



SOUTH ASIA
BIOSAFETY PROGRAM

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SABP

The South Asia Biosafety Program (SABP) is an international developmental program initiated with support from the United States Agency for International Development (USAID). The program is implemented in India and Bangladesh and aims to work with the local governments to facilitate implementation of transparent, efficient and responsive regulatory frameworks that ensure the safety of new foods and feeds, and protect the environment.

SABP is working with its in-country partners to:

- Identify and respond to technical training needs for food, feed and environmental safety assessment.
- Develop a sustainable network of trained, authoritative local experts to communicate both the benefits and the concerns associated with new agricultural biotechnologies to farmers and other stakeholder groups.
- Raise the profile of biotechnology and biosafety on the policy agenda within India and address policy issues within the overall context of economic development, international trade, environmental safety and sustainability.

DEVELOPMENT OF METHODOLOGIES FOR EVALUATING ALLERGENICITY POTENTIAL OF GENETICALLY MODIFIED (GM) FOODS

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Under the USDA-FAS sponsored Borlaug Fellows programme in Agricultural Science and Technology, a research programme on development of methodologies for evaluating allergenicity potential of genetically modified (GM) foods was undertaken at the Department of Food Science and Technology of the University of Nebraska Lincoln (UNL), from February 1 to March 21, 2008. The objective of the research programme was to gain knowledge and expertise and to understand the utility and limitations of various methods currently applied for evaluation of allergenicity potential of novel proteins introduced into GM crops/foods.

ISSUES ADDRESSED

Testing the allergenicity potential of GM foods has become an important international issue because of introduction of novel proteins into the food. The potential for allergenicity of GM foods is assessed from a number of risk factors using *in silico*, *in vitro* and *in vivo* approaches. The Codex guideline for safety assessment of GM foods emphasizes use of a weight of evidence approach for allergenicity evaluation, as no single method has been predictive so far. The focus of Codex guideline is to ensure that allergy risks from GM foods are not greater than those from non-GM varieties, to evaluate the possibility that the introduced protein is an allergen and its potential for cross reactivity and also evaluate the physic-chemical characteristics of the new protein compared to known food allergens. The study undertaken aimed at application of established bioinformatic methods

(*in silico*) and *in vitro* pepsin digestibility for assessment of allergenicity potential of novel food proteins which are also part of the newly evolved ICMR/DBT guidelines and protocols for safety assessment of GM foods and are mandatory for approval of marketing future GM foods in India.

Following is a brief description of the work carried out using these two approaches.

STUDY OF BIOINFORMATIC METHODS

The objective of the study undertaken was to identify potentially relevant matches in amino acid sequence between the proteins in Golden Rice 1 and 2 (GR1 and GR2) and known and putative allergens, which might indicate an elevated risk of allergic reaction to those with specific existing allergies, if they consume grain or food products made from Golden Rice 1 or 2. The method used consisted of performing amino acid sequence identity matches between the proteins expressed by transgenes inserted in Golden Rice 1 and 2 with known and putative allergens using Allergen Online database of the Food Allergy Research and Resource Program (FARRP) of UNL. The sequence database search strategy consisted of a FASTA3 overall search of Allergen Online with its default search and scoring criteria; a FASTA3 search of Allergen Online by 80 amino acid segments as per Codex criteria for cross reactivity of identity matches >35% over 80 amino acid window; and a BLASTP search of NCBI Entrez to identify matches to any sequences newly added to allergen databases and not entered into Allergen Online database. The observations from the three bioinformatic searches showed that none of the approaches used identified criteria for cross reactivity (Codex >35%, 80 amino acids; FASTA full length >50%). From these observations it was interpreted novel proteins introduced into GR1 and 2 are not expected to have any significant risk of cross reactivity for those who are allergic to known allergens. Use of Ber e 1 as a positive control demonstrated the consistency between bioinformatic methods and helps to demonstrate the utility of evaluating the proteins of Golden Rice by the same methods.

The experience gained from performing bioinformatic methods helped in achieving a good understanding of existing molecular and other databases for assessing cross reactivity/allergenicity. Development of such expertise would aid greatly in setting up infrastructure for bioinformatic studies on novel proteins introduced into GM foods in India. Such an infrastructure would need to address critical issues particularly with respect to development of allergen databases relevant to the Indian context, sequence search strategies to apply and the protocol development, and validation through identification of research centres for networking.

STUDY OF PEPSIN DIGESTIBILITY ASSAY

The pepsin digestibility/stability assay is one study in a weight of evidence approach intended to assess the potential allergenicity of GM crops. The purpose of the assay is to confirm the digestive stability and resistibility of food antigens/allergens using pepsin as model proteolytic enzyme in simulated gastric fluid (SGF). The assay provides a simple *in vitro* correlative assay to evaluate protein digestibility but is not meant to predict digestibility of a given protein. The

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CALENDAR OF EVENTS			
Event	Organization	Date	Place
INDIA			
Brain Storming Session on 'Harmonization of Allergenicity Assessment Protocols for Conventional and Novel Foods & Food Products'	National Institute of Nutrition, Department of Biotechnology (DBT) and USDA Borlaug Fellowship Program	August 20 - 21, 2008	NIN, Hyderabad
National Conference on 'Agri And Food Biotechnology For Food, Feed, Fuel And Farmer Security'	PHD Chamber of Commerce and Industry	August 26, 2008	PHD House, New Delhi
Symposium on 'Safety and Allergenicity Assessment of GM Foods-Indian Perspective'	Institute of Genomics & Integrative Biology (IGIB), Indian Aerobiological Society and Indian College of Allergy, Asthma & Applied Immunology.	August 28, 2008	IGIB, New Delhi
Workshops on 'Confined Field Trials of GE Crops: Guidelines and Standard Operating Procedures'	All India Crop Biotechnology Association (AICBA) and Biotech Consortium India Limited (BCIL)	September 5, 2008	Aurangabad
		September 6, 2008	Hyderabad
Sensitization workshop on 'Registration of Plant Varieties Under PPV & FR Act, 2001'	AICBA, BCIL and PPV & FR Authority	September 19, 2008	Hyderabad
SAU workshops on 'Management and Monitoring of Field Trials of Genetically Modified Crops'	Ministry of Environment & Forests, DBT and BCIL	September - October, 2008	At SAUs in 12 States
INTERNATIONAL			
10th International Symposium on the Biosafety of Genetically Modified Organisms	International Society for Biosafety Research (ISBR)	November 16 to 21, 2008	Wellington, New Zealand

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assay was validated in multilaboratory evaluation that was conducted by ILSI-HESI using a set of common proteins.

The main objective of the study was to familiarize with the *in vitro* digestibility protocols and factors affecting the assay particularly pH, pepsin purity, pepsin to target protein ratio, target protein purity and method of detection. The assay was performed using known food allergens, β -lactoglobulin and ovalbumin.

Important factors addressed *in vitro* pepsin digestibility assay are pepsin activity in simulated gastric fluid (SGF), limit of detection of digested protein and the pepsin digestibility of the test protein. Verification of pepsin activity is considered to be an important factor since formulation of SGF that is used to evaluate digestibility of dietary proteins by pepsin in the allergenicity assessment process is based on units of pepsin activity per mg of test protein. The pepsin activity assay is based on the estimation of the amount of soluble peptides present in trichloroacetic acid (TCA) solution after pepsin digestion of denatured hemoglobin. Undigested hemoglobin is precipitated with TCA and the amount of soluble peptides is estimated by measuring the absorbance at 280nm. The amount of soluble product is a measure of the amount of proteinase activity present. Analysis of two batches of porcine pepsin from Sigma Co. showed that the activity was within the acceptable range of variation. The purpose of determining the limit of detection is to verify detection system specificity and sensitivity for test protein spanning experimental range of 10-100% protein mass. The assay demonstrated a clear pattern of reduced intensity of stained bands with decreasing concentration. The minimum amount of protein that could be detected was 0.036mg which is 2.5% of the target protein concentration of 1.47mg.

The digestion assay is based on estimation and analysis of soluble digestive peptides from the digestion mixture containing pepsin and test protein at ratio of 10,000 units/mg protein at various lengths of time starting from 0 to 60 minutes. The extent of digestion was evaluated by reducing SDS-PAGE with control pepsin and test proteins and analysed by pixel densitometry using Kodak Gel Logic 440 imaging system software. The criteria for evaluating stability of the protein is defined as the time required to achieve 90% digestion which is estimated based on the shortest time-digested sample with a band intensity equal to, or less than the 10% undigested standard well. Proteins with >10% stainable full-length protein band remaining at 60 minutes are considered stable. Proteins reduced to <10% stainable band at 5-30 minutes are considered of intermediate stability. Proteins reduced to <10% stainable band by 2 minutes are considered labile or rapidly digested. The standard operating protocol consisted of various controls for test protein and pepsin to ensure reliability of the assay.

The observations of the digestibility assay showed β -lactoglobulin to be the most stable where >90% of the intact protein was detectable after incubation for 60 minutes with pepsin at 10,000 units per mg test protein at pH 1.2. In the case of ovalbumin the assay demonstrated intermediate proteolytic stability. Digestion time for ovalbumin with 10,000 units of pepsin activity/mg protein at pH 1.2 was observed to be 14 minutes.

The experience gained from the *in vitro* digestibility assay helped in achieving good understanding of the critical factors involved in performing the assay that is required for establishing infrastructure for performing *in vitro* pepsin digestibility technique for assessing allergenicity potential of novel proteins. Some of the issues that need to be considered for establishing the assay in the Indian context are

CREAM OF THE (WEB) CROP

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THIS MONTH'S PICK:

the

GMO Compass website

<http://www.gmo-compass.org/eng/home/>

The GMO Compass website provides excellent information on the use of genetically modified organisms (GMOs) in the agri-food industry in Europe and quickly dispels the notion that this trading block is GM-free. The website was set up in 2005 with funding from the European Commission, industry and the German government. Their editorial principles are clearly laid out, indicating that the website:

- is the work of independent science journalists, who are not contractually restricted and exercise journalistic freedom in the selection and presentation of the content;
- is not "for" or "against" genetic modification, rather, its goal is "to contribute to the informed, autonomous development of opinions and encourage responsible debate";
- aims to present objective, science-based information in a way that is easy to understand and readily accessible for the public; and
- collects materials from reputable, publicly available sources including databases, public institutions, scientific literature, and press agencies.

The GMO Compass home page provides links to the major components of their information outreach. These include:

- the latest news about genetic modification of agri-food products;
- member country reports on the use of GMOs;
- stories with background information on GM topics of interest;
- a grocery shopping section that clearly outlines the presence of GM products in fresh and processed food and food additives;
- updated information on agricultural biotechnology's breeding aims, commercial planting, and field trials in the European Union (EU) member states;
- a GMO database of genetically modified food and feed approved in the EU;
- considerable information on the environmental and human safety of genetically modified products;

- clarification on the regulation of GMOs in the EU; and
- links to other information sources on GMOs.

In addition to these regular items, the website hosts specific activities to foster informed debate. The home page currently has links to two of these: (1) documented input from an online discourse on coexistence of GM crops in Europe that took place in 2006; and (2) the results of a GMO Compass snapshot poll on whether European agriculture should make use of all available technologies – including genetic engineering and biotechnology – if negative effects on the environment could be ruled out and positive effects on world nutrition were possible as a result. This was undertaken in July 2008. Of the 5435 ballots cast in the first five days, 83.9% voted for the utilisation of all technologies, leading the editors to indicate that while snapshot surveys are not representative, this one suggests that the mood on GMOs is changing in the EU.



Animation: The Authorisation Process in Motion!

Applying, consulting, and making a decision: The long and winding road to GMO authorisation in the EU

▶ start animation

Our all time favorite feature of this website is the clever animation that outlines "the long and winding road to GMO authorization in the EU". Start with this link on the right hand side of the home page!

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establishing standard protocol itself and identification of labs for validation, database generation of stability of food allergens of importance to the Indian context and novel proteins in GM foods marketed in future, supply of purified and characterized test proteins and manpower training.

OVERALL IMPRESSIONS ON THE BORLAUG FELLOWS PROGRAMME UNDERTAKEN

The research programme undertaken facilitated capacity building in developing technical expertise in the area of allergenicity assessment methodology of GM crops/foods to a considerable extent. It is hoped that the expertise gained will lead to further research and development networking and interactions through international collaborative programmes at government and industry levels.

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IFPRI LAUNCHES WEB-BASED BIBLIOGRAPHY

The International Food Policy Research Institute (IFPRI) has just released bEcon, a web-based bibliography including peer-reviewed applied economics literature that assesses the impacts of genetically modified (GM) crops in developing economies. The publications are organized into four main categories: the impacts of GM crops on farms, on consumers, on industries/sectors, and on international trade. For inclusion in the database, publications must focus on GM crops in developing countries, and be peer-reviewed journal articles, book chapters and published conference proceedings. Technical reports, working papers and discussion papers are considered if there is some indication of peer review. The database is updated every three months and a CD-ROM is produced annually.

See <http://www.ifpri.org/pubs/becon/beconabout.asp> or contact P.Zambrano@cgjar.org for more information or IFPRI-Library@cgjar.org to request CD-ROM copies of bEcon.

EXPERTS DETAIL HOW RICE ABSORBS SO MUCH ARSENIC

Reuters News Service - July 15, 2008

HONG KONG - Scientists in Japan may have discovered why rice absorbs so much arsenic from the soil, paving the way for fresh efforts to block the potentially harmful element from Asia's staple food.

In the Proceedings of the National Academy of Sciences, they said they had identified two proteins in rice plants that

appeared to transport arsenic from the soil to the grain.

Using mutant paddy that did not have these two proteins, the experts found sharply reduced levels of arsenic.

"We used mutant paddy (in which) these two transporters were knocked out and we saw decreased arsenite in both the stalk and rice grain," said Ma Jianfeng at Okayama University's Research Institute for Bioresources.

See the full article at: http://www.agbios.com/static/news/NEWSID_9797.php



The following paper was published recently and may be of interest to readers of the SABP newsletter.

FOOD AND AGRICULTURAL BIOTECHNOLOGY: A SUMMARY AND ANALYSIS OF ETHICAL CONCERNS

Advances in Biochemical Engineering/Biotechnology, Volume 11 'Food Biotechnology', pages 229 - 264

The range of social and ethical concerns that have been raised in connection with food and agricultural biotechnology is exceedingly broad. Many of these deal with risks and possible outcomes that are not unique to crops or animals developed using recombinant DNA. Food safety, animal welfare, socio-economic and environmental impacts, as well as shifts in power relations or access to technology raise concerns that might be generalized to many technologies. These aspects of the controversy over biotechnology are analyzed below as elements of general technological ethics, and key norms or values pertinent to each of these categories are specified in some detail. However, a number of special concerns unique to the use of rDNA in manipulating plant and animal genomes have been raised, and these are reviewed as well. The chapter concludes by reviewing two broad policy strategies for responding to the issues, one involving labels and consumer consent, the other applying the precautionary principle.

The full report can be purchased and downloaded at <http://springerlink.com/content/a0r2757353719612?p=94e5530738a447c5929a0d2083331b94&pi=5>



We welcome reader comments or suggestions. E-mail your letters to: nringma@agbios.com Mail your letters to: The Editor, SABP Newsletter, P.O. Box 475, Merrickville, Ontario, K0G 1N0 Canada

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