Editor's Choice: Evaluating the Potential for Adverse Interactions within Genetically Engineered Breeding Stacks[1]

Plant breeding has a long history of developing varieties with desirable traits in response to the needs of both growers and consumers. Although the bases for most of these traits are not known genetically or biochemically, conventional breeding combines these multiple traits to create new hybrids and stable varieties that are safe and not generally subject to safety assessment. With the advent of genetic engineering, a tool for incorporating additional traits has become available to plant breeders. The safe application of genetic engineering to food and feed crops is widely acknowledged as a useful tool in addressing global agricultural challenges, including population growth and climate change. As used here, the term genetically engineered (GE) stack refers to a plant in which two or more transgenic events (i.e. single-locus insertions) that have been separately assessed for safety have been combined by conventional breeding (Table I). In recent years, increasing numbers of GE stacks have been planted, the first of which offered combinations of insect and herbicide tolerance genes to combat a wider range of pests and weeds than covered by the single events (Que et al., 2010; James, 2011).

Two main questions arise when considering the food and feed safety of GE stacks: (1) does incorporation of more than one event increase genomic instability, and (2) can potential interactions between the products of the combined events impact safety? A related paper considered the stacking of events in light of the plasticity of plant genomes and concluded that enhanced genetic instability from a transgene or from common sequences in two or more transgenes is remote (Weber et al., 2012). This paper addresses the second question of potential interactions between events and their products combined in a stack, reviews the basic principles of plant breeding and its history of safe use, and extends these principles to the feed and food safety of events combined through the same processes used in conventional breeding of non-GE plants. Potential environmental impacts are outside the scope of food and feed safety.

The new varieties developed through modern biotechnology are identified by a number of terms, including genetically modified (GM), GE, transgenic, biotech, recombinant, and plants with novel traits. The term GE is used here as defined by Weber et al. (2012). For these reasons, the term GE is preferred over the term GM.

There are many methods encompassed by the general term conventional breeding, including wide crosses and selection, mutagenesis, and somaclonal variation. When the parental species are not closely related, the cross may be facilitated by embryo rescue, somatic hybridization, or x-ray-induced translocations.

The term interaction, as used in this paper, refers to an effect, such as a new or modified metabolic activity, resulting from a combination of transgenes. An example of an interaction is protein-protein binding resulting in a novel effect only seen with a specific combination of proteins, for instance, protein cofactors or subunits for the same enzymatic complex or subcellular metabolic binding reaction. Examples can also include a direct metabolic interaction that would inhibit or activate components in a metabolic pathway shared by the proteins newly combined in the GE stack or components of independent metabolic pathways that indirectly interact by way of a common metabolite. Thus, interactions within GE stacks generally refer to the metabolic or physiochemical interplay between the products of transgenes or between the product of one transgene and the second gene, rather than between the two genes themselves.

Interactions and Plasticity Are Ubiquitous and Important Phenomena in Conventional Breeding

Conventional breeding has a long history of safe use despite the presence of antinutritional factors, toxins, and allergens in crops. There is no evidence that a random genomic change in a crop has resulted in a novel food or feed safety issue (Weber et al., 2012). Historically, humans have selected desirable traits that arise from the crop's genomic plasticity and interactions between genes. As breeding became more advanced, new methods were applied to select and combine desired traits, which also modify the genome as a consequence.

Plants produce a multitude of metabolites that provide various functions. These include signaling activities in response to environmental stress or attack from plant pathogens and pests. Some metabolites have beneficial effects, while others are toxic when fed at high levels to sensitive animal species (Ames et al., 1990). Although particular metabolites tend to be specific to some plant families, there are metabolites of concern in a number of common food and feed crops, including apple (*Malus domestica*), apricot (*Prunus armeniaca*), *Brassica* spp., celery (*Apium graveolens*), cucumber (*Cucumis sativus*), lima bean (*Phaseolus lunatus*), potato (*Solanum tuberosum*), cherry (*Prunus avium* or *Prunus cerasus*), and sorghum (*Sorghum bicolor*; Beier,

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Table I. *Definitions of terms as used in this document*

Term	Definition
Genetic engineering (also known as genetic modification)	The process of specific, intentional, and directed physical modification of the genome of a plant by the introduction of recombinant DNA. This is distinct from the random genetic modification of the genome of a plant that occurs in conventional breeding or by mutagenesis.
Event (also known as GE single event, transgenic event, GM event, and single event)	A single-locus insertion of recombinant DNA into the host genome regardless of the number of genes contained on the inserted piece of DNA.
Recombinant DNA	DNA that has been manipulated or altered in the laboratory.
Transgene	Refers to any recombinant DNA in an event.
GE Stack (also known as a GM breeding stack, stacked-event product, or combined-event product)	A plant produced by the conventional breeding of individual events that have been individually safety assessed.
Conventional breeding	Methods of crossing plants with desired characteristics to generate offspring combining those desirable characteristics. Methods described by this general term include selection, crossing, embryo rescue, somatic hybridization, somaclonal variation, mutation, and cell selection. Conventional breeding can involve both non-GE and GE plants.
Interaction (between events)	As used in this paper, this refers to an effect, such as a new or modified metabolic activity, resulting from the combination of transgene-derived products in a GE stack that is not occurring in any of the parental single events.

1990; D'Mello et al., 1991; Stewart, 2009). Examples of such metabolites include alkaloids, lectins, glucosinolates, furanocoumarins, cyanoglucosides, nicotines, and phytoestrogens. Some of these can impart bitter taste, and conventional breeding has repeatedly reduced their levels to acceptable concentrations (Drewnowski and Gomez-Carneros, 2000). There are few documented cases in which breeding led to an unacceptable level of a metabolite. Following reports of bitter taste, the potato var Lenape, which was developed from a cross of potato to a wild *Solanum* species, was found to contain doubled levels of glycoalkaloids (Anonymous, 1970). The only other documented incident of unacceptable metabolite levels associated with plant breeding is that of a disease-resistant celery containing elevated levels of furanocoumarins, which may have contributed to dermatitis among grocery store personnel (Berkley et al., 1986; Seligman et al., 1987). The environment can also play a role in impacting furanocoumarin levels; a later report of dermatitis among celery harvesters in southern Israel was attributed to delayed harvest, and hence more mature plants being handled, due to the Gulf War (Finkelstein et al., 1994). Similarly, reports of toxic squash (Rymal et al., 1984) and zucchini (Herrington, 1983) appear to have been limited to individual plants within otherwise widely grown varieties, suggesting the higher toxin levels were due to environmental conditions or mutations. Despite these few instances, these crops remain safe for food or feed use.

Although breeders recombine tens of thousands of genes with virtually infinite potential interactions, to our knowledge, there has never been a report of a completely novel toxin or allergen appearing in a genus as a result of conventional breeding. The development of an interspecific somatic hybrid of potato (*S. tuberosum* and *Solanum brevidens*) containing demissidine (Laurila et al., 1996) has often been cited as proof that novel toxins can arise during breeding. In fact, Jadhav et al. (1981) documented the presence of demissidine in potato more than a decade before it was found in the hybrid. To guard against unexpected increases in levels of known toxins, breeders have instituted screens for these compounds in new varieties before they are released. Examples include cotton (*Gossypium hirsutum*), potato, lima bean, and canola (*Brassica napus*), because they contain known compounds that can impact food or feed safety. However, breeders cannot and do not screen for de novo compounds. Hundreds of thousands of varieties have been bred without the emergence of any novel allergens or toxins, indicating that the likelihood of such events is virtually zero (Stevens, 1974).

Improved crop varieties produced by conventional breeding are the cornerstone of food production in the world today. Thus, the ways in which risks are managed during conventional breeding of non-GE crops, which includes testing for any known concerns, sets the framework for the food and feed safety assessment of GE stacks. As is the case for any other new variety or hybrid, GE stacks are only considered for market introduction if the combined traits have met the intended thresholds for efficacy and stability.

Single Events Undergo a Rigorous Safety Assessment

Newly developed GE events undergo rigorous safety assessments by the developer and by regulatory agencies prior to their commercial release. These assessments evaluate the impact of both intended and potential unintended effects on food, feed, and other aspects of crop safety. Assessment criteria and objectives are based on national priorities and on international standards, such as those of the Joint Food Standards Program of the Food and Agriculture Organization of the United Nations and the World

Health Organization (FAO/WHO, 1996, 2000), as well as Codex Alimentarius Commission recommendations for conducting food safety assessments of GE crops (Codex Alimentarius Commission, 2009). Codex principles and guidance are widely followed for single events. The key components of GE crop safety assessments include (1) descriptive information on the transgenes and inserted recombinant DNA; (2) detailed characterization of the DNA insert relative to the native genome, in planta concentration and stability of the products of the transgene(s), analysis of plant phenotype, and descriptive information such as efficacy, mechanism or mode of action of the new trait(s), and crop management considerations; (3) evaluation of the safety of the products of the transgene(s) in the context of common commercial crop practices and uses; and (4) comparative safety assessment for suitability as food and/or feed. Such analyses might include a determination of expression levels of known allergens and toxins, and overall composition, including nutrients, antinutrients, and selected metabolites for that crop (FAO/WHO, 1996, 2000; Cellini et al., 2004; Codex Alimentarius Commission, 2009; Thomas et al., 2009).

Assessments of nutrient composition and agronomic characteristics, as well as an assessment of potential allergenicity and toxicity, are widely recognized as significant components of GE crop safety assessments (FAO/WHO, 1996, 2000; Metcalfe et al., 1996; Kuiper et al., 2001; Cellini et al., 2004; Delaney et al., 2008; Codex Alimentarius Commission, 2009; Thomas et al., 2009). Differences between the GE crop and appropriate comparators are evaluated in terms of biological relevance, magnitude of difference, exposure, and impact on food or feed safety to determine the need for further investigation. The comparators may include a closely related non-GE variety and other commercial GE and non-GE varieties. The International Life Sciences Institute's Crop Composition Database (http://www.cropcomposition.org; International Life Sciences Institute, 2006) may also serve as a reference for comparison for some crops (Ridley et al., 2004). These holistic analyses also take into account interactions of the transgenes with endogenous genes and their products.

Agriculturally important plants express approximately 25,000 to 50,000 genes at any given time. The safety assessment evaluates the GE crop phenotype, which includes interactions between the event and the host genome that may impact safety. Once the safety assessment is completed, conventional breeding is used to incorporate the event into different genetic backgrounds, without undergoing additional assessment. The developer of the resulting food and feed products is, however, responsible for the safety of those products and for meeting all relevant statutory and regulatory requirements, as is the case for all foods and feeds.

Even within a single genus having species that differ in ploidy level, the number of genes within a plant can vary. For example, some 400 genes are not common in the historically important maize (*Zea mays*) inbreds B73 and Mo17; each of these genes is found in B73 but not in Mo17 or vice versa (Springer et al., 2009; Lai et al., 2010). Similarly, of the approximately 46,000 of genes in soybean (*Glycine max*), 856 genes were present in some but not all of the soybean genotypes tested (Lam et al., 2010). Of the two potato genotypes with sequenced genomes, 275 genes are found in only one genotype or the other (Xu et al., 2011). Thus, crossing different non-GE genotypes results in novel gene combinations and, in turn, novel interactions, but no safety issues have been identified. Hence, this process of creating novel interactions via crossing is highly unlikely to be influenced by the stacking of transgenes.

Despite the extensive genetic variability in crop plants, most regulatory agencies do not review new non-GE varieties developed by conventional breeding unless there is a known safety concern or novelty associated with the new variety (Association of State Universities and Land Grant Colleges, 1972; U.S. Food and Drug Administration, 1992; Council of the European Union, 2002; Canadian Food Inspection Agency, 2006; Experiment Station Committee on Organization and Policy, 2006; Health Canada Food Directorate, 2006). This practice is based on the principle that conventional breeding is regarded as safe.

Potential Interactions Resulting from Combining Events

As defined above, conventional breeding brings together traits from diverse genetic backgrounds. Nonadditive interactions of genes and their products are commonly exploited in breeding programs to produce new crop varieties. In particular, plant breeders effectively utilize heterosis (hybrid vigor) through crosses that combine different alleles, different epigenetic states (Groszmann et al., 2011) and, in some cases, different genes (Xu et al., 2011). However, the mechanisms underlying heterosis are far from understood and likely vary among hybrids. Recent studies have measured the molecular parameters that might be involved in heterosis (Auger et al., 2005; Keurentjes et al., 2006; Swanson-Wagner et al., 2006; Hochholdinger and Hoecker, 2007; Groszmann et al., 2011); these studies clearly indicate that thousands of nonadditive interactions are occurring when genomes are combined by breeding. Furthermore, these gene interactions have sometimes led to gene silencing, which has been unknowingly selected by plant breeders to obtain desirable traits (Parrott et al., 2010).

Because these nonadditive interactions of existing genes have a long history of food and feed safety, the bases of these interactions are largely uncharacterized. Without the information to posit a valid testable hypothesis, specific interactions between endogenous genes in conventional breeding do not lend themselves to direct experimental testing. As information and validated methods to analyze complex data become available, it may be possible to address such interactions.

By contrast, when combining GE events by breeding, it is possible to make predictions concerning possible

interactions between the events within the GE stack in a highly focused manner, and testable hypotheses can be developed for those specific interactions. Compared with the techniques historically used in conventional breeding to introduce new traits into crop plants, such as wide hybridization, genetic engineering uses a direct method of introducing a DNA sequence that confers a specific trait into a crop. Moreover, the crossing methods by which individual events are combined to produce a GE stack are identical to those used to combine multiple traits in new conventional crop varieties and hybrids.

Because crossing two GE parents does not introduce any greater variation in the genome beyond what is obtained by crossing two non-GE parents (Weber et al., 2012), the safety assessments for the individual events are directly applicable to the GE stack. The only remaining safety question that the individual event assessments do not address is that of interactions between the products of the combined transgenes. Two questions help focus specifically on the potential of adverse interactions between the events brought together in a particular GE stack: (1) does a potential interaction between the products in each single event exist in the GE stack, and (2) would the potential interaction result in a food and feed safety concern? In other words, are there consequences of engineered biochemical/functional changes and any interactions between these that might create risk?

A Model for Safety Assessment of GE Stacks

A safety assessment of a crop produced by conventional breeding of single-event parental lines into a GE stack can incorporate a logical decision-making process that addresses whether interactions of the transgene products are likely, and if so, whether they might affect food and feed safety. A model for this is provided in Figure 1, which depicts the overall development of a hypothesis for potential interactions, beginning with the critical knowledge and understanding of the crop plant, previous characterizations of the single events, and the prior food and feed safety assessments on the single-event products. The initial question in the assessment is this: Is it expected or probable that the transgene expression products of the single events will interact in the stack? The only form of interaction different in a GE stack from that seen in conventional breeding of non-GE plants is a metabolic or physiochemical interaction from the combination of the transgene products. All other interactions are identical to those in conventionally bred non-GE plants.

Second, if it is probable that novel interactions will occur in the GE stack, then can the interaction impact the safety of the GE stack? This is a critical step; if there is no basis for a potential interaction, or if a potential interaction exists that does not affect safety, the prior safety assessments on the individual events are sufficient for the GE stack. If a plausible hypothesis can be developed for an interaction that may affect either food or feed safety, then further questions should focus on the likelihood, nature, and significance of the interaction.

A number of relevant points should be considered to develop a testable hypothesis about the presence and nature of interactions leading to a potential safety concern. Although the types of questions asked will depend on the transgenes in the GE stack, these questions should be sufficiently narrow to address food and feed safety. Examples of questions that can be employed to develop a testable hypothesis are highlighted in Table II; these questions focus on direct gene product interactions, gene expression patterns, and metabolic products of the transgenes.

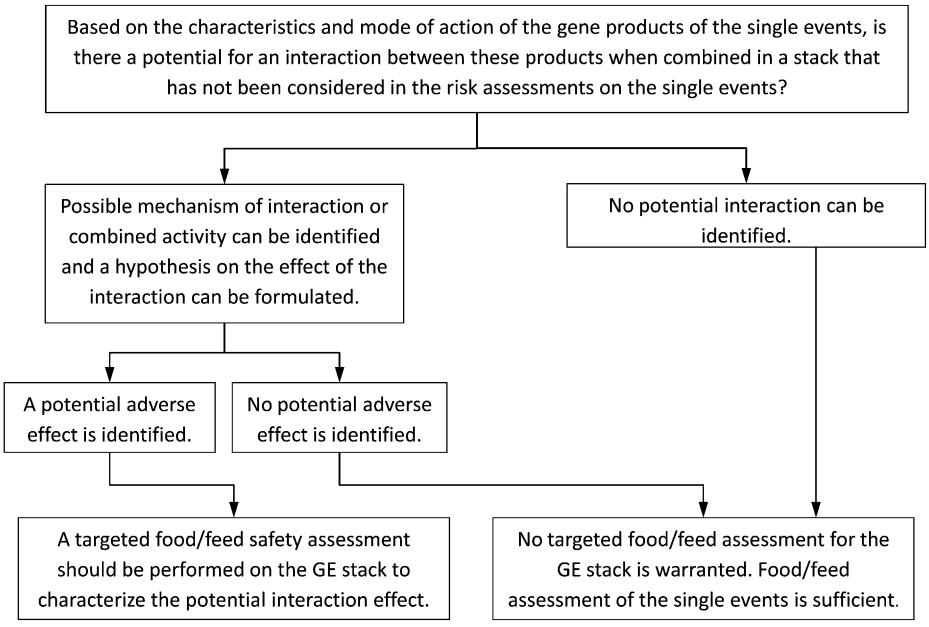

Figure 1. A decision-making process for the safety assessment of a food and feed crop with a GE stack produced by conventional breeding of two or more safety-assessed events.

Table II. *Examples of questions to evaluate the existence of possible interactions*

Possible Interactions	Questions
Direct Interactions	Do the products of the transgene act on nucleic acid to modulate gene expression? Is it reasonable to expect that the products of one event affect transcript stability or gene expression, or promote/alter silencing, of the other event?
	Could the expressed proteins form heteropolymers with unique biochemical activity? Is there a physical interaction between the proteins?
	Do any of the expressed proteins act as activators and/or inhibitors in pathways affected by the products of the other transgene(s)? Is the effect of such an interaction only additive, resulting in a stronger metabolic effect without any further safety implications, or is the resulting effect different in a biologically relevant way from the effects of each of the single events?
Gene Expression Patterns	
	Do the physical or temporal expression patterns of the transgenes overlap? Is it reasonable to expect, based on the characteristics of the gene promoters used and on gene expression patterns, that the products of the stacked events are present in the same tissues and at the same developmental stages increasing the probability of interaction?
	Is there translocation of the molecules produced by the expressed transgenes? Are the products associated with either transgene (e.g. metabolites or small RNAs) transported across cells such that they might reasonably be expected to interact with the transgenic products of the other event?
Metabolic Pathway Participation	
	Do the combined products of the transgenes introduce new, unique biochemical pathway(s) into the plant? Is it reasonable to expect that the introduced products confer new biochemical properties to the transgenic plant, based upon their known mode of action, that are not seen in either single event?
	If the combined transgenes are part of a pathway, are there common metabolites? Is it reasonable to expect that any of the introduced proteins could convert a substrate to new metabolites or lead to levels of known metabolites not seen in either single event?
	Do the combined transgenes produce metabolites that may reasonably be expected to interact? Could the metabolite(s) produced interact with each other or with other metabolites within the stack?

The same logic applies to genes affecting expression of other genes, such as transgenic transcription factors and gene constructs leading to the silencing of endogenous genes. While these types of transgenes may affect expression of batteries of other genes, the types and nature of changes caused by each transgene would be documented before each single event is used in commerce. Testable hypotheses could then be formulated from the knowledge of the specific effects of each transgene.

Interestingly, many products of conventional breeding of non-GE plants depend on phenotypes now known to have been created from alterations in transcription factors or from gene silencing (Parrott et al., 2010). To the extent that conventional breeding has involved crossing plants with unique phenotypes associated with silencing or changes in transcription factors, there is no indication that altered metabolite pools have resulted in any novel risk to food or feed safety. In either transcriptional activation or silencing, there has not been the appearance of novel toxins related to these metabolic alterations.

Regardless of the specific types of transgenes being considered, assessment of the combined information from the single events can help establish (Fig. 1; left) or eliminate (Fig. 1; right) a hypothesis for possible interactions. The need for further safety assessment of the potential interaction is then evaluated on a case-by-case basis. Examples of how the process can be used to arrive at clear safety assessment decisions are provided below.

Example 1: No Expected Interactions

An interaction between the products of transgenes is not expected whenever the single events combined in a GE stack encode proteins that are not known to directly interact or that act on different metabolic pathways with no known connection or common metabolite between them. Currently commercialized GE stacks that combine insect resistance and herbicide tolerance are an example. In these crops, insect resistance is conferred by one or more Bt genes (encoding crystal toxin proteins from *Bacillus thuringiensis*) that are not native to plants, but have a general history of safe use in the food chain as foliar insecticidal sprays in conventional and organic agriculture and in GE crops. The insecticidal proteins exert no known metabolic activity in plants and are either expressed in the cytoplasm or targeted to chloroplasts. Herbicide tolerance is typically conferred by introducing a gene that encodes an herbicide-insensitive version of a target enzyme or that catalyzes a reaction that makes the active ingredient of the herbicide nonherbicidal. For a Bt × herbicide tolerance stack, is there a possibility that an interaction between these events will occur? The GE stack does not create a new biochemical pathway. The herbicide tolerance may or may not serve a metabolic function in the plant, and Bt binds insect gut proteins. As herbicide tolerance may be specifically engineered to be delivered and expressed in the chloroplast or cytoplasm, theoretically, both Bt and the protein conferring herbicide tolerance could be found in the same cell organelles. Colocalization of the two

transgenic traits raises the possibility of interaction in the GE stack; however, these proteins are not physiologically related, are not active in the same metabolic pathway, and share no common metabolites. Because there is no biological pathway in which these gene products would directly or indirectly interact, there is no plausible or testable hypothesis for the interaction of these proteins or their products in the GE stack that creates the need for a new food/feed safety evaluation (Fig. 1; right).

The same logic applies to the stacking of transgenic transcription factors with other transgenes in crops with events that have already undergone a safety assessment. If the stacked transgenes are not expressed in the same tissues or their products are not translocated to the same tissues, there would be no new food or feed safety concerns. Likewise, if the transgenic event simply alters the amount of endogenous transcription factor(s), again, there is no plausible hypothesis for a food or feed safety concern.

Example 2: Expected Interactions between the Events in a GE Stack, with No Impact on Food or Feed Safety

Based on an understanding of the mechanisms of action, some event combinations may have the potential to produce products that interact, at least hypothetically. For example, if individual events produce enzymes within intersecting metabolic pathways or produce enzymes that can compete for the same substrate in a GE stack, then a potential for interaction exists (Fig. 1; left). Relative to the number of possible interactions, there are presently only a handful of demonstrated direct interactions between metabolic enzymes and metabolic pathways, which are manifested as altered flux through a pathway. Importantly, a potential interaction does not necessarily imply that food or feed safety would be affected. Only a small percentage of metabolites in plant biochemical pathways have been shown to have a role other than as reactants in plant metabolism (Buchanan et al., 2000).

An example illustrating a potential interaction within a pathway that does not impact food or feed safety can be found in the starch biosynthetic pathway. A rate-limiting step in starch biosynthesis is the heterotetrameric enzyme ADP-Glc pyrophosphorylase (AGPase; for review, see Hannah, 2005, 2007; Hannah and James, 2008). The product of the reaction, ADP-Glc, is used for starch biosynthesis. Plant AGPase enzymes are composed of two identical small subunits and two identical large subunits. The two subunits are encoded by different genes. In a hypothetical GE stack, one event would encode an engineered small subunit of AGPase capable of synthesizing higher levels of starch, and the second event would encode an engineered large subunit of AGPase also capable of synthesizing higher levels of starch. In both single events, the increased level of starch is due to an increased level of ADP-Glc from the introduced enzymes. Breeders would naturally be interested in combining these traits to look for an additive effect on starch levels.

In this example, the two genes would be expressed, allowing for their direct interaction. Both genes exhibit identical developmental expression profiles, and the proteins are involved in the same metabolic pathway and affect the same enzymatic activity (Fig. 1; left). Importantly, interaction between the transgenes in these events is required for the intended effect of further altering starch biosynthesis in a GE stack. In this case, the GE stack does not introduce a new biochemical pathway or metabolites (it only augments existing starch metabolism), and although threshold effects are possible, there is no potential impact on food or feed safety.

Example 3: Identification of an Interaction between Two Events in a GE Stack Leading to a Potential Impact on Food or Feed Safety

It is extremely difficult to identify a realistic safety issue arising from a GE stack. One purely hypothetical example involves phytoanticipins, which are constitutively produced plant secondary metabolites employed in plant defense systems. They can be classified into four major classes: avenacosides, cyanogenic glucosides, benzoxazinoid glucosides, and glucosinolates. Phytoanticipins become toxic following activation by cleavage by a β-glucosidase, and they participate in the natural plant response to attacks from pests and pathogens (for review, see Morant et al., 2008). The phytoanticipin is normally stored in the vacuole, whereas the β-glucosidase is stored in the plastid or apoplast. In some cases, the phytoanticipin and its cognate β-glucosidase are synthesized in different, but spatially close, tissues. Due to the physical separation of phytoanticipin and β-glucosidase, no toxin is present in undamaged tissue. However, following cell rupture (e.g. from insect chewing) and mixing of cellular constituents, enzymatic hydrolysis of the glucosidic bond in the phytoanticipin precursor produces a compound toxic to the insect.

In this hypothetical scenario, event 1 enhances the level of a particular phytoanticipin that is normally present in the plant at low levels, resulting in a variety with greater resistance to pests and pathogens. Although event 1 elevates the level of this particular biochemical, the intact phytoanticipin itself is nontoxic and, if found within the range of existing levels in this crop, would raise no food or feed safety issues during the safety assessment of event 1. Event 2, which is also in a variety with improved pest resistance, encodes a β-glucosidase with enhanced activity that is linked to a vacuolar targeting sequence. The insertion of this transgene into a host plant with low levels of the particular phytoanticipin substrate of this enzyme presents no health issues, because the inserted vacuole-targeted β-glucosidase activity lacks the substrate

needed for toxin production. The safety assessment of each individual event would be expected to include an analysis of the safety of the event under various environmental conditions. Therefore, the scenario of cell damage that causes enzymatic hydrolysis of the glucosidic bond in the phytoanticipin precursor to produce a toxic compound would have already been evaluated during the safety assessment of the individual events. However, for the sake of discussing the stacked combination of these two events, it is reasonable that this hypothetical scenario could be even more closely examined in the stacked event than had been done with the individual events (Fig. 1; left). In such cases, an additional targeted food/feed assessment of the GE stack would be warranted.

CONCLUSION

In over a century of plant breeding, there are no known de novo adverse effects generated from the breeding process itself, which has provided conventional plant breeding with a history of safety for food and feed. Whatever adverse effects have happened have been totally predictable. Numerous parallels can be made between combining endogenous genes in a conventional breeding program and combining transgenes by using conventional breeding methods. The major difference between the unknown endogenous genes combined via conventional breeding and transgenes combined through conventional breeding is the breadth of knowledge about the transgenes and their products. This knowledge base allows the development of testable scientific hypotheses concerning food and feed safety.

A safety assessment for a food or feed crop produced by conventional breeding of GE events should first address the likelihood of interactions and, if they do occur, whether they might affect safety. If the events are unlikely to interact, no additional assessment should be needed to make a safety determination for the GE stack, because each individual event has already undergone extensive independent safety assessments.

When interactions are hypothesized to occur, the effects can be predicted based on knowledge and safety assessment of the individual parental events. When there is a biological basis to predict an interaction, the nature of the interactions should be evaluated to determine if there could be an impact on food or feed safety. If there is no likely impact of the interaction on food or feed safety, then no additional testing should be necessary to assess the GE stack.

Finally, if the identified interaction could reasonably be expected to impact food or feed safety, the GE stack would require a targeted hypothesis-based food and feed assessment, focusing on the specific interaction identified. The need for such a further assessment and the approach used should be determined on a case-by-case basis, focusing on the specific interaction identified and taking into account the Codex Alimentarius principles for safety assessment (Codex Alimentarius Commission, 2009).

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Henry-York Steiner
Herbicides, Arysta Life Science, Cary, North Carolina 27513

Claire Halpin
Division of Plant Sciences, University of Dundee at the James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, United Kingdom

Joseph M. Jez
Department of Biology, Washington University, St. Louis, Missouri 63130

John Kough
United States Environmental Protection Agency, Washington, District of Columbia 20460

Wayne Parrott
Center for Applied Genetic Technologies, University of Georgia, Athens, Georgia 30602

Lynne Underhill
Bureau of Nutritional Sciences, Food Directorate, Health Canada, Ottawa, Ontario, Canada K1A 0K9

Natalie Weber
Pioneer Hi-Bred International, Wilmington, Delaware 19880

L. Curtis Hannah
Horticultural Science Department, University of Florida, Gainesville, Florida 32611

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