



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 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.

OVERVIEW OF TRANSGENIC MUSTARD IN INDIA

Prof. Deepak Pental, Vice Chancellor, University of Delhi

Oilseed mustard (*Brassica juncea*), is grown in the rabi season in the dryland areas of north western parts of India. It is the second most important oilseed crop after groundnut with an acreage of 6.33 mha and production of 73.3 lakh tonnes during the year 2008-2009. (Source: Economic survey of India, Ministry of Finance, 2008-2009). Oil extracted from seeds of rapeseed-mustard is extensively used as a cooking medium and the seed meal is used as an animal feed. The use of its leaves and tender stems is popular in north Indian cuisine as a vegetable.

In spite of significant economic importance of the crop, yield of mustard has been more or less stagnating for the last 25 years. India continues to remain one of the major importers of the edible vegetable oil, with an annual import of 86.6 lakh tonnes during the year 2008-2009. One of the major constraints limiting the productivity of mustard crop is the lack of pollination control system to develop hybrids. Although, some attempts have been made in the recent past to develop cytoplasmic male sterility/restorer (CMS) system, only limited success has been achieved.



Brassica juncea wild mustard

Genetic engineering-based techniques have been successfully used to develop perfect male sterility/restorer system worldwide leading to significant increases in productivity. Transgenic technology of male sterility/restorer system approved by the regulatory authorities is being widely used in *B. napus* (rapeseed) in Canada, USA and Australia.

To meet the national need of higher productivity in mustard, Centre for Genetic Manipulation of Crop Plants (CGMCP), University of Delhi South Campus (UDSC), initiated work on the development of pollination control mechanism using genetic engineering techniques in *B. juncea* in the year 1994. With the intensive research efforts over a period of 15 years, UDSC has now successfully developed the male sterility and fertility restorer system using two genes viz. *barnase* and *barstar* for pollination control and demonstrated its on-field efficacy for heterosis breeding in *B. juncea*. As regards the R & D achievements, a ribonuclease gene, *barnase*, under the control of tapetum specific promoter TA29 has been used to obtain male sterile plants. A method was developed to achieve high frequency generation of male sterile lines by using an insulator that separates the herbicide resistance conferring gene from the *barnase* gene to achieve precise expression of the latter. Both national and international patents have been obtained for this construct. Similarly, *barstar* gene has been introduced into another line for fertility restoration in the hybrids. The two lines, one male sterile with the *barnase* gene and the other containing fertility restoration *barstar* gene, have been crossed and fully fertile F1 hybrid, i.e. DMH-11 [Varuna (*barnase*) X EH2 (*barstar*)] has been obtained containing both the *barnase* and the *barstar* genes.

The *barstar/barnase* genes express only in the tapetum tissue that surrounds developing micropores/pollen. In the hybrid the effect of the *barnase* gene is negated by *barstar* protein by formation of a *barstar-barnase* complex. As a consequence hybrids are fertile with viable pollen and set seeds. The *bar* gene derived from *Streptomyces hygroscopicus*, encoding an enzyme phosphinothricin acetyl transferase (PAT), which detoxifies the compound DL-Phosphinothricin (PPT), the active compound of the herbicide Basta, by acetylation has been used as a marker gene in both the *barnase* and the *barstar* lines. The expression of the *bar* gene is not intended for herbicide tolerance and will be used only for selection of *barnase* lines during the seed production.

The results of this work have been published in peer reviewed national and international journals including "Molecular Breeding" in 2001 and "Current Science" in 2002. The use of hybrid seed helps in significant increase in the yield. In a series of greenhouse and field evaluations, the transgenic mustard hybrid DMH-11 developed by UDSC using *barnase/barstar* has been reported to give 20 to 30 per cent yield increase over commercially cultivated varieties.

Increase in mustard production is a national priority in view of increase in demand (expected to be 240 lakh tonnes by the year 2020) and there is an urgent

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CALENDAR OF EVENTS

Event	Organized by	Date and Venue	Website
INDIA			
A practical training course on Techniques in Plant Tissue Culture, Genetic Engineering and Molecular Biology	CCS Haryana Agricultural University, Hisar, Haryana	May 12 – June 23, 2010 Hisar	http://hau.ernet.in/cobs/bmbtraining3_2010.pdf
Bangalore India Bio 2010	Department of Information Technology and Biotechnology, Government of Karnataka	June 2 – 4, 2010 Bangalore	http://www.bangalorebio.in/BIO2010/index.php
TERI-ITEC Courses 2010-11 - Applications of Biotechnology and its Regulation	The Energy and Resources Institute (TERI)	August 2 - 22, 2010 Gurgaon	http://www.teriin.org/index.php?option=com_events&task=details&sid=307
Biosafety Course on Introduction to Risk Analysis of GMO	Kuwait Institute for Scientific Research	April 25 - 29, 2010 Safat, Kuwait	http://www.icgeb.org/meetings-and-courses.html
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/

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need for attaining self sufficiency in edible oils production which can be achieved through the use of hybrids. Hybrids based on the barnase/barstar system in *B. napus* are being extensively grown in Canada. The system developed by CGMCP needs to be deregulated in India. There is an urgent need to bring in more clarity in the regulatory requirements for generation of required data on environmental safety and food and feed safety so that the hybrid DMH-11 based on transgenic technologies can reach the farmers.

PLANT BIOTECHNOLOGY RESEARCH ACTIVITIES AT BCSIR LABORATORIES, DHAKA

Dr. Shahina Islam, Head, Plant Tissue Culture Section, Biological Research Division, BCSIR

The plant tissue culture section of Bangladesh Council for Scientific and Industrial Research (BCSIR) began doing research on plant tissue culture techniques in 1993. Since then it has developed into one of Bangladesh's most well-established. Its scientists focus their efforts on plant regeneration and multiplication from tissue culture. The main objectives of the research are:

- Establishment of micropropagation protocols for important medicinal plants, fast-growing fuel wood trees, orchids, ornamental flowers, fruits, vegetables, natural dye producing and timber plants.
- Introduction of genetic transformation to improve plants.
- Conducting training programs on basic tissue culture and commercial tissue culture techniques.
- Establishment of tissue culture based industries for commercial production.

Past research has included:

Horticulture Biotechnology: *In vitro* propagation and breeding of *Citrus macroptera*, *Citrus grandis*, *Citrus aurantium*, *Musa sapientum* *Malus pumila*, year round *Artocarpus heterophyllus*, *Lycopersicon esculantum* and *Solanum tuberosum*,

Tree Biotechnology: *In vitro* propagation and breeding of *Sesbania grandiflora*, *Erythrina variegata*, *Syzygium cumini*

***In Vitro* Propagation and Breeding of Ornamental Plants:** Chrysanthemums, Roses, Orchids

***In Vitro* Technology for Medicinal Plants:** *Azadiracta indica*, *Rauvolfia serpentina*, *Moringa oleifera*, *Aegle mermilos*, *Centella asiatic*, *Adhotoda basica*.

Molecular Biology Research: Detection of potato viruses and viroids through molecular methods.

Present research activities include:

- DNA fingerprinting technology for variety validation of cultured plants.
- Molecular markers for fingerprinting and breeding of ornamental plants.
- Breeding of new ornamental plants through development of advanced technologies such as genetic transformation.
- Application of DNA markers for studying genetic variability in fruit plants.
- Application of RT-PCR and real time PCR for detection and identification of plant viruses and viroids.

Ongoing research includes:

***Bixa* (*Bixa orellana* L.):** North America and Europe import *Bixa* dye from this South American plant to use as a food coloring for margarine, cheese, microwave popcorn and other yellow or orange foodstuffs. In Bangladesh this could reduce the use of chemical dyes that are harmful for human health that are used in foodstuffs. Commercial planting and production

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of Bixa dyes would provide enough for use in food products. Imported food colour could be reduced and natural dye production industries could be set up in Bangladesh. *Research progress:* Field trial for cultivation of Bixa has been completed and the "Development of a cultivation technology of *Bixa orellana* L" process was accepted in 2007. Research continues to analyse the dye and to develop an appropriate technique for dye collection from the seed pulps.

Stevia: *Stevia rebaudiana* is a plant with carbohydrate-based compounds; the main sweet carbohydrate is called stevioside, which is 200-300 times sweeter than sugar. Stevia is a natural sweetener that contains no calories. Stevia does not affect blood sugar levels because it has no calcium cyclamate, no saccharine, no aspartame nor does it have the neurological or renal side effects of some artificial sweeteners. Many global soft drink manufacturers have not exploited this herbal sweetener. The beverage industry can blend



Bixa orellana seed pods



Stevia leaves with tea and coffee which adds value while easing the preparation of the drink.

Centella: *Centella asiatica* (L) Urban is a member of the family Apiaceae. It is used as a tonic in the treatment of leprosy, asthma, bronchitis dropsy, leucorrhoea, skin disease and urethritis. Tissue culture for rapid multiplication of elite clones and germplasm conservation is crucial to exploiting its medicinal properties as a stable supply of its bioactive secondary product has become a priority. The present study is an attempt to develop a method for rapid multiplication of the species by shoot tip and stolon culture to meet the requirements of the pharmaceutical industry.

Strawberry: The common garden strawberry is a plant of the genus *Fragaria*, which is cultivated worldwide for its fruit. Artificial strawberry aroma is widely used in industrialized food products. Developing methods for rapid, efficient and large scale multiplication of strawberry through tissue culture has great commercial potential.

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The Reading List

... new and notable articles

DECOMPOSITION DYNAMICS AND STRUCTURAL PLANT COMPONENTS OF GENETICALLY MODIFIED Bt MAIZE LEAVES DO NOT DIFFER FROM LEAVES OF CONVENTIONAL HYBRIDS

C. Zurbrügg, L. Hönemann, M. Meissle, J. Romeis and W. Nentwig

The cultivation of genetically modified Bt maize has raised environmental concerns, as large amounts of plant residues remain in the field and may negatively impact the soil ecosystem. In a field experiment, decomposition of leaf residues from three genetically modified (two expressing the Cry1Ab, one the Cry3Bb1 protein) and six non-transgenic hybrids (the three corresponding non-transformed near-isolines and three conventional hybrids) was investigated using litterbags. To elucidate the mechanisms that cause differences in plant decomposition, structural plant components (i.e., C:N ratio, lignin, cellulose, hemicellulose) were examined. Furthermore, Cry1Ab and Cry3Bb1 protein concentrations in maize leaf residues were measured from harvest to the next growing season. While leaf residue decomposition in transgenic and non-transgenic plants was similar, differences among conventional cultivars were evident. Similarly, plant components among conventional hybrids differed more than between transgenic and non-transgenic hybrids. Moreover, differences in senescent plant material collected directly from plants were larger than after exposure to soil for 5 months. While the concentration of Cry3Bb1 was higher in senescent maize leaves than that of Cry1Ab, degradation was faster, indicating that Cry3Bb1 has a shorter persistence in plant residues. As decomposition patterns of Bt-transgenic maize were shown to be well within the range of common conventional hybrids, there is no indication of ecologically relevant, adverse effects on the activity of the decomposer community.

Transgenic Research (2010) 19(2):257-67

BIODEGRADATION OF GENETICALLY MODIFIED SEEDS AND PLANT TISSUES DURING COMPOSTING

T. Reuter, T.W. Alexander, W. Xu, K. Stanford and T.A. McAllister

The increasing global market of genetically modified (GM) crops amplifies the potential for unintentional contamination of food and feed with GM plants. Methods proposed for disposal of crop residues should be assessed to prevent unintended distribution of GM materials. Composting of organic material is inexpensive and location-independent. The objective of this study was to determine the effectiveness of composting for disposal of GM plants in terms of reducing seed viability and promoting the degradation of endogenous as well as transgenic DNA. RESULTS: Duplicate samples of corn kernels, alfalfa leaves, and GM canola seeds, meal and pellets were sealed in porous nylon bags and implanted in duplicate 85 000 kg (initial weight) feedlot manure compost piles. Samples were collected at intervals over 230 days of composting. Canola seeds and corn kernels were not viable after 14 days of composting with temperatures in the piles exceeding 50 degrees C. In all samples, PCR analyses revealed that plant endogenous and transgenic fragments were substantially degraded after 230 days of composting. Southern blotting of genomic DNA isolated from canola seeds identified differences in the persistence of endogenous, transgenic, and bacterial DNA. CONCLUSION: Composting GM and non-GM plant materials with manure rendered seeds non-viable, and resulted in substantial, although not complete, degradation of endogenous and transgenic plant DNA. This study demonstrates that composting could be effective for disposing of GM crops in the event of their inadvertent entry into the food or feed chain.

Journal of the Science of Food and Agriculture (2010) 90(4):650-7

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Orchids: With colorful and long lasting blooms orchids are popular and unique gifts. Bangladesh has some hardy, attractive and colourful indigenous varieties that were collected from the Dhaka Botanical Garden, which are being regenerated through tissue culture for commercial purposes.

The Tissue Culture Section is presently constrained by inadequate laboratory and power generation facilities and personnel with appropriate training to carry out or assist biotechnological research needs to be increased. It would like to pursue collaborative research with national and international research institutes/universities with a focus on:

- DNA fingerprinting technology for purity analysis of cultured plant molecular markers;
- genetic transformation of important crops and plants;
- application of DNA markers to studying genetic variability in fruit plants; and
- application of RT-PCR and Real Time PCR for detection and identification of plant viruses and viroids.

BANGLADESH SEMINAR ON BIOTECHNOLOGY IN AGRICULTURE AND HUMAN WELFARE

Bangladesh Association for Plant Tissue Culture and Biotechnology (BAPTC&B) organized a seminar on the application of biotechnology in agriculture and human welfare at the University of Dhaka on March 29, 2010. The invited speakers included Prof. Hans-Jörg Jacobsen from the Institute of Plant Genetics, Leibniz University of Hannover, Germany who presented his paper on Agricultural Biotechnology vs Organic Farming and Current Practices in the Developing Countries, and Prof. Alan McHughen from University of California (Riverside) presented his paper on Agricultural Biotechnology: A Worldwide Perspective.



Prof. Dr. Syed Hadiuzzaman, President, BAPTC&B welcoming the speakers and the participants. Guest speakers (from left), Prof. Dr. Hans-Jörg Jacobsen, Prof. Dr. Alan McHughen, Prof. Dr. Abul Hassan, Chairman, Department of Botany, Dhaka University

After giving an overview of current and projected human population, food shortages and global soil, water and air pollution Dr. McHughen warned that to feed the ever increasing population current agricultural practice is unsustainable. He questioned how to safely increase food supplies with fewer land, water and other resources, without causing additional environmental damage in an ethically sound and sustainable manner. He advocated for the judicious application of genetic modification (GM) to contribute to a 'better', more sustainable and ethically sound human future. He clarified this by saying 'judicious' meant recognizing that GM, like any tool, might be potentially harmful if misused.

Dr. McHughen went on to describe how GM technology could be used to make agriculture more sustainable through increased water use efficiency; losses due to insects, diseases and spoilage; reduced harvest loss; removal of allergens, toxins and antinutrients; and enhanced nutrient profiles. While recognizing that the main technical risks with GM arise from the food and feed safety health threat and the potential danger of ecological damage he stated there has been NO verified documentation of any harm to either health or the environment because of GM crops and foods.

He concluded by acknowledging that GM technology may serve objectives of environmental, agronomic, and economic sustainability but is not a panacea nor can it be categorically rejected or ignored.

Prof. Jacobsen also began his talk by outlining the dim future of food security. He then went on to summarize some recent contradictory reports about the role organic farming can play in addressing increasing food requirements.

He pointed out that GM crops have provided notable successes, which have increased optimism amongst farmers and other food producers. Losses due to various biotic stresses, production costs are lower and in future, by using drought resistant crops it has been estimated that water efficiency can be substantially increased. Prof. Jacobsen cited Bt brinjal as an example noting that it could provide:

- Better quality thus more profit to farmer.
- More production per acre.
- Less use of pesticides therefore fewer farmer inputs and less accumulation of toxins in the fruits. GM crop production does not pose a risk for the accumulation of Mycotoxin as it does in organic farming.

As proof of the impact of GM crops Prof. Jacobsen noted the acceptance of GM crops by farmers is increasing.

He then posed the question that if GM crops promise such sustainable agriculture then why was Golden rice still not under cultivation even after ten years of its development. He surmised that public perception needs to change. Bangladesh's citizens need to accept that to ensure adequate food and food security the country must explore all the tools to increase its food production.

Both presentations generated many questions from the audience which comprised about 200 scientists, faculty and students of various research institutions and public and private universities.

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