The Golden Rice "Tale"[1]

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Golden Rice is, to date, a popular case, supported by the scientific community, the agbiotech industry, the media, the public, the Consultative Group on International Agricultural Research (CGIAR), the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), official developmental aid institutions, etc, but equally strongly opposed by the opponents of genetically modified organisms (GMOs). The first group likes Golden Rice because it is an excellent example of how genetic engineering of plants can be of direct benefit to the consumer, especially the poor and the disadvantaged in developing countries, where GMOs offer many more opportunities for the improvement of livelihood than for those living in well-fed developed nations. The GMO opposition, however, is concerned that Golden Rice will be a kind of "Trojan Horse", opening the developing countries to other applications of GMO technology, and for improving acceptance of GMO food. Indra Vasil persuaded me to write the Golden Rice Tale because the background behind this success, which is embedded in numerous failures and obstacles, and which covers the entire history of the development of plant genetic engineering, might be of interest to those who are faced with the numerous specific problems of strategic research, where the target is set at the outset, where no attractive alternatives to existing academic questions are available, where success is measured in relation to the original target, and not in relation to possible attractive academic solutions.

Motivation and technique development (1972 to 1987)

My scientific career and my interest in "genetic engineering" began in 1970 with the first protoplast experiments with *Petunia* in the laboratory of Professor D Hess in Stuttgart-Hohenheim, Germany. We regenerated fertile plants from mesophyll protoplasts (Durand et al. 1973), introduced isolated nuclei (Potrykus and Hoffmann 1973) and chloroplasts (Potrykus 1973) into protoplasts, and treated protoplasts with naked DNA in an attempt to transfer genes (Hess et al., 1973). In one exciting experiment we used DNA from a dominant red-flowering pure line of *Petunia* to transform protoplasts of a recessive white flowering pure line. We expected pink-flowering plants in case of success. When we finally recovered a greenhouse full of pink-flowering plants, we realized that something had gone wrong. As far as we could reconstruct, we had taken leaves for protoplast isolation from a population of young heterozygous plants that were grown in the same greenhouse to take advantage of the heterozygous state for anther culture experiments. We fortunately had not published, but on the basis of this experience I was very skeptical when Peter Carlson reported about his famous *N. glauca* x *N. langsdorfii* "somatic hybrids". Already at that time (1972) there were claims (from those working with tobacco and petunia) that the new technology would contribute to food security in developing countries. Obviously, to contribute, one would have to *work* with important crop plants and not only talk about them. Even at the peak of success of the Green Revolution it was clear that feeding the exploding population in developing countries would require intensive new scientific research. I therefore began in 1973 to work towards the development of the new technology for cereals (beginning with barley) and tried to repeat what had been so easy with *Petunia*. Our efforts gained the attention of the late Professor G. Melchers who arranged for the opportunity to establish a small research group at the Max-Planck-Institute for Plant Genetics in Ladenburg/Heidelberg. With Emrys Thomas and Gerd

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Wenzel I had enthusiastic colleagues and with Horst Lörz and Christian Harms very motivated graduate students. We all were focusing on cereal tissue and cell culture of wheat, maize, barley, and rye (Potrykus et al., 1976, 1977, 1979). However, a mesophyll protoplast regeneration system could not be developed despite the fact that I challenged, with a most sophisticated microdrop array cell culture protocol, more than 120,000 protoplast culture conditions including up to 7-factor gradient mixtures of all known growth substances in a wide range of concentrations (Potrykus et al., 1978). The best I could achieve with wheat mesophyll protoplasts was the formation of ca. 60-celled "globular proembryos", which, however, refused to develop further. This cereal work found the attention of the Agrodivision of Ciba-Geigy which intended to complement the Pharma-oriented research in the recently established Friedrich Miescher-Institute (FMI) in Basel, Switzerland (a foundation for basic research) with agrobiotechnology-oriented research (yes, already in 1975!). The institute offered me in 1976 the task of establishing three Plant Biology groups, which I tried to coordinate around genetic engineering, mutagenesis, and haploids of cereals with my colleagues Patrick King and Emrys Thomas. As the mesophyll protoplast approach remained very recalcitrant, we studied the loss of competence during the course of leaf differentiation. We found that beyond the basal 3 mm of a young cereal leaf cells are terminally differentiated. We thus looked for alternatives such as somatic embryos inducible from the basal leaf segments, especially effective in *Sorghum* (Wernicke et al., 1981, 1982), and the re-meristematizing response of maize tissue to *Ustilago maydis* infection.

At the beginning of the 1980s it became evident that the crown gall tumor was based on a natural transformation process. Not surprisingly, many laboratories focused on the development of a transformation protocol based on T-DNA transfer. As this was based on a wound-response leading to a wound-meristem to allow for the proliferation of T-DNA transformed cells, and as we knew that graminaceous species have a wound response leading to death of wound-adjacent cells, we could not believe in a future for *Agrobacterium* and cereals (Potrykus 1990, 1991). Consequently, we were focusing on the development of a vector-independent transformation system: "direct gene transfer" via incubating protoplasts in DNA. This was strongly supported through two hard-core molecular biologists who joined the FMI: Barbara and Thomas Hohn were attracted by the scientific potential of the new research area in the institute. This close collaboration between the tissue culture specialists and the molecular biologists soon produced the *Agrobacterium*-independent transformation technique (Brisson et al., 1984, Paszkowski et al., 1984, Schocher et al., 1986). Instrumental in this was Jerszy (Jurek) Paszkowski who joined my group as a fresh PhD from Warsaw (Poland) and who was the perfect link between the two groups. Only half a year after the first *Agrobacterium*-mediated tobacco was reported we could publish the first "direct gene transfer"-derived tobacco. However, this was tobacco and not any cereal. It took two more years to produce the first transgenic maize cell culture, but this then was a non-morphogenic cell line (Potrykus et al., 1985). The next breakthrough came from Indra Vasil's concept of preventing differentiation in cereals by establishing "embryogenic suspensions". This eventually led to the development of the embryogenic callus-suspension culture-protoplast systems for cereals, which as shown later, played a critical role in the production of transgenic cereals (Vasil 1999). Attempts to transform embryogenic cultures with *Agrobacterium* did not yield convincing results. However, this was no longer necessary because by then John Sanford and Ted Klein had invented the "crazy" biolistic transformation method (Sanford 2001), which was used successfully for the regeneration of transgenic plants in tobacco, cotton, etc. Embryogenic suspensions were the ideal material for biolistic treatment and it was to be expected that, with the necessary effort, it would produce transgenic cereals. Embryogenic suspensions were, however, also the only source of totipotent protoplasts of cereals (Vasil and Vasil 1992). We chose this approach for our work. At the end of 1985, I was offered a full professorship at the Swiss Federal Institute of Technology (ETH) in Zürich.

I was responsible for building, together with the professor in crop physiology, Josef Nösberger, a new institute, combining both basic and applied research. This Institute of Plant Sciences was the ideal setting for my intentions (which increasingly focused on the development and use of genetic engineering technology to contribute to food security in developing countries), and it provided longer range independent and stable financing for continued approaches to genetic engineering of cereals. At the same time Swapan Datta joined my group and this was the beginning of our work with rice and an important turning point.

Focusing on rice as the outstanding food security crop (1987-1999)

Swapan Datta was accompanied by his talented wife Karabi. Swapan had joined me to learn from our protoplast experience but I was following at that time another idea. As the embryogenic response was rather genotype-dependent and as I wanted a generally applicable technique, I decided to challenge early sexual embryos down to isolated zygotes. For transformation I wanted to optimize the micro-injection technique (Kost et al., 1995, Lusardi et al., 1994). I convinced two scientists with exciting experience in microinjection (Gunther Neuhaus and German Spangenberg) to join me in Zürich for this purpose, and I invested the entire group, except for Jerszy Paszkowski who was free to work with his group on homologous recombination (Paszkowski et al., 1988, Baur et al., 1990), on the isolation, culture and microinjection of sexual proembryos from wheat, maize, rice, and later *Arabidopsis*. After many years of enormous effort, we found much to our disappointment that the microinjection system worked fine with protoplasts, but was extremely bad with cells surrounded by a cell wall. With Christof Sautter, we also focused on the development of microtargeting as a further genotype-independent transformation technique (Sautter et al., 1991, 1995, Leduc et al., 1994), which too proved to be ineffective for routine transformation. It was fortunate, therefore, that Swapan Datta asked for permission to work part-time (over the weekends) on the embryogenic protoplast transformation approach. And the Dattas made it! There came hygromycin-resistant rice, the first transgenic Indica rice (Datta et al., 1990, 1992, Peterhans et al., 1990, Linn et al., 1995), all from embryogenic protoplasts and direct gene transfer. Swapan introduced me to Gary Toenniessen and the Rockefeller Foundation, which by that time had already spent considerable funds in the Rice Biotechnology Program on colleagues many of whom were not really using these funds for rice work. By 1990 we were also receiving Rockefeller funding (for development of Indica rice transformation protocol) and producing the first insect-resistant Indica rice (Wünn 1996). We had been unsuccessfully using wild type Bt and it took us one year of bargaining until we were allowed to use the synthetic Ciba-Geigy gene. At the beginning of 1990 I had also learned that "food security for developing countries" not only had a quantity aspect, but also a quality component. The major malnutritions were identified as iron > iodine > vitamin A, and this was the beginning of the Golden Rice adventure, and another major turning point.

The problem of iron- and vitamin A-deficiency and traditional solutions

Iron deficiency anemia (IDA) is one of the most common and serious human nutritional disorders in the world. This malnutrition affects more than two billion humans, predominantly women and children. Consequences are millions of birth-related deaths of mothers and children. It impairs physical and intellectual development, the immune system, and general fitness of people of all ages. In infants and young children even mild anemia can impair intellectual development. Anemia in pregnancy is an important cause of maternal mortality, increasing the risk of hemorrhage and sepsis during childbirth. Infants born to anemic mothers often suffer from low birth weight and anemia themselves. An inadequate dietary iron intake

is the main cause of IDA. According to UNICEF, nearly two billion people are estimated to be anemic and about double that number, or 3.7 billion are iron deficient, the vast majority of them women. In Africa and Asia UNICEF estimates that IDA contributes to approximately 20 per cent of all maternal deaths.

Each year more than one million VAD (vitamin A deficiency) associated childhood deaths occur. And, according to the World Health Organization, as many as 230 million children are at risk of clinical or subclinical VAD, a condition which is largely preventable. VAD makes children especially vulnerable to infections and worsens the course of many infections. Supplementation with vitamin A is estimated by UNICEF to lower a child's risk of dying by approximately 23 percent. VAD is also the single most important cause of blindness among children in developing countries, about 500,000 per year.

Rice plants do not produce carotenoid compounds in the grain as consumed by humans. Consequently VAD often occurs where rice is the major staple food. The amount of bioavailable iron is dependent both on the level of dietary iron consumption and on iron absorption during the digestive process. Dietary iron in developing countries consists primarily of non-heme iron of vegetable origin, whose poor absorption is considered a major factor in the etiology of iron deficiency anemia. Also legume staples and grains, including rice, are high in phytic acid, which is a potent inhibitor of iron absorption. Foods that enhance non-heme iron absorption, such as fruits and vegetables rich in ascorbic acid, are often limited in developing countries. Heme iron, which is relatively well absorbed by the human intestine, is found primarily in foods containing blood and muscle. Due to their expense and lack of availability, heme iron-rich foods are often a negligible part of a typical developing country diet.

Interventions to reduce both IDA and VAD involve so far (a) supplementation (eg distribution of vitamin A capsules), (b) food fortification (eg adding iron to wheat flower), and (c) dietary education and diversification. In a FAO/WHO World Declaration on Nutrition (1992) the following strategy has been advocated: "Ensure that sustainable food-based strategies are given first priority particularly for populations deficient in vitamin A and iron, favoring locally available foods and taking into account local food habits." "Supplementation should be progressively phased out as soon as micronutrient-rich food-based strategies enable adequate consumption of micronutrients." And Per Pinstrup-Andersen, Director General of the International Food Policy Research Institute, has pointed out that a sustainable solution of the problem will come only when it will be possible to improve the content of the missing micronutrients in the major staple crops. This was exactly what we were trying to achieve. As the necessary genes for such an improvement were not available in the rice gene pool, genetic engineering was the only technical possibility. As rice endosperm did not contain any provitamin A, the task was to introduce the entire biochemical pathway. As rice endosperm contains very little iron and considerable amounts of a potent inhibitor of iron resorption, and as resorption from a vegetarian diet is generally poor, the task was to increase the iron content, reduce the inhibitor content, and add a resorption-enhancing factor.

Solving the scientific problems: Golden Rice (1992 to 1999)

Peter Burkhardt joined my group in 1991 for a PhD thesis work and it was not difficult to motivate him for the provitamin A project. I approached Nestlé, the world's biggest food company, for funding but the company was (fortunately) not interested. This was "fortunate" in retrospect because it kept the project open for public funding with its important consequences for later free distribution to developing countries. With Peter Beyer at the nearby University of Freiburg, Peter Burkhardt found the ideal scientific supervisor, and I found a perfect partner. Peter Beyer was studying the regulation of the terpenoid pathway in

daffodil and was working on the isolation of those genes we would need to establish the pathway in rice endosperm. We approached the Rockefeller Foundation for funding and Gary Toenniessen responded with the organization of a brainstorming session in New York (1992). Many of the participants thought that such a project did not have much chance of success, but because of its potential importance it was worth trying. Peter Burkhardt found out that the last precursor of the pathway in endosperm was geranlygeranyl-pyrophosphate and consequently, theoretically it should be possible to reach beta-carotene via four enzymes: phytoene synthase, phytoene desaturase, zeta-carotene desaturase, and lycopene cyclase. There were hundreds of scientific reasons why the introduction and coordinated function of these enzymes could not be expected to work, and that it may cause many problematic side effects. Those with the necessary scientific knowledge were right in not believing in the experiment. When we finally had Golden Rice I learned that even my partner, Peter Beyer and the scientific advisory board of the Rockefeller Foundation, except for Ralph Quatrano, had not believed that it could work. This exemplifies the advantage of my ignorance and naivete: with my simple engineering mind I was throughout optimistic, and therefore, carried the project through, even when Rockefeller stopped funding Peter Beyer's group.

Altogether it took eight years but the first breakthrough came when Peter Burkhardt recovered phenotypically normal, fertile, phytoene synthase-transgenic rice plants, which produced good quantities of phytoene in their endosperm (Burkhardt et al., 1997). This demonstrated two important facts: it was possible to specifically deviate the pathway towards beta-carotene, and channeling a lot of GGPP away from the other important pathways had no severe consequences on the physiology and development of plants. This success encouraged me to motivate another PhD student, Paola Lucca, with an MS in pharmacy, to work on the problem of iron deficiency. More on this later, when the provitamin A story has been completed. The next gene to follow was phytoene desaturase, and this caused problems for more than a year. Peter Burkhardt could obtain only heavily distorted transgenic plants. As he left the lab I transferred the continuation of the project to my postdoc Andreas Klöti, who had done excellent work towards engineering RTBV tungro disease resistance (Fütterer et al., 1997, Klöti et al., 1999) and gene silencing, and was happy about an "easier" task. Andreas continued with single gene transformations and the concept was to combine the genes via crossing. We had used biolistic transformation of embryogenic suspensions and precultured immature embryos and had the typical complex integration pattern, and this caused problems with gene stability and plant fertility. When we finally had transgenic plants for all genes separately, we were able to combine genes pairwise but all this did not look too promising. By that time Andreas left the lab and the project was transferred to Xudong Ye, who had done a PhD in forage grass biotechnology in my group, under the supervision of German Spangenberg (Takamizo et al., 1992; Wang et al., 1992). Xudong had survived a tough training and he had learned that success in strategic research may require hard work. Xudong wanted to invest only one year because he had plans to go to the US. He analyzed the situation and decided, after discussions with Salim Al-Babili from Peter Beyer's group and our man behind many constructs, and Andreas, to try a radical change in the approach: (a) change from biolistic to *Agrobacterium*-mediated transformation, (b) use the *Erwinia* double desaturase (*crtI*), and (c) introduce all genes together in a single co-transformation experiment. Xudong recovered ca. 500 independent transgenic lines. As our glasshouse had space for only 50 of them he discarded 450 and grew the 50 best looking ones to maturity. Peter Beyer polished the seeds, analysed them with HPLC, took beautiful photographs and presented them to me at the farewell symposium I had organized on 31 March 1999, the date I had to retire because I had passed the age limit. At this symposium Xudong Ye presented the results for the first time to the public: the endosperm contained good quantities of provitamin A, beautifully visible as "golden" color of different intensity in different lines. The best provitamin A line had 85% of its carotenoids as beta-carotene. Other lines had less beta-

carotene, but interesting levels of lutein and zeaxanthin, both substances of nutritional importance because they have positive effects with regards to macula degeneration (Ye et al., 2000).

Development of high iron rice (1995-2000)

At the same farewell symposium Paola Lucca reported about her "high iron rice". In respect of rice as the major staple there are three key problems: (a) it contains the smallest amount of iron among all major food crops, (b) phytate, the phosphate storage for seed germination, is an extremely efficient inhibitor of iron resorption (up to 98% of available iron can be blocked), and (c) resorption from a vegetarian diet is rather poor. Our scientific advisor for the project was Richard Hurrell, ETH professor for human nutrition, with specialization in iron nutrition. Paola developed an experimental approach to address all three of the above problems. Knowing that only 5% of the iron in the rice plant is in the seed she created a sink for iron storage in the endosperm by expressing a ferritin gene from *Phaseolus* (our request for funding was turned down with the argument that we better study iron uptake into the rice plant!). This led to a 2.5-fold increase in endosperm iron content. As feeding studies with peptides from muscle tissue had shown that cystein-rich polypeptides enhance iron resorption, Paola expressed an appropriate gene, a metallothionin-like gene from *Oryza,* and achieved a 7-fold increase in endosperm cystein. As it appeared unwise to interfere with phosphate storage (in the form of the iron resorption inhibitor phytate) prior to germination, Paola decided to approach inhibitor degradation after cooking. Thanks to the permission from Hoffmann LaRoche, Basel, we could use a thermotolerant mutant of a phytase from *Aspergillus fumigatus*, which refolded to 80% activity after 20 minutes at 100ºC. To prevent activity which could interfere with germination, the enzyme was excreted into the extracellular space. One transgenic line expressed the phytase to levels 700-fold higher than endogenous phytase. In small intestine simulation experiments the phytase degraded phytate to zero levels within one hour at 37ºC. However, much to everybody's surprise, in the transgenic situation the enzyme did not refold properly after cooking and had lost it's thermotolerance. New transgenic plants are meanwhile maturing, where the enzyme has been targeted to the phytase storage vesicles to reduce the phytate content directly. With the experience meanwhile available from low phytate mutants, we hope that this will not too much affect germination. The three "iron genes" are combined with the "provitamin A genes" by crossing. Vitamin A supply is strategy No 4 against iron deficiency, as it has been shown that vitamin A deficiency indirectly affects iron resorption (Lucca et al., 2001).

Attaining public recognition (2000)

The vitamin A rice project was considered a scientific breakthrough because it was the first case of pathway engineering, and it represented considerable technical advancement. We felt it also was a timely and important demonstration of positive achievements of the GMO technology. GMO technology had been used to solve an urgent need and to provide a clear benefit to the consumer, and especially to the poor and disadvantaged. To make the information available to a wider audience for a more balanced GMO discussion, we submitted the manuscript to Nature with a cover letter explaining its importance in the present GMO debate. The Nature editor did not even consider it worth showing the manuscript to a referee and sent it back immediately. Even supportive letters from famous European scientists did not help. From other publications in Nature at that time we got the impression that Nature was more interested in cases which would rather question instead of support the value of genetic engineering technology. Fortunately, Peter Raven (Missouri Botanical Garden, St. Louis, MO,

USA), had heard about Golden Rice, and asked for more information, and invited me at the last minute to present the work at the XVI International Botanical Congress, August 1999 in St Louis, Missouri. He also took care of a press conference and encouraged Science to look at the manuscript. Science was interested in publishing both the pro-vitamin A as well as the iron work in a single publication, but the space it could provide was too narrow for both (Ye et al. 2000). The iron-rice publication is soon coming in Theoretical Applied Genetics (Lucca et al. 2001). The press conference in St Louis, the presentation at the Nature Biotechnology Conference in London, the Science publication with the commentary (Guerrinot 2000) and the feature story in TIME Magazine all led to an overwhelming coverage of the Golden Rice story on TV, radio, and in the international press. A simple example, however, illustrates the difference in attitude between Europe and the rest of the world. When the feature story came out in TIME Magazine (31 July 2000) it was planned that it would appear in the European edition the following week. It did not show up until 12 November 2000.

The challenge of donating a GMO to poor countries (1999 to open-ended)

Golden Rice was developed for the vitamin A-deficient and iron-deficient poor and disadvantaged in developing countries. To fulfil this goal it has to reach the subsistence farmers free of charge and restrictions. Peter Beyer had written up a patent application and the inventors, Peter and myself, were determined to make the technology freely available. As only public funding was involved this was not considered too difficult. The Rockefeller Foundation had the same concept, the Swiss Federal Institute of Technology supported it, but the European Commission had a clause in it's financial support to Peter Beyer, stating that industrial partners of the "Carotene Plus" project, of which our rice project was a small part, would have rights on project results (The IVth and Vth framework of EU funding forces public research into coalitions with industry and thus is responsible for two very questionable consequences: Public research is oriented towards problems of interest to industry, and public research is losing its independence). We did not consider this too big a problem because the EU funding was only a small contribution at the end of the project. But we realized soon that the task of technology transfer to developing countries, the international patent application, and the numerous Intellectual Property Rights (IPRs) and Technical Property Rigths (TPRs) we had used in our experiments, were too difficult to be handled properly by two private individuals. We urgently (because of the deadline of the international patent application) needed a powerful partner. In discussions with industry the definition of "subsistence farmer" and "humanitarian use" was the most difficult problem to be solved. We wanted a definition as generous as possible, because we not only wanted the technology to be free for small-scale farmers, but we also wanted to contribute to poverty alleviation via local commercial development. Very fortunately the company which agreed to the most generous definition was also the company which had legal rights because of its involvement in the EU project. This facilitated the agreement, via a small licensing company (Greenovation), with Zeneca. Zeneca received an exclusive licence for commercial use and in return supports the humanitarian use via the inventors for developing countries. The cut-off line between humanitarian and commercial use is US$ 10,000 income from Golden Rice. This agreement also applies to all subsequent applications of this technology to other crop plants. It turned out that our agreement with Zeneca and the involvement of our partner in Zeneca, Adrian Dubock, was a real asset to the development of the humanitarian project. He was very helpful in reducing the frightening number of IPRs and TPRs and he organized most of the free licences for the relevant IPRs and TPRs such that we are now in the position of having reached freedom to operate for public research institutions in developing countries to go ahead with breeding and de novo transformation into best adapted local varieties. Publicity sometimes can be helpful: only a few days after the Golden Rice story had appeared on the cover of TIME Magazine, I

had a phone call from Monsanto offering free licences for the company's IPR involved. A really amazing quick reaction of the PR department to make best use of this opportunity.

Making best use, not fighting patents helps the poor and underprivileged

At this point it is appropriate to add a more general comment on patents and the heavy opposition against patenting in life sciences. As we did not know how many and which intellectual property rights we had used in developing Golden Rice, and as further development for the humanitarian purpose required freedom to operate for the institutions involved, The Rockefeller Foundation commissioned an IPR audit through ISAAA. The outcome was shocking (ISAAA briefs No 20-2000). There were 70 IPRs and TPRs belonging to 32 different companies and universities, which we had used in our experiments and for which we would need licences to be able to establish a freedom-to-operate situation for our partners, who were keen to begin further variety development. As I was in addition blocked by an unfair use of a material transfer agreement, which had no causal relation with the Golden Rice development, I was rather upset. It seemed to me unacceptable, even immoral, that an achievement based on research in a public institution and with exclusively public funding, and designed for a humanitarian purpose, was in the hands of those who had patented enabling technologies early enough or had sneaked in an MTA in the context of an earlier experiment. It turned out that whatever public research one was doing, it was all in the hands of industry (and some universities). At that time I was much tempted to join those who radically fight patenting. Fortunately, I did a bit further thinking and became aware that Golden Rice development was only possible because there was patenting. Much of the technology I had been using was publicly known because the inventors could protect their right. Much of it would have remained secret if this had not been the case. If we are interested in using all the knowledge to benefit the poor, it does not make sense to fight against patenting. It makes far more sense to fight for a sensible use of intellectual property rights. Thanks to the public pressure there is a lot of goodwill in the leading companies to come to an agreement on the use of IPR/TPR for humanitarian use which does not interfere with commercial interests of the companies. There was a recent satellite meeting in context with the World Food Prize Symposium 2000 at Des Moines, Iowa, which led to agreements on this subject between all participants, including major agbiotech companies (for more information contact C.S.Prakash; e-mail: prakash@acd.tusk.edu).

The challenge of safe technology transfer (2000 to open-ended)

Having solved the scientific problems and having achieved freedom to operate leaves us with technology transfer as the next hurdle. This is a far bigger task than anyone without personal experience should assume. Golden Rice is of course a GMO and this fact is sufficient to cause a series of further problems. All care has to be taken that it is handled according to established rules and regulations (where these do not exist, they have to be established). And, of course, GMOs are faced with emotional and irrational opposition. Rational concerns and questions are taken care of by the established regulations. Let us focus first on safe technology transfer. Again, we realized that we needed help, because this task is beyond the capabilities of a retired professor (a private person) and an already overworked associate professor with no infrastructure and heavy teaching load. We established a "Golden Rice Humanitarian Board" to help make the right decisions, and to have secretarial support. Our decision to work with Zeneca was extremely helpful. Adrian Dubock was willing to provide support for a secretary. We have additional invaluable help from Katharina Jenny from ISCB (Indo-Swiss Collaboration in Biotechnology), an institution jointly financed by the Indian Department of Biotechnology (DBT) and the Swiss Development Corporation. Golden Rice will be

introduced into India in the established organizational framework of ISCB, which has ten years of experience in technology transfer. Thanks to this situation and thanks to the strong commitment of the DBT and the Indian Council for Agricultural Research (ICAR), India will take a leading role and can serve as a model for other countries. The project starts with a careful needs assessment, analyzing and comparing pros and cons of alternative measures and setting a framework for optimal and complementary use of Golden Rice. Of course, there will be bioavailability, substantial equivalence, toxicology, and allergenicity assessments and we are grateful for offers from specialists to help. Careful socioeconomic and environmental impact studies will help to avoid possible risks and make sure that the technology indeed reaches the poor. Care will be taken that the material is given only to institutions which ensure proper handling, according to rules and regulations. Traditional breeding will be used to transfer the trait into locally adapted lines, and again will make sure that varieties important to the poor will be used, and not fashionable varieties for the urban middle class. There will be also direct de novo transformation into important varieties, and this will be done with mannose selection (Lucca and Potrykus, 2001). So far, Golden Rice has a hygromycin resistance gene, and as it has been introduced via co-transformation it is possible to separate it from the pro-vitamin A trait. All this costs a lot of money, which should not affect the free distribution to subsistence farmers. Fortunately, probably the World Bank, ICAR and DBT will share the costs for this development in India. Agreements have been established with several institutions in Southeast Asia, China, Africa, and Latin America and as soon as the a written confirmation of the freedom-to-operate is in the hands of the "Humanitarian Board", the material will be transferred.

The challenge of the GMO opposition (2000-open)

Golden Rice fulfils all the wishes the GMO opposition had earlier expressed in their criticism of the use of the technology, and it thus nullifies all the arguments against genetic engineering with plants in this specific example.

- Golden Rice has not been developed by and for industry.
- It fulfils an urgent need by complementing traditional interventions.
- It presents a sustainable, cost-free solution, not requiring other resources.
- It avoids the unfortunate negative side effects of the Green Revolution.
- Industry does not benefit from it.
- Those who benefit are the poor and disadvantaged.
- It is given free of charge and restrictions to subsistence farmers.
- It does not create any new dependencies.
- It will be grown without any additional inputs.
- It does not create advantages to rich landowners.
- It can be resown every year from the saved harvest.
- It does not reduce agricultural biodiversity.
- It does not affect natural biodiversity.
- There is, so far, no conceptual negative effect on the environment.
- There is, so far, no conceivable risk to consumer health.
- It was not possible to develop the trait using traditional methods.

Optimists might, therefore, have expected that the GMO opposition would welcome this case. As the contrary is the case, and GMO opposition is doing everything to prevent Golden Rice reaching the subsistence farmer, we have learned that GMO opposition has a hidden, political agenda. It is not so much the concern about the environment, or the health of

the consumer, or the help for the poor and disadvantaged. It is a radical fight against a technology merely for political success. This could be tolerated in rich countries where people lead a luxurious life, even without the technology. It can, however, not be tolerated in poor countries, where the technology can make the difference between life and death, and health or severe illness. In fighting against Golden Rice reaching the poor in developing countries, GMO opposition has to be held responsible for the foreseeable unnecessary death and blindness of millions of poor every year.

Opportunities stemming from remaining scientific challenges (the future)

My retirement came too early. We have been working on challenges which might be worth being taken up by other institutions. We have been working on projects to rescue lost harvests and have been successful with insect pest resistance, had success with wheat (Clausen et al., 2000) with fungal resistance, but not with rice despite thousands of transgenic rice lines with genes for most peptides with antifungal effects. We had also no rice lines resistant to RTBV tungro disease despite excellent research of the group of Johannes Fütterer over more than eight years. We were also interested in better exploitation of natural resources and here are two projects I would continue, if the necessary resources were available: We convinced ourselves that engineering of C4 photosynthesis is feasible. We do not think that simple transfer of one or two genes from maize is going to do it. However, we know now that rice (and wheat) have the necessary Krantz anatomy with bundle sheath cells surrounded by mesophyll cells, and we have bundle sheath-specific and mesophyll-specific promoters that work in rice as a basis to engineer the appropriate enzymes cell-specifically to hopefully establish the crucial CO_2 gradient. We also have been working towards nitrogen fixation and our approach was to engineer the *nif*-regulon into the chloroplast genome, maintaining its operon structures and regulating it cell-specifically in such a way that it is activated from its natural regulator gene placed separately into the nuclear genome. Expression would be in the tissue with the lowest possible oxygen tension, the cortex, and the signal to start expression would come from the signal which starts degradation of the photosynthetic apparatus to provide nitrogen to cover the protein requirements during the grain-filling period. The idea would not be to make rice totally independent of external supply of nitrogen, but to provide additional nitrogen during grain filling by rescuing the photosynthetic apparatus for a longer periode of time. To be able to do so we need chloroplast transformation in rice and I had, therefore, established an entire group with Roland Bilang, which did excellent work over five years, but could not establish a functional protocol. And despite an existing publication in Nature Biotechnology, I have the impression that nobody has, so far, such a protocol. As plastid transformation in meristematic or embryogenic cells poses far bigger problems than with chloroplasts in mesophyll cells, I was still interested in the old problem of cereal mesophyll protoplast culture. And, surprisingly, RV Sairam convinced me that this was possible in principle by repeating his work from ICRISAT with *Sorghum* mesophyll protoplasts in my laboratory (Sairam et al., 2000); he could, however, not establish a system efficient enough to be used for chloroplast transformation.

Epilogue

Golden Rice was possible because

- I had stable, public funding over a long period of time, which I could use independent of the opinion of others.
- In Peter Beyer I had the perfect partner, who understood the underlying science and provided the necessary genes and analytical expertise.
- The Rockefelller Foundation was willing to add substantial financial support over a long period of time (special thanks to Gary Toenniessen and Ralph Quatrano).
- The Swiss Federal Institute of Technology supported the concept of strategic research for developing countries.
- The project was supported by an enthusiastic group of coworkers (over 60), all motivated to contribute to food security with their work.
- I was naive enough to believe in its success.

Golden Rice hopefully helps

- to achieve better acceptance of GMO technology,
- to encourage scientists and granting agencies to invest also into projects with no a priori guaranteed success,
- to motivate public research to care more for the problems of food security and less for additional funds from industry,
- to encourage those who have rights in key enabling technologies to make free licences available for humanitarian projects,
- for some scientists to consider that there can be more in a scientific career than the chance for impact factor points, and
- to have some GMO opponents consider whether a differentiated discussion of the GMO technology might not be the better strategy in the long run.

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