Golden Rice & Golden Crops for Human Nutrition
Discovery - Development - Delivery

- Context and Purpose
- Technology Development
- Potential
- Where it stands
- Beyond approval

P. Beyer
We Seed the World, Tutzing, 2013
Population growth - fading resources

Source S. Sanyal „The Nation“ Nov. 1st, 2011
Share of Energy Source & Food Budget in Rural Bangladesh

Staples are cheapest

Philippines Rice Price (Retail)

A trend

Source: FAO, GIEWS food price data & analysis tool

Source: H. Bouis, IFPRI
Context and Purpose

Directly, or indirectly, plants provide all of Humanity’s food

- More of our food comes from fewer species (globally, 54% from corn, rice and wheat).
- Loss of biochemical diversity in the diet.
- Plants, in principle provide all macro- and micro-nutrients required, but the latter are very unevenly distributed.

**Rice, for instance**

<table>
<thead>
<tr>
<th></th>
<th>Provit A</th>
<th>Folate</th>
<th>Iron</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milled grain</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Leaf</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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</tbody>
</table>
Context and Purpose

„Hidden Hunger“ is long known

Supplementation – Fortification - Education – Diversification did not eradicate the problem (despite progress made)

The Copenhagen Consensus 2012
16 investments worthy of investment (decending order of desirability)

1. Bundeled micronutrient interventions to fight hunger and improve education
2. ...Malaria
3. ...Childhood Immunization
4. ...Schoolchildren deworming
5. ...Tuberculosis treatment
6. ...Increase yield enhancement
7 – 13 ...
14. ...HIV vaccine R&D
15. – 16.

The Millenium Goals
189 countries agreed in 1990 to reduce significantly within 25 years suffering from malnutrition and the mortality rate among children under 5 years of age
Biofortification: A complementary intervention

Crop plants are being fortified by means of their own biosynthetic (vitamins) or physiological (minerals) capacities, achievable through

- Breeding techniques (classical, MAS, mutagenesis, wide crosses)
- Recombinant DNA technology

Is all achievable through breeding?

Simple answer: NO!

- Some crops cannot be bred or breeding is very difficult (e.g. banana, cassava)
- Trait variability is insufficient

www.harvestplus.org
Some traits cannot be bred because trait variability is inadequate

Rice, (polished grains), for instance

**Provitamin A:** No „yellow grains“ in germplasm collections

**Folate:** Practically absent

**Iron:** low variability, ranging from 1 – 8 ppm (Target 14 ppm)

**Zinc:** much more important variability, ranging from 16 – 28 ppm (Target: 24 ppm)
Golden Rice cannot be bred

...recombinant DNA technology is necessary

Breeding where possible
Genetic modification where required
The need for vitamin A

A conservative estimation:

- Globally, approximately 670,000 children die every year because they are vitamin A–deficient.
- Another 350,000 children go blind.
- More than 90 million children in Southeast Asia suffer from vitamin A deficiency, more than in any other region.
- More than 190 million preschool children and 19 million pregnant women are vitamin A deficient, globally.

Source: Global prevalence of vitamin A deficiency in populations at risk 1995–2005
WHO Global Database on Vitamin A Deficiency
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WHO Global Database on Vitamin A Deficiency
Vitamin A deficiency in primary target countries

In the Philippines
- Approximately 1.7 million children aged 6 months to 5 years are affected.
- Subclinical vitamin A deficiency affects one out of every ten pregnant women.

In Bangladesh
- 1 out of 5 children aged 6 months to 5 years are estimated to be vitamin A-deficient.
- Among pregnant women, 1 out of 4 are affected by vitamin A deficiency.

Symptoms of Vitamin A Deficiency

- Night blindness
- Irreversible blindness (Xerophthalmia)
- Anemia
- Impaired immunity exacerbates infectious disease (measles)
- Morbidity & impaired development
- Mortality
- Most at risk: Children under 5; pregnant and lactating mothers in rural areas

Providing adequate amounts of vitamin A could reduce overall child mortality by 23-34%.
Assembly-line technologies

Expressed genes
DNA, mRNA

Product

Precursor

Intermediates
Assembly-line technologies

Expressed genes
DNA, mRNA

Precursor
Intermediates
Product
The Golden Rice principle: Filling a gap

Wild-type endosperm

„Absent“

Wild-type endosperm

Burkhardt et al., (1997) Plant J. 1, 1671
Schaub et al. (2005), Plant Physiol. 138: 441
Luckily, there is CRTI

In one step! CRTI substitutes for 4 plant enzymes

*Pantoea ananatis* carotenoid gene cluster

Pathway reconstruction

Wild-type endosperm

„Absent“

Wild-type endosperm

Phytoene Synthase

Phytoene Desaturase

ζ-Carotene Isomerase

ζ-Carotene Desaturase

Lycopene Isomerase

α, β-Lycopene Cyclase

Burkhardt et al., (1997) Plant J. 1, 1071
Schaub et al. (2005), Plant Physiol. 138: 441
Only two biosynthetic transgenes needed

Wild-type endosperm

- Phytoene Synthase (transgene)

- CrtI (transgene)

Wild-type endosperm

GR1: PSY from Daffodil

all-trans-β-carotene

all-trans-α-carotene

α, β-Lycopene Cyclase

E8
Golden Rice: Technology development

Prototypes are not apt for product development

Ye et al., 2000; Science 287:303
Golden Rice 1 was initially considered a product

- Events preselected
- Field trials at Louisiana State University
- Carotenoid content 4.1 – 7.1 μg/g (ca. 4-7 times over prototype)
Towards higher levels: Golden Rice 2

Where’s the bottleneck?

**C₃-Metabolism**

- Precursor shortage?
  - DXS tested

- Phytoene synthase
  - Expression/activity?
    - Different versions tested

- Phytene
  - CrtI
    - Expression/activity?
      - Codon optimized, Promoters tested

- Intracellular storage?
  - Not tested

**β-Carotene**

**α-Carotene**

The key experiment

Since PSY is well expressed in GR1, alternative PSY genes were assayed (Syngenta).

Transformed into rice (Cv. Asanohikare)

Rice and Maize PSY best! Daffodil (used in GR1) least effective!

Paine et al., 2005 Nat Biotechnol. 23, 482-487
Assembly-line technologies

Expressed genes
DNA, mRNA

Product

Too slow!!!
Carried into a production pipeline (Syngenta)

Transform long grain rice variety (Kaybonnet)
Sugar (mannose) selectable marker (PMI)
619 independent events
Screen for seed colour, gene copy number, integration quality, fertility, etc.
Preselect six “Golden Rice 2” events for further screening and development
„Shades of Golden“

Provitamin A increase in GR2:

ca. 10-fold over GR1

c.a. 25-fold over prototype
The essentials apply to various crops, like

1. Cassava (PSY only)

- Ranks 5th among staple crops directly consumed (No. 1 in Sub-Saharan Africa). Provides food security.

- Very low in micronutrients
  Provit A (mostly) 1-5 µg/g; VitE, 1 µg/g;
  Iron 5 ppm, Zinc 1 ppm (fresh weight)

- Varietal recovery very difficult upon breeding (vegetatively propagated)

- Very long breeding cycle, however, provitamin A breeding is underway at CIAT
2. Potato (mini-pathway) CRTB, CRTI, CRY

- Ranks 4th among staple crops directly consumed
- Carotenoid-containing varieties are known
- Breeding is difficult (vegetatively propagated)
- Product development not planned

EC Programs and HarvestPlus*
3. Banana - Plantain (PSY only)

- Ranks 10\textsuperscript{th} among staple crops directly consumed
- Important staple in East Africa (Uganda: 222 kg/person year.
- Very low in micronutrients (Provita 2.7 µg/g; Vit E 1 µg/g; Iron 2.6 ppm, fresh weight)
- Conventional breeding: extremely difficult as bananas are essentially sterile: Most current cultivars are sterile triploids selected from the wild
- Have not been genetically improved for thousands of years
- Huge challenges from global movement of devastating diseases

Courtesy of J. Dale, QUT Australia, GCGH
5. Maize (PSY-CRTI)

Maize is the world’s most important staple crop.

The pathway proceeds beyond beta carotene. The genetic variability for high carotenoid levels is large, but low for provitamin A carotenoids.

A PSY-CRTI combination, as used in GR boosts ß-carotene production in an African white cultivar to 60 µg/g!

Breeding approaches (ongoing) have yielded so far ca.14 µg/g ß-carotene.

Naqvi et al., PNAS, 2009
Harjes et al., Science 2008
Golden Rice breeding from ca. 2005-2010

GR2 events were bred into selected rice cultivars (MAS)

Examples:

- IR64 & IR36: Mega-varieties, broad Asian coverage
- BRRI dhan 29: Most popular boro rice variety in Bangladesh
- PSB Rc82: Most popular in the Philippines
- OS 6561: Most popular in Vietnam
- Chehirang: Leading variety in Indonesia (with IRRC)
- & Stacking Zinc, iron, lysine & agronomic traits

Golden Rice Network

- IRRI (Int.), lead
- Philrice (Philippines)
- BRRI (Bangladesh)
- CLRRI (Vietnam)
- IARI (India)
- TNAU (India)
- DRR (India)
- Huazhong Univ. (China)
- IRRC (Indonesia)
GR Network members are licensees

Some key license terms:

**Granted rights for humanitarian use:**

- In developing countries
- Resource-poor farmers (earning < 10 000 $ p.a.)
- In public germplasm
- No charge for the technology
- Replanting is allowed
- National trade is allowed

**Obligations**

- Regulatory imperative – national or international regulations
- No export (except other licensees)
Breeding led to event selection

GMO regulation is based on the „event“. (There can be only one!)

Starting point: Six GR2 events, all single locus intact integration

- Which event(s) produce consistent levels of provitamin A across cultivars?
- Which event(s) reproduce consistently the characteristics of the recurrent parents?

And the big question:

- Which level of provitamin A must be delivered by GR to be effective?
Bioavailability/Bioconversion of GR

Conducted at TUFTS University.
Five adults.

Very good bioavailability of 3.8 : 1 !

Reg.: https://www.clinicaltrials.gov NCT00680355

Follow-up study in China
63 children, marginally vit A deficient, 6-8 yr.

Unsurpassable!

Spinach 7.5 : 1
β-carotene in oil 2 : 1
Golden Rice 2 : 1
Retinol (reference dose) 1 : 1

Provitamin A in GR is highly bioavailable

50 g (dry weight) of Golden Rice (20 µg/g) can provide ~60% of the Recommended Daily Allowance (RDA) - ca. 100% of the Estimated Average Requirement (EAR) - for vitamin A for 6-8 year old Chinese children.


Greenpeace, 2001
50 grams of Golden Rice can make a large difference

Tony Alfonso
PhilRice
Emerging consent

“Since a large proportion of vitamin A–deficient children and their mothers reside in rice-consuming populations, particularly in Asia, Golden Rice should substantially reduce the prevalence and severity of vitamin A deficiency, and prevent at least hundreds of thousands of unnecessary deaths and cases of blindness every year”.

- Dr. Alfred Sommer -
  Professor and Dean Emeritus
  Johns Hopkins Bloomberg School of Public Health
Ex-ante analyses

Suggest that Golden Rice is most cost-effective
Case studies in India, Bangladesh, The Philippines

\[ D_{\text{DALYs}_{\text{lost}}} = \sum_j T_j M_j \left( \frac{1-e^{-r L_j}}{r} \right) + \sum_i \sum_j T_j I_{ij} D_{ij} \left( \frac{1-e^{-r d_{ij}}}{r} \right) \]

<table>
<thead>
<tr>
<th></th>
<th>Cost per DALY saved Highest efficiency</th>
<th>Cost per DALY saved Lowest efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplementation</td>
<td>134 $</td>
<td>599 $</td>
</tr>
<tr>
<td>Golden Rice</td>
<td>3 $</td>
<td>19 $</td>
</tr>
</tbody>
</table>

WHO effectiveness standard for DALYs saved: $ 620 – 1,850
World Bank  effectiveness standard for DALYs saved: $ 200

Zimmermann & Qaim (2004) Food Pol. 29, 147
Where it stands

The Current GR Project (2011 – 2014)

Implement GR in the Philippines (first launch) and Bangladesh

International Rice Research Institute (IRRI)
- Administration and coordination
- Technical support
- Capacity building, and safety research

Philippine Rice Research Institute (PhilRice)

Bangladesh Rice Research Institute (BRRI)
- Performance and safety research
- Market introduction

Biosafety Resource Network
- Advice on regulatory matters
- Compilation of the Regulatory Dossier

SeedStories & Natl. Partners
- Communication

Helen Keller International (HKI)
- Evaluate the efficacy of Golden Rice to impact vitamin A status

University of Freiburg
- Next generation of GR

GR Projects in India, Vietnam, China and Indonesia run on National funding and are not on the same timelines
Project aims

Move GR through the regulatory process & prove safety

- Molecular data sets ✓
- Screenhouse evaluation ✓
- Confined field trials in one location ✓
- Multilocal field trials, data collection ✓

Examples:
  - Prove in the field that yield, pest resistance, and grain qualities are as before.
  - Data on protein expression

- Cooking and taste tests
- Compositional analyses…..etc.
Project aims

Assessing safety

Golden Rice is being analyzed according to internationally accepted guidelines for the safe use of modern biotechnology.

1. **Codex *Alimentarius* of the FAO and WHO**
   - Guideline for the Conduct of Food Safety Assessment from Recombinant-DNA Plants (CAC/GL 45-2003)
   - Food Safety Assessment from Recombinant-DNA Plants Modified for Nutritional or Health Benefits: Annex 2 (2008)

2. **OECD Consensus Guidelines**

3. **Cartagena Protocol on Biosafety**

Philippines regulations are based on these guidelines.

The Philippines have approved seven GMO crops for food and feed.

([http://agbios.com/dbase.php](http://agbios.com/dbase.php)),

Project aims

Assessing safety

Golden Rice is being analyzed according to internationally accepted guidelines for the safe use of modern biotechnology.

1. Codex *Alimentarius* of the FAO and WHO

   PhilRice and BRRI plan to submit all safety information to their national government regulators during 2013 in the Philippines and 2015 in Bangladesh

2. OECD Consensus Guidelines

3. Cartagena Protocol on Biosafety

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The Philippines have approved seven GMO crops for food and feed. (http://agbios.com/dbase.php),
Beyond GR approval

What do people think?

Consumers

Growers

& additional stakeholders
Talking to people

Beyond GR approval
Perception can change

Introduction of Orange-Fleshed Sweet Potato in Uganda and Mozambique by HarvestPlus
www.harvestplus.org

Reaching those in need

A delivery program is being designed to ensure that Golden Rice is acceptable and accessible in vitamin A deficient communities.

The aim:

- Communities could have ongoing access to Golden Rice without any additional aid or intervention

- Golden Rice would be theirs to choose to grow and consume as they do with other rice.
Demonstrating Efficacy

Does daily consumption of Golden Rice improve the vitamin A status of vitamin A deficient adults?

- The study will be conducted under controlled community conditions to determine if eating Golden Rice every day improves vitamin A status and could therefore contribute to reducing vitamin A deficiency.

- Coordinated by Helen Keller Int. and the University of California, Davis, working with local partners

This study will be carried out only if biosafety is confirmed
Are we being slow?

2000 – Proof of principle

2005 – Technology improvement

2010 – Breeding & event Selection

2013 (?) – Regulatory submission
  Philippines

2015 (?) – Regulatory submission
  Bangladesh

Industries estimate ca. 10 -13 years for this process
Summary

• Vitamin A deficiency remains a serious public health problem.
• Golden Rice and Golden Crops are a potential new and complementing way to address vitamin A deficiency
• Leading nutrition and agricultural research organizations are working together to develop evaluate and introduce these crops.
Financial support

- Bill & Melinda Gates Foundation
- Rockefeller Foundation
- U.S. Agency for International Development
- Department of Agriculture – Philippines
- National funding in additional countries
- No contribution in €
For additional information, see www.goldenrice.org

www.irri.org/goldenrice/