



SOUTH ASIA
BIOSAFETY PROGRAM

August 2010

Vol.6 No.8

NEWSLETTER

for private circulation only - not for sale

www.cera-gmc.org

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.

LABELS FOR GM FOODS: WHAT CAN THEY DO?

Sangeeta Bansal, Jawaharlal Nehru University and Bharat Ramaswami, Indian Statistical Institute

Economic & Political Weekly Vol XLV Nos. 26 & 27: 167-173.

Labelling of genetically modified foods is a contentious issue and internationally there is sharp division on whether such labels ought to be mandatory. This debate has reached India where the government has proposed mandatory labels. Mandatory labelling aims to provide greater information and correspondingly more informed consumer choice. However, even without such laws, markets have incentives to supply labels. So can mandatory labelling achieve outcomes different from the voluntary type? The paper argues that this is not the case in most situations. It goes on to explore the special set of circumstances, where mandatory labels make a difference to outcomes. If these outcomes are intended, mandatory labelling is justified; otherwise not. Although the Indian context provides the motivation, the core arguments given are general and applicable to other country contexts as well.

The full paper can be found at http://cera-gmc.org/index.php?action=sabp_reports

SABP TRAINING WORKSHOP HELD IN BANGLADESH

The South Asia Biosafety Program (SABP) in collaboration with Bangladesh Agricultural Research Council (BARC) and Department of Environment (DOE) of Ministry of Environment and Forests (MOEF) organized a training workshop in Dhaka, Bangladesh on the Safety Assessment of Foods Derived from Genetically Engineered (GE) Plants from July 16 to 20, 2010. Thirty participants, selected from Bangladesh research institutions and government departments, attended the workshop.

The training workshop was conducted by five foreign Resource Persons, Prof. Florida Carino of University of the Philippines, Diliman; Dr. Vasanthi Siruguri, National Institute of Nutrition, India; Dr. Vibha Ahuja, Biotechnology Consortium India Limited; Dr. Donald MacKenzie, Pioneer Hi-Bred, USA; and Dr. Robert Potter, consultant to AGBIOS, Canada.

Inaugurated by Dr. Mihir Kanti Majumder, Secretary, Ministry of Environment and Forests, other guests and presenters included Dr. Wais Kabir, Executive Chairman, BARC; Mr. Md. Abdus Sobhan, Additional

Director General, Department of Environment (DOE); and Mr. M. Solaiman Haider, Deputy Director, DOE and Member Secretary, National Committee on Biosafety (NCB). Prof. Dr. M. Imdadul Hoque, Bangladesh country coordinator for SABP, outlined the development of biotechnology and biosafety regulatory regimes in Bangladesh and gave an account of SABP activities in Bangladesh in the development of biosafety regulatory documents as well as human resource development. Dr. Potter gave a brief overview of the workshop's agenda.

The overarching message in the inaugural ceremony was the hope GM technology would be one of the solutions to the challenge of the need for increased agricultural productivity on decreasing cultivable land in Bangladesh.

The workshop's five-day scientific program began with Dr. Vibha Ahuja's presentation on concepts and principles followed by the safety assessment of foods derived from genetically engineered plants and Dr. Potter's on the role of the risk assessor and the basics of safety assessment including host



Mr. Monowar Islam, Director General, DEO speaking certificate awarding ceremony. Seated on the dais (from left): Prof. F. Carino, Dr. D. MacKenzie, Dr. R. Potter, Dr. M.K.A. Chowdhury and Dr. V. Siriguri.

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WORKSHOP - continued from page 1

organism, donor organism, etc., as well as the risk assessment worksheet and the different methods of molecular characterization of GE events.

Day Two had presentations by Prof. Florida Carino who gave an introduction to the assessment of toxicity and allergenicity of novel proteins derived from GE plants. Dr. Carino also explained the use of bioinformatics in biotechnology: how to use the web-based search tools FASTA and BLAST and how to interpret results from bioinformatics searches. Hands-on exercises in performing bioinformatics analyses were carried out by the workshop participants.

Dr. Donald MacKenzie gave Day Two's final presentation on the expression of novel proteins in GE plants, in which he described different methods of protein analysis, namely, western blots and ELISA. He also described how to quantify protein levels in plants.

Prof. Carino began Day Three by explaining the methods for the direct assessment of potential toxicity of novel proteins produced by GE plants. In her presentation she covered experimental evidence for determining if a protein may be a toxin, including different types of animal testing.

Dr. Vasanthi Siruguri followed with a presentation on the assessment of allergenicity of novel proteins. Evidence from a range of tests was discussed, such as pepsin digestibility, heat stability of novel protein and its abundance, as well as the purpose and limitations of human serum testing and animal models for assessing potential allergenicity. Following some specific exercises completed by the participants, Dr. Potter introduced the case study materials on toxicity and allergenicity.

Day Four had Dr. MacKenzie giving several presentations on the compositional analysis of GE foods in which he covered the general objectives of compositional analysis, concepts of substantial equivalence, detection of unintended effects on key nutrients and anti-nutrients in common crop species, and the role and value of livestock feeding trials. Participants completed a number of hands-on exercises before continuing with the case study.

On the last day participants worked in groups on the case study of an application for approval of food derived from a specific GE plant. The groups prepared their individual decision making letters, which they then presented and discussed.

The workshop concluded with the awarding of certificates to the participants by Mr. Monowar Islam, Director General, Department of Environment (DOE).

BREAKING THE YIELD BARRIER IN RICE: FEASIBILITY OF ENGINEERING 'C₄ RICE'

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Currently almost half of the world's population feeds on rice. Due to increasing demand of rice for consumption, the global

rice production has been steadily increasing up from 126 million tonnes in 1961 to 661 million tonnes in 2008 through adoption of high yielding cultivars and advanced agronomic practices. Cultivation of rice is of immense importance to food security of Asia, where more than 90 per cent of the global rice is produced and consumed. The elite cultivars that dominate the food supplies of the millions of poor people in Asia have approached a yield barrier and rate of production growth is slowing. It is also becoming evident that the gains of the 'Green Revolution' are largely getting exhausted. At the same time, climate change will probably result in more extreme variations in weather with adverse effects on rice production. Hence, feeding the 5.6 billion Asians in the 21st century will require another 'Green Revolution' to boost yields by 50 per cent using less water and fertilizer. It is, therefore, more challenging than before to produce high rice yields with limited resources. Theoretical models have been used to examine this problem and they suggest that this can be done only by increasing photosynthetic efficiency.

Different types of photosynthetic pathways operate in plants. These include: (1) C₃ Calvin-Benson cycle, (2) C₄ Hatch and Slack pathway, and (3) Crassulacean Acid Metabolism (CAM) pathway. In case of C₃ plants, initial fixation of CO₂ is catalyzed by the enzyme RuBisco, while in C₄ and CAM plants, the initial fixation of CO₂ is mediated by the enzyme PEPC. The C₄ plants have evolved independently from C₃ plants at least 45 times. Since the C₃ plants lose considerable amount of fixed CO₂ in the form of photorespiration, nature evolved a mechanism of C₄ photosynthetic system to minimize photorespiratory loss of fixed CO₂ in crop plants like maize, sorghum, sugarcane, etc. The C₄ pathway is catalyzed by a set of enzymes which include: Carbonic anhydrase (CA), Phosphoenolpyruvate carboxylase (PEPC), NADP-malate dehydrogenase (MDH), NADP-malic enzyme (ME) and pyruvate orthophosphate dikinase (PPDK). The C₄

type of photosynthesis takes place in two cells types, i.e., mesophyll cells (MC) and bundle sheath cell (BS) that are morphologically distinct and arranged in concentric circles around veins. This type of arrangement of cells referred to as 'Kranz' anatomy is considered important for the prevention of photorespiration and hence for efficient photosynthetic CO₂ fixation of the atmospheric CO₂ in C₄ plants. Expression of photosynthetic genes encoding the enzymes required for catalysing the C₄ pathway reactions is restricted in a cell specific manner. For instance CA, PEPC, MDH and PPDK genes express in MC, whereas ME expression is restricted to the BS. Since C₄ plants are more productive than C₃ plants and also exhibit higher water and nitrogen use efficiency, efforts are in progress globally to introduce the C₄ pathway genes into rice, a C₃ plant.

Development of C₄ rice transgenics with C₄ genes, such as PEPC, PPDK, MDH and ME has revealed that C₄ genes can be expressed in rice, thereby proving the point that the machinery required for the faithful expression of C₄ genes does exist in MC of rice. In addition, it has been reported that genes encoding all the enzymes involved in C₄ pathway are present in C₃ plants. C₄ photosynthetic features are present in the green tissues around the vascular bundles and also in developing fruits in C₃ plants. On the contrary, patches with mesophyll cells not adjacent to a bundle sheath cell, have been reported particularly in leaf sheaths in C₄ maize. These observations suggest that C₃ and C₄ characteristics are present in a single plant and hence mechanisms to induce high levels of C₄ gene expression are operative in C₃ plants. Moreover, some plants are able to switch between C₃ and C₄ photosynthesis, indicating that the processes governing the



Participants during recent SABP training workshop in Bangladesh.

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The Reading List

. . . new and notable articles

DOES THE USE OF TRANSGENIC PLANTS DIMINISH OR PROMOTE BIODIVERSITY?

P.H. Raven

The protection of biodiversity and of ecosystem services ought to be a top priority, taken into consideration in the course of all human activities, because we depend on it fully now and for the future. In this context, we note that the ecological problems related to the cultivation of GE crops fail to differ in any fundamental way from the ecological problems associated with agriculture in general, except that they usually involve the application of much lower quantities of chemicals and thus tend to leave the environments in and adjacent to where they are grown in better condition than do the conventional ones. Higher productivity on cultivated lands, which is one outcome of growing GE crops, protects biodiversity by sparing lands not intensively cultivated, whereas relatively non-productive agriculture practised is highly destructive to biodiversity, since it consumes more land in an often destructive way, even though more biodiversity may be preserved among the crops themselves than in industrialized, large fields, especially if hedgerows and woodlands are not encouraged in near proximity. The major preservation of biodiversity, however, does not take place among crops! If weeds are present that are closely related to the crops, they may acquire immunity to the effects from which the crops were protected and be more difficult to control among them. The production of superweeds as a result of hybridization between cultivated crops and their wild relatives is essentially a myth. The definition of 'organic' production in the U.S. and elsewhere unjustifiably rules out GE crops, often in such a way as to damage the environment more than would be the case otherwise. Unless the definition of 'organic' is a problem, or close relatives to the crops are weedy among them, there seems to be essentially no ecological risk involved in growing GE crops.

Nature Biotechnology. 2010 Jul 30. [Epub ahead of print]

D-GLUFOSINATE AS A MALE STERILITY AGENT FOR HYBRID SEED PRODUCTION

T. Hawkes, W. Pline-Srnic, R. Dale, E. Friend, T. Hollinshead, P. Howe, P. Thompson, R. Viner, A. Greenland.

Summary A chemical male sterility system based on anther-localized conversion of the inactive d-enantiomer of the herbicide, glufosinate (2-amino-4-(methylphosphinyl)-butanoate) to the phytotoxic l is described. Highly pure d-glufosinate was isolated in >98% enantiomeric excess from the racemate via fermentation with a strain of *Escherichia coli* expressing the PAT (l-glufosinate N-acetyl transferase) gene and purification of the unreacted d-enantiomer from the broth by ion exchange. A modified (F58K, M213S) form of the d-amino acid oxidase (DAAO) (EC 1.4.3.3) from *Rhodospiridium toruloides* was designed, tested *in vitro* and found to efficiently oxidize d-glufosinate to its 2-oxo derivative [2-oxo-4-(methylphosphinyl)-butanoic acid]. Tobacco (*Nicotiana tabacum*) plants were transformed to express this modified oxidase under control of the TAP1 tapetum-specific promoter. A number of the resultant transgenic lines

exhibited complete male sterility that persisted for two or more weeks immediately following foliar treatment with 75 or 200 g/ha of d-glufosinate without exhibiting obvious phytotoxic symptoms or any measurable decline in female fertility. Similarly, plants containing the same construct and, additionally, a PAT gene expressed from a plastocyanin promoter exhibited significantly reduced male fertility and no reduction in female fertility following foliar application of racemic glufosinate. Thus, foliar application of d-glufosinate either purified or as the commercial herbicide, combined with anther expression of a modified DAAO promises to provide a cost-effective conditional chemical male sterility system with the characteristics necessary for practical F(1) hybrid seed production.

Plant Biotechnology Journal 2010 Jul 30. [Epub ahead of print]

DISTANCES NEEDED TO LIMIT CROSS-FERTILIZATION BETWEEN GM AND CONVENTIONAL MAIZE IN EUROPE

L. Riesgo, F.J. Areal, O. Sanvido, E. Rodríguez-Cerezo E

To avoid the economic consequences of admixtures of genetically modified (GM) and non-GM harvests, and to ensure that agricultural production complies with mandatory labeling provisions, the European Union (EU; Brussels) member states have adopted co-existence measures directed to farmers cultivating GM varieties. For GM maize cultivation, regulators have established mandatory isolation distances, which differ between countries and in some cases have been regarded as disproportionate. Taking advantage of numerous field studies conducted by EU researchers in recent years, we report here a statistical analysis of crossfertilization data in maize, showing that separating fields 40 m is sufficient to keep GM adventitious presence below the legal labeling threshold in the EU set at 0.9%.

Nature Biotechnology (2010) 28(8): 780-782

GENE TRANSFER INTO SOLANUM TUBEROSUM VIA RHIZOBIUM SPP.

T. Wendt, F. Doohan, D. Winckelmann and E. Mullins

Agrobacterium tumefaciens-mediated transformation (ATMT) is the preferred technique for gene transfer into crops. A major disadvantage of the technology remains the complexity of the patent landscape that surrounds ATMT which restricts its use for commercial applications. An alternative system has been described (Broothaerts *et al.* in *Nature* 433:629-633, 2005) detailing the propensity of three rhizobia to transform the model crop *Arabidopsis thaliana*, the non-food crop *Nicotiana tabacum* and, at a very low frequency, the monocotyledonous crop *Oryza sativa*. In this report we describe for the first time the genetic transformation of *Solanum tuberosum* using the non-*Agrobacterium* species *Sinorhizobium meliloti*, *Rhizobium* sp. NGR234 and *Mesorhizobium loti*. This was achieved by combining an optimal bacterium and host co-cultivation period with a low antibiotic regime during the callus and shoot induction stages. Using this optimized protocol the transformation frequency (calculated as % of shoots equipped with root systems with the ability to grow

(continued on page 4 - see Reading List)

CALENDAR OF EVENTS

| Event | Organized by | Date and Venue | Website |
|---|--|--|---|
| INDIA | | | |
| Final evaluation workshop on India Agricultural Biotechnology Training Program | United States Trade and Development Agency | September 1, 2010 New Delhi | |
| National Conference on Paradigm Shift in Cotton Research and Cultivation | Navsari Agricultural University | October 20 - 22, 2010 | http://www.nau.in/n.pdf |
| World Cotton Research Conference-5 | International Cotton Advisory Committee (ICAC) and Indian Society for Cotton Improvement | November 7 - 11, 2010 Mumbai | http://www.icac.org/meetings/wcrc/wcrc5/wcrc5_brochure.pdf |
| ICAR Winter School on Molecular Techniques in Gene Isolation and Characterization | National Research Centre on Plant Biotechnology | November 8 - 28, 2010 | http://www.nrpcb.org/brochure_icar_winter_school.pdf |
| INTERNATIONAL | | | |
| ABIC 2010: Bridging Biology and Business | Agricultural Biotechnology International Conference | September 12 - 15, 2010 Saskatoon, Canada | http://www.abic.ca/abic2010/ |
| IBS 2010 - 14th International Biotechnology Symposium and Exhibition | Alma Mater Studiorum - University of Bologna, ADRIA CONGREX and Elsevier | September 14 - 18, 2010 Rimini, Italy | http://www.ibs2010.org/index.asp |
| An Introduction to the Risk Analysis of Current Genetically Modified Organisms (GMOs) and their Products, and to Possible Issues Raised by Novel GMOs in the Future | Biosafety Unit, International Centre for Genetic Engineering and Biotechnology (ICGEB) | September 27 - October 1, 2010 Trieste, Italy | http://www.icgeb.org/meetings-and-courses.html |
| 11th International Symposium on the Biosafety of Genetically Modified Organisms (ISBGMO) | International Society for Biosafety Research | November 15 - 20, 2010 Buenos, Argentina | http://www.isbgmo.info/ |
| BIT's 4th Annual World Congress of GENE-2010: Gene Technology, Environment and Economic Growth | BIT Life Sciences, Inc. | December 1 - 4, 2010 Sanshui, Foshan, China | http://www.bitlifesciences.com/wcg2010/fullprogram.asp |
| 6th International Plant Tissue Culture & Biotechnology Conference | Bangladesh Association for Plant Tissue Culture & Biotechnology (BAPTC&B) | December 3 - 5, 2010 | http://www.bap tcb.org/ptcb_Conference_2010.pdf |

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function of these apparently complex systems must be flexible. In addition to C_3 and C_4 , there are a number of genera that contain C_3 - C_4 intermediate species. The characteristics of these intermediates have been used to hypothesise the sequence of events that occurred as C_4 plants evolved.

Rice is a C_3 plant and a potential target for C_4 pathway engineering. It has been estimated that, the sink is much larger than required for C_3 rice and the evidence suggests that another 50 per cent of the juvenile spikelets could be converted into grains by engineering C_4 pathway in rice (C_4 rice). In contrast to the C_3 rice, C_4 rice will expectedly have higher yield, reduced water loss and increased nitrogen use efficiency, particularly when grown in hot and dry environments. Rice with C_4 photosynthesis could make a major contribution to a second 'Green Revolution'. As mentioned above, all the C_4 genes for C_4 photosynthesis already exist in the rice plant. However, a systematic analysis needs to be carried out to investigate comprehensively the level of expression of the C_4 genes in C_3 plants. Such a study to determine the level of expression of the already existing C_4 genes under optimal as well as in response to different abiotic stress conditions in rice may provide clues to devise strategies for engineering a C_4 rice. Hence, it seems to be an achievable target to tinker with the genes present in rice and tame them to behave like the real C_4 genes as in C_4 maize or sorghum for breaking the yield barrier in rice.

READING LIST - continued from page 3

in rooting media supplemented with 25 µg/ml hygromycin) of the rhizobia strains was calculated at 4.72, 5.85 and

1.86% for *S. meliloti*, R. sp. NGR234 and *M. loti* respectively, compared to 47.6% for the *A. tumefaciens* control. Stable transgene integration and expression was confirmed via southern hybridisation, quantitative PCR analysis and histochemical screening of both leaf and/or tuber tissue. In light of the rapid advances in potato genomics, combined with the sequencing of the potato genome, the ability of alternative bacteria species to genetically transform this major food crop will provide a novel resource to the Solanaceae community as it continues to develop potato as both a food and non-food crop.

Transgenic Research DOI: 10.1007/s11248-010-9423-4

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