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INDIA

Confined Field Trials of Transgenic Maize at the University of Agricultural Sciences, Dharwad

R. M. Kachapur, University of Agricultural Sciences, Dharwad

Trial site for the confined field trials of transgenic maize at the University of Agricultural Sciences, Dharwad.

In 2017, the University of Agricultural Sciences (UAS), Dharwad received an application from a private research organization to conduct confined field trials (CFTs) of a transgenic maize hybrid with stacked events against stem borers and cob borers, as well as herbicide tolerance. The university's Institutional Biosafety Committee (IBSC) granted permission for the trials after thorough discussions and receipt of appropriate permits from the Government of Karnataka.

Dr. R. M. Kachapur was put in charge of the CFTs for these maize hybrids, as he had received training in 2015 on biosafety regulation and how to conduct CFTs for transgenic crops, part of a workshop organized by Biotech Consortium India Limited (BCIL) and UAS, Dharwad under the Phase II Capacity Building Project on Biosafety implemented by the Ministry of Environment, Forest and Climate Change (MoEFCC). The first step was to identify a suitable location with appropriate isolation for the trial. After a thorough survey and discussions with the Director of Research, a field located at the university's Krishi Vigyan Kendra was chosen. Details regarding the trial site, i.e., GPS coordinates, route map, previous crops planted on the field, and crops in adjoining fields were collected, after which a site map was prepared. The trial site was secured with a chain link fence, along with a sign containing details about the trial and contact information of the person in charge. Arrangements were also made for security personnel to monitor the area.

> **Dr. R. M. Kachapur [...] had received training in 2015 on biosafety regulation and how to conduct CFTs for transgenic crops, part of a workshop organized by Biotech Consortium India Limited (BCIL) and UAS, Dharwad under the Phase II Capacity Building Project on Biosafety implemented by the Ministry of Environment, Forest and Climate Change (MoEFCC).**

After ensuring the location's security and spatial isolation, soil samples from the trial site were collected for initial analysis and the results recorded. The land was then prepared through ploughing, harrowing, and opening furrows, after which the field was laid out as per the approved plan, and each of the treatment plots were marked with labels. Meanwhile, the seeds were shipped in a closed container vehicle to the trial site. After receipt, the intactness of both secondary and primary packaging were checked, and a record of transport (ROT) was

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prepared. The seed packets were placed at their respective treatment plots and crosschecked.

The field workers were trained before sowing began. Sowing was completed within the stipulated time and arrangements were made for irrigation. Along all the borders, ten rows of African tall maize (non-transgenic fodder) were sown as guard rows for the main trial. Workers and all equipment were cleaned before leaving the trial site and the process was documented in the record book. Details of the trial and test site were submitted to the Review Committee on Genetic Manipulation within seven business days, with an informational copy sent to the Director of Agriculture, Karnataka State Department of Agriculture, Bangalore.

After 15 days of planting, the treatments were thinned so as to maintain a single healthy seedling per hill. The extra seedlings were removed and buried in a disposal pit inside the trial site and later covered with a layer of soil. At 22-25 days after planting, the buffer rows between treatments were removed and dumped in the disposal pit. All standard operating procedures (SOPs) were followed and all operations were conducted in the presence of Dr. R. M. Kachapur.

The trial site was monitored for spatial isolation once every two weeks for any maize or compatible species, and a record of spatial isolation was maintained. Some of the plants in each treatment were sampled for laboratory estimations. The plant samples were collected at appropriate stages, and a record of sample collection and transport was maintained. Visitors to the trial site were recorded in a log book.

The Central Compliance Committee (CCC) visited the trial site twice and monitored the trial for confinement and compliance with SOPs. The CCC also evaluated all the records for sample collection, transport, date of planting, trial details, spatial isolation, and visitors. Harvesting began at the appropriate husk drying stage and post-harvest observations were recorded. The harvested cobs were shelled manually from individual treatments of all three replications, and the weight of the harvested grains and moisture levels at the time were recorded. All these procedures were conducted in the presence of Dr. R. M. Kachapur and a representative from the Joint Director of Agriculture (JDA), Karnataka State Department of Agriculture, Dharwad.

After recording the yield parameters, all produced seeds and shanks were placed in the disposal pit and burned. Later, the fodder was chopped, heaped, and burned. The remaining incinerated debris was disposed in a pit and covered up with soil. The trial's termination was recorded and soil samples from the root zone area were collected for final soil analysis. Later, the harvested trial site was irrigated to encourage the germination of self-sown maize seeds and observations were made. They were then removed and disposed off in the disposal pit. After ensuring that there were no more volunteers in the trial site, cow peas were sown in all the treatments to look for effects of the transgenic maize crop on subsequent crop germination and establishment, as well as residual toxicity due to herbicide, if any.

Acknowledgements

The author acknowledges MoEFCC and BCIL for training related to biosafety regulations in India and confined field trials of transgenic crops through the Phase II Capacity Building Project on Biosafety, which informed his conduct of confined field trials of transgenic maize, as well as the UAS, Dharwad Administration, for providing the opportunity to participate in the training and CFTs.

Preparation for CFTs of transgenic maize at UAS, Dharwad.

Site visit.

Disposal pit for transgenic maize.

Burning of transgenic maize.

Workshop on Progress and Safety Evaluation of GR2E Golden Rice in Bangladesh

Tahmina Islam, Assistant Professor, Department of Botany, University of Dhaka

On March 6, 2018, a workshop on Progress and Safety Evaluation of GR2E Golden Rice in Bangladesh was held at the CIRDAP International Conference Centre (CICC), Chameli House, Dhaka. The event was organized by the Bangladesh Rice Research Institute (BRRI) and International Rice Research Institute (IRRI). Begum Matia Chowdhury, Member of Parliament and Minister of Agriculture, was the Chief Guest. Mr. Mohammad Moinuddin Abdullah, Senior Secretary at the Ministry of Agriculture, and Dr. Matthew Morell, Director General of the IRRI, were present as Special Guests. The workshop was chaired by Dr. Md. Kabir Ikramul Haque, Executive Chairman of the Bangladesh Agricultural Research Council (BARC).

The workshop aimed to provide an update on the progress of GR2E Golden Rice product development in Bangladesh. The technical briefing consisted of presentations on GR2E regulatory applications that were recently made in Bangladesh, the Philippines, Australia, and New Zealand, along with event selection, nutritional impact, safety assessment, and agronomic data from the GR2E Golden Rice confined field trials in Bangladesh. It allowed participants to examine the rigorous protocols that guide Golden Rice research, as well as discuss the environmental food safety assessment of GR2E Golden Rice in Bangladesh.

Dr. Donald MacKenzie, Regulatory Affairs and Stewardship Leader for Golden Rice at the IRRI, gave an overview of Golden Rice product development and regulatory status, as well as highlighted the potential toxicity and allergenicity risks of Golden Rice. Dr. Nikolaos Tsakirpaloglou, a postdoctoral fellow specializing in molecular biology and biochemistry at the IRRI, discussed the development and molecular characterization of rice event GR2E, providing details about experimental results. Rice event GR2E's nutrient composition analysis was then thoroughly discussed by Dr. Russel Reinke, Healthier Rice Program Lead at the IRRI, after which Ms. Jillian Waid of Helen Keller International talked about its nutritional impact.

Insights from the workshop's morning session set the stage for the policy session in the afternoon, chaired by the BARC Executive Chairman, who noted that the Bangladeshi government has been proactive in adopting biotechnological innovations and works closely with international institutions like the IRRI to the benefit of farmers and consumers. During her speech, the Minister of Agriculture acknowledged the positive impact of biotech crops and their potential to help Bangladesh meet the United Nations Sustainable Development Goal of zero hunger by 2030. Inspired by the success of the country's first commercially released biotech crop in 2013, Bangladesh is now field testing three more crops developed through applications of agro-biotechnology, including Golden Rice. The government has every intention of supporting genetically modified crop cultivation in the future. During the session, key officials and policymakers from various ministries and departments praised the latest developments related to Golden Rice.

The Minister of Agriculture acknowledged the positive impact of biotech crops and their potential to help Bangladesh meet the United Nations Sustainable Development Goal of zero hunger by 2030.

Dr. Md. Shahjahan Kabir, Director General of the BRRI, observed that Bangladesh has emerged as a global model for combating hunger, becoming a country with a food surplus despite a history of chronic food shortages. The current goal is to nutritionally enrich a staple food in order to build a healthier nation that is free of hunger. At present, rice contributes 70% of the daily caloric intake of Bangladesh's population, while the national consumption of vitamin A is estimated at half the recommended daily allowance. About 20% of children and 5% of pregnant women in Bangladesh suffer from vitamin A deficiency and

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Chief and Special Guests seated on the dias.

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Dr. Donald J. MacKenzie giving his presentation.

Dr. Nikolaos Tsakirpaloglou presenting his paper.

Workshop participants.

Workshop participants.

are at risk of blindness. Advances such as beta-carotene fortified GR2E Golden Rice are an important part of an overall strategy to reduce vitamin A deficiency in the country.

The IRRI Director General stated that a robust regulatory landscape is essential to amplifying the impact of Golden Rice and other nutritionally fortified rice varieties such as high zinc rice. He states: "FSANZ's approval process, which ensures the highest standard of health protection, provides a model for decision-making in all countries wishing to reap the benefits of Golden Rice. […] Each regulatory application that Golden Rice completes with national regulatory agencies, including those currently under process in Bangladesh, takes us one step closer to bringing Golden Rice to the people who need it the most."

Recently, GR2E Golden Rice completed its regulatory application with Food Standards Australia and New Zealand (FSANZ). Upon completion of its assessment, FSANZ concluded: "No potential public health and safety concerns have been identified in the assessment of GR2E. On the basis of the data provided in the present Application, and other available information, food derived from GR2E is considered to be as safe for human consumption as food derived from conventional rice varieties."

Golden rice is the first nutritionally enhanced genetically modified rice to receive regulatory approval for food use anywhere in the world, and it's development in Bangladesh is being lead by the BRRI. The application for environmental and food safety assessment of GR2E BRRI dhan29 Golden Rice was submitted to the Ministry of Agriculture on November 26, 2017 and the Ministry of Environment and Forest on December 4, 2017. It is a flagship product of the IRRI's Healthier Rice program.

After the technical and policy sessions, there was a lively discussion, with participants asking questions and offering suggestions. Dr. Humnath Bhandari, IRRI Representative for Bangladesh, delivered the closing comments and vote of thanks, expressing continued support for the project and prospects for the future.

Minister of Agriculture, Begum Matia Chowdhury, delivering her speech.

INDIA

Over Expression of *EaDREB2* in Sugarcane Confers Tolerance to Water Deficit Stress

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Drought is one of the most important factors limiting crop productivity. Lack of water adversely affects plant growth, development, and metabolism at various stages. Water is already a scarce resource in many parts of the world, and predicted climate changes will aggravate the situation in the future.

Sugarcane is an important commercial crop in India. Drought stress is a major limitation for sustainable sugarcane production across tropical and subtropical regions of this country. Sugarcane, being a tropical crop grown throughout the year, faces moisture stress during various growth phases, namely the germination, formative, grand growth, and maturity phases. The water requirement of an annual sugarcane crop is 2000-3000 mm, but water demand varies during germination (200-300 mm), formative (300-600 mm), grand growth (500-1100 mm), and maturity (300-700 mm) phases, as well as between different regions, depending on soil type, climate, and sowing time. Moisture stress during crop growth stages accounts for about 30-70% of sugarcane productivity losses.

Drought stress is a major limitation for sustainable sugarcane production.

It is essential to develop drought tolerant varieties or improve the water efficiency of existing sugarcane varieties ("more crop per drop"). Drought tolerance in plants is a quantitative trait and thus requires manipulation of several genes. The *DREB2* transcription factor is reported to trigger the expression of many dehydration responsive genes associated with drought stress. Screening wild relatives of *Saccharum* sp revealed many fold upregulation of *DREB2* transcriptional factors under low moisture stress when compared to the commercial variety (Augustine et al. Sugar Tech 17:121, 2015).

The *EaDREB2* (1.5 kb) gene was isolated from *Erianthus arundinaceus*, a wild relative of *Saccharum* sp. Cloning of *EaDREB2* was confirmed through polymerase chain reaction (PCR) using gene specific primers, the promoter specific forward primer, and gene specific reverse primers. The pSBI-EaDREB2 construct was used to transform one of the most popular sugarcane varieties (Co 86032) in tropical India through *Agrobacterium*-mediated transformation following the method developed at ICAR-Sugarcane Breeding Institute (Arvinth et al. Plant Cell Reports 29:383, 2010).

The plant selection marker used was hygromycin (*Hpt*), and expression of *EaDREB2* was driven by the *Port Ubi 2.3* promoter cloned from wild rice (*Porteresia coarctata*) (Philip et al. Plant Cell Rep. 32:1199, 2013). Transgene integration was confirmed through PCR, and transgenic events were screened for tolerance to soil moisture stress at the tilling phase by withholding irrigation. Soil moisture content was estimated using the gravimetric method.

The study showed that over expression of the *EaDREB2* gene enhanced drought tolerance in the sugarcane variety under the water deficit stress condition (Augustine et al. Plant Cell Rep. 34:247, 2015). Over expression of the *EaDREB2* gene enabled plants to maintain improved cell membrane thermostability, relative water content, chlorophyll content, and photosynthetic efficiency compared to the untransformed wild-type. The overexpression of transgenes in sugarcane also led to the upregulation of downstream stress tolerance associated genes. The gene of interest (*EaDREB2*) and promoter (*Port Ubi 2.3*) from plant sources are non-allergenic and biologically safe, suitable for biotechnological applications. In summary, the study highlights *EaDREB2* expressed in transgenic sugarcane events, which confers drought tolerance and could be safe for the environment.

INDIA

Genetically Engineered Tomato with Improved Chilling Tolerance

S.R. Kumar, R. Kiruba, and R. Sathishkumar, Plant Genetic Engineering Laboratory, Department of Biotechnology, Bharathiar University, Coimbatore

Tomato (*Solanum lycopersicum* L.) is an economically important crop cultivated throughout the world. Cultivation of tomato has increased sharply and ranks seventh in global production. However, growing environmental stress factors pose a serious threat and could affect tomato production. Chilling, drought, and salinity are the major abiotic factors adversely affecting crop loss worldwide, and they pose a threat to the farming community. Low temperatures (i.e., chilling and freezing stresses) affect plants primarily through dehydration and membrane damage. Chilling tolerance has been achieved in different plants through genetic engineering approaches.

Antifreeze protein genes are highly induced during chilling stress in different plants. Tomato variety PKM1, released by Tamil Nadu Agricultural University, was genetically transformed with the carrot antifreeze protein (AFP) gene. The transgene integration and expression were analyzed in T_0 lines by Southern blotting and reverse transcription-PCR. Accumulation of *AFP* transcripts indicated that the gene was efficiently transcribed in a heterologous host system. Further, at chilling temperatures, ionic leakage from leaves was less in transgenic lines than in control lines. More importantly, transgenic tomato lines did not show any significant change in morphological and agronomical characteristics, including plant growth, height, number of leaves, branches, flowering period, as well as fruit size and shape.

Carrot antifreeze protein protected tomato plants from chilling temperatures through membrane stabilization.

Taken together, the study proved that carrot antifreeze protein protected tomato plants from chilling temperatures through membrane stabilization (Kumar S.R. et al. Acta Physiologiae Plantarum. 36:21-27). It has been found that levels of unsaturated fatty acids were higher in many chilling resistant plants. During chilling stress, the activity of the fatty acid desaturase (FAD) enzyme increases the amount of unsaturated fatty acids in the membrane. This modification allows cell membranes to remain stable at lower temperatures. Thus, desaturation of fatty acids provides tolerance to chilling temperatures.The *Arabidopsis thaliana FAD7* gene, under the control of the COR 15 promoter, was genetically transformed to tomato in an attempt to improve chilling tolerance. Generated transgenic lines were exposed to chilling stress to study their tolerance level. Transcript analysis by reverse transcription-PCR clearly indicated that *FAD7* was induced due to the chilling treatment (as it is under the control of the cold-induced promoter). Further, reduced ionic leakage in transgenic *FAD7* tomato plants indicated improved membrane stability during chilling stress.

Accumulation of osmolytes such as proline and soluble sugars is a common phenomenon. Transgenic *FAD7* tomato plants exposed to chilling stress accumulated more proline and total soluble sugars, revealing that, apart from membrane desaturation, osmolyte accumulation also contributes to chilling tolerance. Similar to our study with AFP, enhancement in chilling tolerance of *FAD7* overexpressing transgenic tomato lines did not affect the yield parameter under laboratory conditions, making it a potential candidate gene for crop improvement. Both our case studies clearly proved that engineered cold-tolerant crops could soon become a reality.

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EVENT	ORGANIZED BY	DATE	WEBSITE
INDIA & BANGLADESH			
State Level Biosafety Capacity Building Workshop, supported by the Phase II Capacity Building Project on Biosafety (Rahuri)	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur and Biotech Consortium India Limited (BCIL)	April 17, 2018 Rahuri, India	http://bcil.nic.in/
Training Workshop on Strengthening Capacities of Enforcement Agencies (Plant Quarantine & Customs Officials) for Transboundary Movement of LMOs, supported by the Phase II Capacity Building Project on Biosafety (Visakhapatnam)	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR)	April 19 – 20, 2018 Visakhapatnam, India	http://www.nbpgr.ernet.in/
State Level Biosafety Capacity Building Workshop, supported by the Phase II Capacity Building Project on Biosafety (Tirupati)	Acharya N. G. Ranga Agricultural University (ANGRAU) and Biotech Consortium India Limited (BCIL)	April 23, 2018 Tirupati, India	http://bcil.nic.in/
State Level Biosafety Capacity Building Workshop, supported by the Phase II Capacity Building Project on Biosafety (Udaipur)	Maharana Pratap University of Agriculture and Technology (MPUAT) and Biotech Consortium India Limited (BCIL)	April 27, 2018 Udaipur, India	http://bcil.nic.in/
INTERNATIONAL			
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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