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Perspective

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Bringing a Transgenic Crop to Market: Where Compositional Analysis Fits

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ABSTRACT: In the process of developing a biotechnology product, thousands of genes and transformation events are evaluated to select the event that will be commercialized. The ideal event is identified on the basis of multiple characteristics including trait efficacy, the molecular characteristics of the insert, and agronomic performance. Once selected, the commercial event is subjected to a rigorous safety evaluation taking a multipronged approach including examination of the safety of the gene and gene product − the protein, plant performance, impact of cultivating the crop on the environment, agronomic performance, and equivalence of the crop/food to conventional crops/food − by compositional analysis. The compositional analysis is composed of a comparison of the nutrient and antinutrient composition of the crop containing the event, its parental line (variety), and other conventional lines (varieties). Different geographies have different requirements for the compositional analysis studies. Parameters that vary include the number of years (seasons) and locations (environments) to be evaluated, the appropriate comparator(s), analytes to be evaluated, and statistical analysis. Specific examples of compositional analysis results will be presented.

KEYWORDS: *agricultural biotechnology, compositional analysis, transgenic crops*

INTRODUCTION

Biotechnology-based solutions to agricultural problems have been among the most rapidly adopted products such that in 2012 >170 million hectares have been planted with these "genetically modified" (GM) or "biotech" crops.[1] These are also the most highly characterized, with respect to safety, food substances consumed. The development of these crops is a lengthy, extensive, and involved process. Thousands of genes and transformation events are evaluated to select the event that will be commercialized.[2] An event is defined as the insertion of the transgene in the nuclear genome of a single cell that regenerates to a fertile plant and passes the insertion to its progeny. Hence, different events would have different insertion sites. The majority of events are not advanced toward commercialization. The ideal event is identified on the basis of multiple characteristics including, but not limited to, trait performance (meeting product specifications), the molecular characteristics of the insert, and agronomic performance. Specifically considered questions are the following: Does the event perform and stably demonstrate the trait efficacy desired? Is extraneous DNA present or are new proteins unintentionally produced? Has the insertion of the new genetic material disrupted plant performance?

Once selected, the commercial event is subjected to a rigorous safety evaluation that takes a multipronged approach. The assessment considers the safety of the gene, the safety of the protein produced by the gene, plant performance, the impact of cultivating the biotechnology crop on the environment, agronomic performance, and the equivalence of the crop/food to conventional crops/food. This last point is partially addressed by compositional analysis, which is composed of a comparison of the nutrient and antinutrient composition of the consumed portions of the crop containing the event, its parental line (variety), and various conventional lines (varieties). Different regulatory agencies in the various geographies have different requirements for the compositional analysis studies. Among the parameters that vary are the number of years (seasons) that should be evaluated, the number of locations (environments), the appropriate comparator(s), the reliance on the ILSI Crop Composition database or other published values, the analytes to be evaluated, and the statistical analysis applied. The purpose of this paper is to describe where in the safety assessment the compositional analysis results fit and to present specific examples of compositional analyses taken from the literature and from recent dossiers.

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PRODUCT DEVELOPMENT

From discovery to commercialization multiple ideas, trait genes, and transformation events are evaluated. A recent survey of the major agricultural biotechnology developers found that on average 10209 genes are evaluated for each product developed. Approximately 500 of those genes will be advanced into proof of concept experiments, and then over 1000 transformation events (per product concept) will be evaluated from which a single event (usually with a back-up event) will be selected for commercialization.[2] It is important to start with such a large number because not all approaches toward developing a commercial event are successful for multiple reasons. The plant may not always perform as anticipated; biology is not always predictable. The trait may be too complex. The gene may not be well expressed in the plant, the plant may not be fertile, or the transformed cell may not readily regenerate into a fertile plant that passes the traits to its progeny in a predictable Mendelian fashion. Furthermore, the insertion site may not be optimal; therefore, many different events may need to be screened to find the best one to move forward. Different approaches have been undertaken to optimize the selection process toward a commercial event. For instance, the genes may need to be codon optimized to express well in the recipient crop, or tissue-specific promoters may be selected to increase the probability of successfully meeting the product specifications.

Selection of the event is guided by the desired product specifications: Does the plant have the desired phenotype and retain the appropriate agronomic performance the growers desire? Regulatory guidelines in some geographies requiring the absence of extraneous DNA, a lack of vector backbone, minimal chromosome rearrangement, and no production of chimeric novel proteins also guide selection of the event. Public acceptance issues affect gene selection and product design. For instance, no animal genes or elements are utilized. The overall expense involved in bringing a product to market also affects which crops will be engineered. This is simple economics; a commodity crop worth billions (such as maize) is more attractive for the investment of the ca. $30 million for a biotech product than a noncommodity crop (such as tomato) whose annual value may not exceed the price tag. This is a partial explanation for the slowness of "second-generation" or nutritionally enhanced plant biotechnology products to be commercialized.[3]

SAFETY ASSESSMENTS

The safety assessment starts long before the event to be commercialized is identified, although once this event has been selected, it will be subjected to a myriad of safety studies. As soon as a gene has been identified as possibly imparting the desired phenotype, a bioinformatics assessment is done even before transformation. Bioinformatics analysis is a useful tool for confirming that the gene has the desired function on the basis of homology to known genes and does not have homology to known allergens or toxins.[4] Also early in the development process of a transgenic product, a digestive fate screen is conducted, also to confirm low potential of allergenicity development or toxicological concerns associated with the newly introduced protein. If no issues are found, the gene enters the transformation pipeline. Event-independent safety studies associated with the gene product, that is, the newly introduced protein, can be undertaken prior to elite event selection. The event-dependent safety assessment is initiated once an elite event is selected. The overall assessment takes a multipronged approach in which the gene, the protein, and the biotech crop are all evaluated. Many publications[5−8] have reviewed the process, which is outlined in Codex Alimentarious[9] but for which individual countries or regions have their own approaches. This process will be described only briefly here.

The safety of the gene and its source are considered. An extensive evaluation of the newly introduced protein is made following the two-tiered approach described in Delaney et al.[5] The first tier takes a weight of evidence consideration of the history of safe use, bioinformatics analysis, expression levels in the crop, mode of action including specificity of the gene product, and stability of the protein to proteases, pH, and heat. Toxicity testing is part of the second tier.

The evaluation of the biotech crop includes the molecular characterization of the insertion site, which is composed of an extensive analysis of the locus as required by some regulatory authorities that includes determining the copy number, number of loci, presence/absence of vector backbone, nucleotide sequence of the insert, nucleotide sequence of genomic region surrounding the insert, bioinformatics assessment of the insertion site and surrounding genomic sequence, and determination of the site of insertion within the crop genome. This latter is dependent on having the genome of that crop already described and available. Although much of this molecular information is interesting, it does not contribute significantly to a risk assessment or to addressing the impact of the insertion on plant performance. The agronomic performance studies in which the crop performance in the field is compared to the parental variety or nontransgenic comparators and other conventional varieties/lines address this last point specifically. Performance parameters, specific to each crop and similar to what a breeder would evaluate, are monitored. If the plant performs normally despite the presence of the transgene, assuming the composition is "normal", then it is concluded that the insertion does not have a negative impact on the plant. In some situations, yield drag may occur; this was the case with the original Roundup Ready soybean lines, but the trait was so valuable to the grower that even a decrease in yield was accepted at that time.[10] An environmental safety assessment is also performed. Both in-laboratory safety studies using either the newly expressed protein or plant materials and surrogate/representative organisms and in-field surveys are performed. The final general area of the safety assessment is nutritional equivalence. Included in this would be the compositional analysis of the crop and of processed fractions. Also included would be nutritional or toxicological studies with the whole food or feed as required by some regulatory authorities. The focus of the following sections will shift from a perspectives approach to the compositional analysis as part of the safety assessment.

MATERIALS AND METHODS

Tissue Sources. Data for Figures 1 and 2 were obtained from grain of seven different maize events and their nontransgenic hybrid comparators that were grown in Iowa in 2007 in a randomized complete block design with three replicate plots. The plants were hand-pollinated using pollen from siblings. These independent events were generated by transformation with the same construct containing the *Escherichia coli* D-serine ammonia-lyase gene under the control of the maize ubiquitin promoter.[11] The presence of this gene allows for

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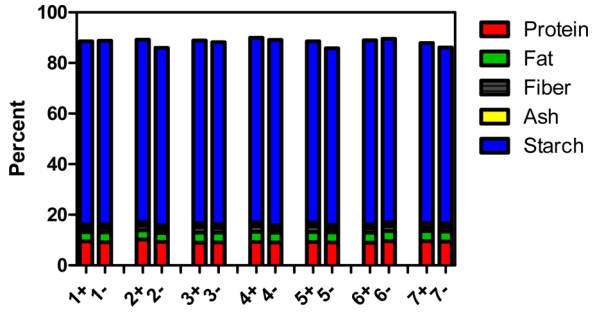

Figure 1. Impact of genetic insert on proximate and fiber composition: proximate and fiber data from multiple maize events (indicated as 1+ to 7+) generated by transformation using the same construct containing the *E. coli* gene for D-serine ammonia-lyase under the control of the maize ubiquitin promoter and grown in parallel with the appropriate nontransgenic hybrid comparator (indicated as 1− to 7−) at the same location during the same growing season.[15] Data are presented as percent dry weight of total grain components.

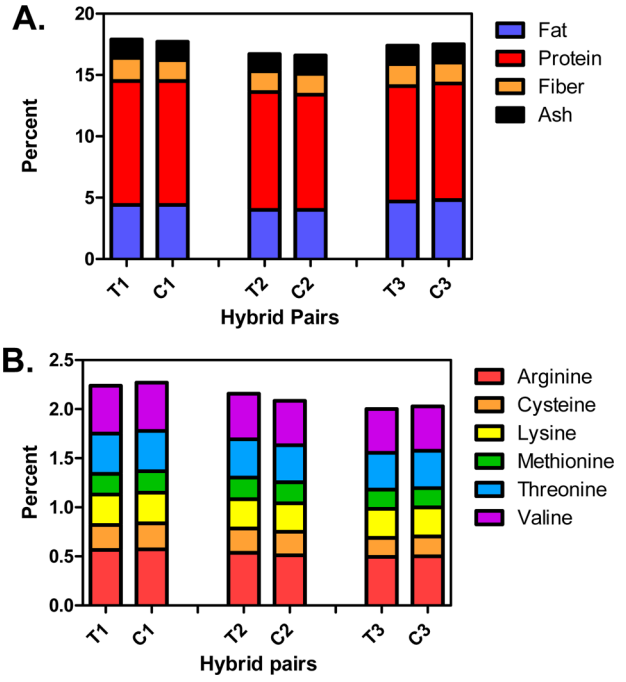

Figure 2. Impact of germplasm background on proximate, fiber, and amino acid composition of a single transgenic event in comparison with its nontransgenic hybrid comparator: (A) proximate and fiber data from a single maize event crossed with three different testers (T1, T2, and T3) and grown in the same growing season together with the appropriate nontransgenic hybrid comparator (C1, C2, and C3); (B) results for key amino acids (important in the nutrient composition of swine and poultry diets) in these same maize hybrids.[11] Data are presented as percent dry weight of total grain components.

selection in tissue culture on D-serine, which is toxic to plant cells lacking D-serine ammonia-lyase.

Data in Figure 3 were obtained from a transgenic potato, event EH92-527-1 (variety Amflora), and three conventional potato varieties (Bonanza, Kuras, and Seresta) grown at five different locations in Sweden, The Netherlands, and Germany during the 2004 growing season. EH92-527-1 is a transgenic potato event that contains high levels of amylopectin (>98%) due to the insertion of a granule-bound starch synthase gene insertion in antisense orientation. This event was granted cultivation approval in Europe in 2010 after a 14 year safety evaluation.[12,13] Data from this event and comparator varieties from the same growing season are also presented in Table 1 and Figure 4.

Data from two additional amylopectin potato events, AM04-1020 (variety Amadea[14]) and AV43-6-G7 (variety Modena[15]) are presented as well in Table 1 and Figure 4. These amylopectin (waxy) potatoes were achieved by transformation of different starch potato mother varieties, Kuras and Karnico, respectively, and with different granule-bound starch synthase RNA interference constructs. The data for Amadea, Kuras, and commercial varieties Agria, Bonanza, Cara, Fontane, Seresta, and Sibu were generated from tubers produced in the 2007 and 2008 growing seasons at 15 locations in Sweden, Czech Republic, and Germany. The data for Modena, Karnico, and commercial varieties Altus, Aveka, Aventra, Axion, Festien, Kuras, and Seresta were from tubers produced during the 2011 growing season at five locations in Sweden, The Netherlands, and Czech Republic.

Compositional Analysis. *Maize Analytical Methods.* Compositional analyses were conducted to measure proximates (fat, protein ash, and starch), fiber, and six key free amino acids.[11] The following analytical methods were used: oil (fat), AOAC[16] method 920.39; fiber, AOAC[16] methods 962.09/4.6.01; ash, AOAC[16] method 942.05; and starch, Corn Refiners Association method G-28/A-20. The amino acid analysis was performed using the Waters AccuTag system on the Acquity UPLC platform after acid hydrolysis. A *t* test was used to determine the difference between transgenic events and nontransgenic hybrid comparators.

Potato Analytical Methods. Compositional analyses were conducted to measure proximates (moisture, protein, ash, and carbohydrate), minerals, sugars, starch, vitamin C, and total glycoalkaloids. Analyses were conducted by Eurofins Scientific Analytics in Nantes, France. Moisture levels were determined in a forced draft oven with controlled elevated temperatures (2 h at 135 °C) (AOAC[16] method 930.15). Protein levels were determined by introducing the sample into the combustion chamber of a protein analyzer, and the generated gas was analyzed for nitrogen content and calculated to the corresponding protein level (AOAC[16] method 990.03). To determine the ash content, the sample was placed in an electric furnace at 525 °C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash (AOAC[16] method 942.05). The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation: % carbohydrates = 100% − (% protein + % fat + % moisture + % ash) (USDA Handbook 74[17]). The sugars were separated by ionic chromatography with NaOH eluant and detected by pulsed amperometry (AOAC[16] methods 906.03 and 923.09). Glycoalkaloids were extracted in boiling methanol/acetic acid and then analyzed by HPLC.[18] Total glycoalkaloids were calculated by summing the chaconine and solanine values for each sample. Vitamin C was extracted using metaphosphoric acid followed by oxidation of ascorbic acid to dehydroascorbic acid and derivatization resulting in an ortho-substituted phenylamine, which is measured fluorometrically (AOAC[16] method 967.22). Starch was analyzed using a MEKU Starch Balance for Amadea, Kuras, and the commercial varieties, Agria, Bonanza, Cara, Fontane, Seresta, and Sibu and by polarimetry for Amflora, Modena, Karnico, and commercial varieties Altus, Aveka, Aventra, Axion, Bonanza, Festien, Kuras, and Seresta. Minerals were analyzed using inductively coupled plasma−atomic emission spectroscopy. Analysis of variance was conducted; significance is indicated at $p < 0.05$ (*). Standard deviations were calculated for the data comparing Amflora with the conventional varieties Bonanza, Kuras, and Seresta (Table 1; Figure 3).

RESULTS

Specific examples are presented to consider factors that may affect compositional variability.

Same Construct Different Events. The questions being asked are as follows: What is the impact of the introduced gene on composition? Does the insertion site make a difference? This can be evaluated by looking at different events that were produced using the same construct for transformation. This

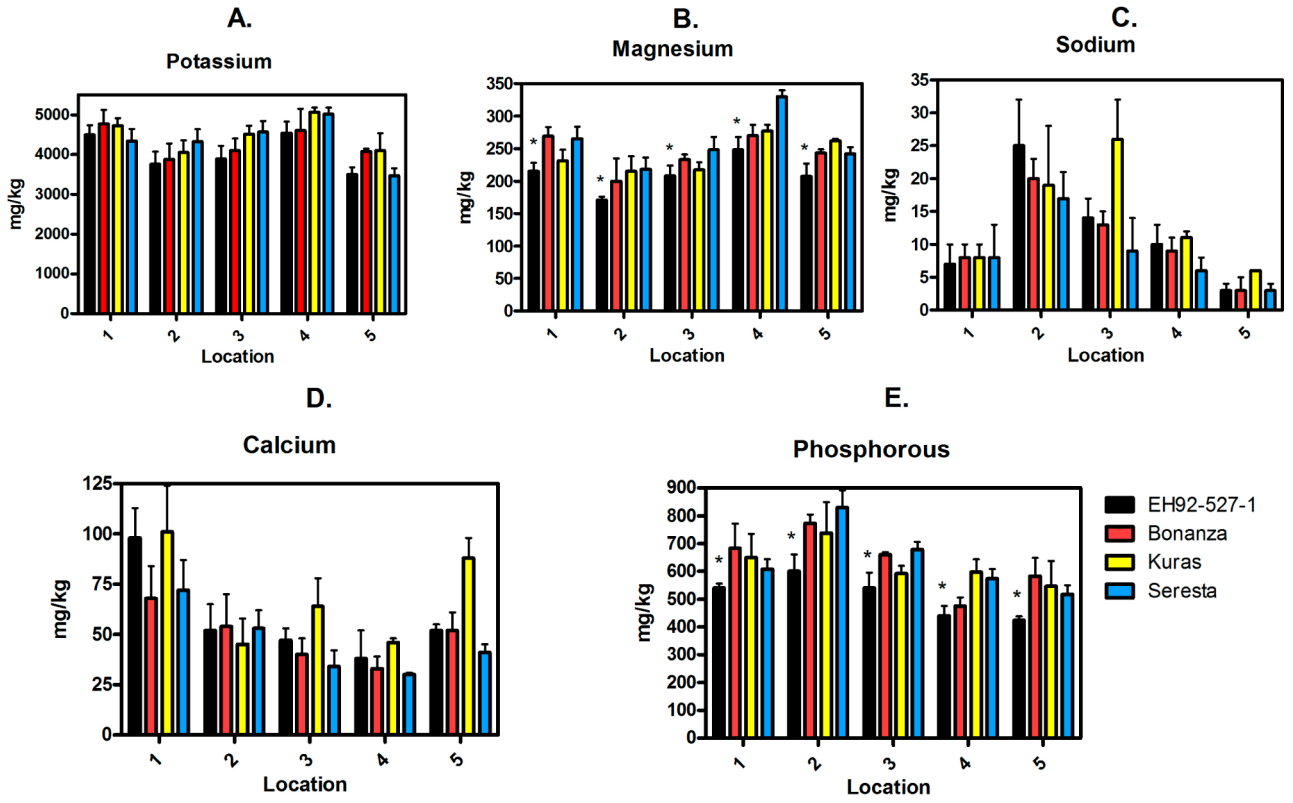

Figure 3. Impact of location on mineral content in a potato event in comparison with nontransgenic comparators. Each panel contains results for a different mineral level for four potato varieties, EH92-527-1 (variety Amflora, amylopectin potato, transgenic) and three conventional starch potato varieties (Bonanza, Kuras, and Seresta) grown at five different locations in a single growing season. Four varieties of starch potatoes including the amylopectin potato clone EH92-527-1 and three conventional varieties (Bonanza, Kuras ,and Seresta) were grown at five locations in three European countries (Sweden, Germany, and The Netherlands) during the 2004 growing season. The potatoes were grown under standard agronomic practices in a complete randomized block design with three or four replicates per block depending upon location (the three sites in Sweden had three replicates, the other two sites in Germany and The Netherlands had four replicates, respectively). Mineral levels were determined in the ash sample by inductively coupled plasma optical emission spectrophotometry. Mean values are presented with standard deviations indicated. The data were analyzed for variances using SAS version 8.2 following two procedures, the General Linear Model and the Mixed Model. Values were considered to be statistically significantly different at the $p < 0.05$ level (indicated by asterisks) when the EH92-521-1 potato event was compared with the conventional potato varieties.

kind of a study is not part of a safety assessment because multiple events are being compared. This information is useful in the consideration of the selection of a novel selectable marker system such as phosphomannose isomerase[19] or D-serine ammoniaplyase.[11] The results for protein, fat, fiber, ash, and starch (Figure 1) allow comparison of the grain composition of different maize events transformed with the same construct into the same genetic maize background and grown at the same location (environment). No statistically significant difference ($p < 0.05$) of the newly introduced gene on grain proximate and fiber composition is seen. Similar results were seen with seven independent maize events produced by the transformation of *E. coli* manA gene encoding phosphomannose isomerase (to allow selection of plant cells on mannose) driven by the maize ubiquitin promoter.[11]

Same Event, Different Germplasm Backgrounds. The questions addressed are as follows: What is the impact of the germplasm background on grain composition in the presence of the transgene? Does the germplasm make a difference? In this experiment a single maize transformation event was crossed with various testers, and the grain produced from these hybrid crosses was analyzed.[11] Results of the proximate analysis are shown in panel A and key amino acids (those that are limiting with regard to nutritional importance in swine and poultry feed) are shown in panel B of Figure 2. Different maize hybrids can be distinguished on the basis of their composition. However, the presence of the transgene, in this case a selectable marker, had no effect on the relative composition.[11]

Same Event, Different Locations. The question being addressed is as follows: What is the environmental impact on the composition of a transgenic crop as compared to conventional comparators? Mineral levels vary by location due to differences in the soil components. Figure 3 shows the mineral levels when a transgenic potato and three conventional potato varieties were grown at five different locations in Sweden, The Netherlands, and Germany. The event analyzed and shown here is the amylopectin potato event EH92-527-1, variety Amflora.[12,13] The results show that there is more variation due to location than due to the presence of the transgenic insertion. For instance, sodium levels in the potatoes were found at a much higher level in all varieties at location 2 than at the other locations. Furthermore, these data also demonstrate the varietal or germplasm differences that can be seen. The potato variety Kuras clearly seems distinct from the other varieties with respect to calcium accumulation. This distinction is not seen for potassium, magnesium, or phosphorus. At location 3, Kuras is different in its sodium

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Table 1. Starch, Sugar, Vitamin C, and Total Glycoalkaloid Levels in Three Amylopectin Potato Events and Comparison to Conventional Varieties[a]

			mean (range)					
variety (year grown)	no. of locations	*N*	starch (g/100 g)	glucose (g/kg)	fructose (g/kg)	sucrose (g/kg)	vitamin C (mg/100 g)	total glycoalkaloids (mg/kg)
Amflora (2004)	5	17	16.2 ± 2.4 (12.6–21.2)	3.1 ± 2.9 (<0.1–9.7)	2.4 ± 2.3 (<0.1–6.8)	3.1 ± 3.1 (<2.0–11.7)	13.3 ± 4.0 (7.1–20.2)	138 ± 47 (72–247)
reference[b]	5	51	18.8 ± 2.5 (13.6–23.7)	1.9 ± 1.7 (<0.1–5.2)	1.2 ± 1.3 (<0.1–4.2)	<2.0 (<2.0)	13.1 ± 4.8 (3.6–25.4)	272 ± 92 (70–474)
Amadea (2007/2008)	15	51	19.6 (16.5–23.7)	6.9[c] (2.9–12.4)	6.8[c] (3.0–12.4)	4.3[c] (0.5–9.0)	10.8 (6.4–18.6)	371 (163–713)
parental (Kuras)	15	51	20.3 (17.2–25.4)	6.0 (2.0–11.9)	5.9 (2.0–11.7)	1.8 (0.5–4.8)	9.3 (6.6–14.2)	553 (341–1246)
reference[d]	15	367	17.6 (8.8–24.5)	5.0 (1.3–11.4)	4.0 (1.2–9.2)	1.9 (0.5–10.2)	9.9 (5.2–14.3)	185 (29–609)
Modena (2011)	5	20	12.8 (10.7–15.1)	5 (2–9)	4 (1–8)	7 (2–23)	10.9 (9.0–13.5)	129 (122–136)
parental (Karnico)	5	20	14.1 (10.7–19.3)	5 (1–11)	4 (1–10)	5 (2–15)	8.6 (5.9–9.9)	132 (103–217)
reference[e]	5	128	15.8 (11.0–20.8)	5 (1–12)	4 (1–11)	5 (2–26)	10.5 (5.8–16.2)	160 (30–599)

[a]Methods used: starch, MEKU Starch Balance for Amadea, Method NF V03-606 for Amflora and Modena; sugars, AOAC Methods 906.33 and 923.09; vitamin C AOAS 967.22, total glycoalkaloids, Hellenas and Branzell.[24] [b]Reference varieties = Bonanza, Kuras, and Seresta. [c]Statistically significantly different ($p < 0.05$) from reference values as determined using analysis of variance comparison. [d]Reference varieties = Agria, Bonanza, Cara, Fontane, Seresta, and Sibu. [e]Reference varieties = Altus, Aveka, Aventra, Axion, Festien, Kuras, and Seresta.

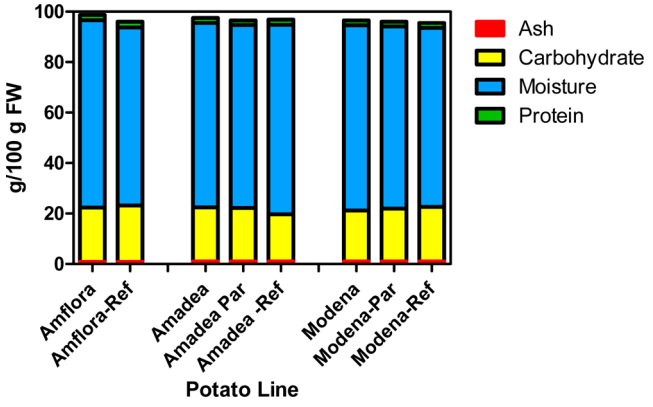

Figure 4. Impact of different events resulting in the same phenotype (amylopectin starch potatoes) on proximate levels. Three different amylopectin potatoes, produced using three different constructs, are compared to conventional potatoes and parental varieties. Data for each event and appropriate comparators were generated at the same time, but the data are from different years for the different events. The three amylopectin potato events are Amflora, Amadea, and Modena. The data for Amflora were from tubers grown in 2004 at five locations, $N = 17$; reference varieties (Ref) , Bonanza, Sibu, and Seresta, $N = 51$. The data for Amadea were from tubers grown in 2007 and 2008 at a total of 15 locations, $N = 15$; parental variety Par), Kuras, $N = 51$; reference varieties, Agria, Bonanza, Cara, Fontane, Seresta, and Sibu, $N = 367$. The data for Modena were from tubers grown in 2011 at five locations, $N = 20$; parental variety, Karnico, $N = 20$; reference varieties, Altus, Aveka, Aventra, Axion, Festien, Kuras, Seresta, $N = 128$.

levels. These results demonstrate the wide natural variability in conventional varieties.

Same Trait or Phenotype, Different Events, Different Constructs. The question being addressed is the following: When the same trait or phenotype is obtained by different approaches, does this have an impact on composition? Figure 4 shows the results of the analysis of proximates for three transgenic potato varieties. All three varieties, Amflora,[12,13] Amadea,[14] and Modena,[15] are amylopectin (waxy) potatoes achieved by transformation of different starch potato mother varieties with different constructs. The proximates (ash, carbohydrates, moisture, and protein) account for the overwhelming majority of the tuber mass and are very much comparable across the different waxy potato events.

Altered Composition As Trait: Impact on Other Components. The question being addressed is as follows: What happens to other components when the phenotype is an altered composition, in this case an altered starch component (inhibition of the synthesis of amylose in the tuber starch resulting in a waxy or amylopectin phenotype)?[20,21] The results shown in Table 1 compare the impact of altered starch composition on other sugars, vitamin C, and total glycoalkaloids. Sugars, vitamin C, and glycoalkaloids are metabolically related to the starch biosynthetic pathway, so it would be anticipated, or at least not unexpected, if differences in levels of these analytes were seen. In two of the three amylopectin potato events, there were increases in sugar and vitamin C levels, although only for Amadea were these significant ($p < 0.05$). For the third event, the trend was less clear and starch levels overall were lower. Each event was obtained by introduction into a different parental line, and this appears to be more influential than the introduction of the inserted DNA that results in the phenotype. So even when it could be anticipated that an indirect effect could occur as the result of an intentional metabolic alteration, this was not always the case and the extent was not always significant. These results reflect

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the plasticity of plant metabolism and the role that the parental germplasm plays.

DISCUSSION

The compositional analysis studies fall under the nutritional equivalence portion of the safety assessment. Over the years, the compositional analysis studies have evolved and expanded immensely. Nevertheless, no event has been rejected or deemed unsafe by regulatory authorities on the basis of its composition. The components that are analyzed are crop-specific, and the selection of components to be analyzed is based on OECD consensus documents,[22] which may not actually recommend the analysis of all the analytes mentioned. The major components include the proximates (moisture, fat, protein, ash); amino acids; carbohydrates (dependent on the crop: crude and dietary fiber, acid detergent fiber, neutral detergent fiber, sugars, starch); fatty acids; minerals; vitamins (crop specific); antinutrients (crop specific); and other crop-specific components such as isoflavones or phospholipids. Examples of antinutrients for soybean would be lectins, trypsin inhibitors, phytate, raffinose, and stachyose; for canola, glucosinolates; for potatoes, glycoalkaloids; for maize, phytate and trypsin inhibitor; for rice, phytate and trypsin inhibitor. At least 95% of the mass of the crop is covered by the components analyzed.[23] Of course, knowing what is "normal" is essential for the comparative analysis. If the natural variability is unknown, it is very hard to make a meaningful assessment of the impact of the transgene on the level of a specific analyte. The ILSI Crop Composition database[24] was compiled to fill this need and currently has data for corn, soybean, and cotton; it is anticipated shortly to include other crops. Prior to the existence of this database, registrants relied on literature sources such as Jugenheimer[25] and other even earlier references, together with the various controls included in the individual studies.

Factors that are considered in the design of compositional analysis studies include (1) number of lines to analyze, (2) appropriate comparator (null/negative segregant, parental variety, reference varieties and how many), (3) number of years/growing seasons, (4) number of locations, (5) number of replicates/location, (6) number of germplasm backgrounds (tester crosses), (7) plot design within location, and (8) statistical analysis to be applied. A summary of the manuscripts on compositional equivalence studies published through 2009 is given in Herman et al.[26] The selection of the appropriate comparator is dictated by the question that is being addressed by the study. If the question is what effect the presence of the transgene has on the composition of the crop, then the most appropriate comparator is the null or negative segregant that differs from the transgenic event by only the newly inserted gene. If the question is if the transgenic event has a composition similar to that of the parental variety, then, obviously, the parental variety is the appropriate comparator. If the question is if the transgenic event has a composition similar to what is normally consumed and the crop is a commodity, then the reference varieties are the best comparators. Can the reference variety be transgenic? Because 95% of the soy and 90% of the maize planted in the United States is transgenic[1] and there is over a decade of safe consumption of foods and feeds from these GM crops (that supports their precommercial safety assessment data), then perhaps the best comparator should be the current commercial varieties that include a GM trait and are transgenic. For products produced by breeding crosses of individual events that need evaluation as per European Union guidelines, the situation becomes even more complicated regarding selection of an appropriate comparator.[27]

Some crops, such as potato and sugar cane, are vegetatively propagated and therefore there would not be a null or negative segregant but only the parental variety that was used for transformation as comparator. For some crops, such as maize and oilseed rape, the parental variety is an inbred and is not normally consumed. Rice, on the other, is consumed as both the hybrid and inbred. Furthermore, some "model" lines that are readily transformed are not normally planted or consumed. For some commodity crops, the best comparator may be samples from the silos; but to cover locational differences, the data in the ILSI Crop Composition database might be extremely useful. An initial question that could also be valuable to answer is the sensitivity of compositional analysis in distinguishing differences and, if there are differences, their biological meaning.

Over the years since the first transgenic crops were approved and introduced, the list of components that have been requested to be analyzed has been steadily growing. There has been much discussion about applying "-omics" technologies to safety assessments. A recent publication by Ricroch[28] reviewed 60 "-omics" studies that compared metabolite levels in GM and non-GM varieties. The main conclusions were that the genetic modification had less impact on gene expression and nutrient/antinutrient composition than conventional breeding. In addition, environmental factors had a greater impact than the use of biotechnology tools. The results presented in this paper concur.

CONCLUSIONS

In conclusion, several points should be reiterated. A thorough selection process is applied during the development and identification of products that are advanced, and only a small fraction of trait genes and events proceed toward commercialization. The safety assessment of the commercial event is extensive and includes a compositional comparison. Compositional analysis is an important element of the multipronged approach for the safety assessment of transgenic crops. Biotech products are the most highly characterized food substances consumed. Germplasm plays the over-riding role in determining the composition of a crop.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) James, C. Global Status of Commercialized Biotech/GM Crops: 2012. ISAAA Brief No. 44. The International Service for the Acquisition of Agribiotech Applications (ISAAA), Ithaca, NY, 2012; http://www.isaaa.org/resources/publications/briefs/44/executivesummary/default.asp

(2) McDougall, P. The cost and time involved in the discovery, development and authorization of a new plant biotechnology derived trait. Crop Life International, 2011; www.croplife.org/PhillipsMcDougallStudy.

(3) Graff, G. D.; Hochman, G.; Zilberman, D. The political l economy of agricultural biotechnology policies. *AgBioForum* **2009**, *12*, 34−46.

(4) Ladics, G. S.; Cressman, R. F.; Herouet-Guicheney, C.; Herman, R. A.; Privalle, L.; Song, P.; Ward, J. M.; McClain, S. Bioinformatics and the allergy assessment of agricultural biotechnology products: industry practices and recommendations. *Regul. Toxicol. Pharm.* **2011**, *60*, 46−53.
(5) Delaney, B.; Astwood, J. D.; Cunny, H.; EichenConn, R.; Herouet-Guicheney, C.; MacIntosh, S.; Meyer, L. S.; Privalle, L.; Goa, Y.; Mattsson, J.; Levine, M. Evaluation of protein safety in the context of agricultural biotechnology. *Food Chem. Toxicol.* **2008**, *46*, S71−S97.
(6) Hammond, B. *Food Safety of Proteins in Agricultural Biotechnology*; CRC Press: Boca Raton, FL, 2008
(7) McHughen, A.; Smyth, S. US regulatory system for genetically modified [genetically modified organism (GMO), rDNA or transgenic] crop cultivars. *Plant Biotechnol. J.* **2008**, *6*, 2−12.
(8) Privalle, L. S.; Chen, J.; Claper, G.; Hunst, P.; Spiegelhalter, F.; Zhong, C. X. Development of an agricultural biotechnology crop product: testing from Discovery to commercialization. *J. Agric. Food Chem.* **2012**, *60*, 10179−10187.
(9) CAC/GL 44-2003 Codex Alimentarius − Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants.
(10) Bohner, H. What about yield drag on Roundup Ready Soybean? 2003; http://www.omafra.gov.on.ca/english/crops/field/news/croptalk/2003/ct_0303a9.htm
(11) Lai, F. M.; Privalle, L.; Mei, F.; Ghoshal, D.; Shen, Y.; Klucinec, J.; Daeschner, K.; Mankin, L. S.; Chen, N.; Cho, S.; Jones, T. Evaluation of the *E. coli* D-serine ammonia lyase gene (Ec. dsdA) for use as a selectable marker in maize transformation. *In Vtro Cell Dev. Biol. − Plant* **2011**, *47*, 467−479.
(12) European Food Safety Authority. http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-070.
(13) European Commission. http://ec.europa.eu/food/dyna/g_register/index_en.cfm.
(14) European Food Safety Authority. http://registerofquestions.efsa.europa.eu/roqFrontend/?wicket:interface=:4:1.
(15) De Vetten, N.; Wolters, A. M.; Raemakers, K.; van der Meer, I.; ter Stege, R.; Heeres, E.; Heeres, P.; Visser, R. A transformation method for obtaining marker-free plants of a cross-pollinating and vegetatively propagated crop. *Nat. Biotechnol.* **2003**, *21*, 439−442.
(16) AOAC International. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th ed.; Horwitz, W., Ed.; Gaithersburg, MD, 2000.
(17) U.S. Department of Agriculture. *Composition of Foods*; Agriculture Handbook 74; Washington, DC, 1973.
(18) Hellenas, K. E.; Branzell, C. Liquid chromatographic determination of the glycoalkaloids α-solanine and α-chaconine in potato tubers: NMKL Interlaboratory Study, Nordic Committee on Food Analysis. *J. AOAC Int.* **1997**, *80*, 549−554.
(19) Reed, J.; Privalle, L.; Powell, M. L.; Meghji, M.; Dawson, J.; Dunder, E.; Suttie, J.; Wenck, A.; Launis, K.; Kramer, C.; Chang, Y. F.; Hansen, G.; Wright, M. Phosphomannose isomerase: an efficient selectable marker for plant transformation. *In Vitro Cell. Dev. Biol. − Plant* **2001**, *37*, 127−132.
(20) Kuipers, A. G.; Jacobsen, E.; Visser, R. G. F. Formation and deposition of amylose in the potato tuber starch granule are affected by the reduction of granule-bound starch synthase gene expression. *Plant Cell* **1994**, *6*, 43−52.
(21) Börnke, F.; Sonnewald, S. Biosynthseis and metabolism of starch and sugars. In *Plant Metabolism and Biotechnology*; Ashihara, J., Crozier, A., Komamine, A., Eds.; Wiley: New York, 2011.
(22) Organization for Economic Cooperation and Development. Consensus Documents for the work on the Safety of Novel Foods and Feeds: Plants; http://www.oecd.org/science/biosafety-biotrack/consensusdocumentsfortheworkonthesafetyofnovelfoodsandfeedsplants.htm.
(23) Harringan, G. G.; Chassy, B. Challenges for metabolomics as a tool in safety assessments. In *Metabolomics*; Roessner, U., Ed.; Intech Publications: Rijeka, Croatia, 2012; pp 331−348.
(24) Ridley, W. P.; Shillito, R. D.; Coats, I.; Steiner, H.-Y.; Shwago, M.; Phillips, A.; Dussold, P.; Kurtyka, L. Development of the International Life Sciences Institute Crop Composition Database. *J. Food Compos. Anal.* **2004**, *17*, 423−438.
(25) Jugenheimer, R. W. Corn: improvements. *Seed Production and Uses*; Wiley: New York, 1976.
(26) Herman, R. A.; Chassy, B. M.; Parrott, W. Compositional assessment of transgenic crops: an idea whose time has passed. *Trends Biotechnol.* **2009**, *27*, 555−557.
(27) Ridley, W. P.; Harrigan, G. G.; Breeze, M. L.; Nemeth, M. A.; Sidhu, R. S.; Glenn, K. C. Evaluation of compositional equivalence for multitrait biotechnology crops. *J. Agric. Food Chem.* **2011**, *59*, 5865−5876.
(28) Ricroch, A. E. Assessment of GE food safety using "-omics' techniques and long-term animal feeding studies. *New Biotechnol.* **2013**, DOI: 10.1016/j.nbt.2012.12.001.