Evaluation of Genetically Engineered Crops Using Transcriptomic, Proteomic, and Metabolomic Profiling Techniques[W]

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A major principle and guiding tool for the food safety assessment of genetically engineered (GE) crops is the concept of "substantial equivalence" according to principles outlined in the Organization for Economic Cooperation and Development (OECD) consensus documents (OECD, 2006) and further elaborated by the Food and Agriculture Organization of the United Nations/World Health Organization. In this safety assessment, GE crop-derived foods and feeds are compared with their counterparts from parental or near isogenic lines in order to identify differences, which are subsequently evaluated with respect to safety for humans and animals as well as nutritional quality. The question addressed is: may the improvement of a plant variety through the acquisition of a new desired GE trait lead to unintended effects (i.e. going beyond that of the original genetic modification) and, if so, does this have an impact on health? Possible mediators of such pleiotropic effects could be altered expression of untargeted genes or metabolic effects of a novel gene product. Current tools to assess the food safety of GE crops include extensive multisite and multiyear agronomic evaluations, compositional analyses, animal nutrition, and classical toxicology evaluations. In the 2000s, new methodologies were developed to allow, in theory, a holistic search for alterations in GE crops at different biological levels (transcripts, proteins, metabolites). These methodologies include cDNA microarrays, microRNA fingerprinting, proteome, metabolome, and toxicological profiling. The term "omics" in relation to food and feed safety appeared for the first time in 2005 (Li et al., 2005). This review highlights the knowledge generated by recently published profiling studies regarding the effect of genetic modification itself, compared with environmental and intervariety variation, for major crops (44 studies) and for Arabidopsis (*Arabidopsis thaliana*) as a reference plant.

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THE LESSONS TO BE LEARNED FROM ARABIDOPSIS

Arabidopsis is a well-established model plant that offers comprehensive resources such as the entire genome sequence, a large collection of natural variants, a number of molecular tools, and several information platforms and databases. In addition, as illustrated below, Arabidopsis provides valuable information about the potential impact of transgenesis.

The first question to be addressed is whether the insertion of genes that are not believed to alter biological processes in plants will lead to transcriptome changes. To answer this question, El Ouakfaoui and Miki (2005) used selectable marker (*nptII*) and reporter (*uidA*) genes. Under controlled growth conditions, they found no reproducible changes for the approximately 24,000 genes screened when comparing transgenic lines with their wild-type progenitor. Their conclusion was that the stable insertion of T-DNA did not cause detectable pleiotropic effects to the transcriptome. This finding was not obvious since, due to the gene density on the Arabidopsis genome, insertion could have been anticipated to cause major disturbances altering gene expression. Strikingly, under abiotic stresses (salt, drought, cold, and heat), the authors found approximately 8,000 genes (35% of the genome) with changed expression in both wild-type and transgenic plants.

In contrast, Ren et al. (2009a) attributed some unintended effects to the presence of a selectable marker gene (*bar*, encoding phosphinotricin acetyl transferase). Metabolic fingerprinting revealed that the major contributors distinguishing the wild type and four transgenic lines were modified levels of Ala and Thr. The authors attributed this trend to the *bar* gene, since it was common to all lines. However, protein analysis by two-dimensional electrophoresis (2DE) on 12 *bar*-containing lines showed no consistent differences (four to 14 protein spots did change in intensity depending on the line, but most of them were different;

Ren et al., 2009b). In that study, cold treatment triggered changes in only 10 protein spots. In another study, Abdeen and Miki (2009) found only four genes differentially expressed in transgenic lines expressing *bar*.

A second question to be examined is whether expression of a protein affecting regulatory processes (e.g. a transcription factor affecting drought tolerance; Abdeen et al., 2010) will necessarily have pleiotropic effects. These authors found no effect on the transcriptome in such plants without drought. As can be expected, in response to drought, changes in the level or timing of expression of some drought-responsive genes occurred between transgenic and wild-type plants.

A third question to address is whether deliberate modification of a metabolic pathway using transgenesis will have pleiotropic effects. Kristensen et al. (2005) inserted one to three genes from a pathway converting Tyr to a cyanogenic glucoside (dhurrin). They found only marginal inadvertent effects on the transcriptome and metabolome when the whole pathway or only the first enzyme was inserted. However, the combination of the first two genes leads to the predicted synthesis of a toxic cyanohydrin intermediate. In this case, plants responded by metabolism and detoxification reactions, as was evident from an altered metabolite profile showing the accumulation of detoxification products and changes in the transcriptome.

Metzdorff et al. (2006) developed and characterized six independent lines transformed with an antisense chalcone synthase gene to decrease flavonoid biosynthesis. The lines differed in the type of integration (site and copy numbers, level of gene silencing). Unintended effects on gene expression included few genes (up to 15 in flower and up to 13 in leaf out of the 1,500 analyzed), and the affected genes were involved in stress response and photosynthesis. Lines differed with respect to the affected genes, and analyses of one such gene by PCR did not show a consistent trend with the microarray data, which the authors explain by a large biological variation in expression for this gene. One conclusion of Metzdorff et al. (2006) is that "it is crucial to have substantial information on the natural variation of crop plants in order to be able to interpret 'omic' data correctly."

Interestingly, Arabidopsis also provides some insight concerning the above-mentioned issue. Ruebelt et al. (2006) qualitatively and quantitatively analyzed its seed proteome and showed that existing natural variability can be important. When various ecotypes were grown side by side in a growth chamber under controlled conditions, the authors found that nearly half of the 2DE-resolved spots were present or absent depending on the ecotype and that 95% of the spots present in all ecotypes varied quantitatively. Twelve transgenic lines were also compared with their parental line as well as with 12 ecotype lines: the genetic modification of Arabidopsis using three different genes and three different promoters did not cause unintended changes to the analyzed seed proteome.

In conclusion, these data on a model plant for research point to a greater influence of genetic background and stress (from the environment or new metabolites) than of transgene insertion itself. To determine whether these conclusions are also valid for crop plants, the following two sections examine the conclusions of profiling strategies in a systematic species-by-species approach.

CROP PLANTS: COMPARISON OF GE VARIETIES WITH IMPROVED AGRONOMIC TRAITS (WITHOUT INTENTIONAL METABOLIC CHANGES) WITH NON-GE VARIETIES

The main data from the publications discussed below are listed in Supplemental Table S1, which also includes data from earlier publications or on other species (cabbage [*Brassica capitata*] and potato [*Solanum tuberosum*]) and on GE plants producing bioproducts (such as antibodies), which are not discussed below. The search strategy used to find these references is presented in Supplemental Table S1.

Barley

Using field-grown barley (*Hordeum vulgare*) lines expressing either a chitinase or a β-glucanase, Kogel et al. (2010) compared changes in the leaf transcriptome and metabolome caused by transgenes, cultivar, or biotic interactions in the root. Transgene effects were negligible in the first case and low in the second, while the difference caused by the genetic background of cultivars (even if down to a low number of alleles) was of a greater magnitude. Effects of exposing roots to the spores of mycorrhizal fungi could be visualized by metabolome but not transcriptome analysis. Based on this result, the authors conclude that the metabolome represents a more immediate probe of the physiological status of the plant.

Maize

When performing transcriptomic studies using in vitro- or field-grown maize (*Zea mays*) plants, Coll et al. (2008, 2009) found differential expression for a minority of transcripts between in vitro-grown MON810 (insect-resistant of *Bt* type) and control lines, and most of these differences were not observed in the field. In real agricultural conditions, under two farming practices (conventional and low-nitrogen fertilization), Coll et al. (2010a) found differential expression for only 0.14% of the analyzed sequences (approximately one-third of the maize genome). Analysis of the expression of a subset of sequences in a different MON810/non-GE pair indicated that varietal differences had the highest impact on gene expression patterns, followed by nitrogen availability, while the MON810 characteristic had the lowest impact.

Coll et al. (2010b) found the grain proteome of two field-grown MON810/non-GE variety pairs to be vir-

tually identical, with very few spots showing variations in the 1- to 1.8-fold range, which were all variety specific. Previously, Albo et al. (2007) had also found limited changes in the grain proteome of two different MON810 varieties (also field grown). Zolla et al. (2008) also used two MON810 variety pairs but found more differences, although environment (field versus growth chamber) induced more changes. To explain the differences resulting from genetic modifications, these authors speculated about genome rearrangement induced by the transformation method but did not consider the possibility that the control lines were certainly not fully isogenic. The discrepancy between these results remains unexplained, especially since one of the two pairs used by Coll et al. (2010b) was the MON810/non-GE pair used by Zolla et al. (2008).

In a first grain metabolome analysis, carried out on a greenhouse-grown MON810 line, Manetti et al. (2006) found differences in the levels of compounds from primary nitrogen metabolism in transgenic grain samples. Using a different MON810 line, grown in a growth chamber, Piccioni et al. (2009) identified 40 water-soluble metabolites and found a higher concentration for five compounds in the GE extracts (all different from those of Manetti et al. [2006]). Leon et al. (2009) found increases in some metabolites from specific metabolisms (purine, amino acid, arachidonic acid, linoleic acid) in three field-grown MON810 lines compared with their controls. There were only 10 metabolites with increased levels when two different technologies were compared. One of them, carnitine, had been proposed in a previous study by the same team (Levandi et al., 2008) to be a biomarker for *Bt* maize (note, however, that both studies analyzed the same samples, which provides no additional perspective). It should be pointed out that these various teams did not find similar results, which may be explained by their use of different genetic backgrounds and/or different growth conditions and also different technologies.

In this context, the work of Barros et al. (2010) is important. Using transcriptome, proteome, and metabolome profiling to compare two GE maize lines (MON810 and glyphosate tolerant) with the respective control lines, they found that the environment (plants were grown over three seasons in one location) affected gene expression, protein distribution, and metabolite content more strongly than the genetic modification. In addition, the authors found distinct profiles for the three locations that were also part of their comparisons during one season.

Natural plant-to-plant variability also exists. Using MON810 and control lines, Batista and Oliveira (2010) compared 2DE-separated protein spots from samples obtained either from individual plants (five different ears of five different maize plants) or from pooled plants. For some spots, they noticed a high variability between individual samples from the same line and that these differences were masked in the pools. For other spots, variability was observed between individual samples and also between pools. The authors concluded that differences not related to the genetic engineering, such as natural plant-to-plant variability, need to be eliminated when using omics.

Harrigan et al. (2010) reviewed compositional data for GE maize and soybean (*Glycine max*) varieties (seven GE crop varieties) from a total of nine countries and 11 growing seasons. From their analysis, which is not based on omic technologies but represents the most comprehensive compilation of GE crop composition data to date, the authors conclude that compositional differences between GE varieties and their conventional comparators are "encompassed within the natural variability of the conventional crop and that the composition of GM and conventional crops cannot be disaggregated."

Pea

Analyzing two pea (*Pisum sativum*) cultivars producing a bean (*Phaseolus vulgaris*) α-amylase inhibitor (AI1), Islam et al. (2009) found around 30 seed protein spots showing changes in abundance in each transgenic/control pair (generally not the same spots, although AI1 was produced at similar levels in both cultivars). While differences were minor for one pair, they were more pronounced quantitatively and qualitatively (appearance and disappearances of 36 protein spots) for the second pair. The authors suggest that differences of "similar magnitude" occur between cultivars. In a different cultivar, Chen et al. (2009) reported that 33 proteins differentially accumulated in AI1-expressing lines compared with the parental line, three of which were associated with the expression of AI1. The remaining 30 proteins were associated with the transformation events. A number of the increased spots corresponded to seed storage proteins. Since such proteins are common food allergens, the authors suggested that these increases might be linked to food antigens detected in mice fed with GE peas (attempts to use 2DE of proteins and proteomics to detect new allergens are listed in Supplemental Table S2).

Rice

Montero et al. (2010) found around 0.40% transcriptomic differences in leaves of in vitro-grown experimental rice (*Oryza sativa*) lines producing an antifungal protein. They could distinguish differences due to transgene insertion (15%), transgene expression (50%), and regeneration (35%). Around half of the genes whose expression was affected by the transgene itself also had their expression affected in non-GE plants after wounding.

Zhou et al. (2009) compared profiles of compounds from primary metabolism in three GE lines (each independently transformed with the same two insect resistance genes; their data were averaged) with those of the wild-type line (field grown side by side). They found three metabolites to be present in greater

amounts in the GE group (up to 3-fold). Differences in other metabolites were within the same range as those of the wild type under various growth conditions (location and/or sowing time). It should be mentioned that wild-type lines planted at different times contained varying amounts of trehalose (up to 40-fold) and change in location influenced the levels of four compounds.

The work by Jiao et al. (2010) provides some perspective on transgenic changes in the context of varietal changes in rice. Comparing two lines with different sets of antifungal genes and one with two insect resistance genes with their respective controls, the authors found decreases or increases, inconsistent between lines, ranging from 20% to 74% for amino acids, 19% to 38% for fatty acids, 25% to 57% for vitamins, and 20% to 50% for elements. These changes were all within the range occurring among varieties (according to OECD values). A 25% reduction in protein content was observed for one antifungal GE line, which was therefore considered by the authors to be less nutritious.

Batista et al. (2008) addressed the following question: which of the mutagenized or transgenic plants are more susceptible to present unintended modification? Gene expression was analyzed in duplicated samples of four types of rice plants (irradiated stable mutants and transgenic plants producing an antibody or developed for improved stress tolerance) and their respective controls. In all cases studied, the modification in transcriptome was greater in mutagenized than in transgenic plants. Since these results were obtained with seedlings grown on tissue culture medium, wider confirmation is necessary.

Soybean

Cheng et al. (2008) found that gene expression in leaves (grown in a growth chamber) differs more between conventional varieties than between two GE glyphosate-tolerant varieties (carrying the same transgenic event) and their closest conventional varieties. The authors also note that the older the soybean variety, the larger the difference in gene expression (recently developed cultivars are more inbred), which raises the question of which varieties should be chosen to create a reference set for the crop species. Also using a glyphosate-tolerant variety (not specified) grown in a growth chamber, but analyzing seeds, García-Villalba et al. (2008) identified and quantified the main metabolites: in general, the same metabolites, in similar amounts, were found in GE glyphosate-resistant soybean and in its corresponding parental line. However, significant differences were observed in some specific cases: among the 45 metabolites examined, higher amounts were found for three and lower amounts for five (one was not detected) in the GE line. At least some of these differences could be explained by modification in the regulation of the shikimate pathway in GE soybean (glyphosate tolerance is conferred by a transgenic 5-enolpyruvylshikimate-3-phosphate synthase enzyme that bypasses the endogenous glyphosate-sensitive enzyme).

The study on natural variation in soybean crop composition and the impact of transgenesis by Harrigan et al. (2010) has been mentioned above.

Using 2DE protein analysis, soybean endogenous allergen expression was found not to be altered after genetic modification (see related refs. in Supplemental Table S2).

Wheat

Gregersen et al. (2005) found that the strong expression of a phytase gene had no significant effect on the overall gene expression patterns in the developing wheat (*Triticum aestivum*) seed. Samples from greenhouse-grown plants were taken at three different seed development times. The slight differences observed concerned primarily genes strongly expressed over a shorter period of seed development. This highlights the necessity of careful interpretation of microarray results when extensive progressive developmental changes occur, as is the case for seeds, and when minor asynchrony is hard to avoid. Ioset et al. (2007) analyzed lines with either a combination of three transgenes or a single one (KP4, of viral origin) for increased defense against fungal pathogens. For greenhouse-grown plants, they found only minor differences in the flavonoid profile between GE lines and their conventional control lines. In contrast, the different genetic background of the control lines resulted in a quantitatively different (up to 2-fold for some compounds) flavonoid content. In a field test, KP4 did not influence flavonoid content either, whether the lines were infected by pathogens or not.

Conclusion

These profiling studies are highly heterogeneous (plant tissues, growth parameters, range of comparators, technologies). They have to be considered as exploratory (i.e. not normalized validated approaches for the routine assessment of GE plants).

This survey on the profiling of GE crop lines with agronomic traits, but without deliberate modifications to metabolic pathways, reveals that some differences exist when compared with control lines. However, the available data on various conventional lines consistently show more differences. This has to be linked to the fact that GE lines have been selected by a process based not only on the suitable expression of a new trait but also on phenotypic and compositional equivalence with a close comparator, followed by a number of crosses to introgress the new trait into elite lines. A number of environmental factors (field location, sampling time during the season or at different seasons, mineral nutrition) have also been shown, consistently, to exert a greater influence than transgenesis.

TOWARD ADAPTING THE SUBSTANTIAL EQUIVALENCE CONCEPT TO GE PLANTS WITH ALTERED METABOLIC TRAITS

The substantial equivalence concept encompasses a comparison of biochemical composition with a non-GE line considered to be safe. However, many GE crop lines have been developed to obtain improved feed or food composition. Before examining whether this concept can be used to address the need to assess the safety of these new crops, the following section examines systematically the conclusions of available omic studies. Further details are given in Supplemental Table S3. Some publications not intended to study the unintended effects of transgenesis per se, but nevertheless providing relevant information, are discussed below or listed in Supplemental Table S3.

Maize

Huang et al. (2005) generated maize lines with an elevated content of free and total Lys in the kernels due to the combined deregulation of its synthesis and reduced levels of a Lys-poor storage protein. Kernels from field-grown plants showed, in addition, strong increases in the content of two Lys metabolites and up to 2-fold higher content of other free amino acids but with only marginal changes for total amino acids.

Potato

Lehesranta et al. (2005) demonstrated major qualitative and quantitative differences in the tuber proteome of field-grown varieties and landraces but found only limited quantitative differences between GE lines (affected either in cell wall structure or ethylene/polyamine metabolism) and their controls. Using the same lines, plus related ones as well as lines expressing a sense and antisense fructokinase gene (all grown in pots), similar conclusions were reached using metabolic profiling (Defernez et al., 2004) or targeted compositional analysis (Shepherd et al., 2006). The most obvious differences were found between the two non-GE varieties. Differences were also found between tissue culture-derived tubers and tubers derived from transformation with the empty vector. This raises the possibility that somaclonal variation (known to occur significantly in potato, depending on genotype) may be responsible for an unknown proportion of differences.

Similarly, using field-grown tubers engineered to produce inulin-type fructans, Catchpole et al. (2005) found their metabolite composition to be similar to the progenitor line and variations to be within the range found in classical cultivars, apart from the predictable increase in fructans and derivatives. Baroja-Fernández et al. (2009) found numerous transcriptomic changes in tubers with altered levels of Suc synthase, but their data were not compared with varietal changes.

An additional perspective (i.e. influence of sampling time) is provided by Kim et al. (2009), who found that 1 week of storage significantly modified tuber metabolite patterns, but the constitutive expression of β-amyloid, curdlan synthase, or glycogen synthase triggered neither quantitative nor qualitative differences.

Rice

In seeds of two high-Trp rice lines (field grown), Wakasa et al. (2006) found an increase in the content of other free amino acids (but to a lesser extent than that of Trp) and of indole acetic acid, which was predictable given the relation between the Trp biosynthetic pathway and the production of this growth regulator. However, they found no major change for other phenolic compounds. The same laboratory (Dubouzet et al., 2007) also found limited metabolic and transcriptomic differences in 8-d-old seedlings of lines with high Trp. Beatty et al. (2009) reported limited transcriptional changes in roots and shoots of "nitrogen use-efficient" rice obtained by overexpression of Ala aminotransferase.

Tomato

Le Gall et al. (2003) analyzed metabolic profiles during tomato (*Solanum lycopersicum*) fruit ripening and the potential unintended effects when two transcription factors were simultaneously overexpressed to increase flavonol content. The levels of at least 15 other metabolites were found to be different between the red GE and non-GE tomato types, but according to the authors (who did not specify the growth conditions), these changes are within the natural variation normally observed in a field-grown crop.

Long et al. (2006) found no perturbation in phenolic metabolites in mutant and transgenic lines altered in structural genes for carotenoid biosynthesis, and reciprocally, the down-regulation of ferulate 5-hydroxylase did not affect carotenoid content in red fruit from greenhouse-grown plants.

In a more comprehensive study, but also limited to greenhouse conditions, Fraser et al. (2007) characterized the fruit metabolic changes associated with the overproduction of carotenoids. Specific sectors of metabolism were altered in green fruit, resembling some metabolic changes normally associated with ripening. Ripe fruit showed the least change in overall metabolites, although levels of 43% of the metabolites were altered. Thus, perturbation in carotenoid synthesis has profound regulatory implications for tomato fruit development, but these effects arise without altering the general phenotype of the plant and fruit ripening.

In addition, as expected, several metabolisms can be altered, either in conventional mutants or in transgenic lines, when regulatory genes are affected, such as those involved in light perception (Long et al., 2006; see other refs. in Supplemental Table S3) or growth regulator biosynthesis (Mattoo and Handa, 2008).

Wheat

Baudo et al. (2009) report the transcriptomic comparison of GE and conventionally bred lines (grown in a greenhouse) expressing a given set of seed storage proteins (glutelins) known to determine bread-making quality. Differences in endosperm and leaf transcriptome between GE and parent lines were rare (up to six genes). More differences (up to 527 genes in endosperm) were observed between this parent line and another conventionally bred line. The latter, although of different overall background, contains the same set of glutelins as the GE line and unexpectedly showed fewer differences (up to 154 genes) with the GE line than with the parent of the GE line. Baker et al. (2006) performed metabolomic comparisons also using lines differing in their set of glutelins. They found some differences in polar metabolites between GE and parental lines, but generally, they were in the range of differences caused by the environment (plants grown in fields on different sites and in different years). Larger differences were often observed between two parental lines, between years, and between different sites than between the GE and control lines. Additional articles analyzing wheat or barley lines with a modified set of seed storage proteins are listed in Supplemental Table S3.

Conclusion

Few of these studies brought their results in perspective with the potential effects of the environment. Nevertheless, the available data are noteworthy since they indicate that GE lines with altered metabolic traits do not necessarily exhibit pleiotropic changes. This is encouraging for the future use of transgenesis to improve food and feed quality. However, some pleiotropic effects do occur when certain pathways are modified.

A key consideration for crops with altered composition, in a substantial equivalence perspective, is the choice of a comparator for GE lines. The published omic studies did not yet examine the question of what the appropriate comparator should be (the progenitor or a crop that most closely resembles the new variety with respect to the intentionally altered metabolic trait). On the other hand, it can be stressed that, up to now, choosing a comparator has not posed a major problem. GE crops (as well as conventional varieties) with altered composition have already been assessed and approved by regulators (e.g. crops with high oleic acid content).

DISCUSSION

Divergent Views on Omics

Some authors (for a selection of refs., see Supplemental Table S4) consider that nontargeted profiling provides coverage of gene, protein, and metabolite analysis that cannot be matched by traditional targeted approaches. A so-called "unbiased" analysis of the metabolome, for example, certainly offers new possibilities for plant physiologists and holds promise for a better understanding of the variation in metabolites relevant to human health and nutrition. However, as Lay et al. (2006) pointed out, "bias" does occur with omics (i.e. systematic errors) as well as other problems with "statistics (e.g., number of replicates), methodology and method misuse."

As this review shows, there is an obvious lack of homogeneity in experimental design and methodology, sometimes even within the same laboratory. Most published omic studies lack a biological validation of observed differences between GE crops and their comparators. Some include no biological replicates. Variable patterns in transcriptome, proteome, or metabolome are reported depending on growth conditions, geography, season, or variety. Considering all sources of difficulties in data interpretation, it seems premature to infer precise conclusions from variations assigned to a GE variety, such as the definition of a given compound as a "biomarker" for a given type of GE crop (Levandi et al., 2008). However, as discussed below, the available data valuably point to general trends concerning transgenesis.

Metabolomics Versus Traditional Analytical Chemistry

Current risk assessment of GE crops includes the analysis of 50 to 150 analytes (depending on the crop species) identified by OECD consensus documents (OECD, 2006) as the key compounds for that crop, using validated analytical methods. Following these guidelines, current approaches allow the measurement of 80% of biomass in soybean seed and 95% of nonstarch biomass in maize grain. Metabolomics would measure a few hundred analytes (i.e. the same compounds, plus additional low-abundant metabolite pools, usually extremely variable, some of which are unidentified). Despite the recent publication of numerous omics studies in relation to GE crop assessment, it does not yet seem feasible to propose large-scale methods that can be internationally certified and accepted. Using metabolomics would be a change of paradigm (measuring more analytes but with less precision.) for GE crop assessment but would provide little or no added value for food safety (Chassy, 2010), since it does not yet surpass the currently used analytical methods (Harrigan et al., 2010). In addition, when studies have used different metabolomic technologies simultaneously, discrepancies in the results were obvious (Leon et al., 2009).

Which Omic Approach and When?

As can be seen in Table I, metabolomics is the prevalent approach. Some authors consider that transcriptomics can routinely establish substantial equivalence (Baudo et al., 2009). Others suggest combining

methods (Supplemental Table S4). However, few studies have used different omics side by side; therefore, a comparative assessment of these techniques is still required.

At present, published profiling studies of GE crops represent merely a compilation of data, and mandatory use of these techniques in GE food safety assessment would be pointless. Basic research should be carried out to improve methods and evaluate the reliability of the results. A weight-of-evidence approach for a better determination of the consistency of the observed differences, and determination of their nontransient nature and of their biological relevance, are all recommended. Modeling is needed to analyze observed differences in various pathways. Subsequently, a tiered approach to the potential use of omics could be proposed, which would follow a decision tree incorporating parameters from traditional safety assessments and establish, on a case-by-case basis, whether omics use is helpful or not.

Food safety-oriented cDNA microarrays could be constructed. van Dijk et al. (2009) used this approach to analyze the tuber transcriptome of two different non-GE potato varieties to detect variation due to genetic differences or environmental conditions. The extent of natural variation of gene expression was examined to help future biological and/or toxicological assessments.

Regarding allergenicity predictions, 2DE combined with immunoblotting are used to identify the allergenic spots that bind IgE antibodies. Proteomic and mass spectrometry methods are also able to provide qualitative and quantitative information on the levels of allergens, including new ones (Supplemental Table S2).

Transgenesis in the Context of Existing Variations

Before commercialization, GE crop lines have to be checked for phenotypic and compositional equivalence (for key nutrient, antinutrient, and toxicant contents) to existing varieties (apart from the new trait). Therefore, it seems unlikely from a plant physiology point of view that a new transgenic line that has equivalent key metabolite content, as well as similar growth, flowering, fruit development, seed production, etc., parameters, would exhibit extensively altered gene expression, protein, or metabolite profiles.

Nevertheless, not unexpected from a systems biology point of view, some differences attributed to transgenesis were reported in the published omics studies. However, when a larger set of references was included in the study (i.e. beyond the pairwise comparison of a GE line and its near isogenic line), the most pronounced differences were consistently found between the various conventional varieties, a trend linked to the crop diversity maintained or created by plant breeders. This should be put in perspective, taking into account that conventional breeding is generally regarded as safe, despite the fact that the nature of the changes in new conventional cultivars is usually unknown (Parrott et al., 2010).

Large effects due to the environment were also observed on gene expression, protein, and metabolite levels in some studies (Baker et al., 2006; Zolla et al., 2008; Zhou et al., 2009; Barros et al., 2010). The present knowledge created by profiling approaches illustrates the need to place pairwise differences between GE lines and their direct progenitor in a wider context.

What Conclusions Can Be Drawn Regarding the Substantial Equivalence Concept?

It is important to keep in mind that the standard proposed by the OECD/Food and Agriculture Organization of the United Nations/World Health Organization was substantial equivalence rather than total equivalence and that there is no specific statistical or biological basis to define "substantial" (Hoekenga, 2008). In other words, no "limits of concern" have

Table I. *Number of publications comparing GE and non-GE crop varieties without or with intentional metabolic changes according to omic profiling*

The total number of published studies and the number with transcriptomic (T), proteomic (P), or metabolomic (M) data are given. Some publications reported various profiling approaches.

Plant Species	GE with No Intentional Metabolic Changes				GE with IntentionalMetabolic Changes			
	Total	T	P	M	Total	T	P	M
Barley	1	1	0	1	—			
Cabbage	1	0	0	1	—			
Grapevine (*Vitis vinifera*)	—				2	1	1	0
Maize	11	4	4	5	1	0	0	1
Pea	2	0	2	0	—			
Potato	1	0	1	0	5	1	1	3
Rice	4	2	0	2	5	2	2	2
Soybean	2	1	0	1	—			
Tomato	—				6	2	0	6
Wheat	3	1	1	1	4	2	0	2
Total	25	9	8	12	19	8	4	14

been defined regarding differences. In addition, plant composition is usually variable even within a single variety. Pairwise differences between a GE line and its comparator are usually less than natural variability. Furthermore, near isogenic lines differ by a number of alleles, which could explain a number of differences attributed to transgenesis. Thus, the substantial equivalence concept cannot provide more than a guiding framework for evaluation.

Nevertheless, the experience acquired after 15 years of GE crop commercialization has comforted the validity of this framework. However, considering the highly polarized views on GE crops, it is important to notice that the opinions expressed previously by food safety agencies (i.e. general "equivalence" of authorized GE crops with non-GE comparators) have now been independently corroborated at the transcriptomic, proteomic, and metabolomic levels by recently published omic comparisons (Table I). None of the published omic assessments has raised new safety concerns about marketed GE cultivars.

Which Changes in Regulation for New Crops?

Based on their extensive comparison of compositional data of maize and soybean varieties, Harrigan et al. (2010) proposed that "if regulatory scrutiny is to be commensurate with the potential for compositional deviation, there is no reason to prioritize crops on the basis of genetic modification via transgenesis over crops genetically modified via conventional breeding, chemical mutagenesis or irradiation." Batista et al. (2008) showed, in the case studied, that the observed transcriptome alteration was greater in mutagenized than in transgenic plants. It should be mentioned that as far back as 1987, a report by the National Academy of Science (entitled Introduction of Recombinant DNA-Engineered Organisms into the Environment) had already stated that "there is no evidence that unique hazards exist in the use of recombinant DNA techniques or in the transfer of genes between unrelated organisms" and "that the risk[s]...are the same in kind as those associated with...other genetic techniques."

Today, the fast-accumulating data from targeted approaches as well as nontargeted profiling, consistently indicating that transgenesis has less impact than conventional breeding, should lead at least to a convergence of regulations for various crop breeding methods. Obviously, on a scientific basis, this should mean lowering the current regulatory burden for GE crops (Chassy, 2010). Considering that health problems have not been identified for GE crops after 15 years of commercialization, the time may have come to simplify the risk assessment of modern biotechnology products and therefore reduce cost. This would make risk assessment more affordable for small companies, academic institutions, or low-income countries.

However, considering that regulations ruling GE crop marketing have been strengthened continuously due to political pressure, especially in the European Union (Morris and Spillane, 2010), it is more likely that the non-GE authorization, and first of mutagenized crops, will be brought into line with the GE regulation. In addition, although there is no evidence that more food safety testing is necessary for GE crops, one can predict that a "whatever is possible should be done" policy will push for the use of omics technologies in their mandatory assessment.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. GE varieties with improved agronomic traits versus non-GE varieties.

Supplemental Table S2. References on the use of "omics" to identify food allergens.

Supplemental Table S3. GE varieties with altered metabolic traits versus non-GE varieties.

Supplemental Table S4. References discussing the use of "omics" in food safety assessment.

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