The Golden Rice Project

Jorge E. Mayer, Peter Beyer, Ingo Potrykus*
University of Freiburg, Germany; *Professor emeritus, ETH Zurich, Switzerland.

Biofortification to complement traditional interventions

In the year 2000, more than 792 million people in 98 developing countries did not get enough food to lead a normal, healthy and active life, as estimated by FAO. Nine million children die of malnutrition every year; a vast majority of these deaths is most probably linked to micronutrient deficiencies. The deficiencies with the highest impact on morbidity and mortality are in iron, zinc, iodine and vitamin A.

At its inception, the Golden Rice Project set out to alleviate the vitamin A deficiency (VAD) problem, because of its relevance and potential impact. Yearly, 500,000 people—mainly children—become blind as a consequence of VAD, 50% of which die within a year of becoming blind. VAD severely affects the immune system; hence it is also involved in many of these children's deaths in the guise of multiple diseases. Recently, malaria deaths in children under five years of age have been linked to deficiencies in intake of protein, vitamin A and zinc.[1] Various public and international programmes for supplementation, fortification and diet diversification have achieved substantial improvements but have difficulty in attaining full coverage of the affected population and above all, sustainability. Biofortification—the fortification of crop tissues by means of their own biosynthetic capacity—involves conventional breeding of genetically improved basic staple crops, and offers an opportunity to obtain a more inclusive coverage, especially of the poorest sectors of society. Genetic improvement can be achieved in various ways, including the introgression of wild relatives, mutagenesis and genetic engineering.

VAD is prevalent among the poor who depend mainly on rice for their daily energy uptake, because the rice endosperm—the edible part of the rice grains—does not contain any beta-carotene (pro-vitamin A), which our body in turn converts into vitamin A. Dependence on rice as the predominant food source, therefore, necessarily leads to VAD, most severely affecting children and pregnant women. For the 400 million rice-consuming poor, the medical consequences are severe: impaired vision —in extreme cases irreversible blindness, impaired epithelial integrity, exposing the affected individuals to infections, reduced immune response, impaired hematopoiesis and skeletal growth, among other debilitating afflictions. Rice containing beta-carotene could substantially reduce the problem. This can only be achieved using genetic engineering because there is no pro-vitamin A in the endosperm— the starch storage tissue of the seed—even though it is produced in the leaves of rice plants. No variability for this trait has been detected in the world's most important rice germplasm collections.

[1] Caulfield LE, Richard SA, Black RE (2004) Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old (2004) Am. J Trop Med Hyg 71 (Suppl 2): 55–63.

Scientific breakthrough

Golden Rice has been engineered to contain the genes necessary to make up the biochemical pathway for beta-carotene production.[2] Moreover, the genetic construct was designed to be expressed exclusively in the rice endosperm, ie in the edible part of the seed.[3] A number of lines with different concentrations of beta-carotene have resulted from the work done in the laboratories of Ingo Potrykus and Peter Beyer, in Switzerland and Germany, respectively, with newer developments coming from the company Syngenta. The target of this research is to produce enough beta-carotene in rice to cover the recommended daily requirements.

The *Golden* trait could in principle be directly introduced into many different rice varieties, but because of the stringent biosafety regulation requirements, in the end only one *regulatory clean* event will be used as the starter seed for introgression in multiple breeding programmes.[4]

Reaching out

Golden Rice will be made available to developing countries within the framework of a humanitarian project. This was, from the onset, a public research project designed to reduce malnutrition in developing countries. Thanks to strong support from the private sector and free licences for humanitarian use, the hurdle of extensive intellectual property rights attached to the technologies used in the production of Golden Rice could be overcome. The arrangement opened the way to collaborations with public rice research institutions in developing countries, providing freedom to operate to develop locally adapted Golden Rice varieties.

Once locally developed varieties containing the *Golden* trait have been cleared for biosafety at the national level, they will be made available to subsistence farmers, free of charge. The seed will become their property and they will also be able to use part of their harvest for the next sowing without restrictions. Golden Rice is compatible with the use of traditional farming systems, not requiring additional agronomic inputs. Therefore, no new dependencies will be created. Moreover, as concluded by many experts, the *Golden* trait does not pose any conceivable risk to the environment which would justify delaying its widespread use.

The progress achieved since the scientific initial breakthrough in 1999 would not have been possible without a novel type of public-private-partnership. Thanks to an agreement with Syngenta and other agbiotech industries, Golden Rice is royalty-free for humanitarian use, which, for the purpose of this project, is defined as ‘an annual income generated from the commercialisation of Golden Rice in the range of US$10'000 per farmer, while income beyond that value would require a commercial licence from

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[2] Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, Potrykus I (2002) Golden Rice: introducing the beta-carotene biosynthetic pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutrition 132: 506S-510S.

[3] Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000). Engineering provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287: 303-305.

[4] Hoa TTC, Al-Babili S, Schaub P, Potrykus I, Beyer P (2003). Golden Indica and Japonica rice lines amenable to deregulation. Plant Physiol 133: 161-169.

Syngenta.' Royalty-free humanitarian sublicences are granted by the Golden Rice Humanitarian Board to public rice research institutions. These sublicence agreements ensure that the material is handled according to established biosafety guidelines and regulations, and that the target population—subsistence farmers and the urban poor—receive the material free of charge for the *Golden* trait.

Golden Rice tailored for local consumption

Development of locally adapted Golden Rice varieties and application to national bioregulatory authorities for field testing and deregulation is in the hands of national and international public rice research institutions. To date, the Humanitarian Golden Rice Network includes 16 national institutions in Bangladesh, China, India, Indonesia, South Africa, The Philippines, and Vietnam. The Network is under the strategic guidance of the Golden Rice Humanitarian Board and under the management of a network coordinator, based at the International Rice Research Institute (IRRI), in the Philippines.

The Humanitarian Board is an ad honorem body that benefits from the expertise of international authorities, including Dr Gurdev Khush, retired rice breeder from IRRI (rice breeding); Prof Robert Russell, Laboratory for Human Nutrition, Tufts University Boston (vitamin A malnutrition); Dr Howarth Bouis, Director of HarvestPlus, International Food Policy Research Institute (IFPRI) Washington (biofortification); Dr Gary Toenniessen, The Rockefeller Foundation (food security in developing countries); Dr Robert Bertram, USAID Washington (development in Third World agriculture); Dr Katharina Jenny, Swiss Development Cooperation (technology transfer and trans-sectorial issues); Dr Adrian Dubock, Syngenta (product development and intellectual property rights); Dr Ren Wang and Dr William Padolina IRRI (international cooperation in rice research); Professor Ingo Potrykus (co-inventor), professor emeritus from the ETH Zurich, chairman (public relations and information); Prof Peter Beyer (co-inventor) Univ of Freiburg (scientific advancement in the areas of biofortification for pro-vitamin A and other micronutrients); and the ex officio members Dr Gerard Barry, IRRI (Golden Rice Network Coordinator) and Dr Jorge Mayer, Univ of Freiburg (Golden Rice Project Manager).

Biofortified seeds — a sustainable solution

Biofortification—the genetically based complementation for missing micronutrients—of basic staple crops with the help of genetic engineering is presumably the most sustainable and cost-effective approach to reduce micronutrient malnutrition among poor populations in developing countries. Golden Rice is the first example of such an approach. If we put aside the research investment by Syngenta so far, investment in the public sector has been relatively modest so far (US$2.4 million over nine years). Product development, however, is time-consuming ands requires substantial additional funding. Funds for product development are normally not provided by the public sector since the work involved does not generate predictable discoveries.

While expenses increase even more dramatically when it comes to biosafety assessment—as required for deregulation purposes—once a novel, biofortified variety has been deregulated and handed over to farmers, the system can develop its full potential. From this point on, the technology is built into each and every seed and does

not require any additional investment. Let's consider the potential of a single Golden Rice seed: a single plant will produce in the order of 1,000 seeds; within four generations—or less than two years—that one plant will have generated more than 10^{12} seeds. This represents up to 28-thousand metric tons of rice, which would be sufficient to feed a 100-thousand poor people for one year, and if they were eating Golden Rice they would be automatically supplemented with pro-vitamin A, reducing VAD. This gained protection is cost-free and sustainable. All a farmer needs to benefit from the technology is contained in a seed!

Ignoring the benefits

It took ten years—from 1980 to 1990—to develop the necessary technology to introduce genes into rice. It took another nine years —from 1990 to 1999— to introduce the genes that reconstitute the pathway for pro-vitamin A biosynthesis into the seed. And it took another five years—from 1999 to 2004—to develop Golden Rice. It is taking several more years to advance the first Golden Rice product through the deregulatory process. Considering that Golden Rice could substantially reduce blindness (500'000 children per year) and deaths (2-3 million per year), the parsimony displayed by the responsible bodies after 20 years is hardly understandable.

Notwithstanding the fact that during the last 20 years a vast knowledge base has been accumulated on the production and commercialisation of transgenic plants, the next years will have to be spent on the conduction of the required biosafety assessments to exclude any putative harm by Golden Rice to the environment and the consumer.

The present regulatory practice in a number of countries is based on an overzealous interpretation of the precautionary principle, with little room left for risk management. The position at present is that even the slightest hypothetical risk must be thoroughly tested and might lead to rejection of a registration application. At the same time, potential benefits are being disregarded. Recognised ecologists, including opponents of the technology, have not been able to come up with a realistic hypothetical risk to any agricultural or wild environment stemming from the production and accumulation of beta-carotene in the endosperm of plants which produce high amounts of the same compound in other organs of the plant anyway, and thus will not provide any additional selection advantage to the crop. This shows a substantial level of irrationality in the present system of environmental risk assessment. Despite this fact, the first Golden Rice small-scale field trial took place in the USA, and not in South East Asia, where it should have taken place, the reason being red tape caused by a misunderstood precautionary principle.

An unbearable financial burden

What are the regulatory requirements standing in the way of Golden Rice deployment? First of all, the application should be for a carefully selected, *regulatory clean* transgenic event. Criteria are not necessarily based on scientific grounds; they include a number of requirements pertaining to the introduced genetic construct, eg the inserted DNA fragment should not have undergone multiple integrations or rearrangements, there should be no read-through across the construct borders or any residual *ballast* DNA. This in turn requires the production of many hundreds of transgenic events using the same

DNA construct, from which the regulatory clean event is then selected. The makeup of the construct itself must have been conceived taking into account the requirements imposed by the regulatory authorities. The carefully selected event can then be used to start a series of mandatory biosafety assessment experiments expected to prove or disprove any putative biosafety hazard. The consequence of this approach is that nearly 99% of all transgenic events, and often those with the highest levels of expression, must be discarded. Already, the first step of mass production of many hundreds of similar events and the subsequent destruction of most of them is beyond reach for most public research institutions, in developing as well as in developed countries, and funding agencies are not prepared to take over such costs.

The biosafety assessment starts with event-independent studies, related to the introduced genes and their function, and are valid for all events produced with these genes. These studies are followed by exposure evaluation tests for the novel trait, its intended use and bioavailability, as would be the case for a product like beta-carotene. This study alone takes about three years, because during the pre-field trial phase the materials have to be produced in dedicated plant growth chambers and greenhouses, which is very expensive and production levels are low. Next in line are protein production and equivalence analyses for the proteins encoded by the introduced genes. For this purpose the proteins have to be isolated from the plant, characterised biochemically, and their function confirmed. Further studies include a demonstration of lack of homology to known toxins and allergens, gastric degradation studies, heat stability, acute toxicity tests in rodent feeding experiments.

This all would seem reasonable if it were not for the fact that most people have been eating these genes and their products from a number of other food sources throughout their lives. At one point, somebody even suggested to analyse whether known daffodil toxins had been introduced into Golden Rice along with the daffodil gene used to reconstitute the beta-carotene biosynthetic pathway, which totally lacks scientific basis: what has been transferred is one defined piece of DNA which is analogous to genes in other organisms, and performing the same function, which has no relation to any toxin or allergen. These studies take at least two years of intensive work in a well equipped biochemistry laboratory.

The event-dependent studies are even more cumbersome; they include:

> *Molecular characterisation and genetic stability*: data on single-copy effect; marker gene at same locus; simple integration; Mendelian inheritance, including phenotypic and biochemical evidence for stability over at least three generations; no potential gene disruption; no unknown open reading frames; no DNA transfer beyond borders; no antibiotic resistance gene or origin of replication; insert size limited to the minimum necessary; sequencing of insert and flanking regions.
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> *Expression profiling*: gene expression levels at key growth stages; evidence of seed-specific expression.
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> *Phenotypic analysis*: field performance, typical agronomic traits, yield compared to isogenic lines; pest and disease status must be same as parent (unexpected improvements are not tolerated).

Compositional analysis: data from growing the event over two seasons at six locations in three replicates on proximates, macro and micronutrients, antinutrients, toxins, allergens; data must be generated on modified and isogenic backgrounds.

Environmental risk assessment: this type of analysis takes 4-5 years of work by an entire research team.[5]

It is obvious that no scientist or scientific institution in the public domain has the potential, funding or motivation to perform such lengthy, expensive biosafety experiments. It comes as no surprise then, that virtually all transgenic events that have been carried through the deregulatory process so far are—directly or indirectly—in the private sector and are restricted to high-value crops. Humanitarian projects do not fall into this category, even though they would benefit millions of people.[6] There is a lot of goodwill in the public and in the private sectors worldwide to exploit the potential of green biotechnology for the benefit of the poor. However, without a realistic risk assessment approach, funds for public research will not be capable of doing the trick. Scientific progress would become detached from product development and the population at large would not benefit from progress.

No justification for extreme precaution

There are historic reasons for the present regulatory framework. In the 1970s, when gene technology was still incipient, taking a precautionary approach was sensible, and it was the scientists themselves—who at the time were not working with plants but with human-pathogenic micro-organisms—established regulations based on the premise that the technology could lead to unpredictable genome alterations. More than 20 years of accumulated experience with transgenic plants and their widespread use on over 60 million hectares planted in a number of countries, accompanied by many hundreds of carefully conducted biosafety experiments by prestigious institutions, has led to the conclusion that there is no specific risk associated with the technology beyond that inherent to traditional plant breeding or natural evolution.[7] And yet, we are still facing hard-to-justify calls for further moratoria.

The fact that regulation of transgenic crops has become stricter lately is counter-intuitive. Some people claim that we have to do so to build up trust in the technology with the consumer. However, experience with this strategy over the last 10 years has demonstrated that this approach did not work in Europe and in many developing countries. One reason is that in the general public's perception a highly stringent regulation by the government must be associated with an inherently risky technology.

The guidelines pretend to apply a predictable, risk-based evaluation system based on objective empirical science. The precautionary principle then turns the process into a subjective framework in which the assessment is based on pretend cultural and moral

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[5] It seems rather paradox that after such exhaustive in-depth studies there would still be people arguing that there could be a hidden danger in transgenic crops.

[6] Zimmermann R, Qaim M (2004) Potential health benefits of Golden Rice: A Philippine case study. Food Policy 29:147-168.

[7] For a discussion on the moral imperative of the use of genetically modified crops in developing countries see Nuffield Council on Bioethics, Follow-up Discussion Paper, January 2004, www.nuffieldbioethics.org.

values guided by consumers' fear perceptions. The EU has endeavoured to change the current framework by embedding the precautionary principle into overly stringent health and environment regulations, combined with technical product standards in excess of international standards, and then exporting those regulations and standards abroad down supply chains via international treaties, international standardisation bodies and bilateral technical capacity building initiatives. Examples of this include the recently enacted EU biotech labeling and traceability regulations implemented by the EU obligations under the Cartagena Protocol to the UN Biodiversity Convention, and the proposed EU REACH regulation, which is intended to serve as a template for global chemicals management.[8]

Ideally, we should be able to free the regulatory process from all scientifically unjustified ballast to end up with a set of rational regulatory guidelines. Such a move would require the involvement of institutions and governments which at the moment lack the will to do so or are under undue pressures—many times commercial in nature—that do not allow them to proceed along these lines. Developing countries must make decisions under duress about the adoption of transgenic technologies, being caught in a conundrum between the urgent need to adopt the technology and possible commercial repercussions by doing so. In essence, the EU exports the high regulatory and standardisation costs abroad, resulting in the buildup of de facto trade barriers.

Gene technology has been endorsed by international agencies, such as FAO and UNIDO, to help solve food security problems in developing countries, but yet we are threading at a very low pace. The highest price for the non-adoption of green gene technology is being paid by those voiceless persons who most need it. The great potential of gene technology to reduce hunger and malnutrition and to help protect the environment will only be attained once regulatory frameworks are based on scientific evidence and a proper risk-benefit analysis. Until then the technology will be restricted to "luxury projects", with safe financial returns for the private sector and mostly located in developed countries.

Every year more countries in the developing world don't give in to the fuzzy arguments of opponents and a number of them have started embracing the technology based on hard scientific, health and economic facts. Positive, highly encouraging reports on increased harvests, reduced use of pesticides, a decrease in the number of people intoxicated from using those pesticides and an increase in the number of beneficial insects in the fields are now coming from countries like South Africa and India. For Golden Rice, the latest World Bank report, presenting an ex ante analysis on the potential socioeconomic impact of the adoption of transgenic technology, is particularly encouraging.[9]

Traditional genome meddling

Green gene technology has the potential to support and complement traditional plant breeding. One criticism frequently brought up in relation with genetic engineering is that the insertion of genes can lead to unpredictable genome alterations. In traditional plant

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[8] Kogan L, The National Interest Journal, Number 77, Fall 2004; www.nationalinterest.org.

[9] Genetically Modified Rice Adoption: Implications for Welfare and Poverty Alleviation
K Anderson, LA Jackson, C Pohl Nielsen. World Bank Policy Research Working Paper 3380, August 2004.

breeding, agronomic traits are combined or eliminated by crossing, followed by selection. In plant breeding, existing varieties and landraces are used as starting materials. Many different traits selected for breeding purposes since the inception of agriculture are based on spontaneous, unpredictable mutations. In the course of traditional breeding, which may include wild relatives of crop plants, many unpredictable genome alterations, such as recombinations, translocations, deletions, inversions and horizontal gene transfer, are combined into a new cultivar. These unpredictable, significant genome alterations accumulate at every breeding step and each new, traditionally bred variety is the result of an increasing array of such genome alterations. This statement is valid for all modern crop varieties, including those used in organic farming. Nevertheless, while none of these [*genetically modified*] varieties has ever been assessed for biosafety, mankind has consumed them unharmed and the environment has not been affected by them either. The fact is that actually nobody could survive without eating food from these *genetically modified* crops.

By comparison, the creation of Golden Rice—involving the insertion of two precisely defined genes into a genome that contains fifty-thousand-odd-genes—is by several orders of magnitude more precise than traditional breeding. Why should this variety, despite the fact that the modification is extremely small and exactly defined, be the subject of a comparatively over-the-top scrutiny?

Assuming responsibility

Green biotechnology has the potential to provide solutions to pest and disease control, improve photosynthetic efficiency, nutritional content, furnish plants with adaptation mechanisms for heat, cold and salt tolerance and many more things to come. The benefits of Golden Rice are clear at face value, yet opponents of the technology are posing as saviours of humanity. The blind and the dead are not at risk, they are a reality we have to face in the eye; will any of the opponents of the technology take the responsibility for this preventable tragedy that is being imposed upon innocents?

The Nuffield Council on Bioethics[10] concluded that '[t]he European Union is ignoring a moral imperative to promote genetically modified crops for their great potential for helping the developing world', and '[w]e believe EU regulators have not paid enough attention to the impact of EU regulations on agriculture in developing countries.'

[10] The Use of Genetically Modified Crops in Developing Countries. Nuffield Council on Bioethics, January 2004.