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Genetically Engineered Crops: From Idea to Product

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Annu. Rev. Plant Biol. 2014. 65:769–90

First published online as a Review in Advance on February 21, 2014

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev-arplant-050213-040039

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Keywords

biotechnology, GE crops, GM crops, transgenic plants

Abstract

Genetically engineered crops were first commercialized in 1994 and since then have been rapidly adopted, enabling growers to more effectively manage pests and increase crop productivity while ensuring food, feed, and environmental safety. The development of these crops is complex and based on rigorous science that must be well coordinated to create a plant with desired beneficial phenotypes. This article describes the general process by which a genetically engineered crop is developed from an initial concept to a commercialized product.

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1. INTRODUCTION

The greatest service which can be rendered any country is to add an useful plant to its culture.

—US President Thomas Jefferson, 1800 (49)

Modern plant genetic engineering began in the 1980s, when techniques utilizing *Agrobacterium tumefaciens*, particle bombardment, and electroporation facilitated the insertion of useful genes into plant genomes (4, 40, 46, 47, 51, 87). Plant transformation accelerated the progress of basic research in plant biology in model systems such as *Arabidopsis thaliana* (94) and was immediately identified as a useful tool for improving agriculture through genetically engineered (GE) crops.

The ability to transform genes into plants allowed the creation of agronomic traits (e.g., insect resistance or herbicide tolerance) and the first transgenic crops: corn, soybean, and cotton (43, 59, 98). Researchers and technology developers introduced bacterial genes into crops to provide protection against pests and diseases, leading to reduced application of pesticides (as reviewed in 41). Development of crops tolerant to herbicides expanded growers' weed-management options and facilitated the adoption of more environmentally benign farming practices that improved soil quality and decreased greenhouse-gas emissions and fertilizer use, while improving the quality of water runoff to streams, rivers, lakes, and aquifers (10, 11, 34, 58, 77, 79, 90, 92).

Even in the early days of GE plant development, researchers envisioned crops with a variety of benefits to both consumers and farmers. These benefits included increased yield and tolerance to abiotic stresses such as drought or salinity (100); improved oilseed nutritional composition, resulting in better vegetable oils (52); enhanced amino acid profiles of grains; and increased vitamin and mineral content (57). The production of industrial enzymes, medically active components (22), and bioplastics was also highlighted and pursued (75), as previously reviewed (13).

Bringing a new GE crop product to the commercial market can be a challenging, long-term, and expensive enterprise, costing an estimated average of USD 136 million and 13 years from product concept to product launch (60). Ideally, the corresponding market opportunity will be large enough to warrant the investment in product discovery, development, and completion of the required regulatory reviews, as well as providing capital for reinvestment in new technology development. The cost of conducting regulatory safety evaluations and securing global registration and authorizations has been estimated to average USD 35 million for GE crops introduced in the 2008–2012 time frame (60). The high cost of developing and gaining regulatory approvals for

GE crops limits the use of GE technology to broad-acre row-crop improvement and restricts opportunities to improve minor crops that are staples in developing countries.

The widely successful commercialization in 1996 of Roundup Ready® (glyphosate-tolerant) soybean serves as an example of the product development process. Efforts to create glyphosate-tolerant crops began in the early 1980s primarily because they held the potential to create value and dramatically improve the ability of farmers to control a broad spectrum of weeds by spraying Roundup®, a nonselective herbicide, on the crop (50, 70). Because soybeans are grown on a large global area (100 million hectares in 2012) (48), the market for such a trait was sufficiently large to warrant the investment; moreover, a viable technical solution was achievable. Additional herbicide-tolerant and insect-resistant crops were commercially introduced in subsequent years, during which they were rapidly adopted relative to other agricultural technologies. In 2012, GE crops were grown in 28 countries and on more than 170 million hectares of land, consisting mainly of corn, soybean, canola, and cotton (48).

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2. THE PRODUCT DEVELOPMENT PROCESS: EARLY PHASES

The process of GE crop development has evolved over time and comprises several phases, each of which takes between two and five years to complete, with certain activities overlapping multiple phases (**Figure 1**). Each phase advancement represents a financial commitment on behalf of technology developers of between USD 10 million and USD 46 million (60). Therefore, all aspects of each trait development program must be thoroughly assessed before a phase transition. Only products that meet technical and safety standards (see sidebar The Theoretical Framework for Safety Evaluation of Genetically Engineered Crops) and have positive business prospects proceed toward commercial introduction.

2.1. Gene Discovery

Once a concept for a commercial product has been formulated, the gene discovery process begins. At this early development stage, candidate genes are drawn from a wide variety of sources, including analysis of the genomes of plants and microorganisms, study of naturally occurring biological processes, and review of relevant scientific literature. In addition, collaborations with

THE THEORETICAL FRAMEWORK FOR SAFETY EVALUATION OF GENETICALLY ENGINEERED CROPS

GE crops and derived products are evaluated for safety globally by regulatory agencies through a framework of science-based risk assessment and risk management measures. The safety of crops produced through biotechnology must meet assessment standards established by regulatory agencies around the world, a process that is composed of four steps: hazard identification, hazard characterization, exposure assessment, and integrative risk characterization (16, 25, 27). As with conventional crops, the safety of GE crops is determined based on the standard that there must be "a reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption" (65, p. 10). This standard is important because no food, either conventional or genetically engineered, is safe in absolute terms. For example, many conventional foods, such as potatoes, soybeans, and celery, contain toxins and/or antinutrients, and, like most traditional foods, they have not been subject to food or feed safety studies. Instead, the safety of these crops and foods has been established by virtue of their long history of safe use (20).

	Discovery Gene/trait identification	Phase 1 Proof of concept	Phase 2 Early development	Phase 3 Advanced development	Phase 4 Prelaunch
Average duration	54 months	27 months	30 months	37 months	49 months
Average cost	USD 31 million	USD 28.3 million	USD 13.6 million	USD 45.9 million	USD 17.2 million
Key activity	• High-throughput screening • Model crop testing	• Gene optimization • Crop transformation	• Trait development • Preregulatory data • Large-scale transformation	• Trait integration • Field testing • Regulatory data generation • Product development	• Regulatory submission • Seed bulk-up • Premarketing • Product development

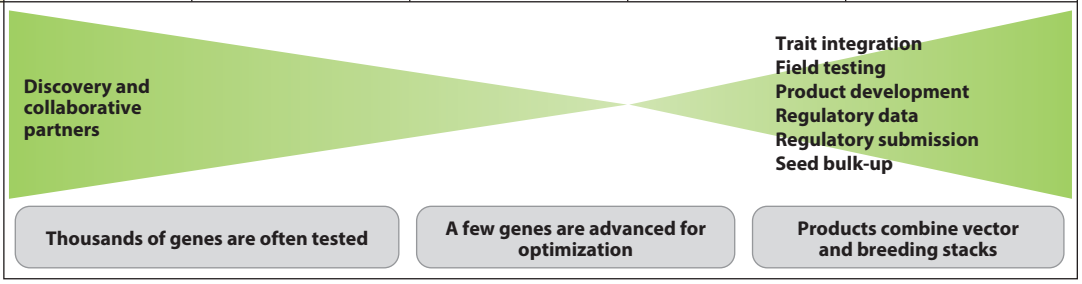

Figure 1

Overview of the development process of a genetically engineered crop, including activities, durations of those activities, and costs. Durations and costs are industry averages (60). Because various activities overlap, the cumulative total of each phase does not reflect the actual duration of the overall research and development process.

other companies or academic groups can lead to discoveries of new candidate genes or genes that have proven and desired activity (**Figure 1**).

High-throughput screens are essential during the discovery phase. Thousands of candidate genes are often evaluated and screened in model transgenic plants such as *Arabidopsis thaliana* or in plant cell culture, both of which have a more rapid life cycle than crops. Significant advances in automation have enabled rapid screening that utilizes, in the case of insect-resistance traits, high-throughput insect-feeding assays with artificial diets to test for insecticidal activity. The goal of the gene discovery phase is to identify candidate genes that have the desired activity and that can then be tested in the target crop.

The soil bacterium *Bacillus thuringiensis* (Bt) serves as a case study for the identification of candidate genes. Bt has been used for decades as a spray application to control certain insects during the cultivation of vegetables such as tomato, broccoli, cabbage, cauliflower, and lettuce, and it has even been used during the cultivation of fruits and nuts and in forestry (reviewed in 88). More recently, Bt has been identified as a source for numerous genes that can confer insect-resistance traits to GE crops (84, 91). Initially, a forward genetics approach was used to identify the toxin-encoding gene that conferred the insecticidal properties of a particular Bt strain against the target insect pest. The gene coding for the insecticidal toxin (Bt toxin) was then inserted into a crop plant, making it resistant to feeding damage by the target pest (23, 86, 99). The advent of advanced sequencing and bioinformatics technologies has changed the nature of Bt toxin discovery, enabling the use of a high-throughput approach to sequence multiple Bt strains in a short time and to analyze the sequence information for predicted Bt toxins (102). Predicted Bt toxins can be expressed in a heterologous expression system and tested in artificial diet bioassays for activity

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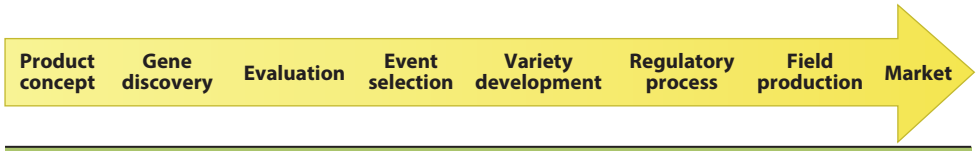

Choice of genes/proteins	Agronomic assessment	Characterization of gene product and comparative analysis	Postmarket assessment
• Source • Initial molecular characterization • History of safe use • Mode of action	• Greenhouse to field • Agronomic performance • Phenotypic screening of events • Event selection (<1%)	• Toxicity • Allergenicity • Nutrition • Compositional analysis • Environment • Further molecular characterization	• Postmarket surveillance • Supplemental food/feed studies, as needed

Figure 2

Overview of the safety assessment process that occurs throughout the development of genetically engineered crops. Some events occur in parallel.

against insect pests of crop plants. Candidate genes can then be engineered to produce proteins with increased spectra of activity based on their structure and function (6).

2.2. Phase 1: Proof of Concept

During phase 1, genes that were identified during the gene discovery process are transformed into the target crop. This phase also marks the beginning of a thorough safety assessment of GE crops, which begins early and spans the entire product development process (**Figure 2**).

Prior to transformation of host plants, a bioinformatics approach is used to screen open reading frames to determine whether the predicted amino acid sequence is homologous to known or putative allergens and/or toxins. This comparison follows internationally accepted standards and guidance from regulatory agencies for evaluating proteins in GE crops (17, 39). This initial analysis of transgene-produced proteins is designed to identify potential concerns from the perspective of food and feed safety and eliminate some genes from further advancement.

2.2.1. Vector design and plant transformation. For plant transformation, a plasmid vector is designed to transfer the candidate gene into the crop plant genome, along with expression elements such as promoters and untranslated regions upstream and downstream of the candidate gene, which enable efficient transcription, mRNA stability, and translation. The expression cassette must be carefully chosen to ensure that the transgene is expressed at the desired level, accumulates in the desired cell compartment, and is present in the desired plant tissues and at the desired time. Multiple expression cassettes and rounds of optimization may be required to ensure optimal establishment of the trait without undesired effects such as yield reduction. The development of Roundup Ready® herbicide-tolerant crops serves as an example: Selection of the promoter to drive 5-enolpyruvylshikimate 3-phosphate synthase expression was critical in providing glyphosate tolerance. In this case, it was desirable to express the protein in vegetative as well as reproductive plant tissues and thereby extend the Roundup® application window (12, 45).

In addition, the coding sequence of the candidate gene might need to be optimized to better match the codon usage of the plant while removing unnecessary elements such as mRNA-destabilizing sequences and palindromic sequences, which can result in reduced mRNA stability

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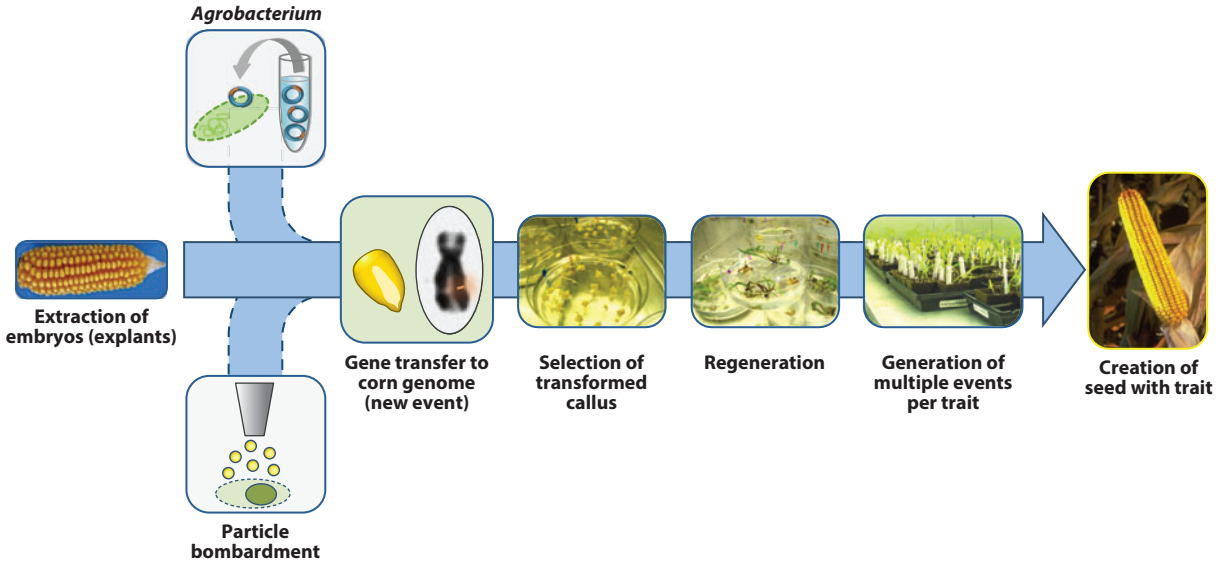

Figure 3

Overview of primary methods used for plant transformation. During the transformation process, either *Agrobacterium tumefaciens* or particle bombardment is used to transfer the desired gene(s) into individual plant cells. These transferred genes then become integrated into the genome of some recipient cells. After selection, whole new transgenic plants are regenerated from transformed cells, giving rise to a transgenic line, referred to as an "event." Thousands of events are generated at this stage to enable testing of multiple variants of the transgene and its expression elements.

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or translation. This optimization process has a beneficial impact on protein expression levels (73, 95). Furthermore, the expression cassette, comprising the expression elements and protein-coding region, must be carefully inspected via bioinformatics analysis to minimize the chances that the transgenic plant will produce unintended transcripts or peptides.

Agrobacterium-mediated transformation and transformation via bombardment with DNA-coated particles are two commonly used methods that can be employed to insert the transgene into the plant genome (1, 97) (**Figure 3**). Along with the transgene expression cassette, the transformation vector often contains a cassette with a selectable marker that allows for selection of plant cells that contain the transgene. Transformed plant cells are regenerated into plants, giving rise to a transgenic line, referred to as an "event." Thousands of events are generated at this stage to enable testing of multiple variants of the transgene and its expression elements.

2.2.2. Transgenic plant testing. Controlled-environment testing in a greenhouse or growth chamber allows for rapid screening of many transgenic events. For example, transgenic plants expressing a protein with insecticidal properties can be screened via a high-throughput insect assay in a greenhouse, and events that are being developed for herbicide tolerance can be treated with the appropriate herbicide to select for events that exhibit the desired level of tolerance. The controlled-environment screening can be combined with high-throughput imaging systems to measure and quantify plant growth and development under these specific conditions.

Transgenic events that meet performance criteria under controlled conditions are then tested in field trials and closely monitored under regulated conditions. These trials include efficacy tests to evaluate the performance of the developed trait; in the case of insect-control traits, the events are tested by infesting test plants with the target insect, either artificially or via natural insect pressure,

and then scoring the level of damage on the plants after insect feeding. The agronomic performance of the event is tested in addition to trait efficacy. To be considered for commercialization, an event that shows excellent protection against insect-feeding damage must also demonstrate agronomic and phenotypic characteristics that are equivalent or superior to those of a nontransgenic isoline in the absence of or with minimal insect pressure.

2.2.3. Gene optimization. At this stage, additional optimization may be necessary to enhance the desired trait within the target crop. Because of the intricacies of effectively expressing a transgenic trait in a crop plant, multiple approaches can be taken to improve performance. A protein that shows promising insecticidal properties when fed to insects in an artificial diet, for example, may not be as effective when expressed in target plants, or may adversely impact the proper development of the plant, reducing yield. These potential issues can be mitigated by changing the expression cassette or the protein-coding region to improve the stability of the expressed protein in the crop plant. For example, codon optimization and chloroplast targeting of the insecticidal Cry1Ah protein in transgenic tobacco resulted in greatly increased expression levels and protection against insect damage (56). Any necessary optimization might then require further analysis via an additional proof-of-concept stage.

2.3. Phase 2: Early Development

The goal of phase 2 is to produce and select events with agronomic performance similar to or exceeding commercial standards while maximizing the probability of success for timely regulatory reviews. Additional studies to describe the mode of action of a candidate gene are also performed.

2.3.1. Commercial vector development and transformation. Once the desired transgenic trait has been observed reproducibly in the greenhouse and in field trials, and no obvious agronomic or phenotypic issues are observed, a new round of transformation begins. The number of transgenic events now increases significantly, with an additional 500–2,000 events generated per transformation vector (60) (**Figures 1** and **2**).

Before the expansion of target crop transformation and increase in total number of transgenic events, the transformation vector is analyzed again by complete DNA sequencing to ensure that it codes for a protein with an amino acid sequence that is identical to the sequence that was identified and tested during previous phases. The encoded protein sequence is again subjected to a bioinformatics evaluation to assess for homology to putative allergens or toxins, as described above for phase 1.

2.3.2. Molecular characterization. A molecular analysis performed on multiple successive plant generations ensures that the integration of the transgene into the crop genome is stable (12, 45, 99). This analysis focuses on selecting events that have a single intact copy of the T-DNA and lack other fragments such as vector backbone sequences or partial gene cassettes, which occasionally are inserted during the *Agrobacterium*-mediated transformation process. The inserted T-DNA and the regions that flank the insertion site are sequenced and characterized. Ideally, no host genes or regulatory elements would be present in close proximity to the transgenic T-DNA. Cases where the T-DNA appears to disrupt a native gene or expression element rarely pose a safety concern, although additional analysis is required to ensure that plant growth, development, and yield are not affected.

The DNA sequence information of the insertion site is also used to develop a molecular assay that can specifically detect the unique event, and it can be used in seed quality-control tests (such as

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tests to determine the genetic purity of a seed batch) and to track the integration of the transgene during the process of incorporating a specific trait into a variety of germplasms (see Section 3.3). The mRNA transcript and size of the translated protein product(s) are also characterized to ensure that they are not changed by hypothetical interactions with surrounding genomic regions. These molecular characterization steps are performed on plants grown in isolation to ensure that seeds derived from these plants contain only the intended transgene. The genetically pure seed lot produced at this stage is used for subsequent safety studies as well as for trait integration and breeding.

2.3.3. Field evaluation. Phase 2 also includes rigorous evaluation of the candidate transgenic events in the field. The agronomic performance of the trait and crop is tested across multiple representative geographic locations and growing seasons, which ensures that the transgenic events display the expected phenotype regardless of environmental conditions and that unintended effects are not present relative to a nontransgenic control line. At this stage, the transgene can be introduced through conventional breeding into a limited selection of commercial germplasms to enable the testing of trait performance across different germplasm backgrounds. The efficacy of a given trait can be tested by exposure to the stress (insect pest, herbicide, or environmental stress) against which it is designed to protect. Events that show the intended trait in these extensive field trials during multiple growing seasons have potential to advance to phase 3.

2.3.4. Mode-of-action studies. If a particular candidate gene shows promising results during field evaluation, then the next step is to perform studies to describe its mode of action—the process through which a particular transgene enables the GE crop to display the desired phenotype. The purpose of these studies is to provide information in support of submissions required for product review as well as to further the development of similar candidate genes or modes of action. A review of the scientific literature is conducted to help researchers understand the role of the candidate gene in its native organism. For traits identified via high-throughput screening assays, experiments are performed to test hypotheses of specific modes of action. Studies on the mode of action of herbicide tolerance and insect resistance may include the characterization of the transgenic protein and its interaction with the target pest. Ideally, these experiments identify different modes of action that can be combined to minimize the development of resistance in the target pest.

3. THE PRODUCT DEVELOPMENT PROCESS: LATE PHASES

The late phases of the product development process play an important role in ensuring that technology developers will gain global freedom to operate through the safety assessment and proper regulatory approvals for GE crops (**Figures 1** and **2**). Phases 3 and 4 involve a series of processes that occur in parallel and contribute to the development of commercialized GE crops (**Figure 1**). Only events that meet the criteria for field performance of the desired trait and that pass the molecular selection criteria described above can advance to these phases. Because submissions to regulatory agencies are made for individual transgenic events, normally only one or two of the highest-quality events advance to phase 3. The product concept, efficacy, market potential, and safety of the trait are once again reviewed, along with all available molecular, field trial, and mode-of-action data on the event(s) to be advanced.

In phase 3, additional data for further risk assessment are generated to thoroughly assess the safety of the GE crop (see sidebar A Comparative Approach to Risk Assessment); these data are part of regulatory submissions that occur in phase 4. Phases 3 and 4 also involve integrating the transgene into a variety of commercial germplasms through conventional breeding methods. Before commercialization, the agronomic and phenotypic performance of the GE crop is also assessed.

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A COMPARATIVE APPROACH TO RISK ASSESSMENT

The process of determining the safety of a GE crop historically begins with a comparative assessment, also referred to as the concept of "substantial equivalence" (14, 65). This approach helps identify similarities and differences between the newly developed crop and a conventional counterpart that has a long history of safe use (20, 53). Any actual or suspected differences then become the focus of the food, feed, and environmental safety assessment, which then indicates whether the GE crop can be considered as safe as the conventional crop and treated in the same manner (19, 37, 38, 65). Typically, comparative safety assessments have used a non-GE counterpart, such as a parental line with a similar genetic background, as a conventional comparator. In cases where an appropriate non-GE comparator does not exist—for example, in the case of retransformation—either a negative segregant (a line where the transgenic event has been segregated away through breeding) or the recipient GE plant may be considered as comparators (31).

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3.1. Phase 3: Advanced Development

Phase 3 entails the rigorous and strategic assessment of the food, feed, and environmental safety of the GE crops (see sidebar Safety Assessment Strategy for Genetically Engineered Crops). The safety data are collected in dossiers that are submitted to regulatory agencies around the world based on a careful strategy that guides the scope and timing of each submission.

3.1.1. Food and feed safety assessment. Multiple internationally recognized comparative and toxicological approaches and guidelines are followed to ensure the food and feed safety of a GE crop (17, 18, 19, 28, 33, 37, 66). An important part of the food and feed safety assessment of GE crops is the evaluation of the nature and safety of the introduced protein. The protein, including its amino acid sequence, is assessed again to ensure that it has no similarity to known human or animal protein allergens or toxins. The introduced protein is also tested by exposure to simulated gastrointestinal fluids as well as heat treatment to ensure that it is rapidly digested and has a low allergenic potential (2, 44, 76, 96). Acute oral toxicity studies are conducted in mice using various concentrations of the purified protein to evaluate potential toxicity and treatment-related effects on survival, body weight, food consumption, and gross pathology. Based on a weight-of-evidence

SAFETY ASSESSMENT STRATEGY FOR GENETICALLY ENGINEERED CROPS

The product development process for GE crops follows a rigorous process for ensuring the safety of the crops as food, as animal feed, and as an integrated part of the environment. The safety studies performed throughout the development of these crops have contributed to several important conclusions. For example, GE crops undergo a safety assessment that is more rigorous and thorough than assessments of any other food crop in history, including conventional foods available in supermarkets. The safety assessment strategy ensures that the safety of GE crops is reviewed by multiple regulatory agencies in accordance with different risk assessment strategies and with national and international safety assessment guidelines (71). Two fundamental questions must be addressed: Is the food/feed safe for humans and animals to consume? And are the plants safe for the environment? Technology developers and regulators use the highest standards of food, nutritional, and environmental safety to evaluate any potential effects of introduced traits in GE plants.

approach, it is then concluded that introduced proteins in currently commercialized GE crops do not pose an allergenic or toxicity risk for humans and animals.

Another part of the food and feed safety evaluation of a GE crop is assessing any potential compositional differences when compared with a conventional control. This includes assessing the levels of numerous nutrients, antinutrients, toxins, allergens, and other important compounds normally present in crops—e.g., macronutrients such as carbohydrates, proteins (including all amino acids), oils (including fatty acids), and vitamins and minerals—to ensure that the GE crops are compositionally equivalent to conventional varieties (67–69). The particular set of key analytes to be evaluated in specific crops is guided by internationally approved crop composition documents developed by regulators and food/feed safety experts (67–69).

Animal-feeding studies are performed on a case-by-case basis to assess whether the novel GE crop is as wholesome and nutritious as a conventional crop with a history of safe use. This is done by assessing the growth of test animals when they are fed a diet containing high levels of a GE grain or crop-derived ingredient. These studies commonly use broiler chickens because of their rapid growth and nutritional sensitivity to diet alterations. The growth performance of animals fed nutritionally balanced diets containing feed derived from the GE crop is measured against that of animals fed nutritionally balanced diets containing feed derived from the conventional counterpart. A GE crop is considered as wholesome and as nutritious as a conventional control when animals fed a diet containing GE crop–derived feed have growth characteristics similar to those fed conventional feed (14).

To satisfy the requirements of some regulatory authorities, 90-day animal toxicity studies adapted from Organisation for Economic Co-operation and Development (OECD) Test Guideline 408 are also performed using the grain or another harvested component of the GE crop (66). These studies are conducted by feeding rodents relatively high levels of the GE crop or conventional comparator (e.g., the GE crop at levels at or above one-third of the total diet). To identify potential toxicologically significant diet-related differences relative to the conventional comparator, standard toxicity endpoints are monitored during the study, including body weight, food consumption, clinical findings, clinical chemistry parameters (hematology, serum chemistry, and urinalysis), macroscopic observations at necropsy, organ weights, and microscopic findings at histopathological examinations. To date, 90-day feeding studies using high-quality, approved testing methods and facilities have revealed no diet-related toxicological hazards in animals fed GE crops (36, 93). In fact, with the weight of evidence from studies characterizing the molecular, agronomic, compositional, and nutritional equivalence, the usefulness of 90-day feeding studies has been questioned, and their use has even been discouraged by some regulatory agencies and other organizations (30, 55, 63, 82, 93).

3.1.2. Environmental risk assessment of genetically engineered crops. Each GE crop product undergoes an environmental risk assessment (ERA) prior to commercialization to assess potential harmful effects on the environment. The ERA considers the potential ecological impact of the modified plant and the introduced trait(s) (**Figure 4**). The ERA approach or framework, as developed by the US Environmental Protection Agency and used effectively for environmental assessments of chemical stressors, is flexible, adaptable, and appropriate for ERAs of GE crop products (24). The guidance from the US Environmental Protection Agency is consistent with other international scientific guidance documents (3, 9, 29, 64) and publications for ERAs of GE crops (62, 80, 81, 101).

Similarly to the compositional evaluation in the food and feed safety assessment, the ERA includes a comparative assessment under diverse geographic and environmental conditions in which the GE plant is compared with an appropriate conventional control plant that is

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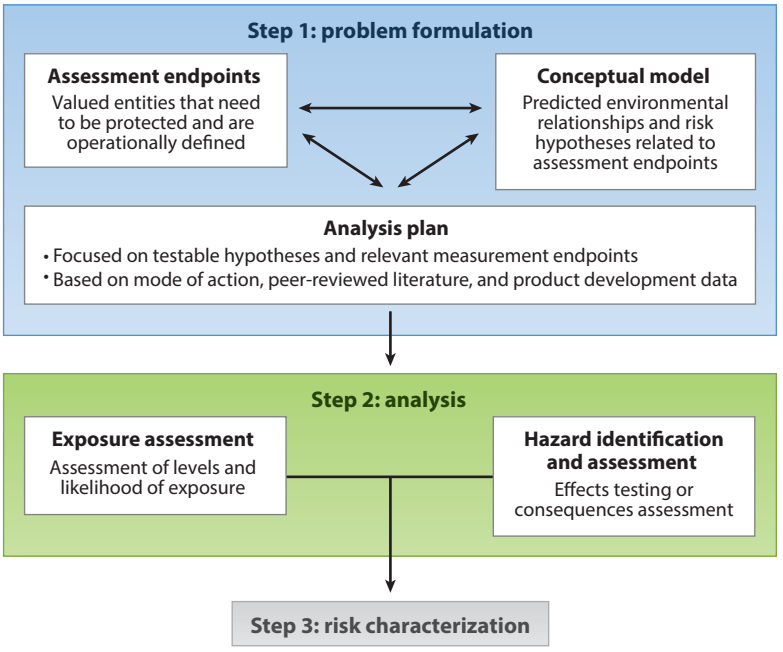

Figure 4

Environmental risk assessment framework for genetically engineered crops.

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genetically similar but lacks the introduced trait. Specific phenotypic, agronomic, and ecological characteristics are measured in the GE plant and the conventional control to determine whether the introduction of the GE trait has resulted in any changes that might cause ecological harm in terms of altered weed characteristics, susceptibility to pests, or adverse environmental impact.

Problem formulation, the critical first step in the ERA, directs the assessment based on the product concept, crop, trait(s), intended use (e.g., import versus cultivation), receiving environment, and potential interaction among these factors. Assessment endpoints, which are specific entities that need to be protected in the environment and that are operationally defined, measurable, and ecologically relevant, are identified in this step. The assessment endpoints are based on broader goals such as protecting agricultural productivity, ecological function, or biological diversity and are typically set by law, regulation, or guidance (89). An example of an assessment endpoint for an insect-resistant GE maize product could be the conclusion that there is no ecologically significant difference in the abundance of relevant beneficial insects that can be reliably evaluated in a genetically modified (GM) maize field compared with a conventional maize field. Within problem formulation, plausible risk hypotheses are formulated based on reasonable exposure scenarios that can be tested in the analysis plan using existing information or new experimental data. A risk hypothesis that may require testing for an insect-resistant GE maize product could be its potential adverse effects on nontarget organisms compared with conventional maize.

Risk hypotheses tested in the analysis plan for the ERA of a GE plant may include assessments of the ability of the crop to become a weed on agricultural land or in unmanaged plant communities, or the potential consequences of gene flow between a crop species and a sexually compatible relative. Additionally, environmental interaction data are collected in field experiments for the GE crop and

the conventional comparator to evaluate potential adverse effects; these data include measurements of disease and insect susceptibility and crop damage under natural infestation pressure.

For insect-resistant GE crops, a stepwise approach with surrogate species is used to assess potential adverse effects on nontarget organisms in many regulatory frameworks (83, 85). Tiered testing is designed to first represent highly conservative, worst-case exposure scenarios, and then progress to real-world field scenarios only if the earlier tiered tests conducted at elevated dose levels fail to indicate adequate certainty of acceptable risk. Tests in the first tier can be well standardized, are conducted at exposure concentrations several times higher than the highest concentrations expected to occur in the field, and provide a high level of certainty that adverse effects are unlikely at realistic field exposure scenarios. Surrogate species are chosen to represent ecologically and economically important taxa of different functional groups (pollinators, predators, parasitoids, detritivores) for that crop that are likely to be directly or indirectly exposed to the insecticidal trait in the field.

Risk characterization, the final step of the ERA, integrates the exposure and hazard assessment information produced in the analysis plan in order to evaluate the likelihood of any potentially harmful ecological effects from the release of the GE plant into the environment. The ERA for a GE crop is an iterative process. If at any stage there is significant remaining uncertainty for the risk conclusion or relevant new information becomes available, additional testing of risk hypotheses is possible. Therefore, the ERA approach effectively leads to conclusions with high certainty that the commercial release of a GE crop will pose minimal environmental risk.

3.1.3. The dossier submission strategy. The safety data gathered throughout the product development process are contained in dossiers (or submissions) presented to regulatory agencies worldwide in order to secure freedom to operate in all desired regions. These regulatory dossiers present a "narrative description" that comprehensively provides the weight of evidence that a particular GE crop is as safe as its conventional counterpart for use in the environment and/or for use as food and feed. General features of risk assessments of GE crops and the contents of regulatory dossiers have been extensively reviewed (15, 18, 21, 54, 64, 65).

Even before the collection of safety data begins, a regulatory submission strategy is developed to guide the specific scope of the data to be collected. Because regulatory requirements vary between countries according to national biosafety laws and frameworks (5, 71), one prerequisite is to identify the countries that have regulatory relevance for a particular GE crop. Technology developers conduct the relevant studies using methods or protocols designed to meet different countries' regulatory requirements and submit dossiers to the appropriate authorities for review. Another important consideration is the extent to which data generated in one country will be used and accepted in another country. This is especially true for data collected to support submissions requesting authorization for cultivation. Some countries also require a local field trial or local feeding study to support an authorization to allow the import of GE grain intended for processing.

Developing a submission strategy requires an understanding of commodity flows and national regulations to ensure market access and unhindered global freedom to operate, as many countries have established independent biosafety frameworks to regulate the import of foods and feeds derived from GE crops. Technology developers conduct assessments to understand export, import, and production trends of key crops and their by-products in different countries and regions of the world. The assessment identifies key export markets for commodities such as corn, soybean, canola, and cotton and ensures that regulatory authorizations will be obtained in the production country (or countries) and in key import countries with functioning regulatory systems, so that grain or by-products derived from GE crops may be lawfully imported as commodities (48) (**Figure 5**). Some countries import GE crops but do not currently have a functioning regulatory framework.

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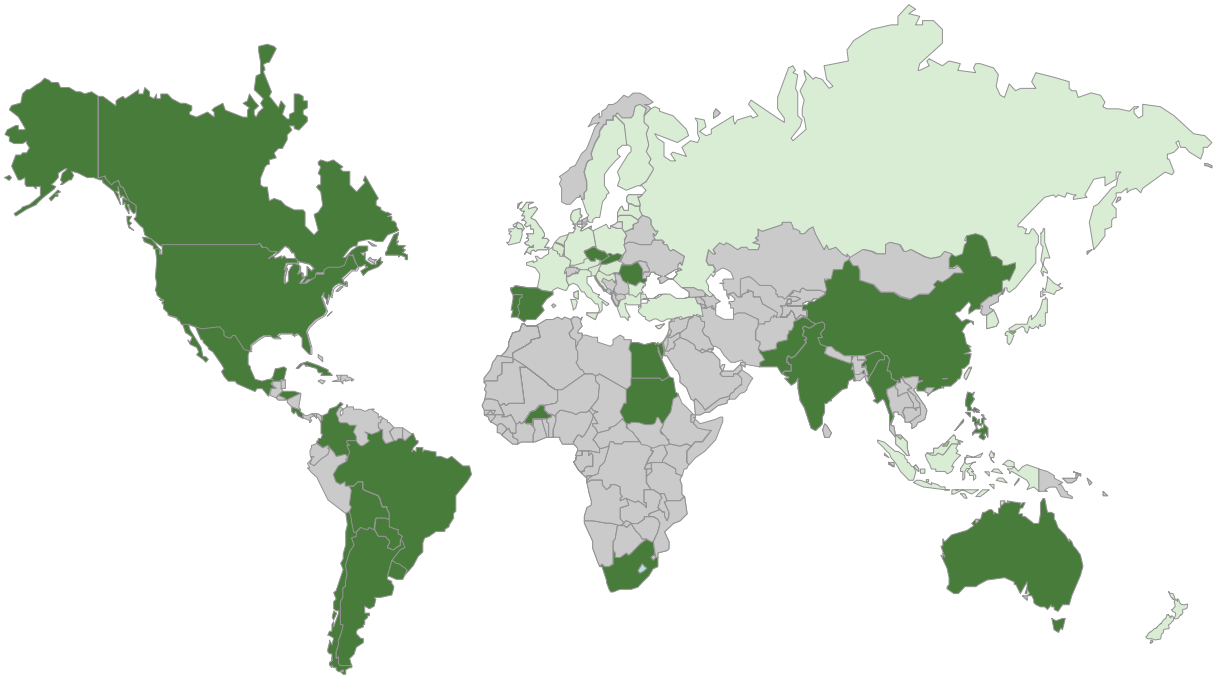

Figure 5

Map of countries cultivating or exclusively granting import approvals for genetically engineered (GE) crops in 2012 (48; Monsanto data). Countries that cultivate genetically engineered crops (*dark green*): Argentina, Australia, Bolivia, Brazil, Burkina Faso, Canada, Chile, China, Colombia, Costa Rica, Cuba, the Czech Republic, Egypt, Honduras, India, Mexico, Myanmar, Pakistan, Paraguay, the Philippines, Portugal, Romania, Slovakia, Spain, South Africa, Sudan, the United States, and Uruguay. Countries that do not cultivate but grant import approvals for genetically engineered crops (*light green*): Indonesia, Japan, Malaysia, New Zealand, Russia, Singapore, South Korea, Switzerland, Taiwan, Thailand, and member states of the European Union EU28 (except for the five countries that cultivate). Some countries import GE crops but do not currently have a functioning regulatory framework.

At the conclusion of the trade assessment, the key countries requiring production and import approvals are identified, and planning for regulatory data development and the drafting and timely submission of regulatory dossiers can commence.

3.2. Phase 4: Prelaunch Regulatory Submissions

As with phase 3, phase 4 includes multiple processes that are performed in parallel. These processes include additional regulatory data generation; global regulatory dossier submissions, discussed in this section; and agronomic performance assessment, discussed in Section 3.3 (**Figure 1**).

Given the large number of regulatory submissions that are made around the world for a given GE crop, there is a great deal of diversity in how the regulatory safety data are compiled and presented to regulators. The subsequent review of regulatory dossiers is likewise diverse in terms of process, length of time to complete, and scope of review. Authorizations from regulatory agencies are, however, a necessary prerequisite for commercial introduction of a GE crop and a developer's freedom to operate, though the process for gaining authorizations can be highly unpredictable. As described in greater detail below, there are many causes for the current unpredictability in regulatory reviews in many countries at a national level and asynchrony of authorizations at an international level (61). These include shifting regulatory data requirements, process challenges

and redundancies in data reviews, a lack of resources or biosafety expertise in some countries, and entanglement of the regulatory process with political and legal issues, all of which have contributed to slowed and less predictable regulatory reviews.

3.2.1. Dossier preparation and submission. To support global trade of GE crop-derived grain and by-products, dossiers must be submitted to authorizing agencies globally (**Figure 5**). Many countries have not just a single regulatory authority but rather multiple agencies, each responsible for assessing a particular aspect of safety. For example, five agencies are responsible for assessing safety in the Republic of Korea: The Ministry of Food and Drug Safety focuses on human safety for food use, the Korea Center for Disease Control and Prevention focuses on human safety except for food use, the Rural Development Administration focuses on environmental safety for agricultural ecosystems, the National Fisheries Research and Development Institute focuses on environmental safety for marine ecosystems, and the National Institute of Environmental Research focuses on environmental safety for natural ecosystems. Making submissions to multiple agencies in multiple countries for each new GE crop poses substantial logistical challenges.

Each agency has its own formatting and review process, resulting in significant differences between countries in the amount of time taken between submission and authorization. Thus, the volume of information and data under review is often quite disparate. In many countries, regulatory submissions are mandated to be reviewed within time frames defined by statute or regulation (e.g., 180 days for the US Department of Agriculture, 270 days for the Korean Rural Development Administration). However, although many biosafety frameworks do have timelines for completion of regulatory reviews, these timelines are typically not met, as regulators often ask multiple rounds of questions, which allows them to stop the clock on the review process while the technology developer prepares answers.

3.2.2. Dossier review/Q&A. Once submitted to regulators around the world, regulatory dossiers are subjected to review by independent risk assessors and scientists across a wide range of disciplines. It is important to note that in many countries, the experts that review safety data often come from academia or government laboratories. Many biosafety frameworks specify the range of disciplines that must be represented on evaluative biosafety panels or committees, and they often include nonscientists as part of the decision-making process. There is usually significant product-by-product and country-by-country variation in the range of questions posed by regulators. Often a request for additional data or information on one product leads to similar data requirements for subsequent products. This successive increase in data requirements is another factor that raises costs (60) and decreases the predictability and transparency of the regulatory review process.

In the EU, the GM authorization framework has two distinct and separate phases. The first is the risk assessment phase, which conforms with the European Commission Implementing Regulation for review of GM food and feed (26). This involves a scientific risk assessment by independent scientists operating under the European Food Safety Authority (EFSA). At the end of this step-by-step assessment, EFSA provides a scientific opinion to the European Commission on a specific product. This is followed by a risk management and political decision-making phase during which the European Commission and the member states take into account EFSA's scientific opinion together with other considerations and propose a decision of whether to authorize the GM product. This politicization of the science-based system has led to a highly unpredictable regulatory process.

3.2.3. Stacked traits. Commercial products increasingly contain multiple GE traits in combination. So-called stacked traits, which are combined GE traits that are brought together in a single

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hybrid or variety via conventional breeding, require authorizations in certain countries for use separately from single-trait products (74). As with single-trait data, there is a wide degree of variation in the data requirements for stacked-trait products. In some countries, the review or approval of stacked-trait products follows the review or approval of the single-trait product and requires additional data and review by regulatory authorities. This again lengthens the time frame needed to gain authorizations for, and thus the ability to launch commercially, the products planted by farmers.

3.3. Trait Development and Integration

Before commercial launch, a GE trait must be introduced into a set of genetically diverse conventional germplasms to meet the requirements of different geographical growing regions. This process, often referred to as conversion, occurs during phases 3 and 4 (**Figure 1**) and ensures the trait functionality and agronomic performance of the commercial product.

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3.3.1. Trait integration. The process of integrating a trait into commercial germplasms can be divided into several strategic activities. The first consideration of a successful breeding strategy should be to determine whether the trait(s) will be introduced through forward or backcross breeding. Forward breeding involves the development of a new variety or inbred line while concurrently selecting for the trait presence from a breeding population. In the case of backcross breeding, a trait is incorporated into a finished variety or inbred line through several generations of crosses to parental lines while selecting for progeny containing the trait. The decision is driven by the ease with which cross-pollinations are made in the target crop and the value placed on maintaining a nontraited germplasm base. For example, cross-pollination is difficult and costly in soybeans, pushing developers toward a forward breeding model, whereas backcross breeding is common in corn, in which cross-pollination is relatively simple and efficient.

The ideal breeding strategy will produce an isoline of the selected germplasm containing the target trait(s) and no other donor genetic material. Therefore, standards must be established to determine the acceptable amount of donor genetic material that remains. Considerable effort may be required in the first generation of new trait integration to minimize the presence of trait-linked donor DNA and avoid potential negative agronomic impacts on crop performance. Once strong donors are developed in a desired germplasm, less stringent criteria may be acceptable in subsequent breeding cycles.

The donor strategy is particularly important when creating stacked traits. In this case, separate integrations for each trait are performed in parallel, and individual conversions are then cross-pollinated in successive cycles until all desired traits are combined in a single plant (72). The conversion is finalized by self-pollinating and selecting for homozygosity of the traits of interest. A drawback to this approach is the potentially prohibitive time required to stack several traits relative to concurrent introgression of multiple traits through a single donor. Finished conversions must be tested to confirm that incorporated traits meet agronomic performance criteria and that no undesired agronomic impact has resulted from the conversion process.

3.3.2. Field testing. After the trait is integrated, lines that have demonstrated satisfactory trait efficacy and yield potential advance to large-scale field testing. Performance testing of the GE crops is done on genetically and geographically diverse germplasms to ensure proper yield and display of the expected phenotype regardless of environmental conditions. This is the final stage before market launch and is used to demonstrate product benefits to growers.

Researchers from technology developers across the world implement prelaunch test protocols. They identify locations to cover different agricultural environments with different soils and

weather. In some cases, sites may be selected to include pressure from a particular insect, disease, or weed. For example, corn hybrids with two or three stacked insect-resistance traits are evaluated in multiple locations, aiding in the determination of a product's fitness for a specific geography as well as the best protection for the level of insect pressure present in that location. All plots are planted and maintained using local agronomic practices. Crop and environmental data are collected according to a predetermined schedule and the protocol requirements. During the growing season, measurements are submitted to a centralized data collection system for real-time or postharvest analysis.

The number and location of research plots are determined by the geography where an insect pest or weed is present as well as the variability of the germplasm that contains the specific trait. For example, to evaluate an insect-resistance trait, at least 20 locations are normally used, whereas around 120 locations are normally used to evaluate traits that respond to environmental pressure. Each field test protocol includes approximately 50 testing locations. Protocols also detail the phenotypic characteristics to collect, such as emergence, pollination, insect and/or herbicide tolerance or control, and harvest measurements. To evaluate an insect-resistance trait, for example, researchers may measure variables such as where in the plant the damage is present, the amount of damage, and at what growth stage the damage occurred.

The performance evaluation is conducted using plots of varying sizes. For example, hundreds of ministrips (4 m^2) are used to evaluate germplasm/trait interactions; large-scale trials of up to 90 hectares (90,000 m^2) can be necessary when there is high environmental variability. The most common plot size of tested events at this stage is 20 m^2. Most of these plots are implemented with the assistance of commercial growers. This field testing evaluation usually takes two years and is tied to the completion of all regulatory approvals, which can extend for the duration of this phase.

The agronomic performance data collected during prelaunch field evaluations are summarized and presented in technical documents that are made available to growers around the world. Products are then launched based on geography, how they perform on an extensive set of attributes, and the added value they bring to growers.

4. CONCLUDING REMARKS

The development of a GE crop through commercialization is a complex, lengthy, and costly process that is accomplished through the coordination of multiple phases and through a robust product pipeline. The scientific knowledge required for and generated throughout this process has benefited farmers and society by contributing to higher crop output, lower resource input, improved pest control, economic gains, and farming practices that dramatically reduce the impact of agriculture on the environment (7, 8, 35, 48). GE crops facilitate simpler and more effective weed and insect-management practices. Engineered insect-resistance traits allow farmers to potentially reduce the number of insecticide sprays they use, and herbicide-resistance traits may allow them to spray broad-spectrum herbicides directly onto the crops while maintaining excellent crop safety (8, 42, 48). Biotechnology can undoubtedly help address the changing environment and contribute to poverty reduction and food security in the developing world (78).

We must recognize the importance and necessity of developing further harmonization of global data requirements. Such a process would facilitate the development of innovative and useful products by driving more technology investment in improved crop technologies that can deliver benefits to farmers and society by reducing the cost of development and the time to commercialization while increasing the predictability, clarity, and transparency of the regulatory process. An improved regulatory process will promote public–private partnerships, shorten innovation cycles,

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increase the rate of productivity gain, conserve limited environmental resources, and contribute to more sustainable agricultural systems.

The product development process described in this review illustrates the thorough safety assessment of GE crops. Each new trait that enters the regulatory approval process represents thousands of pages of safety data submitted to more than two dozen countries worldwide with functioning regulatory systems.

Technology developers have committed to following rigorous stewardship standards to prevent GE crops from entering commodity markets without appropriate approvals (32). Safety assessment strategies and regulatory guidelines are complex and costly and vary widely between countries, and yet they all aim to achieve a common goal: to ensure the safety of GE crops and the foods derived from them (71).

SUMMARY POINTS

1. The development of a commercial genetically engineered (GE) crop product necessitates the coordination of multiple complex phases that often occur in parallel.
2. Numerous steps throughout the development of a GE crop ensure that the final product is safe for use as food/feed and for use in the environment.
3. Farmers' needs, commercial opportunities, and technical feasibility all drive the development of a product concept and eventually a commercialized GE crop.
4. The final production of a commercial hybrid or line requires years of field trials assessing the performance of the product in multiple environments against traditional breeding metrics.
5. GE crops have brought benefits to farmers and society by increasing agricultural productivity and reducing food costs while providing numerous economic, environmental, and nutritional benefits.
6. Lack of global data harmonization and agency synchronization creates obstacles for entry to market, reduces investment in technology expansion, and creates trade barriers by increasing the complexity of the regulatory system.
7. A technology developer's global freedom to operate is crucial for the success of a commercialized GE crop and necessitates a streamlined development process, including a robust safety assessment, intellectual property rights, a regulatory submission strategy, and stewardship after commercial launch.

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DISCLOSURE STATEMENT

The authors are employees of Monsanto Company. They are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors would like to acknowledge Tracey Cavato, Nordine Cheikh, Bradley Comstock, Yong Gao, Jerry Hjelle, Aimee Hood, Michael Koch, Tom McBride, Tom Marvin, Michael Horak, Steve Levine, Rashmi Nair, Jim Roberts, Eric Sachs, Pamela Sisson, John Vicini, and Ty Vaughn for manuscript revisions and comments.

LITERATURE CITED

1. Altpeter F, Niranjan B, Beachy R, Bock R, Capell T, et al. 2005. Particle bombardment and the genetic enhancement of crops: myths and realities. *Mol. Breed.* 15:305–27
2. Astwood JD, Leach JN, Fuchs RL. 1996. Stability of food allergens to digestion in vitro. *Nat. Biotechnol.* 14:1269–73
3. Aust. Gov. Dep. Health Ageing Off. Gene Technol. Regul. 2009. *Risk analysis framework April 2009*. Commonw. Aust., Canberra
4. Barton KA, Binns AN, Matzke AJM, Chilton M-D. 1983. Regeneration of intact tobacco plants containing full length copies of genetically engineered T-DNA, and transmission of T-DNA to R1 progeny. *Cell* 32:1033–43
5. Bendiek J, Buhk H-J. 2010. Risk assessment and economic application of the Cartagena Protocol on Biosafety: GMO approval and import on a world-wide scale. In *Genetic Modification of Plants: Agriculture, Horticulture and Forestry*, ed. F Kempken, C Jung, pp. 631–48. Berlin: Springer-Verlag
6. Bravo A, Gómez I, Porta H, García-Gómez BI, Rodriguez-Almazan C, et al. 2013. Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity. *Microb. Biotechnol.* 6:17–26
7. Brookes G, Barfoot P. 2013. The global income and production effects of genetically modified (GM) crops 1996–2011. *GM Crops Food* 4:74–83
8. Brookes G, Barfoot P. 2013. Key environmental impacts of global genetically modified (GM) crop use 1996–2011. *GM Crops Food* 4:109–19
9. Can. Food Insp. Agency. 2012. *Assessment criteria for determining environmental safety of plants with novel traits.* Dir. 94–08, Can. Food Insp. Agency, Ottawa, Can.
10. Cerdeira AL, Duke SO. 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. *J. Environ. Q.* 35:1633–58
11. Cerdeira AL, Duke SO. 2007. Environmental impacts of transgenic herbicide-resistant crops. *CAB Rev.* 2:14
12. Cerny RE, Bookout JT, CaJacob CA, Groat JR, Hart JL, et al. 2010. Development and characterization of a cotton (*Gossypium birsutum* L.) event with enhanced reproductive resistance to glyphosate. *Crop Sci.* 50:1375–84
13. Chappell J. 1996. Plant biotechnology comes of age—again. *Mol. Breed.* 2:1–6
14. Chassy B, Hlywka JJ, Kleter GA, Kok EJ, Kuiper HA, et al. 2004. Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology. *Compr. Rev. Food Sci. Food Saf.* 3:38–104
15. Chesson A. 2001. Assessing the safety of GM food crops. In *Food Safety and Food Quality*, ed. RE Hester, RM Harrison, pp. 1–24. Cambridge, UK: R. Soc. Chem.
16. Codex Alimentarius. 2001. *Codex Alimentarius Commission: Procedural Manual*. Rome: World Health Organ. and Food Agric. Organ. UN. 12th ed.
17. Codex Alimentarius. 2003. *Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA plants.* Doc. CAC/GL 45-2003, World Health Organ. and Food Agric. Organ. UN, Rome
18. Codex Alimentarius. 2003. *Principles for the risk analysis of foods derived from modern biotechnology*. Doc. CAC/GL 44–2003, World Health Organ. and Food Agric. Organ. UN, Rome
19. Codex Alimentarius. 2009. *Foods Derived from Modern Biotechnology*. Rome: World Health Organ. and Food Agric. Organ. UN. 2nd ed.
20. Constable A, Jonas D, Cockburn A, Davi A, Edwards G, et al. 2007. History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. *Food Chem. Toxicol.* 45:2513–25
21. Craig W, Tepfer M, Degrassi G, Ripandelli D. 2008. An overview of general features of risk assessments of genetically modified crops. *Euphytica* 164:853–80
22. Davies HM. 2010. Review article: commercialization of whole-plant systems for biomanufacturing of protein products: evolution and prospects. *Plant Biotechnol. J.* 8:845–61
23. Donovan WP, Rupar MJ, Slaney AC, Malvar T, Gawron-Burke MC, Johnson TB. 1992. Characterization of two genes encoding *Bacillus thuringiensis* insecticidal crystal proteins toxic to *Coleoptera* species. *Appl. Environ. Microbiol.* 58:3921–27

Annu. Rev. Plant Biol. 2014.65:769-790. Downloaded from www.annualreviews.org
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24. Environ. Prot. Agency. 1998. *Guidance for the reregistration of pesticide products containing Bacillus thuringiensis as the active ingredient.* Publ. 540/RS-89-023, Environ. Prot. Agency, Washington, DC
25. Eur. Comm. 2000. *First report on the harmonisation of risk assessment procedures*, Part 1: *Report of the Scientific Steering Committee's Working Group on Harmonisation of Risk Assessment Procedures in the Scientific Committees advising the European Commission in the area of human and environmental health 26–27 October 2000.* Rep., Eur. Comm., Brussels
26. Eur. Comm. 2013. *Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006*. Off. J. Eur. Union 8.6.2013, No. L 157/1, Eur. Comm., Brussels
27. Eur. Food Saf. Auth. 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. *EFSA J.* 99:1–100
28. Eur. Food Saf. Auth. 2008. Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. *Food Chem. Toxicol.* 46(Suppl. 1):S2–70
29. Eur. Food Saf. Auth. 2010. Guidance on the environmental risk assessment of genetically modified plants. *EFSA J.* 8:1879
30. Eur. Food Saf. Auth. 2011. Guidance for risk assessment of food and feed from genetically modified plants. *EFSA J.* 9:2150
31. Eur. Food Saf. Auth. 2011. Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. *EFSA J.* 9:2149
32. Excell. Through Steward. 2009. *Guide for stewardship of biotechnology-derived plant products*. Guide, Excell. Through Steward., Washington, DC
33. Faustman EM, Omenn GS. 2008. Risk assessment. In *Toxicology*, ed. CD Klaassen, pp. 107–28. New York: McGraw-Hill Medical
34. Fernandez-Cornejo J, Hallahan C, Nehring R, Wechsler S, Grube A. 2012. Conservation tillage, herbicide use, and genetically engineered crops in the United States: the case of soybeans. *AgBioForum* 15:231–41
35. Finger R, El Benni N, Kaphengst T, Evans C, Herbert S, et al. 2011. A meta analysis on farm-level costs and benefits of GM crops. *Sustainability* 3:743–62
36. Flachowsky G, Schafft H, Meyer U. 2012. Animal feeding studies for nutritional and safety assessments of feeds from genetically modified plants: a review. *J. Verbrauch. Lebensm.* 7:179–94
37. Food Agric. Organ. UN, World Health Organ. 1996. *Biotechnology and food safety: report of a joint FAO/WHO consultation, Rome, Italy, 30 September–4 October 1996.* Rep., Food Agric. Organ. UN, Rome
38. Food Agric. Organ. UN, World Health Organ. 2000. *Safety aspects of genetically modified foods of plant origin: report of a joint FAO/WHO expert consultation on foods derived from biotechnology*. Rep., World Health Organ., Geneva
39. Food Agric. Organ. UN, World Health Organ. 2001. *Evaluation of allergenicity of genetically modified foods derived from biotechnology*. Rep., Food Agric. Organ. UN, Rome
40. Fromm M, Taylor LP, Walbot V. 1985. Expression of genes transferred into monocot and dicot plant cells by electroporation. *Proc. Natl. Acad. Sci. USA* 82:5824–28
41. Gatehouse JA. 2008. Biotechnological prospects for engineering insect-resistant plants. *Plant Physiol.* 146:881–87
42. Gianessi LP. 2008. Economic impacts of glyphosate-resistant crops. *Pest Manag. Sci.* 64:346–52
43. Gordon-Kamm WJ, Spencer TM, Mangano ML, Adams TR, Daines RJ, et al. 1990. Transformation of maize cells and regeneration of fertile transgenic plants. *Plant Cell* 2:603–18
44. Hammond BG, Jez JM. 2011. Impact of food processing on the safety assessment for proteins introduced into biotechnology-derived soybean and corn crops. *Food Chem. Toxicol.* 49:711–21
45. Heck GR, Armstrong CL, Astwood JD, Behr CF, Bookout JT, et al. 2005. Development and characterization of a CP4 EPSPS-based, glyphosate-tolerant corn event. *Crop Sci.* 45:329–39
46. Herrera-Estrella L, Depicker A, Van Montagu M, Schell J. 1983. Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. *Nature* 303:209–13
47. Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, Rogers SG, Fraley RT. 1985. A simple and general method for transferring genes into plants. *Science* 227:1229–31

Annu. Rev. Plant Biol. 2014.65:769-790. Downloaded from www.annualreviews.org
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48. James C. 2012. *Global status of commercialized biotech/GM crops: 2012*. Brief 44, Int. Serv. Acquis. Agri-Biotech Appl., Ithaca, NY
49. Jefferson T. 2005 (1800). Summary of public service [after 2 September 1800]. In *The Papers of Thomas Jefferson*, Vol. 32: *1 June 1800–16 February 1801*, ed. BB Oberg, pp. 122–25. Princeton, NJ: Princeton Univ. Press. **http://founders.archives.gov/documents/Jefferson/01-32-02-0080**
50. Kishore GM, Padgette SR, Fraley RT. 1992. History of herbicide-tolerant crops, methods of development and current state of the art—emphasis on glyphosate tolerance. *Weed Technol.* 6:626–34
51. Klee H, Horsch R, Rogers S. 1987. *Agrobacterium*-mediated plant transformation and its further applications to plant biology. *Annu. Rev. Plant Physiol.* 38:467–86
52. Knauf VC. 1987. The application of genetic engineering to oilseed crops. *Trends Biotechnol.* 5:40–47
53. Kok EJ, Keijer J, Kleter GA, Kuiper HA. 2008. Comparative safety assessment of plant-derived foods. *Regul. Toxicol. Pharmacol.* 50:98–113
54. Kuiper HA, Kleter GA, Noteborn HPJM, Kok EJ. 2001. Assessment of the food safety issues related to genetically modified foods. *Plant J.* 27:503–28
55. Kuiper HA, Kok EJ, Davies HV. 2013. New EU legislation for risk assessment of GM food: no scientific justification for mandatory animal feeding trials. *Plant Biotechnol. J.* 11:781–84
56. Li XY, Li SY, Lang ZH, Zhang J, Zhu L, Huang DF. 2013. Chloroplast-targeted expression of the codon-optimized truncated *cry1Ah* gene in transgenic tobacco confers a high level of protection against insects. *Plant Cell Rep.* 32:1299–308
57. Lucca P, Poletti S, Sautter C. 2006. Genetic engineering approaches to enrich rice with iron and vitamin A. *Physiol. Plant.* 126:291–303
58. Marra MC, Piggott NE, Carlson GA. 2004. *The net benefits, including convenience, of Roundup Ready soybeans: results from a national survey*. Tech. Bull. 2004-3, NSF Cent. Integr. Pest Manag., Raleigh, NC
59. McCabe DE, Swain WF, Martinell BJ, Christou P. 1988. Stable transformation of soybean (*Glycine max*) by particle acceleration. *Nat. Biotechnol.* 6:923–26
60. McDougall P. 2011. *The cost and time involved in the discovery, development and authorisation of a new plant biotechnology derived trait*. Consultancy Study for Crop Life International by P McDougall, Midlothian, UK
61. McHughen A. 2012. Introduction to the GM crops special issue on biosafety, food and GM regulation. *GM Crops Food* 3:6–8
62. Nickson TE. 2008. Planning environmental risk assessment for genetically modified crops: problem formulation for stress-tolerant crops. *Plant Physiol.* 147:494–502
63. Nicolia A, Manzo A, Veronesi F, Rosellini D. 2014. An overview of the last 10 years of genetically engineered crop safety research. *Crit. Rev. Biotechnol.* 34:77–88
64. Organ. Econ. Co-op. Dev. 1993. *Safety considerations for biotechnology: scale-up of crop plants.* Rep., Organ. Econ. Co-op. Dev., Paris
65. Organ. Econ. Co-op. Dev. 1993. *Safety evaluation of foods derived by modern biotechnology—concepts and principles*. Rep., Organ. Econ. Co-op. Dev., Paris
66. Organ. Econ. Co-op. Dev. 1998. *Repeated dose 90-day oral toxicity study in rodents.* Guidel. Test. Chem., Sec. 4, Test 408, Organ. Econ. Co-op. Dev., Paris
67. Organ. Econ. Co-op. Dev. 2001. *Consensus document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients*. Doc. ENV/JM/MONO(2001)15, Ser. Saf. Nov. Foods Feeds 2, Organ. Econ. Co-op. Dev., Paris
68. Organ. Econ. Co-op. Dev. 2002. *Consensus document on compositional considerations for new varieties of maize* (Zea mays): *key food and feed nutrients, anti-nutrients and secondary plant metabolites.* Doc. ENV/JM/MONO(2002)25, Ser. Saf. Nov. Foods Feeds 6, Organ. Econ. Co-op. Dev., Paris
69. Organ. Econ. Co-op. Dev. 2004. *Consensus document on compositional considerations for new varieties of cotton* (*Gossypium hirsutum and Gossypium barbadense*): *key food and feed nutrients and anti-nutrients*. Doc. ENV/JM/MONO(2004)16, Ser. Saf. Nov. Foods Feeds 11, Organ. Econ. Co-op. Dev., Paris
70. Padgette S, Re D, Barry G, Eichholtz D, Delannay X, et al. 1996. New weed control opportunities: development of soybeans with a Roundup Ready gene. In *Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects*, ed. SO Duke, pp. 53–84. Boca Raton, FL: CRC

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71. Paoletti C, Flamm E, Yan W, Meek S, Renckens S, et al. 2008. GMO risk assessment around the world: some examples. *Trends Food Sci. Technol.* 19(Suppl. 1):S70–78
72. Peng T, Sun X, Mumm R. 2014. Optimized breeding strategies for multiple trait integration: II. Process efficiency in event pyramiding and trait fixation. *Mol. Breed.* 33:105–15
73. Perlak FJ, Fuchs RL, Dean DA, McPherson SL, Fischhoff DA. 1991. Modification of the coding sequence enhances plant expression of insect control protein genes. *Proc. Natl. Acad. Sci. USA* 88:3324–28
74. Pilacinski W, Crawford A, Downey R, Harvey B, Huber S, et al. 2011. Plants with genetically modified events combined by conventional breeding: an assessment of the need for additional regulatory data. *Food Chem. Toxicol.* 49:1–7
75. Poirier Y, Nawrath C, Somerville C. 1995. Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers, in bacteria and plants. *Nat. Biotechnol.* 13:142–50
76. Privalle L, Bannon G, Herman R, Ladics G, McClain S, et al. 2011. Heat stability, its measurement, and its lack of utility in the assessment of the potential allergenicity of novel proteins. *Regul. Toxicol. Pharmacol.* 61:292–95
77. Qaim M. 2005. Agricultural biotechnology adoption in developing countries. *Am. J. Agric. Econ.* 87:1317–24
78. Qaim M. 2010. Benefits of genetically modified crops for the poor: household income, nutrition, and health. *New Biotechnol.* 27:552–57
79. Raven PH. 2010. Does the use of transgenic plants diminish or promote biodiversity? *New Biotechnol.* 27:528–33
80. Raybould A. 2006. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. *Environ. Biosaf. Res.* 5:119–25
81. Raybould A. 2007. Ecological versus ecotoxicological methods for assessing the environmental risks of transgenic crops. *Plant Sci.* 173:589–602
82. Ricroch AE. 2013. Assessment of GE food safety using "-omics" techniques and long-term animal feeding studies. *New Biotechnol.* 30:349–54
83. Romeis J, Meissle M. 2011. Non-target risk assessment of *Bt* crops—Cry protein uptake by aphids. *J. Appl. Entomol.* 135:1–6
84. Romeis J, Meissle M, Bigler F. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat. Biotechnol.* 24:63–71
85. Romeis J, Raybould A, Bigler F, Candolfi MP, Hellmich RL, et al. 2013. Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops. *Chemosphere* 90:901–9
86. Rupar MJ, Donovan WP, Groat RG, Slaney AC, Mattison JW, et al. 1991. Two novel strains of *Bacillus thuringiensis* toxic to coleopterans. *Appl. Environ. Microbiol.* 57:3337–44
87. Sanford JC, Klein TM, Wolf ED, Allen N. 1987. Delivery of substances into cells and tissues using a particle bombardment process. *Part. Sci. Technol.* 5:27–37
88. Sansinenea E. 2012. Bacillus thuringiensis *Biotechnology*. Dordrecht, Neth.: Springer
89. Sanvido O, Romeis J, Gathmann A, Gielkens M, Raybould A, Bigler F. 2012. Evaluating environmental risks of genetically modified crops: ecological harm criteria for regulatory decision-making. *Environ. Sci. Policy* 15:82–91
90. Scheffe L. 2008. Benefits of conservation tillage. In *Integrated Cropping Systems and Water Management Handbook* (*AGRO-76*), Sec. 10a. Washington, DC: US Dep. Agric. Nat. Resour. Conserv. Serv.
91. Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, et al. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62:775–806
92. Shah DM, Horsch RB, Klee HJ, Kishore GM, Winter JA, et al. 1986. Engineering herbicide tolerance in transgenic plants. *Science* 233:478–81
93. Snell C, Bernheim A, Berge JB, Kuntz M, Pascal G, et al. 2012. Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: a literature review. *Food Chem. Toxicol.* 50:1134–48
94. Somerville C, Meyerowitz E, eds. 2007. *Arabidopsis*. Cold Spring Harb. Monogr. 27. Cold Spring Harbor, NY: Cold Spring Harb. Lab. Press

Annu. Rev. Plant Biol. 2014.65:769-790. Downloaded from www.annualreviews.org
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95. Streatfield SJ. 2007. Approaches to achieve high-level heterologous protein production in plants. *Plant Biotechnol. J.* 5:2–15
96. Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, et al. 2004. A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. *Regul. Toxicol. Pharmacol.* 39:87–98
97. Tzfira T, Citovsky V. 2006. *Agrobacterium*-mediated genetic transformation of plants: biology and biotechnology. *Curr. Opin. Biotechnol.* 17:147–54
98. Umbeck P, Johnson G, Barton K, Swain W. 1987. Genetically transformed cotton (*Gossypium hirsutum* L.) plants. *Nat. Biotechnol.* 5:263–66
99. Vaughn T, Cavato T, Brar G, Coombe T, DeGooyer T, et al. 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. *Crop Sci.* 45:931–38
100. Wang W, Vinocur B, Altman A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218:1–14
101. Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, et al. 2010. Problem formulation in the environmental risk assessment for genetically modified plants. *Transgenic Res.* 19:425–36
102. Ye W, Zhu L, Liu Y, Crickmore N, Peng D, et al. 2012. Mining new crystal protein genes from *Bacillus thuringiensis* on the basis of mixed plasmid-enriched genome sequencing and a computational pipeline. *Appl. Environ. Microbiol.* 78:4795–801

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