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In This Issue:

PLANT RESEARCH NEWS

Development of Rice Seed Based Edible Vaccine for Allergic Immunotherapy

Fumio Takaiwa

More than 30% of the population in developed countries is afflicted with IgE-mediated type I allergic diseases, such as seasonal and perennial rhinitis, asthma, and atopic dermatitis. The majority of these IgE-mediated type I allergies are treated symptomatically by blocking the release of chemical mediators, such as histamine, and leukotrienes and prostaglandins, which cause allergic inflammation. Therefore, although a complete cure of allergic disease is desired, only allergen-specific immunotherapy through desensitization to allergens is currently available.

Allergen-specific immunotherapy is a technique by which tolerance to allergens is developed over time by repeatedly exposing a person to native allergens or allergen extracts in increasing amounts. The treatment usually requires about three years to complete. Pain may accompany the subcutaneous injections, and there is a risk of side effects, such as anaphylatic shock. Therefore, the development of a safe, convenient, and time-saving immunotherapy treatment is desired.

Peptide immunotherapy using a T cell epitope peptide, which directly targets inactivation of specific T cells, is the second generation line of treatment for allergies. This immunotherapy method involves interrupting T cell activation at the initiation step of the immune response during presentation of the antigen. The technique is considered safe and effective for the control of IgE-mediated allergic diseases, because only parts of the allergen, called T cell epitopes, are used as tolerogen. Consequently, binding of the allergen to the allergen-specific IgE antibody can be avoided, and high dose administration of epitope peptide is possible. Furthermore, when T cell epitope peptides are orally administrated, mucosal and systemic immune tolerance to the allergen is expected to be specifically induced. Therefore, peptide immunotherapy is relatively simple and is potentially much safer than subcutaneous injections.

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Because an allergic response results from the downstream effects of Th2-type cytokines produced by T cells in response to an allergen, the goal of immune tolerance therapy (desensitization to allergen) is therefore to either induce inactivation (anergy) or deletion of the allergen-specific Th2 cells, or to achieve the active suppression of cytokine production by regulatory T cells, such as Th3 or Tr1 cells, that release transforming growth factor (TGF)-β or IL-10. The determination of which immune suppression mode results is dependent on the applied dose of allergens.

We recently presented a new cost-effective, simple, oral immunotherapy that uses a seed-based peptide vaccine. When predominant T cell epitope peptides, which were derived from Japanese cedar pollen allergens, were specifically expressed in rice seed and delivered to the mucosal immune system, the development of an allergic immune response of the allergen-specific Th2 cell was suppressed. Furthermore, not only were specific IgE production and release of histamine from mast cells suppressed, but the inflammatory symptoms of pollinosis, such as sneezing, were also suppressed. These results suggest the feasibility of using an oral immunotherapy agent derived from transgenic plants that accumulate T cell epitope peptides of allergens for allergy treatment.

Expression of T cell epitope peptides in rice seed has several advantages over expression in other tissues, such as leaf or root. When the seed expression system is used as a platform for foreign protein production, substantial amounts of recombinant proteins can be accumulated, because the seed is a natural storage organ for accumulating the starch, protein, and oil required for seedling growth. Also, artificial peptides or proteins accumulate in seed, which is in remarkable contrast with other tissues.

Because of these advantages, we developed a production platform for accumulating recombinant proteins in rice seed. We first characterized the seed promoters suitable for the expression of foreign genes in appropriate sites of the rice seed. After demonstrating that the individual promoters of the storage proteins glutelin, globulin, and prolamin directed high levels of expression at different sites in the endosperm, we decided to use primarily the major seed storage protein glutelin GluB-1 promoter for expression of foreign genes. In addition, we demonstrated that the accumulation levels of the foreign protein were enhanced when expressed within the genetic background of a low storage protein character.

Japanese cedar pollen allergic disease (pollinosis) is the most predominant pollen allergy in Japan. About 20%

of Japanese (23 million patients) suffer from this pollinosis from February to April each year. However, because 50% to 60% of Japanese people are sensitized to IgE (IgE antibody-positive to cedar pollen allergens), the number of pollinosis patients is expected to gradually increase. Therefore, there is a national demand to control this allergic disease in Japan.

Two major pollen allergens, designated Cry j 1 and Cry j 2, have been isolated and characterized, and more than 90% of patients have IgE antibodies specific to both Cry j 1 and Cry j 2 allergens. Immuno-dominant multiple T cell epitopes have been identified for humans and mice, and synthetic genes coding for these epitopes have been created using codons corresponding to the individual amino acids that are preferentially used in rice seed storage protein genes. For mice, the codon-optimized T cell epitopes were expressed as a component of the soybean seed storage protein glycinin. Seven predominant human T cell epitope peptides were linked and produced as a hybrid peptide (numbers three and four were derived from Cry j 1 and Cry j 2, respectively). It should be noted that mice and human T cell epitopes are not essentially overlapped. Therefore, since the efficacy of rice seed containing human T cell epitopes can not be examined using the mice system, we investigated whether oral feeding of transgenic rice containing mice T cell epitopes could induce oral immune tolerance.

The predominant two T cell epitopes derived from cedar pollen allergens, Cry j 1 and Cry j 2, were inserted into variable regions of acidic and basic subunits of soybean glycinin. Since the short peptides cannot be directly expressed, it is reasonable to assume that they were expressed as a fusion protein with the soybean seed storage protein, glycinin. When this fusion protein gene was expressed under control of the glutelin GluB-1 promoter, the modified glycinin-containing T cell epitopes were specifically accumulated in the seed at 0.5% of total seed protein (about 7µg in one grain).

Oral administration of 10 grains containing 70µg of modified glycinin to mice every day for one month prior to the systemic challenging with cedar pollen allergen inhibited the development of allergen-specific serum IgE and IgG antibodies and the CD4+ T cell proliferative response. The levels of CD4+ derived cytokines, such as IL-4, IL-5, and IL-13, associated with Th2 allergy and histamine release in serum were significantly suppressed. It should be noted that development of pollen-induced clinical symptoms such sneezing was significantly suppressed in the experimental mouse model. These results indicate the potential of transgenic rice seeds to produce and deliver allergen-specific T cell epitope peptides to the mucosa for the induction of an oral tolerance to pollen allergens. Therefore, we conclude it is feasible to develop an effective peptide-based oral vaccine for allergy treatment using cereal seeds.

We have created transgenic rice plants that accumulated the seven linked major human T cell epitope peptide (7Crp) derived from the Cry j 1 and Cry j 2 allergens. This 7Crp peptide was highly accumulated in the endosperm tissue, at up to 50-60µg /grain, accounting for about 4% of total seed protein. This high accumulation level was achieved by fusing signal peptide and KDEL ER-retention signals at the N and C termini of the 7Crp peptide, under control of the glutelin GluB-1 promoter. The presence of the glutelin signal peptide was essential for accumulation of the 7Crp peptide. The 7Crp peptide is mainly deposited in protein bodies I and II, in which rice seed storage protein prolamin and glutelin or globulin are stored, respectively.

Traditional vaccines, produced using animal or bacterial cells, must be extracted and purified. However, when the vaccine is produced in edible parts of cereal crops, the complex purification process is not required. We show that oral immune tolerance can be easily induced by direct administration of rice seeds containing the T cell epitope peptide. This oral immune tolerance was associated with the reduction of specific T cell proliferative activities, which resulted in the suppression of release of allergen specific cytokines, IL-4, IL-5, and IL-13, and led to alleviation of clinical symptoms through the reduction of specific IgE and histamine production. Taken together, our results indicate that these seeds are effective as a tolerogen for desensitization to allergy disease.

It is important to note that the T cell epitope peptide, which accumulated in rice seed, is

heat resistant, and that T cell proliferative activity (immunogenicity) is retained even after boiling the grains at 100 °C for 20 min., i.e., delivery from steamed rice is possible. This is in remarkable contrast to traditional plant-incorporated edible vaccines against pathogens of infectious diseases, which require delivery in raw form because of sensitivity to heat. Furthermore, compared to other production systems, the seed production system has a low risk of contamination of animal pathogens (animal virus and virion). Recombinant proteins accumulated in seed are highly stable for more than one year, even stored at room temperature, and thus have no need for cold chain (refrigeration) for delivery. There is no requirement for specific equipment or facility for production. The seed system can be easily scaled up, and control of the production scale is easily dependent upon demand. These advantages allow a low cost production platform for seed-based edible vaccine for allergies.

However, there are many hurdles to overcome before this technology is ready for practical use. A primary concern is the contamination of non-transgenic rice seeds by out-crossing in the field or in the process of transportation. Until we are able to assure the security of food rice, we have to pay close attention to the probability and severity of the potential risks of edible vaccine rice in all phases of production, distribution, and use. Avoidance of contamination into the food chain requires establishing reliable methods of traceability.

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Progress in Molecular Approaches to Drought Tolerance in Crop Plants

Tawanda Zidenga

Introduction – drought and agriculture

Dehydration stress is one of the most serious yield-reducing stresses in agriculture. Drought stress is especially important in countries where crop agriculture is essentially rain-fed. In sub-Saharan Africa, drought years have a devastating effect on regional food security. While irrigation is the method of choice in averting drought stress in many areas of the world, alternative low-input approaches are being explored, and biotechnology offers a promising array of tools that may be useful in achieving drought tolerance in plants. One such tool is the low input approach to crop production by which crops are modified to suit the environment in which they are growing, rather than modifying the environment to meet the needs of the crop. This approach is advantageous in areas where water supplementation by irrigation is either difficult or unaffordable.

What happens to plants during drought?

Drought stress causes an increase in solute concentration in the environment, leading to an osmotic flow of water out of plant cells. This in turn causes the solute concentration inside plant cells to increase, thus lowering water potential and disrupting membranes along with essential processes like photosynthesis. These drought-stressed plants consequently exhibit poor growth and yield. In worst case scenarios,

the plants completely die. Certain plants have devised mechanisms to survive under low water conditions. These mechanisms have been classified as tolerance, avoidance, or escape.

ROSes may be bad

Central to signal transduction pathways related to drought and other stresses are reactive oxygen species (ROS), which are molecules formed by the incomplete one-electron reduction of oxygen. Under stress, ROS formation is usually exacerbated. Drought stress leads to the disruption of electron transport systems; therefore, under water deficit conditions, the main sites of ROS production in the plant cell are organelles with highly oxidizing metabolic activities or with sustained electron flows: chloroplasts, mitochondria, and microbodies.[1] ROS are generally damaging to essential cellular components, and plants have evolved various ROS scavenging mechanisms. These include the enzymes superoxide dismutase (SOD), catalase, and peroxidases, as well as oxidized and reduced glutathione.[1] **Fig. 1** shows the relationship between ROS-scavenging mechanisms, stress, and the damage to cellular membranes and macromolecules.

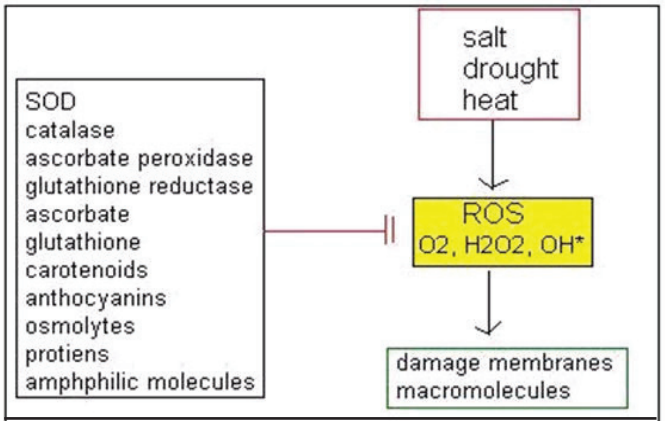

Figure 1. Reactive oxygen species pathway. (*Source: http://dragon.zoo.utoronto.ca/~B03T0301D/: Drought Tolerance in Agriculture*[3])

Researchers have focused on expressing genes for enzymes involved in ROS scavenging to enhance plant protection against oxidative stress. Transgenic alfalfa (*Medicago sativa*) expressing Mn-superoxide dismutase cDNA tended to have reduced injury from water-deficit stress, and this improvement was also seen in field trials in yield and survival.[5]

Secrets of resurrection

What does it take to rise from the dead? This is a question scientists working on resurrection plants have been exploring recently. Resurrection plants can tolerate almost complete water loss in their vegetative parts.[2] At the University of Cape Town in South Africa, researchers are trying to unlock the secrets of the resurrection plant *Xerophyta viscosa* in an attempt to achieve drought tolerance in crops.[1] These plants can be a source of drought tolerance genes for transgenic crop improvement. To withstand periods of drought, resurrection plants practically "die" (by losing all their vegetative parts) and then "rise again" when water becomes available. Their vegetative tissues lose all free water and then rehydrate once water becomes available again. Resurrection plants minimize ROS formation and also upregulate various antioxidant protectants during drying and rehydration.[1] The group has identified a novel stress-inducible antioxidant enzyme, *XvPer1*, by differential screening of a cDNA library of *X. viscose*.

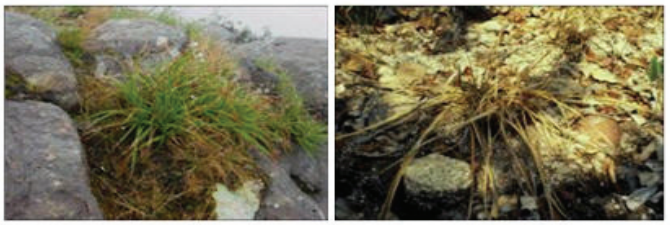

Figure 2. *X. viscosa* plants in their natural habitat are shown fully hydrated (left) and dehydrated (right). (*Source: http://www.scienceinafrica.co.za/2003/october/drought.htm*[4])

Osmoprotectants

Osmolytes are involved in signaling/regulating plant responses to multiple stresses, including reduced growth that may be part of the plant's adaptation against stress. In plants, the common osmolytes are proline, trehalose, fructan, mannitol and glycinebetaine.[6] The protection mechanisms are not yet fully understood, but they are thought to work via osmotic adjustment, stabilizing macromolecules, and scavenging ROS. One proposed transgenic strategy has been to overproduce osmolytes. However, transgenic plants overproducing osmolytes often exhibit impaired growth.

Trehalose, a non-reducing disaccharide, protects biological molecules in response to different

stress conditions in many microorganisms.[7] Plant biologists are interested in channeling trehalose metabolism (**Fig. 2**) to enhance stress tolerance in plants. Trehalose is made from UDP-glucose and glucose-6-phosphate via a two step process.

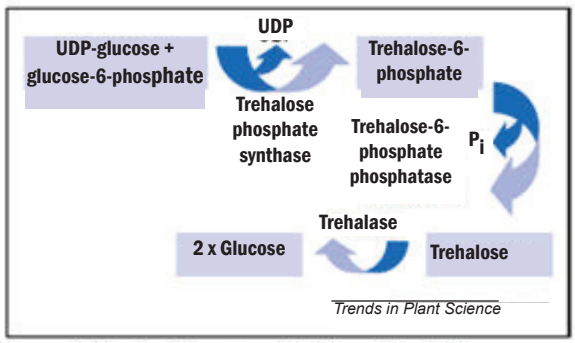

Figure 3. Synthesis and metabolism of trehalose. (*Source: Trends in Plant Science Vol.8 No.8, August 2003*)

The conversion of UDP-glucose and glucose-6-phosphate to trehalose-6-phosphate is catalyzed by trehalose-6-phosphate synthase, encoded by the bacterial *otsA* gene. In the second step, glucose-6-phosphate is converted to trehalose by a phosphatase encoded by the bacterial *otsB* gene. Tobacco plants transformed with bacterial *otsA* have a greater ability to retain water and a greater ability to photosynthesize under water stress.[8]

Protection only when needed

Genes imparting protection from drought stress can be expressed in plants in two ways: they can be expressed all the time, whether or not the plant is under stress; or they can be engineered to express only when there is drought stress. The second method is more favored, as it limits the side effects of the manipulations. One of the challenges biologists face in trying to engineer for drought tolerance is that drought tolerance and/or resistance traits are often negatively correlated with productivity. To achieve protection only when needed, scientists use promoters that are stress-inducible, typically abscisic acid (ABA) inducible promoters.

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Risk Assessment News

Considerations of Cross-Fertilization between GM and Non-GM Maize

Yann Devos, Dirk Reheul and Adinda De Schrijver

With the inscription of 17 genetically modified (GM) maize (*Zea mays* L.) varieties derived from the event MON810 in the common catalogue of varieties of agricultural plant species of the European Union (EU) on 8 September 2004, the acreage of MON810 hybrids increased in Germany, France, and Spain, and their commercial cultivation expanded to the Czech Republic and Portugal in 2005. On 14 December 2005, Germany accepted the listing of 3 GM MON810 hybrids in the national catalogue, and on 30 December 2005, 14 additional Spanish GM MON810 hybrids entered the common EU catalogue. These evolutions may further boost the adoption of transgenic maize by European farmers and illustrate the urgent need for legal and practical frames dealing with coexistence in order to maintain conventional, organic, and genetically modified (GM) crop production, and to guarantee a high degree of consumer choice. In the EU, specific tolerance thresholds have been established or are discussed for the adventitious and

technically unavoidable presence of approved GM material in non-GM produce: 0.9% for food and feed, 0.3-0.7% for seeds (crop specific), and 0.1-0.9% for organic produce (country specific). In addition to the mentioned thresholds, the product needs to be labeled as consisting of, containing, or produced from a genetically modified organism (GMO). In the case of maize seeds, a threshold of 0.3% is currently proposed.

Member states will impose strict technical management measures to keep the adventitious presence of GM material in non-GM produce below the labeling thresholds. As maize is a cross-pollinated crop relying on wind for dispersal of its pollen, on-farm measures may rely on spatial isolation.[1] The task may be difficult, since various biological, physical, experimental, and analytical parameters with varying levels of importance have been identified to play a role in the study of cross-fertilization in maize. The number of variables and their variability may hamper the comparison between research results and make it difficult to define the appropriate length of isolation distances and/or pollen barriers. How some of the parameters can hamper the comparison between research results is addressed below.[1]

- **Definition of isolation distance and pollen barrier**: Although the terms isolation distance and pollen barrier (or buffer zone) are clearly distinct, they are regularly confused in the scientific literature. An isolation distance separates fields by a zone of open ground or a zone with low growing crops, while a pollen barrier consists of plants that are sown or planted around the source or recipient field. If outer parts of fields function as a barrier, the distance between inner parts increases. Barriers may also produce competing pollen (if the barrier is of the same species as the crop) and/or may serve as a physical barrier to air flow and consequently pollen flow. A pollen barrier of maize has been proven to reduce cross-fertilization levels more effectively than an isolation distance of the same length.[2] For the future, it might be advisable to match the common vocabulary to similar definitions.

- **Measuring cross-fertilization**: Cross-fertilization is measured in different ways. Out-crossing may be noted in the following ways: (1) in the hybrid ears by phenotypic markers (e.g., xenia); (2) by detecting off-types in hybrid progeny; (3) by exposing the seedlings to an appropriate selection pressure (e.g., herbicide treatment in case of herbicide-resistant plants); and (4) by the qualitative detection of transgenic DNA and/or proteins in the seeds or seedlings. None of these methods quantifies the share of transgenic DNA. A quantitative DNA analysis expresses the GMO content as a percentage of haploid genomes. However, the latter results differ depending on the genetic constitution of the analyzed tissue (zygotic or maternal), the relative shares of these tissues in the sample, the ploidy levels of the tissue (triploid endosperm *vs.* diploid maternal tissue), the moment of sampling (early or late stage of kernel development), the copy number of transgenic DNA, and the DNA extractability, which may differ between plant tissues (**Fig. 1**).[3]

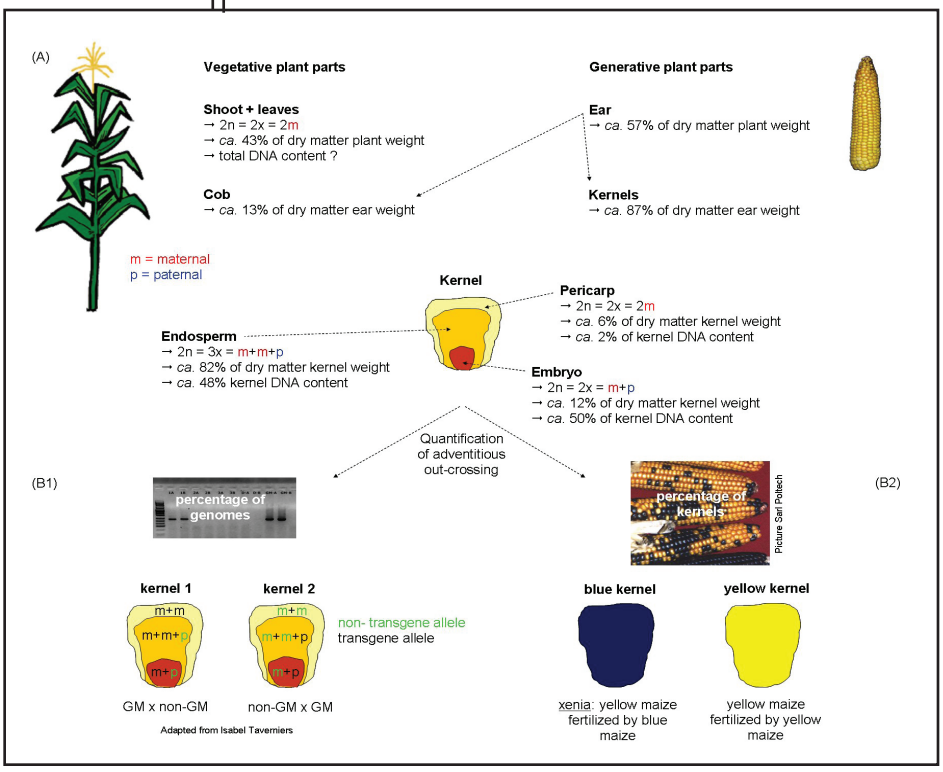

Figure 1: (A) Morphological composition, genetic constitution and its origin, and DNA content of different parts and structures of the maize plant. Indicated fractions are weight averages for varieties grown in Belgium and/or DNA content averages adapted from Trifa & Zhang (2004)[4] with significant variation between varieties and influenced by growing conditions. (B) Cross-fertilization in maize kernels expressed as a percentage of genomes (B1) or as a percentage of kernels detected by phenotypic markers (B2).

As a consequence, results based on quantitative DNA analyses are not smoothly convertible to results based on qualitative analyses.

- **Hemizygosity**: In the production of current GM hybrid varieties, the transgene generally is present in either the seed parent or the pollinator: as a result GM hybrids are hemizygous for the transgenic trait. Hence only half of the pollen produced on the hybrid carries the transgene, and only half of the cross-fertilization is measured compared to a pollen donor that is homozygous for the screened trait (**Fig. 2**).

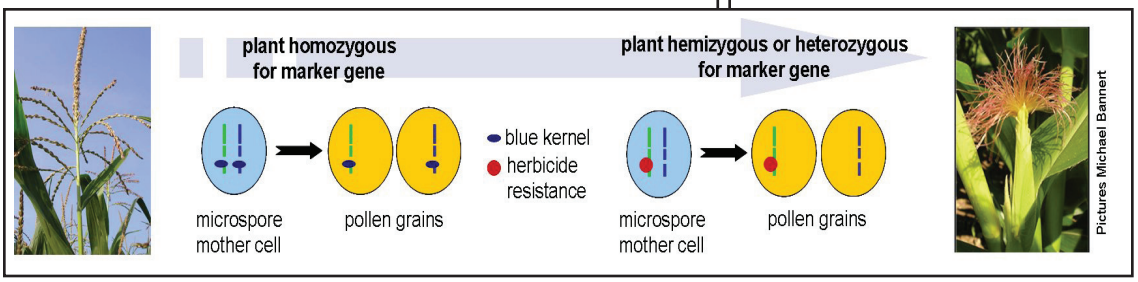

Figure 2: Genetic constitution of non-transgenic and transgenic hybrids and their pollen.

- **Analyzed plant tissue**: The material to be analyzed for the adventitious presence of GM material depends on the use of maize. In grain maize, adventitious mixing is restricted to the grain fraction of the plant: the cross-fertilization level is expressed per grain lot. In corn cob mix and in fodder maize, transgene presence is diluted if expressed as a percentage of genomes since vegetative plant parts (maternal tissue) are included in the harvested material (**Fig. 1**). In non-processed fresh sweet maize, cross-fertilization is expressed per individual ear.

- **Experimental design**: The results of field trials will differ according to the implemented design. In different studies, small recipient plots or even individual plants have been planted at various distances from a source in order to measure how far viable maize pollen can successfully fertilize a maize ovule. Such designs do not reflect the real agricultural situation and are not suited to quantify the adventitious GMO content of recipient fields of commercial size. Individual plants or small recipient plots are much more prone to cross-fertilization than large recipient fields, which may result in an overestimation of the out-crossing level when making extrapolations. Recent studies carried out in France[5], Germany[6], Spain[7], and the UK[8] mimicked worst-case commercial on-farm situations (e.g., pollen source next to or completely surrounded by a recipient field) with a trend towards out-crossing studies in real agricultural situations.[9] As the probability of cross-fertilization diminishes with increasing distances, sampling was performed at different positions within the recipient fields in order to calculate the average percentage of cross-fertilization over the whole field. The recommendations previously made for isolation distances and/or pollen barriers, based on discrete out-crossing levels, may therefore be too conservative and thus larger than the ones actually needed.

Apart from the previously discussed parameters, out-crossing is also affected by the distance between the pollen source and recipient; size, shape, and orientation of the pollen source and recipient; wind characteristics; rain; local environment; pollen viability; water status of pollen; climatic conditions; male fertility; and flowering synchrony.[1] When research results are compared in order to define the appropriate isolation distances and/or pollen barriers limiting out-crossing, the various parameters at play should always be considered.

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REGULATORY NEWS

Inspector General Fires Warning Shot at APHIS

Phillip B. C. Jones

Twenty years ago, the U.S. Department of Agriculture assumed responsibility for regulating field tests of new genetically modified (GM) plants and for ensuring that regulated GM plants, GM pollen, and GM seeds do not persist in the environment. Since then, the agency has approved over 10,000 applications for more than 49,000 field sites of GM plants.

The USDA's Office of Inspector General had doubts about whether the Animal and Plant Health Inspection Service's efforts to regulate GM plants have kept pace with the ever-increasing number of approved field test applications. To evaluate oversight of releases and movements of regulated GM plants, the OIG visited 91 planted or harvested field test sites in 22 states between May 2003 and April 2005.

On December 22, 2005, the Inspector General issued an audit report on the office's findings. The OIG concluded that weaknesses in APHIS' regulations and internal management controls increase the risk that regulated GM organisms will inadvertently enter the environment before the agency considers them sufficiently harmless to merit unregulated status. The Inspector General offered recommendations to improve three broad aspects of APHIS' oversight: in the accountability for GM plants; in the agency's inspections and enforcement of rules; and in guidance for containing GM plants and seeds.

Shoring up Accountability for GM Crops

APHIS uses two mechanisms to authorize field tests: permits and notifications. The agency considers certain GM crops to pose a high risk, such as plants engineered to produce pharmaceutical and industrial compounds, or plants engineered with human genes. Field tests of these GM plants require the issuance of permits. Based on its experience, APHIS deems that certain GM plants do not present novel plant pest risks. To field test a low-risk GM plant, applicants can use a streamlined notification process.

APHIS requires permit applicants to submit written protocols for review. In contrast, the agency does not require notification applicants to submit written containment protocols that describe how they plan to meet performance standards for preventing the escape of GM test plants into the environment. Rather, APHIS allows notification holders to supply protocols verbally if APHIS selects their field test sites for inspection. Since notifications comprise the vast majority of field test authorizations, OIG argues, this policy undermines both the field test approval and inspection processes.

The Inspector General also sees deficiencies in APHIS' monitoring of concluded field tests. At the end of a field test, APHIS does not require permit holders to report on the final disposition of GM plants that produce pharmaceuticals or industrial chemicals. The OIG discovered two harvests of GM

pharmaceutical crops that had been stored at field test sites for over a year without APHIS' knowledge or approval of the storage facilities.

APHIS sometimes lacked information about the precise locations of GM field test sites. The OIG found that, after authorizing a field test, APHIS did not consistently follow up with permit and notification holders to find out exactly where they had planted their GM crops or even if they had planted them. In the OIG's view, APHIS cannot effectively monitor permit and notification holders' compliance with field test requirements without knowing the locations of planted field test sites, including global positioning system coordinates.

To ensure accountability for regulated GM crops, OIG recommended that APHIS should require applicants to provide more information before and during a field test, including global positioning system coordinates of all planted field test sites. APHIS should also require all applicants to file copies of scientific protocols for conducting field tests.

Bolstering Inspections and Enforcement of Rules

The OIG found room for improvement in APHIS' procedures for inspecting test fields. The audit report describes a lack of coordination between the two APHIS units responsible for the inspection program: Biotechnology Regulatory Services (BRS), responsible for overall management of the program; and Plant Protection and Quarantine (PPQ), which performs most of the inspections of GM field test sites. According to the Inspector General, BRS lacks a formal, risk-based process for selecting individual sites for inspection, while PPQ officers do not complete all of the inspections BRS requests, including inspections of high-risk pharmaceutical and industrial crops.

In addition, neither BRS nor PPQ kept track of the total number of completed inspections and their outcomes. The OIG found 11 violations unrecorded in BRS' compliance infractions database at the time of the audit, even though the violations had been reported to BRS or could have been identified from available information.

To strengthen monitoring of field test sites, the OIG recommended that APHIS formalize its inspection process and coordinate the responsibilities of BRS and PPQ. APHIS also needs to develop a comprehensive management information system to track the receipt and to review of all information associated with release permits and notifications.

Neutralizing Terminated Test Fields

The Inspector General office found weaknesses in APHIS' guidance for preventing the persistence of GM crops outside the field test zone. The OIG discovered, for example, that APHIS did not specify when GM crops must be destroyed after a field test. As a result, harvested crops can remain in a field test site for months (**Fig. 1**).

The OIG recommended that APHIS should obtain reports on the final disposition of high-risk pharmaceutical and industrial harvests. The office also suggested that APHIS should draft guidance on deadlines for destruction of test crops.

Figure 1. Cut rows of GM crops found lying in a field up to a month after the conclusion of a test. (*Source: OIG's audit report.*)

APHIS Concurs and Counters

In a letter dated November 2, 2005, W. Ron DeHaven, APHIS' administrator, stated that the Biotechnology Regulatory Services has completed, or has begun implementing, 23 of the 28 recommendations. APHIS disagreed with most of the remaining recommendations.

OIG's suggestions for modifying the notification process created the most noteworthy cause of disagreement. The OIG insists that APHIS should obtain copies of notification applicants' scientific protocols for conducting field tests and allow the agency's biotechnologists to review the protocols to ensure that they meet performance standards. Otherwise, APHIS relinquishes its regulatory responsibility in favor of a system in which notification applicants merely certify that they will meet the performance standards.

"While we do evaluate written protocols for permits," DeHaven argued, "we believe that the current system of performance–based regulatory standards for notifications is effective at protecting American agriculture." DeHaven emphasized APHIS' familiarity with crops eligible for notification, an expertise that

justifies the agency's decision to omit a review of written protocols prior to approval. "The intent of the notification procedure," he said, "is to provide an administratively-streamlined process for trials of crop-trait combinations with which APHIS already has a great deal of experience and familiarity."

A copy of the audit report, "Animal and Plant Health Inspection Service Controls Over Issuance of Genetically Engineered Organism Release Permits," is available at the OIG website (http://www.usda.gov/oig/rptsaudits2005.htm).

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USDA APHIS Seeks Comments on Environmental Risk Assessments:

1. GE Pink Bollworm
2. GE Tall Fescue and GE Italian Ryegrass

Environmental Assessment for Genetically Engineered Pink Bollworm

The USDA Animal and Plant Health Inspection Service (APHIS) is advising the public that an environmental assessment has been prepared for a proposed field trial of pink bollworm genetically engineered to express green fluorescence as a marker. APHIS proposes to use this marked strain to assess the effectiveness of lower doses of radiation to create sterile insects for its pink bollworm sterile insect program. This program, using sterile insect technique, has been conducted by APHIS, with State and grower cooperation, since 1968. Data gained from this field experiment will be used to improve the current program.

Environmental Assessment of GE Tall Fescue and GE Italian Ryegrass

APHIS is likewise advising the public that an environmental assessment has been prepared for a proposed field trial using three transgenic grass lines. The trial consists of tall fescue plants that are genetically engineered for hygromycin resistance and that express the marker beta-glucuronidase, Italian ryegrass plants that are genetically engineered for hygromycin resistance, and Italian ryegrass plants that are genetically engineered to lower the expression of the pollen allergen gene, Lol p1, and that are also hygromycin resistant and express the marker beta-glucuronidase. The purpose of the field trial is to study pollen viability, outcrossing, and hybridization between the two types of grasses. The study will also examine the effect of down-regulating the Lol p1 gene. Data gained from this field experiment will also be used to evaluate current confinement practices for these species of transgenic grasses.

The environmental assessments for both field trials are available to the public for review and comment. APHIS will consider all comments received on or before March 15, 2006.

Comments may be submitted by either of the following methods:

- Federal eRulemaking Portal: Go to http://www.regulations.gov and, in the "Search for Open Regulations" box, select "Animal and Plant Health Inspection Service" from the agency drop-down menu, and then click on "Submit." In the Docket ID column, select APHIS-2006-0015 for the pink bollworm risk assessment or APHIS-2006-0016 for the fescue and ryegrass risk assessment to submit or view public comments and to view supporting and related materials available electronically. After the close of the comment period, the docket can be viewed using the "Advanced Search" function in Regulations.gov.
- Postal Mail/Commercial Delivery: Please send four copies of your comment (an original and three copies) to Docket No. APHIS-2006-0015 or APHIS-2006-0016, Regulatory Analysis and Development, PPD, APHIS, Station 3A-03.8, 4700 River Road Unit 118, Riverdale, MD 20737-1238. Please state that your comment refers to Docket No. APHIS-2006-0015 or APHIS-2006-0016.

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Sources:
USDA APHIS February 13 Federal Register
For Pink Bollworm: http://a257.g.akamaitech.net/7/257/2422/01jan20061800/edocket.access.gpo.gov/2006/E6-1972.htm
For Fescue and Ryegrass: http://a257.g.akamaitech.net/7/257/2422/01jan20061800/edocket.access.gpo.gov/2006/E6-1992.htm