IFIS core food information

Safety and potential allergenicity of foods derived from genetically modified crops

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Abstract

Since its first large-scale introduction in 1996, the commercial cultivation of genetically modified (GM) crops has steadily increased. Before any GM crops are allowed on the retail market, they must be assessed for their safety. This safety assessment is comparative and follows an internationally recognized consensus approach, focusing on the differences between a GM crop and its conventional counterpart. A number of issues are commonly addressed during this safety assessment, such as the potential allergenicity of newly introduced proteins. A weight of evidence approach is recommended by Codex Alimentarius for the assessment of potential allergenicity, with bioinformatics-based studies comparing the structure of a novel protein to that of allergenic proteins being part of this approach. Recent studies have investigated the outcomes of the *in silico* procedures recommended by Codex Alimentarius, while websites such as Allermatch™ (http://www.allermatch.org) offer access to the bioinformatics procedures used.

Before any GM crops are allowed on the retail market, they must be assessed for their safety

1. A brief overview of GM crop development

Genetic modification of plants was first reported by scientists in the 1980s, with large-scale commercial breeding programmes of genetically modified (GM) crops being introduced in Northern America in the second half of the 1990s. Since then, the area of arable land covered by GM crops has steadily increased in a growing number of countries around the world. In 2004, the total global area of GM crops amounted to 81 million hectares (James 2004); this is equivalent to twice the land area of Sweden or three times that of New Zealand.

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Most commercial GM crops have been bred for agriculturally important traits, such as tolerance to herbicides or insect resistance. In the case of herbicide tolerance, any herbicide applied on the field will not affect the GM herbicide-tolerant crop, but will be lethal to conventional crops as well as any weeds present in the field. Herbicide tolerance in GM crops is acquired by the introduction of enzymes into the plant that either resist the toxic inhibitory action of the herbicide, or detoxify the herbicide itself.

In the case of insect resistance, the crop expresses a protein from the soil bacterium *Bacillus thuringiensis*, which is also used as a biological ingredient of pesticide sprays in conventional agriculture. Upon ingestion by insect larvae, the insecticidal protein causes damage to the insect's intestinal cells; this mode of action is very specific to certain insect species and does not affect humans and animals. In a similar fashion, most of the other GM crops have been modified with newly introduced 'foreign' proteins at low levels.

2. The various safety assessments of GM crops

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Before GM crops are allowed onto the retail market in most countries, they must receive regulatory approval; as part of this approval procedure, they must be assessed for their safety. To this end, the company intending to introduce the GM crop onto the market must submit a report containing safety data to the appropriate regulatory authorities.

The safety assessment of GM crops in general follows an internationally recognized consensus approach known as the 'comparative safety assessment', or 'substantial equivalence' (Kok and Kuiper 2003; Kuiper et al. 2001). Using this approach, safety of the GM crop is established relative to its conventionally grown counterpart, which usually has a history of safe use.

An example of this assessment would be to compare insect-resistant GM maize containing a foreign gene/protein from *B. thuringiensis* with conventionally-grown maize. Such a comparison would include crop field behaviour, agricultural characteristics and plant composition. This comparative analysis might reveal, for example, that the presence of the foreign gene and its derived protein product in the GM maize is the only difference between the GM and non-GM maize. Further evaluation would then focus on this difference, which would entail assessments of the safety of the foreign gene and its product.

Safety assessments of GM crops commonly address a number of issues which include:

- Data on the genetic material introduced and its expression in the crop;
- Substantial equivalence, entailing a comparison of the GM crop with a conventionally-grown counterpart, including its composition. Possible differences between each type of crop should be further investigated for reasons of safety.
- Toxicity – the potential adverse effects on health of newly introduced or changed components;
- Allergenicity – the type of immune reaction that occurs in sensitized individuals after exposure to a novel allergenic component;
- Potential for gene transfer, in case the newly introduced DNA is transferred to other organisms, e.g. possible gene transfer from a GM crop to bacteria present in the human gut;
- Nutrition – changes in the content of nutrients or their bioavailability in a crop as a result of genetic modification;
- Any unintended effects resulting from genetic modification.

An international consensus regarding the approaches taken when assessing the safety of GM crops, including the issues outlined above, was recently formalized by the publication of Codex Alimentarius guidelines on the safety of foods derived from GM crops (Codex Alimentarius 2003). This means that

such Codex Alimentarius standards must be implemented by Codex Member States (i.e. most nations), since they can serve as a frame of reference in cases where there are disputes over internationally traded foods.

3. GM crops and potential allergenicity

The remainder of this article will focus on one of the issues used to evaluate the safety of a GM crop, namely the assessment of its potential allergenicity. In Western countries, the prevalence of allergies among different populations is increasing, although the cause for this has not yet been established. Various types of allergy exist and include inhalant allergy (e.g. birch pollen allergy), contact allergy (e.g. nickel allergy) and food allergy (e.g. peanut allergy). The allergic reactions produced may include nausea, vomiting, hives on the skin, or even life-threatening shock. Such reactions are mediated, in part, by immunoglobulin type E (IgE) antibodies, which bind with the allergen; mast cells then release substances such as histamine which cause the symptoms of allergic reactions.

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Since all known food allergens are proteins, it is important to assess the potential allergenicity of newly introduced proteins in GM crops. Furthermore, there is a possibility that if a conventionally-grown crop is already allergenic in its own right, subsequent genetic modification of the crop might have (albeit unintentionally) affected this property as well.

In its guidelines for assessing the safety of foods derived from GM crops, Codex Alimentarius has devoted an annex to evaluation of the potential allergenicity of new proteins introduced into these crops. Since no single assay can provide conclusive evidence on whether a protein is an allergen or not, Codex recommends an approach that considers the 'weight of evidence', as derived from a number of assays.

The assays used for evaluating the potential allergenicity of a novel protein commonly include:

- Allergenicity of the organism serving as the source of the transferred gene coding for the novel protein (e.g. a foreign gene derived from peanut).

- Comparison of the structure of the novel protein with the structures of known allergenic proteins using 'bioinformatics' computer methods. By comparing the amino acid sequence of the novel protein with those of known allergens, certain similarities can be identified. The Codex Alimentarius recommends two types of comparison. Firstly, short identical sequences with a minimum size of six to eight contiguous amino acids are identified, since these stretches might be binding sites for IgE antibodies. Secondly, overall similarities between stretches of 80 amino acids, of which at least 35% are identical, are identified.
- Digestion of the novel protein in simulated stomach fluid. To this end, an *in vitro* (test tube) method is used in which the novel protein is incubated with the protein-degrading stomach enzyme pepsin in a diluted hydrochloric acid solution. The rationale behind this *in vitro* assay is that resistance to degradation by pepsin is considered an indication of increased likelihood of allergenicity.
- Specific serum screening using sera from patients sensitive towards a specific allergen. This is recommended if the known allergen and the transferred gene which codes for the novel protein in question are derived from the same organism, or if the allergen proves to show some similarities to the novel protein in the bioinformatics assay.

The Codex foresees that, in future, additional methods such as animal models and more broadly focused serum screening may become available for use during assessments of the potential allergenicity of GM proteins.

Recently, we have employed bioinformatics to search for short stretches of at least six contiguous amino acids shared by known allergens and the novel proteins expressed in either commercial GM crops or conventional crops. It was observed that, irrespective of the nature of the crop, the identities of six to seven amino acids matched those found in known allergens in the majority of the novel proteins tested. As a follow-up procedure, we recommended comparison of amino acid sequences from these novel proteins with short stretches of allergen sequences that have been reported in the literature to be bound by antisera. In addition, computer programs were used to predict the most

likely site where antibodies will bind within the novel or allergenic protein. After these refining steps had been carried out in our studies, a few selected proteins of interest were identified that should be scheduled for further testing, such as use of serum screening tests (Kleter and Peijnenburg 2002, 2003).

Several websites provide the tools necessary to carry out the bioinformatics procedures recommended by Codex Alimentarius. For example, the Allermatch™ website (www.allermatch.org) has been developed by us in collaboration with bioinformaticians from Plant Research International, which, like our institute (RIKILT-Institute of Food Safety), is part of Wageningen University and Research Center. Allermatch™ offers users a database of allergen sequences, as well as easy-to-use search facilities and concise representation of results, links to more detailed material are also provided (Fiers *et al.* 2004).

In conclusion, GM crops are compelled to undergo a rigorous pre-market safety assessment, which covers a range of issues, including potential allergenicity. Publicly accessible facilities, such as those provided by Allermatch™, will contribute to the transparency of this assessment.

GM crops are compelled to undergo a rigorous pre-market safety assessment, which covers a range of issues, including potential allergenicity

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