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Golden Rice: Effectiveness and Safety, A Literature Review

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INTRODUCTION

If you are like most Americans, you probably consume genetically modified organisms (GMOs) every single day, and likely even at every meal. GMOs are defined as living organisms that have had their genetic material altered in a laboratory using genetic engineering (GE).[1] This laboratory process creates types of plant, animal, bacterial, and viral genes that do not occur naturally.[1] Potatoes, tomatoes, corn, and soybeans are some of the most common GMO crops. [1]

GE produces new varieties of crops that have desirable characteristics and can withstand conditions that non-GE crops cannot. For example, potatoes that do not absorb as much fat when fried. [1]

Possible pros of GMOS include: more nutritious, better tasting food, increased crop yields, resulting in increased food supply at a lower cost, resistance to droughts and disease, insecticides, decreased use of pesticides, increased shelf life, and faster growing crops. [2]

Though there are not sufficient studies showing the long-term effects of GMOs on health, potential cons may include unexpected, or even harmful, changes in crop DNA.[2] GE crops may be bred with other natural organisms, causing unknown effects or even leading to the extinction of the original organism. GMO crops may become more vulnerable to certain pests as a result of increased resistance to other pests. [1] This literature review will investigate the effectiveness and safety of golden rice. Methodology for this paper includes agriculture and food science databases in addition to keyword searches.

GOLDEN RICE: WHAT IS IT?

Golden rice is a genetically modified (GM) rice fortified with β-carotene, or provitamin A. Golden rice was developed to prevent vitamin-A deficiency (VAD) in developing countries.[3] VAD is one of the most harmful deficiencies in the world, in addition to iron, iodine, and zinc.[4] VAD is common in poor countries where diets consist mainly of rice and other foods rich in carbohydrates, but lacking in micronutrients. [4] Vitamin A is vital for the maintenance of the epithelial lining throughout the body, therefore a deficiency compromises the integrity of this lining, causing it to deteriorate. This includes the lining surrounding the eye, which can cause blindness. The is just one of the many possible outcomes of VAD.[4]

VAD is most common in Southeast Asia and Africa and most critically affects small children and pregnant women. Rice lacks β-carotene, which is converted to vitamin A in the body (Vitamin A Deficiency). A 2012 report by the World Health Organization (WHO) indicated that roughly 250 million preschool-age children were impacted by VAD and up to 2.7 million deaths, 1/3 of deaths under the age of 5, could have been prevented with adequate amounts of vitamin A. [4] VAD weakens the immune system, increasing both the chance and severity of typical childhood infections, often leading to death. VAD can cause impaired vision, ultimately leading to irreversible blindness. [4] Other possible effects of VAD include disabled skin integrity, increased exposure to infection due to decreased immune response, and reduced ability to transport oxygen via blood. [4]

Naturally occurring rice seeds lack provitamin A, so only GE makes it possible for golden rice to have the potential to alleviate VAD. Thousands of varieties of rice have been tested for this trait without success.

GOLDEN RICE: BACKGROUND & DEVELOPMENT

In the early 1990's, Ingo Potrykus of Switzerland and Peter Beyer (University of Freiburg, Germany), pitched the idea of a project to genetically engineer the provitamin-A pathway into rice endosperm to the Rockefeller Biotechnology Program in New York. At the time, Beyer was studying the regulation of terpenoid (terpens-class of organic hydrocarbons supplied by a diverse group of plants, termed "terpenoids" when they become denatured by oxidation during drying and curing) pathway in daffodils[3] The isolation of genes needed for this pathway are necessary to initiate the same pathway in rice endosperm. Potrykus possessed the necessary engineering technology and belief that the project was doable. [3]

The goal of the project was to "initiate a project to genetically engineer the provitamin-A pathway into the rice endosperm". [3] The Rockefeller Biotechnology Program organized a brainstorming session, although "the verdict of this initial session was that such a project had a low probability of success, but that it was worth trying because of its high potential benefit." [3]

Through research, the duo discovered that the last antecedent of the pathway in endosperm was geranlygeranyl-pyrro-phosphate (Burkhardt et al., 1997). In theory, because of this, it should have been possible to obtain β-carotene by way of four enzymes: phytoene synthase, phytoene desaturase, ζcarotene desaturase, and lycopene cyclase (Burkhardt et al., 1997), though hundreds of cases of scientific rationale said otherwise. [3] Eight years after presenting their idea to the Rockefeller Program, the first breakthrough finally came when Peter Burkhardt, who was part of Potrykus' laboratory team "recovered phenotypically normal, fertile, phytoene synthase-transgenic rice plants, which produced good quantities of phytoene in their endosperm" (Burkhardt et al., 1997). [3] This development demonstrated the following: diverting the pathway directly toward β-carotene was possible, and directing a substantial amount of geranlygeranyl-pyrrophosphate away from other vital pathways did not have any detrimental effects on physiology or development. [3]

Xudong Ye, another member of Potrykus' laboratory team, performed the most pivotal experiment to date: "cotransformation with two *Agrobacterium* strains containing all the necessary genes plus a selectable marker". [3] Ye's experiment resulted in a yellow-pigmented endosperm that contained provitamin-A, exhibiting that it was indeed possible to manipulate the complete biochemical pathway (Ye et al., 2000). [3] Salim Al-Babili, a third member of Beyer's lab group, was successful in creating the first actual strains of golden rice. The strain with the highest amount of provitamin-A contains enough of the provitamin, 1.6 $\mu g\ g^{-1}$ endosperm, to anticipate relief of VAD. [3] Tests needed to be done involving bioavailability and feeding examination. At the time, these types of tests could not be performed due to the small amount of rice produced in the laboratory team's greenhouse. Proper testing would require hundreds of kilograms of rice and therefore could only be grown in a field. [3]

THE GOLDEN RICE PROJECT

The Golden Rice Project resulted from the meeting with the Rockefeller Foundation, bringing professor Potrykus and Beyer together.[5] The project's home page indicates that extending the project to small farmers of target countries "requires a highly professional and interdisciplinary team." [5] A Humanitarian Board was created to make this easier. The Board is composed of a group of international experts from various prestigious institutions with the goal of providing guidance to the project. The Project involves research and education institutions from the following countries: the Philippines, India, Vietnam, Bangladesh, China, Indonesia, and Germany. [5]

A letter from Potrykus, Chairman of the Board, indicates that the Board, so far, is mainly concerned on further developing the technology needed for the project. [5] Potrykus also notes that there are many sources that provide misleading information about both GM technology and golden rice. The Golden Rice Project prides itself on its thorough webpage, which includes the latest information on "the science, product development, and progress in regulatory approval of *Golden Rice* to all interested audiences." [5]

EFFECTIVENESS OF GOLDEN RICE

The effectiveness of golden rice is a key factor. If it is not found to be effective in treating VAD, there is no reason to continue studying or growing it. A study in the Hunan province of China examined the effectiveness of golden rice in a group of 68 healthy elementary school children between the ages of 6 and 8.[6] Subjects were randomly assigned to three different groups: spinach, golden rice, or β-carotene in the form of an oil capsule.[6]

The children each ate prepared meals for 14 days. All meals were consumed at school during the week and instructions were given to parents regarding provitamin-A or vitamin-A rich foods to avoid while consuming meals at home during the weekend. [6] The contents of at-home meals were recorded in a diary by the participants' parents. Lunches consumed at school included pork, cabbage, and egg and tomato soup totaling 560 calories. This was in addition to the β-carotene capsule, 30g of spinach, or 60g of golden rice. [6] The spinach and β-carotene groups were given white rice to ensure they received the same amount of calories as the golden rice group. The average daily intake of vitamin A was ~240 μg retinol per person, which is 34% of the Chinese Recommended Nutrient Intake for this age group (700 μg). [6]

Fasting blood samples were attempted in the mornings of day 1, 3, 7, 14, and 21. KaliendaGraph by Synergy Software was used to determine the AUC of the total serum retinol response from each of the intervention doses. [6] This measure was then compared to the AUC of the vitamin A reference dose (0.5 mg [$^{13}C_{10}$]retinyl acetate; molecular mass = 338). Concentration of blood retinol was measured using High performance Liquid Chromatography (HPLC). The retinol derived from participants was used to determine the total-body serum volume. The quantity of retinol formed from each β-carotene dose was calculated. [6]

For the purposes of this study, the B-carotene to vitamin A conversion factor was defined as follows: "the amount of labeled β-carotene contained in a given oral dose of GR, spinach, or oil capsule, compared with the amount of vitamin A derived from each dose." [6]

Results of the study indicated that spinach, golden rice, and β-carotene in oil capsule form can each provide children with vitamin A nutrition. [6] Of these 3 provitamin A sources, golden rice was as effective as the pure β-carotene. Both of these sources of provitamin A were much more effective than spinach at providing vitamin A. Ultimately, the results provide evidence that golden rice has the potential to be an effective source of vitamin A. [6] The study notes, "on the basis of our current findings, a single 50-g dry-weight serving of GR (a bowl of ∼100 to 150 g cooked weight) can provide ∼1 mg β-carotene, which would then be converted to ∼435 μg retinol." [6] This amount constitutes approximately 60% of the Chinese Recommended Nutrition Intake of vitamin A for a 7-year-old child. [6]

Another study, which took place in Thailand, was conducted with the goal of determining the "vitamin A value of intrinsically labeled dietary Golden Rice in humans".[7] Golden rice was fed to 5 healthy adult volunteers, three women and two men, along with 10 grams of butter. Each participant was given a reference dose of retinyl acetate (.4-1.0 m) oil one week prior to the ingestion of golden rice.[7] Blood samples were drawn over a period of 36 days. "Results showed that the mean (6SD) area under the curve for the total serum response to [2 H]retinol was 39.9 6 20.7 μg d after the Golden Rice dose."[7] This was then compared with the curve of the retinyl acetate reference dose. Golden Rice supplied between 0.24 and .94 mg retinol, making the conversion factor of golden rice to retinol 3.8 ± 1.7 with a range of 1.9 to 6.4 to 1 by weight.[7] This means that 3.8μg of golden rice provides 1μg of retinal, indicating a very efficient bioconvenversion of β-carotene to vitamin A.[7]

In an examination of the safety of golden rice, which will be discussed in the next section of this paper, it is noted that GR could supply between 11 and 86% of the recommended daily allowance (RDA) of vitamin A (Zimmerman and Qaim).[8] Notably, golden rice was intended as a supplement and 100% RDA of vitamin A is not imperative to alleviate symptoms of VAD. In fact, just 25% RDA of vitamin A is needed to prevent blindness and death.[8] The study notes that the main issue regarding the effectiveness of golden rice is centered around the bioavailability of GR. Those in opposition of the effectiveness of golden rice say that just one molecule of retinol would be produced from 25 molecules of β-carotene, a 1:25 ratio. This issue was resolved when it was discovered the conversion ratio is actually close to 1:4 in humans, meaning golden rice can successfully contribute to vitamin A intake.[8]

SAFETY OF GOLDEN RICE

Safety is another key factor to the potential success and governmental approval of golden rice. Golden rice is a GMO and GMOs are often feared by consumers because of assumptions that they are unsafe and cause detrimental health effects. However, in the Hunan study that was previously mentioned, no side effects or abnormalities were observed during the course of the study. This study also followed up with participants for a period of one year in which again, no complaints were reported.[6]

Bruce Chassy (2010) of the University of Illinois Food Science and Human Nutrition Department evaluated the safety of golden rice. He notes a second version of golden rice, golden rice 2 (GR2) "was constructed using the phospho-mannose-isomerase (PMI) marker system that allows the simple sugar mannose to be used to select transformants."[8] This system has been previously used in the development of other transgenic crops and has been approved by regulating authorities.[8]

Next, Chassy evaluated the protein safety of golden rice. He notes that rice allergies are uncommon and rice does not contain any major anti-nutrients or toxins.[8] The protein sequences of golden rice and GR2 were compared to all known toxins, anti-nutrients, and food allergens and no similarities were found. The maize phytoene synthase protein incorporated into GR2 is a protein that both humans and animals consume on a regular basis.[8] Large quantities of the protein will be needed for animal toxicity studies, while small amounts will be necessary for digestibility studies. Meticulous protein safety tests will be required by regulating authorities although the quantities of proteins present are likely far lower than would be required for known toxins to exert an effect. Since rice is cooked at high temperatures over long time periods, proteins become inactivated and denatured, making it unlikely to pose any threat to humans or animals. [8]

The structure of transgenic crops is commonly evaluated in order to demonstrate that there are no losses in nutritional value and no unintended changes have come about. Since golden rice and GR2 have each been crossed with other varieties of rice grown by farmers in Asia, many distinct compositional profiles exist.[8] No other rice varieties are required to undergo composition testing since rice basically lacks all nutrients, meaning golden rice would not have any less nutritional value than traditional rice. This type of testing is both expensive and time consuming and many have questioned its value.[8]

The International Rice Research Institute (IRRI) is “the world’s premier research organization dedicated to reducing poverty and hunger through rice science; improving the health and welfare of rice farmers and consumers; and protecting the rice-growing environment for future generations.”[9] According to the Institute, a number of food safety-related studies have been done regarding golden rice and three major conclusions can be drawn from them. First, β-carotene is a safe source of vitamin A.[9] It is found in various foods eaten around the world on a daily basis, like fruits and vegetables. Second, the β-carotene in golden rice is identical to the β-carotene found in other food sources.[9] The third and final conclusion the IRRI mentions is that the proteins from the new GM genes in golden rice do not cause and toxic or allergenic effects.[9]

A study done by the Food Allergy Research and Resource Program (FARRP) at the University of Nebraska (2006) evaluated the three proteins expressed by the genes in GR2. The purpose was to “identify any potential sequence matches to allergenic proteins that might indicate an elevated risk of allergic cross reactivity in consumers.”[10] The study used two different sequence alignment and similarity scoring methods to compare these sequences. A FASTA algorithm (Pearson, 2000) was used in addition to the default scoring matrix (BLOSUM 50). These programs were used to evaluate the alignment of each sequence and compare them to AllergenOnline, "looking for matches of low *E* sore values (<1e-7) and/or greater than 50% identity as an indication of potential cross-reactivity."[10] AllergenOnline is a database that includes 1537 known allergens. Lastly, BLASTP was used to pinpoint any significant similarities to newly reported "allergen" sequences, which were not found in AllergenOnline, version 6.0.[10]

Results from the three search methods were negative, meaning "the criteria for suspected cross-reactivity were not reached."[10] This indicates that there is no expected significant risk of cross-reactivity for those with allergies to known allergens,[10] similar to one of the conclusions made by the IRRI.

CONCLUSION

In conclusion, each of the studies discussed regarding the effectiveness of golden rice concluded that it was indeed effective at providing vitamin A. Though it is not possible for golden rice to provide 100% of the RDA for vitamin A, it can provide between 11 and 86%. Just 25% of the RDA is needed to prevent the most severe symptoms of vitamin A deficiency, blindness and death. Results from the Hunan, China study indicated that golden rice was just as effective as pure β-carotene in the form of an oil capsule.

The studies examined for this literature review did not show any negative effects relating to the health or safety of golden rice. The Hunan study included a one year follow-up period in which no health issues occurred. Chassy's evaluation of the safety of golden rice found no issues in either of the following areas: protein safety and structure of transgenic crops.

The IRRI noted that thus far, three conclusions can be drawn from research regarding the safety of golden rice: β-carotene is a safe source of vitamin A, β-carotene in golden rice is identical to the β-carotene found in other food sources, and proteins from the genes in golden rice do not cause and toxic or allergenic effects. Similarly, The University of Nebraska study found that there was no significant risk of cross-reactivity of the proteins in GR2 compared to known allergens.

None of the studies reviewed included consumer acceptability or texture evaluation of golden rice, which go hand-in-hand. Brown rice and white rice have notably different textures and consumers typically prefer one to the other. The color or texture of golden rice may prove to be off-putting for those who will be consuming it. In order for golden rice to reach its full potential, it *must* be acceptable enough for consumption.

As with most scientific studies, further research needs to be conducted. The small sample sizes of the studies discussed are reason for concern and may cause some to be doubtful of golden rice. Longitudinal research with large sample sizes is needed in order to fully understand both the effectiveness and safety of golden rice. These studies should also include a variety of age groups, ethnicities, and both males and females.

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