JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Perspective

pubs.acs.org/JAFC

Compositional Analysis of Genetically Modified (GM) Crops: Key Issues and Future Needs

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ABSTRACT: Effective symposia need two strong legs to stand upon: informative presentations of recent research paired with lively discussion of these topics. Although it is easy for the organizers of a symposium to predict the usefulness of the former, as they select the speakers and their topic areas, guaranteeing productive discussion is a far more difficult task. For the Crop Composition Workshop sponsored by the International Life Sciences Institute's Committee on Food and Biotechnology (ILSI IFBIC), the organizers scheduled four roundtable discussions with preselected questions and with rapporteurs drawn from governmental organizations and public-sector research institutes (the authors). It was also the organizers' intent to let these discussions flow on the basis of the experiences of the participants and pressing issues within the overall debate on the role of crop compositional analysis within safety assessment of biotechnology as it exists now and in the future. The goal of this perspective is to summarize the issues raised, providing references when possible, and to describe the consensus statements reached through the course of these discussions.

KEYWORDS: *genetically modified, transgenic, breeding, safety assessment, appropriate comparator, unintended effects, inherent variability*

INTRODUCTION

Effective symposia need two strong legs to stand upon: informative presentations of recent research paired with lively discussion of these topics. Although it is easy for the organizers of a symposium to predict the usefulness of the former, as they select the speakers and their topic areas, guaranteeing productive discussion is a far more difficult task. For the Crop Composition Workshop sponsored by the International Life Sciences Institute's Committee on Food and Biotechnology (ILSI IFBIC), the organizers scheduled four roundtable discussions with preselected questions and with rapporteurs drawn from governmental organizations and public-sector research institutes (the authors). Participants in the roundtable discussions included invited experts and stakeholders from industry (19 participants), government (46), and universities (28) in both the Global North (51) and South (42). It was hoped that these roundtable discussions would actively engage a majority (or at least a plurality) of the diverse participants in the workshop. It was the organizers' clear intention to have frank and open discussions of these topics, without the requirement of having to speak on behalf of a particular interest group. Rather, it was hoped that participants would help each other to make evidence-based decisions based on their research and share their experiences from both sides of the food and feed regulatory review process. Thus, participants in a particular discussion will be described using aggregate descriptors, but their comments will be anonymous. It was also the organizers' intent to let these discussions flow on the basis of the experiences of the participants and pressing issues within the overall debate on the role of crop compositional analysis within safety assessment of biotechnology as it exists now and in the future. The goal of this perspective is to summarize the issues raised, providing references when possible, and to describe the consensus statements reached through the course of these discussions. The questions for the four discussions are listed in Table 1.

Sessions A and B occurred simultaneously, dividing the 91 workshop participants into two groups. Although the two discussions had different topics and focusing questions, both groups covered largely the same thematic ground. This illustrates the power of multidisciplinary approaches, in that multiple scientific disciplines and perspectives when faced with apparently different problems can reach the same conclusion.

SESSION A: METHODS OF PLANT IMPROVEMENT

The purpose of this discussion was to explore how the methods of plant improvement influence crop composition, whether the improvement be through conventional plant breeding, genetic modification (or transgenesis, hereafter GM), or some combination of methodologies. In this discussion, 22 of the 45 participants verbally expressed an opinion, with 13 from the Global North and 9 from the Global South. University scientists were the most common (10) active participants, followed by governmental scientists (9) and corporate scientists

Special Issue: Safety of GM Crops: Compositional Analysis

Received: March 13, 2013
Revised: May 20, 2013
Accepted: June 7, 2013
Published: June 7, 2013

Table 1. Discussion Questions for the Crop Composition Workshop

session	questions
A	How does transgenic methodology affect the resultant progeny compared to the methodology employed during traditional plant breeding? Is the likelihood of generating unintended effects inherently greater with one methodology compared to the other? If so, is the difference great enough to merit a safety assessment? (Are there circumstances when crop composition would not be considered as "necessary" in the safety assessment?)
B	How does the inherent variability of crop components affect data interpretation and the subsequent safety evaluation? What role does inherent variability play in evaluating the safety consequences of any unintended effects? How can crop composition databases be used to define inherent variability in composition?
C	What is the appropriate comparator to use in a compositional analysis study to support the safety assessment? What defines history of safe use/safe consumption? (What do the science, the data, and the experiences of the past two decades tell us we need for risk assessment?)
D	What factors are to be considered when determining what tissues and what components should be included in the analysis? Are current OECD guidelines adequate?

(3). The rapporteurs started the discussion with a reminder that every method for plant improvement can result in unintended effects, whereas the significance of these effects would be a key question for discussion.[1] The rapporteurs reminded the group of the use of mutagenesis and its importance and widespread acceptance in conventional plant breeding, including circumstances when the desired effect (e.g., extended shelf life/delayed ripening in tomato) has negative unintended but not unexpected effects on product quality (e.g., reduced palatability).[2] The rapporteurs challenged the group to reflect on the past 15 years of research and provide an example of a negative, unintended effect that was detected through compositional analysis that prevented the product from entering the marketplace. No examples could be cited by the participants. The original intention for composition analysis was placed in context, as 15−20 years ago there was little empirical information from which to make a regulatory decision. One of the issues raised several times during the ILSI IFBIC workshop was the need for not only a common purpose but also a common vocabulary and knowledge base among those involved in the risk assessment process. It is hoped that the presentations and conversations among such a multidisciplinary group of scientists helped to reinforce the need for common vocabulary and expand the knowledge base.

One of the key discoveries in plant genetics of the past 15 years discussed during the workshop was the high degree of heterogeneity and plasticity of plant genomes. Far better estimates for natural genetic diversity are now possible, using a combination of high-throughput/next-generation DNA sequencing technologies and hybridization-based approaches, in both low genetic diversity crops such as soybean and high genetic diversity crops such as maize.[3,4] It is now clear that different varieties of the same species can carry different collections of genes while appearing to be visually identical. Hybrid crops such as maize have as many as 10 million single-nucleotide polymorphisms (or SNPs, the most common form of genetic variation) between the two parental varieties and differ by 10−20% in the number of genes present in their genomes (i.e., 5−10000 genes).[4] The impact of mutagenesis to genomes, an acceptable source of introducing genetic variation to conventional plant breeders, has also been visualized with much higher accuracy and precision.[5] Chromosome-level occurrences, wherein segments of chromosomes are duplicated, deleted, or rearranged, are all commonly observed in response to mutagenizing radiation, as are the more commonly considered creation of SNP variants.[5] These developments made clear that commodity crops (such as maize and soybean) are heterogeneous mixtures as presented to the processor or consumer. Whereas not every permutation of these genetic variants would occur within a single farmer's field, it is clearly false to assume compositional homogeneity for each crop in the food supply.

The scale and scope of resources available to public and private sector scientists were common topics during the session A roundtable, to share information between the participants and make facts well-known within one group obvious to all. One such example, highlighted during discussions, was the measured productivity of a public-sector breeding program from the Global South. One research group developed 20 varieties of rice over a 10 year period, which represented the output from an efficient and productive team. However, to release this number of improved cultivars required creating and evaluating ∼100,000 distinct genetic varieties (0.02% of the total). The vast majority of the effort expended was not ultimately productive, as improving yield, crop quality, disease resistance, or some combination of traits is a quite rare occurrence and requires many attempts to create a variety better than those already in the marketplace. The varieties that were discarded were not unsafe, merely not of commercial quality. A breeding program working in a corporate environment, especially in the Global North, would be orders of magnitude larger in scale, but the probability of success (0.02%) is likely quite similar on the basis of traditional line development approaches.

A second discussion on scale and scope addressed the increased capability for compositional analysis, given technical advancements over the past 15 years. Although a larger number of metabolic features can be measured with increased precision and accuracy, understanding the relevance and need for these analyses from a standpoint of *food safety* rather than *fundamental biology* is a central challenge. The goal for a safety assessment is to assess the risks to consumers and the environment; compositional analysis was intended as part of the regulatory assessment to support this goal rather than to catalog every reproducible difference between the samples. Whereas compositional differences may exist between novel varieties and those already in the marketplace, international guidance documents indicate which compounds are of greatest importance to ensure safety.[6] Compositional differences may be real, but not relevant to the issue of safety, and these differences are also seen between different conventional varieties of the same crop. Scale and scope have increased for crop compositional analysis, but similar changes have not been seen on the regulatory side of the system. Regulatory scientists spoke of increasingly long queues, as more GM plants enter their national approval systems and the accompanying dossiers themselves are also longer, as more compositional data are included for review.

The scale and scope of plant transformation technologies have also changed over the past 15 years. Increased understanding of DNA repair mechanisms, which can be

exploited to edit DNA sequences using sequence-specific nucleases and are collectively referred to as "genome editing", represent new methods for making GM organisms.[7] Genes can be rewritten in place, eliminating random insertion effects and permitting the flexibility to introduce small changes to the DNA sequence or induce larger changes, such as the deletion of a gene or the insertion of one or more new genes.[8] Other advancements in modifying the genes in plants, such as transformation vectors that are synthesized from DNA sequences found only in plants, represent more incremental improvements in transformation technology. However, this latter development exposes a gap in the regulatory process, at least in the United States. At present, GM risk assessment is a process that can involve the U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration (FDA), and the Animal Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture. Specifically, the regulatory oversight given to APHIS hinges upon the use of DNA sequences found in plant pathogens as reagents necessary for plant transformation. Without these sequences, APHIS does not have oversight on a GM plant, whereas EPA and FDA may still.[9] Although neither of these developments in transformation technology directly effect crop composition, both of them illustrate how the science has advanced over the past 15 years while the regulatory environment has remained largely static. International standards, such as those from the Organization for Economic Cooperation and Development (OECD), are highly valuable resources for national regulatory committees and agencies.[6] These standards require a great deal of time and effort to establish; however, aspects may become irrelevant to some greater or lesser degree given the pace at which our collective knowledge advances relative to the pace of modifying regulatory practices.

The Session A discussion closed with a collective agreement on the following statement:

> *Based on worldwide experience, there is reason to reconsider what information is required for compositional analysis within deregulation of particular transgene/crop combinations, as some combinations now have a history of safe use. This is an evidence-based decision and would assist regulatory bodies around the world make more effective use of their potentially [limited] resources.*

SESSION B: SOURCES OF VARIATION IN CROP COMPOSITION

There were 21 active participants in Session B, 15 from the Global North and 6 from the South. In this discussion, government scientists were the most common active participants (10), followed by corporate (6) and university (5) researchers.

The Session B discussion opened with a reminder of the role that compositional analysis plays in the risk assessment process. Compositional analysis addresses a key safety concern of consumers and regulators, as unintended effects on crop composition and quality may have arisen due to plant transformation or another step in GM crop development. The hypothesis here is that the unintended effect would change flux patterns through metabolism toward proteins, lipids, carbohydrates, or other key quality indicators that would be detected through quantitative analysis of these features. However, assigning the titles of "unintended effect" and/or "risk to safety" when the expected range and causes of variation for a particular compound in a specific crop are not well understood is nigh on impossible, and an unintended effect rarely causes a risk to safety.[1] Participants in the discussion were firm in their affirmation of the present risk assessment process, as no GM crop has been identified through review in any country that posed a risk to safety due to an unintended effect to composition. This issue is still a hot topic in many consumer groups, such that attention must be paid. Given the importance of broad stakeholder support for the process of safety assessment, it is worthwhile to make clear how safety assessment works as an evidence-based decision process so that producers, consumers, regulators, and others have confidence in safety assessment.

The concept that the food supply is heterogeneous by nature was also raised here and that the food supply has drastically changed due to stakeholder preferences over the past 15 years. As farmers seek higher yielding varieties, the ratio of carbohydrates to proteins within grain has been the target of selection by the seed industry, as total carbohydrates drive yield but are negatively correlated to total proteins.[10] Thus, the composition of the food supply has subtly but steadily shifted over time, although this shift is much less than the variability that is seen even within a single year as a result of environmental and genotype impacts on composition. However, this subtle shift is present in the commodity crops such as maize that is ubiquitous in processed foods and as a source of energy for animal agriculture, although animal diets are typically balanced for key constituents during formulation. This shift in composition was raised in the context of the problem it creates for compositional analysis, as the information in infrequently updated databases may not have representation from current conditions in the marketplace. For example, the vast majority of corn acreage in the United States is now GM (88% for 2012),[11] such that newly released cultivars with improved agronomic performance do not have a non-GM counterpart; thus, non-GM cultivars may likely not be truly representative of the corn crop. Additionally, USDA maintains a Nutrient Database that analyzes product composition using samples available from the marketplace.[12] The entry for "yellow corn meal" is an aggregate of samples that were available to consumers for a window of time and summarize average quality observed for key nutrients and components. As farmer preference has shifted greatly toward GM maize varieties, one imagines that the samples analyzed by USDA have likewise shifted in identity as the food supply has shifted. ILSI maintains a Crop Composition Database that reports similar but more specific data, as it can be queried to analyze variance due to specific factor (i.e., variety, year, country of origin).[13] Both databases are useful to help define the extent of normal variation in crop composition and a history of safe consumption, but each exists in historical time and defines crop composition as it was, rather than how it will be.

The typical response to the problem of compositional analyses is simple to understand but difficult to implement: perform a better experiment. Compositional analyses now involve more reference varieties, locations, seasons, and compounds of interest. However, it is difficult to assign value to these increases in study size to detect smaller differences, when it is clear that much larger compositional differences can be caused by environment and germplasm. As described above, a "better" experiment may be one that includes GM varieties as comparators to better represent what is on the market, but some current regional regulations prevent this from being a viable study design. The costs associated with compositional

analysis are significant and borne by the developer.[14] Session B made a point parallel to Session A, in that well-studied crops now have a very deep knowledge base (e.g., GM maize), but with the expansion of GM technologies into new markets and crops, the lack of historical data presents an additional hurdle for developers to clear. Stakeholders in emerging markets, such as India, Brazil, and Malaysia, are creating compositional databases for crops of high local value that do not appear in other information systems. Perhaps these new databases for cowpeas, common bean, and oil palm can take advantage of the knowledge we have gained over the past 15 years on compositional variability and its sources. This could serve to better inform the compositional analysis portion of safety assessment and potentially increase the efficiency of this process. However, for less-studied crops, the boundaries of normal compositional variation, that which is observed among genetically diverse, conventionally bred cultivars grown over a number of seasons and in a variety of localities and cropping systems, will need to be established before GM varieties can be evaluated in a biologically meaningful way.

An expansion of the topic that the food supply is heterogeneous was the notion that new GM varieties may depart from the substantially equivalent paradigm, as compositional changes are intended and predicted to be beneficial (e.g., changing lipid profiles or increasing essential amino acid content).[15] The role of compositional analysis in such a case was discussed. One possibility discussed was to partition out the beneficial/intended outcome on composition and look at the rest of the picture; here came a reminder that composition analysis is meant to look for unintended effects and not to measure natural variation. As analytical methods to describe plant genomes have improved dramatically in the past 15 years, so have methods to analyze crop composition. However, the gulf between observations and expectations is at least as large with respect to chemistry as genetics, with an even larger need for interpretation to bridge the gap. Thus, much as scientists and regulators need to make their reasoning clear to consumers, advances in different scientific disciplines make it equally important for consensus opinions generated among scientific experts. Given the pace of scientific advancement, it is increasingly difficult for nonexperts to appreciate the strengths and limitations of other approaches, while at the same time it is critical for the chemists, breeders, agronomists, and regulators involved in these decisions to be aware of what is experimentally possible, practical, desirable, and/or necessary for a safety assessment for this process to be effective.

SESSION C: DEFINING THE APPROPRIATE COMPARATOR

Sessions C and D were held on the last day of the workshop and helped to summarize all of the topics presented and discussed. Session C had 20 active participants, with 16 from the Global North and 4 from the South. Government scientists were the most common participants (9), followed by corporate (7) and university (4) scientists. Session C had two questions listed in the agenda, but a third was added by the rapporteurs in response to the topics discussed during the workshop (Table 1, in parentheses). This discussion also highlighted how regulations have remained static while the seed industry has advanced: if 90% of a crop is GM, does using a non-GM comparator make sense as a representative variety already on the market? This question recalled elements of both Sessions A and B, as the goal of compositional analysis is to support the risk assessment. The importance of experimental design was reaffirmed as was the intent of the experiment, whether to find **any** difference between the experimental variety and its comparator or to evaluate whether the difference sits within (or outside) the boundaries established to be acceptable by previous experience. This difference in intent, between the questions driven by scientific interest (the former) and those important to protect the safety and quality of the food supply (the latter), was an often identified stumbling block.

This sentiment was summarized by the rapporteurs, who offered the following:

> *Can we now try to get some consensus on where we have arrived? Do we agree that there is scope for crop specific consideration of what the comparator should be or whether in fact we need any comparator based on how well characterized the crop is, and how much experience we have had with that crop and the events that have been introduced into it? Do we now at least have enough data on the broad natural variability of corn, for example, and on the multiple traits from multiple developers and multiple events, none of which have ever led to an adverse compositional outcome, to conclude that there is scope to consider first whether compositional data is required at all? And second, if it [compositional data] is required that all of the data from the gene pool [a broad survey of varieties rather than a single specific variety] is the appropriate comparator? Do we agree that the science suggests this? Does anyone disagree with this proposition?*

Although there were no specific objections to these propositions, the discussion that followed expanded on these ideas. As in the Session A discussion, it was suggested that crops with extensive experience for particular types of transgenes (e.g., corn with insecticidal proteins from *Bacillus thuringiensis*) may require less review if regulations were to be revised. Eliminating the compositional review component would not engender trust from all stakeholders, but an acknowledgment that 15 years of experience with an increase in knowledge of compositional variability and without any negative outcomes should factor into the decision-making process. On the other hand, other crop and transgene combinations would require the same regulatory thresholds now and in the future. This thought was expanded with a brief review of crop and trait combinations that present known health hazards (e.g., glycoalkaloids in potatoes).[1] These sorts of obvious pitfalls, however, represent areas for crop improvement that can make use of the technological advances of the past 15 years (glycoalkaloid biosynthesis is well understood now), such that selection using molecular genetic markers could remove individuals with unacceptable genetic combinations long before field testing or agronomic evaluations become issues (whether for traditional varieties or GM crops). This led to a second series of summary statements from the rapporteurs, which again did not provoke objection from the participants:

While compositional analysis is not a safety assessment per se, it is a pivotal data source for safety assessment. Can we agree then that what matters is not so much equivalence per se, but to what extent compositional differences, if they are observed at all, fall outside the variability seen in the gene pool and if so, is the departure from the gene pool of such magnitude that it raises questions as to how that would have occurred? There is scope, then, for crop specific, stratified data requirements to deliver the data that are actually required to determine if the GM crop is safe, that take into account the extent of characterization of the crop, experience with the traits [or genes] used in that crop, natural variation, experience with the crop in general, and thus reduce the regulatory burden where justified. Could making these changes to the regulatory process perhaps promote agricultural progress?

SESSION D: DEFINING THE SCOPE OF COMPOSITIONAL ANALYSIS

Much as they had on the previous day, the simultaneous sessions had different stated questions but considered largely the same issues. Session D had 21 active participants, with a more even break between North (10) and South (11) than Session C. Government scientists were again the most common participants (10), followed by university (8) and corporate scientists (3). Discussion of the first question posed to participants of Session D got off to a rapid start as a strong premise was advanced: to remove the requirement for broad compositional analysis for certain crop and trait combinations, on the basis of the enhanced knowledge gained since the inception of GM crop improvement. This point repeated the idea advanced in Session A and was highly similar to the discussion occurring in Session C.

Discussion on the nature of compositional analysis then moved into the importance of national end-use preferences. Not every crop is used identically in every market, such that each nation's regulatory agencies need to take international consensus documents as a starting point but then apply local knowledge and priorities to individual risk assessment processes. Patterns of end-use were discussed, including the possible concentration or dilution of key nutrients or toxicants due to processing (e.g., milling, cooking). Whereas it may not be necessary to obtain compositional data for any or all processed fractions, it was noted that national safety assessment decisions need to be cognizant of how processing may affect composition and if differences have the potential to be altered through the processing stream. A specific example was given for The Philippines, where a national nutritional survey describes consumer preferences and consumption patterns.[16] Thus, the potential impacts of small changes within the food supply on mega-staples such as rice could be modeled to assist in the safety assessment process, although this specific issue may illustrate the divergence between safety assessment and nutritional quality/equivalence. This portion of Session D highlighted the importance of national decision-making influenced by both international guidelines and local impact. However, the discussion of scale and scope from Session A should be recalled here, in that the true risk or implication to food safety should be considered and not just scientific curiosity.

The responses to the second question, on the adequacy of OECD guidelines, reinforced another key theme of the workshop. Many advances have been made in the past 15 years, with respect to the number and nature of GM crops, the area in which they are planted, and the fractions of the world food supply they represent. In parallel, our collective knowledge of biochemistry, genetics, agronomy, and nutrition are all substantially larger now than before. These issues illustrated that risk assessment guidance documents, such as those assembled by OECD, have a life span. These documents are drafted at a particular moment in time, based on the information available to the creators, and are influenced by the concerns and needs of stakeholders. Information, concerns, and needs change. For example, genome editing was not yet discovered in 2002 when the OECD consensus document for maize was created, but this guidance document was not intended to be a permanent resource. As scientific knowledge evolves, guidance documents that assist in evidence-based decision-making should evolve as well. Although revising these documents and governmental regulations is time-consuming, the outcomes are worthwhile and the process engages the international community to develop a "continuous" document. It was also reiterated that it is important for developers to align with the regulatory requirements of individual countries and to meet with the regulatory agencies involved in the development and use of these documents.

SUMMARY

The ILSI IFBIC workshop on crop composition illustrated several key points:

(1) Much has changed since the first GM crops were released in the early 1990s:[1] our collective scientific knowledge has grown substantially, GM varieties for particular commodity crops are now central (nearly exclusive) in many major markets, and the tandem need of increased agricultural efficiency balanced with environmental sustainability requires that GM technologies be widely accessible.

(2) Many things have not changed since the first GM crops were released: scientists are committed to the safety of their fellow citizens and national food supplies, such that safety assessment is still a key activity.

(3) Increased demands on the regulatory environment, coupled with the lack of negative outcomes and the gain in the knowledge base, suggest that as consensus guidance documents expire that their replacements take heed of the lessons learned.

(4) Workshops that involve a broad cross section of stakeholders and experts can provide valuable insights into decision-making processes, their conclusions, and related concerns that span world areas and global markets.

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Notes

The authors declare no competing financial interest.

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