I. Golden Rice

Golden Rice" seeks to solve malnutrition in developing countries

- Golden rice is a genetically modified, biofortified crop. Biofortification increases the nutritional value in crops. Golden rice is genetically modified in order to produce beta carotene, which is not normally produced in rice. Beta carotene is convereted into Vitamin A when metabolized by the human body. We need Vitamin A for healthier skin, immune systems, and vision

- The Golden Rice Project was first introduced in 1999, when two professors Ingo Potrykus and Peter Beyer, proposed their project to Rockefeller Foundation to genetically engineer rice to increase its nutrients. Rockefeller Foundation supported their goal to provide a sustainable biofortification approach to combat vitamin A deficiencies in developing countries.

- Vitamin A deficiency (VAD) is prevalent in developing countries whose diets are dependent on rice or other micronutrient-poor carbohydrate foods, which do not contain vitamin A. The World Health Organization estimates that about 250 million preschool children are affected by VAD and about 2.7 million children die because of the deficiency. VAD can have numerous negative health effects such as dryness of the eye that can lead to blindness if untreated.

- It is based on the fact that rice plants possess the whole machinery to synthesise β-carotene, and while this machinery is fully active in leaves, parts of it are turned off in the grain. By adding only two genes, a plant phytoene synthase (psy) and a bacterial phytoene desaturase (crt I), the pathway is turned back on and β-carotene consequently accumulates in the grain.
- The first breakthrough in the development of Golden Rice was the result of a collaboration between Peter Beyer and Ingo Potrykus, who proved that

β-carotene could be produced in the rice grain. Also, only phytoene synthase and carotene desaturase (CRTI) were needed to get the pathway going, while lycopene cyclase was not required.

- Thus, Golden rice 1 encoded a plant phytoene synthase (PSY), which utilises the endogenously synthesised geranylgeranyl-diphosphate (GGPP) to form phytoene. The second gene encoded a bacterial carotene desaturase (CRTI) . he combined activity of PSY and CRTI leads to the formation of lycopene, which gets further converted to α- and β-carotene

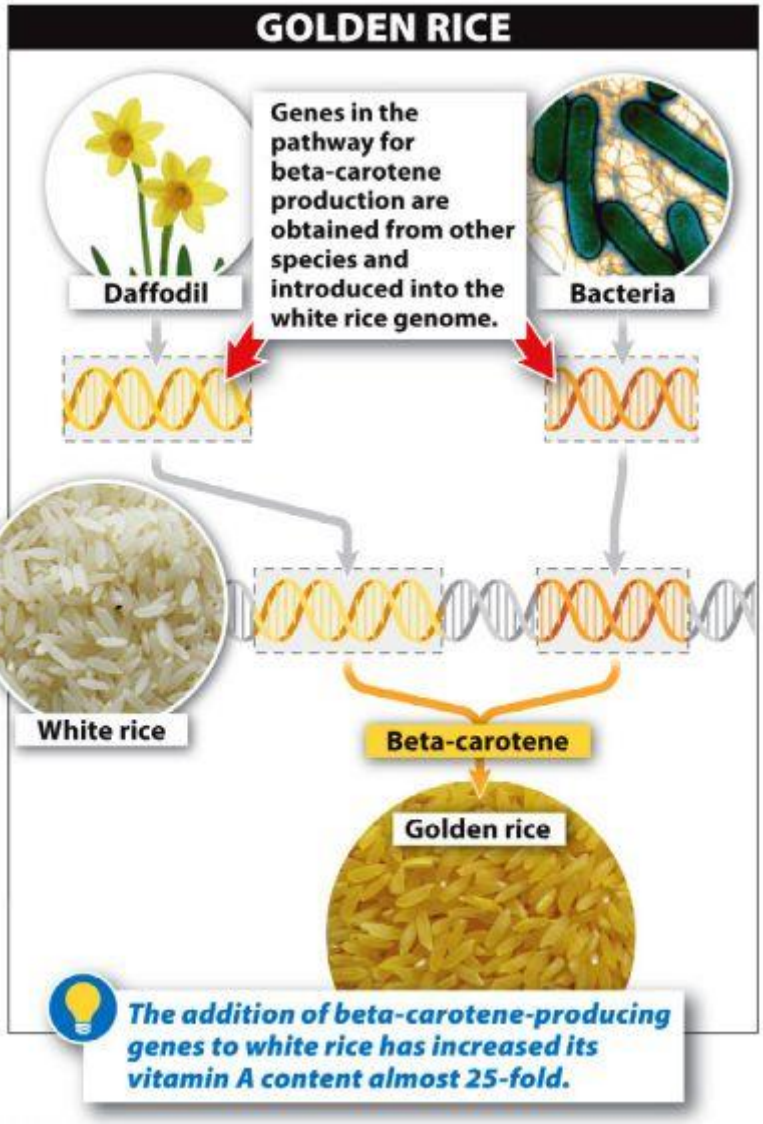
- The first generation of Golden Rice (Golden rice 1),. only contained the phytoene synthase (Psy) gene from daffodil and the carotene desaturase (CrtI) gene from the bacterium Erwinia uredovora. Both the transgenes were expressed only in the rice endosperm (by placing the genes under the control of the endosperm-specific gt1 promoter). The levels of carotenoids obtained in the field amounted to an average of 6 μg/g (about 4 times higher than the untransformed rice)

- Golden Rice 2, was constructed using the same genetic strategy applied in the construction of Golden Rice 1, with the exception that the phytoene synthase gene from Zea mays replaced the gene isolated from Narcissus pseudonarcissus. Golden Rice 2 contains approximately 20-fold more carotenoids . It was found that phytoene synthase (the rate limiting step in carotenoid biosynthesis) from maize as the most efficacious source, resulting in thegreatest accumulation of total carotenoids

- Golden Rice 1 contains about 1.6 g of total carotenoids per gram of dry weight of grain. Golden Rice 2 contains as much as 37 g total carotenoids per gram of dry weight of grain, of which 31 g/g is -carotene.

- Designing Golden RiceRice (Oryza sativa) is a global food staple. It is eaten often and in high amounts but essential nutrient content is low. .

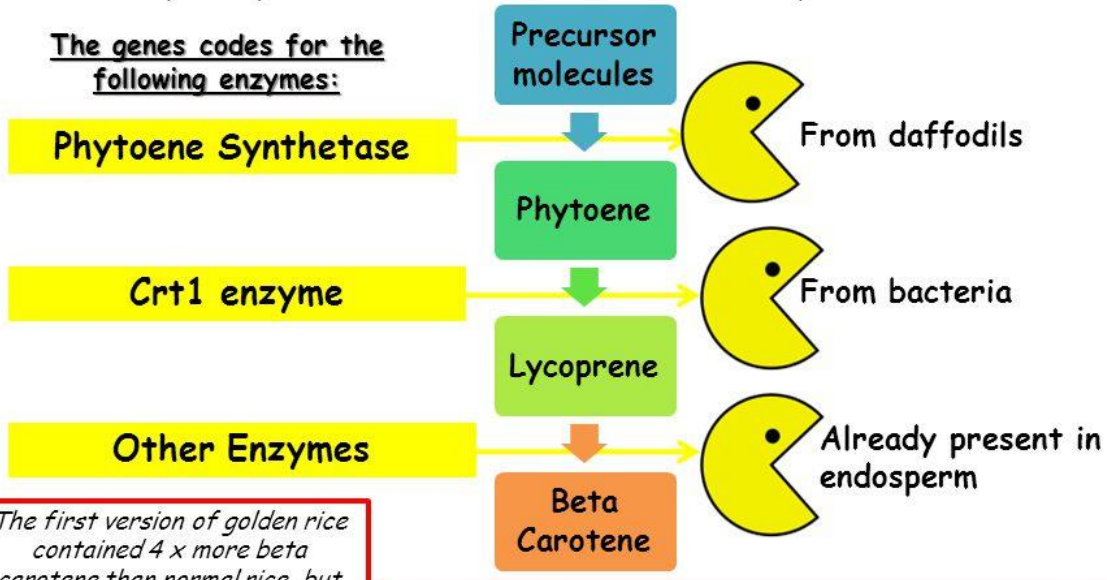
Rice endosperm does not naturally produce-carotene; instead, it produces geranylgeranyl diphosphate (GGPP) which is an early precursor of carotene. Therefore it was necessary to use recombinant genetic techniques, not conventional breeding, to develop a rice endosperm that would produce beta carotene. To convert geranylgeranyl diphosphate to beta-carotene, four additional plant enzymes were needed: phytoene synthase, phytoene desaturase, carotene desaturase, and lycopene cyclase. These enzymes were identified and their genes were isolated from various plants and bacterium. In 2000, Ye et al. put all this information together. The phytoene desaturase and carotene desaturase were circumvented by using a bacterial enzyme, carotene desaturase, that gave the combined result. The entire -carotene biosynthesis pathway (three genes on three vectors) were transformed into rice endosperm using Agrobacterium. The result were yellow endosperms and gained the name Golden Rice. The yellow color was from the beta-carotene formed in the endosperm . .The resulting Golden Rice yielded 1.6 – 2.0 μg beta carotene/g of dry rice. With a conversion factor of 6 μg of beta carotene to 1 μg of retinol, 200 g/day of rice would yield 70 μg /day of retinol which is not enough to fulfill the recommended daily allowance of retinol (1000-800 RE) .


GOLDEN RICE
Genes in the pathway for beta-carotene production are obtained from other species and introduced into the white rice genome.
Daffodil
Bacteria
White rice
Beta-carotene
Golden rice
The addition of beta-carotene-producing genes to white rice has increased its vitamin A content almost 25-fold.

Case Study 2: Golden Rice

Most of the enzymes needed for the synthesis of beta carotene were already present in the endosperm. The **insertion of 2 genes into the rice genome** was needed in order for the metabolic pathway to be activated in the cells of the endosperm.

The genes codes for the following enzymes:



*The first version of golden rice contained 4 x more beta carotene than normal rice, but this still meant that you'd have to **eat large amounts of rice every day in order to prevent vitamin A deficiency**.*

*Further genetic engineering added **stronger promoters** to the vitamin A genes. These promoters **increased the transcription** of the beta-carotene synthesising enzymes, so 'Golden Rice 2' contained over 20x the original amount of beta carotene in the endosperm.*

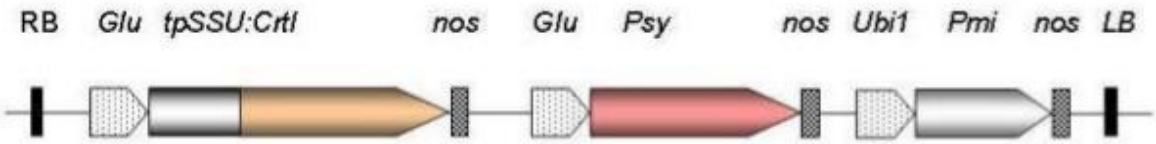


Figure: Gene construct used to generate *Golden Rice-1*.

RB, T-DNA right border sequence; **Glu**, rice endosperm-specific glutelin promoter; **tpSSU**, pea ribulose bis-phosphate carboxylase small subunit transit peptide for chloroplast localisation; ***crtI*** *Carotene desaturase* from the soil bacterium *Erwinia uredovora;* **nos**, nopaline synthase terminator; **Psy**, phytoene synthase gene from *Narcissus pseudonarcissus* (GR1) or Zea mays (GR2); **Ubi1**, maize polyubiquitin promoter; **Pmi**, phosphomannose isomerase gene from *E. coli* for positive selection (GR2); **LB**, T-DNA left border sequence.

- Lycopene is then cyclized to beta-carotene by the endogenous cyclase in Golden Rice.

(The insertion of a *lcy* (lycopene cyclase) gene was thought to be needed, but further research showed it is already being produced in wild-type rice endosperm.)

- **Six GR2 events in *Kaybonnet*, a US variety** (Paine *et al.* 2005)
  - All single-locus, and single insert
  - Carotenoid levels up to 25 ug/g (screen-house samples)
  - Event-specific markers to assist back-crossing into varieties for the Philippines, India, Bangladesh and elsewhere.
- **Only one event will be released/go through regulatory approval**
- **The final event and lines will be chosen on the bases of:**
  - retained level of β-carotene (product performance).
  - agronomic performance (farmer needs)