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South Asia Biosafety Program

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BANGLADESH

Advancing Agricultural Technology to Ensure Sustainable Agriculture in South Asia

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The 3rd Annual South Asia Biosafety Conference was held on September 19-20, at the BRAC Centre Inn in Dhaka, Bangladesh. This conference gave a platform to scientists of the South Asian region to discuss the use of modern biotechnology and advanced transgenic research on agriculture, livestock, forestry, human health and food production. Without using biotechnology there is little hope to ensure food security for the ever growing world population. Therefore, scientists have dedicated themselves to develop stress tolerant, nutritionally enhanced, disease resistant, and high yielding varieties of different genetically modified (GM) crops, in instances where traditional breeding has failed. Evaluation of such new varieties of GM crops is important to ensure safety of both humans and the environment.

There is an urgent need for the advancement of agricultural technology to ensure sustainable agricultural production in the South Asian region to face the major challenges like food security and protecting the environment. Biotechnology has become one of the prominent options to overcome this problem. Currently, genomics, recombinant gene technologies and genetic engineering are some of the tools used to improve crop tolerance for abiotic and biotic stresses as well as nutritional quality of food by biofortification. They are also used for introducing new traits to make crops resistant to pests and to break yield barriers. Consequently, GM crops could contribute to increasing food production and higher food availability in this region.

Some GM crops like *Bt* cotton have already been released in India and Pakistan, which is the first step of introducing biotechnology in the SAARC region. Other GM crops like brinjal, castor, groundnut, mustard, papaya, potato, rice, rubber, sugarcane, wheat, maize, soybean, chickpea, sorghum and tomato are in the pipeline for release in the near future. However, a section of the region still hesitates to take GM crops because of their lack of scientific knowledge. The safety of GM crops must be ensured to dispel fears that it would hamper human health or biodiversity. To assess and minimize the potential risk of GM crops, specific protocols and strong laws have already been prepared, many of which were discussed at this conference. Such activities will both ensure biosafety and encourage people to accept GM crops. The most recent updates on biosafety regulation for GM crops and how to prepare regulatory documents for submission of newly produced transgenic crops, animals and insects in South Asian countries were shared during this conference.

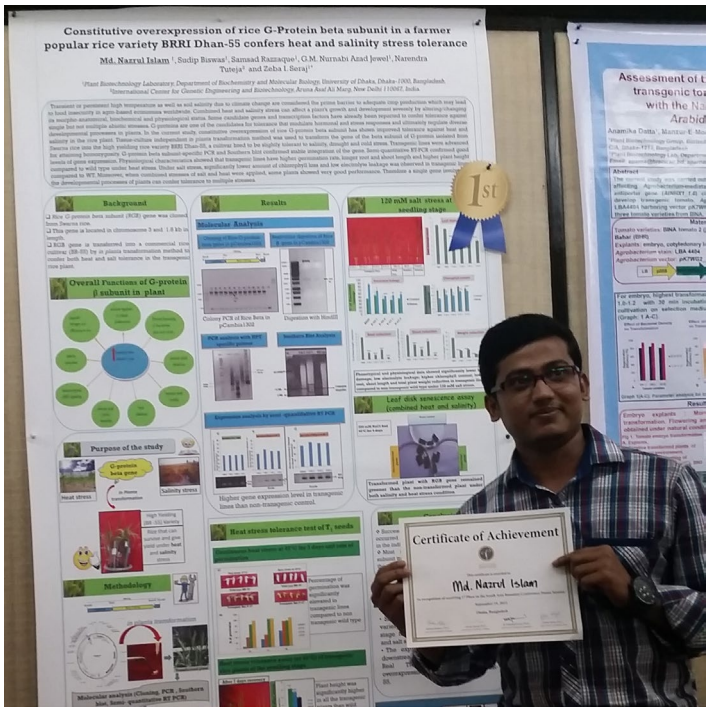
Depending on the specific transgenes introduced, the development of GM varieties may indirectly affect the diversity of other organisms and can cause emergence of secondary pests, the resistance to pesticides and development of super weeds. Therefore, management practices have also been developed so that these adverse effects do not occur. Knowledge about such practices is available on the OECD and SABP websites (www.oecd.org and www.cera-gmc.org/sabp). Further research on the impact of the transgenics already released will show any possible reduction in biodiversity.

Establishment of appropriate policy and legislation for GM crop production at both national and regional levels is at different stages of preparation and implementation in the various Southeast Asian countries. The law enforcement system will define how to process the requests for authorization as well as the mechanism for monitoring and promoting GM crops. Also, guidelines must be prepared and followed strictly for production, internal transport and import of GM crops.

"We hope that by bridging the information gap between the public and harmonizing the research and regulatory processes, transgenic research will show us the way of achieving food sufficiency."

Although *Bt* cotton is already widely accepted, unfortunately the release of *Bt* brinjal is stuck in the dilemma of government bureaucracy. The exception is in Bangladesh, where it has recently been released on a limited scale. We hope that by bridging the information gap between the public and harmonizing the research and regulatory processes, transgenic research will show us the way of achieving food sufficiency. It is our expectation that such conferences, like the South Asia Biosafety Conference, will shorten our time to achieve the desired goal.

Spotlight on the 1st Place Winner of the South Asia Biosafety Conference Poster Session



The South Asia Biosafety Program (SABP) is pleased to announce its inaugural Poster Competition, which took place during the South Asia Biosafety Conference in September. All posters were eligible for the competition, and two prizes were awarded. First prize consisted of a two year membership for the International Society of Biosafety Research, a USD $100 cash prize, and an award certificate. Second prize consisted of a two year membership for the International Society of Biosafety Research, a USD $50 cash prize, and an award certificate.

The posters were judged on clarity and correlation of the abstract to the poster; scientific content and quality of the research; and display, organization and effective use of space.

Congratulations to Md. Nazrul Islam and his team for winning 1st place for the poster on Constitutive Overexpression of Rice G-Protein Beta Subunit in a Farmer Popular Rice Variety BRRI DHAN-55 Confers Heat and Salinity Stress Tolerance.

1st PLACE POSTER WINNER: Md. Nazrul Islam

POSTER CO-ORGANIZERS: Sudip Biswas, Samsad Razzaque, G.M. Nurnabi Azad Jewel, Narendra Tuteja and Zeba I. Seraj

POSTER TITLE: *Constitutive Overexpression of Rice G-Protein Beta Subunit in a Farmer Popular Rice Variety BRRI DHAN-55 Confers Heat and Salinity Stress Tolerance*

POSTER ABSTRACT: Transient or persistent high temperatures as well as soil salinity due to climate change is considered the prime barrier to adequate crop production which may lead to food insecurity in agro-based economies worldwide. Combined heat and salinity stress can affect a plant's growth and development severely by altering/changing its morphoanatomical, biochemical and physiological status. Some candidate genes and transcription factors have already been reported to confer tolerance against single but not multiple abiotic stresses. G-proteins are one of the candidates for tolerance that modulate hormonal and stress responses and ultimately regulate diverse developmental processes in plants. In the current study, constitutive overexpression of rice G-protein beta subunit has shown improved tolerance against heat and salinity in the rice plant. Tissue-culture independent in planta transformation method was used to transform the gene of the beta subunit of G-protein isolated from Swarna rice into the high yielding rice variety BRRI Dhan 55, a cultivar bred to be slightly tolerant to salinity, drought and cold stress. Transgenic lines were advanced for attaining homozygosity. G-protein beta subunit-specific PCR and Southern blot confirmed stable integration of the gene. Semi-quantitative RT-PCR confirmed good levels of gene expression. Physiological characteristics showed that transgenic lines have higher germination rate, longer root and shoot length and higher plant height compared to wild type under heat stress. Under salt stress, significantly lower amount of chlorophyll loss and low electrolyte leakage was observed in transgenic lines compared to WT. Moreover, when combined stresses of salt and heat were applied, some plants showed very good performance. Therefore a single gene involved in the developmental processes of plants can confer tolerance to multiple stresses.

BANGLADESH

Training for Researchers, Officers and Laboratory Staff on the Biosafety of *Bt* Cotton

DR. MD. FARID UDDIN, EXECUTIVE DIRECTOR AND MR. MD. AKHTERUZZAMAN, ADDITIONAL DIRECTOR, COTTON DEVELOPMENT BOARD, DHAKA

Bangladesh has a glorious tradition of cotton and textile production. Before independence, the raw cotton requirement of the local textile industry was met from West Pakistan. The importance of local production of cotton was felt after the independence of Bangladesh in 1971, when the supply of raw cotton was suspended from Pakistan. The textile industry of Bangladesh faced serious problems because of the lack of supply of raw cotton. Due to these circumstances, the Cotton Development Board (CDB) was established under the Ministry of Agriculture in 1972 to promote cotton production in the country. CDB started functioning during 1974-1975 and began growing American Upland Cotton (*Gossypium hirsutum*) on an experimental basis. An extensive program of Upland Cotton production was taken up in 1976-1977 with the introduction of a new variety from the United States of America. The responsibility of cotton research was transferred from the Bangladesh Agriculture Research Institute (BARI) to CDB in 1991.

Since this transfer of responsibility, CDB has been conducting research on different aspects of cotton production. The main focus of cotton research in Bangladesh includes development of hybrid and short duration high yielding cotton varieties with desirable fiber characteristics, agronomic management technologies to increase productivity, integrated management of organic and inorganic fertilizers to improve soil fertility, and identification of bio-pesticide in controlling cotton insect pest and cotton disease management. Additionally, research on stress management has been prioritized to expand cotton cultivation in the hill, char, saline and drought areas combining traditional knowledge and skill with biotechnology tools.

Cotton hybrids have been cultivated in Bangladesh for a couple of years through a public-private partnership for cotton research and development in Bangladesh. Within this initiative, the Supreme Seed Company Ltd. has imported hybrid cotton seed from China. CDB has conducted research on adaptability and generating production technologies. Cotton hybrids were widely accepted by the farmers due to its high yielding capacity (3-4 t/ha seed cotton).

Recently, the National Committee on Biosafety (NCB) approved conducting contained trials of *Bt* hybrid cotton in July 2015 at the Sreepur Research Farm. With the permission of the Ministry of Agriculture, a material transfer agreement has been signed between CDB and the Hubei Provincial Seed Group Company Ltd. in China to make *Bt* hybrid seed for contained trials available. The ultimate goal of CDB will be to make *Bt* hybrid cotton seeds available to the farmers in the near future after proper biosafety assessments. CDB, in collaboration with BARI, has successfully managed its first contained greenhouse trial of *Bt* cotton planted on July 13, 2015.

Acroynm Guide

BARI	Bangladesh Agriculture Research Institute
BRRI	Bangladesh Rice Research Institute
BSO	Biological Safety Officer
CDB	Cotton Development Board
IBC	Institutional Biosafety Committee
NCB	National Committee on Biosafety

As part of the capacity building activities of CDB, two training workshops on biosafety and risk management in *Bt* cotton research were held at the CDB Headquarters located at Khamarbari, Farmgate, Dhaka. The events were organized by the *Bt* Cotton Gene Identification and Efficacy Determination Research Programme of the CDB, and funded by the Ministry of Agriculture.

The first training was held from September 29-October 3, 2015 for researchers and officers. The second training was held October 3-5 for laboratory and field assistants. The training focused on the biosafety regulatory process in relation to the ongoing *Bt* cotton contained greenhouse trials. The training also included building biosafety administration and communication capacity for the members of the Institutional Biosafety Committee (IBC), the Biological Safety Officer (BSO), the *Bt* cotton working group scientists and staffs of CDB. About 20 participants were selected from the CDB of Bangladesh to attend. During these workshops, participants were introduced to:

- Dimensions of cotton research in Bangladesh
- Bangladesh government policy on biotech crops
- Programme implementation, monitoring and reporting
- Agricultural biotechnology in Bangladesh
- Current status of *Bt* cotton research in Bangladesh
- Cartagena protocol on biosafety
- Biosafety guidelines of Bangladesh
- Biotechnology risk management
- Safe work procedures
- Biotechnology risk management: record keeping and risk reporting
- Progress of biotech crop research in Bangladesh
- A journey to *Bt* brinjal release in Bangladesh

Continued on page 4

Continued from page 3

- Contained trial data requirement for the National Biosafety Committee
- *Bt* transgenic crop for cotton bollworm management
- Bioassay technique for *Bt* cotton
- Target and non-target pests
- Steps of biotech crop release in Bangladesh: role and responsibilities of IBC
- Purpose of contained and confined field trials of transgenic crops
- Integrated management of cotton pests
- Resistance management of *Bt* cotton
- Biotechnology for the expansion of cotton in stress areas
- Biosafety issues in contained and confined trials
- Biosafety issues in laboratory experiment
- CDB work plan in *Bt* cotton release
- Future direction of biotech cotton research in Bangladesh

Among the nationals experts on biosafety regulatory authorities of Bangladesh, Prof. Dr. M. Imdadul Hoque, University of Dhaka and South Asia Biosafety Program Country Coodinator, Mr. Mohmmed Solaiman Haider, Department of Environment, Dr. M. Enamul Haque, BRRI, Dr. Partho Sarothi Biswas, BRRI, Dr. Syed Nurul Alam, BARI, and Dr. Md. Aziz Zilani Chowdhury, BARC, provided valuable training to the participants on biosafety issues about contained trials of *Bt* cotton.

The inaugural ceremony of these training events was presided by Dr. Md. Farid Uddin, the Executive Director of CDB. Dr. Abul Kalam Azad, the Executive Chairman of the Bangladesh Agricultural Research Council inaugurated the workshops as the Chief Guest. The concluding session was chaired by Mr. Md. Akhteruzzaman, Additional Director. Dr. Md. Tasdiqur Rahman, Deputy Director and Dr. Md. Kamrul Islam, Program Director of CDB, were present as Special Guests.

Overall, these two events provided an important platform for the interaction between CDB researchers, officers and staff with national experts on biosafety regulatory authorities of Bangladesh to review the progress made and to identify opportunities for strengthening its capacity in broad areas of biosafety in agricultural biotechnology. These training events successfully created a link among the national experts, regulators and scientists. It was very helpful to identify the need in safety and efficacy assessment as well as to speed up the commercialization process.

The events underlined the need for continued engagement and backstopping by partners as Bangladesh moves forward to testing and adopting safe and effective genetically modified *Bt* cotton. During the training, a visit was arranged to see the facility and work activities on contained greenhouse trials at BRRI in Joydebpur, Gazipur.

Commercialization of Genetically Modified Crop and Conflict Among the Experts: The Case of *Bt* Brinjal in Bangladesh

DR. AMIR AHMED, SENIOR RESEARCH ASSOCIATE, ENVIROWATER SYDNEY, AUSTRALIA

Commercialization of *Bt* brinjal, the first genetically modified (GM) crop in Bangladesh encountered huge criticism from a section of green groups and environmentalists despite its enormous agronomic potentials claimed by the scientist and biotechnology industry. In order to address the underlying reasons of this controversy and identify the domains of conflict among various stakeholders, an investigation was carried out involving experts in Bangladesh. This qualitative research incorporates findings from 35 interview data analysed using Nvivo. The findings reveal that experts are, in general, positive towards the introduction of *Bt* brinjal in the agricultural system of Bangladesh because of its proven agronomic advantages over conventional varieties.

A considerably small segment of experts, mostly the supporters of GM-free farming, is skeptical regarding the approval of any GM crop. This skepticism seems to be linked with a distrust of the regulations of GM organisms (GMOs). Reassurance of strict GMOs regulation by the authority may gradually improve the situation. Issues relating to GMOs regulation and trade-related economic and political threats are more contentious components of the GM debate in Bangladesh compared to the threat of biosafety and health risk. Fear of export loss and dominance of foreign companies over the seed market appeared less significant and unrealistic as these are based on assumption rather than rational justifications. It gives an impression that the rumour of *Bt* brinjal failure reported by local media was ostensibly a manoeuvre of particular interest groups. The result also indicates that a considerable doubt exists among the experts about proper labelling of *Bt* brinjal at farm and retail level. Mandatory labelling would be more effective and meaningful in tandem with mass consumer awareness about the benefit of GM crops.

Type of risk and its magnitude would be the determinant factors to answer whether or not benefits of *Bt* brinjal would outweigh the possible risk of GMOs if any such risk comes into view in the future. The analysis also recommends that the government should take initiatives to invite all interested parties, including representatives of private organisations on the same platform to attain a shared understanding about the biosafety framework, regulatory actions and risk management measures of GM crops in Bangladesh. The findings of the study would provide significant insight to the policy planners, researchers and biotechnology industries to comprehend various aspects of the conflicts and help them to find out possible cooperations for fostering sustainable commercialisation of GM crops in Bangladesh.

For more information about this pending journal article, please contact Dr. Ahmed at aaff1273@gmail.com.

PAKISTAN

Nutrition Management May Enhance the Durability of *Bt* Cotton Against Insect Pests

DR. SHAUKAT ALI, PRINCIPAL SCIENTIFIC OFFICER, DR. SIFFAT ULLAH KHAN, RESEARCH FELLOW AND DR. G.M. ALI, CHIEF SCIENTIFIC OFFICER, NATIONAL INSTITUTE FOR GENOMICS & ADVANCED BIOTECHNOLOGY (NIGAB), NATIONAL AGRICULTURAL RESEARCH CENTRE (NARC), ISLAMABAD

Cotton (*Gossypium hirsutum* L.) is one of the major cash crops of Pakistan and serves as a backbone for our country economy. Globally, Pakistan occupies the fourth position among the top five cotton producing countries. Cotton is susceptible to attack from more than fifteen economically key pests, in particular Lepidoptera pests.

The efficacy of *Bt* cotton is dependent upon the expression of Cry genes through the synthesis of insecticidal protein that remains inconsistent throughout the plant life cycle. To address the challenge of resistance development in targeted insect pest complexes, the functional characterization of *Bt* genes is vital and demands better understanding in the context of variations in efficiency of *Bt* genes and their mechanism.

This report scrutinizes the quantitative estimation of the *Cry1Ac* toxin content in Pakistani *Bt* cotton under the influence of various doses of N-P fertilizers. Three local *Bt* cotton varieties (MNH-886, FH-113, *Bt*-121) were evaluated to investigate the expression of the *Cry1Ac* toxin under various fertilizer levels at two different growth stages, specifically 80 and 120 days after sowing (DAS).

The pot experiment was conducted in complete randomized design (CRD) using three replications. Nine different levels of fertilizer treatments formed by a combination of 3 levels of nitrogen fertilizer (50, 100 and 150 kg ha^{-1}) along with three levels of phosphorus fertilizer (25, 50 and 75 kg ha^{-1}) were applied as shown in figures 1 and 2. Urea (46% N) and DAP (46% P + 18% N) were used as the sources of N and P fertilizers respectively.

Quantification of the *Cry1Ac* encoded toxin was carried out through sandwich ELISA at 80 and 120 DAS following the instructions of the kit manufacturer (Envirologix Inc. USA).

At 80 DAS, leaf samples were collected from each plant treated with a specific dose of NP fertilizers along with a control, which was no treatment. Results revealed that the *Cry1Ac* toxin level ranged from 0.6912-0.8829 µg/g in the control, while the level of *Cry1Ac* toxin under the influence of various fertilizer levels ranged from 0.8279-2.5038 µg/g in all the three tested varieties. The highest toxin expression level (2.5038 µg/g) was obtained at fertilizer level F_9 ($N_{150}P_{75}$ kg ha^{-1}) followed by fertilizers level F_8 ($N_{100}P_{75}$ kg ha^{-1}) (1.9575 µg/g) and F_6 ($N_{150}P_{50}$ kg ha^{-1}) (1.8742 µg/g), while the lowest mean of toxin level (1.0260 µg/g) was obtained at fertilizer level F_1 ($N_{50}P_{25}$ kg ha^{-1}) as shown in the figure 1.

Leaf samples were recollected from each specified plant and the toxin level was quantified by sandwich ELISA at 120 DAS. A significant reduction in the toxin level was noticed as compared to 80 DAS (figure 2). The *Cry1Ac* toxin level ranged from 0.5810-0.7518 µg/g in control, while the *Cry1Ac* gene expression under the influence of various fertilizer levels ranged from 0.7068-2.3083 µg/g (fresh tissue weight) in all the three tested varieties. At this stage, again the highest treatments mean (2.3083 µg/g) was obtained at fertilizers level F_9 ($N_{150}P_{75}$ kg ha^{-1}) followed by fertilizer level F_8 ($N_{100}P_{75}$ kg ha^{-1}) (1.7052 µg/g) and F_6 ($N_{100}P_{75}$ kg ha^{-1}) (1.7087 µg/g), while the lowest mean of toxin level (0.8511 µg/g) was obtained at fertilizer level F_1 ($N_{50}P_{25}$ kg ha^{-1})as shown in figure 2. Among the three tested varieties, the highest *Cry1Ac* toxin level was found in variety FH-113 followed by *Bt*-121 and MNH-886.

"In order to achieve the *Bt* toxin level for durable plant resistance (>1.5) at an economically feasible, the NP fertilizer rate should be shifted slightly from the recommended dose."

Statistical analysis revealed significant differences in *Bt* toxins expression under the influence of variable doses of NP fertilizer treatments and also among varieties. The treatments and varieties were significantly different from one another at $p \leq 0.001$ while interaction between varieties and treatments were significant at $p \leq 0.05$ at both stages (80 and 120 DAS) of analysis.

The expression level of the *Cry1Ac* toxin increases with the gradual increase in the level of fertilizers indicating that NP fertilizers have significant positive impact on *Cry1Ac* gene expression in transgenic *Bt* cotton. Based on overall results, it is suggested that in order to achieve economically feasible levels of the Bt toxin for durable plant resistance (>1.5) at an economically feasible, the NP fertilizer rate should be shifted slightly from the recommended dose ($N_{100}P_{50}$ Kg ha^{-1}) to ($N_{150}P_{50}$ Kg ha^{-1}). At the revised level of NP, a good level of transgene expression against Boll worm is achieved and it will benefit the farmers to achieve better levels of insect pest protection. This will further enhance the durability of *Bt* cotton varieties against the target insect pests.

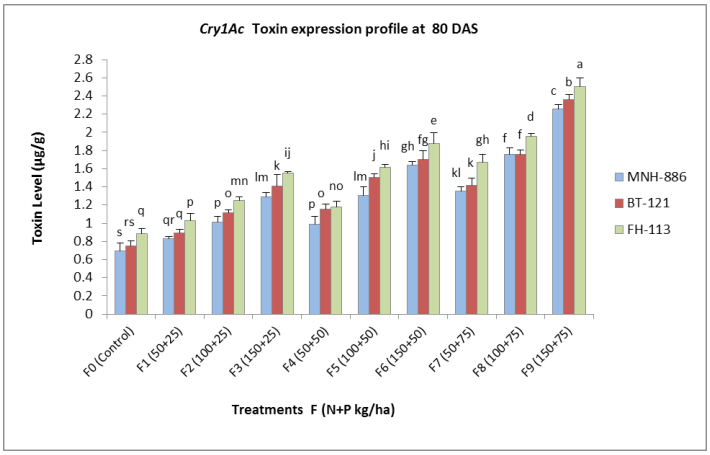


Figure 1

Cry1Ac expression profile as impacted by various fertilizer levels (F_1-F_9) in variety in three *Bt* cotton varieties sampled at 80 DAS.

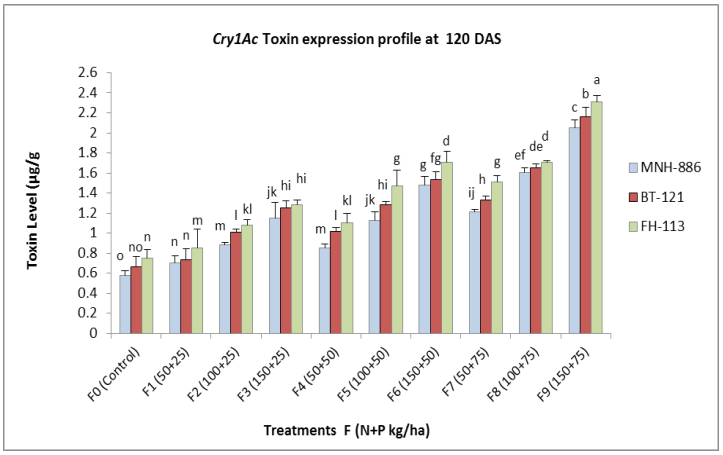


Figure 2

Cry1Ac expression profile as impacted by various fertilizer levels (F_1-F_9) in variety in three *Bt* cotton varieties sampled at 120 DAS.

CALENDAR OF EVENTS

EVENT	ORGANIZED BY	DATE	WEBSITE
INDIA			
International Rice Symposium: Rice Science for Global Food and Nutritional Security	Indian Institute of Rice Research, Central Rice Research Institute, International Rice Research Institute, PJ Telangana Agricultural University, ANGR Agricultural University and the Society for Advancement of Rice Research	November 18-20, 2015 Hyderabad	www.irs2015.in
Workshop for Strengthening the Management and Monitoring of Confined Field Trials of Regulated GE Crops	Biotech Consortium India Limited (BCIL)	November 19, 2015 Hyderabad	www.bcil.nic.in/
Workshop for Strengthening the Management and Monitoring of Confined Field Trials of Regulated GE Crops	University of Agricultural Sciences, Dharwad and Biotech Consortium India Limited (BCIL)	December 16, 2015 Hyderabad	www.bcil.nic.in/
National Seminar on Plant Genomics and Biotechnology Challenges and Opportunities in 21st Century	Department of Agricultural Biotechnology, College of Agriculture, and Orissa University of Agriculture & Technology	January 23-24, 2016 Bhubaneswar	www.ouat.ac.in/ForthEvents.aspx
INTERNATIONAL			
2nd International Conference on Crop Improvement (ICCI 2015)	Universiti Putra Malaysia	December 2-3, 2015, Universiti Putra, Malaysia	www.icci2015.upm.edu.my/home.php
International Symposium on "The Role of Agricultural Biotechnologies in Sustainable Food Systems and Nutrition"	Food and Agriculture Organization of the United Nations	February 15-17, 2016 Rome, Italy	www.fao.org/about/meetings/agribiotechs-symposium/en/

Interested in contributing to the SABP Newsletter?

The SABP Newsletter, published monthly, is distributed to over 10,000 regulators, scientists, policy makers and other stakeholders interested in agricultural biotechnology in South Asia. Each edition includes editorials, information about biosafety regulation and policy developments in India, Bangladesh and Pakistan, SABP and other capacity building activities in the region, and related science or news stories. All contributions to the newsletter should have a clear connection to the mission of SABP, relate to South Asia and cannot be promotional. For more information or for your article to be considered, please email Libby Williams at lwilliams@ilsi.org.

SOUTH ASIA BIOSAFETY PROGRAM

The South Asia Biosafety Program (SABP) is an international developmental program implemented in India, Bangladesh and Pakistan with support from the United States Agency for International Development. SABP aims to work with national governmental agencies and other public sector partners 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.

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