Genetically modified crops: methodology, benefits, regulation and public concerns

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The genetic modification of crop plants from the methodology involved in their production through to the current debate on their use in agriculture are reviewed. Techniques for plant transformation by *Agrobacterium tumefaciens* and particle bombardment, and for the selection of transgenic plants using marker genes are described. The benefits of currently available genetically modified (GM) crops in reducing waste and agrochemical use in agriculture, and the potential of the technology for further crop improvement in the future are discussed. The legal requirements for containment of novel GM crops and the roles of relevant regulatory bodies in ensuring that GM crops and food are safe are summarized. Some of the major concerns of the general public regarding GM crops and food: segregation of GM and non-GM crops and cross-pollination between GM crops and wild species, the use of antibiotic resistance marker genes, the prevention of new allergens being introduced in to the food chain and the relative safety of GM and non-GM foods are considered. Finally, the current debate on the use of GM crops in agriculture and the need for the government, scientists and industry to persevere with the technology in the face of widespread hostility is studied.

What is genetic modification?

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Plant genetic modification (also known as genetic engineering) may be defined as the manipulation of plant development, structure or composition by the insertion of specific DNA sequences. These sequences may be derived from the same species or even variety of plant. This may be done with the aim of altering the levels or patterns of expression of specific endogenous genes, in other words to make them more or less active or to alter when and where in the plant they are 'switched' on or off. Alternatively, the aim may be to change the biological (*i.e.* regulatory or catalytic) properties of the proteins that they encode. However, in many cases, the genes are derived from other species, which may be plants,

animals or microbes, and the aim is to introduce novel biological properties or activities.

Farmers and plant-breeders have been changing the genes of crop plants for thousands of years. However, genetic modification differs from conventional plant breeding in the precision of gene transfer. Conventional breeding is based on the crossing of genotypes containing literally tens of thousands of expressed genes and the selection of progeny that combine the best features of the two parents. In some cases the progeny may contain almost equal numbers of genes from each parent. In others, an attempt may be made to incorporate a single gene from parent *a* into parent *b* by production of a hybrid followed by repeated backcrossing with parent *b* and selection of a desired trait over many generations. However, even after repeated backcrossing, it is inevitable that many undesired genes will also be transferred, and it is almost impossible to identify all of these and their products.

Conventional breeding is limited by fertility barriers that allow only plants of the same, or closely related, species to be crossed. However, 'wide crossing' with more distantly related species can be achieved if 'embryo rescue' is used to culture and regenerate embryos that would normally abort. Similarly, mutagenesis with chemical or physical mutagens can be used to induce new variation in the species of interest. Both wide crossing and mutation breeding can result in the expression in crop plants of many novel or modified genes, the effects of which cannot be assessed readily. However, both approaches are considered to be 'conventional', with no requirement for detailed assessment of the plants produced before they are introduced into the food-chain.

DNA delivery and selection of transgenic plants

Plant transformation can be divided broadly into two stages: DNA delivery and plant selection and regeneration. Two methods are widely used to deliver DNA into plant cells. The first[1] exploits nature's own genetic engineer, the naturally occurring soil bacterium *Agrobacterium tumefaciens,* which infects wounds on some plants to form a tumourous growth called a 'crown gall'. The tumour formations (Fig. 1A) result from integration of a DNA fragment (the T-DNA) from the *Agrobacterium* into the plant genome. As well as inducing tumour growth, genes present in the T-DNA cause the tumourous cells to produce compounds on which the bacteria feed. The T-DNA is present in a plasmid (the Ti or tumour-inducing plasmid), a closed circle of extra-chromosomal DNA, rather than the bacterial chromosome. This means that it can be isolated and manipulated to remove the genes that

would be inserted into a plant by wild *Agrobacterium* and replace them with novel genes. After infection of plant material with the modified *Agrobacterium*, whole plants can be regenerated from the resulting genetically modified tumour-like cell clumps (callus) by application of plant hormones.

The second widely used method is particle bombardment, in which the DNA is coated onto the surface of microscopic gold particles which are then shot into plant cells using a burst of helium gas. Some of the DNA is washed off the particles and becomes integrated into the plant genome (Fig. 1C,D). As with the first method, whole plants can be regenerated from genetically modified cells by careful culturing and the application of plant hormones. This method, which has acquired the unfortunate name of biolistics, has been particularly successful in the production of genetically modified cereals[2].

A current limitation to plant genetic modification is that only some of the cells in the target tissue are genetically modified, irrespective of the method of transfer. It is, therefore, necessary to kill all of the cells that are not modified and this requires that the gene of interest be accompanied by at least one other gene that acts as a selectable marker. In practice, this is usually a gene which makes the transformed cells resistant to an antibiotic (*e.g.* kanamycin; Fig. 1B) or herbicide (*e.g.* phosphinothricin, the active ingredient of Basta; Fig. 1C) which is toxic to untransformed cells. The use of antibiotic resistance genes is discussed further below, but the presence of a selectable marker gene is currently the minimum requirement for plant transformation. A scoreable marker gene may also be present to allow the transformed cells to be visualised, and the bacterial *UidA* (gus; Fig. 1D) or the jellyfish green fluorescent protein (GFP) genes[3,4] may be used for this. However, the presence of these genes is not essential and it is accepted that they should be avoided when producing transgenic plants for food or animal feed.

Benefits of GM crops currently in commercial production and future prospects

The UK is a world leader in this technology, but at present the growing of genetically modified (GM) crops is limited to a few selected test sites. In comparison, 49 million acres of GM crops were planted in the US alone in 1998. However, three imported products from GM crops grown commercially elsewhere in the world have been approved for food use in the UK: slow-ripening tomatoes; soya that is tolerant of a broad-range herbicide (weedkiller) called glyphosate; and insect-resistant maize.

The tomatoes are used to make tomato paste, reducing waste and processing costs, and 2 million tins of clearly labelled GM tomato paste have been sold in the UK since its introduction in 1996. It has a clear consumer benefit in that it is cheaper than its non-GM competitors and of a thicker consistency. Glyphosate-tolerant crops enable farmers to use a

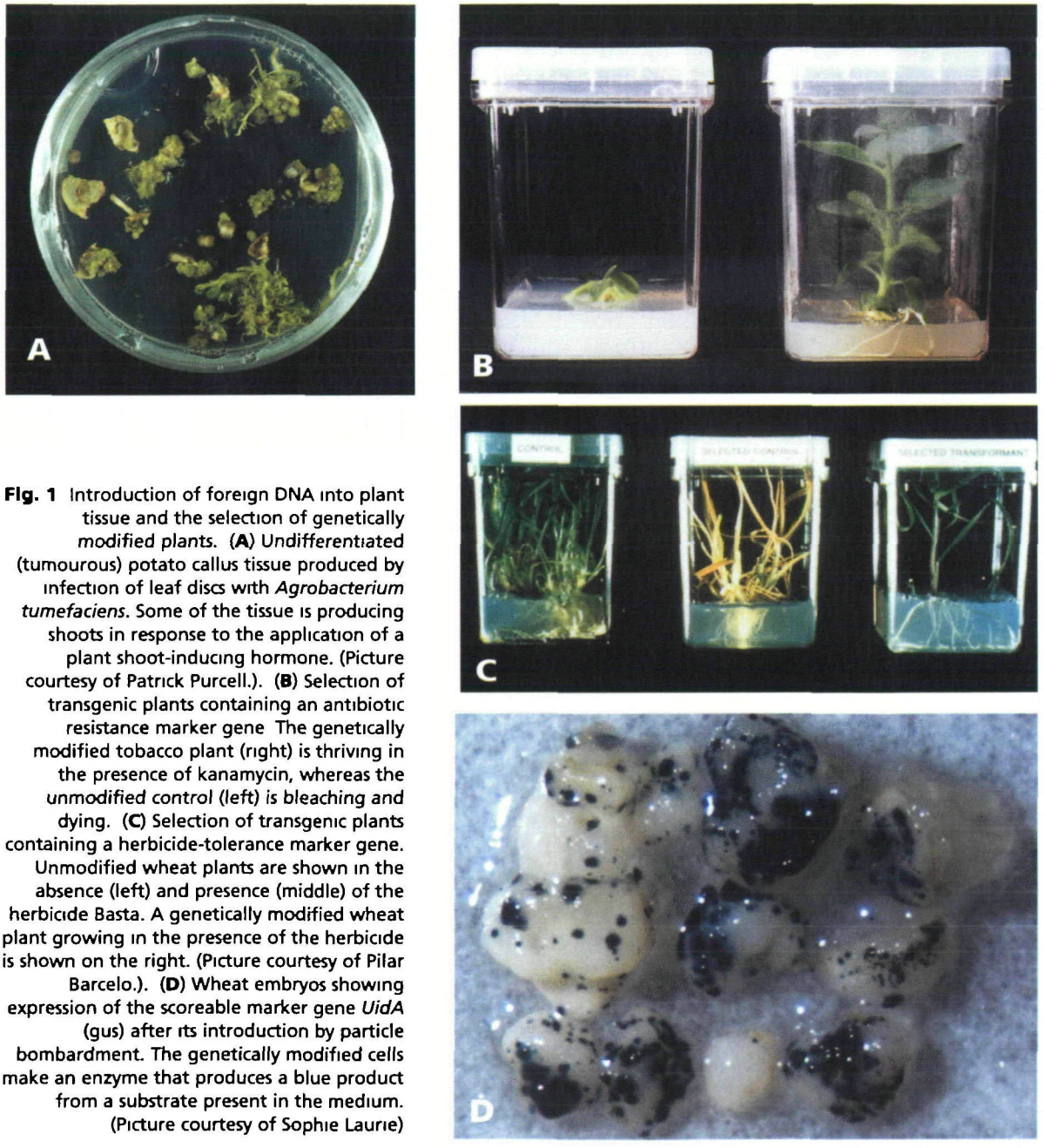

Fig. 1 Introduction of foreign DNA into plant tissue and the selection of genetically modified plants. (**A**) Undifferentiated (tumourous) potato callus tissue produced by infection of leaf discs with *Agrobacterium tumefaciens*. Some of the tissue is producing shoots in response to the application of a plant shoot-inducing hormone. (Picture courtesy of Patrick Purcell.). (**B**) Selection of transgenic plants containing an antibiotic resistance marker gene The genetically modified tobacco plant (right) is thriving in the presence of kanamycin, whereas the unmodified control (left) is bleaching and dying. (**C**) Selection of transgenic plants containing a herbicide-tolerance marker gene. Unmodified wheat plants are shown in the absence (left) and presence (middle) of the herbicide Basta. A genetically modified wheat plant growing in the presence of the herbicide is shown on the right. (Picture courtesy of Pilar Barcelo.). (**D**) Wheat embryos showing expression of the scoreable marker gene *UidA* (gus) after its introduction by particle bombardment. The genetically modified cells make an enzyme that produces a blue product from a substrate present in the medium. (Picture courtesy of Sophie Laurie)

single, safe, rapidly-degrading herbicide instead of a battery of more expensive, more poisonous and more persistent herbicides, reducing total herbicide use by almost half in some cases[5]. They also allow farmers to use no-till agriculture, leaving the soil and weed cover undisturbed over winter, greatly reducing soil erosion and loss of groundwater, as well as providing habitats for insects and birds. Herbicide tolerant soya made up half of the US and 70% of the Argentine crop in 1998, and is popular with farmers throughout the Americas. However, the lack of a clear benefit to the consumer and the widespread, erroneous belief that use of herbicide tolerant varieties would lead to an increase in the amounts of herbicide being applied has made consumer acceptance difficult to achieve in the UK.

The maize approved for food use in the UK contains a gene (commonly called the bt gene) from a bacterium, *Bacillus thuringiensis*. The protein produced by this gene is toxic to some insects, mainly caterpillars, and the bacteria themselves have been used as an insecticide by organic farmers for decades. Variants of the bt gene have been introduced into several crops grown in the US[6], including cotton, sugar beet and potato, as well as maize. The effect of its use in cotton has perhaps been the most striking. Conventional cotton is very susceptible to insect damage and one quarter of US insecticide production is used on this one crop, including 'hard', persistent and completely unselective insecticides such as organophosphates. GM cotton on average requires 15% of the insecticide used on conventional cotton and in some areas of the US in 1996–1998 was not sprayed with insecticide at all. The bt protein does not affect bees or many other benign insects, and has no toxicity to mammals, birds or fish.

A recent study found that caterpillars of the monarch butterfly (which is not a pest species) that were forced under laboratory conditions to eat large quantities of pollen from bt maize (they would not normally eat pollen) suffered higher mortality levels than caterpillars that were not fed the pollen[7]. However, it should be remembered that spraying caterpillars and other insects with pesticide, which equates with the regimen used in the field for almost all non-GM maize, kills them all outright. Use of 'hard' pesticides, such as organophosphates, has been reduced greatly or eradicated altogether with the introduction of GM varieties.

These crops represent the first generation of GM plants in agriculture, but there are many other targets for crop biotechnology. These include other agronomic traits, such as virus resistance[8] and new quality attributes such as nutritional value, including levels of vitamins and other micro-nutrients, such as iron, iodine and folate, as well as colour and flavour. Crops will soon be available that contain modified oils[9], either tailored to meet the specific requirements of processors, or with pharmaceutical[10] or other industrial uses, such as the production of

biodegradable plastics. The use of genetic modification to improve the bread-making quality of UK wheat varieties (UK and European wheats are poor in this respect) is already well advanced[11] and wheat, potato and maize are also being modified to produce starch for industrial uses. Other non-food targets include pharmaceuticals, fragrances, pigments and safe, cheap, edible vaccines[12]. The latter have already reached the human testing stage for vaccines against diarrhoea *Escherichia coli* and hepatitis B, and is obviously most relevant to those areas of the world where drugs, clean needles and syringes are not readily available.

Containment, safety assessment and the role of regulatory authorities

While recognising that GM technology is already benefiting agriculture elsewhere in the world and that the potential benefits of the technology in the UK justify supporting and investing in it, the government, scientists and industry are aware that, as with all new technologies that impact upon the environment and consumer, it should be introduced carefully. For this reason, any organization that seeks to use genetic modification, even in contained conditions, must first obtain approval from the Health and Safety Executive (HSE)[13,14]. A successful application requires that the facilities meet certain standards to ensure that GM organisms are contained, that procedures are in place for sterilization of GM material, and that there is sufficient experience in handling potentially hazardous biological material amongst the staff. Typically GM plants are kept in a greenhouse with filtered negative air pressure ventilation, sealed drains and a chlorination treatment system for drainage water.

There is also a legal requirement for the organization to assess, before beginning a GM project, whether the plants that will be produced could represent a risk to humans, other plants or the environment, including the chances and consequences of cross-pollination with other plants. The HSE inspects organizations regularly to ensure that this assessment process is being carried out satisfactorily.

Before any GM plants can be planted outside of a containment facility in the UK, permission has to be granted by the Department for the Environment, Transport and Regions (DETR)[15,16]. Applications are considered by the Advisory Committee for Release into the Environment (ACRE), an independent committee of experts who consider a similar set of questions on the safety of GM crops to those detailed above, on a case-by-case basis. As well as a detailed risk assessment, the Committee has available the data on the genetic stability and performance of the crop, obtained under contained conditions, usually over several years prior to release.

Assessment of the safety of GM foods is undertaken in the UK by the Advisory Committee on Novel Foods and Processes (ACNFP), another independent committee of experts, with members from universities and research institutes. Any GM food, no matter where it is produced, must be approved by this Committee before it is permitted to enter the UK food-chain. ACNFP requires that information be provided on the composition of materials, effects of production, stability, nutritional characteristics and the likelihood of genetic transfer. It has been argued that GM foods should be subjected to the same testing and approval procedures as medicines (*i.e.* clinical trials). The Government's view, which we share, is that this is impractical and that the methods recommended by the World Health Organization[17,18] are adequate to ensure that any possibility of an adverse effect on human health from a GM food can be detected.

Public concerns

Segregation of GM and non-GM crops and the environmental impact of cross-pollination between GM crops and wild species

Unless GM food is accepted universally, which seems unlikely in the foreseeable future, it is important that alternatives remain available to allow consumers to exercise choice. For imported food-stuffs, UK suppliers will have to contract farmers overseas to grow non-GM varieties. This means paying a guaranteed price to a farmer to use old-fashioned varieties and high chemical inputs. The additional cost will be passed on to the consumer and non-GM soya is already 40% more expensive than the GM alternative. For crops grown in the UK, the main issue will be segregation of GM and non-GM crops and food. Segregation could break down through accidental mixing of GM and non-GM seed for planting, by cross-pollination between GM and non-GM crops (which is less of a problem for inbreeding species such as wheat) or by mixing of the product between the farm gate and the consumer. Some inadvertent mixing is almost inevitable and the production of certifiably GM-free food is, therefore, likely to be expensive. Clearly, a farmer who had paid for expensive GM seed in order to produce a high-value product would wish to avoid pollination from a nearby non-GM crop, and segregation is likely to be a subject of considerable dispute.

The potential environmental impact of cross-pollination with wild species has to be assessed case-by-case, taking into account the species and genes involved. Wheat, maize and potato, for example, do not cross with any wild species in the UK (although forced crosses can be made

between potato and black nightshade in the laboratory). Sugar beet crosses with wild and weed beet, but this poses little threat to agriculture. Indeed, the only major crop in which cross-pollination could be a problem is oilseed rape. This will cross with other cultivated and wild *Brassicas*, including Chinese cabbage, Brussels sprouts, Indian mustard, hoary mustard, wild radish and charlock. The extent of such crossings in agricultural systems is the subject of continuing research, but it does not necessarily mean that GM oilseed rape represents a threat, as this will also depend on whether the gene involved could confer a competitive advantage on a plant that acquired it. The issue of cross-pollination with wild species is reviewed in more detail by Raybould[19].

Antibiotic resistance marker genes

Another topic that has generated much debate, some of it wildly overblown, is the use of antibiotic resistance genes as selectable markers. The use of marker genes to select cells that have been modified with genes of interest is discussed above, and antibiotic resistance genes have been extremely valuable in the development of GM technology. Many scientific bodies around the world, including the World Health Organization and regulatory committees set up by the European Union and several national governments have considered the safety of antibiotic genes in food and have concluded that those that are being used do not represent a health threat. The British Medical Association, however, has expressed reservations[20], and the ACNFP has called for the development of alternative marker systems[21].

The main reason for believing that antibiotic resistance genes in GM crops do not represent a health threat is that they already occur in natural microbial populations, indeed they are widespread amongst soil bacteria[22]. Those that are used most frequently confer resistance to antibiotics that are not used at all in oral medical formulations, such as kanamycin and neomycin, although one notable exception to this is the insect-resistant GM maize of Novartis, which contains a gene for resistance to ampicillin. Further re-assurance can be taken from the fact that horizontal transfer of a gene from ingested plant material to bacteria has never been demonstrated, and there is no indication that it has ever occurred during evolution. The probability that it could occur is, therefore, considered to be so low that it is not relevant when compared with the natural occurrence of antibiotic resistance genes. Antibiotic resistant strains of pathogenic bacteria do represent a health threat, but they arise naturally and thrive because of the sloppy management of antibiotics in human and animal medicine, not because of the use of antibiotic resistance marker genes in biotechnology.

Allergenicity

It has been suggested that consumption of GM foods could lead to increases in toxicity and allergenicity. This is particularly relevant to the use of protective proteins to confer resistance to pests and pathogens as these can reasonably be expected to also show some toxicity to humans. In addition, there was a widely reported case where a methionine-rich 2S albumin storage protein from Brazil nut was expressed in soybean in order to increase the methionine content for animal feed[23]. The protein was subsequently shown to be an allergen, as are a number of related 2S albumins from other species. The plant breeding programme was, therefore, discontinued as it would be difficult to guarantee that the GM soya would not enter the human food chain. This case certainly illustrates the potential for introducing allergens and toxins by genetic modification. However, the fact that the problem was identified before commercial material was produced, and appropriate action taken, demonstrates the high level of awareness of such problems in the plant

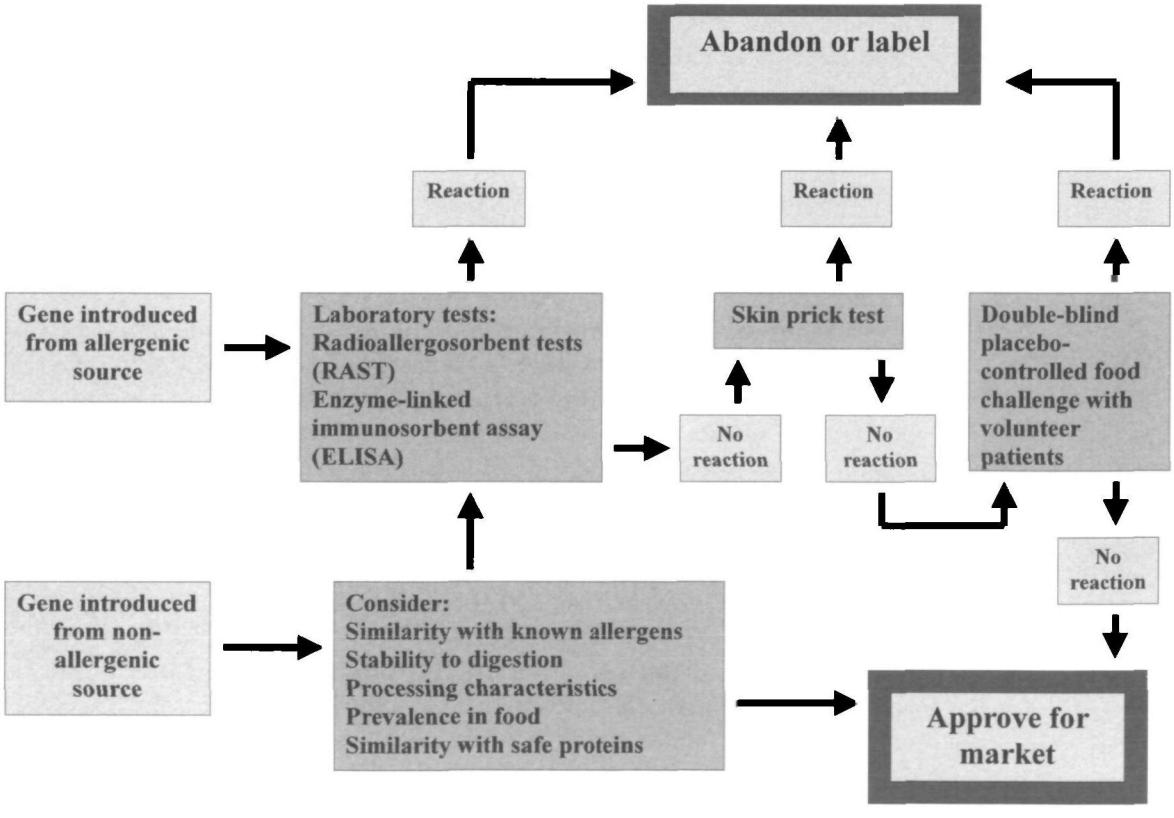

Fig. 2 Flow diagram showing the assessment and testing of possible allergenicity in GM foods. Redrawn from Astwood *et al*[24], with the publisher's permission

biotechnology industry and the effectiveness of 'in house' screening programmes. A typical procedure used to test for the presence of food allergens in transgenic plants is summarized in Figure 2, while the application of this procedure to the GM soybeans containing the Brazil nut 2S albumin is described by Nordlee *et al*[23]. The biotechnology industry takes the view that release of new allergenic products into the food chain is entirely avoidable[24] and the legal requirements for feeding trials provide an additional, effective safety net. Consequently, GM foods may well prove to be safer than those produced by conventional plant breeding, as discussed below.

Relative safety of GM foods compared with 'traditional' foods

The major arable and horticultural crops grown and consumed in Western Europe have been developed using conventional breeding methods, often over centuries or even millennia. Consequently, they are assumed by the consumer to be safe and wholesome. However, most, if not all, of these crops contain compounds that are potentially toxic or allergenic. In most cases, these compounds have probably evolved to provide protection against animal predators or pathogenic micro-organisms and it is, therefore, not surprising that they are also toxic to humans. Furthermore, they are particularly abundant in seeds and tubers, whose rich reserves of proteins, starch and oil are particularly attractive to pests and pathogens. Well-known examples are glyco-alkaloids in potatoes, cyanogenic glycosides in linseed, glucosinolates in *Brassica* oilseeds and proteinase inhibitors in soybean and other legume seeds. It is very doubtful whether these, or many other generally accepted foods, would be approved for food use were the toxins introduced by genetic modification. Similarly, the introduction of new types and varieties of food crops produced by conventional breeding requires no specific testing for the presence of allergens and toxins, although genes may have been introduced from exotic varieties or related wild species. Toxins can also be produced by fungal activity before harvesting or during storage. Ironically, these mycotoxins, including dangerous carcinogens such as aflotoxin, are particularly prevalent in organic food, which has not been treated with fungicides.

It is clear that the public requires a higher level of assessment of the safety of GM food than conventional or organic foods and this is only possible because genetic modification is such a precise process. The products of the introduced genes are readily identified and their expression levels determined. The products may also be isolated in a pure form, either from the species of origin, from the transformed plant or after expression in a micro-organism. The pure protein can then be tested in

detail and its presence in processed foods monitored. In contrast, it is virtually impossible to identify and characterize the changes in food composition that may result from conventional plant breeding. Consequently, we would argue that GM foods may be safer than food derived from non-GM varieties as the risks are readily quantified and monitored and GM foods are examined under a rigorous assessment system that goes beyond that applied to other foods.

Concluding remarks

Despite the safeguards applied to GM crops and foods, and the clear benefits that they are bringing, public acceptance in the UK is currently low. There are several reasons for this, including unease about food safety in general, caused by factors (BSE, *E. coli*, Salmonella, *etc*) that have nothing to do with GM, lack of information on the benefits, a well-organized anti-GM campaign lead by professional, multinational pressure groups (who have no responsibility for food production), and a succession of wild scare-stories. Much of the debate revolves around products that are not, and may never be, commercially available, the infamous 'terminator' crops and tomatoes containing fish genes being good examples. The industry itself must also accept much of the blame for public hostility, since the lack of labelling of GM products between late 1996 and 1999 led to the perception amongst the public that the technology was being imposed on them.

Governments continue to support research and development in plant biotechnology in the face of this hostility, and there are strong arguments to support this position. The most obvious is that GM crops are now well-established and very successful in large areas of the world, particularly the Americas and China, and other European countries are spending heavily in order to catch up with the UK and US in the science. GM crops are already playing a part in increased yields, improving nutritional quality, increasing the profitability of agriculture and reducing its dependence on high chemical inputs. It is almost inconceivable that this revolution in agriculture could be reversed at this late stage. Sooner or later, we will have to allow UK farmers to grow GM varieties if they are to compete.

References

1 Bevan M. Binary *Agrobacterium* vectors for plant transformations. *Nucl Acids Res* 1984; 12: 8711–21

2 Barcelo P, Hagel C, Becker D, Martin A, Lorz H Transgenic cereal (*Tritordeum*) plants obtained at high efficiency by microprojectile bombardment of inflorescence tissue. *Plant J* 1994, 5: 583–92

3 Jefferson RA, Kavanagh TA, Bevan MW. Gus fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* 1987; 6: 3559–64
4 Tsien RY. The green fluorescent protein. *Annu Rev Biochem* 1998; 67: 509–44
5 Dewar AM, Haylock LA, Bean KM, May MJ. Delayed control of weeds in glyphosphate-tolerant sugar beet and the consequences on aphid infestation and yield Pest Manag Sci 2000, 56: 345–50.
6 de Maagd RA, Bosch D, Stiekema W *Bacillus thuringiensis* toxin-mediated insect resistance in plants. *Trends Plant Sci* 1999; 4: 9–13
7 Losey JE, Rayor LS, Carter ME. Transgenic pollen harms monarch larvae. *Nature* 1999; 399; 214
8 Sangare A, Deng D, Fauquet CM, Beachy RN. Resistance to African cassava mosaic virus conferred by a mutant of the putative NTP-binding domain of the Rep gene (AC1) in *Nicotiana benthamania. Mol Breeding* 1999; 5: 95–102
9 Napier JA, Michaelson LV, Stobart AK. Plant desaturases harvesting the fat of the land. *Curr Opin Plant Biol* 1999; 2: 123–7
10 Sayanova O, Smith MA, Lapinskas P *et al.* Expression of a borage desaturase cDNA containing an N-terminal cytochrome b(5) domain results in the accumulation of high levels of delta(6)-desaturated fatty acids in transgenic tobacco. *Proc Natl Acad Sci USA* 1997; 94 4211–6
11 Barro F, Rooke L, Bekes F *et al* Transformation of wheat with high molecular weight subunit genes results in improved functional properties. *Biotechnology* 1997; 15: 1295–9
12 Mor TS, Gomez Lim MA, Palmer KE. Perspective. edible vaccines - a concept coming of age. *Trends Microbiol* 1998, 6: 449–53
13 Health and Safety Commission *Genetically Modified Organisms (Contained Use) Regulations 1992* (SI 1992 No. 3217). London: HSE Books, 1992
14 Health and Safety Commission. *Genetically Modified Organisms (Contained Use) (Amendment) Regulations 1996* (SI 1996 No. 967). London HSE Books, 1996
15 Department of Environment. *Genetically Modified Organisms (Deliberate Release) Regulations 1992* (SI 1992 No. 3280). London: DoE, 1992
16 Department of Environment. *Genetically Modified Organisms (Deliberate Release) (Amendment) Regulations 1996* (SI 1996 No. 304). London: DoE, 1996
17 Jonas D, Kaferstein FK. Genetic modification and food safety. *Biotech Dev Monit* 1995; 25: 11–2
18 World Health Organization. *Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology* Report of a WHO Workshop. WHO/FNU/FOS/95 1. Geneva: WHO, 1995
19 Raybould AF. Transgenes and agriculture – going with the flow? *Trends Plant Sci* 1999; 4: 247–8
20 British Medical Association. *The Impact of Genetic Modification on Agriculture, Food and Health*. London: BMA, 1999
21 Advisory Committee on Novel Foods and Processes. *Report on the use of antibiotic resistance markers in genetically modified food organisms*. London ACNFP, 1994
22 Bushby HVA. Quantitative estimation of rhizobia in non-sterile soil using antibiotics and fungicides. *Soil Biol Biochem* 1981; 13 237–9
23 Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK. Identification of a Brazil nut allergen in transgenic soybeans. *N Engl J Med* 1996; 334. 688–92
24 Astwood JD, Fuchs RL, Lavrik PB. Food biotechnology and genetic engineering. In: Metcalfe DD, Sampson HA, Simon RA (Eds) *Food Allergy*, 2nd edn. New York: Blackwell, 1996