Several new breeding programmes have recently been commissioned, which now must easily place SCRI as having the largest combined soft-fruit breeding genetics programme in the world. In 1996, we had *c.* 20,000 raspberry, 25,000 blackcurrant and 18,000 strawberry seedlings in the field. Whilst the raspberry and blackcurrant programmes are likely to be maintained at these levels, it is anticipated that the strawberries will be increased to about 38,000 seedlings with the three different breeding programmes (Kentish Garden, Pernod Ricard and MRS). This increased activity, particularly with strawberry, has enabled us to undertake the training of three young fruit breeders (one each from Scotland, France and America), which is vital to future genetic advancement in these crops. It has also enabled us to actively increase our international participation in co-operative research and exchange programmes. We have recently formally signed a Memorandum of Scientific Co-operation with Norway, joined the European Strawberry Cultivar Testing Network and provided reference material under the EC Plant Marketing Directive.

We have also started, on a very small scale, an apple selection and evaluation programme with the major objective of identifying a high-quality eating apple that is adapted to Scottish conditions. The first crosses were made in 1996 between locally adapted genotypes and quality desert types. The acquisition of additional new bud wood and root stocks was also initiated, together with training courses for staff and industry. Whilst a long-term project, there is every confidence that this will bring a new dawn to the rebirth of a speciality apple industry in Scotland

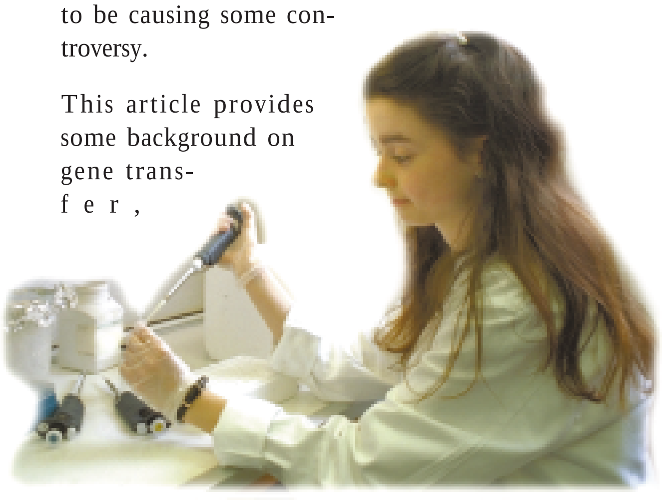
Genetically modified food

J. Graham

Meeting the demands of a consumer society Bigger (and smaller), sweeter, bright red fruit, fewer chemical sprays, more vitamins, longer shelf-life, cheaper, higher yielding and with better resistance to pests and diseases. The demands of consumers, growers and retailers are many, and the timescale in which these have to be delivered gets shorter. Even if genetic material to satisfy all of these criteria was available, a breeding programme may take up to 20 years to achieve some of these goals. By this time consumer demand and preference may have changed, pest and disease problems may have altered, and the industry could be under threat.

The only way to satisfy the various, sometimes conflicting demands on SCRI, is to continue the highly successful breeding practices, and to integrate into these, new technologies which can overcome some of the limitations associated with plant breeding. One technology still in its infancy but with great potential is gene transfer (sometimes referred to as genetic manipulation or genetic engineering). Like most modern molecular genetic techniques, though, it appears to be causing some controversy.

This article provides some background on gene transfer,



what it involves and what it can achieve. While soft fruit is used here to illustrate the potential of this technology, it could apply to any crop species.

From shots in the dark to controlled crossing For millennia, whether knowingly or not, humans have manipulated genetic material to their own ends. To increase food production, the hit or miss nature of early breeding attempts with no knowledge of genetics, led to the development of crops such as wheat from grass seed. For instance, many early blackberries were dioecious but through selection, have become monoecious. In the latter, the male and female flowers are on the same plant, resulting in greatly improved levels of fertility and hence fruit quality.

Unravelling the mysteries of heredity began in the 1850s with the work of the Austrian monk Gregor Mendel. He performed experiments on peas which led to a better understanding of how information is transferred from generation to generation. As early as1868, Menscher suggested what Mendel's physical units of inheritance were. However it took another 80 years or so before Avery, McCarty and McCloud carried out a classic experiment which proved that genetic information is carried by DNA. Once DNA was confirmed as the genetic material or 'units of inheritance' which transfer from one generation to the next, the structure of DNA was examined. In 1953, Watson and Crick described the now familiar, double helix, molecule which unzips and reproduces itself to pass information down the generations.

Since that discovery, scientists have been able to carry out breeding experiments with a greater knowledge of how new combinations of characteristics are achieved. This has been very successful and has led to the production of a wide variety of crops on a commercial scale.

The need for change Although plant breeding has been very successful, plant breeders are faced with a number of obstacles to the production of new, improved, cultivars. These obstacles include a lack of desirable traits in the breeding material, so that unless a source of a gene is available (for example, for pest or disease resistance), the trait cannot be transferred into the offspring. There is also a lack of control over which characteristics are transferred to future generations, leading to the passage of both desirable and non-desirable traits to the offspring. Any breeding process results in a reshuffling of genes, and it is therefore virtually impossible to make single, specific changes to a valuable cultivar. In soft fruit, there are additional complications. The length of time between generations slows down the breeding process and a number of different forms of each gene exist, requiring the evaluation of large seedling populations to identify the desirable ones. Plant breeding cannot offer a rapid solution to new problems, new preferences or new ideals. In fact, there are many examples where it cannot offer a solution at all.

a)

b)

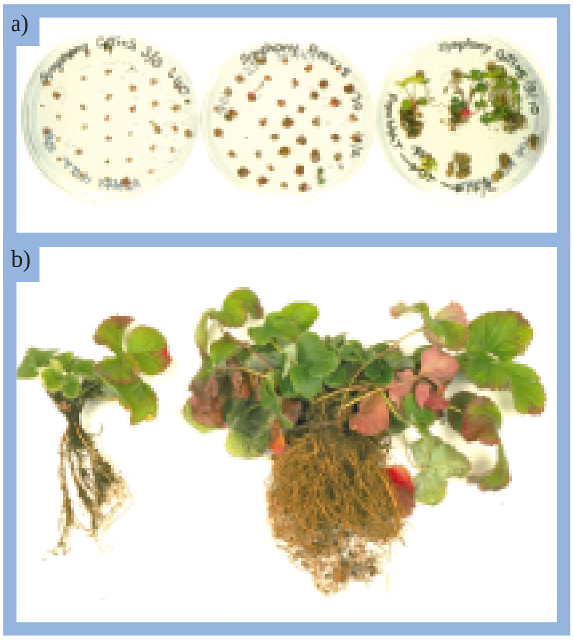


Figure 1 a) The transformation process. b) Non-transgenic (left) and transgenic strawberry plants after attack by vine weevil larvae.

A soil borne bacterium provides a new tool to transfer DNA Gene transfer refers to techniques which enable DNA to be moved from one organism to another by means other than conventional plant breeding. The information represented in DNA is recognised by all living things from Man down to the simplest virus and every organism in between. Each gene contains the information to control a particular function, or groups of genes work together to control more complex processes. Any form of genetic improvement therefore requires the transfer of information from an organism with the desired characteristic, to one without that characteristic. For a characteristic to be transferred to a plant, the gene controlling it must be inserted into the plant's chromosome in a recognisable form on which the plant can act.

In fact, nature has come up with the solution. A common soil bacterium, known as *Agrobacterium*, has the ability to transfer its own DNA into plants, manipulating them to produce a food source for itself. Strange as it may seem, this process has probably been going on for many, many thousands of years - so gene transfer isn't really terribly new. We can therefore use this natural 'genetic engineer' to transfer the traits we require into plants by first substituting a desired DNA sequence for part of the bacterium's own genetic makeup.

Getting the gene into the whole plant is the most difficult part. *Agrobacterium* easily transfers the genes into any cell it infects. At the whole plant level though, it would be almost impossible to get *Agrobacterium* to infect every cell of a plant, and so a process of whole plant regeneration is used. *Agrobacterium* is used to transfer the gene of interest into just a few plant cells; these are then encouraged to divide forming a new plant in which every cell contains a copy of the originally inserted DNA. This DNA should behave as a normal plant gene.

As the genetic code is universal, the fact that a desired characteristic does not already exist in the breeding material does not mean that it cannot be introduced. The gene for a specific characteristic can be identified and copied from another source and inserted into the chosen plant using the *Agrobacterium* technique described above.

Gene transfer provides unlimited possibilities
Resistance to pests and diseases, reduction in chemical usage, better flavour and colour, improved nutritional value, lower processing costs, edible vaccines and novel fuel sources, are just some of the realities of gene transfer.

Resistance to insect pests and the fungal and viral diseases is an important area where gene transfer can make an impact in the short term. These cause major problems in soft fruit crops such as strawberry, raspberry and blackcurrant. The problems have been tackled by a range of approaches, including plant breeding, chemical control and biological control, with varying degrees of success. The following three examples will outline specific problems and how gene transfer can offer a solution to them.

An insect pest The vine weevil is now the major insect pest of strawberry, where the larvae damage root systems and result in severe loss of yield or plant death. Since the withdrawal of persistent organochlorine insecticides, control of adults and larvae has become difficult. Few of the currently available products are

Figure 2 Typical damage caused by vine weevil to a field plantation of strawberry.

particularly effective under field conditions. Biological control has proved ineffective in many areas due to low summer soil temperatures. This, in addition to the lack of resistance in wild and breeding material, has led to the investigation of alternative control strategies, including gene transfer. By providing the plant with its own genetic-based, self-defence mechanism, the use of pesticides may be greatly reduced. One possibility for resistance to vine weevil is a gene from the tropical legume, *Vigna unguiculata*, the cowpea, which encodes an enzyme which inhibits the ability of the insect to digest food. Thus, prolonged feeding on plants with this enzyme will result in a reduction in fecundity of the insect, and eventual starvation. This gene has been shown to confer significant resistance against vine weevil in glasshouse studies on genetically modified strawberry. The gene occurs naturally in an edible plant and has been shown to be non-toxic to humans and mammals. Our ultimate aim is to have this enzyme expressed only in those plant parts on which the insect feeds and not others, thus eliminating any perceived risk to non-target organisms. We are at present studying the effect of this gene under field conditions, and examining any potential risk this gene may pose under the environmental conditions in which strawberry is grown.

A fungal pathogen The fungal disease of greatest concern in raspberry and strawberry is grey mould, caused by *Botrytis cinerea*. The fungus accounts for 50% of all fungicides applied to field-grown soft fruits. The pathogen is difficult to control because there are multiple infection sites and no sources of resistance available to fruit breeders. At present, the crop is sprayed at 7 to 10-day intervals from first flower until shortly before fruit ripening. However, many flowers are missed by sprays and therefore left unprotected. More

Figure 3 Grey mould.

frequent spraying is unacceptable because it is imperative that maximum residue levels are not exceeded and to prevent the development of strains of *Botrytis* which are resistant to the few approved fungicides. The UK soft fruit growers have an excellent, substantiated, record of following the guidelines. However, alternative control strategies are required to reduce the dependency on agrochemicals. Gene transfer can offer a number of possibilities. A number of anti-fungal genes have been identified in plants with the potential to give resistance. One route which we have taken is in switching a natural fruit gene back on (SCRI Ann. Rep. 1995, 115-116). This particular gene is naturally switched off as the fruit ripens, just at a time when *Botrytis* infects. By turning the gene back on so that the product is present as the fruit ripens, we hope to reduce infection and fungal development. A series of such genes could potentially remove the need for fungicides.

A virus disease The virus currently causing the greatest concern is Raspberry Bushy Dwarf Virus (RBDV), for which at least one resistance-breaking strain has emerged. The resistance-breaking isolate can overcome the gene which until recently gave protection against this virus. If the resistance-breaking strain of the virus enters an area of intensive cultivation, it could prove devastating and possibly wipe out an entire industry. Because this virus is pollen-borne, conventional control is virtually impossible. At the moment, the only strategy available to tackle this is the use of gene transfer technology to confer resistance. Control of viruses has involved the use of resistant varieties, where available, or the elimination of the vector, again using chemicals. The phenomenon of virus cross-protection has also been used, whereby previous infection of the plant by a mild strain of virus reduces severity of a later infection with a severe strain. The presence of parts of the mild virus is responsible for cross-protection and, through gene transfer, we can transfer just the parts required to achieve protection.

These examples show how gene transfer can be used to protect against insects, fungal pathogens and viral diseases. In the future we can change the gene insert as required to avoid resistance-breaking strains.

Perception is everything! Research by the Consumer's Association shows that few people appear to know what genetic modification actually is, even though the first products of this technology are already available on the supermarket shelves. Because it sounds so scientific and technological, it can conjure up all sorts of images e.g. 'playing with nature'. Ideas derived from Science Fiction and vociferous minority groups, make people uncomfortable with the whole gene transfer technology. Obviously, we do not all have the time or inclination to become familiar with molecular biology and shouldn't need to. The information on a genetically modified food should be freely available. Identification of such food should be seen as a very positive move, and should be accompanied with relevant information explained in simple, but accurate, terms. The issue of information transfer in an accessible form to the public domain is a real one, which can only be to the benefit of science, technology and the public in general. By and large however, the consumer simply wants the benefits of a process and if these can be explained, identified and justified, then acceptance should follow naturally.

It appears that genetically modified plants may be under threat in the short term, not because they are unsafe, but because of their nature. There is nothing inherently dangerous about the process of genetic modification. However small the risks of gene transfer appear to scientists, public perception may be very different for a whole array of reasons. These differences between the public and scientific community will polarise around notions of safety and risk, labelling and openness. A large factor in this is again the fact that information on scientific matters in general, and biotechnology in particular, is not easily accessed by most consumers. As a result, the potential for misunderstanding is enormous. Scientists must make every effort to accept people's fears of new technologies in general, and proceed in a cautious and responsible way, to ensure that all risks are examined and acted upon. As with any new technique, it needs to be carefully monitored. There is no evidence that genetically engineered food is any less safe than conventional food. Obviously, care and consideration has to be given to what gene(s) is being inserted, for what purpose, and what effects, intentional or otherwise, it could have. It has to be evaluated very much on a case by case basis.

Risks *Crossing the species barrier* A major issue associated with the use of this technology is that it allows genetic information to cross the species barrier. However, it is not safe to assume DNA does not already cross species barriers. In nature, DNA does transfer within and between species, as is the case with *Agrobacterium* described above. DNA also transfers from viruses to bacteria, or viruses to humans. It is only now that these issues have been opened that we are starting to look and find more and more natural examples of it. A number of techniques that enable greater genetic exchange than is possible through plant breeding, have been in common practice for a number of years, and have not caused concern. However, although gene transfer can do the same job but in a much more controlled fashion, it is viewed differently. It is, however, very difficult to draw a line between a genetically modified plant and one produced using other less sophisticated or 'natural' breeding techniques. 'Natural' however is very difficult to define, as this varies between countries depending on the development of their technologies and cultural practices. Immunisation is now a natural technique for protecting people from disease in affluent countries. Edible vaccines could become the norm in developing ones (SCRI Ann. Rep. 1995, 135-137).

Gene escape Genes do not 'escape', but may transfer by normal, predictable means in pollen. Some people fear that widespread use of plants with altered genetic characteristics may threaten the environment by disturbing the existing balance between organisms. It is important, however, to realise that any genetic balance is a dynamic one, with gene mutations and rearrangements occurring as normal events in all living organisms. Transgenic technology does expand this scope, and careful examination of the transgenic plant is required before it goes into large-scale release and forms part of the food chain. Each plant and gene construct must be considered in its own right. For example, in the UK, a genetically modified strawberry cannot transfer its genes into the wild, as they are not sexually compatible. This is not the case in raspberry, so here the rate and extent of gene transfer into the wild needs to be taken into much more careful consideration.

Toxicity and allergenicity Food safety obviously has to be of great concern to both scientists and the public. In recent years, this subject has had a high profile, following a number of food scares from BSE to virulent strains of *Escherichia coli*. Extreme care must be taken during early establishment of this new technology, as irresponsible releases could set developments back by a decade. Extensive testing is essential if only to reassure the lay person of the care being taken by the scientific community.

Antibiotic resistance Most gene transfer methods rely on a second gene, known as a marker gene, to enable selection of transgenic plants. In the majority of cases to date, this marker gene has been the neomycin phosphotransferase (NPTII) gene. NPTII inactivates and provides resistance to the antibiotics kanamycin and neomycin. Some concern has been raised about the safety of the gene product. Given that humans consume an estimated 1.2 x 10^6 kanamycin-resistant microorganisms daily, it is unlikely that the NPTII gene product is toxic. In addition, NPTII has been shown to be non-toxic to mammalian cell lines and when produced intracellularly in *vivo*. Concern has also been raised as to whether eating NPTII may compromise oral kanamycin and neomycin therapy. However, NPTII is rapidly inactivated and degraded in the digestive system, with proteins only rarely absorbed. In addition, NPTII requires ATP in order to catalyse the inactivation of kanamycin or neomycin, and this is present only in extremely low concentrations being unstable at low pH. It has been estimated that less than 0.5% of these antibiotics administered are for oral or gastrointestinal tract use. Transfer of the gene from plant to pathogenic bacteria and the possible consequences, have also been raised as objections to its use. Considering however that the majority of pathogenic bacteria live in the gut and are already exposed to the NPTII gene, transfer from other bacteria is much more likely than transfer from plants.

Despite the fact that there is no evidence against the use of antibiotic resistance genes, public perception may weigh heavily against them. Methods have been devised to eliminate marker genes from the crop plant if deemed necessary. It might also be worth considering that as more and more desirable traits become available for transformation, this may become almost essential. The use of a marker gene in one transformation precludes its use for subsequent modification. Thus unless markers are removed, new markers will continually be required for each new trait to be inserted.

Legislation for safety The United Kingdom was one of the first countries to introduce controls on modern biotechnology. These controls were introduced in 1978, not in response to any identified health or environmental problems, but rather because of the lack of familiarity with the behaviour of GMOs and the need to ensure safety. The current legislation governing the release and marketing of GMOs aims to prevent or minimise damage to the environment. Part IV of the Environment Protection Act 1990 and the regulations made under it, are in line with recommendations made by the Royal Commission on Environmental Pollution and implement the EC Directive on Deliberate Releases into the Environment of GMOs. No GMO can be released or marketed without prior consent of the Secretary of State, acting jointly with the Minister of Agriculture, Fisheries and Food. In every case, the Secretary of State seeks expert advice from the independent Advisory Committee on Releases to the Environment, a committee composed of public and private sector experts, including representatives from environmental groups. UK regulations require a full assessment of the environmental impact and risk of any intended release, and every consent holder has to monitor the environmental effects of a release. Before GMOs can be marketed, they must also be approved at European Community level with all member states able to raise objections. In January 1997, the European parliament approved the text of the European Novel Food Regulation and this became law at the end of April. This definition of Novel Food includes food or food ingredients containing or consisting of GMOs or that have been produced from but not containing GMOs. The regulations are aimed at ensuring that food covered by this Regulation does not present a danger to the consumer, does not mislead the consumer, and does not nutritionally disadvantage the consumer.

In summary Genetically modified plants have great long-term potential and we are only in the early stages of utilising this. For public acceptance in the short term, there is a need to clearly explain the benefits of gene transfer technology. These benefits must not only be in terms of the profitability of firms and farmers; consumers must be aware of the benefits to them in terms of cost, food quality and the environment. It is clear that agriculture has to be efficient, particularly in terms of limited resources and has to be as free from negative effects on the environment as is possible. It must also meet the consumer demands who require fresh produce regardless of season. With this understood, the benefits of genetically modified foodstuff should be clear.

In conclusion therefore, genetic manipulation is not something we should fear but is a process which should be harnessed in a positive manner for not just our good, but for the good of our environment.

Rubus breeding and genetic research

R.E. Harrison, R.J. McNicol & S. Jennings

Historically, raspberry production in Scotland has been for processing through preservation as pulp for jam manufacture, canning or freezing. Although fresh fruit production in Scotland is slowly increasing, it remains a small part of the industry (c. 6 % in 1995). Current Scottish raspberry production remains focused on processing, although the market has changed dramatically. Whereas most fruit was processed as pulp in decades past, only 38% of the crop went to pulp in 1995 and 20% in 1996. The shift away from pulp is due to economics. The best prices for processed fruit now come from Individually Quick Frozen (IQF) fruit and other novel processed products.