Review

Facts and fiction of genetically engineered food

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The generation of genetically engineered (GE) foods has been raising several concerns and controversies that divide not only the general public but also the scientific community. The fear and importance of the new technology, as well as commercial interests, have supported many of the ongoing discussions. The recent increase in the number of GE foods approved for import into the European Union and the increasingly global commercial food trades justify revisiting the facts and fiction surrounding this technology with the aim of increasing public awareness for well-informed decisions. Techniques that have recently become available for assessing food quality and its impact on human health, as well as the wealth of scientific data previously generated, clearly support the safety of commercialized GE products.

Genetically modified commercialized crops – an overview

Although biotechnology emerged more than 8000 years ago, public awareness of biotechnology and genetic engineering concepts is a relatively recent phenomenon (Figure 1). Occasionally, biotechnology and genetic engineering have been used as synonyms, even though the definition of biotechnology is considerably wider. Genetic engineering comprises a set of modern biology techniques used to manipulate an organism's genetic endowment by introducing, modifying or eliminating specific genes. Biotechnology is any technological application that uses biological systems, living organisms or derivatives thereof to make or modify products or processes for specific uses.

Genetic engineering allows gene transfer between unrelated species. As a result, a genetically engineered organism (GEO) contains additional, or modified, characteristics encoded by the introduced gene(s) (Figure 2).

The first successful genetically engineered (GE) plant was reported in 1983, when an antibiotic resistance gene was inserted into a tobacco plant [1], and the first GE food approved for human consumption was the so-called Flavr Savr tomato produced by the Californian company Calgene. This tomato acquired rotting resistance via the antisense gene of polygalacturonase [2]. It was first sold in 1994 in the USA and, although the Food and Drug Administration (FDA) stated that there was no evidence for health risks and that the nutritional content was unchanged, it was only comercialized for a few years. The failure of Flavr Savr has been attributed to Calgene's major management mistakes and inexperience. Additionally, in 1996, Calgene was aquired by Monsanto, who where primarily interested in Calgene's ventures into cotton and canola oil.

During 2007, and after 14 years of commercialization (1994–2007), globally, transgenic crops were produced on 114.3 million hectares (282.4 million acres), 12% more than in 2006. Transgenic crops are now cultivated in 23

Glossary

5-Enol-pyruvylshikimate synthase from *Agrobacterium* sp. CP4 (CP4EPSPS): confers resistance to the herbicide glyphosate (marketed under the trade name Roundup). Glyphosate herbicide inhibits 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the synthesis of the aromatic amino acids tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe). Consequently, glyphosate impacts the synthesis of hormones and other crucial plant metabolites. The *epsps* gene from *Agrobacterium tumefaciens* CP4 strain, *cp4epsps*, codes for an EPSPS that has been shown to be insensitive to glyphosate, thus creating an alternative pathway that is not affected by the herbicide.

***cry* genes**: genes isolated from *Bacillus thuringiensis* (also known as *bt* genes), that have been used to confer resistance to some commercial crops. *cry* genes encode protoxins that solubilize in the alkaline environment of the insect midgut, where they are proteolytically converted by either crystal-associated or larval-midgut proteases into a toxic core fragment of 60 to 70 kDa. This activated toxin interacts with the midgut epithelium cells of susceptible insects and generates pores in the cell membrane, which disturb the osmotic balance and lead to cellular swelling and finally lysis.

Genetically engineered organism (GEO): an organism whose genome has been altered by genetic engineering techniques, also known as recombinant DNA technology. This technology allows the introduction of specific genes into a specific organism that will, consequently, exhibit new, silenced or modified traits.

***npt* II (or *neo*) gene**: gene isolated from the Tn5 *E. coli* transposon that is able to catalyse ATP-dependent phosphorylation of the 3′ hydroxyl group of the aminohexose ring of certain aminoglycoside antibiotics, for example neomycin, kanamycin, genetycin (G418) and paramomycin. This gene facilitates the selection of transgenic plant cells in tissue culture upon the supplementation of the culture media with an aminoglycoside antibiotic.

Phosphinothricin acetyl transferase (PAT): confers tolerance to the L-phosphinothricin herbicide (also called PPT, ammonium glufosinate, BASTA and Bialaphos). L-phosphinothricin inhibits glutamine synthetase, which results in a rapid increase of ammonia levels and a concomitant depletion of glutamine and several other amino acids in the plant. These effects are accompanied by a rapid reduction of photosynthetic CO_2 fixation and are followed by chlorosis and desiccation. Phosphinothricin acetyl transferase, first isolated from *Streptomyces viridichromogenes* or *Streptomyces hygroscopicus*, is able to degrade L-phosphinothricin, thereby counteracting its function.

Promoter: a segment of DNA, usually occurring upstream from a gene coding region, that controls the expression of a gene. Most of the promoters used in the development of the commercialized transgenic plants are constitutive promoters, thus leading to a continuous transcription of the respective genes. To date, the most commonly used promoter has been the cauliflower mosaic virus 35S promoter (CaMV35Spro), which is known to result in high levels of gene expression in plants.

Selectable marker genes: genes that allow the identification or isolation of cells expressing cloned DNA, as well as monitoring and selection of the transformed progeny.

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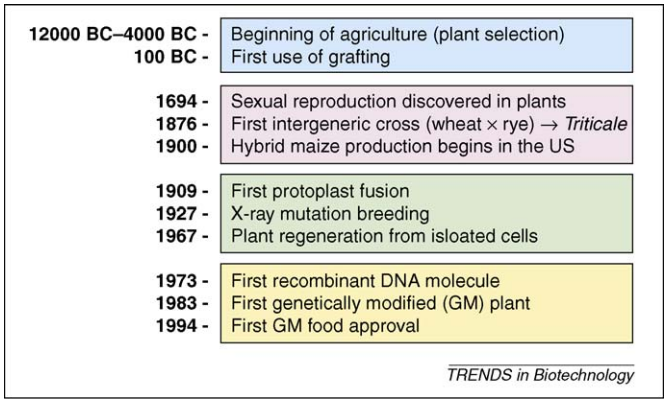

Figure 1. Tools used for plant improvement.

countries, comprising 12 developing and 11 industrial countries, and the number of transgenic crop traits and planted hectarage are predicted to double between 2006 and 2015, in what will be their second decade of commercialization [3]. Table 1 presents an overview of the currently approved transgenic crops for food use, in Europe, as of September 2008.

Benefits of commercialized GE crops

The main traits introduced into commercialized food products from GE crops are herbicide resistance/tolerance and insect resistance. These characteristics have brought several positive farm impacts. The introduction of herbicide resistant/tolerant (HR/HT) crops has, for example, been found to be more environmentally benign than the weed management technology it replaces, leading to substantial reduction in contamination of ground water, soil and air [4,5]. In addition, transgenic crops have facilitated the adoption of either reduced tillage practices [6] or its abandonment, thus protecting the soil from heat, preserving soil moisture and preventing erosion, which has also led to savings in time, energy, equipment and human labour and, consequently, to a reduction in carbon dioxide emissions [7].

The introduction of insect resistance has also had measurable positive effects, including decreased use of insecticides [7,8] and improved health and safety for farmers and farm workers from the reduced handling of pesticides [9]. It has also resulted in savings in energy and machinery used and in an improvement in crop quality (e.g. lower levels of mycotoxins) [10,11]. Moreover, economic studies clearly showed that farmers in developing countries can benefit from transgenic crops [12]. However, in spite of all the scientific studies reporting obvious advantages of using transgenic crops, their beneficial effects are not widely acknowledged. For example, Benbrook [13] claimed that HT and Bt (*Bacillus thuringiensis*) transgenic varieties only led to a reduction in pesticide use in the first three years of commercial use and that this tendency has changed since 1999.

Undoubtedly, the controversy over the costs, risks and benefits of agricultural biotechnology is still inflamed. Despite the reported benefits of HR/HT and insect resistant (IR) GE crops to farmers and the environment,

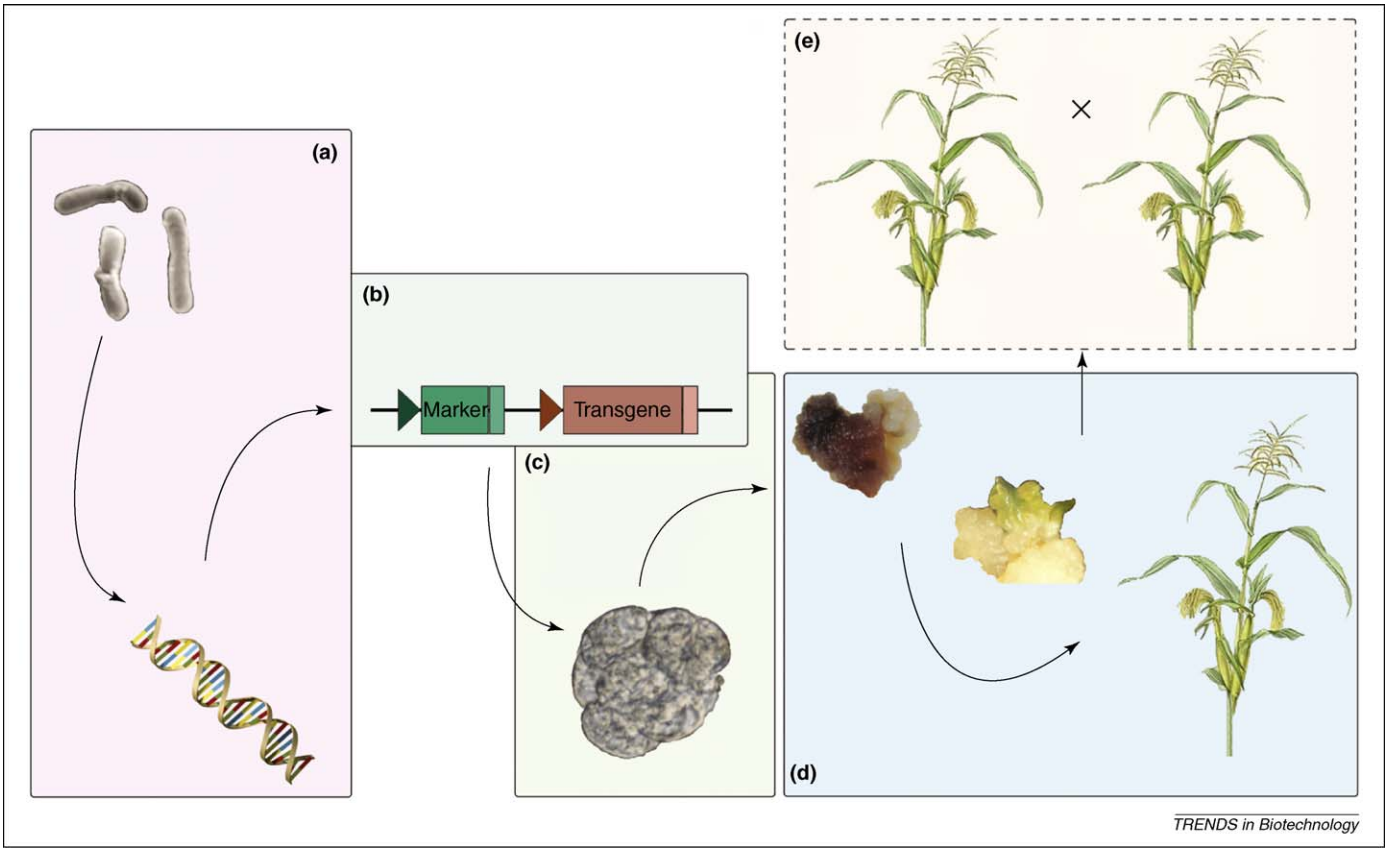

Figure 2. Main stages required for production of GE plants. One or several genes of interest are isolated from a donor organism **(a)**, cloned in appropriate vectors **(b)** and transferred into the receptor organism **(c)**. The receptor plant cells are then selected and regenerated to obtain complete GE plants **(d)**. Finally, the obtained GE plants are crossed with other, already improved plants **(e)**. The regenerated plants and their progenies are analysed at various levels throughout this process.

Table 1. Overview of the currently approved transgenic crops for food use[a]

GE crop	Developer	Trait	Date
Genetically modified soya			
GTS 40-3-2	Monsanto	Herbicide resistance (*cp4epsps* gene)	1996 (Food)
A2704-12	Bayer CropScience	Herbicide tolerance (*pat* gene)	2008 (Food)
Genetically modified maize			
T25	Bayer CropScience	Herbicide tolerance (*pat* gene)	1998 (Food and processed food – starch, oil, meal)
MON 810	Monsanto	Insect resistance (*crylAb* gene)	1998 (Food and food additives)
MON 809	Pioneer Hi-Bred	Insect resistance (*crylAb* gene)	1998
Bt11	Syngenta	Insect resistance (*crylAb* gene) Herbicide tolerance (*pat* gene)	1998 (Food and food additives) 2004 (Sweet maize, food produced freshly or in preserved foods and food additives)
MON 863 × NK603	Monsanto	Herbicide resistance (*cp4epsps* gene) Insect resistance (*cry3Bb1* gene)	2003 (Food additives)
NK603	Monsanto	Herbicide resistance (*cp4epsps* gene)	2005 (Food, food additives)
GA21	Syngenta	Herbicide resistance (mEPSPS protein)	2008 (Food)
MON863	Monsanto	Insect resistance (*cry3Bb1* gene)	2003 (Food additives) 2006 (Food)
DAS1507	Pioneer and Dow Agro Sciences	Herbicide tolerance (*pat* gene) Insect resistance (*cry1F* gene)	2006 (Food)
DAS1507 × NK603	Pioneer and Dow Agro Sciences	Herbicide resistance (*pat* and *cp4epsps* genes) Insect resistance (*cry1F* gene)	2007 (Food)
NK603 × MON810	Monsanto	Herbicide resistance (*cp4epsps* gene) Insect resistance (*cry1Ab* gene)	2007 (Food)
DAS 59122	Pioneeer Hi-Bred and Dow Agro Sciences	Herbicide tolerance (*pat* gene) Insect resistance (*cry34Ab1* and *cry35Ab1* genes)	2007 (Food/food ingredient)
Genetically modified rapeseed			
GT73	Monsanto	Herbicide resistance (*cp4epsps* and *goxv247* genes)	1997 (Refined oil, food additives)
T45	Bayer CropScience	Herbicide tolerance (*pat* gene)	1998 (Food additives)
MS8 × RF3	Bayer CropScience	Male sterility Barnase and barstar genes Herbicide tolerance (*pat* gene)	1999 (Refined oil) 2007 (Food)
Genetically modified cotton			
MON1445	Monsanto	Herbicide resistance (*cp4epsps* gene)	1997 (Food additives) 2002 (Food)
MON531	Monsanto	Insect resistance (*crylA(c)* gene)	1997 (Food additives) 2002 (Food)
MON15985	Monsanto	Insect resistance (*crylAc* and *cry2Ab2* genes)	2003 (Food additives)
MON15985 × MON1445	Monsanto	Herbicide resistance (*cp4epsps* gene) Insect resistance (*crylAc* and *cry2Ab2* genes)	2003 (Food additives)
Cotton MON 531 × MON1445	Monsanto	Herbicide resistance (*cp4epsps* gene) Insect resistance (*crylA(c)* gene)	1997 (Food additives)
Genetically modified sugarbeet			
H7-1	KWS SAAT AG/Monsanto	Herbicide resistance (*cp4epsps* gene)	2007 (Food – sugar and melasses)

[a]In Europe, as of September 2008 (http://europa.eu.int/comm/food/food/biotechnology/authorisation/index_en.htm and http://www.gmo-compass.org/eng/home/).

consumer organizations have claimed that these benefits are not perceived by most consumers as being advantageous to them. The so-called 'next generation of GE plants', currently under development, promises to invert this tendency by being able to provide foods that are more easily recognizable as being healthier, such as food products with enhanced vitamin content or longer shelf time, as well as improved pharmaceutical compounds, such as anticancer agents, therapeutic agents and edible vaccines.

Health concerns of GE food products

Although the vast majority of studies have shown no risks, a few reports have raised concerns regarding the potential horizontal transfer of antibiotic resistance markers (ARMs); the ingestion of 'foreign' DNA and the potential non-expected alterations in nutritional composition, allergenicity and/or toxicity of the new GE food products. Considering that a 0% risk is statistically impossible to prove, the claimed potential health effects are discussed in detail below.

Potential transgene horizontal transfer

Horizontal gene transfer (HGT), or lateral gene transfer (LGT), is any process by which an organism transfers genetic material to another organism other than its offspring and which is followed by integration and expression of the genetic material. This process occurs widely among prokaryotes and crucially influences the evolution of bacterial genomes and the diversification and speciation of the enterics and other bacteria [14]. Various mechanisms exist

for HGT between microorganisms, such as phage transduction, conjugation and transformation by free DNA [14]. The possible scenario for gene transfer between GE crops and microorganisms is, however, limited to transformation by free DNA.

Despite the natural prevalence of ARM genes, which are used in transgenic plant development, among soil and enteric bacteria (which eventually might also be present in our food), concern has been raised regarding the putative transfer of these marker genes to bacteria colonizing our mouth, stomach and gut or to bacteria that we eat together with foods. Van den Eede *et al.* [15] discussed the possibility of this event and speculated about the potential danger it poses to human health by compromising the therapeutic value of antibiotics. However, if we consider the potential horizontal transfer of the marker genes, any gene present in our food, be it GE or not, should have the same potential to be transferred.

Anyhow, there are numerous factors that limit the transfer, uptake and stabilization of plant DNA in bacteria. The breakdown of DNA during food processing before consumption [16,17] and during its passage through the gastrointestinal tract, as well as the existence of bacterial restriction enzymes that typically cleave foreign DNA, are some of the known barriers. Moreover, the recipient bacteria would have to be able to take up and integrate the DNA into their genome, either as a linear fragment (which would require extensive sequence homology), or by formation of an independent replicon. Furthermore, the expression of the integrated gene in bacteria would also require that it becomes associated with appropriate regulatory promoter sequences. Finally, to be maintained in the bacterial population, the transferred trait would have to confer a competitive advantage.

Some scientific studies have reported that food processing, and/or digestion, might be potentially insufficient for the total degradation of ingested DNA. For instance, it has been shown that orally ingested M13 DNA not only survives transiently in the mice gastrointestinal tract but can also enter into the bloodstream, reaching the nuclei of leukocytes, spleen and liver cells, and it might even penetrate the placental circulatory system, thus reaching the offspring of pregnant animals [18,19]. In addition, plasmid DNA that had been previously exposed to human or sheep saliva was still capable of transforming competent bacteria *in vitro* [20,21]. More recently, it was demonstrated that the *epsps* transgene (see Glossary) from GE soya survived during passage through the small intestine [22]. These studies have been taken as evidence that ingested functional DNA could exist in the human gut. Furthermore, although it has been shown that potential integration of foreign DNA with low or no sequence homology under natural conditions is a rare event in bacteria [23], it could be demonstrated that the integration of any heterologous DNA might be significantly increased if it had been linked to a DNA sequence that is homologous to the recipient genome [24]. Nevertheless, such integration was never demonstrated to take place in environments in which the DNA is released from the plant genome, and there is so far no evidence for uptake of ingested DNA by gastrointestinal bacteria as a consequence of the consumption of food.

The potential inactivation of an orally administered antibiotic by the marker gene product and the potential transfer of the ARM genes to gut epithelial cells have also raised concern [25].

npt II (see Glossary) is the most common ARM gene used in the production of GE foods under commercialization in the EU. Fuchs *et al.* [26] verified that the corresponding enzyme is rapidly inactivated by stomach acid and degraded by digestive enzymes, thus preventing its availability for a putative inactivation of an orally taken antibiotic dose. It is also important to note that the *npt* II marker gene is ubiquitous in nature because it comes from *E. coli* bacteria, and it confers resistance to antibiotics that have no therapeutic relevance in human and veterinary medicine [15]. Thus the argument of a putative increase in antibiotic resistance of gastrointestinal bacteria due to a potential horizontal transfer of this marker gene is clearly unacceptable.

The FDA has the following view on the issue of potential transfer of ARM genes to gastrointestinal cells, that, even if it was possible, it would not compromise public health because gastrointestinal epithelial cells do not divide and also have a relatively short life span (seven days). This fact alone would avoid the unlikely compromise of antibiotic therapy [25].

The existence of public concerns, even when no expert panel has ever identified a significant risk associated with the use of ARM genes in plant genetic engineering, has notwithstanding led to efforts aimed at producing ARM-free GE plant products. Such strategies involve various techniques, including the removal of these genes after transgenic plant selection and before commercialization [27,28]. The inevitable increase in costs and development time of the final product, inherent in this process, as well as the long history of safe use of the ARM genes in biotechnology food applications has led some to question the real benefits of marker gene excision [27]. Moreover, the excision technique is not easy to transpose to a wide range of plants and has been restricted to some few model plants.

However, a recently discovered plant gene that confers resistance to kanamycin could be a viable alternative to *npt* II [29]. The extremely low sequence homology of this gene to bacterial DNA could further decrease any hypothetical recombination and functional integration in bacteria. The use of intron-containing marker genes could also minimize putative functional transfer from GE plants to microorganisms by avoiding RNA processing and subsequent translation in prokaryotes [30].

Consumption of 'foreign' DNA

One of the typical concerns consumers express is that of the presence of DNA originating from 'foreign' species (i.e. viruses or bacteria) in the genome of a food plant. However, such 'foreign' DNA intake occurs every time we eat because bacteria and viruses are always present in our food. Moreover, because all DNAs are chemically equivalent, any potential risk associated with DNA consumption would not depend on the species of origin, but only on its sequence [31].

One of the DNA sequences that has raised concern is the cauliflower mosaic virus 35S promoter (CaMV35Spro). The

CaMV35Spro was one of the first plant promoters identified that showed the capacity to direct constitutive expression of heterologous genes in a variety of plants. The exhaustive investigation of its behaviour in many plant species contributed to its widespread use in the development of GE plants for research and agronomic applications.

Kohli *et al.* [32] showed that CaMV35Spro contains a 19-bp palindromic sequence, including the TATA box of the CaMV35Spro, which can act as a hotspot for recombination. Therefore, it has been suggested that the widespread use of a promoter such as the CaMV35Spro, which is very efficient and can function in a wide range of organisms, might lead to inappropriate overexpression of genes in species to which it is transferred and promote horizontal transfer and recombination [33]. In addition, it has been proposed that it might even recombine with dormant endogenous viruses, leading to putative new infectious viruses [33]. Indeed, retroviruses have accumulated in the genomes of many organisms, including humans, throughout evolution, where they comprise ~8% of the total DNA [34]. It is known that some human endogenous retroviruses (HERVs) might produce intact viral particles *in vitro* [35], and some human diseases, such as schizophrenia [36] and some forms of cancer [37], have been associated to endogenous retrovirus expression. However, almost all genomes of HERVs contain lethal mutations and all known endogenous retroviruses, so far, have been unable to produce virus particles *in vivo,* or to replicate. As mentioned above, several barriers exist that would limit the potential interaction of the CaMV35Spro with human DNA. It should also be noted that CaMV (a plant retrovirus) has been ingested by humans in small quantities for thousands of years because it is present in ~10% of cabbages and cauliflowers [38]. The virus infects most plant cells and produces ~105 particles per cell, each particle containing one molecule of viral genome with one copy of each of the two promoters (19Spro and 35Spro) [38]. Thus, the consumption of any CaMV-infected vegetables would result in the ingestion of far more copies of the 35S promoter than the consumption of transgenic plants carrying this promoter.

Unexpected alterations in nutritional composition

Because improved nutritional quality is one of the potential benefits of genetic engineering of food plants, a crucial question is whether these modifications might cause any unexpected and/or undesired effects in the nutritional composition of the final product. It is important to notice that although this concern has been essentially raised for GE food products, it is equally valid to pose this question for plants obtained by conventional breeding techniques.

Soybean (*Glycine max*) has been one of the target crops for genetic modification, and ~64% of worldwide consumed soybean is GE [3]. Although there is no absolute consensus regarding the health benefits of soybean products and soybean isoflavones (antioxidant polyphenols), which might act as phyto-oestrogens in mammals, it seems that they could have beneficial effects in post-menopausal women with regard to bones, cardiovascular risk and hot flushes [39], as well as in prostate cancer [40] and breast cancer [41]. It is therefore not surprising that consumers of transgenic soybean, such as Roundup Ready soya, which has been genetically engineered for glyphosate herbicide resistance, would like to be assured that it contains similar amounts of isoflavones as conventional soya. Soybean isoflavones are shikimate pathway products, which is the target pathway of glyphosate herbicide. Glyphosate has been shown to reduce the levels of isoflavone-related compounds in non-transgenic soybeans. Because it was found that glyphosate resistant (GR) soybeans are not completely resistant and that the resistance level is influenced by environmental conditions, another concern is whether glyphosate might affect the levels of oestrogenic isoflavones in GE soybeans. Lappé *et al.* [42] reported an overall reduction of 12–14% in the oestrogenic isoflavones of GR soybean varieties as compared to conventional varieties grown under similar conditions. However, other studies had opposing results and were able to demonstrate a similar range of natural variability in isoflavone content for GR soybeans as compared to the conventional varieties [43]. Moreover, it was also shown that there were no remarkable effects of glyphosate applications on levels of oestrogenic isoflavones in GR soybean varieties [44]. The major conclusion that could be drawn from these studies only confirmed what soybean experts already knew, that the isoflavone content of any soybean variety, GE or not, is highly variable and greatly influenced by environmental factors such as weather and soil.

Additional nutritional assessment studies have also compared other genetically modified (GM) plants, such as GR maize [45], IR potatoes [46] and IR rice [47], with conventional varieties and have confirmed that GM varieties were of equivalent composition to the non-transgenic varieties. Furthermore, several studies that assessed novel foods, such as transgenic potatoes with soybean glycinin and GR soybeans, for animal nutritional value revealed that the nutritional and biochemical characteristics of the transgenic varieties do not significantly differ from those of the non-transgenic products [48–50].

Allergenicity and/or toxicity

Another issue that needs to be addressed in GE food safety is whether the products of introduced genes might represent allergens and/or toxins or might induce unintended effects on plant metabolism that could lead to upregulated expression of allergens and/or toxins. One of the most controversial studies that addressed the potential toxicity of GE food was conducted by Ewen and Pustzai in 1999 [51]. This study reported several injurious effects in the gastrointestinal tract of rats that had been fed with GE potatoes expressing the lectin *Galanthus nivalis* agglutinin (GNA), a compound with insecticide activity. Ewen and Pustzai were, at that time, severely criticized by the scientific community, not only for seeking publicity by mass media but also for the obvious deficiencies in the presented experimental design, such as the inclusion of too few animals per diet group, uncertainty about the differences in chemical composition between non-GE and GE potato varieties, lack of essential controls and application of inappropriate statistical techniques for the analysis of results [52]. In an effort to set the record of GE food toxicity

straight, Poulsen *et al.* [53] recently performed a 90-day rat feeding study designed to assess the safety of GE rice expressing the same GNA lectin. They found several significant differences in the composition of GE and non-GE rice and also between the rats fed with GE rice and the control rats with regards to their blood biochemistry, haematology, immunological parameters and organ weights. Nevertheless, none of these observed effects was considered to be adverse and, moreover, most of them seemed to be caused by the increased water intake of the rats fed with GE rice, which can be explained by the higher iron content in the GNA rice (+144%) and consequent increased need for iron excretion. It should be highlighted that the potential toxicity and/or allergenicity of lectins has been widely documented (including by the Pustzai group), and for this reason no GE product carrying lectin genes has ever been targeted for commercialization.

The toxicological evaluation of other GE products has also led to contradictory results. Some studies with mice that have been fed on GE Roundup Ready soya claimed significant modifications of some nuclear features of their hepatocytes. Based on these data, and the fact that liver is a primary site for biotransformation of digestion products and that liver-cell nuclei might reveal cellular activity, these authors suggested that GE soybean was able to modify hepatocyte metabolic activities [54]. The same authors further suggested that a GE soybean diet can influence the function of pancreatic acinar cells in mice. These cells have a particular role in the synthesis, storage and regulated secretion of the different enzymes presented in pancreatic juice [55]. In addition, Vecchio *et al.* [56] used testis of mice fed on GE soybean to monitor any potential toxic effects. The authors reported an inhibition of transcription, which occurred in parallel with a decrease in nuclear pore density and consequently led to the accumulation of RNA in the form of perichromatin granules in the testicular cells.

However, all these studies on GE soybeans are tainted with important flaws. For instance, they did not provide any information on the source, nutritional composition or kind of processing of the soybeans used, nor did they discuss the appropriateness of the control used, such as whether it was a near isogenic line that was grown in the same field and under the same environmental conditions. One piece of crucial information would be the isoflavone content of the GE soybean versus the control, because the oestrogenic effect of isoflavones *per se* could be responsible for changes in cell nuclear trafficking [57].

In contrast with the data of Vecchio *et al.*, Brake *et al.* [58] were able to demonstrate that neither Roundup Ready soya nor Bt maize [59] had any negative effects on the testicular development of foetal, postnatal, pubertal and adult mice. The lack of adverse effects of other GE foods, such as GR soybeans [60], IR MON810 and MON863 maize

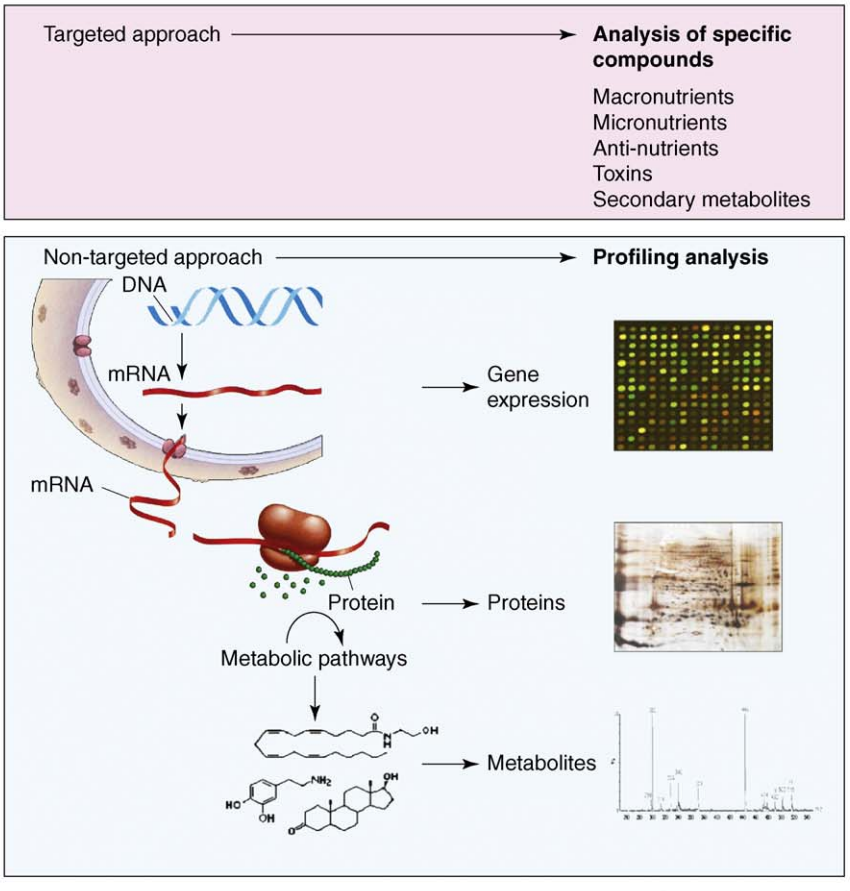

Figure 3. Strategies suggested for analysis of unintended effects of GE food crops. Two approaches have been suggested for detecting putative unintended effects of GE food crops. One approach is targeted, focusing on detection of specific key nutrients, whereas the other one is non-targeted and based on profiling methods. In the second case, potential alterations in the GEO occurring at the genomic level, as well as at the levels of gene expression, translation and metabolic pathways, are evaluated.

lines [61,62], IR and phosphinothricin tolerant 1507 maize [63] and IR KMD1 rice [64], was also confirmed.

Because most commercialized GE foods result from the introduction of genes that originated from sources with unknown allergenic potential, a major concern regarding GE food safety remains the potential allergenicity of the resulting products. However, it is important to note that genetic engineering has also been highlighted as a powerful tool to reduce allergenicity in important food plants, such as soybean [65], tomato [66] and rice [67].

So far, and after several years of testing of novel GE foods, post-market allergenicity problems have only emerged in two cases; one concerns a Pioneer Hi-Bred GE soybean variety and the other the Aventis Starlink GE maize variety. Pioneer Hi-Bred soya was modified to improve its nutritional value by transferring a gene from Brazil nut coding for a methionine-rich protein (2S albumin), which in conventional varieties is a rare amino acid. However, it was verified that 2S albumin from the Brazil nut, as expressed in transgenic soybeans, was able to bind immunoglobulin E (IgE) from people who are allergic to Brazil nuts and, consequently, although this product was only ever intended for use as animal feed, it was never commercialized [68].

StarLink maize, a particular Bt maize, contains Cry9C, an insecticidal protein from Bt bacteria. This maize had been approved by the Environmental Protection Agency for use as animal feed but not for food, because the Bt Cry9C protein (68 kDa) had some attributes of an allergenic protein after its expression in maize. In September of 2000, traces of Starlink maize were detected in some food products (taco shells) (http://europe.cnn.com/2000/FOOD/news/09/18/food.corn.reut/). Additionally, the Cry9C protein was also detected in maize seeds from another GE variety. After the publication of these occurrences by the media, some consumers have reported adverse effects presumably related to the consumption of maize-containing food products. However, after extended evaluations conducted by various independent institutions [69,70], a direct implication of Cry9C in these putative incidents was not confirmed.

Although the cases of the Pioneer Hi-Bred soybean and the Starlink maize have raised doubts regarding the safety of the food chain, the truth is that they also proved that the existent regulatory system was running efficiently. To date, no experimental evidence has supported a higher degree of allergenicity of approved GE foods as compared to their non-transgenic counterparts [71–75].

New profiling methods for evaluating the safety of GE food

Before market introduction, GE food products, like any other novel food product, are subjected to extensive

Table 2. Main goals and conclusions from GE food-safety assessments with profiling methods

Approach	Main objective	Major conclusions	Refs
2D polyacrylamide gel electrophoresis; mass spectrometry	Comparison of protein profiles of a GE tomato and the same unmodified hybrid for resistance to tomato spotted wilt virus	No significant differences, either qualitative or quantitative, were detected	[81]
	Comparison of tuber protein profiles of potato varieties, landraces and GE lines	Less variation between GM lines and their non-transgenic counterparts compared with variations found between different non-GE varieties and landraces	[82]
2D polyacrylamide gel electrophoresis; western blotting; mass spectrometry	Comparison of the IgE response of soya-allergic individuals both to GM Roundup Ready soya and its non-transgenic control	Allergen expression was not altered by genetic modification	[71]
Microarrays	Monitoring of the extent of unexpected transcriptome modifications in rice obtained by conventional plant breeding and by γ-irradiation in comparison with rice obtained through genetic engineering	Conventional breeding of rice mutants caused higher modification at the transcriptome level than the introduction of transgenes by genetic engineering	[83]
	Comparison of endosperm and leaf gene-expression profiles of transgenic and conventionally bred wheat lines expressing additional genes encoding for high molecular weight subunits of glutenin	Greater differences in expression profiles amongst related lines of wheat that were produced by conventional breeding than in a comparison between transgenic and untransformed lines	[84]
	Comparison of gene-expression profiles in developing seeds of wild-type wheat and wheat transformed for endosperm-specific expression of an *Aspergillus fumigatus* phytase	The expression of the *A. fumigatus* phytase gene in the wheat seed had no significant effects on the overall gene expression patterns in developing seeds	[85]
	Comparison of transcriptome profiles of commercialized MON810 maize varieties and their non-GE near-isogenic counterparts	Variation between GE and non-GE plants was lower than observed amongst lines produced by conventional breeding approaches	[86]
Metabolite profiling	Comparison of metabolite profiles of conventionally bred wheat lines and transgenic lines that expressed additional high-molecular-weight subunit genes	Differences between the control and transgenic lines were within the same range as the differences amongst the control lines that had been grown on different sites and in different years	[87]
	Metabolite profiling to assess the compositional changes occurring in potato tubers after genetic modifications to different metabolic pathways	Encountered changes were not significant in the context of the variability within the entire dataset	[88]
	Comparison of the metabolite profiles of Bt GE maize and their non-transgenic parental lines grown under identical conditions	Metabolism of the transgenic organism showed unexpected variations compared with the wild line. L-carnitine was pointed out as an example of a good candidate for markers of transgenic Bt maize	[89]

assessment of their potential effects on human health. Konig *et al.* [76] organized an overview of test methods developed for the assessment of the safety of foods derived from GE crops. Although risk assessment of predictable effects is easily attained through specific *in vitro* and clinical tests, some groups have highlighted the need to also estimate any unpredictable and unintended effects [76,77], although there is no indication that such effects are more likely to occur in GE crops than in conventional ones. There are two different approaches for this purpose [78]. One is a targeted approach that is regularly used to evaluate new commercialized GE foods. Here, several key nutrients are analysed that, if inadvertently altered, could influence the nutritional value and eventually the safety of the modified product. This approach does not consider any unknown anti-nutrients and natural toxins. The second strategy is a non-targeted approach that is based on profiling methods, in which potential alterations in GEOs that occur at the genomic level, as well as at the levels of gene expression, translation and metabolic pathways, are evaluated [78] (Figure 3).

Although target approaches for evaluating potentially hazardous effects of genetic modification remain in regular use, several recent studies have begun to explore profiling methods with the aim of increasing the probability of detecting any unpredictable unintended effects and, consequently, improving the efficiency of GE food safety assessment (Table 2). Profiling techniques could be a potentially powerful tool to complement the targeted safety assessment of GE food, offering the capacity to perform a broad screening for possible changes of the modified organism at different integration levels of cells or tissues in a non-selective, impartial manner. However, there are still some limitations that have to be addressed. One of these limitations is the interpretation of the observed differences with respect to their biological relevance and toxicological significance. It is crucial to take into account the natural variation in the levels of certain compounds in crops when assessing the statistical significance of a detected unintended effect. Furthermore, much work still has to be done regarding standardization of sample collection, preparation and extraction procedures and validation of methods [79].

Conclusion and outlook

There has been an extensive worldwide debate over GE foods and related issues, such as the consumer's 'right-to-know', labelling, safety and ethical or putative religious concerns. Clear division between and amongst the public and the scientific community has led to heated discussions (which so far have not resulted in a consensus over the topic) and has even raised questions on the impartiality of the review process in some scientific journals [80].

In this review we have presented several scientific studies that have been performed with the aim of addressing and clarifying the issues of safety of GE foods. From these, it is clear that there is no unequivocal evidence supporting adverse effects of any of the currently commercialized GM food products. Based on scientific data, the European Food Safety Authority (EFSA) has been providing recommendations on transgenic plants and their products that EU countries should follow.

Genetic engineering is a very recent technology. Every new technology raises fear, and it is understandable that consumers have doubts about potential health, environmental and ethical implications. Nevertheless, the beneficial effects of genetic engineering are unquestionable, not only in developing novel crops but also in developing new medical products. Across human history, we have already faced several similar situations, such as the discovery of electricity and antibiotics and the invention of cars and planes, to name a few, and despite the potential risks, which undoubtedly exist, we have always decided to go ahead in the name of progress. In this respect, genetic engineering should simply be seen as another human-made discovery that has tremendous potential not only for developing but also for developed countries.

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