



Review article

Biological and biomedical aspects of genetically modified food

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Abstract

Genetically modified (GM) foods are the product of one of the most progressive fields of science—biotechnology. There are major concerns about GM foods in the public; some of them are reasonable, some of them are not. Biomedical risks of GM foods include problems regarding the potential allergenicity, horizontal gene transfer, but environmental side effects on biodiversity must also be recognized. Numerous methods have been developed to assess the potential risk of every GM food type. Benefits of the first generation of GM foods were oriented towards the production process and companies, the second generation of GM foods offers, on contrary, various advantages and added value for the consumer. This includes improved nutritional composition or even therapeutic effects. Recombinant probiotics and the principle of alternative gene therapy represent the latest approach of using GM organisms for biomedical applications. This article tries to summarize and to explain the problematic topic of GM food.

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1. What are genetically modified organisms?

Along with introducing of genetically modified organisms (GMO) into daily life the GMO give rise to safety concerns. Have we really any reason to be afraid? Firstly, we must precisely know what do GMO represent. GMO are organisms that are generated by combining genes of different species using recombinant DNA (rDNA) technology. Resulting organisms are then said to be transgenic, genetically engineered or genetically modified (GM). This group of organisms includes microorganisms, plants and animals. We can meet GMO in different spheres of our life such as medicine, agriculture and, of course foods. This issue is interesting and much discussed. GMO can resolve problem of nutrition in the third world, reduce vitamin and nutrition deficiency. For example by using GM technology it is possible to create rice with an increased synthesis of vitamin A

precursor [1], plants resistant to herbicide and insecticide or plants able to grow in extreme weather conditions. Recently, GM crops are being used in agricultural production in 18 countries around the world [2]. Although there is no direct evidence that GMO have any harmful effect on human's health many people are sceptical about. Reason for this sceptical attitude is lacking knowledge and negative propagation of GMO in the media. It is often being told only about risks associated with GMO but not about benefits. Nevertheless, some authors predict that 21st will be the century of biotechnology. Genetic modifications are often compared to traditional plant breeding (TPB). TPB can be defined as "applied plants genetics" [3]. Principal mechanism of TPB is chromosomal recombination. In comparison to TPB the GM technology enables transfer of single genes, while TPB manipulates with hundreds of genes. In other words we are not able to predict how much genetic information will be transferred by TPB. In addition, GM technology allows transfer between species, while TPB does not allow overcoming of interspecies barriers. Finally, GM technology results in small proteome changes in comparison to TPB.

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2. What is the history of GMO?

In prehistoric times man used to find food in nature and after that he went for domestication of the plants for further cultivation. With the help of scientists and naturalists he began to utilize the techniques such as hybridization and produced plants through natural breeding between related varieties. After 1900 by using Gregor Mendel's genetic theory of crop species improvement developed through classic selection, a plant of one variety is crossed with a related plant to produce desired characteristics. With the discovery of three-dimensional double helix structure of DNA by James Watson and Francis Crick in 1953, scientists gained the possibility to identify and "splice" genes from one kind of organism and transfer them into another. In 1973, Herbert Boyer and Stanley Cohen combined their research to create the first successful rDNA organism [4]. In 1980, the US Supreme Court in *Diamond* puts genetically patenting law into force. This decision allowed the Exxon Oil Company to patent an oil-eating microorganism. In 1982, US Food and Drug Administration (FDA) approved the first genetically engineered drug, Genentech's Humulin, a form of human insulin produced by bacteria. This was the first consumer product developed through modern bioengineering. In 1992, the first GM food crop was tomato produced by Calagene, named Flavr Savr. Calagene submitted this to US FDA for its safety evaluation, and FDA declared the tomato as safe for human use. Finally, it was released for the market in 1994. The antisense gene in Flavr Savr tomatoes delays its ripening and allows better storage. The first test of transgenic potato was conducted in 1986 in Belgium and it was conducted in United States the next year. Subsequent GM food crops involve virus-resistant squash, a potato variant that includes an organic pesticide called Bt, strains of canola, soya-bean, corn and cotton engineered by Monsanto to be immune against their popular herbicide Roundup and Bt corn. In 1994, The European Union's first genetically engineered crop, tobacco, was approved in France and in 2000 International Biosafety Protocol has been approved by 130 countries at the Convention on Biological Diversity in Montreal, Canada. The protocol agrees upon labeling of genetically engineered crops, but it still needs to be ratified by 50 nations prior to gaining validity and effect.

3. Which generations of transgenic plants are known?

The application of modern genomic research techniques to plant species promises an explosion of new knowledge and information that may lead to important new advances in agricultural production and the quantity, quality and variety of food products. Although various classifications can be found in the literature, two principal classes of GM foods exist (Fig. 1).

Roundup Ready soybeans are parts of the "first generation" of biotechnologically derived seeds, which offer farmer benefits such as lower chemical input and reduced farming costs. Other seeds, which are part of this "first generation", include those with specific pest, disease or virus resistance. For example, papaya-ringspot-virus-resistant papaya has been grown and

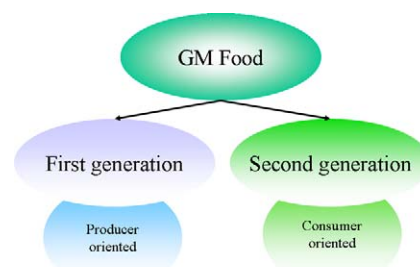


Fig. 1. GM food can be divided into two groups.

The first generation brings various improvements in the production. GM food from the second generation is oriented towards the consumer and brings better nutritional value or even therapeutic effects.

commercialized in Hawaii since 1996 [5]. There may also be a benefit for the environment, if the use of pesticides will be reduced. For instance, transgenic crop containing insect-resistant genes from *Bacillus thuringiensis* have made it possible to reduce significantly the amount of insecticide applied on cotton in the United States.

The "second generation" of seeds now being introduced offer processor, end-user and consumer specific benefits such as increased levels of protein, modified and healthier fats, modified carbohydrates, improved flavor characteristics and increased levels of desired phytochemicals. Modification of qualitative and quantitative characteristics, such as the composition of proteins, starch, fats or vitamins by modifications of metabolic pathway could increase the nutritional status of the foods and might help to improve human health by addressing malnutrition and under-nutrition. For example, iron deficiency causes anemia in pregnant woman and young children. Anemia has been identified as a contributing factor in over 20% of maternal deaths (after giving birth) in Asia and Africa [6]. Transgenic rice with elevated levels of iron has been produced using genes involved in the production of an iron-binding protein and in the production of an enzyme that facilitates iron availability in the human diet [7,8].

The next vital step toward realizing the full potential of genomic advances to neutralize bio-threats is to translate GMO's genetic codes into a deeper understanding of how these organisms infect and survive in the host and how they cause toxic effects. This information is needed to fully understand what makes a biological agent a threat and how it can best be found and counteracted. In cases of bioterrorist attack such as the recent anthrax outbreaks, decision makers and law enforcement officials need to understand the situation quickly. Early detection and identification of the biological organism and its source are crucial for minimizing the potentially catastrophic human and economic costs of such an attack.

4. How are GMO made?

The rDNA techniques and genetic "engineering" is expected to bring about great progress in the improvement of breeding technology and development of new plant varieties showing high quality or producing higher yields. This will improve nutritional, flavor or storage properties, such as those with excel-

lent pest and disease resistance or stress tolerance. So how can you prepare GM plants? If you can identify and isolate an appropriate gene, that has the trait you need, you can transfer this gene from one organism to another using the rDNA technology bypassing biological species. The introduced gene usually serves two distinct purposes. One of the transgenes is a selectable marker, meaning that it confers the transformed cells a distinctive phenotype that can be used to select few GM among a sea of unmodified cells. These marker genes typically confer resistance to a selective agent, often an antibiotic. The other transgene is meant to confer a desirable phenotype, which is often agronomic (herbicide, pest, stress resistance) or related to food quality (shelf-life, taste, nutritional value).

There are four main approaches in which plants can be manipulated by genetic “engineering” (Fig. 2). These are:

- Gene insertion using a bacterial vector—*Agrobacterium tumefaciens*. To achieve a genetic modification using this technique, a restriction enzyme is used to cut non-virulent plasmid DNA derived from *A. tumefaciens* and thus create an insertion point, into which the gene can be ligated. The engineered plasmid is then put into a strain of *A. tumefaciens*, which contains a “helper” plasmid and plant cells are treated with the recombinant bacterium [9]. The helper plasmid assists the expression of the new gene in plant as these grow in culture. The utility of *A. tumefaciens*-mediated gene transfer is illustrated by its use to develop potato plants that are resistant to Colorado beetle [10]. The inserted cryIIIa gene encodes for an insecticidal protein from soil bacterium *B. thuringiensis*. When expressed in potato plants this protein is toxic to Colorado beetles by combining with a protein found within their digestive epithelium to form a membrane channel that results in cell lysis.
- In microballistic impregnation or gene gun the target gene is applied to minute particles of gold or tungsten, which are fired into plants tissue at high velocity. Cells that incorporate the target and marker genes can then be selected in culture and grown [11,12].
- Electroporation is the application of a pulsed electric field that allows the transient formation of small pores in plant cells, through which genes can be taken up and in the form of naked DNA incorporated into the plant genome [13,14].
- Gene neutralization by means of antisense technology, homologous recombination (gene knock out) and gene replacement. Antisense technology involves introduction of the non-coding strand sequence of a particular gene to inhibit the expression of the desired gene. The application of antisense technology has resulted in a consumer food product, the FlavrSvr™ tomato, in which shelf-life is enhanced by suppression of the enzyme polygalacturonase [15]. A similar increase in the shelf-life of tomatoes can also be achieved using antisense technology against the enzyme 1-amino-1-cyclopropanecarboxylic acid synthase [16]. In traditional breeding technology, the development of a new variety was only possible using particular species and crossing its known types. Thus, researchers have prepared technologies such as antisense technology to develop new varieties. And the results are insect-resistant, herbicide tolerant, virus-resistant crops.

5. How are GMO detected in food?

EU legislation for GM crops marking from year 1998 and 2000 (EU Regul. 1139/98 and 49/2000) determines, that crops containing more than 0.9% GMO have to be marked to ensure the deducibility from “farm to fork”. Nevertheless, it is still not clear, whether this number means DNA/DNA or weight/weight ratio. Though, if the introduced DNA and its product have been degraded during the production process, there was no need to mark the final product. However, new legislation extends the “marking law” to all foodstuffs for better monitoring of the fate of food during its processing (EU Regul. 1829/2003 and 1830/2003). There are some current projects that focus on:

- development of novel detecting methods for determining GM crop derived foods (RTD-project),
- development of quantitative methods (QPCRGMFOOD),
- detection using microarray technology (GMOCHIPS).

QPCRGMFOOD project is also focused on real-time methods development and determination of suitable reference genes in polymerase chain reaction (PCR) techniques for the cardinal GM crops food [17]. For detection of GMO content in foodstuffs, there are two approaches used:

- Those based on correlation of GMO content with GM DNA content in food.
- Methods based on correlation of GMO content in food with GM protein (or GM mRNA) content in product. However, the interrelation GMO/GM protein has a lower information value [18].

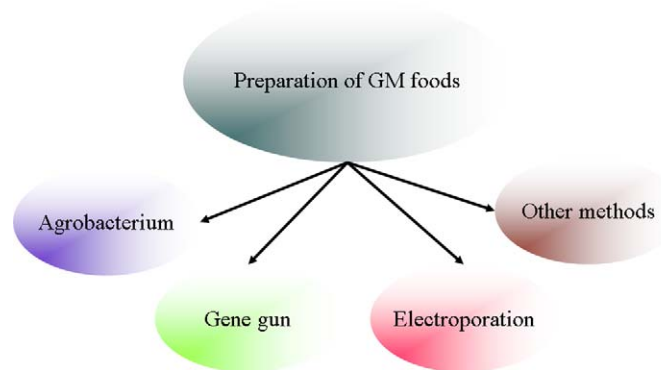


Fig. 2. Genetic modifications of plant cells can be achieved by various mechanisms like electroporation, gene gun, homologous recombination etc., but the mostly used method is the use of *Agrobacterium* sp. for the delivery of the transgene into the host plant cells.

The most frequently used quantitative methods for determination of GMO content in product are Southern blot, quantitative end-point PCR and quantitative real-time PCR [19].

One of the most fundamental procedures of GM DNA identification is sample preparation, which can be divided into several steps: 1.) determination of appropriate sample amount and sample size, 2.) selection of representative samples, 3.) removal of additional ingredients (salt, sugar, spice etc.), 4.) homogenization, 5.) pretreatment (neutralization, removal of fat and starch), 6.) lysis (buffers, SDS, EDTA, guanidium-HCl, CTAB, magnetic beads), 7.) separation/purification (enzymes, CTAB, phenol/chloroform, DAEA, silica, isopropanol), 8.) DNA analysis (PCR). The sample preparation protocol for work with protein is being changed from step 5.) extraction (special buffers binding agents) 6.) centrifugation (removal of other substances), 7.) protein analysis [20].

Methods for GM DNA identification can be divided according to their specificity into these groups:

- screening methods (analyzing promoters and terminators specific for GMO);
- gene specific methods (analyzing the specific gene);
- construct-specific methods (that focus on analysis of naturally not existing junctions, for example connection between promoter and gene);
- event-specific methods (focusing on unique junction between inserted DNA and recipient genome) [18].

These methods are usually based on PCR. This involves PCR as well as ligase chain reaction (LCR), nucleic acid sequence-based amplification (NASBA), but also fingerprinting techniques such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD). Further techniques include probe hybridization, electrochemiluminescence polymerase chain reaction (ECL-PCR) [21], multiplex PCR/membrane hybridization assay (MPCR-MHA) [22] and liquid phase hybridization (LPH) PCR enzyme-linked immunosorbent assay (ELISA) technique for specific detection of PCR products [23]. The microarray technology, matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF MS) and surface plasmon resonance (SPR) are also alternative methods for DNA-based methods. Protein-based methods can be divided into two groups: 1.) protein detection methods such as one-dimensional SDS gel electrophoresis (SDS-ELPO), two-dimensional SDS gel electrophoresis (2D-SDS-ELPO), 2D-SDS-ELPO combined with immunological methods, Western blot analysis, ELISA and a variation on ELISA lateral flow strip method, and 2.) methods based on the detection of enzymatic activities.

6. What are the societal aspects of GMO?

It is essential that we improve food production and distribution in order to feed and free from hunger a growing population, while reducing environmental impacts and providing productive employment in low-income areas. This will require a

proper and liable utilization of scientific discoveries and new technologies. The developers and overseers of GM technology applied to plants and microorganisms should approve their efforts address such needs. The effects of any new technology introduced on the scale anticipated for biotechnology extend beyond the factories and research centers influencing our everyday lives. Biotechnology has, for example, made it possible to detect, and in some cases to treat, diseases such as sickle-cell anemia, Tay-Sachs disease, diabetes, and cystic fibrosis. GM organisms currently produce many therapeutic molecules such as insulin, cytokines, interferons, interleukins etc.

Consumer acceptance of genetic engineering (GE) has been found to vary between different types of applications of the technology, but also within the same category of application [24]. Consumers have been shown to hold more positive attitudes towards the use of GE for medical purposes than for food production purposes [25,26].

Following initial apprehension that GE could give rise to infectious organisms—the spread of which would be difficult to restrict—a stringent set of guidelines was drawn up by governments and leading scientists in the mid-1970s to regulate research in this field. Though it is not possible to eliminate the risk of a GE accident completely, the experience of the last 10 years of research has indicated that the chances of constructing a disease-producing organism by accident are very low. This is because such pathogens require an extremely complex set of distinct characteristics, and are effective only when all are present.

7. What are the risks of GMO?

The issue of GMO crops, foods derived from GMO crops and genetic manipulations generally are very controversial. Nevertheless, GMO bring many benefits, on other hand many concerns and risks related to GMO impact on human's health in relationship with allergy, toxicity and nutrition value are associated with GMO. There are concerns that transgene protein may provoke allergic reaction in sensitive individuals. Many studies show that GMO crops nutrition value is comparable with their traditional counterparts. Much discussed is the potential risk of GMO related to environment such as extension of antibiotics resistance genes [27], horizontal gene transfer (HGT), spontaneous formation of superweed or GMO adverse effect on biodiversity. The purpose of many studies is to investigate the fate of ingested foreign DNA. For production of economically viable yields, reliable weed control is essential. The elimination of weed may potentially result in disruption of essential food sources for depending organisms [28] and subsequently in extinction of some food chains. Other risk of GM crops using is introducing of herbicide tolerance to weed followed by formation of superweeds.

On the other hand, farmers could achieve higher yields by the use of GM crops. Other advantage of GM crops is no need for tractor hoeing which improves the habit for ground-nesting of farmland birds. Using of GM crops resistant to herbicide and insecticide lead to reduced use of externally applied agriculture chemicals. In other words, GMO crops are environment-

friendly. It is necessary to improve and develop new techniques for GMO safety assessment related to human health and environment.

8. How are the risks tested and evaluated?

Current analyses are based on the international principles of food safety FAO/WHO 1995 and 1997. There are three basic approaches determining the toxicity, which include: 1.) the testing of interaction between structure and function to assess the potential toxicity and allergenicity, 2.) *in vitro* assays using enzymes, receptor proteins or cultured cell lines, 3.) *in vivo* animal experiments [29]. Most valuable information provides the analysis 3.), which is based on 1.) and 2.) investigation. Approach 1.) involves the computer analysis of gene product, for example testing the relationship and relativity between sequence or structure of the gene product and well known allergen. Second 2.) concept elicits the risks resulting from the function of given molecule, whether the molecule does induce any toxic processes on the cell level. Test 3.) serves for the simulation of expected human responses on animal model, using the monitoring of immunological and endocrinal toxicity, individual organ-toxicity and toxico-kinetics, which illuminates the fate of the substance in the body of experimental animal. Rarely, there are also volunteer-tests performed. The whole process of risk-testing involves also the post-marketing monitoring, which can uncover the formerly undetected toxicity and allergenicity mainly in the early phase after marketing of the product. Post-marketing monitoring observes and detects contingent allergic responses, other side effects and also consequences of long-term consumption of the product on the random-sampled individuals.

To evaluate the safety of GMO crops-containing food, there are test methods developed that represent these steps: 1.) Characterization of primal original plant. The detailed description of primal plant had to be performed via determination of: identity, phenotype, agronomic performance, geographical distribution, history of use and compositional analysis of nutrients, anti-nutrients, toxins, allergens. 2.) Characterization of donor organism and delivery process. Description of donor involves its classification, taxonomy studies and definition of possible toxicity, allergenicity or pathogenicity. Particularly vector, transgene delivery, inserted DNA sequence and also insertion site had to be characterized. 3.) Testing the safety of gene product. Nature and properties of gene product such as structure identity and characterization, mode of action, specificity, toxicity and allergenicity are being defined. 4.) Direct testing of the GMO crops-derived food. This step includes determination of identity, phenotypic and agronomic analysis of GMO crops, compositional analysis, nutrient analysis and safety analysis of food based on animal studies [29].

There are also in-process methods aimed at comparing the primal and GM plant. These techniques can be divided into targeted and non-targeted approaches. Targeted approaches monitor directly the consequence of novel gene product presence on the GM plant phenotype. Also changes in chemical composition are being detected. Non-targeted approaches con-

sist of three basic levels: functional genomics, proteomics, metabolomics. Functional genomics contain methods like mRNA fingerprinting and DNA microarray. On the level of proteomics, the protein composition of original and GM plant is being compared using methods such as two-dimensional electrophoresis (2-D ELPO) [30] and its modification or 2-D ELPO in connection with MALDI-TOF MS analysis [31]. For testing two samples on the same gel, a method called difference gel electrophoresis (DIGE) [32] is being used. The metabolomics level of analysis tries to identify and quantify the maximum amount of particular components. This involves separating methods like gas chromatography (GC), liquid chromatography (LC), high performance liquid chromatography (HPLC) combined with various detection methods such as nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), mass spectroscopy (MS), flame ionization detector (FID).

9. What is the HGT?

HGT refers to the exchange of the genes between two different organisms without reproduction, which is the opposite to the vertical gene transfer that occurs between related organisms by sexual reproduction. By HGT the organisms acquire a foreign gene. Allowing the transfer of genes between unrelated species, HGT increases genetic diversity and thereby the newly introduced genes into the genome speed up the genome innovation and evolution, though not necessarily gene evolution [33]. Three mechanisms of gene transfer are known in bacteria (Fig. 3). They include 1.) transformation, involving the uptake and incorporation of naked DNA, 2.) conjugation, a cell contact-dependent DNA transfer mechanism found to occur in most bacterial genera and 3.) transduction, whereby host DNA is encapsulated into a bacteriophage which acts as a vector for its injection into a recipient cell. These mechanisms serve as an important tool for the development of GE technology. The general concern was the hypothetical danger of the release of genetically manipulated bacteria and the possible

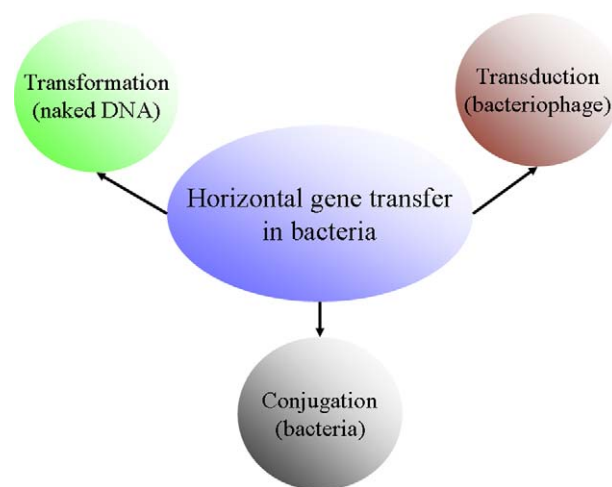


Fig. 3. HGT in bacteria occurs principally through three different mechanisms: conjugation between bacteria, transduction during the infection with a bacteriophage and transformation after the intake of naked foreign DNA.

gene transfer to other microorganisms. For the detection of the genetic transfer numerous methods are available. Almost all the methods involve the selection for specific genetic characteristics or phenotypes and this selection imposes a bias on the kind of genes that can be demonstrated to be transferred in situ. More recent work, using green fluorescent protein by Dahlberg et al. [34] has removed some of the constraints imposed by the need to select the particular genes. In addition, microcosm systems may permit the manipulation of physicochemical variables (temperature, pH, humidity, carbon, nitrogen, and phosphorus sources). The ability to detect gene transfer is dependent on the fate of the transferred DNA after entering the recipient cell. Many bacteria possess DNA restriction systems, which destroy foreign DNA, but bacteriophage and wide host range plasmids have developed their system to overcome the restriction system barrier by reducing the number of restriction cleavage sites they contain or by the production of restriction protection systems. Even though bacteria escape the restriction system barrier, they must possess the capability to replicate and maintain in the host cell for its survival. In case of transposons, the gene should be successfully integrated into the host genome and replicated. The efficiency of integration by homologous recombination depends upon the degree of homology of the donor and recipient DNA regions and this is monitored by the mismatch repair system (mutS and mutL in *Escherichia coli*). For example, recombination between *E. coli* and *Salmonella typhimurium* is enhanced 1000-fold when the mismatch repair system is inactivated [35].

10. How to get GMO allergy?

In the past few decades the amount of allergic diseases, like allergic rhinitis, asthma and eczema significantly increased [36]. Very high portion from this number represents cases induced by allergenic foods [37]. Since every kind of foods can be allergenic, the GM foods, can be too. There are risks not only from the parent crop, but in addition the new transgenic product can be an allergen. In most cases allergic reactions occur when allergens cross-link IgE antibodies and cause mast cell degranulation. These allergenic substances from foods can act through ingestion, cutaneous contact, inhalation or injection. The problem of food allergens involves not only the consumers, but also individuals who are exposed to these food-stuffs, e.g. farmers, packers. Therefore these products are strictly controlled. There are some possible situations in terms of allergenic potential of GM crops:

- unmodified plant gene from non-allergenic crop inserted into target crop;
- unmodified plant gene from crop known to cause allergy inserted into target crop;
- unmodified gene from non-plant source inserted into target crop;
- modified gene from non-plant source inserted into target crop;
- existing gene from target crop modified and reinserted;

- gene expression up- or down-regulated in non-allergenic or known allergenic crop [38].

Food allergies tend to increase their prevalence. Between 1 and 2% of adult population suffers from food allergies and within children this number increases to 2-8% [39]. This issue in relationship with GM food is very interesting and actual. Food allergy may be defined as hypersensitivity and implies immunological reaction to food or breakdown intolerance [40]. It is important to differ between allergic reaction and allergic sensitization. Sensibilisation is the process whereby immune system becomes reactive to an allergen. Clinical cross-reactivity exists between aeroallergens and food allergens. For example people with grass pollen allergies are often allergic to peach, celery and tomato. Many people are allergic to eggs, peanut, cow's milk, soy, wheat in children, shellfish, fish, nuts, peanuts in adult. These results lead to conclusion that many foods contain proteins, which may provoke allergic reaction in sensitive individuals. Food allergies do not need to be life-long diseases. Studies have demonstrated the loss of food allergy in up to one third of children in 1-3 years of age.

There are attempts to compare GM crop to its traditional counterparts and significant differences are investigated with regard to allergenicity. Although GM food brings benefits, there are concerns that transgene proteins may induce sensitization, cross-reactive with allergen and thus provoke allergic reaction or change expression of allergen already expressed in the host plant. Probability of introduction of allergen by genetic modification is not greater than by conventional breeding. Many studies show different causes and factors resulting in food allergies such as industrialized lifestyle [41], cigarette smoke, exposure to viral and bacterial infections [42], changes in feeding practices. GM soy bean with transferred 2S albumen protein from Brazil nut [43] gives evidence that allergenicity assessment of transgene product is very important. The purpose of this modification was to improve the content of soybean with cysteine and methionine. But a side effect was that patients allergic to Brazil nut were allergic to GM soy. What is allergen in one country needs not be an allergen in other. For example rice in Europe and North America does not provoke allergic reaction, but in Japan it poses a problem. Simultaneously with extension of knowledge about food allergy it is possible to achieve enhancement techniques for GMO safety assessment in relationship with allergenicity.

11. What are the environmental effects of the GMO?

The environmental risks include plant invasiveness or dispersal of the plant itself into the native ecosystem causing indirect impacts on the diversity of crops, gene flow through pollen transfer or through HGT with associated microorganisms, development of resistance in target organisms and non-target effects on native flora and fauna including effects on the biodiversity of beneficial and antagonistic microorganisms [44]. The interaction between transgenic plants and their root-associated bacteria is highly specific. The introduction of the new varieties may affect some important microorganisms, for

example glyphosate applied to Roundup Ready soybean cultivars enhances colonization by several bacteria. This interaction could reduce the benefit of cultivars in crop rotation. Many researches have which been conducted to study the effect of transgenic plants on the whole microbial community showed that its effect was smaller in comparison with environmental factors such as sampling date and field site. The minor affect in the diversity of the microbial community, such as the removal or appearance of specific functional groups of bacteria like plant growth promoting rhizobacteria, phytopathogenic organisms or key organisms responsible for nutrient cycling processes, could affect the soil health and ecosystem functioning. The impact that plant variety may have on the dynamics of rhizosphere microbial population might also affect plant growth and health. The main interaction zone between the plant and soil is the rhizosphere. Root exudates can promote or retard the growth of rhizosphere microorganisms, An example can be transgenic bird foot trefoil (*Lotus corniculatus*) plant which produces root exudates that favors opine degrading rhizobacteria [45].

The impacts of GMO on biodiversity has been focused on possible gene escape and gene flow to wild relatives and the replacement of traditional crop varieties with GM crops and possible effects on non-target eukaryotic organisms such as effects of Bt corn pollen on the monarch butterfly. The selectable marker gene, present in the transgenic plants could inactivate oral doses of the antibiotic and another concern is that the antibiotic resistance gene could be transferred to the pathogen microbes in the gastrointestinal tract or soil, rendering them resistant to treatment with such antibiotics [46].

A major problem in evaluating the environmental effects of transgenic crops is the lack of sufficient long-term baseline information on the variation in soil nutrient cycling in diverse agroecosystems to compare with agroecosystems, in which transgenic crops have been introduced. In addition, knowledge of the complex diversity of soil microorganisms is limited since only a small portion of soil microbial populations can be cultured and identified using standard analytical methods, although some new methodological approaches, including the use of molecular biology techniques, show some promise in helping to understand the impact of transgenic crops on soil microbial ecology. The effects of transgenic plants on phytopathogens are obviously intentional because the very purpose of the introduced genes is to protect the plant by reducing the effect of the pathogens on their hosts. There have been no reports as yet of disturbances in pathogen communities or of an emergence of new pathogens in transgenic plants.

12. What are the benefits of GMO?

Much attention in the public debate is paid to the risk of GMO use. This attention is, of course, needed; however, we shall not forget the primary reasons for developing transgenic organisms. Except easier, higher and more effective production, the second and third generations concentrate on the consumer benefits. The use of GMO was restricted to the improvement of production like adding flavors, conservative additives,

amino acids and similar [47]. But the modern biotechnology has focused on new techniques developed to use viable GM organisms for direct consumption. Recombinant probiotics, increased nutritional value of meat and plants and even the use of GMO for vaccination will definitely change the future of mankind. We, however, also should not forget the possibilities of GMO for partly solving the hunger problem in the third world. Crops resistant to drought, GM potatoes resistant to nematodes and GM soybeans resistant to herbicides are now improving the quality of life of millions of people in many countries around the world [48].

13. How can GMO be a functional food?

The term functional food is widely used but its meaning lacks precise definition. Mostly, functional food includes probiotics, plants and meat that act preventively against various diseases, but some authors include also food with improved nutritional value, or even GMO from the first generation.

Nevertheless, the most popular functional foods are probiotics. Firstly described as “good microbes” that help to restore local normal microbial flora, they have developed to highly sophisticated therapeutics. Especially Lactobacilli and Bifidobacteria are often used in the milk industry and added to the final products. Accepted indications of these naturally occurring bacteria include lactose intolerance, diarrhea and even helicobacter-induced gastritis. The induction of immune system explains the preventive effect of probiotics against various infections. Direct ecological competition with local pathogens is the cause of antimicrobial effects during gastrointestinal infections [49]. Still questionable is, on the contrary, the anticarcinogenic effect of naturally occurring probiotics. GM probiotics will be discussed in section about alternative gene therapy (AGT).

Although reports about GM fish are rare, this group of vertebrates is used in GE for more than a decade. The possibilities and targets of genome tailoring in fishes have been reviewed recently [50]. The simplest approach came out to enhance the growth by increasing growth hormone production using various methods with very good results (increase in weight up to 200%). The same methods were applied when genes from Antarctic fishes that are resistant to freezing were transferred to industrially used species like the Atlantic salmon. The cold resistance is caused by an anti-freeze protein that influences membrane fluidity. However, the expression of this protein is still too low to improve the quality of consumed meat. In contrast, successful experiments focused on affecting the fertility of fishes were completed. Sterile individuals produce quantitatively and qualitatively improved meat. The sterility is induced by the production of triploid zygotes or by influencing the gonadotropin axis. Further advances were achieved in DNA vaccination of fishes against various diseases. Although this might improve the biological safety, the method must be tested and controlled prior to industrial application.

Livestock and poultry have been modified with similar methods like fishes; especially successful were experimental modifications to growth hormone axis and myostatin. In live-

stock specifically milk production was the target of interest. Transgenic lactase in milk digested lactose and, thus, improved the symptoms of lactose intolerance, which is very frequent in Western population [51,52].

Human vaccination—one of the greatest invention in the history of medicine—will probably be changed in the near future. Biotechnologists have prepared tobacco and lettuce plants that express HBs antigen [53]. Volunteers consuming 150–200 g of HBs antigenic lettuce three times during 2 months produced corresponding antibodies. However, the interindividual variability in response to this kind of vaccination requires further research.

Transgenic plants belonging to the second and third generation of GMO have various potentially positive effects for the health of the consumer. One of the most interesting approaches is the production of direct therapeutic proteins by transgenic plants. Soybean proteins were mutated to produce an ovokinin analogue after digestion. Ovokinin as a hexapeptide is a potent but due to methodological problems rarely studied antihypertensive drug [54]. Similarly, other oligopeptides with known endocrine or paracrine functions could be transferred into plant proteins. Of course, the consumption of these GM plants must be restricted to therapeutic use under tight medical control.

Other approaches are concentrating on the micronutritional value of the plants. And the results are supporting this approach as very successful. One of the striking successful example is a transgenic rice with increased beta-carotene production and iron accumulation. The so-called golden rice is very useful in Southern Asia where a big part of the population suffers from iron and vitamin A deficiency [55].

14. What is AGT?

GE based on the knowledge of molecular biology begun in Petri dishes using *E. coli* and other bacteria. It is obvious, that the most astonishing progress in this field has been also made in bacteria. Lactic acid bacteria used for centuries in fermentation processes are useful organisms for genetic modifications. A number of strains are widely used as probiotics, but their effects were improved by the preparation of so-called recombinant probiotics [56]. Although still the major application of lactic acid bacteria is their use in the food industrial processes of fermentation [57], in a well-designed experiment Steidler have proved the use of transgenic bacterial expression system for the production of interleukin-10 inside the gastrointestinal tract in an experimental model of chronic inflammatory bowel disease [58].

Other bacteria like *Listeria*, *Salmonella* and *Escherichia* are used for the so-called bactofection—the bacteria-mediated delivery of genes into human cells. The bacterial vectors are destroyed in the target cells and the DNA can be either integrated into the human genome or remain as episomes outside of the chromosomal DNA [59]. However, the detailed mechanism of DNA transfer from the bacteria to the target cell is not known [60]. The applications are wide, including infection therapy [61], therapy of cystic fibrosis [62] or even cancer chemotherapy [63]. Another specific application is the use of transformed

bacteria for vaccination [64,65]. This method has made the biggest progress in GMO use in biomedicine [66]. One of the reasons is the simple vaccination, since some of them can be applied orally [67].

Gene therapy has changed the perspectives in various disease entities [68]. The process of gene transfer can be divided into in vitro and in vivo. In its classical forms the vectors used are usually viruses, cationic liposomes or the DNA is applied as naked [69]. However, these vectors mostly lack the possibility of any gene expression regulation. This can be in part solved by the use of bacteria as vectors [59]. Unfortunately the type of regulation can be only positive. AGT is an approach to improve the gene expression regulation. The main difference lies in the producing cells. In the classic gene therapy the producing cells are the cells of the patient—these eukaryotic cells are, however, difficult to regulate. In AGT, the producing cells are the transformed bacteria, while the therapeutic protein is produced in situ. This method has been proved for experimental angiogenesis using bacteria producing vascular endothelial growth factor [70]. The main advantage lies in the improved regulation, while the expression can be up-regulated using low molecular weight inducers and stopped using antibiotics, as the used bacteria have a defined resistance spectrum against antibiotics.

15. What is the conclusion?

The introduction of GM food in the market is controlled and especially in the EU strictly regulated. The process of tolerance and toxicity testing implies in vitro and in vivo experiments as well as post-market monitoring. The future will bring improvements in molecular techniques for preparing GM organisms. Gene delivery into the parental organism will be much better controlled and monitored, selection markers will be eliminated in the final product. The wide debate about GM food is needed and should involve all participating societal groups, including the consumers. Nevertheless, the decision must be based on objective facts, information from experts and not on subjective emotionally affected opinions, which is often currently the case. The risks but also the possible benefits of GM foods should be assessed and evaluated individually for every GM organism. Future will show how quickly and how much will biotechnology change our lives... actually, it has already begun to do so.

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References

- [1] Burkhardt PK, Beyer P, Wunn J, Kloti A, Armstrong GA, Schledz M, et al. Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. *Plant J* 1997;11:1071–8.

- [2] James D, Schmidt AM, Wall E, Green M, Masri S. Reliable detection and identification of genetically modified maize, soybean, and canola by multiplex PCR analysis. *J Agric Food Chem* 2003;51:5829–34.
- [3] Cellini F, Chesson A, Colquhoun I, Constable A, Davies HV, Engel KH, et al. Unintended effects and their detection in genetically modified crops. *Food Chem Toxicol* 2004;42:1089–125.
- [4] Hughes SS. Making dollars out of DNA. The first major patent in biotechnology and the commercialization of molecular biology, 1974–1980. *Isis* 2001;92:541–75.
- [5] Gonsalves D. Control of papaya ringspot virus in papaya: a case study. *Annu Rev Phytopathol* 1998;36:415–37.
- [6] Conway G, Toennissen G. Feeding the world in the twenty-first century. *Nature* 1999;402:C55–C58.
- [7] Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F. Iron fortification of rice seed by the soybean ferritin gene. *Nat Biotechnol* 1999;17:282–6.
- [8] Lucca P, Hurrell R, Potrykus I. Fighting iron deficiency anemia with iron-rich rice. *J Am Coll Nutr* 2002;21:184S–190S.
- [9] Dandekar AM, Fisk HJ. Plant transformation: agrobacterium-mediated gene transfer. *Methods Mol Biol* 2005;286:35–46.
- [10] Perlak FJ, Stone TB, Muskopf YM, Petersen LJ, Parker GB, McPherson SA, Wyman J, Love S, Reed G, Biever D, et al. Genetically improved potatoes: protection from damage by Colorado potato beetles. *Plant Mol Biol* 1993;22:313–21.
- [11] Baum K, Groning B, Meier I. Improved ballistic transient transformation conditions for tomato fruit allow identification of organ-specific contributions of I-box and G-box to the RBCS2 promoter activity. *Plant J* 1997;12:463–9.
- [12] Christou P. Rice transformation: bombardment. *Plant Mol Biol* 1997;35:197–203.
- [13] Bates GW. Electroporation of plant protoplasts and tissues. *Methods Cell Biol* 1995;50:363–73.
- [14] Terzaghi WB, Cashmore AR. Plant cell transfection by electroporation. *Methods Mol Biol* 1997;62:453–62.
- [15] Sitrit Y, Bennett AB. Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: a re-examination. *Plant Physiol* 1998;116:1145–50.
- [16] Hamilton AJ, Fray RG, Grierson D. Sense and antisense inactivation of fruit ripening genes in tomato. *Curr Top Microbiol Immunol* 1995;197:77–89.
- [17] Kuiper HA, Konig A, Kleter GA, Hammes WP, Knudsen I. Safety assessment, detection and traceability, and societal aspects of genetically modified foods. European Network on Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD). Concluding remarks. *Food Chem Toxicol* 2004;42:1195–202.
- [18] Miraglia M, Berdal KG, Brera C, Corbisier P, Holst-Jensen A, Kok EJ, et al. Detection and traceability of genetically modified organisms in the food production chain. *Food Chem Toxicol* 2004;42:1157–80.
- [19] Ahmed FE. Detection of genetically modified organisms in foods. *Trends Biotechnol* 2002;20:215–23.
- [20] Terry CF, Harris N, Parkes HC. Detection of genetically modified crops and their derivatives: critical steps in sample preparation and extraction. *J AOAC Int* 2002;85:768–74.
- [21] Liu J, Xing D, Shen X, Zhu D. Detection of genetically modified organisms by electrochemiluminescence PCR method. *Biosens Bioelectron* 2004;20:436–41.
- [22] Su W, Song S, Long M, Liu G. Multiplex polymerase chain reaction/membrane hybridization assay for detection of genetically modified organisms. *J Biotechnol* 2003;105:227–33.
- [23] Liu J, Deng P, Fang S, Zhao J. Studies on protein-based identification method of genetically modified capsicum. *Wei Sheng Yan Jiu* 2003;32:134–7.
- [24] Frewer L, Lassen J, Kettlitz B, Scholderer J, Beekman V, Berdal KG. Societal aspects of genetically modified foods. *Food Chem Toxicol* 2004;42:1181–93.
- [25] Braun R. People's concerns about biotechnology: some problems and some solutions. *J Biotechnol* 2002;98:3–8.
- [26] Harlander SK. Safety assessments and public concern for genetically modified food products: the American view. *Toxicol Pathol* 2002;30:132–4.
- [27] Halford NG, Shewry PR. Genetically modified crops: methodology, benefits, regulation and public concerns. *Br Med Bull* 2000;56:62–73.
- [28] Squire GR, Brooks DR, Bohan DA, Champion GT, Daniels RE, Haughton AJ, et al. On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philos Trans R Soc Lond B Biol Sci* 2003;358:1779–99.
- [29] Konig A, Cockburn A, Crevel RW, Debruyne E, Grafstroem R, Hammerling U, et al. Assessment of the safety of foods derived from genetically modified (GM) crops. *Food Chem Toxicol* 2004;42:1047–88.
- [30] Gorg A, Obermaier C, Boguth G, Weiss W. Recent developments in two-dimensional gel electrophoresis with immobilized pH gradients: wide pH gradients up to pH 12, longer separation distances and simplified procedures. *Electrophoresis* 1999;20:712–7.
- [31] Andersen JS, Mann M. Functional genomics by mass spectrometry. *FEBS Lett* 2000;480:25–31.
- [32] Unlu M. Difference gel electrophoresis. *Biochem Soc Trans* 1999;27:547–9.
- [33] Jain R, Rivera MC, Lake JA. Horizontal gene transfer among genomes: the complexity hypothesis. *Proc Natl Acad Sci USA* 1999;96:3801–6.
- [34] Dahlberg C, Bergstrom M, Hermansson M. In situ detection of high levels of horizontal plasmid transfer in marine bacterial communities. *Appl Environ Microbiol* 1998;64:2670–5.
- [35] Matic I, Taddei F, Radman M. Genetic barriers among bacteria. *Trends Microbiol* 1996;4:69–72.
- [36] Sampson HA. Food allergy. *JAMA* 1997;278:1888–94.
- [37] Emmett SE, Angus FJ, Fry JS, Lee PN. Perceived prevalence of peanut allergy in Great Britain and its association with other atopic conditions and with peanut allergy in other household members. *Allergy* 1999;54:380–5.
- [38] Lack G. Clinical risk assessment of GM foods. *Toxicol Lett* 2002;127:337–40.
- [39] Bock SA. The natural history of adverse reactions to foods. *N Engl Reg Allergy Proc* 1986;7:504–10.
- [40] Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, et al. Clinical and laboratory investigation of allergy to genetically modified foods. *Environ Health Perspect* 2003;111:1114–21.
- [41] Hirsch T. Indoor allergen exposure in west and East Germany: a cause for different prevalences of asthma and atopy? *Rev Environ Health* 1999;14:159–68.
- [42] Martinez FD. Role of respiratory infection in onset of asthma and chronic obstructive pulmonary disease. *Clin Exp Allergy* 1999;29(Suppl 2):53–8.
- [43] Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK. Identification of a Brazil-nut allergen in transgenic soybeans. *N Engl J Med* 1996;334:688–92.
- [44] Giovannetti M. The ecological risks of transgenic plants. *Riv Biol* 2003;96:207–23.
- [45] Mansouri H, Petit A, Oger P, Dessaux Y. Engineered rhizosphere: the trophic bias generated by opine-producing plants is independent of the opine type, the soil origin, and the plant species. *Appl Environ Microbiol* 2002;68:2562–6.
- [46] Daniell H, Muthukumar B, Lee SB. Marker free transgenic plants: engineering the chloroplast genome without the use of antibiotic selection. *Curr Genet* 2001;39:109–16.
- [47] von Wright A, Bruce A. 7. Genetically modified microorganisms and their potential effects on human health and nutrition. *Trends Food Sci Technol* 2003;14:264–76.
- [48] Atkinson HJ, Green J, Cowgill S, Levesley A. The case for genetically modified crops with a poverty focus. *Trends Biotechnol* 2001;19:91–6.
- [49] Zubillaga M, Weill R, Postaire E, Goldman C, Caro R, Boccio J. Effect of probiotics and functional foods and their use in different diseases. *Nutr Res* 2001;21:569–79.
- [50] Maclean N. 5. Genetically modified fish and their effects on food quality and human health and nutrition. *Trends Food Sci Technol* 2003;14:242–52.
- [51] Sang H. 6. Genetically modified livestock and poultry and their potential effects on human health and nutrition. *Trends Food Sci Technol* 2003;14:253–63.

- [52] Vilotte JL. Lowering the milk lactose content in vivo: potential interests, strategies and physiological consequences. *Reprod Nutr Dev* 2002;42:127–32.
- [53] Mercenier A, Wiedermann U, Breiteneder H. Edible genetically modified microorganisms and plants for improved health. *Curr Opin Biotechnol* 2001;12:510–5.
- [54] Matoba N, Doyama N, Yamada Y, Maruyama N, Utsumi S, Yoshikawa M. Design and production of genetically modified soybean protein with anti-hypertensive activity by incorporating potent analogue of ovokinin (2-7). *FEBS Lett* 2001;497:50–4.
- [55] Bouis HE, Chassy BM, Ochanda JO. 2. Genetically modified food crops and their contribution to human nutrition and food quality. *Trends Food Sci Technol* 2003;14:191–209.
- [56] Ahmed FE. Genetically modified probiotics in foods. *Trends Biotechnol* 2003;21:491–7.
- [57] Renault P. Genetically modified lactic acid bacteria: applications to food or health and risk assessment. *Biochimie* 2002;84:1073–87.
- [58] Steidler L, Hans W, Schotte L, Neiryck S, Obermeier F, Falk W, et al. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 2000;289:1352–5.
- [59] Pilgrim S, Stritzker J, Schoen C, Kolb-Maurer A, Geginat G, Loessner MJ, et al. Bactofection of mammalian cells by *Listeria monocytogenes*: improvement and mechanism of DNA delivery. *Gene Ther* 2003;10:2036–45.
- [60] Loessner H, Weiss S. Bacteria-mediated DNA transfer in gene therapy and vaccination. *Expert Opin Biol Ther* 2004;4:157–68.
- [61] Shen H, Kanoh M, Liu F, Maruyama S, Asano Y. Modulation of the immune system by *Listeria monocytogenes*-mediated gene transfer into mammalian cells. *Microbiol Immunol* 2004;48:329–37.
- [62] Fajac I, Grosse S, Collombet JM, Thevenot G, Goussard S, Danel C, et al. Recombinant *Escherichia coli* as a gene delivery vector into airway epithelial cells. *J Control Release* 2004;97:371–81.
- [63] Fu GF, Li X, Hou YY, Fan YR, Liu WH, Xu GX. *Bifidobacterium longum* as an oral delivery system of endostatin for gene therapy on solid liver cancer. *Cancer Gene Ther* 2004;12:133–40.
- [64] Dietrich G, Spreng S, Favre D, Viret JF, Guzman CA. Live attenuated bacteria as vectors to deliver plasmid DNA vaccines. *Curr Opin Mol Ther* 2003;5:10–9.
- [65] Michael A, Stratford R, Khan S, Dagleish A, Pandha H. Novel strains of *Salmonella typhimurium* as potential vectors for gene delivery. *FEMS Microbiol Lett* 2004;238:345–51.
- [66] Medina E, Guzman CA. Use of live bacterial vaccine vectors for antigen delivery: potential and limitations. *Vaccine* 2001;19:1573–80.
- [67] Page DT, Cudmore S. Innovations in oral gene delivery: challenges and potentials. *Drug Discov Today* 2001;6:92–101.
- [68] Mountain A. Gene therapy: the first decade. *Trends Biotechnol* 2000;18:119–28.
- [69] Klink D, Schindelhauer D, Laner A, Tucker T, Bebok Z, Schwiebert EM, et al. Gene delivery systems—gene therapy vectors for cystic fibrosis. *J Cyst Fibros* 2004;3(Suppl 2):203–12.
- [70] Celec P, Gardlik R, Palffy R, Hodossy J, Stuchlik S, Drahovska H, et al. The use of transformed *Escherichia coli* for experimental angiogenesis induced by regulated in situ production of vascular endothelial growth factor—an alternative gene therapy. *Med Hypotheses* 2005;64:505–11.