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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 national governmental agencies to facilitate the implementation of transparent, efficient and responsive regulatory frameworks for products of modern biotechnology that meet national goals as regards the safety of novel foods and feeds and environmental protection.

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 Bangladesh and address policy issues within the overall context of economic development, international trade, environmental safety and sustainability.

DESIGN CONSIDERATIONS FOR LABORATORY NON-TARGET STUDIES USED TO SUPPORT ENVIRONMENTAL RISK ASSESSMENT

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NON-TARGET RISK ASSESSMENT OF GE CROPS

Genetically engineered (GE) plants, and food and feed products derived from them, are strictly regulated by governments internationally. Environmental risk assessment of GE plants is designed to answer very specific questions about the potential risks of introducing such plants into the environment. Problem formulation directs the scope of risk assessment and defines explicit expressions of the environmental entities that are to be protected (termed assessment endpoints) against a potential stressor. A typical assessment endpoint that concerns non-target arthropods (NTAs) is "beneficial arthropod abundance." Problem formulation further generates testable scientific hypotheses and endpoints to measure (termed measurement endpoints) that are relevant for decision-making and are subsequently addressed in the analytical phase of the risk assessment (Raybould 2006; Wolt *et al.* 2010). Problem formulation should culminate in a conceptual model delineating how harm can occur by a particular stressor on the assessment endpoint, leading to an analysis plan that is consistent with the risk hypotheses and should establish the relationship between the stressor and the ecological impacts of concern. A typical risk hypothesis related to NTA effects of an arthropod-resistant GE plant is: "The expressed protein is not toxic to NTAs at the concentration present in the field" (Raybould 2007; Romeis *et al.* 2008). The risk hypotheses are then typically addressed following a tiered framework that is conceptually similar to that used to assess the environmental impact of conventional chemical plant protection products (Hill and Sendashonga 2003; Garcia-Alonso *et al.* 2006; Rose 2007; Romeis *et al.* 2008).

Based on the risk hypotheses, early-tier laboratory experiments are conducted under worst-case exposure conditions where species representative of NTAs present in the receiving environment that are likely to be exposed to the arthropod-active protein are exposed to concentrations of the protein in excess of exposure in the field. This increases the likelihood of detecting adverse effects on NTAs, if present. If no adverse effects are seen under these worst-case exposure conditions, the risk can be characterized as being acceptable and there may be no need to conduct any further testing because of the minimal probability of adverse effects in the field where NTAs are exposed to much lower concentrations of the arthropod-active protein. Early-tier testing thus allows elimination from further consideration risks that are negligible, and allows assessors to focus resources to address more significant risks or uncertainties.


Photo: M. Meissle (Agroscope ART)

The applicability of the tiered risk assessment framework to GE plants has become evident from the experience with GE crops expressing Cry proteins derived from *Bacillus thuringiensis*; a recent meta-analysis of published studies on non-target effects of such Bt crops has confirmed that laboratory studies "…predicted effects that were on average either more conservative than or consistent with effects measured in the field" (Duan *et al.* 2010).

LABORATORY STUDY DESIGN CONSIDERATIONS

Good study design is critical for early-tier laboratory studies because it contributes to the robustness of, and confidence in, environmental risk assessments for GE plants (Romeis *et al.* submitted). Good study design seeks to minimize the probability of erroneous results: false negatives – the failure to detect adverse effects of substances that are potentially harmful in the field, and false positives – the detection of adverse effects when the substance is unlikely to be harmful in the field. Erroneous results may arise if the conduct of the test introduces bias, or exposes the test NTAs to conditions that are significantly different from those under which the test is known to be reliable.

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DESIGN - *continued from page 1*

Early-tier laboratory studies should accurately determine the effects on surrogate non-target arthropods of known concentrations of the test substance. In most cases, the test substance will be a purified protein produced in microbial expression systems, or, alternatively, GE plant tissue.

Unbiased and reliable test systems reduce the probability of false positives and negatives by a combination of several test protocol design criteria:

a) The test substance must be well characterized and described. This includes the source and purity of the arthropod-active protein, and its stability and homogeneity in the carrier through which it is provided to the test organism.

b) The test substances must be biochemically and functionally equivalent to the protein or other active ingredient produced in the GE crop.

c) The bioactivity of the test substances, as provided to the test organisms, must be established (*e.g.*, in sensitive insect bioassays).

d) Test organisms should be exposed to high concentrations of the test substance relative to predicted exposures in the field (if possible) or dose-response studies should be performed.

e) Exposure of the test organisms to the test substance should be confirmed by, for example, use of a positive control and diet analysis to measure the concentration of the test substance.

f) Endpoints should be measured that are likely to indicate the possibility of adverse effects on the abundance of NTAs or other assessment endpoints. Risk assessors should agree on how to interpret and use these data in the risk assessment. Determination of the measurement endpoint(s) should consider the knowledge about the impact of the arthropod-active protein on the target organisms, knowledge about the biology of the selected NTA species and life-stages, and the availability of reliable test protocols.

g) A sufficient number of replicates need to be included in the study so that effects can be detected with a certain statistical power.

h) Negative control treatments must be included to assess the suitability of the test system, the organisms (*e.g.*, health) and the test conditions, and to evaluate potential effects of the matrix or formulation in which the test substance is delivered. Test results from assays with unacceptable high negative control mortality should be discarded.

Conclusions

Confidence in a conclusion of no adverse effect on a surrogate species, and confidence in extrapolating that conclusion to other species, depends upon the ability of the laboratory study to detect such effects. Thus emphasis is placed on attributes that reduce the likelihood of false negatives (criteria b-g above). Adhering to the principles and recommendations outlined in Romeis *et al.* (submitted) should increase confidence in the results of early-tier laboratory studies, and thereby reduce data requirements for stressors that pose low risk. The guidance provided should facilitate the reproduction of a study, peer review of such tests by others in the scientific community, and in general benefit regulatory authorities by enhancing the quality of information generated for use in risk assessments. High confidence in the results of early-tier laboratory studies is a precondition for the acceptance of data across regulatory jurisdictions and should encourage agencies to share useful information and thus avoid redundant testing.

This paper can be downloaded from http://cera-gmc.org/index.php?action=publications

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BANGLADESH PLANT TISSUE CULTURE & BIOTECHNOLOGY CONFERENCE

The 6th International Plant Tissue Culture & Biotechnology Conference was held December 3 to 5, 2010 at the Department of Botany, University of Dhaka, Bangladesh. It was organized by the Bangladesh Association for Plant Tissue Culture & Biotechnology (BAPTC&B) in collaboration with the Dhaka University, Bangladesh Agricultural Research Council (BARC), Ministry of Science and Information & Communication Technology and National Institute of Biotechnology (NIB) with many international and national organizations acting as sponsors and co-sponsors. Participants included 35 foreign and more than 200 local biotechnologists from universities, national agricultural research institutes, private and NGO sector biotech laboratories as well as policy makers.

With a theme of the "Role of Biotechnology in Food Security and Climate Change" the conference included paper presentations, scientific sessions and a poster session covering various aspects of plant biotechnology, biosafety, food security and climate change. South Asia Biosafety Program (SABP) sponsored the "Biosafety and Public Acceptance of Genetically Modified Plants" scientific session.

The inaugural ceremony included remarks from the Bangladesh Minister of Agriculture, Begum Matia Chowdhury, indicating the government's support of the safe introduction of biotechnology in agriculture, as well as remarks from the Vice Chancellor of the University of Dhaka and the Executive Chairman of BARC. The keynote address was provided by Dr. Swapan Datta, Deputy Director General of the Indian Council for Agricultural Research and, like previous remarks, focused on the need to increase production of food and feed while

(continued on page 4 - see Bangladesh)

The Reading List

. . . new and notable articles

CONSTITUTIVE EMISSION OF THE APHID ALARM PHEROMONE, (E)-BETA-FARNESENE, FROM PLANTS DOES NOT SERVE AS A DIRECT DEFENSE AGAINST APHIDS

Kunert G, Reinhold C, Gershenzon J.

BACKGROUND: The sesquiterpene, (E)-beta-farnesene (EBF), is the principal component of the alarm pheromone of many aphid species. Released when aphids are attacked by enemies, EBF leads aphids to undertake predator avoidance behaviors and to produce more winged offspring that can leave the plant. Many plants also release EBF as a volatile, and so it has been proposed that this compound could act to defend plants against aphid infestation by 1) deterring aphids from settling, 2) reducing aphid performance due to frequent interruption of feeding and 3) inducing the production of more winged offspring. Here we tested the costs and benefits of EBF as a defense against the green peach aphid, *Myzus persicae*, using transgenic Arabidopsis thaliana lines engineered to continuously emit EBF.

RESULTS: No metabolic costs of EBF synthesis could be detected in these plants as they showed no differences in growth or seed production from wild-type controls under two fertilizer regimes. Likewise, no evidence was found for the ability of EBF to directly defend the plant against aphids. EBF emission did not significantly repel winged or wingless morphs from settling on plants. Nor did EBF reduce aphid performance, measured as reproduction, or lead to an increase in the proportion of winged offspring.

CONCLUSIONS: The lack of any defensive effect of EBF in this study might be due to the fact that natural enemy attack on individual aphids leads to a pulsed emission, but the transgenic lines tested continuously produce EBF to which aphids may become habituated. Thus our results provide no support for the hypothesis that plant emission of the aphid alarm pheromone EBF is a direct defense against aphids. However, there is scattered evidence elsewhere in the literature suggesting that EBF emission might serve as an indirect defense by attracting aphid predators.

BMC Ecology (2010) 10(1):23. [Epub ahead of print]

IMPROVED FOREIGN GENE EXPRESSION IN PLANTS USING A VIRUS-ENCODED SUPPRESSOR OF RNA SILENCING MODIFIED TO BE DEVELOPMENTALLY HARMLESS

Saxena P, Hsieh YC, Alvarado VY, Sainsbury F, Saunders K, Lomonossoff GP, Scholthof HB.

Endeavours to obtain elevated and prolonged levels of foreign gene expression in plants are often hampered by the onset of RNA silencing that negatively affects target gene expression. Plant virus-encoded suppressors of RNA silencing are useful tools for counteracting silencing but their wide applicability in transgenic plants is limited because their expression often causes harmful developmental effects. We hypothesized that a previously characterized tombusvirus P19 mutant (P19/R43W), typified by reduced symptomatic effects while maintaining the ability to sequester short-interfering RNAs, could be used to suppress virus-induced RNA silencing without the concomitant developmental effects. To investigate this, transient expression in *Nicotiana benthamiana* was used to evaluate the ability of P19/R43W to enhance heterologous gene expression. Although less potent than wt-P19, P19/R43W was an effective suppressor when used to enhance protein expression from either a traditional T-DNA expression cassette or using the CPMV-HT expression system. Stable transformation of *N. benthamiana* yielded plants that expressed detectable levels of P19/R43W that was functional as a suppressor. Transgenic co-expression of green fluorescent protein (GFP) and P19/R43W also showed elevated accumulation of GFP compared with the levels found in the absence of a suppressor. In all cases, transgenic expression of P19/R43W caused no or minimal morphological defects and plants produced normal-looking flowers and fertile seed. We conclude that the expression of P19/R43W is developmentally harmless to plants while providing a suitable platform for transient or transgenic overexpression of value-added genes in plants with reduced hindrance by RNA silencing.

Plant Biotechnology Journal (2010) Nov 16. doi: 10.1111/j.1467-7652.2010.00574.x. [Epub ahead of print]

ADVANCES IN PLANT MOLECULAR FARMING

Obembe OO, Popoola JO, Leelavathi S, Reddy SV.

Plant molecular farming (PMF) is a new branch of plant biotechnology, where plants are engineered to produce recombinant pharmaceutical and industrial proteins in large quantities. As an emerging subdivision of the biopharmaceutical industry, PMF is still trying to gain comparable social acceptance as the already established production systems that produce these high valued proteins in microbial, yeast, or mammalian expression systems. This article reviews the various cost-effective technologies and strategies, which are being developed to improve yield and quality of the plant-derived pharmaceuticals, thereby making plant-based production system suitable alternatives to the existing systems. It also attempts to overview the different novel plant-derived pharmaceuticals and non-pharmaceutical protein products that are at various stages of clinical development or commercialization. It then discusses the biosafety and regulatory issues, which are crucial (if strictly adhered to) to eliminating potential health and environmental risks, which in turn is necessary to earning favorable public perception, thus ensuring the success of the industry.

Biotechnology Advances 2010 Nov 27. [Epub ahead of print]

CALENDAR OF EVENTS

Event	Organized by	Date and Venue	Website
INDIA			
TERI-ITEC Courses 2010-11: Applications of Biotechnology and its Regulation	The Energy and Resources Institute	January 3 – 21, 2011 Gurgaon	http://www.teriin.org/index.php?option=com_events&task=details&sid=302
Global Agricultural Biotechnology Summit 'Agbio 2011'	Tamil Nadu Agricultural University and Maryland India Business Round Table	January 31 – February 2, 2011	
ICPACC 2011: International Conference on Preparing Agriculture for Climate Change	Punjab Agricultural University	February 6 - 18, 2011 Ludhiana	http://www.pau.edu/int_conf/program.htm
3rd International Group Meeting on Wheat Productivity Enhancement Under Changing Climate	University of Agricultural Sciences, Dharwad and Directorate of Wheat Research, Karnal	February 9-12, 2011 UAS, Dharwad	http://www.uasd.edu/3rdIGM/wheatintmeet2.pdf
International Conference: Leveraging Agriculture for Improving Nutrition and Health	International Food Policy Research Institute (IFPRI)	February 10 - 12, 2011 New Delhi	http://www.ifpri.org/2020-agriculture-nutrition-health
Indian Seed Congress 2011	National Seed Association of India (NSAI)	February 22 - 23, 2011 Hyderabad	http://www.nsai.co.in/ISC_2011_Delegate.pdf
Bio Asia 2011: The Global Bio Business Forum	Government of Andhra Pradesh, Federation of Asian Biotech Associations, All India Biotech Association, University of Hyderabad	February 21-24, 2011, Hyderabad	http://www.bioasia.in/
South Africa – Argentina Joint Regional Biosafety Workshop and Seminar: Biosafety of GM Crops: Emerging Issues and Challenges Affecting Regulatory Decision Making	The International Centre for Genetic Engineering and Biotechnology in collaboration with the South African Department of Agriculture, Forestry and Fisheries & Argentinean Secretariat of Agriculture, Livestock and Fisheries (SAGyP)	March 7 - 11, 2011 Pretoria, South Africa	http://www.icgeb.org/tl_files/Meetings/2011/ICGEB%20Workshop%20SA%202011-%20Notification.pdf

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protecting the environment and dealing with the challenges of climate change and increased population.

There were nine scientific sessions covering the topics

- large scale production of different economically important crops through *in vitro* techniques
- conservation of biodiversity through biotechnology
- biosafety and public acceptance of genetically modified plant
- use of molecular markers in crop improvement
- development of plants resistant to biotic stress
- functional genomics and proteomics
- biotechnology in waste management
- plant genetic transformation and progress and prospect of commercialization of *in vitro* derived plants and value-added products

Five presentations were made at the SABP sponsored session. Presenters included Dr. Andrew Roberts, Deputy Director, Center for Environmental Risk Assessment (CERA), Dr. Vibha Ahuja, General Manager, Biotech Consortium India Ltd., Professor Imdadul Hoque, SABP Bangladesh Country Coordinator, Mr. Mohammed Solaiman Haider, Deputy Director, Department of Environment & Member Secretary, National Committee on Biosafety, Mr. Monzur Morshed Ahmed, SSO, Institute of Food Science & Technology, BCSIR Labs., Dhaka and Member of the Biosafety Core Committee. Topics included an introduction to biosafety and the South Asia Biosafety Program, an overview of SABP's activities in the development of biosafety regulatory regimes in India, the development of biosafety regimes in Bangladesh and the elements of the National Biosafety Framework of Bangladesh.

In his remarks at the conclusion of the conference Mr. Md. Abdur Rob Howlader, Secretary, Bangladesh Ministry of Science and Information & Communication Technology (ICT), pointed out the Government of Bangladesh's keenness to patronize research and development in the field of biotechnology. He disclosed that the government had already decided to establish a Biotechnology Cell under the Ministry of Science & ICT headed by a Joint Secretary. He assured that the Cell would soon start functioning. He also said his ministry had already taken steps to update the Science and Biotechnology Policies of Bangladesh and has been offering more fellowships in the field of biotechnology to attract more young researchers than before.

The conference ended with a vote of thanks offered by Prof. M. Imdadul Hoque, Secretary, Organizing Committee. A set of recommendations was adopted during the concluding ceremony.

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