crop rotation (see Box 2). Insecticides such as organophosphates are effective in reducing damage caused by cotton bollworms, but they may also kill beneficial insects that are necessary for the pollination of various crops and wild plants. Insecticide residues can build up in the soil and some insecticides harm predators through the process of **bioaccumulation**. Before Bt cotton became available, it is estimated that insecticides accounted for up to a third of the cultivation cost of cotton in India. Since the introduction of Bt cotton to some Indian farms in 2002, the national use of chemical insecticides on cotton has been reduced by approximately half.

The use of Bt cotton has had a beneficial effect on the yield of cotton in India. The estimated mean increase in yield experienced as a result of using Bt cotton is 34%. Licensed Bt cottonseed is up to three times more expensive than conventional hybrid cottonseed. However, the increase in yield more than compensates for this, and farmers planting transgenic crops generally benefit from significantly higher profits. National cotton yield in India has almost doubled in the last 10 years, and at least half of this increase is attributable to the use of Bt cotton. Since the introduction of the transgenic crop, India has improved its position in worldwide cotton trading from being the third largest importer of cotton in 2002 to now being the second largest exporter (see Figure 4). This represents a huge economic boost, bringing dramatic improvements in the Indian rural economy.

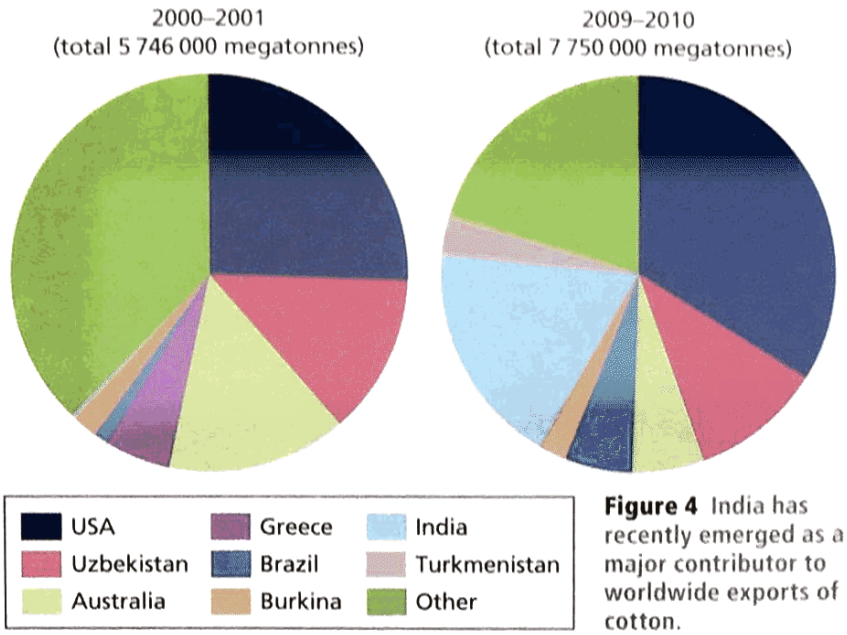


Figure 4 India has recently emerged as a major contributor to worldwide exports of cotton.

Table 1 Increase in the use of Bt cotton in India since 2002

Year	Area of land planted with Bt cotton/thousand hectares
2002	50
2003	100
2004	500
2005	1300
2006	3800
2007	6200
2008	7605
2009	8301

FURTHER READING

http://tinyurl.com/dxpmtlz
http://tinyurl.com/bpn7p23

The widespread use of Bt cotton exposes large populations of invertebrates to the effects of the Cry1Ac toxin. Various studies suggest that the effect on non-target insect populations of using Bt cotton is significantly less damaging than the effects observed when using chemical pesticides. No long-term effects of Bt cotton have been observed in non-target species, and the Cry1Ac toxin does not build up in the soil or demonstrate bioaccumulation. Minor reductions in the population of other insect species have been observed, but these are likely to be due to the effects of reduced numbers of cotton bollworm. As part of a complex food web, any change in the cotton bollworm population is likely to have an effect on the population of its predators and on alternative insects that the predators eat.

BOX 2

Pest control by crop rotation

Growing the same crop in one area repeatedly can lead to the build-up of pests which infest that crop. Crop rotation can be used to reduce the damage caused by cotton bollworms. Farmers will grow cotton for 1 year in a particular field and then in the following year they may grow an alternative crop such as maize, which is not damaged by the cotton bollworm. When the alternative crop is grown, there is less food available for the cotton bollworm, so this strategy can help prevent the population of the cotton bollworm from building up year on year.

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