



Genetic modification

SUMMARY Genetic modification (GM) is the application of modern techniques of molecular biology to plant breeding. It enables plant breeders to achieve new combinations of genes that are not possible by conventional methods. The first commercial GM crops have modifications for herbicide tolerance and insect pest resistance, but major developments are expected in many other areas, such as disease resistance and enhanced nutrient content. The techniques have raised two particular technical concerns. Firstly, unexpected side effects of the modifications might occur, allowing synthesis by the plant of unpredicted and possibly undesirable compounds. There is evidence that this may occasionally occur, although routine safety checks have so far successfully detected the problem and prevented commercial release. Secondly, modified gene combinations may spread into the environment and recombine with other plants, again leading to unpredicted novelties. Though there is evidence for spread in some cases, there is no sign of adverse impacts. There is also controversy about the commercial and ethical implications. Opposition to GM crops is strong in Europe but plantings are increasing rapidly elsewhere.



Carnation 'Moonshadow' genetically modified by Florigene Ltd with a gene from a pansy to enhance blue coloration and commercially available in some countries, but not currently within the EU (photo courtesy of Florigene Ltd, Australia)

RHS policy statements

- 1 The Royal Horticultural Society recognises increasing public interest in the potential of genetic engineering to enhance cultivated plants. It is aware of concerns about the practical and ethical implications of such practices.
- 2 RHS scientific staff will keep abreast of the technical developments and interpret them to help gardeners and horticulturists to understand them.
- 3 The RHS is committed to trial and demonstrate genetically manipulated plants in the same way as conventionally produced plants when and if presented for consideration for the Award of Garden Merit or other RHS awards. This will be done in a responsible manner, paying due attention to current legislation and providing appropriate information.
- 4 RHS scientists use some biotechnology techniques for tasks such as plant identification and disease diagnosis. It is not part of the Society's policy to use these techniques to produce genetically modified plants.
- 5 The RHS recognises the legitimate rights of organic gardeners to practise gardening according to organic standards and the legitimate rights of the majority of gardeners who are not adherents of the organic movement to grow GM plants when and if they are approved for sale, and the need to find an accommodation where these rights may conflict.

Genetic modification

First thoughts

In the recent debate on genetic modification where extreme positions have been taken it is difficult to arrive at a consensus, but society will have to make a value judgement. The RHS has no position for or against genetic modification but is committed to providing its members with objective information. Without objective data there is no basis for rational discussion and decision.

What is genetic modification?

Genetic modification or 'GM', also known as genetic engineering or genetic enhancement, applies the modern techniques of DNA technology to the science of breeding. These techniques make possible new combinations of genes that were impossible to achieve by conventional methods. In this leaflet we examine the techniques of genetic engineering, what they can achieve and the issues arising from their use.

What is genetics?

Genetics is the science of heredity, the process whereby the blueprint for an organism is passed through the egg, seed or spore to the next generation. The process is very conservative, ensuring that offspring resemble their parents. Changes to the blueprint in nature usually occur slowly over many generations, by the process of evolution under pressure of natural selection. The information that makes up the blueprint is coded in a chemical molecule called deoxyribonucleic acid (DNA).

In higher organisms, the cells have a nucleus containing sets of chromosomes, usually two sets but sometimes more in polyploids. The DNA is bound up with proteins in the chromosomes and there are therefore usually two sets of information in every cell, one set inherited from each parent.

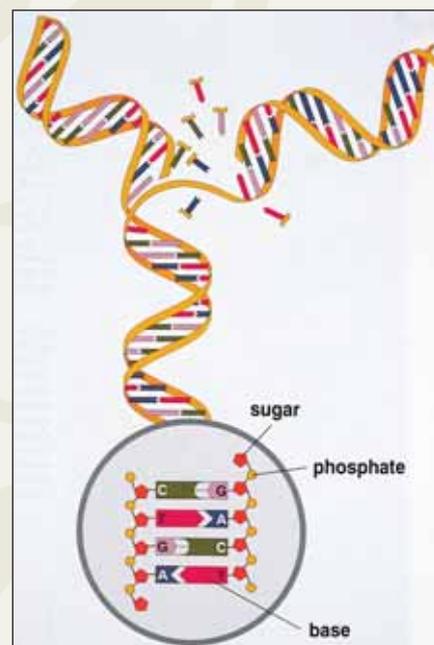
Half the total passes into every gamete (pollen grain, sperm, ovule or ovum) and the full set is thus restored after fertilisation.

DNA is constructed of very long sequences of four related but distinct chemical subunits called bases, as shown in the diagram.

Those sequences that code for specific information are called genes; other sequences do not code directly but may modify how and when genes work, a process known as 'gene expression'. The total information content of the DNA is called the genome. In this leaflet we refer mainly to plants, but many of the basic processes of genetics are universal to all living things and much of the information and discussion also applies to animal and micro-organism genetics.

The sequence of the bases in the DNA molecule is unique to every organism; the degree of similarity between genomes defines the species and, ultimately, the individual – though 'individuality' also has other determinants. The sequence in the gene defines the sequence of amino acids in the protein for which that gene codes – as molecular biologists say, 'genes code for proteins' – and these are fundamental to the structure and function of the cell, and hence, of the organism. Proteins include the enzymes that control the organism's chemical activities, such as the synthesis of component materials and the chemical processes by which the cells liberate energy.

In order to accomplish all these processes according to the 'blueprint' in the DNA and via the activities of enzymes, the cell uses the base sequences in the gene to synthesise complementary molecules, also made of bases, called ribonucleic acids (RNA).



Each DNA molecule consists of two long strands made up of sugar and phosphate subunits and wound round each other in a double helix. Strung along these 'backbone' strands are four nitrogen-containing molecules called bases: adenine, cytosine, guanine and thymine (A, C, G and T). These bases pair off across the helix like rungs of a ladder in a strict pattern, A with T and C with G. Thus the two strands of the molecule are complementary. The sequence of the bases is the language of the genetic code and the sequences that code for proteins are called genes. To duplicate DNA for cell division, the DNA molecule unwinds, breaking the rather weak links between the complementary bases. New bases and the associated 'backbone' units from the cell then link to the bases on each strand, which rewind. Because of the complementary pairing, each of the two reformed molecules is an exact copy of the parent molecule. A similar process occurs when the cell transcribes the sequence during messenger RNA synthesis.

There are several types of RNA and they transcribe, or 'read', the DNA sequences, gather the amino acids from the cell and assemble them in the coded order to synthesise proteins. These processes occur outside the nucleus in cell structures called ribosomes, often likened to cell 'factories', and the RNA is the means whereby the instructions in the nucleus are transmitted to the main body of the cell and put to use.

Conventional genetic manipulations

Humans have been rearranging the genomes of organisms, that is, making

alterations to the information stored in the nucleic acid sequences, for thousands of years. They have done this by the traditional processes of breeding, which have generated the range of domesticated plants, animals and microorganisms in use today. By these conventional methods, plant breeders have selected plants with vastly improved characteristics of yield and flavour compared to the wild relatives. They have used two principal methods. Firstly, they have selected superior specimens from the natural range of variation available and propagated and bred from them. Secondly, by hybridising plants that will do so naturally, or can be persuaded to do so by artificial pollination methods, they have created progenies within species and between species which have new and desirable combinations of genes not available in the wild. More recently, novel techniques such as embryo rescue have also been used for this purpose.

The progenies of conventional breeding combine characteristics of each parent, both desirable and undesirable, and selecting a new variety is a slow and complex process. For example, a wheat breeder will take eight to ten plant generations to select, stabilise, multiply and register a new variety.

The developments that are possible by these methods are astonishing and the wild ancestors may be unrecognisable. For example, a combination of selection within the variation shown by the wild cabbage *Brassica oleracea* and crosses between different geographical provenances of this plant has produced cabbages, cauliflowers, Brussels sprouts and kohlrabi. The wild ancestor is an insignificant weedy species without head, curds, sprouts or swollen stem.

More recently, breeders have used special techniques such as chemically induced chromosome doubling with colchicine, or alterations to the DNA (mutations) induced by chemicals or UV light, to extend the range of gene

exchanges. All these techniques, however, have been limited to exchanges of genes between relatively closely related organisms, because exchanges between more distant or non-relatives are prevented by naturally evolved barriers or subsequent infertility. Thus, for example, it has not been possible to produce a blue rose by traditional breeding methods because no genes for blueness exist in roses or related species in the family and no way could be found to hybridise such genes successfully into roses from other plants.

Genetic modification (GM)

Until the 20th century, conventional breeding techniques worked with genes by observing the effects they have on the organism, but without knowing how those effects were produced or indeed, even that genes existed. Following the work of Mendel and others, the inheritance of some characters like seed colour was revealed to follow simple mathematical ratios and the concept evolved of genes as 'strings of beads' which were heritable in combinations that were predictable, but there was still little idea of how they functioned. Then as biochemical knowledge increased, genes and gene expression became associated with certain biochemical pathways and products, but the way genes controlled these biochemical processes was still obscure.

In the past fifty years, the science of molecular biology has opened the 'black box' of the genome, revealed the instructions it contains and gained many insights into the ways that these instructions are carried out by the cell. Scientists now understand much about how the processes of heredity work, and have an ever-expanding range of techniques whereby those processes can be investigated and modified. This has resulted in radical changes to traditional views of heredity and what constitutes 'natural' processes. Consider the following facts:

1. The great majority of DNA sequences (the basic information) are common to all organisms: >90% of human DNA is homologous to that of the amoeba and at least 98% to that of the chimpanzee, so a very large range of basic functions are common to a huge variety of organisms. Of course, the numbers and kinds of genes made up from DNA, and their functions, do also differ considerably.

2. Individual DNA sequences (genes) can now be moved, in principle, from any organism to any other, and made to function in the recipient (though not all genes are yet known, not all moves are yet possible and the blue rose has yet to appear).

3. Characterised DNA sequences and genetically modified ('transgenic') organisms can be patented, although this is subject to considerable international discussion.

The arrival of these new technologies has raised many possibilities for genetically altering plants in ways that were previously inconceivable. This new technology is loosely described as 'genetic modification' ('GM') or 'genetic engineering'. It is an extension of the older technology of plant breeding and is one example of the larger science of 'biotechnology' which has been defined as:

"any technique that uses living organisms or substances from those organisms, to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses." (Office of Technology Assessment, US Congress).

Again, this very wide definition of biotechnology includes some conventional and even ancient technologies, such as brewing, but it is specifically the developments in molecular biology in the past fifty years that have given biotechnology massive new impetus in most of its applications. Using the modern techniques of gene

transfer and cell cloning, plants have been, or might be engineered to the following ends:

- enhanced and novel resistance to pests and diseases
- resistance to herbicides in plants, and to insecticides in beneficial insects
- facilitated hybridisation, for example by producing male-sterile parents that do not produce pollen and therefore do not need protecting from self-pollination
- modifications to oil/starch/protein synthesis, eg to produce starch as a raw material for industry, to improve the quality of vegetable oils and to improve the breadmaking quality of wheat
- vitamin enrichment: eg 'golden rice' which has been modified with a gene for Vitamin A synthesis to alleviate deficiency of this vitamin
- reduced seed shedding to facilitate harvesting for crops such as oil seed rape
- longer shelf life or cut flower retention, eg in tomatoes and carnations
- novel colours, fragrances and flavours
- improved plant architecture, eg to increase photosynthesis or mechanise harvesting
- modified fruit/tuber structures
- increase stress tolerance, eg cold, salt, drought
- plant 'factories' to produce specialised products, eg vaccines
- bioremediation, eg enhanced uptake of heavy metals to detoxify polluted soil

The ability, actually or potentially, to carry out these previously impossible modifications has raised enormous interest among some biologists, who see the technology as a solution to intractable problems, for example, enhanced and novel resistance to plant

virus diseases. The techniques also open up entirely new possibilities such as using plants as 'factories' to synthesise animal vaccines. However, the technology also raises issues of scientific and moral concern, particularly in the areas of environmental impact, food safety and commercial ethics.

The issues

Three general considerations underlie all the recent debates about genetic modification. These are environmental safety, food safety, and ethical and commercial issues.

1. Environmental safety

Does exploitation of the GM plant on the relevant commercial scale – field crop, horticultural specialist crop, in the open, under glass, in hydroponic systems – or in gardens by amateurs, pose any unacceptable hazard to the environment?

Concerns have been expressed over two main issues: firstly, that GM plants *per se* or parts of them such as their pollen may be more persistent or invasive, or possibly have adverse effects on other organisms and thereby be environmentally disruptive; and secondly that genes from GM plants could flow into natural populations of related plants by normal processes of cross pollination, with unforeseeable and irreversible consequences. The issues are distinct, but both must be considered in every case.

The complexities surrounding risk assessment in cases of potential environmental impact from the GM plants *per se* can be clarified by considering the following three questions, taking into account the nature of the introduced gene and the characteristics of the host plant:

1. will the inserted gene(s) make the host plant more persistent?
2. will these gene(s) make the host plant more invasive?
3. will being more persistent or invasive,

or having other adverse effects on other organisms, make the modified plant or parts of it more undesirable in the environment?

To assess the environmental impact of the second potential hazard, gene flow, a further two questions should be asked:

4. will the inserted gene(s) be transferred to other organisms?
5. if yes, what risks are revealed when the previous questions about persistence, invasiveness and desirability are applied?

For example, oil seed rape has been modified to contain a gene making it immune to the effect of the broad-spectrum herbicide glyphosate, allowing cost effective and efficient weed control. The possibility of enhanced invasiveness or persistence must be considered (questions 1-3 above), particularly if this herbicide was used more widely and more frequently. A different concern is that this gene could enter wild relatives by cross pollination, leading to the production of 'superweeds' with resistance to this herbicide (questions 4-5 above).

For a 'superweed' with glyphosate resistance to arise by gene flow (question 4), the GM plant must be able to cross-pollinate a wild plant relative, produce a fertile cross and then produce progeny which can survive and multiply. Only if all these steps are possible is it then necessary to ask question 5 and assess whether the "superweed" does pose a risk.

In the case of the herbicide resistant oil seed rape, cross-pollination with wild relatives leading to fertile progeny is possible. The possibility that these progeny can survive and multiply is the subject of research, though the presence of the herbicide resistance gene *per se* could only pose a problem in situations where herbicide is used: the threat of the 'superweed' is therefore likely to be to agriculture, not to wild ecosystems.

Herbicide tolerance is not a new



A genetically engineered rose containing a gene from rice which enhances the rose's resistance to blackspot disease.

Acknowledgements: R Marchant, J. A. Lucas, M. R. Davey, J. B. Power (supported by DEFRA).

phenomenon and there are many instances where weeds have acquired tolerance through natural selection pressures and processes. Again, these problems are agricultural and do not affect wild ecosystems. With respect to other crops that may be genetically modified for herbicide tolerance, potatoes, maize and wheat do not produce fertile progeny with any wild relatives in Britain, but sugar beet might do so. As in other areas, hazards must therefore be assessed on a case-by-case basis.

There are also concerns that the use of GM plants such as the glyphosate-resistant oil seed rape could lead to a reduction in biodiversity in the fields, since the herbicide would then kill all plants except the crop, destroying both wild weedy plants and their insect fauna which are an important food source for birds and other wild animals.

Proponents of the technology argue that it will lead to a reduction in overall herbicide use, since farmers currently attempt to achieve weed-free fields with more intensive herbicide applications. Opponents argue that survival of volunteer plants with single or multiple herbicide resistance could lead to a greater use of herbicides. The potential

impact on biodiversity in agricultural systems is an important issue and is the subject of a number of major scientific studies, including the recently completed Farm Scale Evaluations discussed on p7. Data from these studies will help to inform debate and decisions. For a more detailed discussion of the issues relating to GM crops and conservation, consult English Nature or visit their website on <http://www.english-nature.org.uk/news/statement.asp?ID=14>

2. Food safety

Do the products of these engineered plants pose unacceptable hazards to human health?

Concerns that food containing ingredients from GM plants may be unsafe centre principally around two possibilities. Firstly, genes for antibiotic resistance have been used as 'markers' to indicate to researchers when cells have been successfully transformed: cells containing the resistance genes survive exposure to the antibiotics (see definitions: **Tissue culture** and **Marker genes**). If these genes pass into food, it has been suggested that they might pass into gut bacteria and spread undesirable resistance to antibiotics. There is very little evidence that these genes pose a threat to human health, but the concerns have led to the development of technologies to remove them from the final GM plant and also to find replacement markers not based on antibiotics.

Secondly there have been concerns that genes, inserted into a new cell environment along with the accompanying DNA that forms the expression system, may act in unpredictable ways, to form higher and possibly dangerous levels of plant products not present in the unmodified plant, for example, proteins with allergenic properties. It is also feared that such genetic transformations could activate genes not normally functioning in those tissues, so that the tissues

contain plant products not normally found there. Where such changes occur – and there is evidence that they may occasionally do so – toxic products may be detected and then eliminated by normal toxicological tests or by analytical techniques designed to detect proteins and compare them with known examples.

3. Ethical and commercial issues

Should ethical, practical or commercial considerations limit the use of GM technology?

Ethical issues include:

- the rights of other species not to be manipulated in these ways by man
- religious objections to interfering with nature
- the unacceptability to some faiths of the use of genes from taboo animals in food
- the exclusion of GM crops from organic production in Europe under Council Regulation (EC) No. 1804/1999 which regulates organic farming, and the unacceptability of GM plants to some other organisations and individuals
- the freedom to choose 'GM free' food, organic or not, which includes the freedom to choose food that has been genetically modified.

Practical issues include:

- whether genetically modified plants have an advantage to society that outweighs the possible risks in their use
- whether the use of plants genetically modified for pest or disease resistance, for example the maize modified to contain the insecticidal toxin of *Bacillus thuringiensis*, reduces sales of conventional Bt products
- concerns that, if a serious problem arises subsequent to release, the released organism cannot be recalled.

Commercial issues include:

- the acceptability of patents on genes or organisms and concerns about the 'theft' of genes and genomes
- the concentration of commercial power into fewer, larger multinational companies
- the commercialisation of genes taken from developing countries without adequate financial compensation
- the imposition of commercial constraints (to buy seed and herbicides) on developing world farmers.

Thus the debate about the human and environmental safety of GM plants has become linked to wider concerns about the ethics of the commercial concerns involved in their production. These concerns are outside the scope of this leaflet; some pre-date the current GM debate.

Regulation of GM plants

The EU and the UK have detailed legislation governing the deliberate release of GM crops, either for research or for marketing. Releases for research purposes are authorised by each Member State within its own territory, but consent for marketing and commercialisation is a European Union responsibility and must be agreed by the EU as a whole.

In the UK independent expert committees consider all applications on a case by case basis and advise Ministers: the Advisory Committee on Releases into the Environment (ACRE) evaluates the risk to the environment and human health while the Advisory Committee on Novel Foods and Processes (ACNFP) evaluates all issues relating to food safety. GM crops with herbicide tolerance are also subject to pesticide safety legislation. Only if Ministers are content with the safety of a release will consent be granted and, for research releases, that consent is subject to strict and enforced conditions. To date all UK

releases have been for research purposes and no commercial GM crops have been grown.

The Department for Environment, Food and Rural Affairs is the lead ministry with regard to the regulation of genetically modified organisms. It is also committed to transparency and publishes a wide range of information on its website: www.defra.gov.uk/environment/gm/index.htm

None the less the move from research scale to widespread commercialisation would be significant and Ministers have recognised the importance of considering the wider issues inherent in the potential adoption of GM technology. For that reason they established the Agriculture and Environment Biotechnology Commission which allowed all stakeholders to have a say in this debate.

It is clear from the very wide range of potential outputs that may be achieved with genetic modification, and the very wide range of uses for those outputs, that there can be no single policy for all GM. Some outputs have been widely accepted for many years: the production of human insulin from genetically modified bacteria for example. Others are controversial and require cautious appraisal, for example the drift of herbicide tolerance into potentially weedy species. Yet others can appear morally distasteful: for example the so-called 'terminator' genes could be used to prevent farmers from saving their own seed, although such technology could also be beneficial in ensuring that GM plants do not establish in the wild and become a conservation problem. Thus while all new uses of GM must be considered with care, each can only be approved or rejected on a case by case basis and forming only one of many other approaches to making agriculture and horticulture more sustainable.

Current global plantings of GM plants

Since the first commercial plantings of GM crops in the mid 1990s the global rate of uptake has accelerated. In 2005 there were 90 million ha, which is about 6% of the global cultivable crop land (www.isaaa.org). The countries with the largest areas are USA (55% of the total), Argentina (19%), Brazil (10%), Canada (6%) and China (4%). There are 14 countries growing >50,000 ha of GM crops, the great majority of which had only two types of modification: herbicide tolerance (oil seed rape and soybean) or Bt toxin expression, sometimes 'stacked' with herbicide tolerance (maize and cotton). There are claims for very large economic benefits resulting from lower pesticide inputs and the resulting smaller environmental 'footprint' (<http://www.agbioforum.org/v8n23/v8n23a15-brookes.htm>); for a more sceptical view of genetic technologies, see <http://www.genewatch.org>

The modification for herbicide tolerance allows crops to be treated with a broad spectrum herbicide, which results in improved weed control but will sometimes reduce biodiversity. The Bt toxin is a crystalline protein produced by the bacterium *Bacillus thuringiensis*. The one most widely used is specifically toxic to caterpillars, including important pests of maize and cotton. The toxin is also used as a conventional pesticide spray, but by inserting the gene into the crop, spraying is rendered unnecessary and targeting of the pest is much improved. However, resistance to the toxin has already arisen naturally in some caterpillars. Exposing insects to the toxin, regardless of whether they are causing significant damage, will increase the risk of selecting resistant insects.

In addition to these four major crops, several others have approvals for herbicide tolerance and two (potato, tomato) have approvals for Bt toxin-mediated insect resistance in 2005. The

following additional modifications have also had approvals in one or more countries:

Carnation Florigene 'Moon' series:

modified colour and delayed senescence

Papaya: papaya ringspot virus-resistance

Tomato: 'Flavr Savr' prolonged ripeness without softening and delayed ripening

Potato: potato leaf roll virus resistance

Approval may not lead to commercial planting and some approved crops may be subsequently withdrawn. Modified potatoes and the 'Flavr Savr' tomato are not currently grown (www.agbios.com).

There are many other modifications under development, including rice modified with resistance to various pests and diseases and with enhanced nutrient levels. The EU has funded a five-year research programme called Pharma-Planta which will research modifications to crop plants to produce therapeutically valuable proteins, such as neutralising antibodies to HIV and rabies, a process sometimes called 'phytopharming'. There is disquiet in some quarters about food crops being used for this purpose and they would be subject to extensive biosafety and environmental risk assessment before approval could be given for commercial use.

Within the EU, the five-year moratorium on planting GM crops announced in 1998 is over. The EU approved three varieties of GM maize and one of GM oil seed rape for planting in 2005, but there has been no significant commercial planting except in Spain. Several member states, including France, have banned some EU-approved varieties and are in dispute with the EU over the legality of this ban. However, France is also reported to have had a small (500 ha) commercial planting of GM maize in 2005. Spain approved GM maize before the EU moratorium was imposed and planted 60000 ha in 2005. Several requests for consent for commercial

planting are currently pending in the EU, including one from The Netherlands to grow the Florigene GM blue carnation.

The UK government has given approval for commercial planting of one GM maize variety, but this has subsequently been withdrawn from sale and was never grown. The Minister responsible for approvals stated in September 2005 that commercial plantings are unlikely in the UK before 2008.

Update on the UK farm-scale evaluations

The Farm Scale Evaluations (FSE) were announced by the UK government in 1998, in order to assess the impact on the environment of commercial planting of three GM crops, oil seed rape, sugar beet and maize, which had been modified for herbicide tolerance. These modifications would allow farmers to achieve a very high level of weed control with a single herbicide and there were concerns that such use would adversely affect the weed and invertebrate populations in fields, and in turn the birds and other wildlife that feed on that biodiversity.

The FSE were carried out on experimental plots at commercial farms between 1999 and 2004. Researchers selected single fields at a large number of farms and planted half of each field with the GM crop, managed with the herbicide to which it was tolerant, and the other half with the same crop unmodified and managed with conventional herbicide inputs. Over the evaluation period the farmers rotated their crops in the conventional way, so the comparisons were made under conditions reflecting normal practice. The final reports and a non-technical summary are available:

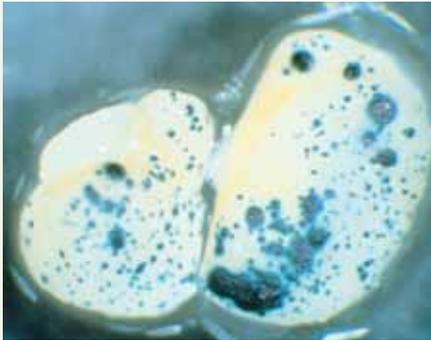
<http://www.defra.gov.uk/environment/gm/fse/index.htm>

The researchers measured weed frequencies and types, weed seed output and populations of a range of

invertebrates, including visiting honeybees. They found that GM crops tended to have lower weed populations or, in a few instances when this was not the case, they had a higher proportion of grass weeds. In the rape and beet GM crops there were overall less insects, though not in some groups. In the GM maize, there were generally more insects. The researchers emphasise that the population differences can be explained by the differences in herbicide use and are not a direct effect of the genetic modifications on the environment. In particular, the timing of herbicide application affected invertebrate populations. For example there were more springtails in the GM crops, because they feed on decaying plants and in the GM crops weeds were allowed to grow larger before the herbicide was applied, leading to more rotting plant material. Conversely, there were fewer honeybees in GM beet and some rape crops, because there were less broad-leaf weeds with flowers for them to visit. The reports discuss many more such examples.

The government announced that as a result of these trials it would not support any EU decision to allow the commercial planting of the GM rape and beet. After the trials were completed it was announced that the herbicide atrazine, which was used in the management of the conventional maize plots, was to be withdrawn from sale in the EU. In future, the perceived benefit to wildlife of planting GM maize may need to be reassessed if this results in a different herbicide regime for conventional maize.

Other leaflets in the RHS Guidelines series can be read and downloaded from www.rhs.org.uk/publications. They can be obtained by post by sending an A4 SAE to A W Mailing Services Ltd, PO Box 38, Ashford, Kent TN25 6PR (£1.14p postage for the full set).



Expression of a GUS marker gene results in blue colour in the tissues to show that successful transformation has taken place.
Acknowledgement: Photograph courtesy of IACR Rothamsted, Harpenden, Herts, U.K.

DEFINITIONS

Clones of an organism are genetically identical copies. Cuttings and other types of plant offshoot are clones – all Cox's Orange Pippins are clones of the original tree – but seeds are not because the sexual processes of seed production introduce variability. In molecular biology, the process of gene cloning is fundamental to the techniques of genetic engineering. The term refers to the processes by which a gene is first identified and then multiplied to make a large number of identical copies, sufficient for study and manipulation.

Enzymes are proteins which control the chemical reactions in the cell. These include the synthesis of DNA and RNA, the synthesis of the cell building materials such as cellulose, and all the reactions by which the cell gains energy.

Marker genes are used in genetic engineering to identify transformed cells. A popular technique is to attach a gene for antibiotic resistance to the gene

which a biotechnologist wishes to insert. If the insertion is successful, the cell will be transformed and also antibiotic resistant. When exposed to the relevant antibiotic, this cell will survive but those without the marker will not. Because of concerns about the possible transfer of antibiotic resistance to other organisms, including humans, alternative markers are now in use. Another example, the GUS (glucuronidase) marker, is illustrated left: expression of the marker gene causes a blue colour, indicating successful transformation.

RNA is ribonucleic acid, a very large molecule with similarities to DNA. It consists of a single strand of pentose sugar with a chain of bases. It is synthesised in the nucleus from the DNA template and so carries a transcription of the DNA base sequence. There are three main types of RNA. Messenger RNA (mRNA) carries the information of the gene – the base sequence – to the ribosomes. Transfer RNA (tRNA) has much shorter base sequences, sufficient to code for one of the 20 amino acids: tRNA carries the amino acids from the cell contents to the ribosomes where the amino acids assemble into proteins. Ribosomal RNA forms part of the structure of the ribosome and aligns the mRNA to allow accurate translation of the sequence.

Ribosomes are small structures in the cell which control the synthesis of proteins from amino acids using the mRNA templates to determine the amino acid sequence and tRNA to bring the amino acids to the template. As the

chain of amino acids lengthens, it assembles into the three dimensional structure of the protein.

Tissue culture is a cloning technique. Individual plant cells, if separated from the parent plant and provided with nutrients and light, will grow to form a disorganised mass of cells which can be easily subdivided. Under the influence of the correct levels of plant hormones, these cell masses will differentiate back into whole plants. This allows the production of whole plants from individual cells. The technique is crucial in genetic modification because it allows huge numbers of individual cells to be subjected to the required transformation techniques – some of which have very low success rates – and then the regeneration of transformed plants from the successfully altered cells.

Vectors are used to insert new genes into cells. For example, the plant pathogenic bacterium *Agrobacterium tumefaciens* naturally inserts part of its genome into the host genome as part of the infection process. This part, called a plasmid, can be modified to contain the new gene, so that the bacterium vectors it into the recipient cell; for this purpose the bacterium can be disabled so that infection does not occur. Viruses can also be used as vectors. DNA can also be successfully inserted into cells simply by coating tiny pellets of metal with the required gene sequences and “firing” them into the cells – a technique known as “biolistics.”



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