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Safety aspects of novel foods

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Abstract

Cultivation of genetically modified (GM) crops with benefits to farmers (pest resistance, herbicide tolerance) has rapidly gained adoption since 1996. Future GM crops are envisioned that will also provide health benefits to their consumers. To assess the food safety of GM crops, internationally recognised strategies have been developed. These strategies focus at the characteristics of the genetic modification process, the properties of newly expressed compounds and at possible alterations in composition of the new crop. Substantial equivalence has been proven to be an adequate guidance principle in order to identify relevant safety issues of GM crops. New *profiling* methods are described for the detection of unintended effects, which may occur in GM foods as a result of the genetic modification. These profiling techniques are of particular relevance for those food crops in which profound changes in metabolic pathways may have taken place as a result of the genetic modification process.

Keywords: Substantial equivalence; Genetically modified (GM) crops; New profiling methods; Food safety; Genomics; Proteomics; Metabolomics

1. Cultivation of genetically modified (GM) crops

Improvement of crops using recDNA technology has primarily been focused on the identification and isolation of genes involved in the control of important *agronomical* traits of food plants. Crops have been modified through the insertion of new traits or the inhibition of existing gene functions, resulting in improved tolerance for herbicides, and/or increased resistance against pests, viruses or fungi.

Commercial cultivation of GM crops started in 1996 and covered in 1999 approximately 40 million hectares of arable land (James, 1998, 2000). Most of the transgenic crops are grown in USA (72%), Argentina (17%), and Canada (10%), in descending order of area: soybean, corn/maize, cotton, canola/rapeseed, potato, squash and papaya, with soybeans and corn accounting for, respectively, 54 and 28% of the crop acreage, and cotton and canola each occupying 9%. Herbicide resistant plants have been generated through the introduction of genes which code for enzymes with either a reduced affinity for the herbicide, or that degrade the herbicide. Target herbicides include glyphosate, glufosinate, phosphinothricin, and bromoxynil. Genes coding for insecticidal proteins derived from micro-organisms and plants, e.g. the bacterial Cry proteins, have been inserted in food crops to enhance insect resistance in plant species.

2. New healthy diets and major uncertainties

The main GM crops that are currently cultivated have (potential) benefits to farmers cultivating these crops. These crops may be joined in the near future by others with health benefits for their consumers. Such crops are currently in development or field tested. Some of these new crops may contribute to combat dietary deficiencies, like vitamin A or iron deficiency, while others may be safer due to a reduction/elimination of allergenic compounds or the detoxification of contaminating mycotoxins. For further details see Kleter, Noordam, Kok, and Kuiper (2000).

In addition, many intrinsic plant compounds have been linked to positive health effects and would therefore be an attractive target for plant engineering. There is substantial evidence mainly from epidemiological studies that diets rich in vegetables and fruit may prevent human cardiovascular diseases and cancer (Steinmetz & Potter, 1996). Potentially anti-carcinogenic

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compounds have been identified in vegetables and fruit, like allium compounds, carotenoids, isoflavones, isothiocyanates and others, while flavonoids seem to lower the incidence of cardiovascular diseases (AICR/WCRF, 1997; Hollman & Katan, 1999). Some of the presumed effects may be ascribed to the anti-oxidant activity of selected compounds, but the direct relation with health protection is complicated, given the potentially pro-oxidative behaviour of compounds due to the formation of intermediate reactive oxygen species, and catechol-type metabolites as for instance has been demonstrated in case of isoflavonoids (Metodiewa, Jaiswal, Cenas, Dickancaite, & Segure-Aguilar, 1999; Rice-Evans, Miller, & Paganga, 1996; Zhu, Ezell, & Liehr, 1994).

On the other hand, some of these beneficial compounds have been shown to exert adverse effects as well. The potential adverse and beneficial effects of new foods with increased levels of such beneficial compounds, i.e. food safety and nutrition respectively, should therefore be evaluated concomitantly rather than separately (OECD, 2001). It should be noted that there is still a lack of knowledge on the effects of many health-positive, intrinsic plant compounds, such as on the following issues:

- which compounds exert beneficial effects
- mechanisms of health promoting effects and of potential toxicity
- dose-response relationships of protective and adverse effects
- bioavailability of compounds of interest
- matrix effects on bioavailability and metabolism
- interaction between bio-active compounds
- current non-nutrient levels and variability
- losses or modifications of compounds through food processing

3. Food safety of GM crops

Various international bodies like the Organisation for Economic Co-operation and Development (OECD), the Food and Agriculture Organisation/World Health Organisation (FAO/WHO), and the European Union have designed strategies for the safety evaluation of genetically modified foods or food ingredients (EU, 1997a, 1997b; FAO/WHO, 1991, 1996, 2000; OECD, 1993, 1996). Food safety issues of GM crops are summarised in Table 1.

The safety assessment of foods is a complex issue since foods contain a myriad of compounds like essential macro- and micro-nutrients and other presumably health protecting compounds, but also natural compounds which may exert adverse effects on humans and animals. Straightforward feeding tests with pesticides, food additives and pharmaceuticals, using laboratory animals, are routinely performed in order to assess their safety. However, it would be in many cases difficult, if not impossible, to perform such animal testing with whole complex foods. Due to their bulk and specific nutritional composition, animal feeding studies with large amounts of test material cannot be carried out because of limited dose ranges of test materials, while furthermore many confounding factors may be present in the test material. This makes the interpretation of results obtained from animal feeding trials and the safety assessment at least questionable and in many cases impossible (Kuiper & Noteborn, 1996; Kuiper, Noteborn, & Peijnenburg, 1999; Noteborn et al., 1995). This has led to the development of a *comparative* approach for the safety assessment of GM food crops, i.e. the concept of *substantial equivalence* (OECD, 1993). Investigation of the agronomical, chemical, biochemical

Table 1
Food safety issues pertaining to GM crops

Issue	Study methods
Characteristics of the genetic modification (identity, source, stability of integration)	DNA analysis (Southern, sequencing), trait inheritance
Expression of new gene products, their toxicological properties, and transfer via animal feed to edible animal-derived products	mRNA—and protein (Western blotting, proteomics)—analysis; amino acid sequence comparisons to toxins; animal tests (acute oral, subchronic oral)
Potential alterations in levels of nutrients, non-nutrients and toxicants (unintended effects)	Composition analysis, metabolomics
Gene transfer between the GM plant food and the gut microflora of humans or animals	Screening for the insertion of foreign DNA, including bacterial and antibiotic resistance genes
Potential allergenicity	Amino acid sequence comparison to allergens, in vitro digestibility assay, skin prick tests, animal testing, double blind placebo controlled food challenge
Potential intake and dietary impact of the GM crop	Nutrient composition analysis, estimation of consumption, nutrient intake of (sub)populations, and associated health effects
Effect of processing/cooking	Assay of enzymic activity, analysis of protein integrity, compositional analysis
Formation/toxicity of new metabolites of herbicides/insecticides applied to GM crops and possible changes in patterns and levels of residues	Pesticide residue analysis, metabolomics, bio-assays, in vitro toxicology, animal toxicology

and nutritional characteristics of the genetically modified crop relative to those of the existing traditional food, and a toxicological characterisation of newly expressed compounds may establish whether the GM food crop is as safe as its traditional counter part, if available. Application of the concept of substantial equivalence is a *starting point* for the safety assessment, and provides guidance through identification of potential differences between the GM crop and its counter part. As a result of this approach no further safety testing of the GM crop under investigation may be needed in case substantial equivalency applies, while specific safety testing may be necessary according to the identified differences between the GM and traditional product. Within the EU's procedures for novel foods, this is currently implemented as the *notification*- (product is substantially equivalent) versus the *authorisation*- (product cannot be considered substantially equivalent) route. Application of the substantial equivalence concept is not a safety assessment per se, and does not provide absolute safety of the new product. It is considered as a useful tool to carry out a relative safety assessment of GM crops and has proven to be adequate for the assessment of GM crops now admitted to the market (FAO/WHO, 2000).

With respect to the assessment of the potential allergenicity of GM foods, an internationally agreed decision-tree approach has been designed (FAO/WHO, 2000). In case the new gene is derived from a known allergenic source, the safety assessment is focused on the immunochemical reactivity of the new protein with serum IgE from patients allergenic to the original source. In case of negative results, in-vivo skin tests and food challenge tests may be performed to confirm the absence of allergenicity. In case the source of the gene is not (known to be) allergenic, a sequence homology search with known allergens, and a characterisation of the physical chemical properties of the new protein should be performed. No single test parameter is predictive for allergenicity, but a combination offers reasonable reassurance for not introducing new allergenic foods. However other criteria (protein expression levels, protein functionality) may still be developed and validated, together with validated animal test models.

4. Unintended effects

One of the safety concerns regarding GM foods is possible altered expressions of macro- and micro-nutrients, naturally occurring toxins and anti-nutrients, or the formation of novel toxins, as a result of the molecular transformation process. A number of *single* key macro- and micro-nutrients and specific toxins are measured in the GM plant and compared with those measured in the traditional counterpart. Such an analysis however may reveal secondary effects only by chance or if anticipated. Limitations of this approach are (1) the availability of a counterpart with a similar genetic background to compare compositional data with, (2) the availability of data on the composition and limited information on natural variations and (3) lack of specific analytical detection methods. Therefore, it is desirable to develop new methods which allow for the *simultaneous* screening of potential changes in the physiology of the GM food crop at different biological integration levels, e.g. at the genome during gene expression, at the proteome during protein translation, or at the metabolome during metabolic pathway processing.

4.1. Analysis of the insertion place(s)

Characterisation of the place(s) of insertion is the most direct approach to predict and identify the occurrence of (un)intended effects of the transformation. However, knowledge of a crop plant's genome is still limited, and even if it would be established that the introduced sequence has not interrupted a coding gene sequence, it cannot be predicted whether the insertion has affected regulatory elements that can be involved at large distances from the actual gene location. In the near future, site-directed insertion by using homologous sequences may reduce the risk of unintended side effects of the genetic modification.

4.2. DNA micro-array technology

The DNA microarray technology (i.e. genomics) is a very promising method to study gene expression. It is based on hybridisation of mRNA to a high-density array of target sequences, immobilised on small glass surfaces. The major advantage of this technology is that it allows small-scale analysis of expression of a large number of genes at the same time in a sensitive and quantitative manner (Schena, Shalon, Davis, & Brown, 1995; Van Hal et al., 2000). At RIKILT the tomato has been chosen as a model crop, and informative cDNA libraries have been obtained by subtraction, consisting of cDNAs that are specific for the *red-ripe* stage, and of *green-specific* cDNAs (Kok, Keijer, Kramer, Van der Wal-Winnubst, & Van Hal, 2000). In the green stage, metabolic pathways leading to anti-nutritional factors, e.g. glyco-alkaloids are presumably active, whereas in red-ripe tomatoes metabolic pathways leading to nutritional factors, e.g. vitamins, are presumably more active. Subsequent hybridisation with mRNAs isolated from a number of varieties under investigation, including genetically modified ones, showed highly reproducible fluorescence patterns, which are subject of further interpretation. This method may effectively be used to screen for altered gene expression and provide information on the *nature* of detected alterations.

4.3. Proteomics

Correlation between mRNA expression and protein levels is generally poor, as rates of degradation of individual mRNAs and proteins differ. Understanding of the biological complexities in the plant cell can further be improved by exploiting proteomics, a technique which analyses many proteins simultaneously (Abbott, 1999). The current methodology for protein expression studies consists of 2D-gel electrophoresis, followed by excision of protein spots from the gel, digestion into fragments by specific proteases, and subsequent analysis by mass spectrometry. No comparative protein analysis of GM food crops and counterparts with a similar genetic background has been published till now.

4.4. Metabolomic profiling

A multi-compositional analysis of biologically active compounds in plants may indicate whether (un)intended effects have taken place as result of genetic modification. Recently it has been shown that the use of chemical fingerprinting techniques, using off-line LC-NMR, provides information on possible changes in plant matrices, due to variations in environmental conditions (Lommen, Weseman, Smith, & Noteborn, 1998). Furthermore a chemical fingerprint was established by taking ^{1}H NMR spectra from different water and organic solvent extracts from genetically modified tomato varieties, such as the antisense RNA exogalactanase fruit, and from their non-modified counterpart(s) (Noteborn, Lommen, Van der Jagt, & Weseman, 2000; Noteborn, Lommen, Weseman, Van der Jagt, & Groenendijk, 1998).

4.5. ENTRANSFOOD

Across Europe a consortium of laboratories, regulatory offices, industries, and consumer groups, funded within the 5th Frame work program, Quality of Life Management of Living Resources, Key Action 1, is currently involved in the safety assessment of GM food crops. The Network, ENTRANSFOOD, clusters research activities of four EU-RTD projects and consists of four Working Groups. Research is focussed on (1) development of improved safety testing methods for GM foods, (2) detection of unintended effects, (3) gene transfer, and (4) detection and traceability of GM food crops (EU contract no. QLK1-1999-01182; ENTRANSFOOD, 2000).

5. Conclusions

Modern biotechnology can contribute in designing new foods with specific health protective properties, but given the relatively poor state-of-the-art with respect to knowledge on working mechanisms, joint research in epidemiology, nutrition, and food toxicology is in the first place needed in order to select relevant compounds and to demonstrate their beneficial action. It should be kept in mind that it may not be possible to identify single 'miracle' compounds given the complexity of foods and the interactions with living organisms. To this end the potential usefulness of modern techniques like genomics, proteomics, and metabolomics in modern plant breeding should be further explored.

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