Review Article

Constantly evolving safety assessment protocols for GM foods

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The introduction of GM foods has led to the evolution of a food safety assessment paradigm that establishes safety of the GM food relative to its conventional counterpart. The GM foods currently approved and marketed in several countries have undergone extensive safety testing under a structured safety assessment framework evolved by international organizations like FAO, WHO, Codex and OECD. The major elements of safety assessment include molecular characterization of inserted genes and stability of the trait, toxicity and allergenicity potential of the expressed substances, compositional analysis, potential for gene transfer to gut microflora and unintentional effects of the genetic modification. As more number and type of food crops are being brought under the genetic modification regime, the adequacy of existing safety assessment protocols for establishing safety of these foods has been questioned. Such crops comprise GM crops with higher agronomic vigour, nutritional or health benefit/ by modification of plant metabolic pathways and those expressing bioactive substances and pharmaceuticals. The safety assessment challenges of these foods are the potential of the methods to detect unintentional effects with higher sensitivity and rigor. Development of databases on food compositions, toxicants and allergens is currently seen as an important aid to development of safety protocols. With the changing global trends in genetic modification technology future challenge would be to develop GM crops with minimum amount of inserted foreign DNA so as to reduce the burden of complex safety assessments while ensuring safety and utility of the technology.

Key Words: GM foods, safety assessment, protocols, allergenicity, toxicity

INTRODUCTION

Developments in science and technology of GM food production have increased considerably since their introduction in the food chain. As a result GM food safety assessment systems involving policies, laws and guidelines, are constantly evolving in attempts to keep pace with technology developments and their risk benefit evaluations. The cultivation and marketing of GM foods for human consumption is based on a premarket food safety testing paradigm that establishes safety of a GM food relative to its traditional or conventionally bred counterpart.[1] Under such a paradigm a reasonable degree of safety could be established for the currently marketed GM foods with simple modifications involving single genes and traits. New plant varieties being developed consist of more complex genetic modifications with more number of genes and by modification of a large number of metabolic pathways to achieve improved/ enhanced agronomic properties particularly abiotic stress resistance, food quality such as processing and storage properties, nutrition and health benefits such as enhanced vitamins and minerals and expression of pharmaceuticals for human and veterinary use.[2] Genetically modified organisms other than plants under active consideration are GM animals including fish, which are likely to enter the food chain shortly. These developments pose an inevitable challenge for the capability of current GM food safety assessment strategies and protocols for evaluating future GM foods. The present paper attempts to assess the evolution of safety assessment protocols for GM foods in the light of these developments.

EXISTING STRATEGIES FOR FOOD SAFETY ASSESSMENT OF GM FOODS

Various strategies have been designed by international for a like FAO, WHO, OECD and the Codex to assess the safety of GM crops. The Codex Alimentarius Commission of the UN has evolved an international guideline for food safety assessment of GM foods on the basis of risk analysis concepts and principles, which came into force in 2003.[3,4] The WTO also refers to these guidelines for trade related issues in food safety. These guidelines are based on an integrated stepwise case-by-case evaluation of safety relative to the traditional or conventionally bred counterpart. The assumption is that traditional food has a history of safe consumption and thus can be used as a baseline for safety assessment of GM crops/foods. To facilitate this comparative approach the concept of substantial equivalence (SE) has been introduced by the international fora and has now become the corner stone of the GM food safety assessment paradigm. SE is the

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starting point in the safety assessment process where significant differences in compositional, agronomical and morphological characteristics are first identified which then becomes the focus of further safety evaluations to assess any potential for adverse effects in humans. Using this approach 3 scenarios are provided for safety testing, viz., SE, except for the new trait and not SE. Most of the currently marketed GM varieties come under the second scenario.

THE SAFETY ASSESSMENT PROCESS

Using the SE concept the safety assessment of GM foods is carried out from the parent to the transformed crop generating a comprehensive set of information on the source, type and target of potential hazards likely to occur. This is facilitated through molecular characterization of the inserted DNA and the expressed gene products, compositional and morphological changes, toxicity and allergenicity of new protein expressed, safety and nutritional evaluation of whole GM food, and gene transfer to gut flora of humans or animals and identification of any unintentional effects of GM on plant metabolism that could have adverse effects.[1] A combination of database screening, in silico, in vitro and in vivo animal tests are deployed in the testing strategy to provide for a comprehensive evaluation of phenotypic and agronomic equivalence, compositional equivalence, safety and nutritional equivalence in comparison to a conventional counterpart.

NEW CONCEPTS/AREAS TARGETED FOR FUTURE SAFETY TESTING OF GM FOODS

As more food crops are being brought under the GM regime and the range of modifications are increasing, current safety assessment systems are being revisited to assess their utility for assessing future GM foods and identify areas for improvement in safety assessment methods. While WHO has expressed the opinion that the GM foods available on the market are not likely to present risk for human health it also stresses that the benefits of the technology of GM foods can be realized only if properly assessed before marketing through broad, coherent, evidence-based evaluation.[5] Some of the targeted areas for improvement include compositional analysis for SE studies, detection of unintentional effects, biomarkers for allergenicity assessment and safety assessment of foods with enhanced nutritional or health benefits. Guidelines are also being enhanced for assessing GM foods with stacked traits. New guidelines are being framed for the safety assessment of foods derived from GM animals. Advances in molecular biology are being exploited to evolve more efficient transformation methods.

COMPOSITIONAL ANALYSIS FOR SE STUDIES

Current emphasis is on international standardization and harmonization and provide peer reviewed databases of the list of key components including nutrients, antinutrients, toxins and allergens, secondary plant metabolites that could have health impact, with appropriate ranges of natural variation so as to aid in comprehensive justification of substantial equivalence. The OECD has evolved consensus documents on composition of various foods/crops that are used as reference guides for comparative studies.[6] In addition ILSI has developed a Crop composition database, which serves as a supplement to OECD documents.[7] Recently the need for comparative food compositional analysis of staple foods for use in developing countries has been recognized.

DETECTION OF UNINTENTIONAL EFFECTS-USE OF PROFILING TECHNIQUES

To a large extent targeted compositional analysis of defined constituents have been used for detection of unintentional effects of GM in plants. Recently it is being deliberated whether the targeted approaches could detect potential unintentional changes that could be introduced by the future GM of crops particularly modification or introduction of new metabolic pathways. Thus non-targeted approaches using profiling techniques are being focused to compliment targeted approaches in future GM food safety assessments.[8] These techniques are gene expression analysis by microarray techniques, 2-D protein gel electrophoresis followed by MS, and analysis of chemical compounds by LC-MS-NMR to assess metabolism. The applicability of these methods is based on generating background data on natural variations in compositional patterns. The current challenge is to evolve criteria for interpreting potentially observed differences with respect to their biological relevance and toxicological significance.

BIOMARKERS FOR ALLERGENICITY ASSESSMENT

The primary focus of allergenicity assessment is on the safety of any newly expressed protein, to prevent the transfer of a major allergen or crossreactive protein into a different food crop. Systematic approaches have been evolved over time by experts under ILSI, IFBC and FAO/WHO in the form of decision trees, which involve stepwise assessment of source organism of the protein, structural similarity/homology to known allergens (bioinformatics), in vitro (physicochemical properties) and in vivo tests (specific and targeted serum screening) using sera from allergenic patients and animal models.[9] However since no single test could reliably predict an allergenic response, the Codex has recommended a weight of evidence approach using data from all sources in order to achieve the best possible approach.[3] Current international focus is on understanding the relationship between protein structure, resistance to proteolytic digestion, function and IgE mediated allergic sensitization so as to evolve appropriate biomarkers for allergenicity assessment of GM foods.

Bioinformatics is evolving as a major thrust area for rapid screening and a priori assessment of allergenicity potential of the new proteins in GM crops and various specialized food allergen and allergen sequence databases and algorithms are being developed. The use of appropriate animal models has been opined as a necessary element for assessment of allergenicity potential by various working groups.[10] Among the animal models proposed, the Balb/c mouse and the Brown Norway rat model have been extensively studied but are yet to be validated.

DEVELOPMENT OF ANIMAL FEEDING STUDIES /MODELS FOR SAFETY AND NUTRITIONAL TESTING OF WHOLE GM FOODS

Animal feeding studies for assessing toxicity and nutritional effects of GM foods are recommended where SE could not be established between the GM and non-GM counterpart. Due to complexity of whole GM foods, the goal of animal studies has been to establish safety relative to the traditional counterpart rather than absolute safety. Current testing protocols for GM foods are based on a battery of standardized toxicity tests elaborated by OECD for defined chemical substances. While single dose acute toxicity testing have been used for demonstrating adverse effects of the recombinant proteins or metabolites introduced into GM crops various sub-chronic toxicity tests in rodent models have been used for testing whole GM foods. Various limitations of adapting conventional animal toxicity studies to safety testing of GM crops/foods have been recognized particularly with respect to obtaining sufficient amount of purified recombinant protein from the GM plant for acute toxicity testing and the complex and bulky nature of foods that limits their application at high levels without compromising nutritive value and balance of the diet. On the other hand a sub-chronic 90-day rodent feeding study on whole GM food is considered appropriate for testing safety of long-term consumption of GM foods.[11] Designing such feeding experiments that could indicate whether observed effects are caused by inherent gene product, the expressed protein or any secondary effects of GM has been found to enhance considerably the utility of currently used toxicity tests.[12] Long-term livestock feeding studies in target animals such as chicken, pigs and cow are being recommended for assessing growth performance particularly for GM foods with enhanced nutrition.

SAFETY ASSESSMENT GUIDELINES FOR FOODS WITH ENHANCED NUTRITION OR HEALTH BENEFITS

GM foods with nutritional enhancement that are under active consideration particularly for developing countries include rice with enhanced β-carotene and iron and potato with enhanced protein.[2] Safety and nutritional assessment of these foods are of particular importance from developing countries' perspective as consumption of such crops may cause significant changes in dietary intake patterns. Most of the genetic modifications targeted for nutritional enhancement involve complex modification of metabolic pathways in plants with the objective of increasing the content of or alteration in composition of a single nutrient with no concomitant change in overall macronutrient composition, changes in bioactive components with expected benefits to health, reduction in the content of a antinutrient, toxin or allergen and change that affect the bioavailability, absorption, or utilization of a nutrient. Safety testing strategies and methods for these GM foods are being evolved by Codex with reference to characterization of the change introduced in relation to stability of the level of expression and impact of post harvest practices on stability, and methods for testing bioavailability of the modified nutrient particularly in staple crops under the normal practices and customs of each country, exposure assessment, animal feeding studies to assess nutritional impact and identification and characterization of risks and benefits.[13]

GM FOODS WITH STACKED TRAITS

GM crops with stacked genes are obtained from crosses of 2 or more GM events. Currently existing ones are a combination of 2 or 3 events of insect resistance and herbicide tolerance traits in maize, cotton and soybean. In future it is highly likely that more traits with specific values like insect resistance, herbicide tolerance and various quality traits may be introduced. The safety testing protocols/ methods involve robust molecular characterization methods that are capable of assessing molecular equivalence between the stacked and single events at the insert and protein level to subject to further safety evaluation.[14]

FOODS DERIVED FROM GM ANIMALS

The possible commercialization of recombinant DNA animals including fish in a foreseeable future led to the initiation of developing safety assessment guidelines for this group of foods on the basis of risk analysis principles earlier developed for GM plant foods by the Codex.[15] Issues identified for safety assessment namely, the nature of recombinant construct and its expression product, health status of the recombinant DNA animal and composition of foods produced from GM animals including key nutrients, food storage and processing and intended nutritional modification.

DEVELOPMENTS IN TRANSFORMATION TECHNOLOGIES AFFECTING SAFETY ASSESSMENT PROTOCOLS

Advances in molecular biology are facilitating improvements in gene delivery methods with increased efficiency and stability. Transformation methodologies are being explored that can reduce the amount of genetic elements inserted so as to simplify and reduce uncertainties in safety assessment process.[1] Considerable work is in progress to introduce plant based selectable markers like the phosphomannose isomerase system that could replace currently used antibiotic resistant marker genes in the transformation process.

DEVELOPMENT OF SAFETY ASSESSMENT PROTOCOLS FOR DEVELOPING COUNTRIES: NEEDS AND CHALLENGES

International trade regulations concerning food safety are becoming more complex which is likely to place considerable burden on developing countries with respect to importing or exporting GM foods. Many developing countries have yet to formulate food safety assessment frameworks for GM foods, which require considerable capacity building initiatives. The urgency of this demand is reflected as more food crops are being brought under the GM regime. Added to this are considerable concerns, opinions and differing risk perceptions among various countries developing/producing/ marketing/ consuming GM foods, on the extent and nature of risk/safety assessment data to be generated for GM food approvals which are resulting in asynchronous regulatory approvals. Under such a scenario developing countries are faced with the

challenge of evolving safety assessment protocols under a framework taking into consideration the types of GM foods to be cultivated and marketed, the safety issues to be addressed and approaches for their assessment, development of guidelines, identification of basic data required for risk assessment and the extent of capacity building required particularly for risk analysis. Evolving safety testing protocols for GM staple foods with altered nutrient levels/ composition, agronomic properties like insertion of insecticidal genes, identifying unapproved GM varieties for their toxic and allergenic properties in food aid supplements as well low level presence of GM varieties that are still undergoing field testing are some of the major tasks to be addressed.

CONCLUSIONS

The evolution of safety assessment protocols for GM foods is a dynamic process. As more foods undergo GM and with more complex traits the demands for robust and sensitive methodologies together with elements of rapidity, efficiency and cost-effectiveness are likely to increase. Such demands would be considerably met with advances in molecular biology and improvements in GM technology particularly in transformation methods that could reduce the amount of foreign DNA to be inserted so that safety assessments are simplified while ensuring the safety and utility of the technology.

AUTHOR DISCLOSURES

Sesikeran B and Vasanthi Siruguri, no conflicts of interest.

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