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South Asia Biosafety Program

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BANGLADESH

Highlights from the Annual Plant Tissue Culture and Biotechnology Conference & Training Workshop on Biosafety Regulatory System and Network Development

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Guests during the inaugural ceremony of the BAPTC&B Conference.

The Bangladesh Annual Plant Tissue Culture and Biotechnology (BAPTC&B) Conference and Training Workshop, part of the UNEP-GEF funded Implementation of the National Biosafety Framework (INBF) Project, was held on April 6-7, 2018 at the Bangladesh Sugarcrop Research Institute (BSRI) in Ishurdi, Pabna. It was organized by BAPTC&B, in collaboration with BSRI, the INBF Project, and Krishi Gobeshona Foundation (KGF), with other national organizations acting as sponsors and co-sponsors. About 150 participants representing universities, public colleges, national agricultural research institutes, nongovernmental organizations, and private laboratories attended this conference, which included presentations in different scientific sessions and a poster session covering various aspects of plant tissue culture, plant genetic transformation, marker assisted breeding, etc.

The inaugural ceremony was held at the BSRI auditorium. The conference was inaugurated by Prof. Dr. Md. Alauddin, Vice Chancellor of Mawlana Bhashani Science and Technology University, who also acted as the Chief Guest. Prof. Dr. K. M. Nasiruddin, Vice Chancellor of Bangabandhu Sheikh Mujiur Rahman Science and Technology University, was also present as the Special Guest. The inaugural ceremony was chaired by Prof. Dr. Rakha Hari Sarker, President of the Bangladesh Association for Plant Tissue Culture & Biotechnology.

The inaugural ceremony started with a welcome address by Dr. Md. Amzad Hossain, Director General of BSRI. Dr. Kuasha Mahmud, Head of the BSRI Biotechnology Division, gave the keynote address. In his presentation, Dr. Mahmud highlighted the research and development activities being carried out in different research divisions of BSRI. He also briefly described the achievements of BSRI and their contribution to the national agricultural research systems.

Besides chairing the inaugural ceremony, Prof. Dr. M. Imdadul Hoque, Dean of the Faculty of Biological Sciences, University of Dhaka and

INDIA

Comments/Suggestions Invited on Draft Guidance Documents

The Review Committee on Genetic Manipulation (RCGM), housed in the **Department of Biotechnology (DBT)**, has placed the following two documents for comments/suggestions from stakeholders:

1. **"Environmental Release of Genetically Engineered Microorganisms (GEM): Regulatory Requirements for Risk Assessment and Approval in India, 2018"** - Regulatory requirements for risk assessment of genetically engineered microorganisms (GEM) and/or its derived product under research, manufacture, import, or processed for environmental release purposes that includes applications like biopesticide, bioremediation, biomass conversion, crop quality improvement, waste treatment, biomining, mineral leaching, oil recovery, desulfurization of fossil fuels, biofertilizers and biosensors.
2. **"Guidelines and Protocols for Analysis of Effects of GE Plants on Soil Microorganisms 2018"** - Methodologies and data requirements for assessing the effects of GE plants on soil microorganisms.

A proforma has been provided for sending comments/ suggestions by **May 25, 2018** via email to **bsu.dbt@nic.in**.

For further information please visit:
http://www.dbtindia.nic.in/dbt-invites-comments-suggestions-on-draft-documents/

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Participants at the conference.

Guests during the inaugural ceremony of the Training Workshop on Biosafety Regulatory System and Network Development.

Bangladesh Country Coordinator for the South Asia Biosafety Program (SABP) also gave a brief speech highlighting the contribution of BAPC&B toward popularizing plant tissue culture and modern biotechnology in Bangladesh. The inaugural ceremony ended with a vote of thanks by Professor Dr. Mihir Lal Saha, General Secretary of BAPTC&B.

On the first day of the conference, about 30 research papers were presented in three different scientific sessions—Plant Genetic Transformation, *In Vitro* Morphogenesis of Economically Important Plants, and Marker Assisted Breeding & Microbial Biotechnology. Additionally, there were seven poster presentations, mostly by young plant biotechnologists.

The Training Workshop on Biosafety Regulatory System and Network Development took place on the second day of the conference. It was inaugurated by Dr. Sultan Ahmed, Director General of the Department of Environment (DOE). The short inaugural ceremony commenced with a welcome address by Mr. Mohammed Solaiman Haider, Director (Planning) of the DOE and Project Director of the INBF Project. Prof. Dr. M. Imdadul Hoque also spoke during the inaugural ceremony, highlighting the development of biosafety related activities, as well as the South Asia Biosafety Program's contribution toward developing biosafety regulatory systems in Bangladesh. The inaugural ceremony was chaired by Dr. Md. Amzad Hossain.

After the inaugural ceremony, Mr. Mohammed Solaiman Haider gave a presentation titled *Biosafety: National and International Perspective; Biosafety Systems as per Bosafety Rules and Biosafety Guidelines; Risk Assessment, Management, and Communications*. The second presentation on the *Biosafety Concerns in Contained, Confined and Open Field Trials of Genetically Engineered Crops* was delivered by Prof. Dr. M. Imdadul Hoque. Finally, Mr. Mohammed Solaiman Haider conducted an exercise on network development. There were lively discussions on the presented papers, and participants were very much enthusiastic about the status of regulatory systems in Bangladesh, as well as the status of a released GE crop (Bt-brinjal) in Bangladesh. Some farmers who have been cultivating Bt-brinjal were also present during the workshop.

INDIA

Composite Transgenic Plants: An Advantageous Tool for Elucidating Gene Function in Difficult to Transform Trees

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Concerted efforts by researchers to generate and analyze mutants of model plants have led to the unraveling of genetic determinants of different aspects of plant growth and development. The advent of gene silencing and genome editing tools has added more precise and powerful tools for directed disruption of genes. Model plants have been primarily used to better understand the molecular basis of a phenotype due to their relative amenability to reverse genetic approaches. Next generation sequencing techniques have helped generate complete genome sequence information from non-model trees like Eucalyptus, and gene expression analysis have given insights on gene function in these species. The unique genomic context of non-model plants implies that the functions ascribed to these genes need to be ascertained via transgenic tools. However, the time-consuming and often difficult tissue culture dependent genetic transformation methodology in non-model species have meant that high throughput gene function studies have not kept pace with the gene sequence information deluge in these species.

The composite plant strategy in combination with RNAi approaches has been successfully used in elucidating the function of nodulation genes in the difficult to transform tree species.

During the last decade, composite transgenics approaches using *Agrobacterium rhizogenes* have been used to circumvent these limitations by providing a rapid and less technically demanding tool for analyzing the function of the genes involved in root growth and development. It involves the generation of transgenic hairy roots on non-transgenic shoots, thereby providing an *in vivo* environment for evaluating the function of genes involved in root traits like symbiotic and parasitic interactions, nutrient and water uptake, and salt tolerance. Transgenic hairy roots are characterized by high lateral branching and negatively geotropic behavior. The root inducing Ri plasmid of the *A. rhizogenes* strains are co-transformed with a binary vector harboring the desired gene construct along with a green fluorescent protein marker gene for tagging transgenic roots. The non-GFP roots are dehisced to obtain a composite plantlet with transgenic roots generated on a non-transgenic shoot. The composite plant strategy in combination with RNAi approaches has been successfully used in elucidating the function of nodulation genes in the difficult to transform tree species of the Casuarinaceae family (Gherbi et al. Proc. Natl Acad Sci USA.105: 4928-4932, 2008; Svistoonoff et al. PLoS ONE. 8: e64515, 2013).

At the Institute of Forest Genetics and Tree Breeding, in collaboration with the Institut de Recherche pour le Développement, the composite plant strategy with GFP roots were generated for the first time in the difficult to transform tree *Eucalyptus camaldulensis* (Balasubramanian et al. BMC Proc. 5 (7): 45, 2011). The technique has been successfully applied by Placensia et al. (2015) for validating the function of the lignin biosynthesis gene *EgCCR1* in *E. grandis* (Plasencia et al. Plant Biotechnol J. 14(6):1381-93, 2015). For engineering a polygenic trait like salt tolerance, screening to identify genes that significantly contribute to a trait becomes crucial. At the Institute of Forest Genetics and Tree

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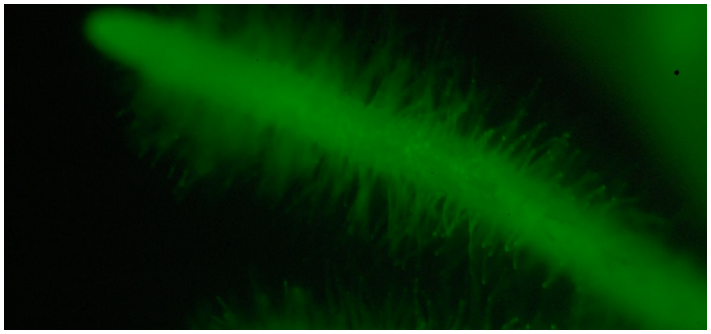
Composite transgenic plantlet of Eucalyptus camaldulensis



GFP expressing transgenic hairy roots generated on non-transgenic shoots.

Breeding, the composite plant strategy was used in combination with RNAi to elucidate the contribution of a sodium transporter gene in determining salt tolerance in *E. camaldulensis* (unpublished).

The advent of CRISPR/Cas9 gene-editing technology offers powerful high-throughput options and has already been used in conjunction with the composite plant strategy for elucidating gene function. In long generation trees species like Eucalyptus and Casuarina, information on the genetic determinants of phenotypes can significantly hasten breeding for desired traits. Composite transgenic strategy thus has the potential application of rapidly identifying genes for genetic modification or in marker assisted breeding programs for desired traits like drought, flooding, and other less studied traits in trees.

INDIA

Development of Transgenic Chickpea and Pigeon Pea Plants for Biotic and Abiotic Stress Tolerance

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Globally, chickpea and pigeon pea are the third and fifth most important pulse crops mainly grown in developing countries. South Asia accounts for the bulk of production of both these pulses. India is the largest producer of pulses in the world, with a 25% share of global production. While chickpea is the topper among pulses, occupying 39% of the pulse area, pigeon pea follows with a 21% area share. Despite being a major chickpea producing country, India still imports around one million tons of chickpea every year. Pod borer (*Helicoverpa armigera*), Ascochyta blight (*Ascochyta rabiei*), and Fusarium wilt (*Fusarium oxysporum*) are the biotic stresses that lead to approximately 4.8 million tons in yield loss every year. Likewise, drought, salinity, heat, and cold are the major abiotic stresses, leading to approximately 6.4 million tons in yield loss every year for chickpea alone. Soil salinity is an ever-increasing production constraint in pigeon pea, adversely affecting flowering and plant growth, as it is grown predominantly in regions in India where saline soil makes up more than 51%. The limited genomic resources coupled with narrow genetic diversity in the elite gene pool of these legume crops have hampered genetic improvement, either by traditional or molecular methods.

Approaches to generating transgenic crops are an elegant and perhaps most effective delivery system for *Bacillus thuringiensis* (*Bt*) toxins, which apparently provides a relatively long lasting and seed borne solution for the management of the lepidopteran pest pod borer. Most of the legumes are known to be salt sensitive and die before maturity in the field when salinity rises to 100mM NaCl. Hence, it is necessary to make cultivars or transgenic plants tolerant to abiotic stresses through genetic engineering methods for a sustainable increase in the production of these pulse crops.

A *Bt* chickpea (cvs. C-235 and HC-1) was developed using a tissue culture independent *Agrobacterium*-mediated transformation protocol (Patent No. 252590), as well as a *Bt* pigeon pea (Manak variety) with the *cry1Ac* gene. The putative transgenic plants were analyzed using multiple evaluation strategies, such as PCR, ELISA and Southern blotting, to select plants for further advancement. Quantitative assessment of *Bt* Cry toxin using ELISA in the leaves of transgenic chickpea plants showed variation in the expression of the Cry1Ac toxin. High expressing transgenic plants exhibited phenotypic abnormalities, whereas high expressing pigeon pea plants did not survive. Results obtained through Southern blotting using a gene specific probe confirmed the single copy integration of the *cry1Ac* gene into the chickpea and pigeon pea genome. Evaluation of the T_3 generation progenies showed low mortality of larvae (30%-40%) that fed on the *Bt* chickpea plants for three days. However, there was a significant reduction in larval weight, prolongation of the larval period, and reduced adult emergence. In pigeon pea, two promising transgenic lines showed 87.5% and 62.5% mortality, respectively, of *Helicoverpa* larvae when an insect bioassay was conducted for four days on T_4 generation plants.

For salinity tolerance, transgenic chickpea and pigeon pea plants carrying *OsRuvB* and *OsLecRLK* genes individually have been developed. Also, the *Psp68* gene has been introduced in pigeon pea. Southern hybridization analysis of the transgenic plants revealed a single copy of the transgene in all of the transgenic plants for both crops. A physio-biochemical assay of transgenic and control chickpea and pigeon pea plants under 100mM and 75mM salt stress revealed salt tolerance in the transgenic plants, while the non-transgenic plants for both crops did not tolerate the stress.

Significant reduction in larval weight after feeding on Bt chickpea.

Transgenic and control plants.

CALENDAR OF EVENTS

EVENT	ORGANIZED BY	DATE	WEBSITE
INDIA & BANGLADESH			
Second International Conference on Nanobiotechnology for Agriculture	The Energy and Resources Institute	December 6 – 7, 2018 New Delhi, India	http://www.teriin.org/event/second-international-conference-nanobiotechnology-agriculture
13th International Conference on Dryland Development: Converting Dryland Areas from Grey into Green	International Dryland Development Commission (IDDC) Arid Zone Research of India, Jodhpur and Central Arid Zone Research Institute	February 11 – 14, 2019 Jodhpur, India	http://www.13icdd.com/
XIV Agricultural Science Congress – Innovation for Agricultural Transformation	National Academy of Agricultural Sciences and ICAR-Indian Agricultural Research Institute	February 20 – 23, 2019 New Delhi, India	http://ww.14agricongress2019.in & http://www.iari.res.in/files/Latest-News/14ASCFirstCircular_19042018.pdf
INTERNATIONAL			
Regional Expert Consultation on Agricultural Biotechnology – Scoping Partnership to Improve Livelihoods of Farmers in Asia-Pacific	Asia-Pacific Association of Agricultural Research Institutions (APAARI) and Asia Pacific Consortium on Agricultural Biotechnology and Bioresources	May 29 – 31, 2018 Bangkok, Thailand	http://www.apaari.org/web/our-projects/apcoab/upcoming-events/
2018 BIO International Convention	Biotechnology Innovation Organization	June 4 – 7, 2018 Boston, USA	http://www.convention.bio.org/2018/
2nd World Congress & Expo on Biotechnology and Bioengineering	Biocore Conferences	June 25 – 27, 2018 Dubai, UAE	https://biocoreconferences.com/biotechnology2018/
5th International Conference on Biotechnology Engineering (ICBioE)	Department of Biotechnology Engineering (BTE) and International Islamic University Malaysia (IIUM)	September 19 – 20, 2018 Kuala Lumpur, Malaysia	http://www.iium.edu.my/icbioe/2018/
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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