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GOLDEN RICE FOR NUTRITIONAL SECURITY

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INTRODUCTION:

A japonica variety of rice was engineered with three genes necessary for the rice grain to produce and store beta-carotene. These included two genes from the daffodil plant and a third from a bacterium. Researchers used a plant microbe (Agrobacterium) to ferry in the genes into the plant cells. The incorporation of these genes allows the rice plant to modify certain metabolic pathways in its cells to produce precursors of Vitamin A, which was previously not possible. This was considered a technical milestone, as most agronomic traits engineered to date have only required the introduction of a single gene.

- Golden rice is a variety of *Oryza sativa* rice produced through genetic engineering to biosynthesize beta-carotene, a precursor of vitamin A, in the edible parts (endosperm) of rice.
- Genetic engineering was the only way to produce GOLDEN RICE because there was no rice germplasm available capable of synthesizing carotenoids.

The Developers:

- The Golden Rice project, which began in the early 1990's, was a result of a collaborative effort between the Swiss Federal Institute of Technology (ETH-Zurich) and the University of Freiburg, Germany. Ingo Potrykus and Peter Beyer are its main developers. The scientific details of the rice (the product of an eight-year project by Ingo Potrykus and Peter Beyer) were first published in *Science*, Funding was obtained from ETH-Zurich itself, the European Commission's agricultural research program, and the Rockefeller Foundation.

TECHNOLOGY:

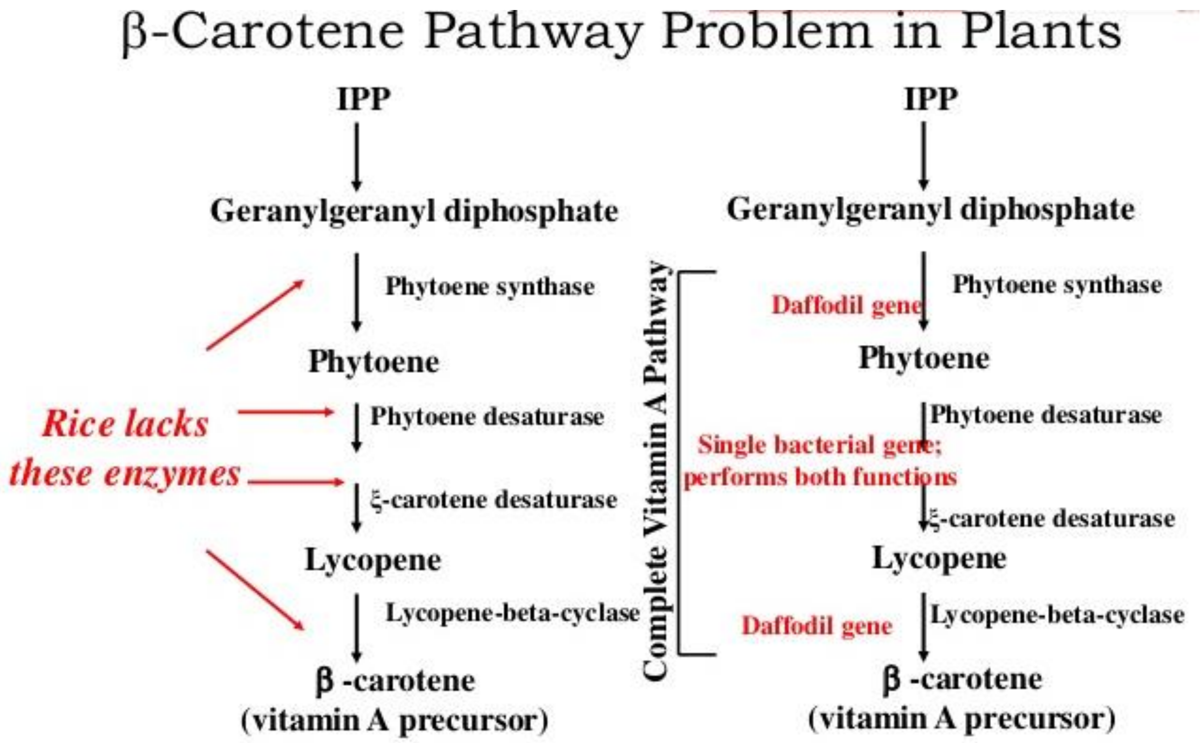
Transgenic approach has become feasible due to two reasons:
1. The rapid progress in the development of rice transformation technologies through biolistic methods as well as using *Agrobacterium.*

2. The availability of almost complete molecular elucidation of the carotenoid biosynthetic pathway in numerous bacteria and plants which provides ample choice of bacterial genes and plant cDNA to select from.

- Vitamin A deficiency often occurs in the countries where rice is the staple food. Since rice grain does not contain provitamin A *i.e.*, β-carotene, Golden rice can cope up the deficiency.
- Rice produces β-carotene in the leaves but not in the grain, where the biosynthetic pathway is turned off during grain development.
- Immature rice endosperm is capable of synthesizing GGDP (geranyl geranyl diphosphate) but subsequent stages of pathway are not expressed in this tissue.

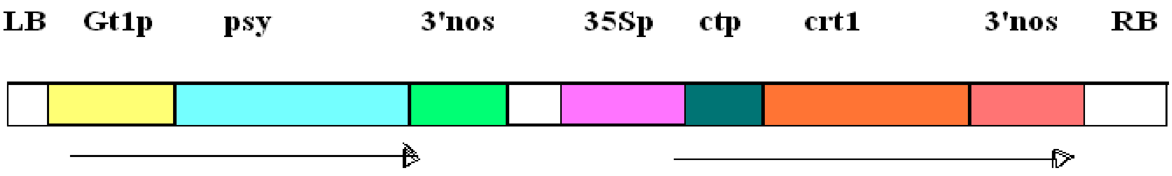
Beta-Carotene Pathway Problem in Plants

- Absence of the enzymes responsible for carotene pathway *i.e.*, Phytoene synthase (psy), Lycopene cyclase gene, Phytoene desaturase and Zeta-carotene desaturase gene.



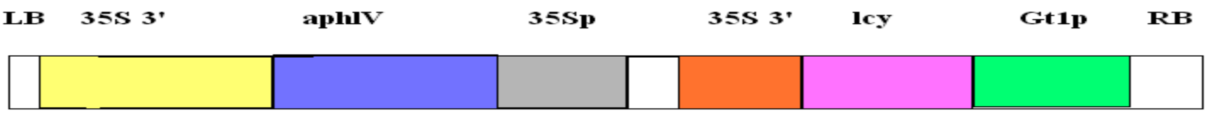
- Thus, in order to solve the Beta-Carotene Pathway problem, genes encoding enzymes Phytoene synthase (psy) and Lycopene cyclase gene were derived from daffodils (*Narcissus pseudonarcissus*) and Phytoene desaturase, Zeta-carotene desaturase gene from soil bacteria *Erwinia uredovora.*
- The daffodil phytoene synthase (*psy)* gene with rice glutelin promoter construct was inserted into the vector pZPsC, along with the bacterial zeta carotene desaturase gene, (*crt*1) controlled by the 35S promoter.

- (a) pZPsc



Both enzymes were targeted to the plastid (the site of GGDP synthesis): *psy* gene by its own transit peptide and the *crt*1 gene by fusion to a pea RUBP (ribulose-1,5-bisphosphate carboxlase/oxygenase) small subunit (rbcs) transit peptide sequence.

(b) pZLcyH



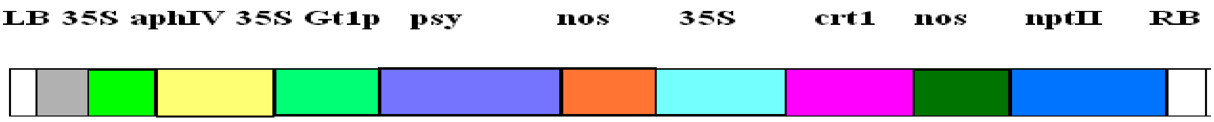
- The lycopene β-cyclase gene with a functional transit peptide was inserted into vector pZLcyH under the rice endosperm-specific glutelin promoter along with hygromycin resistance marker gene.

Co-transformation

- The immature precultured embryos were inoculated with an Agrobacterium mixture of LBA4404/pZCycH and LBA4404/pZLcyH.
- The co-transformed plants were analyzed by Southern hybridization.

To determine the formation of β-carotene, mature seeds from the transform lines and control plants were air dried, de-husked and polished and the colour of the transformed endosperms was observed.

c) pB19hpc



The three vectors constructed. pB19hpc is used in single transformation whereas pZPsC and pZLcyH are used in co-transformation. LB, left borders; RB, right borders; p, promoter; Gt1 glutelin; *psy*, phytoene synthase; *crt*l, bacterial carotene desaturase; l*cy*, lycopene β-cyclase; aphIV, hygromycin resistant gene.

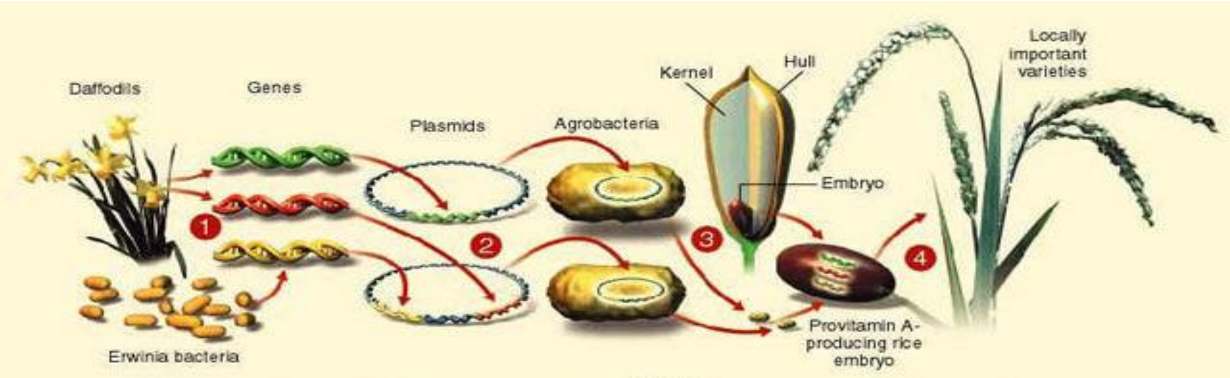
Single Transformation

- The rice immature embryos were inoculated with Agrobacterium LBA 4404/pB19hpc with the presence of hygromycin.

- The hygromycin-resistance plants were analyzed by Southern hybridization for the presence of psy and crtl genes
- The endosperm of these plants' seeds were isolated and appeared yellow, indicating carotenoid production
- High Performance Liquid Chromatography (HPLC) analysis revealed the presence of β-carotene in transgenic endosperm

The Golden Rice Solution

In one transgenic line, β-carotene content was as high as 85% of the total carotenoids present in the grain.



Improvements made to Golden rice

- The *pmi* (mannose 6-phosphate isomerase) mannose-selection gene was substituted to avoid antibiotic selection using the hygromycin-resistance gene.
- The Golden Rice trait was genetically engineered into *indica* rice cultivars, whereas the original Golden Rice was produced using the *japonica* variety Taipei 309.
- Subsequent research indicated that the lycopene beta-cyclase transgene was not required to produce beta carotene in the endosperm.

New Golden Rice generation: Golden Rice 2

- Further work by Syngenta to optimize beta-carotene production showed that the daffodil phytoene synthase was rate limiting and *psy* gene from maize was much more effective in the accumulation of total carotenoids.
- Transformation of rice with the a new construct resulted in a 23 fold increase in carotenoids compared with the original Golden Rice and has been named Golden Rice 2.
- To construct Golden Rice 2, the phytoene synthase gene (*psy*) from maize and the carotene desaturase gene (*crtI*) from *Erwinia uredovora* were inserted into rice.
- The genetic elements present in the Golden Rice 2 construct are shown in Figure 5.1. *Agrobacterium tumefaciens*-mediated transformation was used to introduce the DNA construct into Oryza sativa. The selectable marker used for transformation was the *E. coli* phosphomannose isomerase selection (PMI) system. A polymerase chain reaction-based (PCR) method was used for preliminary identification of events with single copies of the

transferred DNA. No additional molecular characterization of the transformants was reported (Paine and others 2005).

PRESENT STATUS:

Golden rice has got regulatory approval from Australia (2017), Canada (2018), United States (2018), New Zealand (2017) and Philippines (2019). It is being used as food for direct use or used in processing.

ADVANTAGES AND DISADVANTAGES:

The advantage of golden rice is that it can be used in areas where vitamin A deficiency is common, so it can help prevent blindness.

- Golden Rice 2, which is deep orange in color, was developed to greatly increase the β-carotene of rice. It contains as much as 37 µg total carotenoids/g dry weight of grain, of which 31 µg/g is β-carotene. Originial Golden Rice (GR1) does not produce enough ß-carotene (Provitamin A); it produces "only **1.6 µg/gm** of carotenoids; a child would have to eat more than **10kg/day** to get sufficient dose".
- Due to the higher level of - carotene, the consumption of Golden Rice 2 in typical quantities may provide adequate daily intake of vitamin A in countries in which rice is a staple food, assuming cooking losses of -carotene are not excessive.
- If proven effective, Golden Rice 2 could provide adequate levels of vitamin A to large populations that are currently at risk for VAD. Golden Rice 2 may also be effective even in populations where rice is a smaller dietary component. Although not safety related, stability, bioefficacy, and consumer acceptability will need to be established as a practical matter.

However, there are also disadvantages. For example:

- beta carotene levels in golden rice may not be high enough to make a difference
- there are fears that it will cross-breed with and contaminate wild rice
- there are concerns that food from GM plants might harm people
- seed for GM plants can be expensive

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