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A Scientific Audit: The 'Golden Rice'— How Not to Do Science
By Dr. Mae-Wan Ho

The "golden rice"—a GE rice engineered to produce pro-vitamin A—is being offered to the Third World as cure for widespread vitamin A deficiency.

The audit uncovers fundamental deficiencies in all aspects, from the scientific/social rationale to the science and technology involved. It is being promoted in order to salvage a morally as well as financially bankrupt agricultural biotech industry.

The scientific/social rationalization for the project exposes a reductionist self-serving scientific paradigm that fails to see the world beyond its own narrow confines. The "golden rice'" is a useless application. Some 70 patents have already been filed on the GE genes and constructs used in making the "golden rice." It is a drain on public resources and a major obstruction to the implementation of sustainable agriculture that can provide the real solutions to world hunger and malnutrition.

"Golden rice'" is not a "second generation" GE crop as has been claimed. It involves standard first generation technology and carries some of the worst features in terms of hazards to health and biodiversity. Rockefeller Foundation, the major funder of the project by far, has withdrawn support from it. The project should be abandoned altogether.

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A Gift-Horse for the Poor

A report in *Financial Times* states that the creators of "golden rice'" have struck "a ground-breaking deal" with corporate giant AstraZeneca to give Third World farmers free access to the grain while allowing it to be commercially exploited in the developed world. The company will oversee the production of stable GE line(s) and patenting and take the lines through field trials and commercial approval. While farmers in developed countries will have to pay royalties, those in the Third World earning less than U.S. $10,000 will not. But will Third World farmers be allowed to save the seeds for replanting? It did not say.

This "golden rice," not yet available, is already worth its weight in diamonds. The project was funded from four sources of public finance totaling U.S. $100 million: the philanthropic Rockefeller Foundation, whose mission is to support scientific research that specifically benefit the poor, the Swiss Federal Institute of Technology, the European Community Biotech Program, and the Swiss Federal Office for Education and Science.

The announcement failed to mention that there are already 70 patent claims on the genes, DNA sequences, and gene constructs used to make the "golden rice." Will the cost of paying royalties for the previous 70 patent claims be added to the cost of the "golden rice"? Which of the royalties on the 70-odd patents would the Third World farmers be absolved from paying? Rockefeller Foundation, the major funder by far, has reportedly abandoned the project to "shift its agricultural funding focus to support research that will have a more direct benefit to subsistence farmers."

The Scientific/Social Rationale Is Fallacious

Many have commented on the absurdity of offering "golden rice'" as the cure for vitamin A deficiency when there are plenty of alternative, infinitely cheaper sources of vitamin A or pro-vitamin A, such as green vegetables and unpolished rice, which would be rich in other essential vitamins and minerals besides. To offer the poor and malnourished a high-tech "golden rice" tied up in multiple patents, that has cost U.S. $100 million to produce and may cost as much to develop, is worse than telling them to eat cake.

"Golden rice"was engineered to produce pro-vitamin A or b-carotene (the substance that makes carrots orange) in the endosperm, i.e., the part of the rice grain that remains after it has been polished. The scientific paper started with a review of the literature to rationalize why such GE rice is needed and of benefit for the Third World. The paper was accompanied by an unusually long news feature entitled, "The Green Revolution Strikes Gold," which reinforced the rationalization for the project, explaining the remarkable feat of technology involved and stated that the scientists intend to make the "golden rice" "freely available to the farmers who need it most." The last sentence in this glowing report, however, gave the game away: "One can only hope that this application of plant genetic engineering to ameliorate human misery without regard to short-term profit will restore this technology to political acceptability."

What were the reasons for the scientists to embark on the project? It is important to know, as these reasons may have been used to persuade funders to support the project in the first place, and funders ought to bear as much of the responsibility.

The first reason given is that the aleurone layer (in unpolished rice) is usually removed by milling as it turns rancid on storage, especially in tropical areas; and the remaining endosperm lacks pro-vitamin A. The researchers are tacitly admitting that at least some varieties of unpolished rice will have pro-vitamin A. The reason rice is milled is to prolong storage for export, and to suit the tastes of the developed world. So why not give the poor access to unpolished rice? A proportion of every rice harvest could be kept unpolished and either given freely to the poor, or sold at the cheapest prices. But the scientists have not considered that possibility. Unpolished rice is fact part of the traditional Asian diet until the Green Revolution when aggressive marketing of white polished rice created a stigma of unpolished rice. However, most rural communities still consume unpolished rice and now that consumers have become aware of its nutritional value, unpolished rice is becoming sought after.

"Predominant rice consumption," the researchers claim, promotes vitamin A deficiency, a serious health problem in at least 26 countries, including highly populated areas of Asia, Africa, and Latin America. Some 124 million children worldwide are estimated to be vitamin A deficient. (Actually, the latest figures quoted in a press release from the International Rice Research Institute (IRRI) is 250 million preschool children.) The scientists seem to be unaware that people do not eat plain rice out of choice. The poor do not get enough to eat and are undernourished as well as malnourished. The Food and Agricultural Organization (FAO) started a project in 1985 to deal with vitamin A deficiency using a combination of food fortification, food supplements, and general improvements in diets by encouraging people to grow and eat a variety of green leafy vegetables. One main discovery is that the absorption of pro-vitamin A depends on the overall nutritional status, which in turn depends on the diversity of the food consumed.

"Predominant rice consumption" is most likely to be accompanied by other dietary deficiencies. A recent study by the Global Environmental Change Programme concludes that predominant consumption of Green Revolution crops is responsible for iron deficiency in an estimated 1.5 billion, or a quarter of the world's population. The worst affected areas are in rice-growing regions in Asia and South-East Asia where the Green Revolution had been most successful in increasing crop yield.

Research institutions such as IRRI have played the key role in introducing Green Revolution crops to the Third World. IRRI was founded in 1959 under an agreement forged by the Rockefeller and Ford Foundations with the Philippine government, and its lease for operation expires in 2003. At its recent 40th anniversary celebration, hundreds of Filipino rice farmers protested against IRRI for introducing GE crops, blaming IRRI, among other things, for promoting the Green Revolution and causing massive loss of biological diversity in rice paddies throughout Asia.

It is clear that vitamin A deficiency is accompanied by deficiencies in iron, iodine, and a host of micronutrients, all of which come from the substitution of a traditionally varied diet with one based on monoculture of the Green Revolution. The real cure is to re-introduce agricultural biodiversity in the many forms of sustainable agriculture already being practiced successfully by tens of millions of farmers all over the world.

As the scientists know, clinical deficiency can be dealt with by prescription of vitamin A pills, which are affordable and immediately available. "Oral delivery of vitamin A is problematic," they state. Judging from the reference cited they may be referring to the well-known harmful effects of vitamin A overdose. But why would high levels of pro-vitamin A rice in a staple food that people generally consume in the largest amounts in a meal not also cause problems connected with overdose? In particular, vitamin A poisoning has been known to result from excessive b-carotene intake in food.

Finally, why is it necessary to genetic engineer rice? "Because no rice cultivars produce [pro-vitamin A] in the endosperm, recombinant technologies rather than conventional breeding are required." This is the conclusion to the whole fallacious reasoning process. It amounts to this: rice is polished, which removes pro-vitamin. A, therefore a hundred million dollars (much of it tax-payers' money) are needed to put pro-vitamin A into polished rice. A more likely explanation is that the geneticists are looking for funding to do their research, and have constructed, as best they could, a series of rationalizations for why they should be supported. Neither the scientists nor the funders have looked further beyond the technology to people's needs and aspirations, or to what the real solutions are.

The Science and Technology Is Standard First Generation

It took ten years to engineer b-carotene into polished rice because rice naturally does not have the metabolic pathway to make it in the endosperm, perhaps for good biological reasons. Immature rice endosperm makes the early precursor, geranylgeranyl-diphosphate (GGPP). In order to turn GGPP into b-carotene, four metabolic reactions are needed, each catalyzed by a different enzyme. Enzyme 1, phytoene synthase converts GGPP to phytoene, which is colorless. Enzymes 2 and 3, phytoene desaturase and zcarotene desaturase, each catalyzes the introduction of two double-bonds into the phytoene molecule to make lycopene, which is red in color. Finally, Enzyme 4, lycopene b-cyclase turns lycopene into b-carotene. Hereafter, the enzymes will be referred to by numbers only. Thus, a total of four enzymes have to be engineered into the rice in such a way that the enzymes are expressed in the endosperm. Some very complicated artificial gene constructs have to be made. The gene constructs are made in units called expression cassettes (see Box 1)

Box 1

The 'Gene Expression Cassette'–A Unit of Transgenic Construct

The gene for each enzyme never goes in alone. It has to be accompanied by a special piece of genetic material (DNA), the promoter, which signals the cell to turn the gene on, i.e., to transcribe the DNA gene sequence into RNA. At the end of the gene, there has to be another signal, a terminator, to mark the RNA so it can be translated into protein. To target the protein to the endosperm, an extra bit of DNA, a transit sequence, is required. The resulting expression cassette for each gene is as follows:

promoter transit sequence gene terminator

Typically, each bit of the construct: promoter, transit sequence, gene and terminator is from a different source. Several expression cassettes are usually linked in series, or "stacked" in the final construct.

In order to select for the plant cells that have taken up the foreign genes and gene-constructs, "golden rice" makes use of a standard antibiotic resistance gene coding for hygromycin resistance, also equipped with its own promoter and terminator. All these expression cassettes have to be introduced into the rice plant cells. One simplification available is that the reactions catalyzed by two of the enzymes, 2 and 3, could be done by a single bacterial enzyme, let's call it enzyme 2-3, so a total of four expression cassettes have to be introduced, one for each of three enzymes and the fourth for the antibiotic resistance marker.

Unlike natural genetic material which consists of stable combinations of genes that have co-existed for billions of years, artificial constructs consist of combinations that have never existed, not in billions of years of evolution. Artificial gene-constructs are well-known to be structurally unstable, which means they tend to break and join up incorrectly, and with other bits of genetic material, resulting in new unpredictable combinations. This process of breaking and joining of genetic material is referred to as recombination. The more complicated the construct, the more it tends to break and rearrange or form new combinations. The instability of the construct means that it is seldom inserted into the plant genome in its intended form. The inserts are generally rearranged, with parts deleted, or repeated.

In order to make many copies of the construct and to facilitate entry into plant cells, the construct is spliced into an artificial vector, which is generally made from genetic parasites that live inside cells. The artificial vector also enables the construct to be efficiently smuggled into the plant cell and to jump into the genome of the plant cell. The vector used in the case of the "golden rice'" is the one most widely used since the beginning of plant genetic engineering. It is derived from the "T-DNA," part of the tumor-inducing (Ti) plasmid (a genetic parasite) of the soil bacterium, *Agrobacterium.* The Ti plasmid naturally invades plant cells, inserting the T-DNA into the plant cell genome, and causing the cell to develop into a plant tumor or gall. The artificial gene construct is spliced in between the left and right borders of the T-DNA vector. The borders of the T-DNA are "hotspots" for recombination, i.e., they have a pronounced tendency to break and join up, which is ultimately why the vector can invade the plant's genome and carry its hitch-hiker gene construct along with it.

Three different constructs were made. The first consists of the expression cassettes of enzyme 1 from daffodils and enzyme 2-3 from the plant bacterial pathogen, *Erwinia uredovora* , together with the expression cassette of an antibiotic resistance marker gene that codes for hygromycin resistance. Another antibiotic resistance gene (coding for kanamycin resistance) is also present, albeit lacking a promoter. Hygromycin and kanamycin are both aminoglycoside antibiotics that inhibit protein synthesis. The resistance genes originate from bacteria and generally have specificities for more than one aminoglycoside antibiotic. This first construct is the most complicated, but it still does not have all the required enzymes. Enzyme 1 and the hygromycin resistance gene are both equipped with a promoter from the cauliflower mosaic virus (CaMV), which is especially hazardous (see below).

The second construct consists of the expression cassettes of enzyme 1 and enzyme 2-3 as in the first, but without any antibiotic resistance marker genes. The third construct consists of the expression cassette of enzyme 4, again from daffodil, stacked with the hygromycin-resistance marker-gene cassette. The strategy of separating the genes for the enzymes and antibiotic resistance marker into two different constructs is that it overcomes some of the problems of structural instability: the more cassettes stacked, the more unstable is the construct.

Each construct was spliced into a T-DNA vector, and two transformation experiments were carried out. In the first experiment, 800 immature rice embryos were inoculated with the vector containing the first construct, and hygromycin was used to select for resistant plants that have taken up the vector, resulting in 50 GE plants. In the second experiment, 500 immature embryos were inoculated with a mixture of the vectors containing the second and third construct respectively. Selection with hygromycin gave rise to 60 GE plants that have taken up the third construct, but only twelve of these had taken up the second construct as well. The transformation process is well-known to be random, as there is no way to target the foreign genes to precise locations in the genome. There could be more than one site of insertion in a single cell. Furthermore, as mentioned earlier, the actual inserts are likely to be rearranged, or subject to deletions or repetitions. Hence each transformed cell will have its own distinctive pattern of insert (s), and each GE plant, which comes from the single transformed cell, will differ from all the rest.

Note that the GE plants from the first experiment will not have the full complement of enzymes required to make b-carotene, and should give red endosperm from the lycopene present. Only the GE plants from the second experiment which have taken up both vectors would possess all the enzymes needed, and give orange-colored endosperm.

Uncontrollable Technology and Unpredictable Outcomes Raise Questions On Safety

Unexpectedly, transgenic plants from both transformation experiments gave orange polished grains. Chemical analyses confirmed that only b-carotene, in varying amounts, was found in all lines, but no lycopene. This suggests that enzyme 4 may be present in rice endosperm normally, or it could be induced by lycopene, to turn all of the lycopene into b-carotene. Lutein and zeaxanthin, two other products derived from lycopene, were also identified in varying amounts besides b-carotene. All of these were absent from non-GM rice.

In addition, many other uncharacterized, unidentified products were found, which differ from one line to another. What is the nutritional value of the other products? Are any of the known and unknown products harmful? Without thorough chemical analyses and toxicity tests, it is impossible to tell. This highlights the unpredictable, uncontrollable nature of the technology.

Molecular analyses of the GE inserts were not done in any detail. Nevertheless, judging from the evidence presented, there are the usual signs of deletions, rearrangements, and multiple repeats of the constructs inserted due to structural instability of the constructs and the tendency for recombination. There is no guarantee that any of the plants will give stable progeny in successive generations. The instability of GE lines is well-known and is a continuing problem for the industry. Inserted genes can lose their activities or become lost altogether in subsequent generations. There is nothing in "golden rice" to distinguish it from standard first generation GE plants with all the well-known defects and hazards.

'Golden Rice' Is No Technical Improvement And More Unsafe

"Golden rice" exhibits all the undesirable, hazardous characteristics of existing GE plants, and in added measure on account of the increased complexity of the constructs and the sources of genetic material used. The hazards are highlighted below:

- It is made with a combination of genes and genetic material from viruses and bacteria, associated with diseases in plants, and from other non-food species.

- The gene constructs are new, and have never existed in billions of years of evolution.

- Unpredictable by-products have been generated due to random gene insertion and functional interaction with host genes, which will differ from one plant to another.

- Over-expression of transgenes linked to viral promoters, such as that from CaMV, exacerbates unintended metabolic effects as well as instability (see below). There are at least two CaMV promoters in each transgenic plant of the "golden rice," one of which is linked to the antibiotic resistance marker gene.

- The transgenic DNA is structurally unstable, leading to instability of the GE plants in subsequent generations, multiplying unintended, random effects.

- Structural instability of transgenic DNA increases the likelihood of horizontal gene transfer and recombination.

- Instability of transgenic DNA is enhanced by the CaMV promoter, which has a recombination hotspot, thereby further increasing the potential for horizontal gene transfer.

- The CaMV promoter is promiscuous in function and works efficiently in all plants, in green algae, yeast and *E. coli.* The spread of genes linked to this promoter by ordinary cross-pollination or by horizontal gene transfer will have enormous impacts on health and biodiversity. In particular, the hygromycin resistance gene linked to it may be able to function in bacteria associated with infectious diseases.

- Horizontal transfer of transgenic DNA from GE plants into soil fungi and bacteria has been demonstrated in laboratory experiments. Recent evidence suggests that it has also taken place in a field-trial site for GE sugar-beets, in which transgenic DNA persisted in the soil for at least two years afterwards.

- Prof. Hans-Hinrich Kaatz from the University of Jena, has just presented new evidence of horizontal gene transfer within the gut of bee larvae. Pollen from GE rapeseed tolerant to the herbicide glufosinate were fed to immature bee larvae. When the microorganisms were isolated from the gut of the larvae and examined for the presence of the gene conferring glufosinate resistance, it was found in some of the bacteria as well yeast cells.

- All cells including those of human beings are now known to take up genetic material. While natural (unmanipulated) genetic material is simply broken down to supply energy, invasive pieces of genetic material may jump into the genome to mutate genes. Some insertions of foreign genetic material may also be associated with cancer.

- Horizontal transfer of genes and constructs from the "golden rice'"will spread transgenes, including antibiotic resistance genes, to bacterial pathogens, and also has the potential to create new viruses and bacteria associated with diseases.

Conclusion

In conclusion, the "golden rice" project was a useless application, a drain on public finance and a threat to health and biodiversity. It is being promoted in order to salvage a morally as well as financially bankrupt agricultural biotech industry, and is obstructing the essential shift to sustainable agriculture that can truly improve the health and nutrition especially of the poor in the Third World. This project should be terminated immediately before further damage is done.

The "golden rice" possesses all the usual defects of first generation transgenic plants plus multiple copies of the CaMV promoter which we have strongly recommended withdrawing from use on the basis of scientific evidence indicating this promoter to be especially unsafe. A growing number of scientists (318 scientists from 39 countries to date) are calling for a global moratorium on the environmental releases of GEOs until and unless they can be shown to be safe.

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