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South Asia Biosafety Program

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BANGLADESH

Training Workshop on Genetically Modified Organism (GMO) Detection and Good Laboratory Practices

Papia Sultana, Department of Environment (DOE), Bangladesh

Participants at the training workshop on genetically modified organism (GMO) detection and good laboratory practices.

The Department of Environment (DOE), as part of the Implementation of National Biosafety Framework (INBF) Project funded by the United Nations Environment Program Global Environment Facility (UNEP-GEF), organized a training workshop at the DOE GMO Detection Laboratory on February 17-22, 2018. About ten DOE officials participated, of which seven were from DOE Headquarters and three from DOE Divisional Offices, namely, Chittagong, Rajshahi, and Khulna.

The inaugural ceremony was held at the DOE's Chameli Conference Room on February 17. Dr. Sultan Ahmed, DOE Director General, inaugurated the Training Workshop as the Chief Guest. The ceremony started with a Welcome Address by Mr. Mohammed Solaiman Haider, DOE Director (Planning) and INBF Project Director. Mr. Haider explained the overall objectives of the training workshop, which follows up on a previous workshop held for DOE scientists working at DOE Headquarters. In this workshop, officials from DOE Divisional Offices were included, with the aim of updating their knowledge on recent developments related to GMOs and their detection methods. Prof. Dr. Md. Imdadul Hoque, Dean, Faculty of Biological Sciences, University of Dhaka and Bangladesh Country Coordinator, South Asia Biosafety Program (SABP), followed up the address with a short speech highlighting the importance of this kind of training workshop. He expressed his hope that participants will have the opportunity to learn about the development process of genetically modified organisms and their detection using molecular techniques, through theoretical and hands-on training.

Dr. Sultan Ahmed, the Chief Guest, described the objectives of the GMO detection laboratory at the DOE. He also thanked the Project Director for taking the initiative to hold such an important training workshop, with participants from DOE Headquarters and DOE Divisional

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Mr. M. S. Haider, Dr. S. Ahmed, and Prof. Dr. Md. I. Hoque.

Participants during the laboratory training.

Offices. He asked participants to take full advantage of the workshop to learn more about GMOs and their detection methodologies, and particularly how to use the sophisticated equipment installed at the DOE GMO Detection Laboratory. He hoped the five-day workshop will help build confidence for utilizing available resources to detect GMOs and wished everyone success.

Prof. Dr. Rakha Hari Sarker of the Department of Botany, University of Dhaka and GMO Detection Consultant under the INBF Project conducted the training workshop. He was assisted by Dr. Tahmina Islam, Assistant Professor, Department of Botany, University of Dhaka and Mr. Mohammed Solaiman Haider.

During the initial parts of the hands-on training, participants were familiarized with the operations of different laboratory equipment, such as the polymerase chain reaction (PCR) machine, real time PCR machine, gel apparatus, gel documentation system, laminar hood, clean bench, -80° freezer, bench top centrifuge machine, mini centrifuges, PH meter, and vortex machine. They also learned how to prepare necessary chemicals and buffers. Several experiments were performed, including genomic DNA isolation from leaf samples, gel electrophoresis, DNA quality and quantification by gel electrophoresis, PCR experiments with gene specific primers, and gel documentations.

Participating DOE officials had the opportunity to learn various aspects of GMO development, their global status, and how to use the equipment at the GMO Detection Laboratory.

Prof. Dr. M. Imdadul Hoque, Mr. Mohammed Solaiman Haider, Prof. Rakha Hari Sarker, and Dr. Tahmina Islam gave presentations on genetic transformation methods, the global status of GMOs, the chronology of biosafety development in Bangladesh, uses of PCR and real time PCR, and good laboratory practices. Participants also visited the Plant Biotechnology Laboratory of the Department of Botany, University of Dhaka for an overview of different work activities related to plant genetic engineering for different crops. By the workshop's conclusion, participating DOE officials had the opportunity to learn various aspects of GMO development, their global status, and how to use the equipment at the GMO Detection Laboratory.

Group photo of the participants and trainer.

INDIA

Mutated *ASAL* Gene Expressed in Rice Confers Resistance to Sheath Blight

Sampa Das et al., Division of Plant Biology, Bose Institute, Kolkata

Caused by *Rhizoctonia solani,* sheath blight is the most devastating disease for rice after blast disease, accounting for huge economic losses. In India, about 20% of yield is compromised annually due to this disease. Controlling sheath blight is difficult due to the wide host range of this pathogen, high pathogenic diversity, and the fungal pathogen's unique ability to survive in soil as sclerotia for many years. The opportunistic pathogen germinates when the condition becomes favorable and attacks the host. In the past couple of decades, an array of antifungal proteins have been expressed in rice to develop sheath blight resistance, including chitinases, Thoumatin-like protein, lipid transfer proteins, plant defensins, etc., with very limited success.

Transgenic mASAL expressing rice plants demonstrated significant resistance against sheath blight, both in detached leaf and whole plant bioassays

Prof. Das's group at Bose Institute has been working on several mannose binding monocot plant lectins, which have the inherent ability to antagonize various sap sucking insect pests affecting many crop plants. These lectins possess one or more carbohydrate binding domains that bind reversibly to specific mono- or oligosaccharides. The lectins belong to the "monocot mannose binding lectin superfamily" composed primarily of bulb and leaf lectins found in the Amaryllidaceae, Alliaceae, Orchidaceae, Araceae, Liliaceae and Bromeliaceae plant families. Despite strong sequence conservation, they typically vary in the tertiary structure and quaternary organization, which provides the greatest insight into their functionality in a biological system.

Dimeric lectin, i.e., Allium sativum leaf agglutinin (ASAL), has specific antagonistic effects toward insects, whereas tetramers exhibit an anti-retroviral property. For instance, tetrameric snowdrop lectin, or GNA (*Galanthus nivalis* of Amaryllidaceae) is known to be a potent inhibitor of HIV and other retroviruses due to its ability to bind to gp120. While searching for the key factors driving this quaternary association, an amino acid stretch "- DNSNN-" was identified in the ASAL sequence. It forms a bridge between two monomers, resulting in a dimeric lectin. *In vitro* mutagenesis of "-DNSNN-" generated a novel monomeric protein, most strikingly appearing to be antagonistic to *R. solani*. A series of immuno ligand assays of the sub-proteome of affected *R. solani* mycelium followed by LC-MS-MS analyses detected vital proteins acting as interacting partners of the newly designed, mutated monomeric lectin like protein referred to as "mASAL" (Ghosh et al. BMC Microbiol. 15:237, 2015).

Before engineering rice plants with the *mASAL* gene, a detailed biological safety assessment of the mASAL protein was conducted that unraveled mASAL as a thermolabile, pepsin digestible protein. Immuno histochemical analyses using mouse models and human blood sera cross reactivity tests resolved that the protein was a non-allergenic and biologically safe protein suitable for biotechnological application (Ghosh et al. J. Agric. Food Chem. 61:11858-64, 2013). Subsequently, transgenic mASAL expressing rice plants demonstrated significant resistance against sheath blight, both in detached leaf and whole plant bioassays (Ghosh et al. BMC Biotech.16:24, 2016). In summary, the study highlights the efficacy of a novel, biologically safe mASAL protein expressed in transgenic rice plants that confers resistance to sheath blight caused by *R. solani*.

INDIA

Abiotic Stress Tolerant Crops: The Quest for Novel Genes

Prabhjeet Singh, Department of Biotechnology, Guru Nanak Dev University

Drought and heat stress are among the major abiotic factors that limit crop productivity. Identification and functional characterization of genes involved in the stress response is, therefore, a prerequisite to enhancing the stress tolerance of crop plants through conventional plant breeding and modern approaches such as gene editing and genetic modification. Seed is the part of the plant most tolerant to desiccation, capable of surviving more than a 90% loss of original water content at maturity.

The seed, thus, presents an interesting model for understanding the molecular basis of dehydration tolerance. Researchers at the Department of Biotechnology, Guru Nanak Dev University, Amritsar (Punjab), working in collaboration with Prof. J. P. Khurana and Prof. Paramjit Khurana, University of Delhi South Campus (UDSC) and Dr Sanjay Kumar, CSIR-Institute of Himalayan Bioresource Technology, Palampur, have shown that comparative analysis of gene expression in water-stressed grains of wheat can lead to the discovery of novel genes that can potentially be used for enhancing the stress tolerance of crop plants. These studies have resulted in the identification of genes for translation initiation of factor 3 subunit g (*TaeIF3g*) and vesicle-associated membrane protein associated-protein (*TaVAP*) that are regulated differentially by drought. Subsequent analyses have demonstrated that ectopic expression of *TaVAP* and *TaeIF3g* in *Arabidopsis* confers enhanced stress tolerance under different abiotic stress conditions.

The enhancement in drought stress tolerance of the *TaVAP*-overexpressing transgenic plants is not associated with any yield penalty under control conditions, making it a potential candidate for crop improvement.

Ectopic expression of these genes enables plants to maintain improved water status, higher photosynthetic efficiency, and anti-oxidant activity. Increased tolerance to abiotic stresses is often associated with adverse effects in transgenic plants under non-stress conditions. However, the enhancement in drought stress tolerance of the *TaVAP*-overexpressing transgenic plants is not associated with any yield penalty under control conditions, making it a potential candidate for crop improvement.

Furthermore, studies carried out in collaboration with Prof. Ashwani Pareek of Jawaharlal Nehru University (JNU) have also led to the cloning of the wheat cyclophilin gene (*TaCYPA-1*), which possesses peptidyl prolyl *cis-trans* isomerase activity. Peptidyl prolyl *cis-trans* isomerases are the only enzymes that catalyze the conversion of peptidyl prolyl bonds from *cis* to *trans*, making them critical for the correct folding of proteins. Site-directed mutagenesis has shown that it is possible to increase the catalytic efficiency of *TaCYPA-1*.

Functional characterization, using *E. coli* as a model organism, has demonstrated that *TaCYPA-1*, like many other cyclophilins, plays an important role in stress tolerance. A novel stress-regulated calmodulin-binding protein, glycine-rich RNA-binding protein (SbGRBP), has also been identified and characterized in sorghum, which is considered as a model crop for studying drought and heat stress tolerance. Since calmodulin is a Ca^{2+} sensor and regulates activities of diverse proteins such as heat shock proteins, transcription factors, enzymes, etc., this gene may also have important implications for the abiotic stress response of plants. Efforts are now underway to transfer these genes to different crops to improve their stress tolerance.

INDIA

Genetically Engineered Chickpea Resistant to Insect Attack Holds Promise for Indian Agriculture

Bidyut Kr Sarmah and Sumita Acharjee, Department of Biotechnology, Assam Agricultural University (AAU), Jorhat

Chickpea growing in a field.

Chickpea is one of the most popular legumes due to its protein-rich seeds, which are consumed as a source of vegetable protein, especially by India's large vegetarian population. However, the seeds are also eaten by many insect pests, causing huge production losses in both the field and in storage. In the field, pods are damaged by pod borers (*Helicoverpa armigera*), while bruchids (*Callosobruchous* sp) reduce the quality and quantity of dry seeds in storage. There is no germplasm resistant to both of the aforementioned insect pests available in the chickpea gene pool for conventional breeding to use in order to develop resistant varieties. Therefore, farmers depend heavily on insecticides to prevent losses. If chickpeas are not washed properly before consumption as soaked raw seeds, they can cause food poisoning, leading to serious illness and even death.

An option is to introduce resistance genes from other sources using genetic engineering. This was successfully pursued at Assam Agricultural University (AAU), with the introduction of *Bacillus thuringiensis* (Bt) insecticidal genes and a bean a-amylase inhibitor gene to confer resistance to pod borers and bruchids, respectively. The work was done in collaboration with the Commonwealth Scientific and Research Organization (CSIRO), with funding from the McKnight Foundation, Indo-Swiss Collaboration in Biotechnology, Australia-India Strategic Research Funds, Department of Biotechnology at AAU (DBT-AAU), and the Indian Council of Agricultural Research (ICAR).

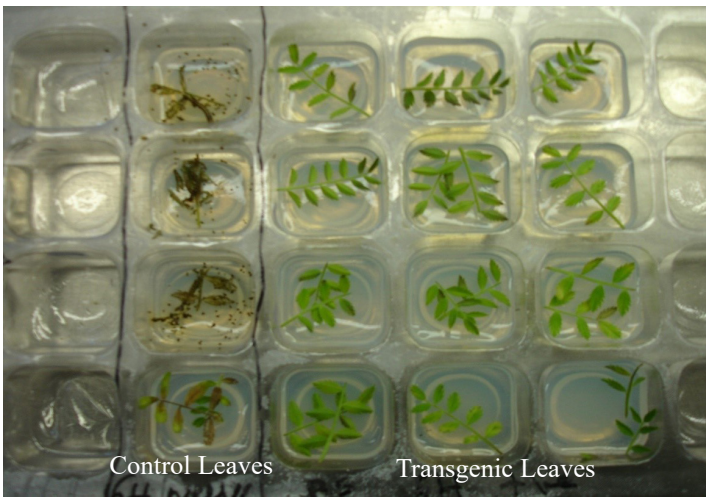
The transgenic chickpea lines harboring either a Bt-*Cry1Ac* or Bt-*Cry2Aa* gene were generated by *Agrobacterium*-mediated genetic transformation. Several (>150) independent lines were generated and characterized for transgene expression, copy number, and segregation of the transgene in subsequent generations. After successful laboratory bioassays, lines with complete resistance to pod borer larvae were tested for whole plant bioassays in the greenhouse. The five best lines were transferred to various public (University of Agricultural Sciences, Dharwad, Punjab Agricultural University, and the Indian Institute of Pulses Research) and private organizations (Sungro Seeds, Ltd.) for introgression breeding and field trials.

Sungro Seeds conducted field trials of Bt chickpea lines having a *Cry2Aa* gene in Andhra Pradesh during 2014–15 and 2015–16 and found very low insect damage and considerable yield advantage. Further field trials will be conducted using *Cry1Ac* lines in the coming years. Gene pyramiding initiatives are also in place to unite *Cry1Ac* and *Cry2Aa* in a single genetic background through breeding, and researchers at AAU are attempting to introduce a third gene, vegetative insecticidal protein (*vip*) in chickpea for enhanced protection to *Helicoverpa*.

If chickpeas are not washed properly before consumption as soaked raw seeds, they can cause food poisoning, leading to serious illness and even death. An option is to introduce resistance genes from other sources using genetic engineering.

In a similar manner, a gene from *Phaseolus vulgaris* (Rajmah), known as an alpha amylase inhibitor (αAI), was isolated and introduced in chickpea to express the inhibitors in the seeds only. The transgenic dry seeds preserve huge amounts of these inhibitors, thus making them resistant to bruchid larvae.

Researchers at AAU and CSIRO plan to determine the nutritional equivalence and agronomic performance of transgenic chickpea expressing either the Bt gene or amylase inhibitor gene according to biosafety guidelines. In addition, researchers at AAU are also engaged in developing insect resistant pigeon pea, which is the second most important grain legume in India, as well as optimizing an *Agrobacterium*-mediated genetic transformation method in another important grain legume, blackgram.



*Chickpea lines expressing a Bt gene is resistant to pod borer (*Helicoverpa armigera*).*

Bruchid resistant chickpea lines expressing a bean a amaylase inhibitor gene

Red Rot Resistant Transgenic Sugarcane Developed through Expression of *β-1,3-glucanase* Gene

Shivani Nayyar, Bipen Kumar Sharma, Ajinder Kaur, Anu Kalia, Gulzar Singh Sanghera, Karanjit Singh Thind, Inderjit Singh Yadav, and Jagdeep Singh Sandhu, Punjab Agricultural University, Ludhiana

Sugarcane (*Saccharum* spp.) is a commercially important crop in tropical and sub-tropical regions, cultivated primarily for the production of sucrose, ethanol, biofuel, and fiber-related commodities. The crop is vulnerable to several diseases and maximum devastation occurs due to red rot caused by the fungus *Colletotrichum falcatum*. The pathogen attacks sucrose-accumulating parenchyma cells of economically important cane stalk, leading to severe losses in sugar recovery (25-75%), extraction (7.1-32.5%), polarity (7.4-38.7%), purity co-efficient (0.5-8.3%), and commercial cane sugar (7.8-39%). Susceptibility to red rot is reported to be a serious constraint for sugarcane production, with annual losses of $500-1000 million, depending on the disease's severity.

C. falcatum spreads through infected cane stalk setts, diseased debris, and resting spores in the soil. The disease can be avoided in this vegetatively propagated crop through the use of healthy planting material. Disease management by chemical treatment is reported to be difficult, whereas the use of biological agents, such as *Trichoderma* spp., has been demonstrated to control red rot in sugarcane. *Trichoderma* secretes various cell wall-degrading enzymes, among these β-1,3-glucanase targeting the β-glucan chain in the *Colletotrichum* cell wall at β-1,3-glucosyl linkages, resulting in swelling, distortion, and lysis of pathogen hypha, thus restricting their growth and activity. The antifungal activity of *Trichoderma* spp. β-1,3-glucanase has been shown to inhibit the growth of a number of fungal pathogens and has been directly associated with disease resistance.

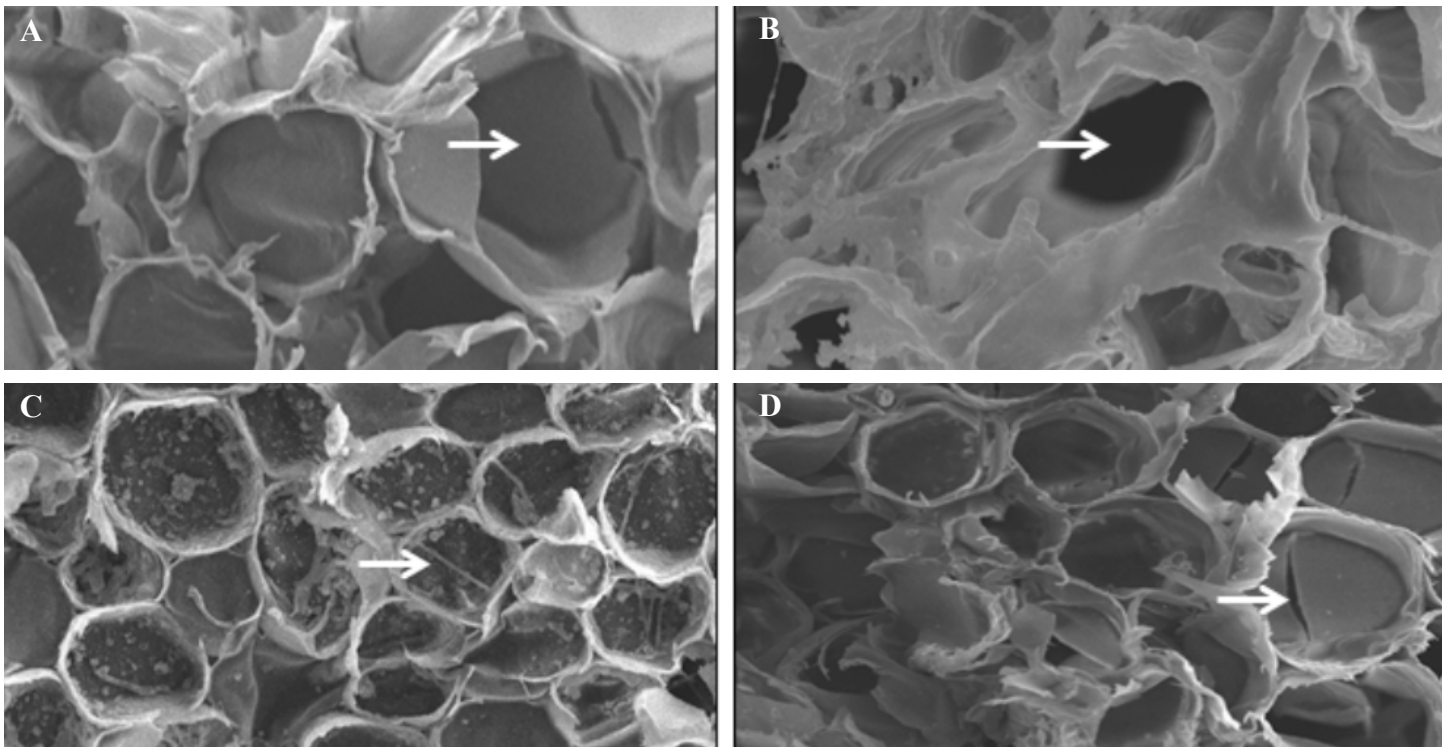
The cloning and characterization of *β-1,3-glucanase* genes from *Trichoderma* spp. demonstrated inhibitory activity against phytopathogenic fungi. Further, their over-expression in transgenic plants resulted in fungal resistance in *Brassica napus,* strawberry, rice, and pearl millet. However, there is no information in the literature about expression of *β-1,3-glucanase* for resistance against fungal pathogens in sugarcane.

We have developed red rot resistant transgenic sugarcane through expression of the *β-1,3-glucanase* gene from *Trichoderma* spp. The transgene integration and expression was analyzed in the first clonal generation raised from T_0 plants by polymerase chain reaction, reverse transcription-PCR, quantitative reverse transcription-PCR, revealing up to 4.4-fold higher expression, in comparison to non-transgenic sugarcane. Bioassay of transgenic plants with two virulent *C. falcatum* pathotypes causing red rot disease, Cf 08 and Cf 09, demonstrated that some plants were resistant to Cf 08 and moderately resistant to Cf 09.

We have developed red rot resistant transgenic sugarcane through expression of the *β-1,3-glucanase* gene from *Trichoderma* spp.

The electron micrographs of sucrose-storing stalk parenchyma cells from these plants displayed characteristic sucrose-filled cells inhibiting Cf 08 hyphae and lysis of Cf 09 hyphae. In contrast, the cells of susceptible plants were sucrose-depleted and defenseless against both pathotypes. The transgene expression was up-regulated (up to 2.0-fold in leaves and 5.0-fold in roots) after infection, as compared to before infection, in resistant plants. The transgene was successfully transmitted to the second clonal generation raised from resistant transgenic plants. β-1,3-glucanase protein structural models revealed that active sites Glutamate 628 and Aspartate 569 of the catalytic domain acted as a proton donor and nucleophile, with a role in cleaving β-1,3-glycosidic bonds and pathogen hyphal lysis.

The transgenic sugarcane developed in our laboratory has the inherent ability to inhibit lysis of stem parenchyma cells upon *C. falcatum* infection. As a consequence, the losses encountered in sugar recovery will be reduced and this will be a major boost for the sugar industry.



Scanning electron micrographs of stalk sections from CG_1 transgenic and Non Transgenic (NT) plants following inoculation with C. falcatum. *(A) Sucrose-filled parenchyma cells of non-inoculated NT plant (control). Arrow indicates the characteristic turgid cell. (B) Presence of normal Cf 09 fungal hyphae in parenchyma cells of susceptible NT plant. Arrow indicates the sucrose depleted cell. (C) Parenchyma cells of transgenic plant moderately resistant to Cf 09. Arrow indicates abnormal fungal hypha and amorphous debris. (D) Sucrose-filled parenchyma cells of transgenic plant resistant to Cf 08 showing absence of hyphae. Arrow indicates the turgid cell.*

CALENDAR OF EVENTS

EVENT	ORGANIZED BY	DATE	WEBSITE
INDIA & BANGLADESH			
Training Program on Management of Plant Genetic Resources	ICAR-National Bureau of Plant Genetic Resources	March 6 – 19, 2018 New Delhi	http://bit.ly/2H0ouno
Workshop: Smart Metabolic Engineering of Plants for Drug Biosynthesis	International Centre for Genetic Engineering & Biotechnology (ICGEB)	March 16 – 17, 2018 New Delhi	https://www.icgeb.org/meetings-2018.html
National Conference on Enhancing Productivity of Oilseeds in Changing Climate Scenario	Indian Society of Oilseeds Research, Hyderabad & ICAR-Directorate of Groundnut Research, Junagadh	March 17 – 19, 2018 Junagadh	http://bit.ly/2FIenTt
State Level Biosafety Capacity Building Workshop Supported by the Phase II Capacity Building Project on Biosafety (Bhubaneswar)	Orissa University of Agriculture & Technology and BCIL	March 19, 2018 Bhubaneswar	http://bit.ly/2FWXnfg
State Level Biosafety Capacity Building Workshop Supported by the Phase II Capacity Building Project on Biosafety (Coimbatore)	Tamil Nadu Agricultural University and BCIL	March 23, 2018 Coimbatore	http://bit.ly/2FWXnfg
International Conference on Agriculture and Allied Sciences: The Productivity, Food Security and Ecology	Bidhan Chandra Krishi Viswavidyalaya	April 13 – 14, 2018 Mohapur	http://bit.ly/2Bi2l3I
INTERNATIONAL			
ICGEB-NASSL "South Asian Biotechnology Conference 2018 - SABC 2018"	National Academy of Sciences of Sri Lanka (NASSL), ICGEB, and the South Asian University (SAU)	March 28 – 30, 2018 Colombo, Sri Lanka	https://www.icgeb.org/meetings-2018.html
2nd World Congress & Expo on Biotechnology and Bioengineering	Biocore Conferences	June 25 – 27, 2018 Dubai, UAE	https://biocoreconferences.com/biotechnology2018/
5th International Rice Congress	International Rice Research Institute	October 14 – 17, 2018 Singapore	http://ricecongress2018.irri.org/
9th Meeting of the Conference of the Parties	Convention on Biological Diversity	November 10 – 22, 2018 Sharm El-Sheikh, Egypt	http://bch.cbd.int/protocol/meetings/

SOUTH ASIA BIOSAFETY PROGRAM

The South Asia Biosafety Program (SABP) is an international developmental program implemented in India and Bangladesh 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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