Experience with the ‘Humanitarian Golden Rice‘ project has shown that ‘extreme precautionary regulation‘ - not IPRs - prevents use of the GMO potential to the benefit of the poor, and that the public domain is incompetent and unwilling to deliver products.
Vitamin A Deficiency and Rice

The problem:
Rice as major staple does not contain any pro-vitamin A.

The consequences:
400 million rice-eating poor suffer from vitamin A deficiency.
6,000 die per day, 500,000 become blind every year.

The transgenic concept:
Introduce, under endosperm-specific regulation, all genes necessary to establish the biochemical pathway.

Why genetic engineering in addition to the traditional interventions?
The genetic basis in the rice gene pool does not offer a basis for a conventional approach.
The majority of the vitamin A deficient depend upon rice which totally lacks provitamin A and is poor in other micro-nutrients such as iron, zinc, and essential amino acids.

In developing countries 500,000 children per year go blind and up to 6,000 per day die from vitamin A malnutrition.

And this will continue year by year, if we do not complement traditional interventions.

‘Biofortification‘ – improvement of the micronutrient content of crops on a genetic basis – can reduce malnutrition in a cost-effective and sustained manner.
Engineering the provitamin A pathway in rice endosperm

Enzymes not active in the endosperm of rice.

Provitamin A Golden Rice

\[
\begin{align*}
&\text{IPP/DMAPP-Isomerase} \\
&\text{Phytoene-Synthase} \\
&\text{Phytoene Desaturase} \\
&\zeta\text{-Carotene-Desaturase} \\
&\text{Phytoene} \\
&\text{Phytofluene} \\
&\zeta\text{-Carotene} \\
&\text{Neurosporene} \\
&\text{Lycopene} \\
&\text{GGPP-Synthase} \\
&\text{Phytoene-Synthase} \\
&\text{Phytoene Desaturase} \\
&\zeta\text{-Carotene-Desaturase} \\
&\text{Lycopene isomerase} \\
&\alpha, \beta\text{-Lycopene Cyclase} \\
&\text{Provitamin A}
\end{align*}
\]
Reconstruction of β-carotene Biosynthetic Pathway in Rice Endosperm by Agrobacterium-Mediated Transformation

GGPP

Phytoene

Lycopene

β-Carotene

GGPP → Phytoene → Lycopene → β-Carotene

**Phytoene Synthase**

**Phytoene Desaturase**

**Lycopene Cyclase**

Restriction Enzymes: a) I-sce I, b) Kpn I. Probe: *

Restriction Enzymes: a) I-sce I, b) Kpn I. Probe: *

Restriction Enzymes: a) I-sce I, b) Spe I. Probe: 

* Restriction enzymes: a) I-sce I, b) Kpn I. Probe: psy

* Restriction enzymes: a) I-sce I, b) Spe I. Probe: cyc

* Restriction enzymes: a) I-sce I, b) Spe I. Probe: cyc

* Restriction enzymes: a) I-sce I, b) Spe I. Probe: cyc

* Restriction enzymes: a) I-sce I, b) Spe I. Probe: cyc
“Golden Rice“ contains the genes required to activate the biochemical pathway leading to accumulation of provitamin A. Proof of concept was complete in 1999.

And this was the end of public funding.
How to get Golden Rice into the hands of the vitamin A-deficient populations?

• Some contact with CGIAR system but ...

• No financial support from public domain.
  • No knowledge about IPR problems.
  • No idea how to get free licences.

• No idea about product development.
  • No idea about deregulation.

• No idea of what was ahead of us.

The way forward:

Public-Private-Partnership with Zeneca / Syngenta.

1980 – Technology Development - 1990 ✓

1990 - Science - 1999 ✓

1999 - Product development - 200? private

Intellectual Property Rights 2000 ✓
Material Transfer Agreements 2001 ✓
GMO-Competent Partner 2001-2002 ✓
Transfer to Indica rice varieties 2002 ✓
“Regulatory Clean Events“ 2002 ✓
“Regulatory Clean“ line at 1.6 µg/g 2003 ✓
“Regulatory Clean“ line at 6.0 µg/g 2004 ✓
Experimental lines at much higher levels 2004 ✓
Agronomic normality in field test 2004 ✓

Deregulation ✓ ✓ ✓ ✓

public private
Golden Rice Humanitarian Board

Robert Bertram, USAID Washington
Peter Beyer, University of Freiburg
Howarth Bouis, IFPRI Washington
Adrian Dubock, Syngenta Basel
Katharina Jenny, SDC Bern
Gurdev Khush, UC Davis, CA
Ingo Potrykus, Switzerland, Chairman
Robert Russell, USDA Boston
Gary Toenniessen, Rockefeller Foundation
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Philippines: National Rice Research Institute (PhilRice)
Vietnam: Cuu Long Delta Rice Research Institute
India: Department of Biotechnology, New Delhi (DBT)
India: Directorate of Rice Research, Hyderabad (DRR)
India: Indian Agricultural Research Institute, Delhi (IARI)
India: University of Delhi South Campus (UDSC)
India: Tamil Nadu Agricultural University (TNAU)
India: Agricultural University Pantagar (GBPUAT)
India: University of Agricultural Sciences Bengalor
Bangladesh: Bangladesh Rice Research Institute
China: Huazhong Agricultural University, Wuhan
China: Chinese Academy of Sciences, Beijing
China: Yunnan Acad. Agri. Sciences, Kunming
Indonesia: Agency for Agricultural Res.& Dev., Jakarta
Germany: University of Freiburg

Humanitarian Golden Rice Network

South Africa: CSIR, Pretoria, sorghum, maize.

With technical support from Syngenta
Introgression of the Golden Rice trait into Vietnamese varieties. High iron/high yield variety Khang Dan and High yielding/good grain quality/aromatic varieties.

The Golden Rice trait expresses in each of the popular, agronomically valuable varieties!
The field test with 6 lines did, so far, not indicate any agronomic or ecological problem and the content of provitamin A was at least as high as in the glasshouse.

The same test had been planned at the same time for India, Vietnam, The Philippines and Bangladesh. Although no ecologist can propose any substantial environmental risk from Golden Rice - and every day 6,000 children die from vitamin A-malnutrition – it took more than two years to get the permission for the first planting in Southeast Asia.
The level of expression can be regulated with the choice of genes. Depending on the source of the gene the total amount of provitamin A, so far, ranges from 0.8 to ca 40 µg/g endosperm.

Effect of five different genes on accumulation of provitamin A. Details in manuscript submitted to Nature Biotechnology.

Genes from proof-of-concept experiment, Science 2000

Details embargoed until publication.
How much GoldenRice has a child to eat to prevent vitamin A malnutrition?

Lines used for subsequent calculation.

Wildtype

SGR 1

SGR2

Further lines with much higher content of provitamin A in the pipeline.

SGR1: 1.6µg regulatory clean; jointly developed by public & private sector.

SGR2: 16µg developed by private sector; donated to the humanitarian project!
Amount depends upon the typical diet. Example Bangladesh

Calculation from International Food Policy Institute:

(1) Share of calorie intake for rural Bangladesh.

- **79% energy intake from rice but no provitamin A!**
- **Provitamin A in vegetables and fruit**
- **Vitamin A in fish & animal food**
50% RDA required to prevent VADD!

Provitamin A-contribution from a typical daily diet:

Calculation from the International Food Policy Research Institute: (2) Vitamin A contribution from nutrient intake.

Conversion factor used: 12:1

No VADD with Golden Rice!

VADD without Golden Rice!

A typical daily diet would prevent vitamin A-deficiency, ...

... but GMO-regulation prevents, so far, use of Golden Rice.
Science is moving towards nutritional optimization, but regulators will not deregulate. Regulation is set to look at risks, not benefits. 24,000 death per day are irrelevant!

Engineered provitamin A pathway in rice endosperm.

GGPP-Synthase

IPP $\xleftrightarrow{\text{PP}}$ DMAPP

Phytoene-Synthase

Phytoene $\xrightarrow{\text{GGPP}}$ GGPP

Phytoene Desaturase

Phytofluene $\xrightarrow{\text{Phytoene Desaturase}}$

ζ-Carotene-Desaturase

ζ-Carotene $\xrightarrow{\text{ζ-Carotene-Desaturase}}$

Lycopene isomerase

α, β-Lycopene Cyclase

Golden Rice

Lycopene $\xrightarrow{\text{Lycopene isomerase}}$

Vitamin A

Vitamin E ✓

γ-Oryzanol ✓

Carotenoids: ✓

β-carotene, lutein, zeaxanthin

Iron & zinc bioavailability:

Ferritin, Phytase,

High-quality protein:

Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val
Cost-effective and sustained production of nutritious food.

1 seed ➢ 1 plant ➢ 1,000 seeds / 20 g

In two years ➢ 1,000,000 seeds / 20 kg
➢ 1,000,000,000 seeds / 20 t
➢ 1,000,000,000,000 seeds / 20,000 t

The potential of one GoldenRice seed:

Each seed has the potential, to produce in two years food for 100,000 poor. And it carries the technology, to reduce vitamin A-malnutrition in a cost-effective, sustained manner.

All a farmer needs to benefit from this technology is one seed. He needs neither additional agrochemicals nor pesticides or novel farming system or seed. He can use part of his harvest for the next sowing. No new dependencies are created. The technology is free up to a yearly income from rice of $10,000 per farmer or local trader.

GMO Regulation, so far, prevents use of this technology by the farmer.

The paper uses the ‘global economy-wide computable general equilibrium model‘ to analyse the potential economic effects of adopting first and second generation GMO crops in Asia.

- ‘The results suggest that farm productivity gains could be dwarfed by the welfare gains resulting from the potential health-enhancing attributes of Golden Rice‘.

- Projected gains from Golden Rice adoption by developing Asia would amount to $ 15.2 bn per year globally.

- Enhanced productivity of Asian unskilled labor in $ bn: China 7.2; India 2.5; Other S+SE Asia 4.1.

GMO Regulation, so far, prevents use of this technology by the farmer and the public domain does not support its completion.
Requirements for Golden Rice deregulation.

- **Deregulation**
  - Exposure evaluation
    - Modelling analysis for intended use.
    - Bioavailability study.
  - Protein production and equivalence
    - Extraction from GMO plant or heterologous source
    - Biochemical characterisation
    - Function/specificity/ mode of action.
  - Protein evaluation
    - No homology with toxins and allergens.
    - Rapid degradation in gastric/intestinal studies.
    - Heat lability
    - No indication of acute toxicity in rodents.
    - Further allergenicity assessments

- Event-independent studies
Requirements for Golden Rice deregulation.

**Molecular characterization and genetic stability**
- Single-copy effect; marker gene at same locus.
- Simple integration; Mendelian inheritance over three generations (minimum).
- No potential gene disruption.
- No unknown open reading frames.
- No DNA transfer beyond borders.
- No antibiotic resistance gene or origin of replication.
- Insert limited to the minimum necessary.
- Insert plus flanking plant genome sequenced.
- Phenotypic evidence for stability over 3 generations
- Biochemical evidence for stability.
- Unique DNA identifier for traceability/detection.

Event-dependent studies
Deregulation

**Expression profiling**
- Gene expression levels at key growth stages.
- Evidence for seed-specific expression.

**Phenotype analysis**
- Field performance, typical agronomic traits, yield - compared to isogenic lines.
- Pest and disease status to be same as isogenic background.

**Compositional analysis**
- Data from 2 seasons x 6 locations x 3 reps. on proximates, macro and micro nutrients, antinutrients, inherent toxins and allergens. Data generated on modified and isogenic background.

**Environmental risk assessment**
- Minimize potential for gene flow.
- Evaluate any change in insect preference – by field survey.

Data submitted must be of scientific publication quality
Product Development:

Repetition of the same experiment, with "regulatory clean" technology, so often until finding the one event (regulatory clean, one insertion, high expression) which can serve as the basis for product development and the deregulatory procedure:

4-8 years of intensive experimental work with no chance for publication, and difficult to finance!

Deregulation:

Exposure evaluation, Protein production and equivalence, Protein evaluation, Molecular characterization and genetic stability, Expression profiling, Phenotype analysis, Compositional analysis, Environmental risk assessment:

5 years of intensive experimental work with no chance for publication, and difficult to finance!

Freedom to operate for intellectual property: easily achieved because of goodwill from the private sector.

Who in the public domain can afford to spend one decade of his/her career on product development and deregulation with no academic recognition?
GMO regulation, so far, prevents use of the technology for the benefit of the poor.

Why do we have ‘GMO-regulation‘?

**History:** precaution was sensible at the beginning of technology development. Key argument: ‘unpredictable genome alterations.’

**Experience:** no specific risks associated with GMOs.

Why do we maintain ‘extreme precautionary‘ regulation?

‘To build trust for acceptance of GMOs‘.

**Experience:** This does not and cannot work.

What is the price developing countries are paying?

Under these conditions GMO technology cannot help to reduce hunger and malnutrition in developing countries.
... there are only 4 cases which contribute to the GMO increase, all developed and deregulated by the private sector in the U.S. and subsequently adopted by developing countries: cotton, maize, soybean, canola with herbicide tolerance and insect resistance.

Novel cases from the public domain have little chance to contribute to this optimistic scenario in the near future if present regulation is maintained.

Clive James
ISAAA 2005
There are hundreds of ‘food-security‘ transformation events, produced in the public domain in Egypt, Kenya, South Africa, Zimbabwe, China, India, Indonesia, Malaysia, Pakistan, Philippines, Thailand, Argentina, Brazil, Costa Rica, and Mexico,

... established in rice, maize, pearl millet, sorghum, wheat, potatoes, cassava, sweet potatoes, melons, cucumbers, squash, watermelons, tomatoes, bananas, plantain, beans, papaya, sunflower, soybean, ground nut, chickpea, oil palm, cabbage, cauliflower, cacao, mango,

... with improved agronomic performance, stress tolerance, and nutritional value, *

*) Data from J.I. Cohen, Nature Biotechnology 23 (1) 2005

... which all will be faced with the same prohibitory regulatory problems as Golden Rice, and the same shortage in financial support, regulatory expertise, governmental support, and radical hostility from numerous so-called “humanitarian“ organizations.
Traditional breeding leads to massive and uncontrolled modifications of the genome!

GMO regulation is justified because of ‘unpredictable and uncontrolled genome alterations’.

Every step and each component is unpredictable and leads to uncontrolled genome alterations!

Ultimate Landrace

New varieties

IR 64

Crossing & selection
The genome of each variety of every crop plant is heavily ‘genetically modified’ – and unregulated!

Nothing justifies extreme regulation!
We have been made to believe that GMO’s are dangerous by those, who now collect a lot of campaign money to protect us from those dangerous GMO’s.

“The Emperors New Clothes” by Christian Andersen.

Two weavers visited the emperor and claimed to be able to make special magic cloth, which foolish persons can not see.

The emperor wanted them to make a suit for him from this magic cloth.

Also the emperor did not want to be considered foolish and pretended the same.

The prime minister was sent, to look at the progress with the magic cloth, but could not see it. As he did not want to be considered foolish, he pretended, to never have seen such beautiful cloth.

The weavers were payed very generously, and disappeared.

We have been made to believe that GMO’s are dangerous by those, who now collect a lot of campaign money to protect us from those dangerous GMO’s.
The trend towards organic food, not GMOs, enhances the problem.
From all our experience with traditionally bred ‘genetically modified’ crop varieties, and from basic biological science we know that there is no sensible argument for specific regulations for ‘genetically engineered’ crops.

The results from extensive ‘biosafety research’ with ‘genetically engineered’ varieties, and experience from all previous deregulations confirm this view.

It does not make sense, therefore, to maintain the present ‘extreme precautionary’ GMO regulation, even if it would not prevent use of the technology for the solution of severe humanitarian problems.

The scientific community has the duty of stressing this point to the media, politicians and the public.

Keeping a low profile is irresponsible.
Basic research projects are often justified – and funded – with the argument that they would help to solve problems of our society. Problems are not solved by basic science, but by delivery of products based on science. This part of the story is ignored once science has been successful. Neither is there any granting system to support product development and deregulation, nor can any public scientist afford to invest into the delivery of a product in our academic environment (‘publish or perish!’). Scientists and funding agencies like to ‘delegate’ this responsibility to the private sector. There is, however, no doubt that humanitarian problems are—and will always remain—under the responsibility of the public sector. Our society should expect the public sector to assume its responsibility!

How?

Establish science-based benefit/risk regulations, the financial basis for product development and deregulation, and academic recognition of respective contributions.
In the early 19th century a Thai princess celebrated her 18th birthday. She fell into the palace pond...

... and drowned in front of hundreds of guests. Nobody helped her out of the water. Why? Because it was “taboo” to touch a member of the “divine” royal family! Everybody likes to believe that he/she would have saved the princess, ... however ...
... in the early 21st century 500,000 children per year become blind and 6,000 die every day from vitamin A malnutrition. This could be prevented.

However, GMOs are “taboo” and there is no support from the public domain for product development and deregulation.
Acknowledgments

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- Groups Peter Beyer & Ingo Potrykus for science.

- Humanitarian Golden Rice Board for guidance and decision-making.

- Humanitarian Golden Rice Network for variety development.

- Syngenta (Adrian Dubock) for key contributions in product development, deregulation, strategic advice.

- Agbiotech companies for IPR donations.


No support from EU or EU national agencies!
Malnutrition kills 24,000 people per day – and who cares?

Golden Rice could prevent vitamin A malnutrition in rice-consuming populations.

Other ‘Golden’ food-security crops are in the pipeline.

Multi-trait nutritional optimization is the next task.

‘Biofortification’ offers cost-effective, sustainable solutions.

GMO regulation prevents, so far, use of the technology, and refuses to consider benefits.

GMO regulation is irrational, opportunistic, and unjustified.

The public, not the private sector is responsible for solutions.

Solutions are based on products, not publications.

The public sector is incapable and unwilling to deliver products.

• Link between science and product missing in the public domain.

Academia is often financed, pretending to work towards solutions.

Contributions to solutions have no support from academia.