



SOUTH ASIA
BIOSAFETY PROGRAM

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SABP

The South Asia Biosafety Program (SABP) is an international developmental program initiated with support from the United States Agency for International Development (USAID). The program is implemented in India and Bangladesh and aims to work with national governmental agencies to facilitate the implementation of transparent, efficient and responsive regulatory frameworks for products of modern biotechnology that meet national goals as regards the safety of novel foods and feeds and environmental protection.

SABP is working with its in-country partners to:

- Identify and respond to technical training needs for food, feed and environmental safety assessment.
- Develop a sustainable network of trained, authoritative local experts to communicate both the benefits and the concerns associated with new agricultural biotechnologies to farmers and other stakeholder groups.
- Raise the profile of biotechnology and biosafety on the policy agenda within India and Bangladesh and address policy issues within the overall context of economic development, international trade, environmental safety and sustainability.

MEANINGFUL BIOSAFETY AND BIO-EFFICACY ASSESSMENT OF GE CROPS IS FEASIBLE ONLY BY MULTI-LOCATIONAL CFTS AND NOT UNDER ARTIFICIAL GREENHOUSE CONDITIONS

Dr. S.R. Rao, Advisor and Dr. Sanjay Kalia, Scientist C,
Department of Biotechnology

In the past two decades, advancement in technologies have contributed immensely to understanding biological processes, cell and tissue culture and genetic manipulation of plants alike. Development of recombinant DNA (rDNA) technologies and genomics has provided a powerful tool for crop improvement through genetic engineering and precision molecular breeding. In contrast to conventional and molecular breeding for crop improvement, rDNA technologies have been seen with suspicion. Suspicion of and opposition to these technologies stems from a concern about their safety, to be consumed as food and feed and their effects on the environment. In order to mitigate these concerns adequate regulatory frameworks stipulating standards for experimental confined field trials (CFTs) are in place.

Apprehensions have been expressed on the imperative/necessity for experimental CFTs for GE crops although such trials are imperative for conventional and molecular breeding programmes. It has been suggested that these trials could be circumvented through greenhouse trials. The selection process of prospective events and hybrids is done in the greenhouse and is integral to crop improvement. Initial

selection data from the greenhouse provides information on survivability, genetic stability and phenotypic performance.

Data thus collected from the greenhouse is limited and needs to be replicated in CFTs as greenhouse conditions can't mimic the natural environment. Greenhouse data is also limited to an extent by the uniform conditions that are provided through all the critical processes of plant development as opposed to natural conditions where the plant is subjected to different environmental regimens. CFTs that are scientifically regulated give data about the performance of crop plants in different agro-climatic regimes where plants are subjected to the diverse interactions of microbes, competition, soil texture and water capacity. Therefore, it is imperative to replicate GE crops in CFTs spread over diverse agro-climatic regions as is the case with conventionally bred crops to give a real time estimate of their performance before they are commercially released. Compromising on this aspect will lead to erroneous speculation on trait issues and could lead to immense losses to farming communities.

Greenhouse-based evaluations of crop performance can lead to misleading calculations as reported in several publications with respect to the identification of promising technologies



Source: Flickr and Penn State

and elite varieties, even in cases of traditional breeding. A specific study called "Transgene x Environment Interactions in Genetically Modified Wheat" has been reported by scientists from the University of Zurich, wherein an experiment with similar design was conducted in the greenhouse and in the field to compare plant phenotypes and the impact of environmental factors on possible unintended effects of the introduced transgene.

The results indicated that in the greenhouse, lines of GM wheat carrying a fungal disease mildew resistance gene had a yield that was up to twice as high as that of control plants, whereas in the field this ratio was reversed for some of the lines. Differences in some other development parameters

(continued on page 2 - see Meaningful)

MEANINGFUL - continued from page 1

were also noted. The results of this study demonstrated that, depending on the insertion event, a particular transgene can have large effects on the entire phenotype of a plant and that these effects can sometimes be reversed when plants are moved from the greenhouse to the field. The complex relationship between plants and their environment is only revealed in field trials.

The evaluation of phenotypic performance in the natural environment is a key component of crop development, but field studies are particularly crucial in the development of new crops through genetic engineering. Field studies enable researchers to evaluate the environmental safety of GM plants and collect biosafety data required by regulatory authorities. In addition, plant material, such as seeds and forage, can be produced using small CFTs and collected to perform compositional analyses and other testing necessary to demonstrate food safety. Greenhouse studies cannot be performed at a scale sufficient to comply with these regulatory requirements. Without this field data, developers cannot make scientifically tenable predictions about the performance of the plants in the field or about the environmental safety of the plants.

CONFINED FIELD TRIALS: AN ESSENTIAL STEP IN THE DEVELOPMENT OF A GE CROP

Dr. O.P. Govila, Former Professor of Genetics, Indian Agricultural Research Institute, New Delhi -110012

Genetically engineered (GE) crops that are being developed for commercial cultivation must be tested under field conditions to study their effects on the environment. A matter of commercial necessity rather than regulatory compliance, developers also use the opportunity to evaluate the performance of GE crops in different agro-ecological conditions.

As GE crops may contain one or several more genes than conventionally bred crops, their field-testing is carried out under conditions that ensure that the materials tested remain within the trial site; and hence, such trials are referred to as CONFINED FIELD TRIALS (CFTs). CFTs of GE crops are similar to field trials done regularly for conventional breeding, except that they are confined to a particular site. Procedures for the conduct of CFTs are intended to accomplish three important goals:

- Preventing the escape from the trial site of novel genes in pollen, seed or other plant parts.
- Preventing GE plant material from being consumed by humans and/or animals.
- Preventing GE plants from establishing and persisting in the environment.

With the achievement of these three goals, novel genes and their products are confined to the field trial sites and their release into the general environment is prevented.

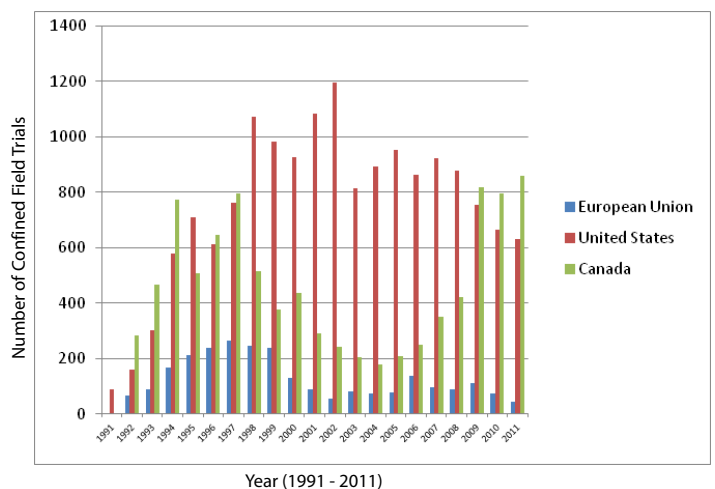
Generally, CFTs are conducted under the supervision of scientists from public or private research institutions after approval from regulatory agencies has been sought. These trials are usually carried out on a small scale, often on an area of not more than one hectare, at experimental stations such as those under the control of national agricultural research systems (NARS), local universities, or private sector research units. In cases where they have to be conducted in a farmer's field, the land has to be leased for a sufficient duration of time to ensure that post harvest monitoring and land use restrictions are assured. Organizations conducting CFTs are expected to be staffed by competent scientists with

sound experience in the safe conduct of field trials who have the capacity to evaluate the performance of new varieties for farmers.

It should be noted that the conduct of CFTs is an **ESSENTIAL** step in the process of developing and commercializing a GE plant. Because of concerns raised by NGOs and some decisions made by regulatory agencies from time to time, conduct of CFTs is becoming extremely difficult for both public and private sector organizations involved in the development of GE crops in this country. It is a common misunderstanding that confined field trials should be subject to essentially the same risk assessment process as for commercial releases, demonstrating that regulators, national biosafety committees, and sometimes capacity builders and trainers do not appreciate that the risk mitigation measures used to confine these trials render more extensive environmental risk assessment unnecessary.

In India the conduct of CFTs is regulated as per "Rules for the manufacture, use, import, export and storage of hazardous micro organisms, genetically engineered organisms or cells, 1989 (generally referred as Rules, 1989)" notified under the Environment (Protection) Act, 1986. An elaborate set of guidelines and standard operating procedures for the conduct of confined field trials of regulated, genetically engineered plants have been in place since 2008. Although the rules and guidelines are in place, concerns have been raised by civil society organizations about the conduct of field trials in the last five years. A public interest litigation (PIL) is also under the review of Hon'ble Supreme Court.

Yearwise Status of Approvals of Confined Field Trials in EU, US and Canada



It is worth noting that while the ultimate commercialization of GE crops depends on a number of factors, CFTs allow the research to move forward to promote scientific progress and inform decision making processes, which is why research is actively continuing in many countries despite controversy. For example, many countries in Europe routinely perform field trials of GE crops. A total of 2,581 notifications for confined field trials of GE crops were recorded in 21 European Union member countries in the twenty years from 1991 to 2011. Of those countries, France alone received 590 CFT notifications, followed by Spain with 569, Italy 295, UK 238 and Germany 183. The USA approved a total of 15,845 notifications for trials between 1992 and 2011. This compares to less than 100 confined field trials approved in India since 2005.

(continued on page 4 - see CFTs)



The Reading List

... new and notable articles

MARKER-FREE SITE-SPECIFIC GENE INTEGRATION IN RICE BASED ON THE USE OF TWO RECOMBINATION SYSTEMS

Nandy S, Srivastava V

Transgene integration mediated by heterologous site-specific recombination (SSR) systems into the dedicated genomic sites has been demonstrated in a few different plant species. This approach of plant transformation generates a precise site-specific integration (SSI) structure consisting of a single copy of the transgene construct. As a result, stable transgene expression correlated with promoter strength and gene copy number is observed among independent transgenic lines and faithfully transmitted through subsequent generations. Site-specific integration approaches use selectable marker genes, removal of which is necessary for the implementation of this approach as a biotechnology application. As SSR systems are also excellent tools for excising marker genes from transgene locus, a molecular strategy involving gene integration followed by marker excision, each mediated by a distinct recombination system, was earlier proposed. Experimental validation of this approach is the focus of this work. Using FLPe-FRT system for site-specific gene integration and heat-inducible Cre-lox for marker gene excision, marker-free SSI lines were developed in the first generation itself. More importantly, progeny derived from these lines inherited the marker-free locus, indicating efficient germinal transmission. Finally, as the transgene expression from SSI locus was not altered upon marker excision, this method is suitable for streamlining the production of marker-free SSI lines.

PLANT BIOTECHNOLOGY JOURNAL (2012) JUN 12. DOI: 10.1111/j.1467-7652.2012.00715.x. [EPUB AHEAD OF PRINT] <http://onlinelibrary.wiley.com/doi/10.1111/j.1467-7652.2012.00715.x/abstract;jsessionid=66BD7823CD916AC1D9D6BB43F79CFC51.d02t01?deniedAccessCustomisedMessage=&userIsAuthenticated=false>

DIVERSE GENETIC BASIS OF FIELD-EVOLVED RESISTANCE TO BT COTTON IN COTTON BOLLWORM FROM CHINA

Zhang H, Tian W, Zhao J, Jin L, Yang J, Liu C, Yang Y, Wu S, Wu K, Cui J, Tabashnik BE, Wu Y

Evolution of pest resistance reduces the efficacy of insecticidal proteins from *Bacillus thuringiensis* (Bt) used in sprays or in transgenic crops. Although several pests have evolved resistance to Bt crops in the field, information about the genetic basis of field-evolved resistance to Bt crops has been limited. In particular, laboratory-selected resistance to Bt toxin Cry1Ac based on recessive mutations in a gene encoding a toxin-binding cadherin protein has been identified in three major cotton pests, but previous work has not determined if such mutations are associated with field-selected resistance to Bt cotton. Here we show that the most common resistance alleles in field populations of cotton bollworm, *Helicoverpa armigera*, selected with Bt cotton in northern China, had recessive cadherin mutations, including the deletion mutation identified via laboratory selection. However, unlike all previously studied cadherin resistance alleles, one

field-selected cadherin resistance allele conferred nonrecessive resistance. We also detected nonrecessive resistance that was not genetically linked with the cadherin locus. In field-selected populations, recessive cadherin alleles accounted for 75-84% of resistance alleles detected. However, most resistance alleles occurred in heterozygotes and 59-94% of resistant individuals carried at least one nonrecessive resistance allele. The results suggest that resistance management strategies must account for diverse resistance alleles in field-selected populations, including nonrecessive alleles.

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA. (2012) JUN 26;109(26):10275-80. EPUB 2012 JUN 11. <http://www.pnas.org/content/early/2012/06/06/1200156109.abstract>

FUNGAL SOIL COMMUNITIES IN A YOUNG TRANSGENIC POPLAR PLANTATION FORM A RICH RESERVOIR FOR FUNGAL ROOT COMMUNITIES

Danielsen L, Thürmer A, Meinicke P, Buée M, Morin E, Martin F, Pilate G, Daniel R, Polle A, Reich M.

Fungal communities play a key role in ecosystem functioning. However, only little is known about their composition in plant roots and the soil of biomass plantations. The goal of this study was to analyze fungal biodiversity in their belowground habitats and to gain information on the strategies by which ectomycorrhizal (ECM) fungi form colonies. In a 2-year-old plantation, fungal communities in the soil and roots of three different poplar genotypes (*Populus × canescens*, wildtype and two transgenic lines with suppressed cinnamyl alcohol dehydrogenase activity) were analyzed by 454 pyrosequencing targeting the rDNA internal transcribed spacer 1 (ITS) region. The results were compared with the dynamics of the root-associated ECM community studied by morphotyping/Sanger sequencing in two subsequent years. Fungal species and family richness in the soil were surprisingly high in this simple plantation ecosystem, with 5944 operational taxonomic units (OTUs) and 186 described fungal families. These findings indicate the importance that fungal species are already available for colonization of plant roots (2399 OTUs and 115 families). The transgenic modification of poplar plants had no influence on fungal root or soil communities. Fungal families and OTUs were more evenly distributed in the soil than in roots, probably as a result of soil plowing before the establishment of the plantation. Saprophytic, pathogenic, and endophytic fungi were the dominating groups in soil, whereas ECMs were dominant in roots (87%). Arbuscular mycorrhizal diversity was higher in soil than in roots. Species richness of the root-associated ECM community, which was low compared with ECM fungi detected by 454 analyses, increased after 1 year. This increase was mainly caused by ECM fungal species already traced in the preceding year in roots. This result supports the priority concept that ECMs present on roots have a competitive advantage over soil-localized ECM fungi.

ECOLOGY AND EVOLUTION 2012 AUG;2(8):1935-48. EPUB 2012 JUL 12. <http://onlinelibrary.wiley.com/doi/10.1002/ece3.305/supinfo>

CALENDAR OF EVENTS

| Event | Organized by | Date and Venue | Website |
|---|---|---|---|
| INDIA | | | |
| 6th International Congress on Legume Genetics and Genomics | International Crops Research Institute for the Semi-Arid Tropics | October 2 - 7, 2012, Hyderabad | http://www.icrisat.org/gt-bt/VI-ICLGG/homepage.htm |
| Silver Jubilee International Symposium on "Global Cotton Production Technologies vis-à-vis Climate Change" | Cotton Research and Development Association and CCS Haryana Agricultural University, Hisar | October 10 - 12, 2012 Hisar | http://crdaindia.com/?view=news&page_id=16 |
| International Symposium on New Paradigms in Sugarcane Research | Society for Sugarcane Research and Development and Sugarcane Breeding Institute | October 15 - 18, 2012 Coimbatore | http://www.sugarcane.res.in/images/sbi/Centenary/1st_circular_int_symposium.pdf |
| Third National Symposium on Agriculture Production and Protection in Context of Climate Change | The Society of Agricultural Professionals, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, and Birsa Agricultural University, Kanke, Ranchi | November 3 - 5, 2012 Ranchi | http://www.baujharkhand.org/Downloads/online%20circular%20for%203rd%20national%20symposium.pdf |
| 44th Annual National Conference of the Nutrition Society of India (NSI) | Nutrition Society of India | November 16 - 17, 2012 Tirupati | http://www.nutritionocietyindia.org/ |
| Winter school on "Molecular breeding approaches for genetic enhancement in oilseed crops" | Directorate of Oilseeds Research | December 1 - 21, 2012, Hyderabad | http://dor-icar.org.in/media/docs/winter-school-dec-2012.pdf |
| AgTech Global Summit – 2012 | Bejo Sheetal Bio-Science Foundation and Maryland India Business Round Table | December 9-13, 2012, Aurangabad | |
| INTERNATIONAL | | | |
| 12th International Symposium on Biosafety of Genetically Modified Organisms (ISBGMO12) | International Society for Biosafety Research | September 16 – 20, 2012 St Louis, Missouri, USA | http://www.isbgmo.com/ |
| Sixth meeting of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety (MOP-6) | Convention on Biological Diversity (CBD) and MoEF | October 1 - 5, 2012 Hyderabad | http://www.cbd.int/doc/?meeting=MOP-06 |
| Eleventh meeting of the Conference of the Parties to the Convention on Biological Diversity (COP-11) | CBD and MoEF | October 8 - 19, 2012 Hyderabad | http://www.cbd.int/doc/?meeting=cop-11 |
| 1st International Conference for GM Crops and Food | Faculty of Agriculture, Cairo University | November 27 – 29, 2012 Cairo, Egypt | http://www.icgmc.com/ |
| International scientific workshop 'Non-target organisms and GM crops: Assessing the effects of Bt proteins' | November 29 - 30, 2012 Amsterdam, The Netherlands | November 29 - 30, 2012 Amsterdam, The Netherlands | http://www.cogem.net/index.cfm/en/symposium/ |

CFTs - continued from page 3

It is to be understood that CFTs are very different from **commercial releases**. Separate questions, separate review and approval processes are followed in every country for the two steps. The focus in CFTs is toward ensuring confinement measures, and not asking for elaborate safety data, as safety data as a prerequisite to their approval is actually generated during the CFTs. These trials are essential to the

collection of biosafety data to meet regulatory requirements for assessing environmental and food assessment data. CFTs finally allow an opportunity to evaluate trait efficacy and agronomic performance in different agro-climatic conditions.

It has been well established globally that confined field trials **CAN** be performed **safely** and **routinely** by focusing on material and genetic confinement measures.

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