JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Symposium Introduction

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Safety of GM Crops: Compositional Analysis

ABSTRACT: The compositional analysis of genetically modified (GM) crops has continued to be an important part of the overall evaluation in the safety assessment program for these materials. The variety and complexity of genetically engineered traits and modes of action that will be used in GM crops in the near future, as well as our expanded knowledge of compositional variability and factors that can affect composition, raise questions about compositional analysis and how it should be applied to evaluate the safety of traits. The International Life Sciences Institute (ILSI), a nonprofit foundation whose mission is to provide science that improves public health and well-being by fostering collaboration among experts from academia, government, and industry, convened a workshop in September 2012 to examine these and related questions, and a series of papers has been assembled to describe the outcomes of that meeting.

KEYWORDS: *GM crops, compositional analysis, safety assessment, genetic engineering (GE), biotechnology, crop breeding, trait development, natural variability, International Life Sciences Institute (ILSI)*

INTRODUCTION

Since 1996, when genetically modified (GM) crops (also known as genetically engineered crops) were first grown commercially, the area planted with GM crops has steadily increased to an estimated 160 million hectares across 29 countries.[1] 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. This technology has been adopted quickly and widely by growers because growing GM crops that contain insect resistance or herbicide tolerance traits has led to lower inputs, convenience, and flexibility in crop management strategies while maintaining or increasing crop yield and quality.[2]

Many countries require that a comprehensive safety assessment of a particular GM crop be reviewed and approved by national or regional regulatory agencies before any food or feed arising from the GM crop may be imported. Additional assessment of environmental safety is often also required, particularly for in-country cultivation. General guidelines for the safety assessment, including which types of data are necessary, were laid out by the Codex Alimentarius Commission in 2003.[3] Although the principles in these guidelines remain in use today, specific regulations vary by country or region, and requirements continue to evolve. Some of the types of studies that may be required for the safety assessment relate to mode of action of the trait, protein expression, allergenicity assessments, evaluation of agronomics, toxicity evaluation, and compositional analysis.[3,4]

The compositional analysis approach to assessing the nutritional status of food and feed from a GM crop is based on the concept of *substantial equivalence*, which "embodies the idea that existing organisms used as food, or as a source of food, can be used as the basis for comparison when assessing the safety of human consumption of a food or food component that has been modified or is new".[5] In this context, two conventionally bred cultivars of a crop having a history of safe use are considered to be substantially equivalent to one another, despite any differences in nutritional status. Although not safety assessments themselves, compositional analysis studies using the concept of substantial equivalence have been considered a "key step in the safety assessment process",[6] by placing the nutritional status of the GM crop into the context of the commercial crop with a history of safe use.

The International Life Sciences Institute (ILSI) is a not-for-profit, international organization whose mission is "to provide science that improves public health and well-being".[7] The primary focus of ILSI's activities is on nutrition and health, food safety, risk assessment, and environment. ILSI fosters collaboration among experts from academia, industry, and government entities and aids in the cooperative conduct, gathering, summarization, and dissemination of science. Within the ILSI organization, the International Food Biotechnology Committee (IFBiC) serves as a biotechnology resource that aids science-based decision-making in food and feed safety assessments for GM crops.[8] Scientific topics of interest to IFBiC are addressed by task forces, which bring together individuals working within government agencies, industry, and academia who have interest and expertise in the area. IFBiC's Task Force 12 took on the task of addressing issues related to crop compositional analysis and its role in the safety assessment of food and feed derived from GM crops.

The task force organized a workshop titled, "Safety of GM Crops: Compositional Analysis", which was held September 13−15, 2012, in Dulles, VA, located just outside Washington, DC, USA. This paper is an introduction to the proceedings of the workshop found in a special section of this issue of the *Journal*. In this introductory paper, the intention is not to discuss the following papers in the series but rather to offer an overview of the topic area to highlight its significance and provide perspective.

APPLICATION OF COMPOSITIONAL ANALYSIS IN GM CROP SAFETY ASSESSMENT

The insertion of a new GM trait into the genome has been hypothesized to result in a gain, alteration of expression, or loss of a trait that may have the potential to influence the composition of the GM crop relative to its non-GM parent line. Therefore, as part of the food and feed safety assessment,

Special Issue: Safety of GM Crops: Compositional Analysis

Received: March 11, 2013
Published: September 4, 2013

compositional studies involve the measuring of levels of key components, “those components in a particular food that may have a substantial impact in the overall diet”,[3] present in the food and feed originating from the GM crop. These can include nutrients, antinutrients, secondary metabolites, and toxicants. A starting point for deciding which crop components to include in the assessment has traditionally begun with the guidelines put forth by the Organisation for Economic Co-operation and Development (OECD) in their Series on Safety of Novel Foods and Feeds, which includes recommendations for various important food and fiber crops, such as for rice.[9] Development of the composition consensus documents is initiated by a proposal from one of the 34 member countries of OECD, an accession country, or an engagement partner. If the proposal is accepted, an ad hoc Expert Group is formed to generate the data and information contained in the document following an established format. Extensive input is received from member countries and academic, government, and industry groups following an iterative review process. Once finalized, the consensus document is declassified and broadly distributed.[10] Suggestions to developers of new crops are provided in two sections: one section for constituents to be analyzed for a new crop variety for food use, and a second section for feed use. However, the nature of the expected changes made to the crop will determine which components are included in the assessment.

Assuming that any apparent changes in levels of crop components in the GM crop are caused by insertion of the GM trait, these differences can be categorized as either intended effects or unintended effects.[11] An intended effect is the aim of the trait or traits being incorporated into the crop. When the intention of the GM trait is to modify one or more crop compositional components, these components should be evaluated in the compositional analysis study. This helps to place the compositional changes in perspective of the conventional crop.

Unintended effects are those that result from insertion of the trait or its action but that were not a part of the original, intended outcome. Possible unintended changes attributable to the GM trait can be divided further into expected and unexpected effects.[6] Occurrence of expected effects can be predicted due to the expected phenotypic characteristics conveyed by the trait(s) being incorporated into the crop. For example, the trait of interest may have particular effects on a metabolic pathway; thus, other changes may be anticipated on the basis of our understanding of crop physiology. Other expected differences could be due to changes in compositional components, such as changes in amino acid content due to differences in protein levels, or a calculated difference in carbohydrates based on a difference in another proximate. Unexpected effects are those that cannot be predicted by current knowledge of crop biology and composition. All unintended effects often are mistakenly attributed to the process of insertion of the GM trait or to the trait itself. However, it is important to note that not all observed differences can or should be attributed to the insertion of a GM trait. For example, if the GM crop and its near-isogenic comparator are not as genetically alike as they are assumed to be, or if a near-isogenic comparator is not available, observed differences may not be due to the presence of the trait. In addition, random variation (i.e., type I errors) frequently can cause apparent differences in composition.

Current approaches may involve assessing the component levels in the GM crop compared with those present in a non-GM comparator. Some regulatory authorities expect the comparators to be near-isogenic lines of the GM crop (in sexually propagated crops, or isogenic lines for vegetatively produced crops), but alternative comparators may be acceptable if sufficient rationale for the choice of an alternative is provided.[12] If no statistically significant difference is observed when the level of a component in the GM crop is compared with that present in the non-GM comparator, then one assumes that the insertion or expression of the event(s) did not result in any meaningful unintended effects, and then no further safety assessment is necessary for that component. However, if a difference is observed, then an additional assessment of the biological relevance is typically made. One approach is to evaluate the observed differences in composition of the GM crop in the context of the composition of crop varieties or hybrids with a history of safe use. These varieties may be grown concurrently with the GM crop and its non-GM comparator, or the data may be those recorded in the literature.

The above approach has been used by many governmental regulatory agencies around the world in establishing a portion of the safety assessment for food and feed originating from a GM crop. Much of the work to date has dealt with traits for insect resistance or herbicide tolerance and combinations or “stacks” of these two traits.[13−15] Compositional analyses of insect-resistant and herbicide-tolerant crops have included maize,[16−22] soybean,[23−27] cotton,[28−30] rice,[31] wheat,[32] and alfalfa.[33] In addition, considerable effort has been made with disease resistance traits in potato,[34] intended changes in nutrient composition in crops such as sweet potato, maize, and rice,[35] and drought tolerance in maize.[36]

COMPOSITIONAL ANALYSIS OF GM CROPS IN THE FUTURE

Over the past 18 years, researchers have gained extensive experience with the compositional analysis approach in GM crops and its importance to the safety assessment. This experience allows for re-examination of the approach to determine if the data generated continue to add value to safety assessments and if the way we are interpreting the data is appropriate.

Extensive composition data assessing the natural variability in individual components in maize[37−39] and soybean[15,40,41] indicate that growing season and geography are more likely to be responsible for variability rather than the presence of a GM trait. These environmental effects may lead to greater changes in levels of particular crop components than the apparent differences between the GM crop and its comparator. For example, Seguin et al.[42] found that α-tocopherol levels in soybean seed could vary by seeding rate, row spacing, and, most dramatically, seeding date; earlier planting dates resulted in α-tocopherol levels as much as 45% greater than at later seeding dates.

The variety and complexity of traits and technologies that will constitute GM crops in the near future also raises new questions about compositional analysis and how it should be conducted to evaluate the safety of these crops resulting from the changing science. Future work will involve such things as more complex “stacks” of traits,[43] further work on environmental stress tolerance, traits that purposefully alter plant physiology or composition, traits involving site-directed gene insertion,[44] and traits that employ RNA interference (RNA*i*) technology.[45] These more complex approaches to crop improvement bring new challenges to the continued implementation and interpretation of the current compositional analysis approach and to the safety assessment in general. For example, the traditional non-GM comparator may no longer be appropriate if a new GM crop is modified with the aim to substantially alter metabolic processes or create a substantially changed fatty acid profile. "In such cases plant composition may be modified to such an extent that for [food and feed] risk assessment an appropriate comparator cannot be identified for the species in question".[12]

Currently, historical data on crop compositional components are compiled from data originating from non-GM varieties with a history of safe use to represent what component levels normally could be found in food and feed in the global market. In 2011, it was estimated that globally 32% of the area planted to maize, 75% of the soybean area, and 82% of the cotton area were planted with GM varieties.[1] This brings into question the current practice of including only non-GM commercial varieties with a history of safe use as an accurate representation of the levels in today's food and feed when a considerable amount or majority of a crop is now derived from GM crops. After 18 years, the authors are not aware of any adverse effects on human or animal health due to consuming products originating from approved GM crops, but currently regulatory authorities have not indicated when they will consider food and feed from a deregulated GM crop to have a history of safe use.

In addition, there is lack of agreement among countries or regions as to how data resulting from compositional analysis studies should be interpreted. Therefore, there are benefits in examining the current approach to compositional analysis and interpretation in light of past experiences and new information about the science. IFBiC Task Force 12 members concluded that a workshop would be the best forum for exploring questions about the future of compositional analysis and for distributing the information globally. This workshop, titled "Safety of GM Crops: Compositional Analysis", was held September 13−15, 2012, in Dulles, VA, located just outside Washington, DC, USA.

■ ILSI WORKSHOP

The purpose of the workshop was to identify and address topics related to crop composition from a scientific perspective and to facilitate an exchange of ideas by bringing together experts in the field and other stakeholders. The workshop was designed to consider experiences over the past 18 years and examine the current knowledge about traditional breeding methods, transgenics, and unintended effects. The current status of the compositional analysis approach was examined with a view to determine if the general application of the substantial equivalence concept is still warranted and, if so, in what cases it should be applied and how should it be conducted to contribute meaningfully to the safety evaluation. The objectives of the workshop were to review traditional breeding methods and consider the effects these methods may have on crop composition and compositional variability; consider compositional analysis with a scientific, nonbiased view; discuss the science behind the current approach to compositional analysis in the framework of the safety assessment; and arrive at a consensus and make possible recommendations regarding the state of the current approach to compositional analysis.

The workshop was attended by participants from around the world interested in issues concerning crop composition: 46 participants were identified as government representatives, 28 as university scientists, and 19 as representatives from industry. After welcoming remarks and clarification of objectives, the workshop opened with a presentation and discussion of the origins of natural variation within a crop, the effects of plant breeding on variability, and crop domestication.[46] The rest of the workshop was divided into four thematic sessions. The first session considered aspects of conventional development of new crop varieties. The topics presented included aspects of traditional and modern breeding,[47] genomic variation in plants recovered through plant cell and tissue culture (presented at the workshop by Dr. John Finer, of The Ohio State University), genomic changes that may take place during domestication and improvement of a crop,[48] and the natural variability present in crop composition.[49] The second session addressed the development of crops using methods of modern biotechnology. Topics in this session covered product development,[50] bringing to market a GM crop,[51] and also resources of crop composition data and their usefulness.[52] Session three provided a discussion of compositional analysis methods, which included the development of methods,[53] the creation of composition consensus documents authored by the OECD, and a review of methods for evaluation of endogenous allergens and their relevance in the safety evaluation of GM crops.[54] The final session was concerned with the interpretation of composition data. Presentations addressed the importance of composition in the evaluation of food safety,[55] a discussion on statistical significance as it relates to biological importance,[56] and perspectives on how composition data are interpreted by regulatory bodies assessing food and feed safety.[57]

Four round-table discussion sessions also took place during the workshop. The purpose of these discussions was to provide the workshop participants with an opportunity to express their thoughts and opinions about issues related to compositional analysis. Some of the specific questions addressed during the discussions were as follows: How does transgenic methodology affect the resultant progeny compared to methodology employed during traditional plant breeding? How does the inherent variability of crop components affect data interpretation and the subsequent safety evaluation? What is the appropriate comparator to use in a compositional analysis study? And what factors are to be considered when determining what tissue types and crop components should be included in the analysis?

A summary of the discussions during the roundtable sessions can be found in this special issue.[58] Some of the key points resulting from the discussions were the following: (1) Scientists working in areas related to the safety of GM crops recognize the important role the safety assessment plays in assuring a safe food supply and that the "safety assessment is still a key activity".[58] (2) The body of scientific knowledge about the evaluation of GM crop safety has greatly increased since the release of the first GM crops, but regulations in general have not changed at the same pace as our learning. (3) Consensus documents that are based on sound scientific knowledge are helpful resources in evaluation of GM crop safety, but such documents need to be updated to reflect the advances in the science.

The organizers of the workshop anticipate that the information presented in the associated series of papers in this *Journal* and the online video of the workshop available through the ILSI Web site (http://www.ilsi.org/FoodBioTech/Pages/2012PlantCompositionWorkshop.aspx, accessed March

6, 2013) will continue to generate further discussion on topics and issues related to compositional analysis of GM crops. As this information is distributed to a wider audience, additional discussions also will generate new ideas that should lead to the employment of the best science in formulating future techniques and strategies to be used in the compositional analysis approach.

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