



ISB NEWS REPORT

COVERING AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY DEVELOPMENTS

MARCH 2004

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NEWS AND NOTES

BIOTECHNOLOGY BIODIVERSITY INTERFACE GRANTS: REQUEST FOR APPLICATIONS

The Program for Biosafety Systems (PBS) is pleased to announce a request for applications for research projects funded by the Biotechnology Biodiversity Interface Grants program. The funding for these research grants is intended to enhance the capacity of developing countries in Asia and Africa to make regulatory decisions concerning the environmental impact of transgenic crops, genetically engineered livestock and fish, and recombinant livestock vaccines. By funding research focused on the needs of developing countries, PBS hopes to support science-based decision making and policy development in those countries. The deadline for applications is **April 15, 2004**. To access the RFA, please navigate to http://www.isb.vt.edu/grants/PBS_rfp.pdf. Applications are welcome from our developing country partners, SROs, crop research networks, IARCs, and U.S. universities, among others.

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REGULATION OF AG BIOTECH CROPS AND FOOD IN THE UNITED STATES

The U.S. Government has established a new website which provides information on the U.S. oversight system for products of modern biotechnology. It includes information on the roles of the regulatory agencies and links to relevant statutes and regulations. The centerpiece of the website is a searchable database containing information on all genetically engineered crop plants intended for food or feed that have completed the recommended or required reviews for food, feed, or planting use in the United States. Construction of the website and database has been a joint effort undertaken by the Department of State (DOS), the U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA) and the U.S. Geological Survey (USGS). The website is located at <http://usbiotechreg.nbii.gov>.

THE ISB NEWS REPORT

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RISK ASSESSMENT

COMPARING ENVIRONMENTAL AND HEALTH BURDENS OF TRADITIONAL VS. GM BEET

Richard Phipps and Richard Bennett

Background

The introduction of new technology has often been controversial. For example, an anti-vaccination society actively campaigned against the use of smallpox vaccine developed by Jenner, while the introduction of pasteurization of milk met with stiff opposition. The use of GM crops is the most recent example of a controversial new technology. Despite the fact that since 1996¹ nearly 250 million ha of genetically modified (GM) crops have been grown in 16 countries worldwide by six million farmers, there is still an ongoing debate, restricted mainly to the European Union (EU), concerning their effect on the environment and human health.

In recent years the reduction in pesticide use in agriculture has been a key policy objective for many governments in the EU, and as such, a reduction in pesticide use is generally considered an environmental benefit. Progress in this area has occurred through the introduction of a range of management and incentive schemes. Several authors have also published work showing that the introduction of GM crops has effected a significant reduction in pesticide use on a global basis and have estimated the reduction that would occur in European and UK agriculture should GM crops be commercialized^{2,3,4,5,6}.

The results of the UK Farm Scale Evaluation of spring-sown GM crops have recently been published in the Philosophical Transactions of the Royal Society (2003) and are a benchmark study in the field of ecology. They compared the effects on the biodiversity and abundance of farmland wildlife of conventional herbicide practice with that used in herbicide tolerant spring sown GM crops. The paper by Champion *et al.*⁷ reported that, in the case of the GM sugar beet, the number of spray applications was reduced from 3.65 to 1.65, the number of active ingredients used was reduced from 8.05 to 1.95, and the weight of active ingredient applied was decreased from 2551 to 1637 g/ha, a reduction of 36%. The same pattern occurred with fodder beet and forage maize but not with spring rape.

While the Farm Scale Evaluations assessed the effects of introducing GM crops on the abundance and diversity of farmland wildlife, the study only considered one aspect of the entire complex system of crop production and did not provide a full picture of the effect of introducing GM crops on the environment and human health. Phipps and Park², while noting changes in cropping management associated with the introduction of GM crops,

recognized that further complex calculations would need to include not only different energy costs of pesticide production but also the fact that the use of less pesticide will require less raw materials and inerts, fewer manufacturing plants, less diesel in the manufacturing process, less fuel for shipment and storage, less water and fuel used in spraying, and, of course, less packaging for their containment and distribution in order to obtain the full impact of this new technology.

As technical advances become available, there is a need for objective assessments of the complete system or processes involved. In the case of assessing environmental impacts of new technologies, use of Life-Cycle Assessment⁸ (LCA) enables ‘the compilation and evaluation of the inputs, outputs, and potential environmental impacts of a product or system throughout its life cycle’ according to internationally agreed standards. Although LCA has been applied to industrial processes and products, it has only recently been applied in agriculture. Following accepted guidelines, the authors applied LCA to estimate the environmental burdens associated with growing GM herbicide-tolerant and conventional sugar beet in the UK and Germany.

Life-Cycle Assessment Methodology and Results

In order to apply an LCA to conventional and herbicide-tolerant sugar beet production systems in the UK and Germany, the systems were first defined and then modeled using PIRA Environmental Management System (PEMS) software⁹. These models included the manufacture, packaging, and transport of the herbicides and the use of farm machinery for field operations. Inventories of inputs and outputs in the form of energy and natural resource use, waste products, and emissions into the environment (comprising around 250 different chemical compounds/categories) associated with each part of the defined systems were compiled on spreadsheets and linked to the PEMS models. Models of spray dispersion were used to estimate the amounts of each spray formulation that were deposited onto plants, reached the soil, evaporated or drifted into the air, were likely to be lost by drainage to groundwater, and were lost by biodegradation in top soil, taking account of the half-life of each active ingredient and the growth stage of the crop at the time of spraying.

The impacts of these emissions, which were produced at various stages of the production cycle, were then grouped together in a range of different impact categories. These categories included the amount of energy used, the effect of carbon dioxide and other gases on global warming, the production of chemicals that could contribute to the depletion of the ozone layer, potential effects on water quality, and the production of sulphur dioxide, which contributes to

the production of acid rain. In addition, the effect of emissions produced on a number of human health impact categories, such as respiratory problems and carcinogenic effects, were also considered.

In each impact category, the LCA showed that the GM production system had lower values than the conventional systems. The qualitative results shown below strongly suggest that, when a holistic approach is taken, the use of GM varieties of sugar beet offers positive environmental and human health benefits when compared with conventional production systems.

Using Impact Categories described above: Is GM Better or Worse?

	+ as good, ++ better, +++ much better
Energy (MJ)	+
Global Warming (kg CO2 equ.)	++
Ecotoxicity (kg Cr equ.)	+++
Acidification (kg SO2 equ.)	++
Nutrition (kg PO4 equ.)	++
Toxic particulates (PM)	++
Carcinogenicity (kg PAH equ.)	++

Conclusions

The potential impact of GM crops on the environment and human health compared with conventional crops is a key topic within the GM debate. In the case of GM herbicide-tolerant sugar beet, results of the LCA suggest that growing the GM variety would produce fewer emissions that can potentially harm either the environment or human health when compared with the typical herbicide regimes currently being used on sugar beet in the UK and Germany.

It is hoped that the preliminary LCA described here, although it relates to just one case study of a GM crop in two countries of Europe, will stimulate interest in the use of LCA to help assess the potential environmental and human health impacts of GM crops and add to the current GM debate.

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P L A N T R E S E A R C H

DNA-BASED METHODS IN SORGHUM DIVERSITY STUDIES AND IMPROVEMENT

Tawanda Zidenga

Introduction

Sorghum is the fifth most important grain crop in the world after wheat, maize, rice, and barley, and the second most important cereal crop (after maize) in sub-Saharan Africa. It is thought to have originated from Northeast Africa (the Ethiopia, Eritrea area), the center of diversity for the crop. Sorghum is cultivated mostly in the developing world (especially in Africa and Asia), although it has become an important industrial crop in many developed countries. Its value in arid climates is due to its ability to withstand dry conditions. The International Crop Research Institute for Semi-Arid Tropics (ICRISAT), located in India, serves as a world center for improving sorghum grain yield and quality.

Genetic improvement of sorghum can help farmers in semi-arid areas where sorghum is a key food crop. In the past, studies have been devoted to assessing patterns of sorghum genetic variation based on morphology or pedigree. However, this approach has its limitations. Complex quantitatively inherited traits are difficult to trace based solely on morphology. For this reason, DNA-based methods have been employed in studies of sorghum genetic diversity and in genetic improvement of the crop.

Molecular markers in sorghum diversity studies

Molecular markers are considered constant landmarks in

the genome. Markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. In contrast to morphological markers, which are based on visible traits, and biochemical markers, which are based on proteins produced by genes, molecular markers rely on a DNA assay.

There are many different kinds of molecular markers including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites, and single nucleotide polymorphisms (SNPs). There are merits and demerits of each type of marker, depending on the specific objectives of the particular study. RFLP is based on detection of variations in restriction fragment length of specific DNA sections among individuals, as determined by the presence or absence of restriction sites. It is possible to locate a gene by looking for RFLPs that are almost always inherited with it. RAPDs are DNA fragments, which are polymorphic in size, generated by PCR using one or two randomly selected oligonucleotides or primers. They are dominant markers and RAPD patterns can be used in strain identification (one of the methods of DNA fingerprinting). In AFLP, DNA treated with restriction enzymes is amplified using PCR. The method allows selective amplification of restriction fragments, giving rise to large numbers of useful markers that can be located on the genome relatively quickly and reliably. This technique, however, is patented, with the patent held by Keygene, a private company in Netherlands (<http://www.keygene-products.com>). SNPs are single base changes in the nucleotide sequence of DNA. The potential number of SNP markers is very high, meaning it should be possible to find them in all parts of the genome, and microarray procedures have been developed for automatically scoring hundreds of SNP loci simultaneously. Microsatellites (or Simple Sequence Repeats (SSRs)) are short DNA sequences, usually two to three bases long, repeated a variable number of times in tandem. They are distributed evenly throughout the genome and are thus frequently used as landmarks for gene mapping.

Diversity studies have been carried out in the Ethiopia/Eritrea area, which, like most areas, is threatened by loss of landraces due to introduction of improved varieties from elsewhere. Evaluating germplasm diversity can help to identify landraces with the greatest novelty and thus are most suitable for rescue or incorporation into crop improvement programs. Genetic distance estimates determined by molecular markers help identify suitable germplasm for incorporation into plant breeding stocks. The greatest

potential for markers perhaps is their potential to accelerate the rate of gain from selection for desirable genotypes and in the manipulation of quantitative trait loci (QTLs). Researchers at Purdue¹ conducted experiments on QTL analysis of drought tolerance in sorghum and identified regions of the sorghum genome that condition the expression of drought.

A set of 15 SSRs has been developed for sorghum. Compared to other markers, SSRs exhibit uniform genome coverage, high levels of polymorphism and co-dominance. They are detected by specific PCR-based assays and can be used for pedigree analysis because they represent single loci and can uniquely define genotypes. Agrama and Tuinstra² at Kansas State University recently compared SSRs with RAPDs. Their results indicated that SSRs were highly polymorphic compared to RAPDs (which had nearly 40% monomorphic fragments). Earlier, Ghebru³ *et al.* also reported genetic diversity of Eritrean sorghum landraces using SSRs.

Genetic transformation of sorghum

Sorghum has trailed behind other cereals in the progress towards genetic transformation. Its transformation has been hampered by tissue culture limitations, including low regeneration frequencies and accumulation of phenolic pigments. Researchers have partially addressed this problem by adopting strategies such as optimization of bombardment, minimization of pigment production, and adjustment of media composition for efficient shoot regeneration. Jeoung⁴ *et al.* demonstrated the use of GFP as a reporter for optimizing the conditions for successful transient expression during *Agrobacterium*-mediated transformation of sorghum. Using GFP, they were able to determine the suitability of different inbreds for *Agrobacterium*-mediated transformation.

Recently, Tadesse⁵ *et al.* reported optimization of transformation conditions and production of transgenic sorghum via microprojectile bombardment. In this study, transgenic sorghum was produced by combined use of optimized bombardment conditions, strong monocot gene promoters, and stepwise antibiotic selection. Tadesse and coworkers have also used the same conditions to generate transgenic sorghum plants expressing the *dhdps-raec1* mutated gene encoding an insensitive form of the dihydrodipicolinate synthase, the key regulatory enzyme of the lysine pathway. It is hoped that overexpression of this gene will lead to sorghum lines with elevated lysine content.

Together, marker assisted selection and genetic transformation will help improvement of sorghum both as a food

crop and as an industrial crop. An important development has been the Sorghum Genomics Project, Texas A & M University (<http://sorghumgenome.tamu.edu/>). The project was initiated out of a realization that sorghum could be a key species for comparative analysis of grass genomes, as well as a source of beneficial genes for agriculture. A comparison of rice, a C3 plant that is well adapted to wet environments, and sorghum, a C4 plant adapted to arid environments, may elucidate the combination of genetic traits required for adaptation to each of these extremes of agriculture. Genetic mapping studies have revealed that genes for various traits are found on the same place on the chromosomes of similar organisms. For example, the genes for traits such as digestibility, dwarfism, and waxy skin (which protects a plant during times of drought) are found in the same approximate location on cereal grains such as wheat, maize, sorghum, and rice. Maize, however, has about three times as much genetic material as sorghum. Therefore, it should be easier to locate a desired trait in sorghum and then find the gene in maize, than it is to search through maize's entire genetic library. Information derived from such an initiative will be of relevance both to crop improvement by transgenesis and plant biology in general.

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REGULATORY NEWS

GMOs SPAWN REGULATORY CHALLENGES

Phillip B. C. Jones

On January 20, the U.S. National Academies' National Research Council announced the release of its report on

strategies for biologically confining genetically modified organisms. The Committee on the Biological Confinement of Genetically Engineered Organisms cautioned that far more work is required to ensure that GMOs do not contaminate the food supply or eliminate natural species.

Commissioned by the Department of Agriculture, the NRC committee considered how bioconfinement techniques can prevent GMOs from escaping into natural ecosystems where they may breed with or compete against their wild-type counterparts or pass GM traits to other species. The committee stressed that confinement options vary with the precise species selected for transformation due to variations in genetics, ecology, and dispersal biology.

What are these bioconfinement measures? An approach for biologically confining GM mollusks is to generate three sets of chromosomes in the animal's cell nuclei; triploidy prevents successful cell division and reproduction. The two main bioconfinement methods used in microbes are the induction of suicide genes and "phenotypic handicapping," a tactic that renders the microbes less suited for competition with indigenous counterparts. For genetically engineered plants, bioconfinement techniques include interference with sexual and vegetative reproduction and the use of a specific artificial stimulus to limit the expression of the transgenic trait. A salicylic acid spray, the report suggests, could provide the sole stimulus for expression of a GM plant's transgene that synthesizes a pesticide protein.

Bioconfinement methods are expected to work best on a small scale; the NRC warns that the efficiency of these strategies decreases with the number of GMOs involved and the size of the area they occupy. Moreover, the probability of failure increases with the amount of time that the GMOs persist in the natural environment.

To ensure confinement efficacy, the NRC recommends that bioconfinement strategies should be devised at the earliest stages of GMO development. And because no single bioconfinement method is likely to be 100 percent effective, developers of GMOs could implement redundant systems to decrease the chance of a failure, such as a combination of biological, physical, and chemical confinement measures. Taking aim at biopharming, the NRC advises that alternative nonfood host organisms should be sought for genes encoding products that must be excluded from the human food and animal feed supplies. Researchers have genetically engineered many nonfood plants, such as tobacco, petunia, and duckweed. In passing, the NRC notes that a decision not to produce a particular GMO is also a form of bioconfinement.

A copy of the NRC report, "Biological Confinement of Genetically Engineered Organisms," is available at The National Academies Press website (<http://www.nap.edu/books/0309090857/html>).

GM Bugs: An Oversight in Regulatory Oversight

The NRC committee report warns that there has been little research on the bioconfinement of GM insects and that the subject is inadequately understood. On January 22, the Pew Initiative on Food and Biotechnology echoed this sentiment with the release of its study on insect engineering. During an online discussion hosted by the *Washington Post*, Michael Fernandez, Director of Science for the Pew Initiative, explained that his organization "published this report in the hopes of jump-starting the dialogue about the benefits, risks, and regulatory challenges of GM insects."

The Pew Initiative report surveys efforts to genetically modify insects to benefit public health, enhance agriculture, and provide new forms of economically useful insects. Public health may be enhanced, for example, by the release of GM mosquitoes that inject antigens into humans to induce an immune response against a variety of diseases. Mosquitoes can also be engineered to produce proteins that disrupt the malaria parasite's life cycle within the insect.

Researchers are investigating a different tactic to stop the spread of Chagas' disease, which is transmitted by infected kissing bugs. In the paratransgenic strategy, an insect is not genetically engineered, but rather harbors symbiotic bacteria that have been genetically altered. To control Chagas' disease, a bacterium that lives in the bug's gut will be engineered to kill the parasite that causes the disease as it passes through the insect's digestive system. A similar scheme may be used to control the spread of sleeping sickness: tsetse flies can be outfitted with paratransgenic symbionts that hinder the development or transmission of trypanosomes.

GM insects may also be used in genetic control programs to fight insect pests as an alternative to pesticides. Radiation or chemical mutagenesis is used to affect the reproductive ability of a target insect population. Mediterranean fruit flies, for example, are mass-reared and sterilized by irradiation prior to the release of males. After liberation into the environment, the sterile males mate with wild-type females, hindering the ability of the females to reproduce.

Another conventional genetic control program targets the pink bollworm, which feeds on cotton plants throughout most of the southwestern cotton-growing states in the United States—except for California's San Joaquin Valley.



Here, a sterile insect technique program protects more than 900,000 acres of cotton. The program requires millions of male pink bollworm moths, which are sterilized by irradiation and released to suppress reproductive success of wild-type females. The sterile moths, weakened by exposure to radiation, need to outnumber wild-type fertile moths by nearly 60:1, resulting in costs that are prohibitive for implementation in other areas. One way to eliminate this need for large numbers of irradiated males is to genetically engineer bollworms to carry a gene that would prevent offspring from developing.

Genetic engineering can also be used to enhance the characteristics of economically beneficial insects, such as the honeybee. Honeybee populations have decreased during the past ten years due to the insect's susceptibility to diseases and parasites. Researchers are devising ways to engineer honeybees that are more resistant to diseases, parasites, and certain insecticides. Another useful insect, the silkworm, can be modified to produce a therapeutic protein or spider silk, a material that could be used to make bulletproof vests, parachutes, and artificial ligaments.

Despite the potential benefits of GM insects, the technology does present concerns about the long-term effects of these designer bugs on the environment, public health, and food safety. GM insects could disrupt ecosystems in unintentional ways and pass on new traits to wild-type insects. As noted in the NRC report, hordes of GM insects will have to be released into the natural environment to have an effect. These engineered insects cannot simply be recalled after they have performed their service. Fortunately, U.S. regulatory agencies maintain a tight watch on insect engineering. Right? Well, not quite.

Established in the mid-1980s, the U.S. Coordinated Framework for Regulation of Biotechnology directs the Food and Drug Administration, the Department of Agriculture, and the Environmental Protection Agency to regulate biotech products according to their intended use. For example, the FDA regulates food and feed; the USDA regulates plant pests, plants and veterinary biologics; and the EPA oversees the use of pesticides and novel microorganisms. GM insects do not fit neatly into any pigeonholes supplied by the regulatory framework.

Moreover, the Pew Initiative found that federal agencies have not indicated if they would regulate GM insects, how regulatory reviews would be performed, which agencies would be involved, or how the agencies would coordinate their efforts. Michael Rodemeyer, executive director of the Pew Initiative, warns that without a clear roadmap for

regulation "the public has little reason to trust that the risks and benefits are being appropriately weighed and measured." The Pew Initiative recommends that the United States should clarify its policies for regulating GM insects before the need arises to advise international bodies on the management of insect engineering.

See the *Pew Initiative on Food and Biotechnology report "Bugs in the System?"* <http://pewagbiotech.org>.

USDA Recommends Regulation Renovation

During a January 22 press conference, Agriculture Secretary Ann M. Veneman announced her agency's intent to update and strengthen biotech regulations for the importation, interstate movement, and environmental release of GMOs. Secretary Veneman stated that the regulatory framework must keep pace with technological advances to ensure that regulations are based on sound scientific principles and mitigation of risk. Moreover, she sees "a system that will place a greater emphasis on risk and additional flexibility for products that have demonstrated their safety." The anticipated result of the regulatory overhaul is that certain biotech products will be subjected to less regulation, whereas products associated with a greater perceived risk would be regulated more stringently.

As a first step, the USDA's Animal and Plant Health Inspection Service will prepare an environmental impact statement evaluating biotech regulations and proposing changes. One USDA proposal is to broaden APHIS' regulatory scope to include GMOs that may be used as biological control agents. The agency defines a "biological control organism" as any organism used to control a plant pest or noxious weed; presumably, certain GM insects would be covered under this category. The USDA promises to consider all comments on its proposals that it receives on or before March 23.

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