

Review Article

Nutritional and safety assessment of foods and feeds nutritionally improved through biotechnology - case studies by the International Food Biotechnology Committee of ILSI

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During the last two decades, the public and private sectors have made substantial research progress internationally toward improving the nutritional value of a wide range of food and feed crops. Nevertheless, significant numbers of people still suffer from the effects of undernutrition. As newly developed crops with nutritionally improved traits come closer to being available to producers and consumers, scientifically sound and efficient processes are needed to assess the safety and nutritional quality of these crops. In 2004, a Task Force of international scientific experts, convened by the International Food Biotechnology Committee (IFBiC) of ILSI, published recommendations for the safety and nutritional assessment of foods and feeds nutritionally improved through modern biotechnology (J. Food Science, 2004, 69:CRH62-CRH68). The comparative safety assessment process is a basic principle in this publication and is the starting point, not the conclusion, of the analysis. Significant differences in composition are expected to be observed in the case of nutritionally enhanced crops and must be assessed on a case-by-case basis. The Golden Rice 2 case study will be presented as an example of a food crop nutritionally enhanced through the application of modern biotechnology (i.e., recombinant DNA techniques) to illustrate how the 2004 recommendations provide a robust paradigm for the safety assessment of "real world" examples of improved nutrition crops.

Key Words: Golden Rice 2, vitamin A**INTRODUCTION**

The United Nations charter declared that freedom from hunger is a fundamental human right, and diets deficient in essential nutrients are a pervasive form of hunger. Substantial international research progress has been made over the last two decades by public and private sector plant scientists towards improving the nutritional value of a wide range of food and feed crops. Nevertheless, more than 400 million people in the world still suffer from the effects of undernutrition. More than half of the undernourished are children, with at least 5 million dying each year.

Improved nutrition crops are being developed by both conventional breeding, which builds on the natural variation in nutrients in crop germplasm, as well as through the tools of modern biotechnology. As newly developed improved nutrition crops come closer to being available to the consumer, deployment will be facilitated if scientifically sound and efficient processes are used to assess their safety and nutritional quality. A Task Force of scientific experts was convened by the International Food Biotechnology Committee (IFBiC) of the International Life Sciences Institute (ILSI) to address the topic of the safety and nutritional assessments of foods and feeds that are nutritionally improved through modern biotechnology. The work of this Task Force culminated in 2004 with a publication that included a series of recommendations for the nutritional

and safety assessments of such foods and feeds.¹ This document has gained global recognition from organizations such as the European Food Safety Agency.² The same Task Force has now applied the key recommendations from this publication to five cases studies of nutritionally enhanced crops currently in development, one of which is Golden Rice 2. The present paper assesses Golden Rice 2 relative to the set of recommendations that are consistent across the safety and nutritional assessment of the five case studies.

BACKGROUND ON THE GOLDEN RICE 2 CASE STUDY

Golden Rice 1 was developed to help control vitamin A deficiency (VAD).³ To construct Golden Rice 2 (GR2), the *phytoene synthetase* gene (*psy*) from maize and the *carotene desaturase* gene (*crtl*) from *Erwinia uredovora* were inserted into rice.⁴ Evaluation of phytoene synthase (the rate limiting step in carotenoid biosynthesis) from several plant sources identified the maize *psy* gene as the most efficacious, resulting in the greatest accumulation of total

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carotenoids and β -carotene.

GR2 contains up to 37 μg total carotenoids per gram of dry weight of grain, of which 31 $\mu\text{g/g}$ is β -carotene. While this level of β -carotene is high, its bioavailability is unknown. GR2 was developed with the expectation that it could make a major contribution to the vitamin A requirement. It is conservatively estimated that a breastfed 1 to 2-year-old child could derive 60% of the of U.S. recommended dietary allowance (RDA) from the consumption of ~ 70 g of GR2 (weight before cooking); an average serving size for a child this age in Thailand is 160 g, by comparison. The level of β -carotene in GR2 raises the possibility that GR2 may help reduce many of the deaths attributed to VAD.⁵

Biofortified GR2 with high levels of β -carotene is in an early stage of development. The published data provide a description of the DNA construct introduced into rice and report the concentrations of both total carotenoids, as well as that of the five major carotenoids present in representative transgenic rice plants.⁴

ASSESSING GR 2 RELATIVE TO ILSI RECOMMENDATIONS

The safety and nutritional assessment of GR2 is discussed relative to each of the key recommendations in the 2004 ILSI publication on the nutritional and safety assessments of such foods and feeds¹.

Recommendation 1

The safety assessment of a nutritionally improved food or feed begins with a comparative assessment of the new crop with an appropriate comparator crop that has a history of safe use.

One overarching conclusion from the ILSI publication that is evident for the GR2 case study is that the comparative safety assessment process is applicable. Absolute safety is not an achievable goal for any human endeavor, and this is particularly relevant with respect to food and feed. The safe use of food or feed has typically been established either through experience based on its common use, or by experts who determine its safety based on established scientific procedures. Starting in the 1990s, the standard applied to biotech food and feed crops has been that they should be as safe as an appropriate counterpart with a history of safe use. This assessment process has been endorsed by many publications and organizations.^{1,2,6,7} The comparative safety assessment process has sometimes been called the substantial equivalence principle.

Key to the comparative safety assessment process is the recognition that the comparative analysis of composition and plant phenotypic and agronomic properties is the starting point, not the conclusion, of the assessment. The similarities noted between the new and traditional crop do not need further assessment because this provides evidence that these aspects of the newly developed crop are as safe as the traditional crop with a history of safe consumption. The identified differences are subjected to further scientific assessment to clarify whether any safety concerns exist. In the case of nutritionally enhanced crops, significant differences in composition are intentional and, therefore, expected to be observed and should not be considered negative findings. Instead, the nutritional and

safety aspects of potentially significant differences must be assessed on a case-by-case basis.

A fundamental aspect of the comparative safety assessment process is the use of a comparator with a history of safe consumption. To date, the comparator has been a traditional crop developed through conventional breeding since a long history of safe consumption exists in such instances. Therefore, it is interesting to note that characterization of the genetic changes during plant evolution, crop domestication, and the many forms of "conventional breeding" have been larger in scale and less well defined than the genetic changes to a species that arise from application of modern biotechnology.⁸⁻¹⁰ Thus, the history of safe consumption of domesticated crops has been possible while those plant genomes have undergone large changes. With GR2, as with all crops needing to be commercially successful, they undergo extensive breeding with elite lines such that $>99\%$ of the germplasm of commercialized lines will be derived from elite lines that have not experienced genetic transformation. This breeding significantly reduces the opportunity for transformation-induced, random genome changes from being in the final product.

Recommendation 2

To evaluate the safety and nutritional impact of nutritionally improved food and feed crops, it is necessary to develop data on a case-by-case basis in the context of the proposed use of the product in the diet and consequent dietary exposure.

GR2 is in an early stage of development, establishing the proof-of-concept.⁴ The relevant genes are now being crossed into selected local cultivars in several Asian countries to allow further evaluation of agronomic efficacy and safety studies. The components found in GR2 have a history of safe use. No novel proteins or metabolites not normally encountered in the human diet are known to be present; any novel proteins present are at exceedingly low concentrations. The safety of carotene desaturase stands out as the single component that warrants further characterization. The concentration of carotenoids is expected to be similar to that encountered in other commonly eaten foods. If no undesirable or adverse compositional changes are encountered upon analysis, GR2 will present insignificant food safety risks.

Recommendation 3

The safety of any protein(s) newly introduced into a crop need to be assessed. It is noted that recommendations for the safety assessment of transgenic proteins that follow a tiered approach are currently being finalized for publication by an ILSI IFBiC protein safety task force.

The development of GR2, like several of the case studies assessed by the Task Force, involves introduction of a protein not currently present in the crop. Therefore, it is important to note that another IFBiC Task Force is developing the scientific basis for the safety assessment of proteins to be published in early 2008 that includes recommendations for a tiered, weight-of-evidence approach to the safety assessment of proteins. Both Codex Alimentarius⁶ and the European Food Safety Authority² have recognized that a weight of evidence approach is

appropriate for the safety assessment of novel proteins as numerous factors (e.g., source of the protein, sequence homology to known allergens and/or toxins, and protein digestibility) contribute to whether it has the potential to be allergenic or toxic.

The presence of increased levels of carotenoids in the rice endosperm is evidence that the introduced enzymes are active; however, the enzymes have not been directly characterized. Sequence comparisons with DNA and protein databases showed that the inserted proteins bear no significant similarity to known toxic proteins or allergens. It is recommended that the molecular form of carotene desaturase and its *in vitro* digestibility be analyzed, but it is considered unnecessary to assess its acute toxicity in animal studies if it is digestible—such studies would add little additional assurance of safety. The level of expression of the introduced proteins should be determined in the grain since that is the portion of the plant consumed by humans. The similarity of the introduced proteins to those isolated from the donor organism should be established, particularly with regard to the potential for post-translational modification.

Recommendation 4

Compositional analysis of crops with known toxicants and antinutrient compounds should include analysis of those specific analytes. If warranted, an evaluation of the targeted metabolic pathway should also be conducted to identify specific metabolites for inclusion in the compositional analysis due to safety and/or nutritional considerations.

In biotech crops with improved nutritional characteristics, metabolic pathways are often modified to achieve the desired nutritional improvement, and a full understanding of the changes that have occurred is important for both the safety and nutritional evaluation of the biotech crop. The carotenoid biosynthetic pathway that is altered in GR2 are well described at the biochemical and molecular level.⁴ Carotenoids are one of many end products of isoprenoid metabolism; however, they constitute a small percentage of the total cellular content (e.g., ~20 – 40 µg/g dry weight, approximately 0.0002 – 0.0004% of dry weight) in GR2, and it is, thus, likely that very little metabolic energy and only small amounts of precursors are invested in their biosynthesis. Although the DNA construct present in GR2 would be expected to have little effect on overall composition, it might be expected to alter the distribution of metabolites that are ultimately derived from geranylgeranyl-diphosphate (GGPP).

GR2 varieties contain 10–40 µg/g of carotenoids;⁴ this represents a negligible change in the overall composition. It is recommended, therefore, to compare GR2 with near isogenic conventional rice varieties in terms of stable intermediary metabolites involved in carotenoid biosynthesis, especially ones derived from GGPP, and the composition analytes recommended by the OECD consensus document on compositional considerations of key food and feed nutrients and anti-nutrients for new varieties of rice (*Oryza sativa*).¹¹

Because the carotenoid biosynthetic pathway is well understood, there would be no need to employ untargeted compositional analysis of GR2. Within one chapter of the

2004 ILSI publication, comprehensive, untargeted compositional analysis techniques, such as metabolomics, proteomics, and transcriptomics, were suggested as potentially useful tools to screen for unintended changes in food and feed crops. However, even if the carotenoid biosynthetic pathways in rice were not well understood, before untargeted compositional analysis would be informative for GR2, efforts need to continue on several fronts: 1) to standardize the reporting structure of such “omics” data, 2) to establish a database of baseline profiling results that reflects the natural variability in the metabolome (e.g., like the ILSI database (<http://www.crop-composition.org>) and 3) to recommend current best practices.

Recommendation 5

The phenotypic properties of the nutritionally improved crop need to be assessed when grown in representative production locations as part of the overall comparative safety assessment process. Further study is warranted if significant unintended and unexplainable differences are identified.

The most likely undesirable effect of the modification present in GR2 is a redistribution of metabolites that arise from GGPP, such as within the carotenoid pool or among carotenoids, tocopherols, and terpenoids.¹² Perturbations in isoprenoid metabolite concentrations subsequent to pathway engineering have been reported.¹² Such changes would have negligible nutritional impact on humans because rice is not a good source of any required nutrients that are formed from GGPP; however, there may be unintended effects on plant metabolism that could, in turn, indirectly affect composition and nutritional value. In this regard, it is encouraging that the phenotype of the transgenic rice plants seems to be indistinguishable from that of conventional counterparts.⁴ Keeping in mind that all plant breeding can lead to unintended changes,¹³ compositional analysis can reveal whether significant and meaningful changes have occurred during gene transfer and subsequent breeding.

Recommendation 6 & 7

Studies in laboratory animals may serve a useful role in confirming observations from other components of the safety assessment, thereby providing a sense of added safety assurance. However, studies in laboratory animals and targeted livestock generally lack adequate sensitivity to reveal unintended minor changes that have gone undetected by targeted analysis. Animal feeding studies should be conducted in target species to demonstrate the nutritional properties that might be expected from the use of the modified crop, crop component, or coproduct.

Though not directly related to safety, there are matters of practical concern that should be addressed as part of a comprehensive release strategy. While theoretically capable of supplying nutritionally relevant levels of β-carotene, the retention of β-carotene after processing, storage, and cooking needs to be determined. Therefore, although animal nutrition studies would not be appropriate, it is recommended that GR2 be tested in premarket studies in free-living humans that would include assessing

the palatability of rice with an appearance altered relative to conventional rice.

Recommendation 8

The premarket assessment will identify safety and nutritional issues before product launch. It is unlikely that any new product with scientifically valid adverse health concerns will be marketed. Postmarket monitoring of nutritionally improved food products may be useful to verify premarket exposure assessments or to identify changes in dietary intake patterns. Postmarket monitoring should only be conducted when a scientifically valid testable hypothesis exists, or to verify premarket exposure assessments.

Any potential postmarket risks that may be identified with GR2 should be balanced against the potential to ameliorate VAD and, thereby, reduce the loss of life and clinical symptoms due to the nutritional deficiency. The magnitude of this potential nutritional impact will only become known for certain if GR2 is adopted.

CONCLUSIONS

Vitamin A deficiency is a major public health challenge. GR2 represents a significant enhancement of total carotenoid production in rice, with β -carotene being the predominant form. GR2 is in an early stage of development, establishing the proof-of-concept. The relevant genes are now being crossed into selected local cultivars in several Asian countries to allow further evaluation of agronomic efficacy and safety studies (see <http://www.goldenrice.org/> for the latest information). The components found in GR2 have a history of safe use. No novel proteins or metabolites not normally encountered in the human diet are known to be present; and the introduced proteins are expected to be at exceedingly low concentrations. The safety of carotene desaturase stands out as the one component that warrants further characterization. It is recommended that the molecular form of the protein and its *in vitro* digestibility be analyzed, but it is considered unnecessary to assess its acute toxicity in animal studies if it is digestible—such studies would add little additional assurance of safety. The concentration of carotenoids is similar to that encountered in other commonly eaten foods. Rice allergy is not common and rice is not a significant source of toxicants or antinutrients. If no undesirable or adverse compositional changes are encountered upon analysis, GR2 will present insignificant food safety risks.

Though not directly related to safety, there are matters of practical concern that should be addressed as part of a comprehensive release strategy. While theoretically capable of supplying nutritionally relevant levels of β -carotene, the retention of β -carotene after processing, storage, and cooking needs to be determined. Moreover, some have questioned if yellow rice will be acceptable to people who traditionally eat white polished rice. All of these points need to be resolved if GR2 is to become an important solution to VAD.

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AUTHOR DISCLOSURES

Kevin C Glenn, no conflicts of interest, except the paper has been authored in the Monsanto Company.

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