Engineering Nutritional Quality

Nutritional quality augmentation through addition of new quality traits, removing or reducing undesirable traits, or other manipulations is an important goal in bioengineering food crops. Crops that feed the world are primarily cereals, roots and tubers, and legumes. Unfortunately, they are nutritionally inadequate in providing certain amino acids required for proper growth and development of humans and animals.

- For example, **cereals are generally deficient in *lysine* and *threonine***, while **legumes are generally deficient in sulfur amino acids (*cysteine* and *methionine*)**. In some species (e.g., rice) where the amino acid balance is relatively appropriate, the overall protein quantities are low. Molecular genetic approaches are being adopted to genetically engineer seed protein. **They may be categorized as follows:**

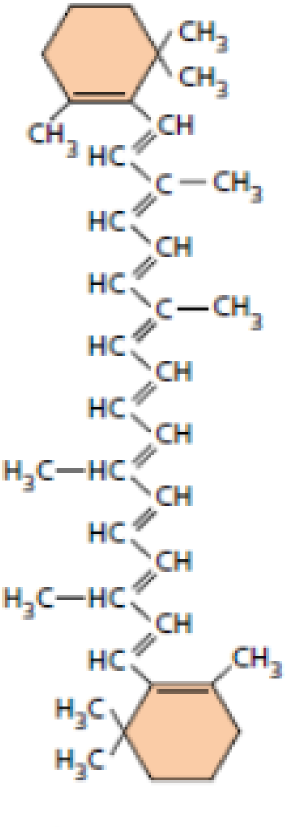
1. Altering the amino acid profile of the seed.
2. Selective enhancement of expression of existing genes.
3. Designing and producing biomolecules for nutritional quality.

The Making of "Golden Rice"

- **"Golden rice"** is so called because it has been genetically engineered to produce **β- carotene** (responsible for the **yellow color** in certain plant parts like **carrot roots**) in its endosperm. This rice produces **β-carotene** or **pro-vitamin A**, the **precursor** of **vitamin A**, which does not occur in the endosperm of rice.

- An estimated **3 billion people** of the world depend on **rice** as a staple food. Of this number, about 10 percent are at risk for **vitamin A** deficiency and the associated **health problems** that **include blindness**, as well as deficiency of other micronutrients like **iron** and **iodine**. The effort to create golden rice was led by **Dr. Ingo Potrykus**, a professor of plant science at the Swiss Federal Institute of Technology.

Ingo Potrykus



β-Carotene

- In 1990, **Garry Toenniessen**, the director of food security for the Rockefeller Foundation, **recommended the use of the sophisticated tools of biotechnology to address the problem of lack of vitamin A in rice**. Later, at a Rockefeller-sponsored meeting, **Potrykus** met **Peter Beyer** of the University of Freiburg in Germany, an **expert on the β-carotene pathway in daffodils**. In 1993, with seed money of $100,000 from the Rockefeller Foundation, the two embarked upon an ambitious project to create a transgenic plant in a manner unlike any before. **After seven years**, the duo announced to the world their outstanding achievement, the golden rice, at a cost of $2.6 million. The bill was partly footed by the Swiss government and the European Union.

The Science Behind Golden Rice

The scientific feat accomplished in engineering β-carotene into rice is that it marks the first time a metabolic pathway has been engineered into an organism. Rice **lacks** the metabolic pathway to **make** β-carotene in its endosperm. **Potrykus** and **Beyer** had to engineer a metabolic pathway consisting of **four enzymes** into rice (see Figure 1). Immature rice endosperm produces **geranylgeranyl-diphosphate (GGPP)**, an **early precursor of β-carotene**. The **first enzyme** engineered was ***phytoene synthase***, which **converts** GGPP to **phytoene** (a colorless product). Enzyme number **2**, called ***phytoene desaturase***, and enzyme number **3**, called ***β-carotene desaturase***, each catalyzes the introduction of two **double-bonds** into the **phytoene molecule** to make **lycopene** (has **red color**).

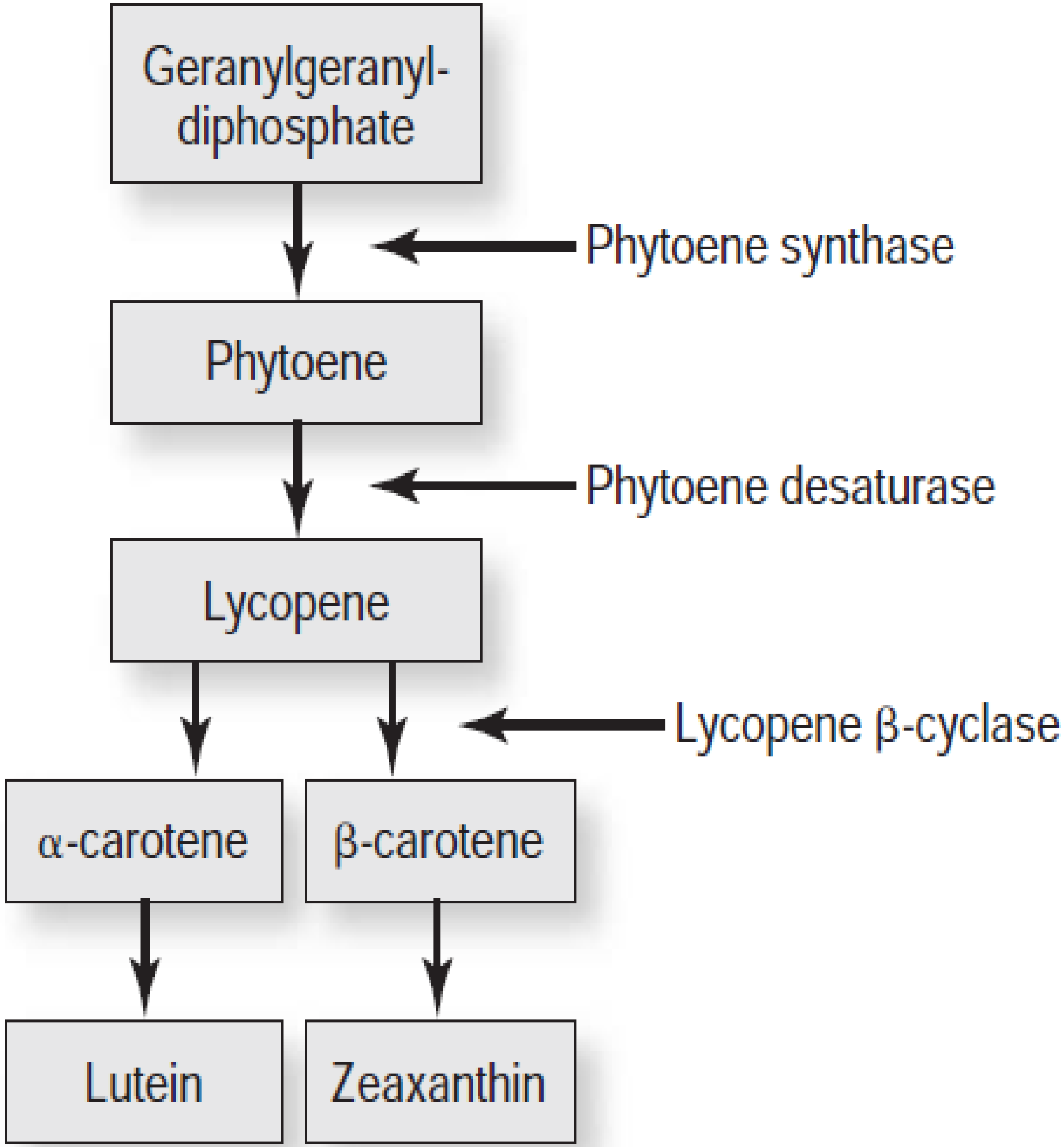
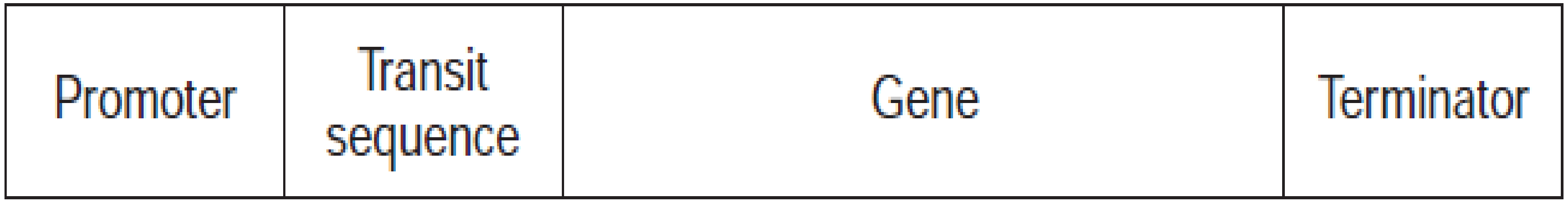


FIGURE 1 A summary of the key steps in the biosynthesis of beta-carotene. Starting from geranylgeranyldiphosphate **(GGPP)**, three enzymes are involved in the conversion of intermediary products to **beta-carotene**, the **precursor of vitamin A**.

FIGURE 2 the general characteristics of an expression cassette. An expression unit consists of the transgene flanked by the transit sequence and a terminating sequence. The transit sequence directs the location of expression of the transgene in the host. The promoter is the engine that drives the expression of the transgene.



- Enzyme number **4**, called ***lycopene β-cyclase***, **converts lycopene** into **β-carotene**. A unit of transgenic construct (called an expression **cassette**) was designed for each gene for each enzyme. These expression cassettes were linked in series or "stacked" in the final construct (see Figure 2).
- The source of genes for enzymes **1** and **4** was the **daffodil**, while genes for enzymes **2** and **3** were derived from the bacterium ***Erwinia uredovora***. Three different gene constructs were created, the **first** and most complex combining enzyme **1** with the enzymes **2–3** combo, together with an antibiotic resistance marker gene that encodes **hygromycin resistance**, along with its **promoter**, **CaMV 35S**.

- The **second** gene construct was like the first, except that it lacked any antibiotic resistance marker gene. The **third gene** construct contained the expression cassette for enzyme **4**, plus the antibiotic marker. By separating the genes for the enzymes and antibiotic resistance marker into two different constructs, the scientists reduced the chance of structural instability following transformation (the more cassettes that are stacked, the more unstable the construct).

- These gene constructs were transformed into rice via *Agrobacterium*-mediated gene transfer in two transformation experiments. **In experiment 1**, the scientists inoculated 800 immature rice embryos in tissue culture with *Agrobacterium* containing the **first** transgenic system. They isolated 50 transgenic plants following **selection by hygromycin marker**. In the **second experiment**, they used 500 immature rice embryos, inoculating them with a mixture of ***Agrobacterium*** **(T-DNA)** vectors carrying both the **second** and **third** constructs. This experiment yielded 60 transgenic plants.

- The **second experiment** was the **one expected to yield the anticipated results of a golden endosperm**. This was so because it **had all four enzymes required for the newly created metabolic pathway**. However, the scientists also recovered transgenic plants with yellow endosperm from **experiment 1**. Subsequent chemical analysis **confirmed** the presence of **β-carotene**, but **no lycopene**. This finding suggests that enzyme **4** may be present in rice endosperm naturally, or it could be induced by lycopene to turn lycopene into β-carotene. Analysis also showed the presence of lutein and zeaxanthin, both products derived from lycopene. None of the above was found in the control (non engineered) plants.

How Much Vitamin A?

- The initial golden rice lines produced **1.6** to **2.0** micrograms of **β-carotene** per gram of grain. The **recommended daily allowance** (RDA) set by health agencies for children is **0.3 mg/day**. Estimates of bioavailability of β-carotene has been put at less than 10 percent in some cases. Activists like Greenpeace continue to strongly protest what they believe to be a hype and overstatement of the benefits of this biological invention. The organization estimates that an adult would have to eat about 9 kg of cooked rice daily to satisfy his or her daily need of vitamin **A**. However, the scientists intend to refine their invention to make it produce three to five times its present level of β-carotene.

White, non-GM rice

Golden, GM rice


Thank you