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Development of an Agricultural Biotechnology Crop Product: Testing from Discovery to Commercialization

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ABSTRACT: "Genetically modified" (GM) or "biotech" crops have been the most rapidly adopted agricultural technology in recent years. The development of a GM crop encompasses trait identification, gene isolation, plant cell transformation, plant regeneration, efficacy evaluation, commercial event identification, safety evaluation, and finally commercial authorization. This is a lengthy, complex, and resource-intensive process. Crops produced through biotechnology are the most highly studied food or food component consumed. Before commercialization, these products are shown to be as safe as conventional crops with respect to feed, food, and the environment. This paper describes this global process and the various analytical tests that must accompany the product during the course of development, throughout its market life, and beyond.

KEYWORDS: *GM, testing, methods, validation*

INTRODUCTION

"Genetically modified" (GM) or "biotech" crops (also referred to as "genetically engineered" crops) have been the most rapidly adopted agricultural technology in recent years as evidenced by a 94-fold increase from 1.7 million hectares in 1996 to 160 million hectares in 2011.[1] The acreage of GM crops planted by country in 2010 is summarized in Table 1.[2] Currently, >10% of the 1.5 billion hectares of the world's crop land are planted with GM crops, an 8% increase in 2011 alone.[1] This represents a very rapid rate of adoption in the 17 years since these products have been introduced. GM crops can provide many benefits to growers including (1) reduction in pesticide use; (2) less soil erosion due to the utility of no- or reduced till practices; (3) decrease in mycotoxin contamination of grain; (4) reduced use of petroleum due to a more infrequent need to enter the field and apply pesticides; and (5) increases in biodiversity.[3]

The development of GM crops as shown in Figure 1,[2] from transformation and event selection through commercial authorization, is a lengthy, complex, and resource-intensive process. This paper describes this global process and the various analytical tests that must accompany the product during the course of development throughout its market life and beyond. We have divided the overall process into two major segments. The first is the development of the product and covers gene discovery through plant transformation to elite event identification. The second segment covers the safety assessment that is performed, which comprises the regulatory

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Table 1. Global Area of Biotech Crops in 2010 by Country

rank	country	area (million hectares)	biotech crops
1	USA	66.8	maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash
2	Brazil	25.4	soybean, maize, cotton
3	Argentina	22.9	soybean, maize, cotton
4	India	9.4	cotton
5	Canada	8.8	canola, maize, soybean, sugar beet
6	China	3.5	cotton, papaya, poplar, tomato, sweet pepper
7	Paraguay	2.6	soybean
8	Pakistan	2.4	cotton
9	South Africa	2.2	maize, soybean, cotton
10	Uruguay	1.1	soybean, maize
11	Bolivia	0.9	soybean
12	Australia	0.7	cotton, canola
13	Philippines	0.5	maize
14	Myanmar	0.3	cotton
15	Burkina Faso	0.3	cotton
16	Spain	0.1	maize
17	Mexico	0.1	cotton, soybean
18	Colombia	<0.1	cotton
19	Chile	<0.1	maize, soybean, canola
20	Honduras	<0.1	maize
21	Portugal	<0.1	maize
22	Czech Republic	<0.1	maize, potato
23	Poland	<0.1	maize
24	Egypt	<0.1	maize
25	Slovakia	<0.1	maize
26	Costa Rica	<0.1	cotton, soybean
27	Romania	<0.1	maize
28	Sweden	<0.1	potato
29	Germany	<0.1	potato
	total	**148.0**	

submissions made around the world through commercialization and includes a discussion on labeling and other trade issues.

BIOTECH PRODUCT DEVELOPMENT

The development process may be divided into two parts: (1) identification of the trait and production/selection of the event to be commercialized and (2) safety assessment of the product for which commercial authorization is pursued. The first step in GM event development involves identification of the gene or genes that impart the trait or desired phenotype. The gene is isolated from the source organism and confirmed that it performs appropriately in the host background. In some instances, the gene may need to be optimized to adopt its codon usage to better align with the host organism. For example, in maize, the genome is GC-rich, so the AT-rich *Bt* genes (from *Bacillus thuringiensis*) were optimized for expression in the maize genome.[5] Care is taken during this step to verify that unintended modifications are not made. An additional consideration in the gene optimization phase is the appropriate selection of the gene promoter to target the appropriate expression level of the gene at the desired plant developmental stages and in the desired plant tissues. For instance, if the trait imparts resistance to root worm, the gene could be driven by a root-specific promoter. Prior to transformation initiation, the genetic constructs (genes of interest and associated promoters/terminators) are inspected using bioinformatic tools and literature references. This ensures that the proteins coded by the transgenes do not have similarity to known and putative allergens and toxins. Once these decisions are made, the transformation process is initiated. Transformation procedures include biolistics, protoplast, and *Agrobacterium*-mediated methods, among others. The transformation process is the delivery of the transgene cassette into the host cell genome. This can be accomplished by biolistics, in which DNA-coated particles are propelled by high pressure to deliver the gene of interest. When plant cell walls are removed, the resulting protoplasts can be opened using electric or osmotic forces that allow DNA from the media to enter the cells. The *Agrobacterium*-mediated method uses a natural delivery system that the bacteria have evolved for depositing the gene of interest into the plant genome.

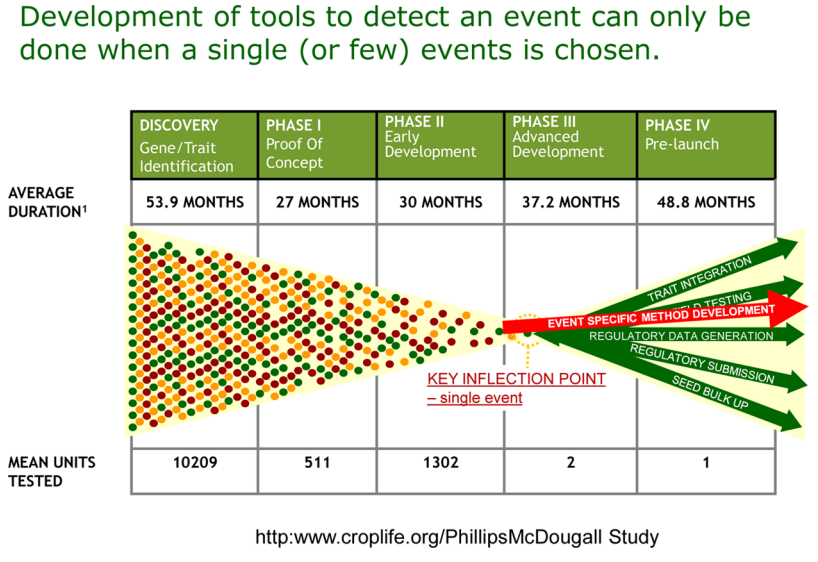

Figure 1. Stages in the development of an agricultural GM product. Mean units evaluated is taken from the Phillips MacDougall Survey in which biotechnology providers were queried and includes both the number of genes screened and the transgenic events evaluated.

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As shown in Figure 1, hundreds to thousands of different transformation events are generated.[4] According to the McDougall survey of the industry's largest biotech companies, currently an average 6204 "units" (candidate genes, constructs, or transformation events) are screened to obtain one commercial product.[4] Following transformation, the host cells containing each event are regenerated into plantlets.

The trait developer must identify events that meet the product specifications, from which one event is selected for commercialization. In addition to trait efficacy, product specifications include (a) that the plant is fertile, (b) that the trait is inherited in a Mendelian fashion (assuming the nucleus is the insertion site of the transgene), (c) that plant performance and appearance are not affected, (d) that the trait is stably expressed across generations, (e) that exogenous DNA is minimized, (f) that no fusion proteins are created by the insertion, and (g) any other parameters that may be specific for the trait of interest. Of these, the plant performance and appearance (phenotype) are extremely important. If the genetic insert has no impact on the plant performance (especially yield) and appearance, this indicates that the insert does not have an adverse effect on the host plant. One or more transformation events may be identified as candidates for commercialization. Regulatory studies are then initiated for the safety assessment.

Each transformation event has a unique and usually random insertion site within the host plant genome that defines it. Trait developers can track this event by this unique "footprint." This is important for much of the testing described later in this paper.

Part I. Trait Development/Event Selection. Following transformation, the transformed plant cells are regenerated into plants. Regenerated plants are then selected on the basis of healthy growth and normal phenotype to continue to seed production and further evaluation. If the regenerated transformed plants (transformants) are sterile or have developmental defects or abnormal phenotypes, they are discarded. Only healthy, normal, efficacious, and fertile regenerated transformed plants are isolated for further evaluation.

The selected transformants are characterized for transgene integration, transgene expression, trait efficacy, and agronomic performance. To confirm the transgene has integrated in the plant genome and to assess the number of copies of the transgene, Southern blot analysis and/or polymerase chain reaction (PCR) analyses are conducted. Segregation analysis is also used to further confirm the transgene insertion site in the plant nuclear genome. The flanking plant genomic borders of the insertion are characterized by various molecular techniques. The sequence of the inserted transgene is compared to the DNA fragment sequence used in the plant transformation, and any changes observed are documented. Bioinformatics tools are used to analyze the flanking genomic region/insert junction to determine whether there was disruption of an endogenous plant gene or if any novel open reading frames (ORFs) have been formed. In cases when the transgene insertion appears to disrupt a putative gene, additional analyses are conducted to evaluate the disruption at the molecular and phenotypic levels. Any transformant with unintended adverse phenotypic effects is discarded. If a novel ORF is created due to the transgene insertion, this novel ORF sequence will be analyzed using bioinformatics comparisons to the allergen and toxin databases to ensure the sequence does not have similarity/homology to any known and putative allergens or toxins harmful to organisms that consume the crop as food/feed. Initial trait efficacy testing is performed in the greenhouse when possible or in small field trials. Transformants that do not perform efficaciously are discarded.

The remaining transformants are evaluated for trait efficacy, trait stability, and agronomic observation in field conditions at multiple geographic locations and over multiple growing seasons. Various PCR-based detection methods and protein-based methods are developed to track the particular event and the expression of the transgene during subsequent plant breeding and trait evaluation. For herbicide-tolerant GM events, bioassays employing the respective chemicals such as glyphosate and glufosinate are routinely used due to their ease of use and high-throughput nature. The transformants with desirable trait efficacy and favorable agronomic characteristics are introgressed into elite germplasm (if the crop is vegetatively propagated, such as potato or sugar cane, these steps are not undertaken) for product development. Usually several rounds of backcrossing are performed to remove any unfavorable agronomic characteristics associated with the original transformed germplasm and to integrate the trait into an elite germplasm background. After each plant cross, testing is performed to ensure seed purity and the selection of offspring that are homozygous for the transgene. Agronomic performance data are collected. Trait stability is examined across different plant generations to ensure the desirable trait efficacy for a product. The transformant with stable and desirable trait efficacy and favorable agronomic characteristics is carried on for further evaluation.

Part II. Safety Assessments of GM Crops. The intent of the safety assessment is to identify new or altered environmental or nutritional impacts and characteristics, relative to the parental conventional counterpart. Comparison to the parental conventional counterpart is a critical component of the safety assessment. Establishment of "substantial equivalence" or "as safe as" the conventional counterpart with the only difference being the introduced trait (unless compositional changes are intended) is essential for regulatory authorization. Throughout this assessment, testing methodologies play an integral role. GM crops are one of the only crops formally reviewed by regulatory agencies for their potential to transfer novel traits to wild relatives and for their potential to become weeds. They are also assessed for other potential risks such as environmental effects on mammals, birds, nontarget insects, and soil organisms.

For food/feed safety, GM crops are assessed with respect to their potential toxicity, potential to cause an allergic response, and possible unintended effects that may result from the insertion of the new genes into the host plant(s). Nutritional value, as determined by compositional analysis of nutrients and antinutrients specific to each crop and wholesomeness studies (e.g., broiler chicken feeding study), is monitored extensively.

Many of the testing methodologies and outcomes are relevant to both the environmental assessment and the food/feed safety. Examples would include the characterization of the genetic modification, the expression of introduced gene products, and compositional analysis.

Testing Methodologies in Environmental Risk Assessment. To assess the environmental effects of insect-tolerant GM crops for cultivation authorization, testing may be conducted on many types of nontarget organisms such as earthworms, fish, beneficial insects, and birds. This helps determine if there are any unintended consequences associated with the introduction of the GM crop. To conduct these tests, a source of the

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introduced protein(s) encoded by the transgene(s) is necessary. Alternatively, the test substance may be pollen, lyophilized leaves, or other appropriate plant parts from the GM crop. Because the introduced proteins are generally expressed at very low levels within the plants (ng to μg/g tissue) and hard to produce in sufficient quantity, the proteins are produced in microbial organisms to provide the amount needed for the testing. This is described in more detail under Protein and Grain Safety Assessments later in the paper. For feeding types of studies, ELISAs are used to quantify the amount of target protein in the feed mixture administered.

Testing Methodologies in Food/Feed Safety Assessment. According to the Codex guideline for food safety assessment (2003), the approach is based on the principle that the safety of foods derived from new plant varieties, including GM plants, should be evaluated relative to the conventional counterpart that has a history of safe use. The assessment takes into account both intended and unintended effects of the introduced modification(s). A variety of data and information are necessary because no one test can detect all possible unintended effects or identify those relevant to food/feed safety.[6] The agronomic and phenotypic data collected by plant breeders provide the initial screen for selecting events for commercialization. Those that pass this initial screen are then moved into the food/feed safety assessment process, when various methods are utilized to further identify and detect any unintended effects.

The Codex (2003) guideline for food safety assessment is a stepwise process that includes relevant factors such as (a) description of the recombinant DNA plant; (b) description of the host plant and its use as a food; (c) description of the donor organism; (d) description of the genetic modification(s); (e) characterization of the genetic modification(s); (f) safety assessment [(i) expressed substances (non-nucleic acid substances); (ii) compositional analyses of key components; (iii) evaluation of metabolites; (iv) food processing; and (v) nutritional modification] (g) other considerations.

Testing methodologies are particularly important in steps e and f of the safety assessment.

Characterization of the Genetic Modification. Characterization of the genetic modification(s) (step e above) provides information on the DNA insertions into the plant genome.[6] The Codex (2003) guideline indicates that sufficient information should be provided on the genetic modification to allow for the identification of all genetic material potentially delivered to the plant. The information may include the characterization and description of the inserted genetic elements; the number of insertion sites; the organization of the inserted genetic material at each insertion site (including copy number and sequence data); and identification of open reading frames (ORFs). Southern blotting has been the method of choice for generating this information; however, in recent years, PCR has played a greater role. PCR amplification with subsequent sequencing of the PCR products has been used to identify junctions of inserts with the plant genomic DNA, to sequence the genetic inserts, and to identify ORFs within the junctions of the insert with the plant genomic DNA. Quantitative PCR has also been used to estimate gene copy number and zygosity in seeds and plants.[7]

Allergenicity Assessment of the Newly Introduced Protein in the GM Plant. A weight-of-evidence approach has been routinely used to determine potential for allergenicity of introduced gene products.[6] Five characteristics that are evaluated: (a) whether the source of the introduced gene(s) is allergenic (e.g., peanut is an allergenic food, and genes from the peanut plant would need to be carefully assessed as to whether their gene products are allergens); (b) whether the introduced gene product or products have sequence homology to known and putative allergens; (c) whether the introduced gene product or products are resistant to digestion in simulated gastric fluid; (d) whether the introduced gene product or products are glycosylated; and (e) whether the introduced gene product or products are heat stable.

The use of SDS-PAGE coupled either with staining or with Western blotting techniques is integral to the digestion testing of introduced proteins.[8] In this evaluation, the introduced protein is exposed to pepsin, and aliquots of the pepsin protein mixture are sampled over specified time points (30 s, 1 min, 5 min, etc.). The protein samples are then evaluated electrophoretically via SDS-PAGE and visualized by gel staining or blotting to membranes. The membranes are probed with antibodies specific to the protein to determine at what time interval the protein can no longer be detected.

Goodman et al.[9] describe in detail the approaches to evaluating the potential allergenicity of proteins not previously in the food chain.

Genetic Stability. Genetic stability of the DNA insert and traditional Mendelian segregation are evaluated in multiple generations of the crop, via both phenotypic assessment and molecular methods confirming the presence of the insert.

Lateral flow immunoassays or ELISAs are often used to identify the seeds or plants that will be utilized in the genetic stability testing because the generations of seeds used may be still segregating. PCR or Southern blotting is used to identify the DNA insertion across generations and within generations of the GM crop plants.

Expression of Transgene Products. For the assessment of the expression of non-nucleic substances (e.g., proteins) [step f(i) above], the Codex (2003) guidelines require information to be provided on all expressed substances in the GM plant that include the gene product(s) (protein or an untranslated RNA); the gene products' function; the phenotype of the new trait(s); the level and site of expression in the plant (particularly in consumed fractions); and the amount of target gene product(s) if the function of the expressed sequence(s) is meant to alter the accumulation of a specific endogenous mRNA or protein. For protein expression, immunoassays offer simple, specific, and sensitive protein detection methods that address a wide range of needs[10] such as determining the amount of the expressed proteins in various plant parts (leaves, forage, pollen, roots, etc.) and seed/grain. Quantitative ELISA provides a specific and high-throughput assay system for analyzing large numbers of samples to provide a "view" of the expression over time in the plant's life cycle and different tissues. The expression values generated are used to determine the exposure to humans, animals, and other nontarget organisms that consume the plant and/or plant products as food or feed.

Compositional Analysis. Historically, the compositional analysis of the GM product was compared to the conventional parental variety using methodologies developed for the nutritional labeling industry. These validated methods include those among AOAC International,[11] the American Oil Chemists' Society (AOCS),[12] and European Standards (EN)[13] or internal methods. The variety of analytes currently being requested by various jurisdictions exceeds the available methods and those for which the natural variation is known. Valuable information on the important analytes is summarized

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in the OECD Consensus documents for each crop.[14] The International Life Sciences Institute maintains a useful database[15] of results from such analyses. The compositional analyses form the basis for evaluating substantial equivalence of the GM crop with conventional varieties.

Protein and Grain Safety Assessments. Delaney et al.[16] describe in depth and present case studies for the protein safety evaluation undertaken as part of the overall safety assessment for GM crops. It is worth noting that testing methodologies include, among others, the use of ELISAs and Western blot analyses during the course of the protein safety assessment.

The potential toxicity of a novel introduced protein is evaluated by comparing the novel protein's amino acid sequence to that of known toxic proteins in public databases and by conducting acute oral toxicity studies with mammals such as mouse. To obtain quantities of an introduced protein sufficient for biochemical and toxicological tests, the proteins are produced microbially in the laboratory. A range of analyses are subsequently undertaken to establish that the microbially produced proteins are equivalent to the proteins produced by the transformed plants. Several characteristics are analyzed, such as molecular weight, immunoreactivity, post-translational modification, and N-terminal amino acid sequence. Usually, mice are fed a diet containing a single dose of the introduced protein that is equivalent to hundreds or a thousand times the level of the estimated dietary intake for human consumption. Observations for mortality and clinical or behavioral signs of toxicity as well as individual body weights are made to identify potential protein toxicity and, when appropriate, determine a NOEL (no observable effect level).

The diets for grain feeding studies are tested for the protein level by ELISA and for their nutrient compositional profile. Oral toxicity feeding studies in rats are carried out with diets containing GM grain, nonbiotech crop with comparable genetic background, or other commercial nonbiotech crop. Observations for body weight, food efficiency, and gross necropsy among other diet-related effects are conducted to identify any potential toxicologically significant diet-related differences. Additional histopathological observations may be made as needed.

Food Processing. Many crops are processed to produce a large variety of food products or components of complex foods. One example is maize, which is processed into flour, high-fructose corn syrup, grits, and oil, among others, which are incorporated into many food products. It may be of value for the food safety assessment to determine the level of the introduced gene product(s) in some of these processed components. Similar to many proteins, the newly introduced protein will probably be denatured during processing. Again, quantitative ELISA is generally the methodology of choice to assess the introduced protein quantity. The ELISA method must be validated on the particular processed matrix to ensure that there is no background matrix interference.

Unintended Effects. GM plants are developed with the objective of conferring a specific trait, that is, intended effect, to the plants via the insertion of defined DNA sequences (typically recombinant genes).[6] In some cases, existing traits of the modified plant(s) could be lost or modified or additional traits could be acquired, that is, unintended effects. The potential for unintended effects is a general and well-known phenomenon in traditional, conventional breeding and selection for traits and is not specific to transformation of plants with recombinant DNA. Unintended effects are not necessarily adverse and may be beneficial or have no effect on the food derived from the plant. An example of an unintended, yet beneficial, effect is the reduction of fungal diseases in insect-resistant corn. There are fewer insect-inflicted wounds to the plant and hence fewer sites for infection by disease-causing organisms and therefore less mycotoxin contamination of the grain.[17] The intent of the safety assessment of GM plants is to reduce the possibility that a food derived from the plant would have an unexpected and/or adverse effect on human health and the environment. The Dutch government contracted a study to summarize all published unintended effects on the environment as a result of planting insect-resistant or herbicide-tolerant crops over the first 10−15 years of cultivation.[18] No unintended effects as a direct result of the genetic modification were found. Some indirect effects on disease susceptibility and minor nutrient uptake specific to herbicide usage were reported. Their overall summation was that "in general, it can be concluded that very few clearly unexpected effects were observed during the large scale post-release growing of herbicide-tolerant crops and Bt crops".

DNA, protein, and composition testing methodologies are integral tools in the food/feed safety assessment process of GM crop plants. The use of testing methodologies provides the necessary information to determine the safety of the food and feed and also assists in the characterization of test materials used in the environmental assessment of the introduced trait(s). To date, no safety issues have been found associated with GM products in food and feed, unlike the very real safety issues associated with pathogens, toxins, and other contaminants that have been/may be found in the food supply.[19] Tolerance levels based on results of appropriate studies are set for mycotoxins, pesticides, and drug residues. On the other hand, despite the lack of any adverse effects, zero tolerance levels for GM products continue to exist in some jurisdictions.

DETECTION OF AGRICULTURAL BIOTECHNOLOGY CROPS, FOOD, AND FEED

Consumer preferences or product claims can make the detection of GM crops in food and feed products desirable or even required, especially in the international trade of goods. Both protein analytical methods, such as ELISA and lateral flow strips, and DNA analytical methods, mainly PCR, have been used for the detection of biotech material in seeds, grains, or food and feedstuff derived thereof. These methods have been in use for over a decade and are still the most commonly used diagnostic tools.[15,20] Newer technologies are also being developed for the detection of biotech crops, to allow (1) additional testing outside a laboratory setting, for instance, isothermal PCR at field sites [there are many variations for isothermal amplification technologies, for example, loop-mediated isothermal PCR (LAMP), helicase-dependent PCR,[21] nicking enzyme amplification (NEAR), and recombinase polymerase amplification (RPA) among others]; (2) higher throughput platforms such as the Array Tape Platform from Douglas Scientific and the BioMark HD System from Fluidigm to enable the reduction of PCR reactions to the nanoliter range (in addition, digital PCR on the BioMark platform has been used in the absolute quantification of DNA copies);[22,23] and (3) testing for RNAi products.

Asynchronous Authorization. If a new GM product is still under regulatory review in one or several importing countries at the time when it is authorized and commercially introduced in an exporting country, detection of the potential

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low-level presence of this new product is typically required before exportation to the destination(s) that have not yet fully authorized it. Once the new product has full regulatory authorizations at all relevant import destinations, the respective testing is no longer required. However, these periods of "asynchronous authorization" are a substantial burden for international trade and may disrupt product streams of food and feed across borders. Similar to the detection of novel GM products during asynchronous authorization, rare incidents of inadvertently released GM events have made the detection of those events required, until they were successfully removed from all commercial product streams or had obtained regulatory authorization in all relevant export markets. Typically "zero tolerance" is applied in these cases. However, moving forward, thresholds of low-level presence may be tolerated, especially if the trait is "familiar" (i.e., present in a similar event already approved) or if the event is approved in a different jurisdiction.[24] In the European Union (EU), for example, there are no such threshold values for the low-level presence of new GM events in seeds or food products in effect. However, a level of 0.1% of mass fraction, also referred to as "technical zero", was recently defined for animal feed products.[24] This very low level, which could arguably be considered a "threshold", applies only to those GM events (a) that are approved in a third country and for which the EU authorization procedure has been pending for more than 3 months and (b) for which the quantitative event-specific detection method has been validated and published by the European Union Reference Laboratory and the certified reference materials are available.

Mandatory Labeling and Tolerance Levels. A huge dichotomy exists between those that recognize the benefits of biotech products and those that advocate for consumers' right to know what is in their food. This results in various regulations that govern mandatory labeling of GM products in some countries. Countries that have adopted mandatory labeling rules for biotech-derived food or feed have typically set tolerances for the adventitious, low-level presence of such material (Table 2). In those countries, PCR is typically employed to determine whether the products need to be labeled. To that end, validated event-specific PCR tests must be supplied to some jurisdictions as part of the application for registration of the new event.

Table 2. Summary of Some International Labeling Regulations for Foods Derived from Products of Modern Biotechnology[a]

country	labeling	labeling threshold for approved GM events (%)
Argentina	voluntary	NA[b]
Australia and New Zealand	mandatory	1
Canada	voluntary	NA
China	mandatory	none
European Union	mandatory	0.9
Japan	mandatory	5[c]
Russia	mandatory	0.9
South Korea	mandatory	3[c]
United States	voluntary	NA

[a]Source: Various USDA Foreign Agricultural Service Attaché reports. [b]Not applicable. [c]Top three ingredients in Japan and top five ingredients in South Korea.

Local food and feed manufacturers and retailers often choose to incorporate raw materials, ingredients, and products that contain GM material below the defined regulatory threshold to avoid labeling their products (Table 3). Food manufacturers and retailers may desire to use labeling for their "non-GM" products, especially in markets that otherwise do not mandate any labeling with regard to GM or non-GM. Typically, grain for such products is produced under non-GM identity preservation programs that segregate it from GM grain. The downstream processing and handling are also aimed at avoiding admixture of biotech ingredients. At various points along the supply chain, products are tested to confirm that they do not contain GM-derived ingredients in excess of the labeling threshold and can meet the specifications for a non-GM claim.

Table 3. Agricultural Products Listed in the Chinese Labeling Regulations[a]

soybean seeds	corn seeds	rape seed for planting	cotton seed for planting	tomato seed
soybean	corn	rape seed		fresh tomato
soybean powder	corn oil	rape oil		tomato jam
soybean oil	corn flour (including corn flour with harmonized schedule codes 11022000, 11011300, and 11042300)	rape meal		
soybean meal				

[a]U.S. Department of Agriculture (USDA). GAIN Report CH7053 (2007). Foreign Agricultural Service Attaché Reports.

Confirmation of Premium GM Crops. For biotech crops with traits that add value for food or feed processors, the livestock industry, or the consumer, it may be desirable to confirm the presence and quantity of the value-added trait in crop or products, especially if those are traded at a premium compared to conventional products. PCR-based analyses, ELISAs, or lateral flow strip technologies are useful for these applications.

QA/QC Commercial Seed Production. Last but not least, routine QA/QC of commercial seed production requires extensive testing, especially for those crops that are abundantly commercialized as GM varieties. This testing is not only required to confirm non-GM status of conventional seed lots but also to confirm the purity of GM seed lots.

PCR or ELISA methods for the quantification of a commercialized GM event are typically validated for seeds, grain, and unprocessed flours. If the methods are applied to additionally processed matrices or products with ingredients derived from multiple plant species, the test results should be interpreted with caution in cases when the appropriate matrix validation has not yet been completed.

An additional confounder is that regulations of commercial non-GM specifications will typically refer to thresholds in terms of "percent GM". "Percent", however, is not a unit of measurement, but merely the ratio between two measurements of the same unit. This leads to different possible definitions of "percent GM". The EU labeling regulations, for example, refer to the ratio of event DNA relative to the respective plant species DNA, whereas Japanese labeling regulations, for example, aim to regulate the mass/mass ratio of biotech material relative to the product. The recent European

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Commission adopted Regulation (EU) No. 619/2011,[24] which introduces a 0.1% threshold for EU nonauthorized GM material in feed, states that the values are in mass fractions. Often, what the two constituents of the percentage calculation for "GM" should be are poorly or not at all defined. This opens the door to different approaches in translating measurements into test results in terms of percent. As a result, there exists the potential for a range of different interpretations of the same set of protein- or DNA-based data.

REGULATORY REQUIREMENTS FOR DETECTION METHODS AND REFERENCE MATERIALS

The development and implementation of various detection methods are an integral part of plant biotech developers' business, and those methods are used in research, development, and commercial operations as well as for grain channels and regulatory authorization purposes. Before biotech crops are approved for commercialization, regulatory agencies require an exhaustive series of regulatory studies to ensure environmental and food and feed safety. In addition, validated diagnostic methods (such as event-specific PCRs and/or a protein method) and reference materials have to be provided as a condition of the regulatory authorization process in some jurisdictions. These methods are intended for evaluation of GM traits in seed, grain, and other agricultural commodities and enforcement of labeling requirements for biotech-derived products in some countries and regions.

Except in the United States, where the U.S. Environmental Protection Agency (EPA) requires a validated protein method for the detection of insecticidal traits, most authorities demand either or both a quantitative and qualitative PCR method. These two DNA-based methods are usually event-specific PCR assays. The quantitative assay is validated to meet the EU's validation criteria.[25] However, the qualitative gel-based PCR assay is defined by Chinese and Korean authorities and has less-well documented criteria.

Reference Materials (RM) are required as reference standards in method development, validation, laboratory proficiency checks, quality control, and calibration of routine application of test methods. They are produced according to international standards and guidelines to ensure quality and traceability. Such materials are provided by the trait developer to regulatory agencies in a controlled manner to ensure proper use and distribution of RM that contains intellectual properties from the seed registrants. For commercialized events, these reference materials are made available globally in the form of flour or purified DNAs through a designated third-party source and may be certified as Certified Reference Materials (CRMs). Currently, CRMs are available globally from either the Institute for Reference Materials and Measurements (IRMM)[26] or AOCS.[27]

GLOBAL AUTHORIZATIONS AND PRODUCT STEWARDSHIP

The plant biotechnology industry is committed to supporting responsible use and management of its products and smooth trade transactions in the global agricultural community. Crop Life International members believe that the global harmonization of detection methods and reference materials for GM crops is necessary to ensure a consistent standard. To support that effort, Crop Life International and its members launched a detection methods Web site[28] hosting their detection methods and related information in a searchable database for commercialized biotech-derived products. These detection methods were developed and validated by the technology providers for their own proprietary technologies and products. The information provided will contribute to the global harmonization for testing, enable smooth and efficient global trade, and meet national labeling and low-level presence requirements.

Today, the regulation of plant biotechnology, including the requirements for detection methods and standardization of testing for biotechnology products, is far from harmonized. The proliferation of detection methods, lack of global standards, and sometimes impractical regulatory requirements all pose great risks to international trade.

Biotechnology providers are committed to the responsible management of a product from its inception to its ultimate use and beyond. For this reason, the industry sponsored the Excellence Through Stewardship (ETS)[29] initiative to promote the global adoption of stewardship programs and quality management systems for the full life cycle of biotechnology-derived plant products. ETS members are required to adopt and abide by stewardship objectives, principles, and management practices, which are verified by third-party audits. To support the development and implementation of stewardship programs and quality management systems, ETS has published five guide documents. They include the *Guide for Stewardship of Biotechnology-Derived Plant Products*, *Guide for Product Launch Stewardship of Biotechnology-Derived Plant Products*, *Guide for Maintaining Plant Product Integrity of Biotechnology-Derived Plant Products*, *Guide for Incident Response Management of Biotechnology-Derived Plant Products*, and *Guide for Product Discontinuation of Biotechnology-Derived Plant Products*.

TRADE ISSUES

GM crop acceptance is varied in different parts of the world. Farmers realize the benefits of biotechnology crops and are eager to plant seeds that are resistant to diseases and insects and tolerant to herbicides because doing so improves efficiencies at the farm level. However, some consumers are exercising their right to choice by making demands of their governments for labeling foods and feeds derived from GM crops. This becomes an issue for trade due to the lack of harmonized rules to facilitate the trade of authorized GM products. Table 2 outlines the diversity regarding labeling regulations around the globe.

These labeling requirements can be misleading to the public as they imply that there are health, safety, and nutritional differences associated with GM products. In comparison, maximum residue limits (MRLs) based on results of safety studies for these compounds have been established in different countries for veterinary drugs and pesticides in food. This contrasts to the "zero tolerance" concept adopted by some countries for GM crops for which no safety issues have been found to date.

Asynchronous authorizations as discussed above can lead to disruptions in trade. Table 4 depicts an abridged segment of asynchronous authorizations for products routinely traded between the United States and European Union. Whereas currently there are about 30 commercialized crop events derived from modern biotechnology cultivated worldwide, the forecast is that by 2015 there will be more than 120.[30] With the potential for up to 54 new GM products being brought to market by Asian companies and public entities in the next few

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Table 4. Asynchrony of Modern Biotechnology-Derived Crop Approval between the United States and European Union[a]

crop and event	United States	European Union
soy, MON 40-3-2	1994	1996
cotton, MON531	1995	1997
cotton, MON1445	1995	1997
maize, T25	1995	1998
maize, MON810	1996	1998
maize, Bt11	1996	1998
maize, GA21	1997	2005
canola, T45	1998	1998
soy, A2704-12	1998	2008
soy, A5547-127	1998	2012
canola, GT73	1999	1996
canola, MS8 x RF3	1999	1999
rice, LL62	2000	assessment
maize, NK603	2000	2005
maize, 1507	2001	2006
cotton, 15985	2002	2003
maize, MON863	2002	2006
cotton, LL25	2003	2008
maize, 59122	2005	2007
sugar beet, H7-1	2005	2007
maize, MON88017	2005	2009
cotton, MON88913	2005	Assessment
soy, MON89788	2007	2008
maize, MIR604	2007	2009
maize, MON89034	2008	2009
maize, 98140	2008	assessment
soy, 356043	2008	2012
cotton GHB614	2008	2011
soy, MON87701	2011	2012
maize, MIR162	2010	assessment completed in 2012

[a]Sources: AgBioForum, 13(2), 2010 pp 173-182 and http://www.gmo-compass.org/eng/gmo/db/ http://www.cera-gmc.org/?action=gm_crop_database.

years, the current acceptance pattern may be altered to reflect approvals in Asia first and then spread to other regions. To date, GM crops developed in Asia have been for domestic consumption, not export. These GM products may be less likely to be submitted to other countries for import approval.

A more recent trade complication is the prevalence of "stacked" traits in commodity crops. Plants with stacked traits contain two or more transgenes, often the result of crossing two transgenic plants. In the United States and Australia, approved events incorporated by breeding crosses into new products do not require additional evaluations. This is not the case in other regions. If all stacked products were to be regulated as new products, then approvals would need to increase exponentially and the number of products would overwhelm the regulatory system. This introduces an entirely new dimension to testing and approving new products and is outside the scope of this paper.

CONCLUSIONS

In conclusion, extensive work goes into selecting both the trait and the GM event expressing the trait long before any decision is made to commercialize a GM product. Once the event is selected, a safety assessment is conducted on the GM crop to confirm that it is as safe as its conventional counterpart. Diagnostic tools and methods play an important role in safety assessment throughout the development process and after commercialization.

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Notes

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