Genetically modified plants and human health

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Summary

Genetically modified (or GM) plants have attracted a large amount of media attention in recent years and continue to do so. Despite this, the general public remains largely unaware of what a GM plant actually is or what advantages and disadvantages the technology has to offer, particularly with regard to the range of applications for which they can be used. From the first generation of GM crops, two main areas of concern have emerged, namely risk to the environment and risk to human health. As GM plants are gradually being introduced into the European Union there is likely to be increasing public concern regarding potential health issues. Although it is now commonplace for the press to adopt 'health campaigns', the information they publish is often unreliable and unrepresentative of the available scientific evidence. We consider it important that the medical profession should be aware of the state of the art, and, as they are often the first port of call for a concerned patient, be in a position to provide an informed opinion.

This review will examine how GM plants may impact on human health both directly – through applications targeted at nutrition and enhancement of recombinant medicine production – but also indirectly, through potential effects on the environment. Finally, it will examine the most important opposition currently facing the worldwide adoption of this technology: public opinion.

Introduction

Plants with favourable characteristics have been produced for thousands of years by conventional breeding methods. Desirable traits are selected, combined and propagated by repeated sexual crossings over numerous generations. This is a long process, taking up to 15 years to produce new varieties.[1] Genetic engineering not only allows this process to be dramatically accelerated in a highly targeted manner by introducing a small number of genes, it can also overcome the barrier of sexual incompatibility between plant species and vastly increase the size of the available gene pool.[1]

Transgenic (GM) plants are those that have been genetically modified using recombinant DNA technology. This may be to express a gene that is not native to the plant or to modify endogenous genes. The protein encoded by the gene will confer a particular trait or characteristic to that plant. The technology can be utilized in a number of ways, for example to engineer resistance to abiotic stresses, such as drought, extreme temperature or salinity, and biotic stresses, such as insects and pathogens, that would normally prove detrimental to plant growth or survival. The technology can also be used to improve the nutritional content of the plant, an application that could be of particular use in the developing world. New-generation GM crops are now also being developed for the production of recombinant medicines and industrial products, such as monoclonal antibodies, vaccines, plastics and biofuels.[2–4]

In 2007, for the twelfth consecutive year, the global area of biotech crops planted continued to increase, with a growth rate of 12% across 23 countries.[5] The principle crops grown are soybean and maize, although cotton, canola and rice are also on the increase. However, genetically modified crops grown in the EU amount to only a few thousand hectares (~0.03% of the world production),[6] which is probably a reflection of European opposition to this technology. In contrast, food derived from GM plants is ubiquitous in the USA. Indeed, many animal feeds used in Europe derived from imported plant material contain GM products. Similarly, GM cotton is widely used in clothing and other products.

Genetically modifying a plant

A number of techniques exist for the production of GM plants. The two most commonly employed are the bacterium *Agrobacterium tumefaciens*, which is naturally able to transfer DNA to plants, and the 'gene gun', which shoots microscopic particles coated with DNA into the plant cell.[1] Generally, individual plant cells are targeted and these are regenerated into whole GM plants using tissue culture techniques. Three aspects of this procedure have raised debate with regard to human health.

- The use of selectable markers to identify transformed cells
- Transfer of extraneous DNA into the plant genome (i.e. genes other than those being studied)
- The possibility of increased mutations in GM plants compared to non-GM counterparts due to tissue culture processes used in their production and the rearrangement of DNA around the insertion site of foreign genes.

To facilitate the transformation process, a selectable marker gene conferring, for example, resistance to an antibiotic (e.g. kanamycin, which will kill a normal non-GM plant cell), is often co-transferred with the gene of interest to allow discrimination of GM tissue and regeneration of GM plants. Critics of the technology have stated that there is a risk of the spread of antibiotic resistance to the bacterial population either in the soil or in the human gut after ingestion of GM food. However, these antibiotic resistance genes were initially isolated from bacteria and are already widespread in the bacterial population. In addition, kanamycin itself has GRAS status (Generally Regarded As Safe) and has been used for over 13 years without any known problems. Studies have concluded that the probability of transmission of antibiotic resistance from plants to bacteria is extremely low and that the hazard occurring from any such transfer is, at worst, slight.[7,8] Nevertheless, other selection strategies that do not rely on antibiotic resistance have been developed,[9] and procedures to eliminate the selectable marker from the plant genome once its purpose has been fulfilled have also been designed.[10]

The second aspect of the plant transformation procedure that has been criticized is that unnecessary DNA is transferred into the plant genome as a consequence of the engineering and transfer process.[11] Of course, there is no reason that DNA *per se* should be harmful, as it is consumed by humans in all foods, but again plant technologists have responded to the criticism by designing 'minimal cassettes' in which only the gene of interest is transferred into the plant.[12]

Finally, it has been claimed that GM plants carry more mutations than their untransformed counterparts as a result of the production method.[13] Genome-wide mutations may be produced by the tissue culture process, generating so called somaclonal variation, and endogenous DNA rearrangements may occur around the integrated transgene.[13] Theoretically, this may mean that plants may be produced with, for example, reduced levels of nutrients or increased levels of allergens or toxins[13] (although the alternative must also hold true, that positive traits may be expressed). Latham *et al*.[13] have stated that mutations around foreign gene insertion sites have not been fully characterized in either experimental or commercialized GM plants. Consequently, these authors have proposed several recommendations involving improved molecular analysis prior to the future commercialization of GM crops.[13]

However, as described in this report, it must be emphasized that GM crops grown to date have been produced under rigorous regulatory frameworks, and have been extensively safety tested prior to commercialization.

Food applications for GM plants

In the developing world, 840 million people are chronically undernourished, surviving on fewer than 8000 kJ/day (2000 Kcal/day).[14,15] Approximately 1.3 billion people are living on less than US$1/day[16,17] and do not have secure access to food. Many of these are also rural farmers in developing countries, depending entirely on small-scale agriculture for their own subsistence and to make their living.[18] They generally cannot afford to irrigate their crops or purchase herbicides or pesticides, leading to a vicious circle of poor crop growth, falling yields and pest susceptibility.[18] In addition, the world's population is predicted to double over the next 40 years, with over 95% of individuals being born in developing countries.[19] It is estimated that to meet these increased demands, food production must increase by at least 40% in the face of decreasing fertile lands and water resources.[20,21] GM plant technologies are one of a number of different approaches that are being developed to combat these problems. Specifically, studies are under way to genetically modify plants to increase crop yields, or to directly improve nutritional content.

Increasing nutritional content

In the developed world the nutritional content of food items is not of major concern, as individuals have access to a wide variety of foods that will meet all of their nutritional needs. In the developing world, however, this is often not the case, with people often relying on a single staple food crop for their energy intake.[18] GM technology offers a way to alleviate some of these problems by engineering plants to express additional products that can combat malnutrition. An important example of the potential of this technology is the 'Golden Rice Project'. Vitamin A deficiency is widespread in the developing world and is estimated to account for the deaths of approximately 2 million children per year.[18] In surviving children it has been identified as the leading cause of blindness.[22] Humans can synthesize vitamin A from its precursor β-carotene, which is commonly found in many plants but not in cereal grains.[18] The strategy of the Golden Rice Project was to introduce the correct metabolic steps into rice endosperm to allow β-carotene synthesis. In 2000, Ye *et al*.[23] engineered rice that contained moderate levels of β-carotene and since then researchers have produced the much higher yielding 'Golden Rice 2'.[24] It is estimated that 72 g of dry Golden Rice 2 will provide 50% of the RDA of vitamin A for a 1–3-year-old child.[24]

Golden Rice was developed for farmers in the poorest countries, and from the beginning, the aim of the scientists was to provide the technology free of charge, which required the negotiation of more than 100 intellectual and technical property licenses.[25] Golden Rice will be given to subsistence farmers with no additional conditions[18] and is an impressive example of a health solution that can be offered by plant biotechnology.

Increasing food production

Crop yields worldwide are significantly reduced by the action of pathogens, parasites and herbivorous insects.[26] Two examples of commercial GM crop growth in this area are the insect-resistant crops expressing the *bt* gene (from the bacterium *Bacillus thuringiensis*) and virus-resistant GM papaya.[27] The first of these has been particularly successful; in the USA, for example, insect resistant GM maize is grown over an area of 10.6 million hectares and comprises 35% of all maize (GM and non-GM) grown in the country.[28] At the laboratory level, resistance has also been engineered to bacterial and fungal plant pathogens.[29,30]

A primary cause of plant loss worldwide is abiotic stress, particularly salinity, drought, and temperature extremes.[31] In the future, these losses will increase as water resources decline and desertification intensifies. Drought and salinity are expected to cause serious salinization of all arable lands by 2050,[32] requiring the implementation of new technologies to ensure crop survival. Although a number of promising targets have been identified in the production of abiotic stress tolerant GM plants, research remains at the laboratory-based level. An example is the study by Shou *et al*.[33] demonstrating that expression of an enzyme in GM maize activates an oxidative signal cascade that confers cold, heat and salinity tolerance.

Are GM foods safe to eat?

GM crops are tightly regulated by several government bodies. The European Food Safety Authority and each individual member state have detailed the requirements for a full risk assessment of GM plants and derived food and feed.[34] In the USA, the Food and Drug Agency, the Environmental Protection Agency and the US Department of Agriculture, Animal and Plant Health Inspection Service are all involved in the regulatory process for GM crop approval.[35] Consequently, GM plants undergo extensive safety testing prior to commercialization (for an example see http://www.efsa.europa.eu/EFSA/KeyTopics/efsa_locale-1178620753812_GMO.htm).

Foods derived from GM crops have been consumed by hundreds of millions of people across the world for more than 15 years, with no reported ill effects (or legal cases related to human health), despite many of the consumers coming from that most litigious of countries, the USA.

There is little documented evidence that GM crops are potentially toxic. A notorious study claiming that rats fed with GM potatoes expressing the gene for the lectin *Galanthus nivalis* agglutinin suffered damage to gut mucosa was published in 1999.[36] Unusually, the paper was only published after one of the authors, Arpad Pusztai, announced this apparent finding on television.[37] The Royal Society has since stated that the study 'is flawed in many aspects of design, execution and analysis' and that 'no conclusions should be drawn from it': for example the authors used too few rats per test group to derive meaningful, statistically significant data.

Is there any *a priori* reason to believe that GM crops might be harmful when consumed? The presence of foreign DNA sequences in food *per se* poses no intrinsic risk to human health.[38] All foods contain significant amounts of DNA and RNA, consumed in the range of 0.1–1.0 g/day.[39] Of potential concern is the possibility that the protein produced by the transgene may be toxic. This would occur if the transgene coded for a toxin that was subsequently absorbed systemically by the host. However, the potential toxicity of the protein expressed in a GM food is an essential component of the safety assessment that has to be performed.[40] Potential allergenicity to the novel gene product is another commonly expressed concern. Allergies to non-GM foods such as soft fleshed fruit, potatoes and soy are widespread. Clearly, new varieties of crops produced by either GM techniques or conventional breeding both have the potential to be allergenic. Concern surrounding this topic relates to two factors; the possibility that genes from known allergens may be inserted into crops not typically associated with allergenicity and the possibility of creating new, unknown allergens by either inserting novel genes into crops or changing the expression of endogenous proteins.

Assessment of the allergenic potential of compounds is problematic and a number of different bodies have produced guidelines and decision trees to experimentally evaluate allergenic potential.[41–43] These are effective at assessing compounds which may prove to be hazardous through a hierarchical approach which includes determining whether the source of the introduced gene is from an allergenic plant, whether GM foods react with antibodies in the sera of patients with known allergies and whether the product encoded by the new gene has similar properties to known allergens. In addition, animal models are used to screen GM foods.[40] Tests are not performed to formally assess any risk posed by inhalation of pollens and dusts; however, this is not assessed for conventionally grown foods and feeds either, and no allergies have been attributed to commercially grown GM pollen to date. Two examples are frequently quoted regarding GM crop allergenicity:

- A project to develop genetically modified peas by adding a protein from beans that conferred resistance to weevils was abandoned after it was shown that the GM peas caused a lung allergy in mice[44]
- Soya bean engineered to express a Brazil nut protein was withdrawn from production after it was also found to be allergenic in tests.[45]

Opponents of GM technology often cite these examples as proof that it is inherently unpredictable and dangerous, although another interpretation would be to say that safety testing of GM plants was effective in both cases, having identified allergenic potential before either product was released to market. It is perhaps a sobering thought, that if conventional plant breeding techniques had been used to achieve the same aims, there would have been no legal requirement for the assessment of allergenicity and the plant varieties could have been commercialized without *in vivo* testing. However, GM technology might also be used to *decrease* the levels of allergens present in plants by reducing expression levels of the relevant genes. For example, research was recently undertaken to identify an allergen in soybeans and remove it using GM technology.[46]

Non-food applications for GM plants

There are also a number of uses for plants outside of the food industry, for example in the timber, paper and chemical sectors and increasingly for biofuels. In all cases, non-GM and GM approaches are both being developed. Of significance to the medical field is the use of GM plants for production of recombinant pharmaceuticals. Molecular farming to produce GM plant-derived pharmaceutical proteins (PDPs) is currently being studied by academic and industrial groups across the world[4]. The first full-size native human recombinant PDP, human serum albumin, was demonstrated in 1990,[47] and since then antibodies, blood products, hormones and vaccines have all been expressed in plants.[48] Protein pharmaceuticals can be harvested and purified from GM plants, or alternatively, plant tissue in a processed form expressing a pharmaceutical could potentially be consumed as an 'edible vaccine'. As the molecular farming industry is still in its infancy, only one product has been approved for use so far – recombinant human intrinsic factor for use in vitamin B12 deficiency (http://www.cobento.dk). However, a number of molecular farming candidates are in clinical trials, including hepatitis B vaccine produced in potatoes and lettuce,[49] vaccines for heat labile toxin produced by *E. coli* and Norwalk virus,[50,51] human pro-insulin[52] and several monoclonal antibodies.[53–57]

Using GM plants as a platform for producing pharmaceuticals has many potential advantages over traditional systems. For example, GM plants can produce complex multimeric proteins such as antibodies that cannot be readily expressed by microbial systems. In addition, pharmaceutical production can potentially be on a vast agricultural scale.[4,58] The latter point is particularly important as it opens the way for many new applications that require administration of large amounts of proteins. These include topical application of antibodies and microbicides on mucosal surfaces for the prevention of infection. Not all applications need be on such a large scale; the hepatitis B vaccine is currently produced in genetically modified yeast, but not enough can be made at an affordable price to meet the demands of developing countries.[58] It has been estimated that 250 acres of greenhouse space would be enough to grow the amount of GM potatoes required to meet the annual demand for hepatitis B vaccine in the whole of South East Asia.[58]

Currently, over three million people die every year from vaccine-preventable diseases, the vast majority in the developing world. The current model of profit-motivated pharmaceutical production by companies in the developed world is ineffective in ridding the developing world of disease. GM plant technology may provide an alternative, as it is relatively low-tech and can be applied locally in the developing world by scientists working in partnership with governments and not-for-profit research funding agencies.

As with all aspects of GM crops, objections have been raised to the use of plants for manufacturing recombinant pharmaceuticals. Of greatest concern is that the pharmaceutical could inadvertently enter the human food chain. Theoretically, this might happen by uncontrolled dispersal of GM seed or by hybridization with a sexually compatible food crop following escape of GM pollen. In 2002, a company called Prodigene was fined and was severely censured for breaches in safety regulation when, due to inappropriate removal procedures, GM maize expressing a PDP was found to be growing in a soybean crop destined for human food consumption in the next growing cycle.[59] Although rare, incidents such as these demonstrate the potential risks of the technology. One proposal is to limit molecular farming to non-food crops, such as tobacco. Whilst feasible, there are significant advantages to the use of food crops for recombinant pharmaceutical production, such as attainment of GRAS status and utilizing well-established agricultural techniques for production. In the next section, the development of techniques to minimize GM gene flow are discussed.

GM plants and the environment

Any adverse effects on the environment through the large-scale growth of GM plants may indirectly affect human health. The following concerns have been expressed with regard to GM plants and the environment:

- That GM plants will sexually hybridize with non-GM plants through the transfer of pollen
- That GM plants may themselves become invasive weeds
- That the conditions required to grow GM plants will affect local wildlife populations.

In 2001, in a highly publicized study, evidence was presented that GM genes from GM maize had, by cross-pollination, contaminated wild maize in Mexico, the global centre for biodiversity of this species.[60] The validity of this work was disputed at the time of publication,[61,62] and later studies have also failed to detect any evidence of transgene spread to Mexican maize growing in the wild.[63] More recently, it has been reported that GM herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L) planted in Oregon, USA, was found up to 3.8 km outside the designated area of cultivation.[64] The authors of the study postulated that this dispersal was a result of both pollen-mediated sexual crossing with plants in the wild, and GM crop seed dispersal.

In 1999, a scientific paper was published which claimed that maize engineered to express the insecticidal Bt toxin was harmful to the larvae of the Monarch butterfly, an iconic species in American culture.[65] It was claimed that larvae reared on their staple diet of milkweed, dusted with pollen from Bt maize, ate less, grew more slowly and suffered higher mortality rates.[65] A number of longer term studies have since investigated the likelihood of Monarch butterfly larvae being exposed to sufficient quantities of Bt maize pollen in nature to illicit a toxic response, and this was found to be insignificant.[66–68]

It is difficult to evaluate the effect of GM crops, or probably more importantly the regime required to grow them, on surrounding wildlife, particularly when considering long-term effects. The UK Farm-Scale Evaluations[69] were the biggest study of the potential environmental impact of GM crops conducted anywhere in the world. In a four-year programme, researchers studied the effect of management practices associated with 'genetically modified herbicide tolerance' on farm wildlife, compared with conventional weed control.[69] The study reported that for three of the four crops tested, the wildlife was reduced in the GM fields compared to non-GM, but in the final crop (maize) the opposite occurred. The researchers stated that this difference did not occur because the crops were genetically modified, but because the farmer was able to employ a different herbicide regime to that used on conventional crops. The study has provided a platform for the government to objectively evaluate the effect of these crops, and even though the results were portrayed by critics of the technology as evidence for environmental hazards of GM, they resulted in government approval for the commercial growth of a herbicide-resistant GM maize in the UK.[69]

GM plants are also being assessed for how they might have a positive role to play in the environment by selective removal of pollutants – a process known as phytoremediation. For example, plants have already been genetically engineered to accumulate heavy metal soil contaminants such as mercury and selenium to higher levels than would be possible for non-GM plants,[70,71] so not only can they grow on contaminated sites but they can also remediate contamination. These plants can be harvested and destroyed, the heavy metals disposed of or recycled, and the decontaminated field re-used.

Gene transfer in the environment

A number of strategies have been proposed to prevent gene flow from GM plants to the wider environment. The transfer of a gene to wild or non-GM crops is a particular concern when it is expressing a protein that is designed for use in industry or pharmaceuticals. It is widely agreed that food should not contain products that have been specifically designed for these applications.[72] Two strategies to prevent this happening are physical isolation and genetic containment. Physical isolation can be difficult and costly and must be carried out at every stage of production. The crop must be bred in isolation and both small- and large-scale field trials should also be carried out in isolated areas.[72] The seed and commercial crops themselves could be grown either in contained greenhouse conditions, or in areas where no weed or food crop relatives are grown.[72] In addition, the ground where the GM crop has been grown and the surrounding fields should be left to 'lie fallow' for a time to ensure no seeds remain and grow in the next crop cycle.[72] In practice, the most likely approach will be to have specified farms where dedicated planting and harvesting equipment, transport, grain-handling, drying and storage systems would be used.[72]

Genetic containment can be achieved at a number of levels by technological means. Existing sterility and incompatibility systems to limit the transfer of pollen can be utilized,[72] as can Genetic Use Restriction Technologies (GURTS) which interfere with fertility or seed formation.[72] Transfer of the foreign genes into the chloroplast genome is another strategy, as in many plant species chloroplasts are maternally inherited and not contained in pollen.[73]

The co-existence of crops for human consumption alongside related varieties grown for industrial products, that would be harmful if consumed by people, is not a new phenomenon, nor one that is confined to GM plants. For example, farmers in Canada grow two varieties of (non-GM) rapeseed – high and low erucic acid producers. The erucic acid extracted from the high producing variety is used as an industrial lubricant and is toxic to humans if consumed, whilst the low producing rapeseed variety, called canola, is used to make cooking oil. Canadian farmers have developed systems to routinely keep the two apart during growth and processing.

GM plants and public opinion

Several NGO and media organizations are implacably opposed to GM plants. Crops that have been designed to help relieve malnutrition in the developing world, such as Golden Rice, are attacked on the basis that it 'tastes awful'[25] and that 'to be of any benefit a child would have to eat approximately 7kg of cooked Golden Rice',[74] an over-estimation by more than 15 times according to the founder of the product.[75] Insect-resistant cotton engineered to produce the Bt toxin requires far less pesticide application and produces higher crop yields than the non-GM counterpart,[76] generating savings of up to $500 per hectare for farmers.[77] Despite this, the crop has been criticized on the unsubstantiated grounds that it 'is killing the natural parasitic enemies of the cotton bollworm and increasing a number of other pests' and that 'its success will be short-lived as the bollworm will become resistant to the insecticide'.[74] These allegations have been made in spite of the fact that Bt bacteria have been widely used as a spray on organic crops by farmers for decades without any resistance developing in insects, in addition to no evidence of any emerging resistance after eight years of growing the GM crop.[76]

In some quarters, GM food is cited as being 'unnatural', although this accusation could be levelled at all of our food, which has been produced over millennia by artificial breeding. Very few commercialized crops would be able to survive unaided in nature. When considering 'natural' food production, it should be recognized that technology has always played an important part in the food industry. For example, antibiotics are widely used in feed in the poultry industry, and modern varieties of wheat were produced with the aid of radiation-induced mutation.[78] Scientists were greeted with expressions of outrage in many quarters when they genetically engineered frost-resistant plants with a gene from a cold water fish[79] – and yet fish and plants have a large proportion of genes in common, as do all living creatures.

Opposition to GM crops is perceived to be greater in the EU compared with other countries such as the USA, where food from GM crops has become part of the normal diet.[37] However, the situation is complex and UK public opinion is perhaps not so set against GM crops as is generally believed. Surveys have reported findings in which only 13% of consumers said they actively avoid GM foods, while 74% were not sufficiently concerned to actively avoid it.[80] This seems surprising considering the amount of anti-GM media coverage. From many of these articles it would seem appropriate to assume that the public as a whole are adamantly opposed to GM foods, but this is not substantiated by the surveys conducted.[80–82]

Nevertheless, considerable opposition to GM crops does exist and scientists must engage with the public to a much greater extent to ensure that the subject is debated rationally. This opposition is having many serious effects, not least because many developing countries that could benefit from the technology will not take it up as long as they believe that there remain significant areas of concern and that they will not be able to export produce to the EU market.[83] The implementation of the improvements to the design of GM crops discussed in this report would also further reassure the general public and pave the way for widespread acceptance of a technology that will be crucial in helping to alleviate current and future challenges in food and medicine supply.

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