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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.

SUGARCANE AS A PLATFORM FOR THE PRODUCTION OF HIGH VALUE PROTEIN MOLECULES: A PROMISING STRATEGY IN BIOPHARMING

N. Subramonian and D.P. Syamaladevi, Crop Improvement Division, Sugarcane Breeding Institute, Coimbatore

The application of GM crops as production platforms for different substances with industrial uses, particularly biopharmaceuticals, has many advantages like production cost savings; the possibility of large-scale production of drugs - free from animal and human viruses; and easier storage and transportation without refrigeration (in seed form).

Plant molecular farming applications can be broadly classified as plant made pharmaceuticals (PMPs), plant made vaccines (PMVs) and plant made industrials (PMIs). Since the first report on the application of plant genetic engineering for the production of novel protein molecules in 1989, this technology has developed and diversified rapidly. The concept behind the use of plants as production platform for diverse biomolecules is the product of combining the production technology of bio-pharmaceuticals with that of agricultural biotechnology. The gene/s coding for the protein of interest is expressed in crops that can be cultivated on a large scale with ease and, subsequently, the protein is extracted and purified.

Various plant production platforms have been developed for molecular farming in plants under green house or confined open field production, including leafy crops (alfalfa, lettuce, *Arabidopsis*, spinach, tobacco) cereals and legumes (barley, maize, pea, pigeon pea, rice, wheat); fruits and vegetables (banana, carrot, potato, tomato, carrots); oil-yielding plants (false of flax, flax, rape, safflower, soybean, white clover, white mustard); and sugar crops (sugar beet).

Each crop species has its advantages and disadvantages as platforms for molecular farming. For example, leafy crops produce a large biomass. As the proteins are synthesized in an aqueous environment, there is a likelihood of degradation through proteolysis after the harvest and hence the need to be transported in frozen conditions to the processing units. In addition, the transgene may interfere with the normal plant metabolism. However, some of these species are found to be excellent platforms for the production of vaccines and antibodies through transient expression.

In seed crops like cereals and grain legumes the proteins can be targeted toward seeds and can be stored at room temperature without degradation allowing long-term storage and also transportability. This also facilitates harvested product batch processing. Fruits and vegetables are being used as platforms for oral vaccine production. With oil seeds the proteins can be targeted to the oil bodies for further downstream processing. Compounds traditionally synthesized from petrochemicals have been produced in field crops like maize and tobacco.

Sugarcane, a monocot C4 plant, has many significant advantages that make it an ideal platform for the production of high value molecules as listed below.

1. One of the highest biomass producers.
2. Mean yield of 40 tons per acre (40,000 canes of 1 kilogram each).
3. One kilogram of cane yields 600 to 650 ml of juice. Hence, 24,000 litres of juice from one acre.
4. Sugarcane juice has a relatively negligible amount of protein (around 0.04 per cent), hence it would be heterologous proteins expressed in juice are simpler to purify.

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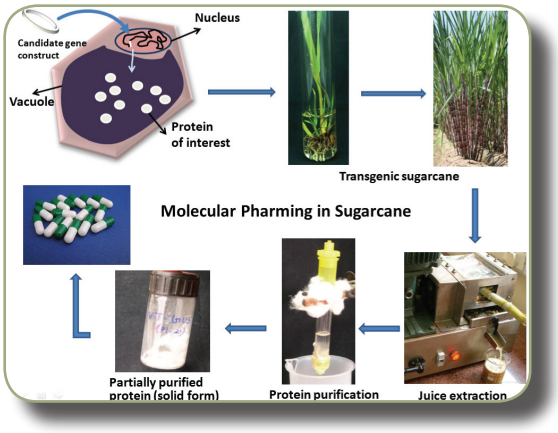
Biopharming - *continued from page 1*

5. Biosafety issues are relatively low because, a) commercially grown sugarcane is vegetatively propagated and sexual seeds are not used for commercial cultivation; b) it does not flower in many parts of the country and, even if it flowers, it does not set seeds; c) no reports of pollen transfer from cultivated sugarcane to its wild relatives; hence, no chance of transgene introgression to wild *Saccharum* sps; d) no weediness observed as sugarcane is vegetatively propagated and requires ideal conditions for establishment and growth; e) cane harvesting before flowering is possible.
6. Morphologically distinct varieties are possible for biofarming requirements (specific variety for production of different products) thus eliminating the risk of cross-contaminationwith commercially available varieties.
7. Due to vegetative propagation, transgenes could be fixed in the first generation and only the expression of transgene stability needs to be assessed in the preceding vegetative generation.
8. Large storage vacuole makes it possible to store even proteins that are phyto-toxic in isolation from other metabolic pathways.



9. Transgenic sugarcane can be grown in isolation without much of a buffer zone or refugea, unlike other seed propagated crops, thus reducing the cost of cultivation.
10. As sugarcane can be grown under diverse agroclimatic conditions it can be grown near the processing units
11. Grown year round in many parts of the country, an assured supply of raw material is possible.
12. Raw sugarcane juice is palatable therefore direct delivery of vaccines or nutricuticles is an option.

However, there could be a few issues that need attention.

1. Low pH (around 5.0) in sugarcane juice could denature certain proteins.
2. Correct required protein glycosylation needs to be confirmed for the desired activity.
3. Codon optimization of the genes for plant expression.

At the Sugarcane Breeding Institute, Coimbatore we have developed a technology that expresses and directs recombinant protein to the vacuoles of the stem parenchyma cells, which is the sugar storage site. With the use of an in-house developed promoter for higher expression in sugarcane clump (stem) and a vacuole localizing signal, we have generated transgenics that express GUS and GFP. Our experiments have shown that GUS with his-tag could be isolated and partially purified from sugarcane juice without any loss of enzymatic activity and, in some selected events, the protein yield (70 per cent purity) was as high as 1 mg per ml of juice. Easy extraction and low protein juice content make downstream processing easier. With a definite advantage over the other crops, sugarcane holds promise as a new candidate crop for biopharming.

GLOBAL STATUS OF BIOTECH/GM CROPS 2012 AND ITS CONTRIBUTION TO FOOD SECURITY IN BANGLADESH

A day-long seminar on the Global Status of Biotech/GM Crops 2012 and its Contribution to Food Security in Bangladesh, jointly organized by the Bangladesh Association for Biotechnology and Genetic Engineering (BABGE) and International Service for the Acquisition of Agri-biotech Applications (ISAAA), was held at the Bangladesh Agricultural University (BAU), Mymensingh on March 25, 2013. The seminar was inaugurated by Prof. Dr. Md. Rafiqul Hoque, Vice Chancellor, BAU, chaired by Prof. Dr. Md. Abdul Khaleque Patwary, Dean, Faculty of Agriculture, BAU, and the welcoming address was given by Prof. Dr. Md. Shahidul Haque, Former Head, Department of Genetic Engineering and Biotechnology, BAU.

Prof. Dr. K.M. Nasiruddin, Country Coordinator, ISAAA; Prof. Dr. M. Imdadul Hoque, Dean, Faculty of Biological Sciences, University of Dhaka and Country Coordinator, South Asia Biosafety Program (SABP); and Dr. G.P. Das, Country Coordinator, Agricultural Biotechnology Support Program II (ABSP II) made presentations. About 100 participants including BAU faculty, post-graduate students and print and electronic media journalists attended.

Dr. Nasiruddin spoke about Clive James's most recent ISAAA publication "Global Status of Commercialized Biotech/GM Crops: 2012". He summarized the report saying that in 2012 a record 170.3 million hectares of biotech crops were grown globally in 28 countries, 20 of which were developing, the remaining 8 were industrialized. He pointed out, according to ISAAA Briefs, two new countries, Sudan and Cuba, planted Bt cotton and Bt maize respectively, for the first time in 2012. Prof. Nasiruddin also reported that, at 52 per cent, for the first time, developing countries grew more global biotech crops in 2012 than industrial countries, who grew 48 per cent.

Dr. Das spoke about the progress of ABSP II including field trials of GMOs in Bangladesh. He highlighted the status of field trials of fruit and shoot borer resistant Bt eggplant and late blight resistant (LBR) potato, which have been carried out, with promising results, at different sub-stations of Bangladesh Agricultural Research Institute (BARI) following the standard biosafety guidelines. Dr. Das said ABSP II is going to launch a new project on salinity and drought tolerant rice at the Bangladesh Rice Research Institute (BRRI).

Prof. Imdadul Hoque presented a paper on Biosafety and Biotech Promotion in Bangladesh. He highlighted the development of biosafety regimes in Bangladesh and he described the obligations of the Cartagena Protocol and the need to develop Biosafety Guidelines and Biosafety Rules of Bangladesh. He described the scope of Biosafety Guidelines of Bangladesh and the functions of the relevant committees, including the National Committee on Biosafety (NCB) and the Biosafety Core Committee (BCC), as well as the role of Institutional Biosafety Committees (IBCs) and Field Level Biosafety Committee. He also pointed out the purpose of the development of the National Biosafety Framework (NBF) of Bangladesh including the main elements of NBF as well as the administrative structures of NBF and the GM crop approval process currently being practiced in Bangladesh.

The seminar was followed by a lively discussion that included many questions from participating journalists about the benefits versus potential risks of GM crops. Invited speakers and some expert audience members replied to the questions as well as taking part in the discussion.

The Reading List

. . . new and notable articles

BRINGING A TRANSGENIC CROP TO MARKET—WHERE COMPOSITIONAL ANALYSIS FITS

Privalle LS, Gillikin N, Wandelt C

In the process of developing a biotech product, thousands of genes and transformation events are evaluated to select the event that will be commercialized. The ideal event is identified based upon multiple characteristics including: trait efficacy, the molecular characteristics of the insert, and agronomic performance. Once selected, the commercial event is subjected to a rigorous safety evaluation taking a multipronged approach including examination of the safety of the gene, and gene product - the protein, plant performance, impact of cultivating the crop on the environment, agronomic performance, and equivalence of the crop/food to conventional crops/food by compositional analysis. The compositional analysis is comprised of a comparison of the nutrient and anti-nutrient composition of the crop containing the event, its parental line (variety) and other conventional lines (varieties). Different geographies have different requirements for the compositional analysis studies. Parameters that vary include the number of years (seasons) and locations (environments) to be evaluated; the appropriate comparator(s); analytes to be evaluated; and statistical analysis. Specific examples of compositional analysis results will be presented.

Journal of agricultural and food chemistry. 2013 Mar 27. [Epub ahead of print] See: http://pubs.acs.org/doi/abs/10.1021/jf400185q?prevsearch=bringing%2Ba%2Btransgenic%2Bcrop%2Bto%2Bmarket&searchhistorykey=

CYSTEINE PROTEASE ENHANCES PLANT-MEDIATED BOLLWORM RNA INTERFERENCE

Mao YB, Xue XY, Tao XY, Yang CQ, Wang LJ, Chen XY

Oral ingestion of plant-expressed double stranded RNA (dsRNA) triggers target gene suppression in insect. An important step of this process is the transmission of dsRNA from plant to midgut cells. Insect peritrophic matrix (PM) presents a barrier that prevents large molecules from entering midgut cells. Here, we show that uptake of plant cysteine proteases, such as GhCP1 from cotton (*Gossypium hirsutum*) and AtCP2 from Arabidopsis, by cotton bollworm (Helicoverpa armigera) larvae resulted in attenuating the PM. When GhCP1 or AtCP2 pre-fed larvae were transferred to gossypol-containing diet, the bollworm accumulated higher content of gossypol in midgut. Larvae previously ingested GhCP1 or AtCP2 were more susceptible to infection by Dendrolimus punctatus cytoplasmic polyhedrosis virus (DpCPV), a dsRNA virus. Furthermore, the pre-fed larvae exhibited enhanced RNAi effects after ingestion of the dsRNA-expressing plant. The bollworm P450 gene CYP6AE14 is involved in the larval tolerance to gossypol; cotton plants producing dsRNA of CYP6AE14 (dsCYP6AE14) were more resistant to bollworm feeding (Mao *et al*. in *Transgenic Research* 20:665-673, 2011). We found that cotton plants harboring both 35S:dsCYP6AE14 and 35S:GhCP1 were better protected from bollworm than either of the single-transgene lines. Our results demonstrate that plant cysteine proteases, which have the activity of increasing PM permeability, can be used to improve the plant-mediated RNAi against herbivorous insects.

Plant molecular biology 2013 Mar 4. [Epub ahead of print] See: http://link.springer.com/article/10.1007%2Fs11103-013-0030-7

EXPLOITING PLANT VIRUS-DERIVED COMPONENTS TO ACHIEVE IN PLANTA EXPRESSION AND FOR TEMPLATES FOR SYNTHETIC BIOLOGY APPLICATIONS

Saunders K, Lomonossoff GP

This review discusses the varying roles that have been played by many plant-viral regulatory sequences and proteins in the creation of plant-based expression systems and virus particles for use in nanotechnology. Essentially, there are two ways of expressing an exogenous protein: the creation of transgenic plants possessing a stably integrated gene construction, or the transient expression of the desired gene following the infiltration of the gene construct. Both depend on disarmed strains of *Agrobacterium tumefaciens* to deliver the created gene construction into cell nuclei, usually through the deployment of virus-derived components. The importance of efficient mRNA translation in the latter process is highlighted. Plant viruses replicate to sustain an infection to promote their survival. The major product of this, the virus particle, is finding increasing roles in the emerging field of bionanotechnology. One of the major products of plant-viral expression is the virus-like particle (VLP). These are increasingly playing a role in vaccine development. Similarly, many VLPs are suitable for the investigation of the many facets of the emerging field of synthetic biology, which encompasses the design and construction of new biological functions and systems not found in nature. Genetic and chemical modifications to plant-generated VLPs serve as ideal starter templates for many downstream synthetic biology applications.

New phytologist. 2013 Mar 4. doi: 10.1111/nph.12204. [Epub ahead of print] See: http://onlinelibrary.wiley.com/doi/10.1111/nph.12204/full

UPDATE ON THE USE OF TRANSGENIC RICE SEEDS IN ORAL IMMUNOTHERAPY

Takaiwa F

Rice seed provides an ideal production platform for pharmaceuticals in terms of high productivity and stability, as well as the scalability, safety and economy that are expected in plant production systems. Furthermore, these therapeutic products are bioencapsulated in protein bodies, which are seed-specific storage organelles that provide protection from digestion by gastrointestinal enzymes during delivery to the gut-associated lymphoid tissue. Thus, rice seed

(continued on page 4 - see Reading List)

CALENDAR OF EVENTS

Event	Organized by	Date and Venue	Website
INDIA			
National Workshop on Strategic Intellectual Property Management for Agriculture	Andhra Pradesh Technology Development & Promotion Centre	April 25 - 27, 2013 Hyderabad	https://www.mycii.in/image/eventimages/eventmainimages/E000014735_Brochure%2015.2.13.pdf
International Consultation on Molecular Genetics: Science, Technology, Regulation	M.S. Swaminathan Research Foundation	April 29 - May 1, 2013 Chennai	http://www.mssrf.org
Agricultural Graduate Student Conference 2013	Tamil Nadu Agricultural University, Coimbatore	May 2 - 3, 2013 Coimbatore	http://tnau.ac.in/agsm2013/
Workshop on Taking Forward Herbicide Tolerant GM Crops: Opportunities and Challenges	Biotech Consortium India Limited (BCIL) and Indian Society of Weed Science	May 2, 2013 New Delhi	
AP-TEC 2013@TIRUPATI	Confederation of Indian Industry	June 6 - 8, 2013 Tirupati, Chittoor, Andhra Pradesh	http://ow.ly/hOefY
XIII National Seed Seminar on Innovations in Seed Research and Development	Indian Society of Seed Technology and University of Agricultural Sciences, Bangalore	June 8 - 10, 2013 Bangalore	http://www.iari.res.in/
INTERNATIONAL			
2013 BIO International Convention	Biotechnology Industry Organization (BIO)	April 22 - 25, 2013 Chicago, Illinois, USA	http://convention.bio.org/
BIT's 6th Annual World Congress of Industrial Biotechnology (IBIO-2013)	Information Research Center of International Talent, SAFEA and China Medicinal Biotech Association	April 25 - 27, 2013 Nanjing, China	http://www.bitlifesciences.com/ibio2013/
World Biotechnology Congress (WBC 2013)	Eureka Conference	June 3 – 6, 2013 Boston, Massachusetts USA	http://www.worldbiotechcongress.com/confprog.htm
Strategic Approaches in the Evaluation of the Science Underpinning GMO Regulatory Decision Making	ICGEB	July 1 - 5, 2013 Trieste, Italy	http://www.icgeb.org/tl_files/Meetings/2013/TS_BIOSAFETY_1-5%20July_2013_Rev7Feb2013.pdf
Biosafety: An International Short Course in Environmental Aspects of Agricultural Biotechnology	Michigan State University College of Agriculture and Natural Resources in Collaboration with the Plant Breeding and Genetics Program	August 4 – 9, 2013 East Lansing, Michigan, USA	http://worldtap.msu.edu/short-courses/biosafety/
International Conference on Genetic Engineering and Genetically Modified Organisms	OMICS Group	August 12 - 14, 2013 Raleigh, North Carolina, USA	http://www.omicsgroup.com/conferences/genetic-engineering-genetically-modified-organisms-2013/

READING LIST - *continued from page 3*

provides an ideal delivery system for the mucosal immune system. Oral immunotherapy using unprocessed transgenic rice seed containing therapeutic products has been demonstrated to induce effective mucosal immune tolerance and immune reactions against allergies and pathogens.

IMMUNOTHERAPY. (2013). 5(3):301-12. DOI: 10.2217/IMT.13.4. SEE: HTTP://ONLINELIBRARY.WILEY.COM/DOI/10.1111/PBI.12007/ABSTRACT

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