Chapter 2

The scientific basis of genetic modification

Introduction

2.1 Genetically modified (GM) crops have come into prominence during the past decade. This is because plant breeders have learnt to apply GM technologies, first developed for plants in 1983, to a wide range of crop species.[1] The first applications of genetic engineering, or genetic modification, as it came to be known, were in human medicine. The need to develop cheaper, safer and more plentiful sources of substances such as insulin and interferon drove developments from 1976 until the early 1980s, and led to the formation of a number of new, small, innovative biotechnology companies, often closely associated with universities. This, in turn, released new scientific knowledge and commercial opportunities which were taken up by leading members of the pharmaceutical industry and, more recently, by a number of agrichemical and seed companies. The initial commercial goals of plant biotechnology were directed at the markets of the developed world. There is, however, a growing realisation in the wider research community that this new set of technologies could make a valuable contribution to the increasingly urgent problem of the global food supply. In this chapter we set out the scientific basis for the genetic modification of plants.

Conventional plant breeding

2.2 Almost all of the crops that we cultivate today are much changed from their wild ancestors. Breeding by selection and saving the best seed for the next generation has been in progress for many thousands of years. In most crops, the incorporation of traits compatible with agriculture, such as free threshing in cereals, was achieved centuries ago. Scientific breeding, however, which followed the rediscovery of Mendel's Laws,[2] has been under way only for the last 90 years.

2.3 Progress has been dramatic, particularly for the cereal staples, wheat, maize and rice. New agricultural methods, particularly chemical fertilisers, herbicides, fungicides and mechanisation, have been developed alongside improved crop varieties to double world food production over the past 40 years. In view of some of the perceived problems associated with GM crops, it is worth pointing out that in conventional breeding, as with any new technology, some mistakes have been made and lessons learnt.

2.4 For example, in the 1950s, a type of maize was used for F1 hybrid[3] production in the US which was subsequently discovered to be associated with susceptibility to Southern corn leaf blight.[4] During the 1970s, wheat varieties with single major gene resistance to fungal diseases were released in the UK, one after another. The best varieties were usable, on average, for only 14 months as the fungus populations repeatedly overcame the disease-resistance genes. The supply of resistance genes became limiting and farmers increasingly had to rely on chemical fungicides to keep their crops free from disease.[5] These instances, although costly and apparently very serious at the time, did not produce significant, long-lasting effects.

1 Genetic modification involves the direct introduction of desirable characteristics by the artificial transfer of foreign or synthetic DNA into a plant. DNA (deoxyribonucleic acid) is the biochemical substance from which the genetic material is made.

2 Mendel's first law states that when a cell (containing two copies of each gene, arrayed on two matching chromosomes) divides to form two sex cells (such as eggs, sperm or pollen), each sex cell will only have one copy of each gene. The second law states that members of different pairs of genes will move into the sex cells independently of each other. Consequently, it cannot be predicted which one of the two copies of each gene will end up in a particular cell. However, the closer the genes are to each other on the chromosome, the more likely they are to be inherited together.

3 F1 hybrid seed is produced by inter-crossing two predefined parental lines. F1 hybrids are favoured by producers because they display hybrid vigour or heterosis. F1 hybrids do not breed true and, therefore, farmers cannot save seed.

4 Pring D and Lonsdale D (1989) Cytoplasmic male sterility and maternal inheritance of disease susceptibility in maize, **Annual Review of Phytopathology**, 27:483–502.

5 Johnson R (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat, **Euphytica**, 63:3–22.

2.5 Many have therefore argued that the effects of the introduction of GM crops on the agricultural environment should, realistically, be assessed in the context of current intensive agricultural methods. These include not only the varieties produced by advanced plant breeding techniques, but also the use of more agrochemicals and water than before, and increased use of marginal lands. Others have argued that both conventional plant breeding and GM crops should be compared with a substantially increased use of organic farming methods. Some of the proposed benefits of GM crops are novel but, in general, the new technology offers only a more efficient or different way of solving old problems. For example, breeders have been using disease- and pest-resistance genes for decades. In effect, the new insect-resistance genes[6] are unlikely to be different from insect-resistance genes already in use, such as the leafhopper resistance used in rice or hessian fly resistance in wheat. 'Natural' tolerance to herbicides was used in maize in the late 1980s, and again, very recently, in Pioneer Hi-bred's 'Smart Canola'.[7] The new GM crops which are tolerant to Roundup[8] are unlikely to be different in their effects on the environment. Thus, although GM crops may pose novel pressures on the environment there is, as yet, no reason to consider GM varieties as qualitatively different from non-GM varieties.

2.6 Over the past half-century the professional plant breeder has assimilated many new technologies. Examples include: 'doubled haploids', where lines can be made pure breeding in a single step; 'induced mutations', where new variation can be generated by irradiation or chemical treatments; 'F1 hybrids', where farmers can benefit from the expression of hybrid vigour, and 'molecular markers', where a breeder can select for a piece of DNA rather than a trait, thereby avoiding expensive and time-consuming tests in selecting the ideal parent or progeny. To the plant breeder, genetic modification is simply the latest technology which breeders hope to bring to bear in their quest for ever-improved crops.

2.7 At the current stage of its development, genetically modified or transgenic technology does not offer the means of targeting where transgenes are integrated into the chromosomes; integration into the plant chromosomes appears to be more or less random. However, conventional plant breeding is usually a matter of putting two sets of about 25,000 genes together, allowing them to segregate at random and then selecting the best. Indeed, entirely new species have been manufactured using this approach. An example is *Triticale*, a synthetic hybrid between wheat and rye grown extensively in Eastern Europe over this century, which is the result of combining 50,000 largely untested genes, 25,000 from each species.

Plant genetic transformation

2.8 'Transformation', 'genetic modification', 'genetic engineering' and 'transgenesis' are all synonyms for the transfer of isolated and cloned genes into the DNA, usually the chromosomal DNA, of another organism. Transformation of micro-organisms was first achieved in 1973 and this was followed by the development of GM technology for animals. Plants, due to the dense nature of the plant cell wall, were more difficult to transform. It was another ten years before the first successful experiments were reported. These first examples involved the use of the crown gall-inducing bacterium, *Agrobacterium tumefaciens*, to transfer genes for antibiotic resistance into tobacco plants (paragraphs 2.14–15).

6 Bt insect-resistance genes come from the bacterium *Bacillus thuringiensis*. These genes code for a variety of toxins, which vary in the extent to which they are toxic to different insects. Organic farmers spray Bt toxins on crops to control pests. In contrast, transgenic plants containing Bt genes produce Bt toxins within their cells, which are eaten by pests preying on the plants.

7 Concar D and Coghlan A (1999) A question of breeding, **New Scientist**, No. 2175:4–5.

8 'Roundup Ready' is the proprietary name given to crops which have been modified to contain resistance genes to the herbicide glyphosate (Roundup).

2.9 Since those early days, almost every significant crop species has been successfully transformed. The technology, initially in the hands of only a few advanced academic laboratories, has been established and refined in the laboratories of most major plant breeding companies. The international movement of research scientists between laboratories, the work of agencies such as the Department For International Development (DFID) in the UK, and initiatives such as the Rockefeller Rice Biotechnology Program[9] have ensured that effective transformation technology is now practised in all major plant breeding research centres in both developed and developing countries.

2.10 Initially, transformation was developed in model broad-leaved plants such as tobacco and tomato. Narrow-leaved plants, which include all the major cereal crops, were more difficult and the first successful transformations, in rice and maize, were not reported until the late 1980s.[10] Consistently successful transformations of the more recalcitrant cereals such as wheat and barley have only been achieved very recently.

The experimental components of successful transformation

2.11 First, for the novel gene to be transferred, the transgene, which will have been isolated as a stretch of DNA, must be linked or spliced to a suitable promoter.[11] The transgene will code for the production of a protein, often an enzyme, which in turn will catalyse a biochemical reaction in the plant. The promoter component of the DNA will determine where, when and to what degree the transgene is expressed in the plant. This engineered construct must then be introduced into the target plant's own chromosomes.

2.12 This is usually carried out on cultured cells which have to be subsequently regenerated into an intact plant. Since transformation can be very inefficient (in some situations only around one in a thousand cells may be transformed), most of the gene constructs used to date have incorporated a selectable marker gene as well as the transgene. Markers, such as antibiotic resistance or herbicide tolerance, allow the breeders to select only successfully transformed cells in culture media containing the antibiotic or plants grown in the presence of the herbicide. However, as transformation efficiencies have increased, the need for such markers has declined (see below).

Transformation methods

2.13 Although several methods of plant transformation have been used, only two are relevant today to the transformation of food crops. These are *Agrobacterium* and the 'gene gun'. Since both these methods have been patented, we can expect that other methods will continue to be developed in order to circumvent these patents. Both methods have advantages and disadvantages, depending on the application and the crop.

2.14 *Agrobacterium:* this bacterium has been called 'nature's own genetic engineer' because it naturally transfers DNA to its plant host. Of course, it also causes disease 'naturally' in plants. However, the attenuated strains used as carriers or vectors by plant genetic engineers have had their plant

9 The Rockefeller Rice Biotechnology Program began in 1984, focusing on Asia. It concentrated first on developing tools of rice biotechnology such as gene-mapping, gene-tagging and genetic transformation. As these tools have been developed, greater emphasis has been placed on training, technology transfer and capacity-building within individual countries.

10 Gordon-Kamm W, Spencer T, Mangano M, Adams T, Daines R, Start W *et al*. (1990) Transformation of maize cells and regeneration of fertile transgenic plants, **The Plant Cell**, 2:603–618 and Fromm M, Morrish F, Armstrong C, Williams R, Thomas J and Klein T (1990) Inheritance and expression of chimeric genes in the progeny of transgenic maize plants, **BioTechnology**, 8:833–839.

11 A promotor is a DNA sequence that regulates the expression of a gene. Each gene has its own promotor which receives specialised proteins that bind and activate a gene.

gall-inducing ability removed. The modified vector is then transformed to carry the engineered gene constructs before being introduced into a host plant cell. The new genes then integrate into the host DNA of the plant. Initially this method was thought only to be applicable to broad-leaved plants, but carriers capable of infecting and transforming cereals have recently been developed.

2.15 This method has the advantage that it is relatively simple and can be applied by any laboratory with suitable tissue culture facilities. Occasionally, DNA from the bacteria may get transferred in addition to the transgene and it is possible that the carrier itself may persist in or on transformed plants for up to a year after transformation. These technical difficulties have been criticised as the inadvertent transfer of genetic material and the introduction of live-engineered bacteria into the environment.

2.16 *The gene gun:* in this method gold or tungsten micro-particles are coated with transgene constructs and fired into target cells or tissues. In the early experiments the guns were powered by gunpowder, but today the particles are accelerated with an electrical discharge or compressed helium gas. One or more copies of the transgene construct are integrated into the chromosomes of the target cells. Such methods initially required a sophisticated laboratory environment. However, portable hand-held guns have recently been developed to make the technology more widely available.

2.17 All plant transformation methods in use today suffer from the fact that the transgene(s) cannot be directed to any particular point on the host chromosomes. Incorporation into the host DNA is more or less at random. Since the location of the transgene in the host's DNA can affect the efficiency with which it is expressed, it is often necessary for the researcher to produce many individual transgenic plants to ensure that an effective breeding group or line with the desired characteristics can be selected from them. These plants will then be bred conventionally.

Selectable markers

2.18 Some of the debate about GM crops concerns the marker genes co-introduced with the transgenes. Several exotic markers have been used as research tools, for instance, GUS, a gene encoding ß-glucuronidase, can be identified in stained material by a blue colour. However, in practical plant improvement programmes, markers have been largely restricted to proteins providing resistance to herbicides or antibiotics. Putative transformants can be sprayed with, or grown on, media containing the appropriate chemical. Transformed plants are identified as those that survive. Critics of GM technology argue that even if marker genes are avoided, the resulting lines are still likely to contain small segments of non-coding, non-native DNA, which initially flanked the construct in the vector. The presence, size and any possible function of such inserts are always considered in the UK regulatory approval process (see Chapter 7).

2.19 Markers are used only to make the detection of transgenic plants easier. Removal of marker genes from such plants is technically possible but extremely difficult, although methods are being developed to do just this. However, in situations where the presence of the transgene itself can be detected easily or when efficiencies in transgenic production become high enough, then the use of markers can be dispensed with. Efficiencies as high as 5% are now being obtained and, at these rates, it is feasible to screen directly for the unique DNA sequence that describes any gene. It is likely, therefore, that selectable markers (which include genes that confer antibiotic resistance) will cease to be an issue with the next generation of transgenic releases.

Applications of plant transformation

2.20 GM plants are used or will be used in a number of different ways. Research applications are increasingly important. For example, GM plants are developed to try to identify gene function by

simply seeing whether the introduced gene has any observable effects. Transformation is now so routine in some model species that it can be used as a tool to identify which fragment of a plant's DNA contains a gene of interest. When other experiments have narrowed the possibilities down to a stretch of DNA which contains, say, 100 genes, the critical gene may be identified by breaking the fragment into smaller pieces and firing these into the plant to see which have the desired effect. These sorts of applications are not considered here in detail.

2.21 The most important use of GM plants is to accelerate plant breeding: here, native genes and promoters, previously isolated from the target species, are placed directly into an otherwise ideal varietal background. Although this can also be achieved by repeated backcrossing,[12] it might take ten generations to meet the purity standards required by the Plant Breeders Rights legislation in the UK. The products of the transgenic strategy will be virtually indistinguishable from those of conventional breeding. However, the stringency of the DUS[13] regulatory procedure means that varieties that have been developed elsewhere may take several years before they can be licensed in the UK, and this applies particularly to the new varieties of oilseed rape that are currently being developed. Quite apart from the issues that are specific to GM plants, these crops will not be available for commercial planting in the UK for several years because of the need, for example, to show that their yield is higher than that of current cultivars (see Box 3.1). One example of using genetic modification to accelerate plant breeding is the manipulation of storage-protein genes in wheat to improve bread-making quality. A further example, soon to be in agricultural use, is the transfer of a bacterial blight resistance gene, *Xa21*, from a wild relative to cultivated rice where it was found to confer resistance against most, if not all, races of the pathogen.

2.22 *Antisense transformation:* this technique eliminates the effects of unwanted genes. If a gene is inserted into a plant in reverse (antisense) sequence, the transcribed antisense RNA (ribonucleic acid) product will often interfere with the function of similar native genes. This property can be exploited to remove or suppress the effects of any gene or group of similar genes. In some situations a similar result can be achieved by mutating the target gene and rendering it functionless, but this conventional technique is much slower and requires considerable resources for the necessary screening. An example of the use of antisense transformation is the development of a transgenic tomato with delayed ripening and longer shelf life. In this case, the gene controlling production of an enzyme which promotes cell wall breakdown after ripening was knocked out by use of the tomato gene in an antisense sequence. As a result the tomatoes stay firmer for a longer period.

2.23 *Transformation with beneficial genes isolated from other plants:* this procedure, also called inter-specific transfer, provides a means of circumventing natural breeding barriers. This application is not in wide usage, simply because the identification and supply of useful genes from other plants is limited. However, the complete DNA sequences of the model plants *Arabidopsis* and rice will soon be available, so increasing the availability of a large number of plant genes (paragraph 3.41). One of the eventual goals of the plant breeder is transfer of the genes conferring apomixis (the ability to produce seed without going through normal sexual reproduction) to crop plants. Other examples include the use of plant genes to modify starches and oils.

2.24 *Transformation using genes isolated from bacteria or viruses:* at present this is a widely used approach because many genes have been identified from these sources. Examples include the insect-resistance genes and herbicide-tolerance genes, currently used in the US in the production of corn, cotton, soya and potato varieties. Although these genes are commonly spoken of as bacterial or viral in origin, the genes that are eventually used to transform crop plants are considerably

12 Backcrossing is the process by which an FI hybrid, made by crossing two parent plants, is crossed back to one of the parents.
13 DUS are the criteria needed for a new inbred variety to be approved for Plant Varieties Rights regulations in the UK. These are: distinctness – is it different from anything already available on the market? uniformity – are all the seeds exactly the same? and stability – is the variety stable over several generations?

modified. DNA is broadly similar in plants, bacteria and animals. However, even between narrow-leaved and broad-leaved plants, there are some differences between the preferred sequence of the DNA components. To accommodate this variation, transgenes are usually reconfigured, or 'optimised' and resynthesised. As a result, transgenes may bear as little as 60% identity to the original gene, although the differences will not often alter the amino acid sequence of the protein that is produced.[14]

New transformation technologies

2.25 *Switch technology:* as outlined in paragraph 2.11, the promoter or regulatory sequence at the beginning of a transgene construct determines where and when in the plant the gene will be turned on. Some promoters will respond to an externally applied stimulus, such as a chemical application. Developments in this area are known as 'switch technology' and offer the possibility of switching genes on only when they are needed, for example when a particular disease is prevalent, or when the weather is such that a decrease in crop quality can be expected. If the switch is a commercially available chemical then the same technology offers farmers the opportunity to use the technology only when needed. The seed producer can, in a similar way, restrict the use of farm-saved seed or the transfer of the gene to other varieties. These techniques have not yet been commercialised.

2.26 *Gene use restriction technology (GURT):* one extension of switch technology is the production of transgenic plants that make lethal proteins late in seed development.[15] This modification, dubbed 'Terminator' ensures that the seed cannot be germinated, at least not without application of a proprietary chemical stimulus. The advantages to the seed producer are obvious, as the farmer must then purchase new seed every year. The use of this or other similar technologies would prevent gene flow into other plants being grown near by, since they would produce no viable seed. GURT is still in the early stage of development (a patent is owned jointly by the USDA (US Department of Agriculture) and Delta and Pine Land Co., a US (United States) company currently under offer from Monsanto). It has been severely criticised as a technology which will disadvantage poor farmers, particularly those in developing countries, who will not be able to afford to buy new seed of this type and will have to rely on conventional sources.[16] A similar situation also arises from the current use of F1 hybrids which are sown in parts of the developing world.[17] Critics also argue that gene transfer to nearby 'non-Terminated' crops, particularly in outcrossing species such as maize, could lower the productivity of farm-raised seed. The risk of this happening will depend on the nature of the gene(s) in GURT. If the gene(s) are dominant, then any hybrid seed produced from low levels of cross pollination with nearby crops will not germinate. If the genes(s) are recessive, there is a possibility of low level accumulation in farm-saved seed from nearby fields. Any future application of GURT technology will need to be carefully monitored to avoid these potential problems.

2.27 The owners of the technology would argue that the protection offered by GURT technology might be the only means by which they could get proprietary genetic improvements incorporated into

14 Fujimoto H, Itoh K, Jamamoto M, Kyozuka J and Shimamoto K (1993) Insect-resistant rice generated by introduction of a modified delta-endotoxin gene of *Bacillus thuringiensis* (Bt), **BioTechnology**, 11:1151–1155. DNA is made up of base-pairs. Groups of three base-pairs code for individual amino acids. The amino acids are then linked together to form proteins.

15 US Patent 5723765, Oliver *et al*. (1998) **Control of Plant Gene Expression**, Delta and Pine Land Co. and USDA.

16 Edwards R (1998) End of the germ line, **New Scientist**, No. 2127:22.

17 F1 hybrids have been developed for a range of crops and are used by farmers despite the fact that seed cannot be saved because such crops can offer multiple disease resistance, superior yields and improved yield ceilings. This is true in both developing countries such as India (where rice F1 hybrids developed by the International Rice Research Institute (IRRI) are proving popular) and developed countries like the US (where F1 hybrids have revolutionised corn production).

both developed and developing country agriculture without loss of their intellectual property. This type of technology could be protected by payment of an 'annual technology fee' in developed countries, a process which would be impractical in most developing countries. Moreover, while national programmes and the Consultative Group on International Agricultural Research centres (CGIAR)[18] continue to breed locally adapted varieties there will always be free choice for farmers, and the decision to grow or not to grow 'Terminated' crops will be a purely commercial one. The Working Party considers that it is very important for this and other reasons, that the CGIAR centres, which have already barred the use of GURT in their programmes,[19] and other national programmes continue to produce new varieties (see paras 4.39–42, 4.74–75, 8.54).

2.28 *Multiple co-transformation:* it is possible to introduce several genes simultaneously. Although the mechanism is not known, it has been observed that multiple groups of transgenes (up to 20), delivered at the same time tend to integrate in tandem at the same location on the chromosome.[20] This will increase the ability of the plant breeder to introduce more than one transgene at a time when multiple genes are needed to produce the desired result.

2.29 *Chloroplast transformation:* it is possible, although still technically difficult, to insert transgenes into chloroplasts and amyloplasts, plastids which are present in many copies in some plant cells, rather than into the nuclear genome.[21] Because of the large numbers of such plastids which would have to be transformed and the potential difficulties associated with controlling gene activity in a non-nuclear location in the cell, chloroplast transformation may not be appropriate for all transgenic applications. However, an advantage is that such transgenes are unlikely to be spread to wild relatives or other crops through the pollen, because pollen carries DNA from the nucleus, rather than from chloroplasts.

Potential problems with GM crops

2.30 *Side-effects:* when a genetic system is perturbed by the introduction of a transgene with a new or modified effect, it is possible that unexpected pleiotropic effects (side-effects) will be encountered. Yet, the situation with transgenes is no different from genes introduced by traditional varietal hybridisation and selection. Moreover, the several years of trials that are necessary prior to crop registration in the UK should allow any such side-effects to be identified and the new variety rejected.

2.31 *Gene silencing:* scientists have, as yet, no control over where in the plant's chromosomes a transgene will integrate. Some regions of the plant genome contain large domains of non-coding DNA, which will be highly methylated.[22] Transgenes inserted into this part of the DNA are prone to become methylated themselves, and eventually to cease to function, although this may take several generations. Gene silencing is effectively non-reversible and the GM plant will revert to the way it was before it was modified.

2.32 *Instability:* in practice, any set of genetic engineering experiments will yield a range of plants, some stable and some less so. The plant breeder will select on the basis of efficiency and stability and then, over several generations, breed the modified plant types into closely related varieties. Then,

18 The CGIAR system comprises sixteen international research institutions, with a principal mandate for increased, more robust and more sustainable agricultural production, especially of food staples in developing countries.

19 Anon (1998) CGIAR acts on 'terminator technology' **CGIAR News**, December, 3.

20 Hadi M, McMullen M and Finer J (1996) Transformation of 12 different plasmids into soybean via particle bombardment, **Plant Cell Reporter**, 15:500–505.

21 A plastid is an organelle which carries its own DNA and is contained in the cytoplasm of a plant cell.

22 Methylation is a natural mechanism by which many species, including humans, regulate when genes are turned on and off in particular cells, tissues or whole organisms. Some of the base pairs in DNA can have additional methyl groups added through the action of cellular enzymes. Such methylated stretches of DNA are then inactive.

before release, the new variety will be tested at many different locations over several years. It will only then be approved if it meets the UK DUS criteria (see paragraph 2.21). These controls alone would ensure that GM plants prone to being silenced would be identified and excluded early in the breeding process. However, it should also be noted that it is not in any company's interest to market an unstable product. To do so would involve them in lawsuits and compensation costs which would be prejudicial to their market share in future years.

2.33 *Resistance breakdown:* disease or pest resistance conferred by a transgene can become ineffective. Many plant disease resistance genes are specific to particular pathogen strains. This means that growing such crops becomes, effectively, an ideal environment for the rare mutant in the pathogen or pest population that can overcome the resistance gene and that such mutants would prosper. Strategies to avoid, or at least delay, this outcome include the use of multiple resistance genes or the cultivation of small areas of susceptible crop varieties to provide refuges in which the non-resistant pathogen or pest may persist. As a result, resistance to the genetic modification will develop more slowly. However, the conventional use of pesticides sprayed on crops encourages resistance in a similar fashion. GM sources of resistance are therefore likely to be no different from conventional resistance genes. Resistance genes derived from Bt, for example, are very specific in their ability to kill certain insect pests but are likely to be overcome by resistant insects in due course.

2.34 In summary, regulatory procedures, outlined in Chapter 7, take account of these problems which may arise during the development of GM crops. Specific concerns about human health are discussed in Chapter 5 while broader environmental concerns are discussed in Chapter 6.

Testing for transgenics

2.35 The controversy concerning the segregation of GM from non-GM products has raised the question about whether reliable tests to identify such transgenic materials are available. For example, there might be a requirement for appropriate testing of plant materials, such as seeds, fruit or leaves, or of plant products, such as sugar or starch. There is no test for products which originate from GM plants, but which do not contain GM DNA or proteins, and are chemically identical to the product from the unmodified plant. For example, sugar produced from GM beet or cane plants cannot be distinguished from that produced by non-GM plants.

2.36 Yet DNA tests, similar to those used by researchers to identify GM plants, are simple to set up and will work on any plant material. These use the polymerase chain reaction (PCR) to amplify a fragment of unique and diagnostic transgene DNA. The test can be sensitive to a fraction of 1% and can be used to test mixtures of foodstuffs. The tests will also work on cooked material because, whilst high temperatures break down DNA, a few, often partially degraded molecules, always remain and are adequate for a PCR test. The key requirement is prior knowledge of the precise DNA sequence of at least part of the transgene.

2.37 This latter requirement is likely to provide a stumbling block as more and more gene constructs are used by commercial breeding companies. Where isolated genes are patented, the complete DNA sequence is published and therefore appropriate diagnostic tests can be devised, although the information may take some years to appear in the public domain. Where an isolated gene is kept and deployed as a trade secret, the sequence may never be in the public domain and a reliable test will not be obvious. Moreover, as more and more transgenes are incorporated into breeding programmes, they are likely to accumulate through the normal process of crossing and selection. The diversity of transgenes and promoters available is likely to make unequivocal testing for the presence of genetic modification impractical in the not too distant future.

How far will the science progress?

2.38 In plants, the first genes to be manipulated were those for herbicide tolerance. There were several reasons for this, the first being that it was possible. The genes for herbicide tolerance are single genes and therefore much easier to isolate and manipulate than the multigene complexes responsible for such important traits as salt tolerance and drought resistance. Secondly, it made sense to the companies who were to finance the research and development (R&D), since herbicide tolerance is about creating a selective herbicide from a non-selective herbicide. Such herbicide-tolerant GM plants are examined for safety by regulatory authorities. Thirdly, although modern selective herbicides are very effective they are expensive and, unlike the broad-spectrum herbicide 'Roundup', can persist in the soil. A number of these GM herbicide-tolerant crops are now being grown, and soymeal from Monsanto's herbicide-tolerant 'Roundup ready' soybeans is already on the European market.

2.39 Biotechnology has the capability of producing many new plant products. A number of different types can be described:

- application of a range of gene-inactivating techniques to reduce the activity of or switch off specific unwanted genes (paragraph 2.22). These might be fruit softening, toxin or allergen genes;
- introduction of new plant genes or enhancement of existing gene action to improve starch or oil yield, modified oils or starches, enhance fruit flavour, colour or nutrition;
- introduction of genes to confer resistance to herbicides, pests or pathogens, or to enhance resistance to environmental stresses like drought, heat or cold;
- introduction of new plant genes to enhance the production of hybrid crops or to modify seed production by inducing apomixis, so that hybrid vigour can be effectively 'fixed' for harvest and resowing (paragraph 3.39).

2.40 It is very difficult to predict exactly when these new developments will become commercially available, but it is possible to arrange them in an approximate time sequence:

- continued development of rapid genetic typing methods to speed conventional plant breeding systems, leading to the identification of genes responsible for desirable traits, and their transfer to other species, for example between cereals;
- continued development of genetic manipulation, along the lines of herbicide tolerance, involving one or more genes, with the production of plants resistant to many herbicides, and a wide variety of pathogens, including viruses, bacteria and fungi, thus greatly reducing or eliminating the huge losses due to these agents;
- continued development of novel fertility systems, leading to the production of new F1 hybrids, with increased yields;
- continued development of fruits and vegetables with longer shelf-lives and better shipping characteristics;
- modification of crops to produce oils with properties more suitable for industrial use, fats more suitable for the human diet and modification of starches and other carbohydrates for either dietary or industrial use;
- isolation of genes that control development to manipulate flower shape and colour for the horticultural industry. Mauve carnations are already available. Other applications are possible such as blue roses, geraniums that smell of roses or lawns that (almost) never need mowing;

- genetic modification of fruits and vegetables with the aim of improving flavour, texture and nutritional content and, in particular, to ensure that levels of the micronutrients that appear to be increasingly important for health are either maintained or introduced at appropriate levels;

- elimination of genes for toxic or allergenic substances (peanuts can cause a fatal allergic reaction in some people); for example, by the use of antisense technology to block the activity of genes;

- isolation and utilisation of more complex genetic systems such as those controlling salt tolerance and drought resistance, making possible the production of plants which can be grown in a much wider range of environments;

- isolation and modification of genes that control plant development and differentiation; for example, the plant's flowering time, so that it may be possible to produce plants that come to maturity more quickly, or plants such as oilseed rape that could be grown further north in countries like Canada and Sweden, and aspen trees that are fertile within the first year. Conversely, it would be advantageous sometimes to delay flowering, in annual non-seed crops such as lettuce and potato;

- as timber and pulp increasingly come from cloned plantations they could be modified for pest and disease resistance, and have their juvenile period substantially reduced to aid breeding programmes;

- production of drugs and vaccines in plants;

- introduction of new genetic systems into the plant to increase yields by, for example, modifying photosynthesis or enabling crops such as wheat to fix nitrogen;

- application of GM technologies to bring orphan crops, particularly in tropical developing country agriculture, into commercial production.

- production of plants for cleaning up polluted areas.

2.41 To take a specific example, genetic modification of potatoes could:

- increase the availability of UK varieties by extending the growing seasons through the introduction of stress tolerance characteristics;

- improve flavour and mash texture through modification of starch and sugar content;

- reduce the water content in potatoes and alter cell-wall composition to limit the fat retained in crisps and chips;

- extend shelf-life by suppressing sprouting and reducing rot;

- reduce chemical residues by introducing herbicide tolerance, disease- and pest-resistance traits.

2.42 During the period from 1986 to 1997, approximately 25,000 transgenic crop field trials were conducted on more than 60 crops with 10 traits in 45 countries. No adverse effects on food safety or the environment have been noted, relative to production in non-GM current varieties. Of this total of 25,000, 15,000 field trials were conducted during the first 10-year period and 10,000 in the last two-year period. Seventy-two per cent of all transgenic field trials were conducted in the US

Table 2.1

Traits already commercialised in field trials, and under development for selected crops in 1997

Crop	Traits already commercialised	Traits in field trials/development
Canola (oilseed rape)	1 Herbicide tolerance 2 Hybrid technology 3 Hybrid technology and herbicide tolerance 4 High lauric acid	1 Improved disease resistance 2 Other oil modifications
Corn	1 Control of Corn-borer 2 Herbicide tolerance 3 Insect protected/herbicide tolerance 4 Hybrid technology 5 Hybrid/herbicide tolerance	1 Control of Asian Corn-borer 2 Control of Corn Rootworm 3 Disease resistance 4 Higher starch content 5 Modified starch content 6 High lysine 7 Improved protein 8 Resistance to storage grain pests 9 Apomixis
Cotton	1 Bollworm control with single genes 2 Herbicide resistance 3 Insect protected/herbicide tolerance	1 Bollworm control with multiple genes 2 Control of Boll Weevil 3 Improved fibre/staple quality 4 Disease resistance
Potato	1 Resistance to Colorado Beetle	1 Resistance to Colorado Beetle + virus resistance 2 Multiple virus resistance (PVX, PVY, PLRV) 3 Fungal disease resistance 4 Higher starch/solids 5 Resistance to potato weevil/storage pests
Rice		1 Resistance to bacterial blight 2 Resistance to rice-borers 3 Fungal disease resistance 4 Improved hybrid technology 5 Resistance to storage pests 6 Herbicide tolerance
Soybean	1 Herbicide tolerance 2 High oleic acid	1 Modified oil 2 Insect resistance 3 Virus resistance
Tomato	1 Delayed/improved ripening	1 Virus resistance 2 Insect resistance 3 Disease resistance 4 Quality/high solids
Vegetables & Fruit	1 Virus resistance	1 Insect resistance 2 Delayed ripening

Source: James C. (1997) **Global Status of Commercialised Transgenic Crops in 1997. ISAAA Briefs No.5**, ISAAA, Ithaca.

and Canada. By the end of 1997, 48 transgenic crop products, involving 12 crops and six traits, were approved for commercialisation in at least one country by 22 owners of technology, of which 20 were private-sector operators.[23] The crops include soybean, cotton, oilseed rape, potato, maize,

23 James C (1997) **Global Status of Transgenic Crops in 1997, ISAAA Briefs No. 5**, ISAAA, Ithaca, New York. ISAAA is the International Service for Acquisition of Agri-biotech Applications. It monitors and evaluates the availability of biotechnology for transfer to the developing world. In addition, work is being undertaken in the developing world to

tomato and pumpkins, and the traits insect, virus and herbicide tolerance, delayed ripening, male sterility and changes in oil composition (Table 2.1).

2.43 There are several other crops where transformation could be agronomically valuable. Wheat has been technically difficult to transform, but GM wheat is expected to enter the market soon. Research on the genetic modification of rice, cassava, yam, pearl millet and sorghum is being undertaken in public-sector institutions.

How far have GM crops entered agriculture?

2.44 The total area planted with GM crops in 1998 is shown in Table 2.2. In 1998 approximately 28 million hectares were planted with transgenic crops, mostly in the US, where 20.5 million hectares were sown, representing 74% of the global total of transgenic crop plantings. This figure was up from 11 million hectares in 1997 and 1.7 million hectares in 1996. These are extremely high adoption rates for a new technology by agricultural standards. Argentina grew 4.3 million hectares of GM crops in 1998, a three-fold increase from 1997.

Table 2.2

Global area of transgenic crops in 1998: by crop (millions of hectares)

Crop	1998	%
Soybean	14.5	52
Corn/Maize	8.3	30
Cotton	2.5	9
Canola (oilseed rape)	2.4	9
Potato	<0.1	<1
Total	27.8	100

Source: James C. (1998) **Global Review of Commercialized Transgenic Crops: 1998. ISAAA Briefs No.8**, ISAAA, Ithaca.

2.45 The principal reported agricultural benefits of these GM crops include more flexibility in crop management, decreased dependency on conventional insecticides and herbicides, and higher yields and cleaner and higher grade of harvested product. In 1997, the economic benefit to US farmers was estimated at US$133 million for Bt cotton, US$119 million for Bt corn and US$109 million for herbicide-tolerant soybean, with an overall total of US$360 million, up from US$159 million in 1996.

2.46 Why is the cultivation of GM crops growing so quickly? US farmers consider that herbicide-tolerant soya offers them real advantages. In the US, where springtime sowing is normal, the use of a post-emergent herbicide has meant some changes in agronomic practice, leading to retention of more soil moisture. This, together with the slightly longer growing season and the effectiveness of the herbicide, has resulted in significantly higher yields. Consequently, farmers in the US will soon be growing GM crops on a wider scale. These new crops will bring a much closer relationship between the farmer and the agrochemical company, which will sell both seed and herbicides, and also a similar closer relationship between the farmer and the retailer, as complete traceability will be essential. There may also be a need for a licensing system to monitor and, if necessary, deal with environmental issues.

assist national programmes, to identify priority needs for biotechnology applications, and to develop and implement priority proposals.

2.47 The situation is very different in Europe where there have been almost no commercial plantings. The EU's approval process for novel crops is slow, causing tensions with the US over the delay in permitting imports of GM food supplies.[24] There have also been difficulties in defining which products have to be labelled and how. These problems are discussed further in Chapter 7.

Issues arising from the introduction of GM plants

2.48 *Antibiotic resistance:* this issue arose in connection with a GM-maize variety produced by Ciba-Geigy (now Novartis). The UK Advisory Committee for Novel Foods and Processes (ACNFP) recommended against authorisation of this product for animal feed, its only projected use. This was because of the perceived risk associated with the transfer of an antibiotic-resistance marker gene (paragraph 2.18) in the maize to the bacterial gut of livestock that had been given the feed. If the antibiotic in question (ampicillin) was present in the animal feed, there was perceived to be an eventual possibility of transfer of the resistance gene to humans through transfer of resistant bacteria to those in contact with the cattle, although this has not been observed. The widespread use of antibiotics in animal feed, coupled with their widespread clinical use has already led to an alarmingly high level of antibiotic resistance in bacteria which infect humans. The debate centred on whether that figure was already so high that a very small increased risk would be of little or no significance, or whether the high level meant that no increase, however marginal, should be permitted. The ACNFP took the latter view, influenced by the potential serious outcome of an event which although very unlikely, was not impossible. The Royal Society, in its recent statement on GM plants for food use,[25] reached a similar conclusion, as did a poll conducted through the Newsletter of the International Society of Chemotherapy[26] where 57% of the 198 Society members who responded opposed the use of this particular antibiotic marker gene, with a further 34% taking the view that the risk of resistance-gene spread was low but finite.

2.49 This recommendation was later overruled by the European Commission (EC) on a majority vote, since the maize was only to be used for animal food, and for production of starch for some processed food products, and such processing degrades the DNA so that it is no longer functional. The EC gave permission to allow marketing of the seed in January 1997, and 1000–2000 hectares were grown that year. However, in February 1998, Greenpeace applied to the French courts to overturn the issuing of the consent. The Conseil d'État issued an injunction preventing the marketing of the maize until the case put by Greenpeace had been resolved by consulting the European Court of Justice. This process is likely to delay the growing of this maize by at least a year, although 15,000 hectares of maize were grown in 1998 in Spain. GM tomatoes, which contain a kanamycin-resistance gene in a form which did not cause concern to regulators, are on trial in Spain, but a commercial permit has not yet been issued.[27]

GM DNA transfer in animals

2.50 Concerns have been expressed that the DNA introduced by genetic modification might be transferred across the wall of the gut to the host, and lead to genetic alteration of that host, despite the fact that we eat large amounts of degraded and undegraded DNA in our everyday diet. Experiments have

24 House of Lord's Select Committee on the European Communities (1999) **EC Regulation of Genetic Modification in Agriculture** (Session 1998–99 2nd Report), p. 46. The Stationery Office, London.
25 The Royal Society (1998) **Genetically Modified Plants for Food Use**, p. 8. The Royal Society, London.
26 Pechére J-C (1997) A β-lactamase gene in a transgenic maize? **Antiobiotics Chemotherapy**, 1:9; Pechére J-C (1998) Concerns about the presence of a β-lactamase gene in a transgenic maize, **Antibiotics Chemotherapy**, 2:16.
27 House of Lords Select Committee on the European Communities, **EC Regulation of Genetic Modification in Agriculture**, p. 11.

shown that DNA consumed in the diet is very unlikely to survive intact beyond the stomach and into the gastrointestinal tract. That DNA which remains after digestion consists of very small fragments which do not contain whole genes. However, some experiments have shown that these fragments may enter the blood stream[28] and that small amounts may even enter cells and attach to cellular DNA.[29] Such DNA fragments would not function in the human or animal because of their small size. Furthermore, no evidence of active ingested genes, even those designed to work in human cells, has been found.[30]

2.51 *Toxins:* a number of plants produce toxins as a protection against insect and fungal pests and it is for this reason that we cook many foods such as potatoes. These are parts of their innate defence systems and, as such, are important to maintain. They are generally present at such low levels that humans and animals are able to tolerate them. Plant breeding, either with or without the aid of genetic modification, may be used to remove toxins or allergens in existing food crops. Such toxins are almost always bred out during development of commercial varieties.

2.52 It is always possible, however, that toxin levels could be increased by such breeding. For example, a hardy new potato variety called Lenape, produced by conventional breeding in the 1960s, owed its unusual burnt flavour to dangerous levels of toxins called glycoalkaloids, and was subsequently withdrawn.[31] Questions about toxins are always asked by UK regulatory committees in the consideration of submissions for entry into the human food chain.

2.53 *Allergens:* there is one documented case where genetic modification involving transfer of a gene from the Brazil nut to soybean also led to transfer of allergenicity.[32] Blood serum from people known to be allergic to Brazil nuts was tested for the appropriate antibody response to the transferred gene. Seven out of nine individuals showed a positive response. This adverse result alerted the company and the work was discontinued so the product was not even submitted to the regulatory authorities. The Working Party notes that the potential allergenicity of proteins expressed by novel genes is now a routine part of safety assessment procedures and that there are many databases of known allergens that could help identify proteins that may be problematic if inserted into food products. However, since the generation of new allergens can never be excluded, the Royal Society, in its report, sensibly recommends that this topic be given particular attention.[33]

2.54 When an application to market a GM variety for cultivation in the EU is submitted, information on likely toxic or allergenic effects must be included in the application. Continued care is needed in this area, and if there is any reason to suspect an allergenicity problem, then the appropriate health network can be alerted. It should be noted that the EU Novel Food Regulations specifically require that products must be clearly labelled if they contain genes that may result in toxicity or allergenicity, particularly if such genes would not normally be expected to occur in the food.

2.55 *Feeding trials with foods from GM crops:* some critics of the use of genetic modification in food production have argued that all such foods should be subjected to the same sort of safety assessment as new drugs. In particular, there have been calls for testing through long-term feeding trials. However, this is not easy and the difficulties have been well explained in a recent article.[34]

28 Schubbert R, Lettmann C and Doerfler W (1994) Ingested foreign (Phage M13) DNA survives transiently in the gastrointestinal tract and enters the blood-stream of mice, **Molecular and General Genetics**, 242:495–504.
29 Schubbert R, Renz D, Schmitz B and Doerfler W (1997) Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA, **Proceedings of the National Academy of Sciences**, 94:961–966.
30 Cohen P (1998) Strange fruit, **New Scientist**, 2158:42–45.
31 Ibid.
32 Nordlee J, Taylor S, Townsend J, Thomas L and Bush R (1996) Identification of a Brazil nut allergen in transgenic soybeans, **New England Journal of Medicine** 334:688–692.
33 The Royal Society, **Genetically Modified Plants for Food Use**.
34 For an example of a criticism of this argument see MacKenzie D (1999) Unpalatable truths, **New Scientist**, No. 2182:18–19.

The article reported that scientists from the 29 industrialised countries of the OECD concluded at a meeting in Paris in December 1998 that a whole new approach would be needed (if such a process were to be developed). For example, it is not possible to test foods at 100–1000 times the likely intake, as is done with new drugs, in order to ensure safety. This is because foods cannot be fed in such exaggerated doses without profound effects on the subject's physiology. The best that can be done is to replace the 'normal' component of the diet with the novel food and look for any adverse effects. Companies have done this a number of times during the regulatory assessment of novel foods by the ACNFP.

2.56 Because of these difficulties, the ACNFP's usual procedure for evaluating a novel food is to compare its composition with that of the conventional food it most closely resembles and which has been in the diet for many years. The question is then asked, 'Are there any differences between the two which might cause a problem?' This is the process called 'substantial equivalence' and is described in more detail in a recent report.[35] The report concluded, *inter alia*, that 'foods derived from GM sources should be assessed in a similar manner to those produced by conventional techniques'.

2.57 This conclusion has been questioned in a series of experiments conducted by Dr Arpad Pusztai at the Rowett Institute which are outlined in detail in Appendix 1. Initial press releases following the experiments suggested that potatoes which had been genetically modified to contain a lectin (a toxin) affected the growth rate and immune function of rats. The Rowett Institute subsequently withdrew the initial claims and apologised for releasing misleading information. At this stage the results of the experiments had not been submitted for peer review. An independent audit of the experimental results did not support the conclusion that the GM potatoes had an effect on growth, organ development or immune function. Dr Pusztai rejected some conclusions of the audit committee and 20 scientists from 14 countries announced their support for him. These events were accompanied by extensive media coverage, most of which highlighted the purported dangers of GM food and called for a moratorium.

2.58 A number of conclusions can be drawn from Dr Pusztai's work. First, the case for damage to rats in long-term feeding trials is, on published evidence to date, at the most 'non-proven'. Secondly, it is irresponsible to conduct science by press-release, rather than by the processes of peer review and criticism that ensure scientific integrity. Thirdly, the relative responsibilities of the scientist and their host institution are unclear in such a situation, in particular when and how scientists should express their own concerns. Fourthly, it is clear that the UK lacks a public forum in which such debates can be carried out and, as a result, issues that should be resolved by debate have instead resulted in parties talking past each other and directly to the public. Such a forum for debate is badly needed. The role of an overarching body in providing such a forum is discussed in paragraph 8.26.

2.59 *The natural/unnatural boundary:* critics of GM technology itself often state that this methodology provides the breeder with the opportunity to make unnatural combinations of genes. Presumably the perceived boundary between natural and unnatural lies at the limits of sexual compatibility, since the introduction of exotic genes from wild relatives of rice, wheat or Brassica crops has raised no difficulties in the past. What then when the technology is used to move native genes more efficiently through a breeding programme? Is this 'unnatural'? Such distinctions lie at the heart of the public debate, and we trust that what we have said elsewhere (paragraph 2.5) will be helpful, but we believe that there are no clear cut solutions, that such issues can only be settled on a case-by-case basis and that this falls within the remit of an overarching body.

2.60 *Environment:* the potential impact of GM crops on the environment has received much attention in recent years from the scientific community and is dealt with in Chapter 6.[36] In Chapter 6 we discuss

35 **The Nutritional Assessment of Novel Foods and Processes** (1993) HMSO, London.
36 See also the discussion in The Royal Society, **Genetically Modified Plants for Food Use**.

studies on the effects of insect-resistant crops on non-target species, the possible development of pest resistance in insect-resistant GM crops, the risk of transfer of genes to wild relatives and non-GM crops and the assessment of risk where GM virus-resistant plants are being developed. In reaching a balanced perspective it is important that any negative effects are judged in relation to those of the conventional insecticides and herbicides which these crops are intended to replace.

Conclusions

2.61 The Working Party concludes that the genetic modification of crop plants does not differ to such an extent from plant breeding as practised in the past as to make the process morally objectionable. GM technology is a new tool which plant breeders are using to achieve their breeding goals more accurately and rapidly. The Working Party accepts that combinations of, for example, bacterial and plant genes are being produced in GM crops which are very unlikely to be found or impossible to realise in nature. However, provided that caution is exercised with respect to potential side-effects such as allergenic reactions, we do not consider that the generation of such new combinations should be further restricted or even prohibited. Yet, the novelty of the technology together with broader public concerns leads us to conclude GM crops should be recognised as such and that specific GM regulations should be maintained for several years.

2.62 Most people lack the opportunity to gain an understanding about the science involved in the creation of GM crops and the differences between them and non-GM crops. They also lack a way of explaining their fears and concerns to those responsible for the development, production and sale of such crops. We suggest below some institutional arrangements that could deal with both these issues.

2.63 We also acknowledge that the credibility of the government information on food safety has been so badly impaired in recent years that it may be more expedient for non-governmental entities, supermarkets and food manufacturers to take on some of the task of informing the public. The public's distrust of information from those with vested interests, however, suggests that companies marketing GM crops carry little weight with them. In fact, companies' efforts to persuade people of the benefits of GM crops are probably counter-productive. This may also be true of attempts by food manufacturers that go far beyond the provision of simple and balanced information.

2.64 The Working Party considers that it is wrong to ignore public unease about GM crops, whatever its basis. We consider it very important that the Government take steps to acquire and disseminate reliable and up-to-date information about the underlying science, and also to respond to public concerns. So we welcome the formation of the Cabinet Ministerial Group on Biotechnology and Genetic Modification and the initiation of a review jointly by the Cabinet Office and the Office of Science and Technology (OST) of the framework for overseeing developments in biotechnology and genetic modification.

2.65 We next urge the scientific community to continue to bear its share of the responsibility for the task. Much has already been done through the OST programme called 'The Public Understanding of Science' initiative, but we believe that many such initiatives have been independent of each other, that they could be better co-ordinated, and that there has been little exchange of best practice. **We also recommend that the Cabinet Ministerial Group on Biotechnology and Genetic Modification initiates a wide-ranging review of the scope, co-ordination and effectiveness of the several current 'public understanding of science' initiatives with a view to achieving the best use of the available resources.**

2.66 But it also very important that the scientific community listens to and understands the concerns and fears of the consumer public, and in this the role of the social scientist is crucial. **The Working Party recommends that the UK Research Councils, COPUS, the Royal Society, the**

Institute of Biology, the UK Life Sciences Committee, and industrial bodies such as the BioIndustry Association and others, examine how they can work together to continue their development of both new and ongoing mechanisms in which scientists would be able to engage better with the public.

2.67 **We further recommend that the Government takes an initiative to bring relevant experts and consumer public together, possibly along the lines of the UK National Consensus Conference on Plant Biotechnology,**[37] **to seek to understand the underlying concerns and to propose a way forward.** However, the most urgent need is to draw together, in a single decision-making process, three different strands: scientific assessment of risk, public perception of this risk and the ethical issues involved. We return to these points at paragraph 8.26.

37 Anon (1994) Final Report of the **UK National Consensus Conference on Plant Biotechnology**, The Science Museum, London.