

Safety assessment of genetically modified food crops

Naveen Arora, Ankita Mishra

*Institute of Genomics and Integrative Biology, Delhi University Campus,
Mall Road, Delhi-110007, India*

Abstract

Genetically modified (GM) food crops are aimed towards improving food quality, quantity and to help protect and preserve the environment by reducing reliance upon chemical pesticides and herbicides. Despite such potential benefits, GM foods obtained from transgenic crops require safety testing for evaluating their tendency to provoke an allergenic reaction, stability in the environment and unintended effects. Transgenic proteins encoded by the foreign genes may act as cross reactive allergens or may alter the level of an intrinsic allergen(s). Therefore, safety assessments are required prior to the release of the GM food in the market for consumption. With the advancing scientific knowledge and better access to the available resources, researchers need to develop improved protocols for identifying potential allergens and more reliable animal models mimicking food allergy in humans.

Keywords: Genetically modified foods, allergenicity assessments, transgenic proteins, food allergy

INTRODUCTION

Biotechnology aims at developing transgenic crops with desired traits. These varieties are superior to their natural counterparts. Transgenes incorporated into GM crops provide resistance against insects, pests, fungi, viruses and bacteria that cause plant diseases; tolerance to environmental imbalances like drought, cold, salinity and abiotic stress^{1,2,3}. GM crops are high yielding varieties having increased shelf life, improved nutritional value and texture. Molecular biology methods applied for GM crop development are precise and introduce only the desired trait into the plant. These methods have the capacity to alter the nutritional profile of the plants. It may also interfere with the already present natural allergens. Hence, there exists an urgent need to assess the safety (allergenicity studies) of the GM crops. Each GM

food undergoes a thorough pre-market safety assessment before it is released⁴. These rigorous safety assessment tests make sure no allergenic variety is made available for human consumption. GM foods that are approved by international regulatory authorities to date have been fully assessed for both allergenicity and toxicity.

Safety evaluations are a pre requisite as 2-8 % of the world population suffers from food allergy and it has shown an increasing trend^{5,6}. Food allergy is defined as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food protein. It is distinct from other adverse responses to food, such as food intolerance⁷, pharmacologic reactions, and toxin-mediated reactions. The individual food allergy is affected by geographical location, culture and life style⁸. In the United States of America some foods are categorized as major food allergens and these include milk, egg, peanut, tree nuts, soy, wheat, fish, and shell fish⁹. Food allergens are the parts of food or ingredients within food (usually proteins) that are recognized by immune cells. Although the structural properties of

Address for Correspondence: Naveen Arora, Ph.D., Room 509, Allergy and Immunology Section, Institute of Genomics and Integrative Biology, Delhi University Campus, Mall Road, Delhi-110007, India, Phone: (011) 27666157, Fax: (011) 27667471, E-mail: naveen@igib.res.in ; navdelhi@hotmail.com

proteins that cause allergic reactions have not been characterized completely, some features include molecular size of 10 to 70 kD, stimulate immune responses (induce the production of allergen-specific IgE), and stable to heat, proteases, acids, processing and digestion¹⁰.

Food allergic reactions may be IgE-mediated, non IgE-mediated (cell mediated), or both. Milk, egg and peanut account for the vast majority of IgE – mediated reactions in young children, whereas peanuts, tree nuts and sea food (fish & shell fish) account for the vast majority of IgE- mediated reactions in adults^{11,12,13}. IgE-mediated (allergic) reaction refers to a hypersensitive reaction in an atopic individual which may not happen in an otherwise normal individual. Type I allergy is one of the four forms of hypersensitivity reaction, characterized by excessive activation of mast cells and basophils by IgE, resulting in an extreme inflammatory response. Common allergic manifestations include eczema, hives, allergic rhinitis, asthma, gastrointestinal tract allergies, and reactions to the venom of stinging insects such as wasps and bees. In some people, severe allergies to environmental or dietary allergens or to medication may result in life-threatening anaphylactic reactions and death. Non IgE-mediated food allergy is associated with the following disorders –

- **Eosinophilic GI disease (EGIDs)** – is an uncommon gastrointestinal disease affecting both children and adults. It is characterized by eosinophilic infiltration in one or more areas of the GI tract and abdominal pain. The symptoms of the disease depend on which part of the GI tract is involved and whether the accumulation of eosinophils is localized or widespread.
- **Food Protein – induced enterocolitis syndrome (FPIES)** - generally affects infants and young children. Symptoms include vomiting, diarrhea (sometimes bloody), dehydration and low-blood pressure. The child or infant can appear lethargic, and have problems gaining weight. Symptoms occur shortly after consuming the culprit food. Vomiting starts within 2 hours after eating the causative food, and diarrhea typically starts within 5 hours of eating.
- **Systemic (whole body) contact dermatitis.**

- **Allergic contact dermatitis (ACD)** – is a form of eczema, onset of the disease is characterized by an allergic reaction to food molecules that occur naturally in foods such as mango. The allergic reaction involves immune cells but not IgE antibodies. Symptoms include itching, swelling and small raised areas on the skin that may or may not contain fluid.

GENETICALLY MODIFIED (GM) FOODS

In the past, selective breeding techniques were used by researchers to improve crop varieties. But these conventional methods were time consuming and often not very accurate. Therefore, genetic engineering gained recognition and offered a wide range of valuable genes and methods of inserting them into the plant genomes. Genetic engineering involves the use of r-DNA technology to alter the genetic makeup of organisms such as animals, plants, or bacteria. When food-crops are genetically modified, one or more genes are incorporated into the crop's genome using a vector containing several other genes along with the transgene, like viral promoters, transcription terminators, antibiotic resistance marker genes and reporter genes. These crop plants possess enhance desired traits such as increased resistance to herbicides, insecticides, improved shelf life, nutritional content, better taste and texture etc.

GM FOOD SAFETY GUIDELINES

An integral part of the safety evaluation is the assessment of the potential allergenicity of the novel proteins being expressed in the GM food crop. Previous studies employed for assessing the safety of chemical additives used in food industry, involved testing single chemical components. But this is not feasible for testing GM foods. Therefore, an alternative approach was required for the safety assessment of GM foods. First food safety assessment report¹⁴ described comparative approach and has laid the basis for later safety evaluation strategies. This led to the development of the concept of substantial equivalence. Substantial equivalence (SE) emphasizes that safety assessment is not a parameter which can be evaluated on an absolute scale. Instead it measures whether the GM food is safe as its traditional counterpart if such

counterpart exists or to an earlier approved GM variety¹⁵. SE analysis includes three levels - complete, partial and not at all¹⁵. Complete - substantially equivalent i.e. the GM food is similar to the native counterpart, partial - substantially equivalent except for the inserted gene and 'not at all' means that the GM food is not at all equivalent to its counterpart and a step by step evaluation of the transgenic food is mandatory^{15,16}. GM and non-GM counterpart used for the comparative studies should be subjected to similar environmental conditions to avoid genotypic and phenotypic differences not related to the transformation process. The non-GM counterpart should be non-transgenic, isogenic line to the GM line. Comparative studies should take into account agronomic, morphological, genetic and compositional aspects in order to make an objective assessment. Further, attention should be paid to the choice of comparator, the design of field trials, and statistical analysis of the generated data in order to obtain authentic results.

Safety assessment strategies involve an integrated, case-by-case approach to be used in the allergenicity assessment of GM foods. The safety evaluation protocols mainly focus on, whether the original source of the GM food protein is allergenic or the GM food protein is similar to any known or reported allergens and lastly whether the GM food protein can remain intact after digestion. Additional testing includes the use of specific immunological methods or animal models, for further evaluation. GM foods are assessed on the basis of several guidelines issued by international scientific organizations such as the World Health Organisation (WHO), the Food and Agriculture Organization (FAO), and the Organization for Economic Cooperation and Development (OECD). With the advancements in the field of science and technology new risk assessment techniques are evolving. FAO/WHO, 2001¹⁷ introduced the decision tree approach for the safety evaluation of GM foods¹⁸. This approach involves assessment of the source of the introduced protein, followed by *in silico* sequence homology studies using allergen databases, assessment of immunoreactivity with sera from allergic patients, physiochemical stability (in vitro digestibility and thermal stability) and animal model studies. Codex Alimentarius Commission (Codex), 25th session,

Rome, Italy, 30 June–5 July, 2003, proposed weight of evidence approach to be followed to evaluate the allergenic potential of novel proteins¹⁹. This approach takes into account several parameters for safety assessment like safety of the gene(s) source; amino acid sequence homology to known allergens; stability to pepsin digestion *in vitro*; and specific IgE binding studies or skin-prick testing.

SOURCE OF THE GENE

The evaluation begins with studying the source of the transgene. The sources may be classified as allergenic, moderately allergic or of unknown allergenic potential. Major food allergens include peanuts, tree nuts, soy and wheat from plant kingdom and milk, egg, fish and shell fish from animal kingdom. These eight major food allergens are referred to as 'Big Eight' in the United States of America²⁰. If the gene is from an allergenic origin or from a moderately allergic source, then a careful evaluation is mandatory in order to conclude that the gene does not encode for an allergen. An historical example is the development of a GM soybean that contained a gene from the Brazil nut, a plant that is known to cause allergy in some people²¹. In most of the cases GM crops obtain genes from organisms that have unknown allergenic potential, like bacteria, viruses, weeds, and ornamental plants.

SEQUENCE HOMOLOGY STUDIES

Amino acid sequence homology studies involve comparison of the transgenic protein sequences with the known allergens to identify probable homologues. BLAST²² and FASTA²³ are popular alignment tools used for the comparison studies they predict functional similarity and clinically important cross reactivity among proteins. The basis of bioinformatic studies is that if proteins possess reasonable linear sequence similarity, they tend to share three dimensional structural motifs^{24,25} and are more likely to share allergenic cross reactive epitopes. Sequence homology studies recommended by FAO/WHO, 2001 and Codex, 2003 evaluate the allergenic potential of novel proteins over an 80 amino acid sliding window, e"35% identity depicts that transgenic protein may elicit allergic reactions in sensitized individuals. Aalberse, 2000

suggests that proteins showing greater than 70% identity over an 80 a.a. sliding window are more likely to share IgE cross reactivity²⁶. According to safety assessment protocols if high degree of similarity is observed among sequences IgE serum screening is mandatory to further validate the safety of the crop. Several allergen databases are available online for homology studies including Food Allergy Research and Resource Program (FARRP)²⁷, Structural Database of Allergenic Protein (SDAP)²⁸, Allermatch²⁹, AlgPred³⁰ and Allergen Database for Food Safety (ADFS)³¹.

Amino acid sequence homology approach has certain limitations firstly the comparisons are restricted to the known allergens reported in the popular databases and scientific literature secondly short segment amino acid exact matches give a high rate of false negatives and lastly food allergens reported in the database are few. Therefore more stringent tests are to be performed to confirm the allergenicity of the proteins. There is no single test that can be relied upon to correctly predict the allergenic response of a protein. An integrated stepwise approach should be followed as it takes into account data obtained from several tests.

HEAT STABILITY

Food allergens are mostly resistant to thermal processing and degradation during the passage through the digestive tract in order to sensitize individuals or elicit allergic reactions. Till date no specific characteristics have been defined which can distinguish an allergen from a non allergen. Fermentation, blanching, refrigeration, heating, pasteurisation and texturizing are some of the techniques employed for food processing³². These procedures may alter the native structure of the proteins and thereby affect the overall allergenicity of the foods. Depending on the degree of distortion the processing method has on the protein structure the allergenic potential may increase, decrease or remain unaffected.

Heat treatments disrupt the native 3-D structure of the proteins leading to loss of conformational epitopes, activation of new epitopes or improving accessibility of cryptic epitopes³³. Proteins with enzymatic or

biological activity are better suited for heat stability assays because the retention of the biological activity after incubation under high temperature is a deciding factor in determining the likelihood of the transgenic protein being allergenic. Optimum temperature range for thermal stability studies is from 25°C to 95°C for up to 30 minutes¹⁸.

PEPSIN RESISTANCE

Pepsin digestibility assays involve *in vitro* studies with simulated digestive fluids to assess the allergenic potential of novel proteins. Mostly food allergens are stable to peptic and acidic conditions of the digestive system and elicit an allergic response on reaching the intestinal mucosa^{34,35,36}. Astwood and group, 1996³⁷ had first proposed simulated gastric fluid (SGF) digestibility studies for safety evaluation of GM foods. Although some researchers^{38,39,40} doubt the efficacy of this assay for allergenicity assessments, Codex, 2003 has proposed pepsin resistance as one of the parameters for safety assessments. Reports have been published stating that food allergens causing systemic reactions are stable in the assay, while non-allergenic food proteins are typically digested relatively quickly⁴¹.

SERUM SCREENING

Serum screening test are performed to evaluate the IgE binding and the range of IgE binding / cross reactivity of the novel proteins. FAO/WHO, 2001 recommends both specific and targeted serum screening to be performed, specific serum screening involves testing the transgenic protein with sera from patients with clinical food allergy to a specific allergen to confirm that the tested protein is not cross reactive with the protein to which the patient produces IgE antibodies. Targeted serum screening involves testing the gene product of interest with sera from patients sensitive to food or aeroallergens from the same broad group. Codex recommendations include specific serum screening as part of the weight of evidence approach to identify potential allergens.

The availability of sera from well characterized patients is important for reproducible, authentic, sensitive and specific results⁴². Sera should be obtained from donors with well documented clinical

history of food allergy. Ideal serum donors develop symptoms within 20 minutes of exposure to the allergen. Common symptoms include acute urticaria, angioedema, bronchial asthma, emesis and diarrhoea. If serum from different donors is pooled for the study, it is important to characterize the individual sera to understand the similarities or dissimilarities. Donors should have significant levels of serum IgE, individuals with unusually high total IgE can dominate the result as the specificity of their IgE is often broad and unrelated to allergic disease. Considerable efforts go in arranging appropriate donors and well characterised positive and negative controls, in order to generate meaningful data accurate for prediction of allergenicity.

ANIMAL MODEL STUDIES

Animal models are commonly used for scientific experiments because they possess the potential to mimic human diseases. These models provide assistance in studying the various stages associated with a disease and also help in designing therapy treatments. Animal studies have been in use for many decades, apart from allergenicity evaluation studies for GM foods, they are also used for assessing safety of different compounds like pesticides, pharmaceuticals, industrial chemicals and food additives. However, in most cases the test substance is a single compound with well characterized elements, of known purity, negligible nutritional value and very low human exposure⁴³. It is therefore relatively easy to feed such compounds to animals at a range of doses in order to identify any potential adverse health effects of importance to humans. But this methodology cannot be followed for foods which are complex mixtures of compounds varying in composition and nutritional value. In addition, a key factor to consider in conducting animal studies on foods is the nutritional value and balance of the diets used, to avoid the induction of adverse effects which are not related directly to the test material itself.

FAO/WHO, 2001 proposed the use of animal models for allergenicity prediction studies. The guidelines recommend the use of two separate species of animals and/or using two different routes of sensitization in one species. None of the animal models developed till date completely mimic the food allergic

conditions occurring in an individual. Some models are helpful in studying the mechanism of the allergic reaction while others evaluate the immunotherapeutic effect of reagents. It is unlikely that a single animal model can effectively predict the potential allergenicity of the novel food antigens because of the complex genetic diversity that predisposes the subjects to allergy and secondly cause food allergy in humans is a complex disorder that involves number of different factors to promote sensitization. Several models have been developed for allergenicity prediction studies like rodents, dogs and swines. Murine models of food allergy that have been studied are – BALB/c, DBA/2, C3H/HeJ, BDF-1, A/J and C57/B16⁴⁴.

Depending on the available literature and the scientific reports it is concluded that further studies are necessary to design better animal models capable of predicting potential allergenicity and imitating human food allergy conditions. Current safety assessment guidelines proposed by Codex, does not include animal models as part of the weight of evidence approach. The main reason for not incorporating such studies is that they lack scientific justification due to non validated predictive values.

SOME COMMERCIAL GM CROPS

GM tomatoes

First commercially grown GM crop plants having license for human consumption were the Flavr Savr tomatoes. These were slow ripening kanamycin resistant varieties, with an antisense gene which interferes with polygalacturonase enzyme responsible for the ripening process⁴⁵. Flavr Savr tomatoes, before their commercialization had undergone rigorous safety testing regime. Calgene Inc. (manufacturers) submitted a report to the FDA stating the safety of the product the results showed no significant alteration in total protein, vitamins and mineral contents of the GM and parent tomatoes. Male/female rats were tube-fed homogenized GM tomatoes and no toxic effects were observed. In addition, organ and body weights, weight gains, food consumption and other blood parameters were comparable for GM-fed and control group. It was concluded that Flavr Savr tomatoes are “substantially equivalent” to their natural counterparts.

GM soybean

Soybean (*Glycine max*) seeds are naturally deficient in methionine to overcome this researchers developed transgenic varieties with improved nutritional quality. Methionine-rich 2S albumin gene from Brazil nut (*Bertholletia excelsa*) was introduced into soybeans. Since Brazil nut is a known food allergen, Nordlee and group²¹ had carried out studies to assess the allergenic potential of these transgenic varieties. Studies had shown that genetically engineered soybeans containing Brazil nut 2S albumin gene could cause allergic reactions in Brazil nuts sensitive humans. Further, when the safety assessment reports were made public growers discontinued the production process.

GM maize

SmartStax is a genetically modified variety of corn with eight desired traits combined or 'stacked' together – six for insect resistance (*Bt*) *cry1A.105*, *cry2Ab*, *cry3Bb1*, *cry1Fa2*, *cry35Ab1* and *cry34Ab1* and two for herbicide tolerance *Pat* and *CP4* epsps. Monsanto and Dow AgroSciences jointly developed this variety, so that the two could share their GE traits. It is believed that this is the largest ever commercial launch of GM corn because it has more than three desired characteristics. At present the transgenic varieties which are commercially grown have just three traits in them⁴⁶. But safety assessments are required in order to explore the potential threat SmartStax can cause to the consumers. In the past, Canada has approved these eight traits separately for commercialization without assessing the safety of all the traits jointly. However, this does not comply with the Codex; the guidelines propose that foods with stacked GE traits should go through a full safety assessment for new unintended effects.

GM Brinjal

Bt Brinjal is a transgenic variety developed by Mahyco (Maharashtra hybrid seed company ltd.) a leading Indian seed company. It has *cry1Ac* gene from a soil bacterium, *Bacillus thuringiensis* inserted into the genome through an *Agrobacterium*-mediated gene transfer. It is the first genetically modified food crop in India that has reached the approval stage for commercialization. Three transgene inserted are namely -

- *cry1Ac* gene which encodes an insecticidal protein

is derived from common soil bacterium *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki* to produce the insecticidal protein. The *cry1Ac* gene is driven by a viral promoter, the cauliflower mosaic virus (CaMV) 35S promoter.

- *nptII* gene for an antibiotic resistance marker, neomycin phosphotransferase-II.
- *aad* gene fanother marker 3" (9) O-aminoglycoside adenyl transferase.

These genes are put together in such a way that they work in tandem to produce insecticidal protein that is toxic to the targeted insects like the fruit and shoot borer *Leucinodes orbonalis* and fruit Borer *Helicoverpa armigera*. There is a severe paucity of literature with respect to the effects of the Bt- Toxin on humans. Long term effects of consuming this crop in terms of health risks are still not known. Genetic Engineering Approval Committee (GEAC), India has cleared Bt Brinjal for commercial release, but the safety assessment studies performed by US firm Monsanto and Indian firm Mahyco lacks authenticity.

GM Pea

Transgenic pea varieties were developed after a ten year long project at CSIRO (Commonwealth Scientific and Industrial Research Organization), Australia. The gene coding for the protein, α -amylase inhibitor-1 (α A1) from common bean (*Phaseolus vulgaris*), was inserted into pea (*Pisum sativum* L.) to make the pea-plants resistant to attack from weevils. Researchers found that the transgenic protein provoked immune reactions not exhibited by the native protein. These varieties when fed to mice cause inflammation in the lungs⁴⁷. The most probable reason behind the transgenic protein causing inflammation is altered processing of the protein in peas. Post-translational modifications of proteins are generally species-specific, and as genetic modification involves cross-species transfer of proteins, as a result transgenic proteins differ structurally from the native proteins. These investigations reveal that diversity in translational and post-translational modification pathways between species could potentially lead to discrete changes in the molecular conformation of the expressed protein and subsequent cellular function and antigenicity. Later use of GM peas was discontinued.

CONCLUSION

Due to changing climatic conditions, escalating population size, enhanced dependency on agrochemicals it has become difficult for the growers to meet the consumer demands. Farmers and consumers both are at the receiving end, former due to high input costs and the latter because of the enormous damage these chemicals cause to the environment and human health.

Agricultural biotechnology has evolved to help growers in every possible manner to overcome major food related problems world over. r-DNA technology lay main emphasis on the development of transgenic varieties (GM) with desired traits. These methods have improved both the quality and quantity of the food grains in a sustainable manner. Molecular biology methods are precise that only a single gene can be transferred from varied sources like bacteria, viruses and other eukaryotic organisms into plants to develop GM varieties. Therefore we can conclude that the development of GM varieties has been beneficial for the consumers and the environment, but safety of transgenic materials is still not completely deciphered.

It is the responsibility of the regulatory authorities to design safety assessment protocols free from lacunae and which can evaluate the allergenic potential of GM food crops. Available literature and experimental data should be extensively referred by eminent researchers and scientist before drafting the safety assessment tests. Transgenic varieties can be easily commercialized and supplied to the consumers if proper tests are available to evaluate the allergenic potential, considering all the parameters responsible for causing allergic reactions in individuals⁴⁸.

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