

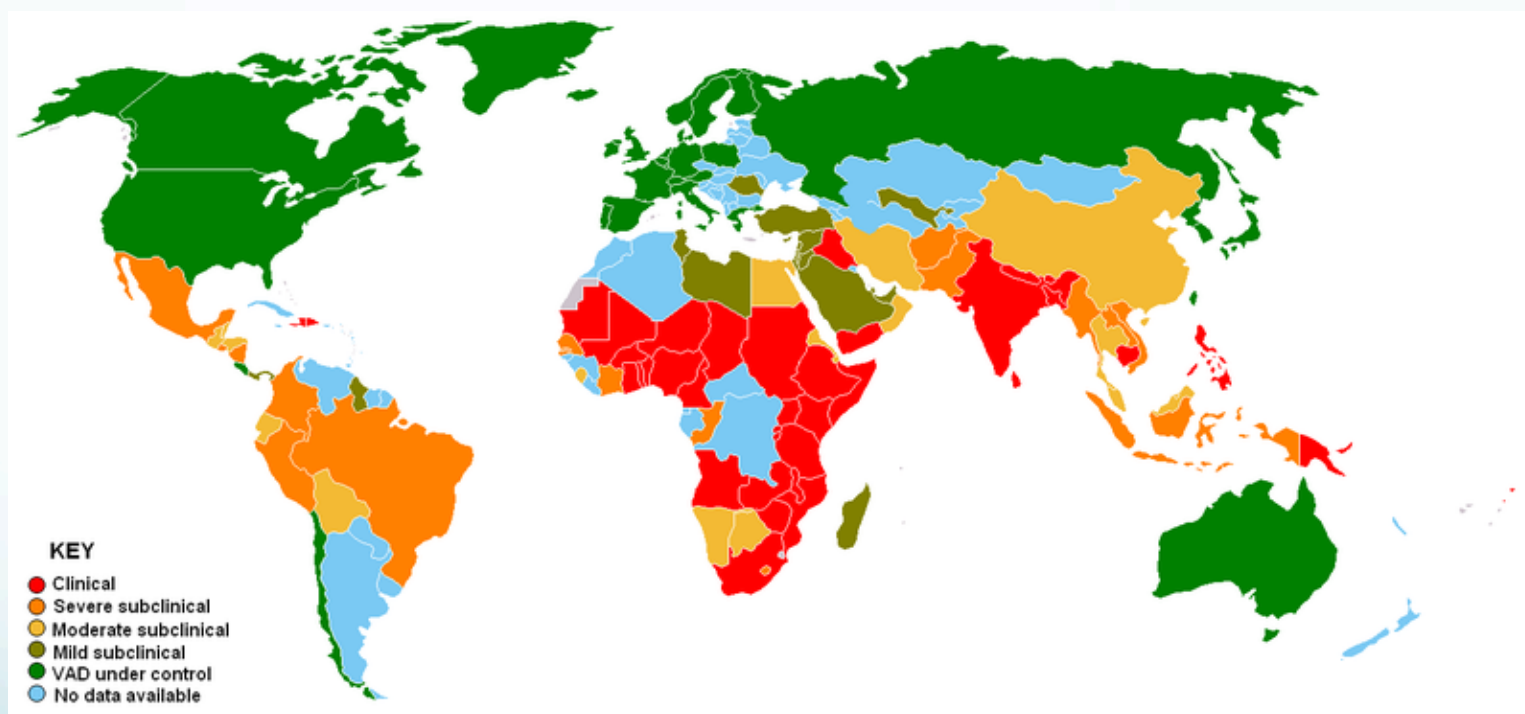
Golden Rice: Introduce Pro- vitamin A in Rice Endosperm

Yingying Wu



OHIO
UNIVERSITY

Vitamin A deficiency



Source: WTO

- Vitamin A deficiency can result in permanent blindness and increase the incidence and severity of infectious diseases.
- It is estimated that a quarter of a million children go blind each year because of nutritional deficiency in southern Asia.
- Success may be got by using recombinant technologies to produce provitamin A in a major staple food, rice. No rice cultivars produce provitamin A in endosperm, which is the edible part of the grain.



Co-creator of Golden Rice: Professor [Ingo Potrykus](#)

What is Golden Rice?

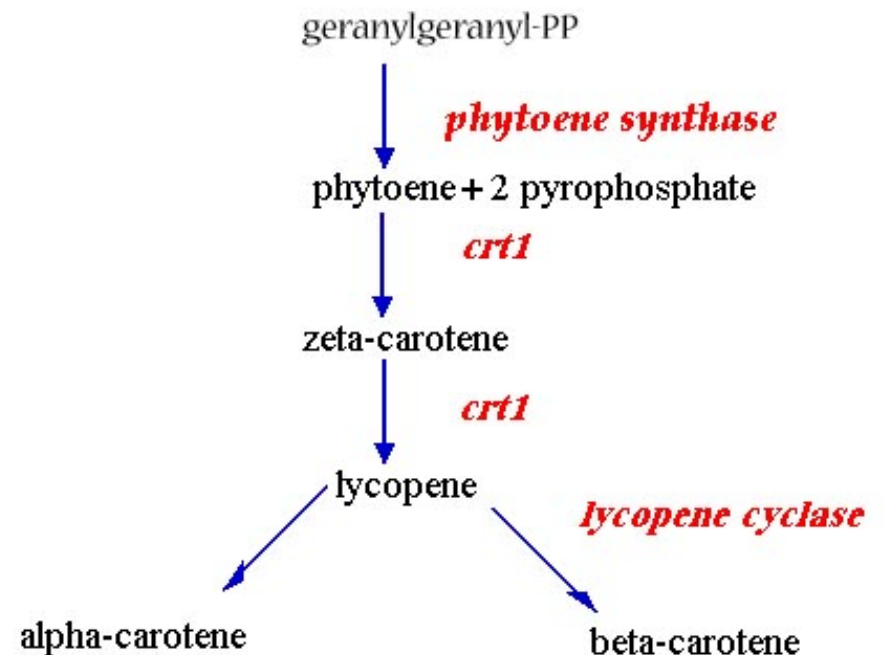
- ‘Golden Rice’ is a kind of rice engineered to produce beta-carotene (provitamin A).
- The carotenoids produced in the endosperm of the grains give rise to a characteristic yellow color.



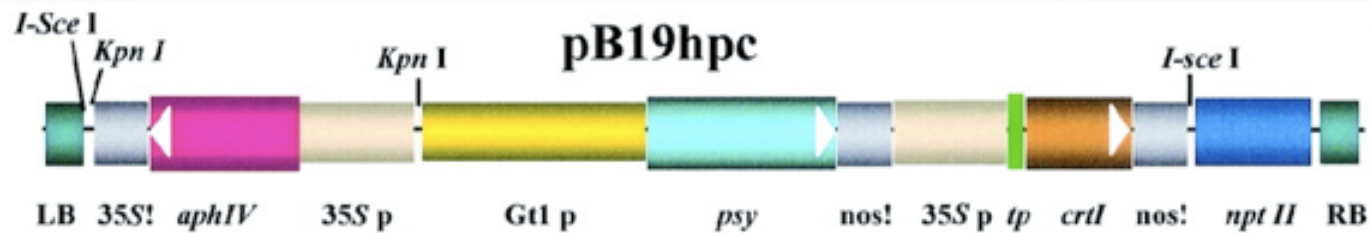
Common white rice and golden rice

Carotenoid Biosynthesis in Golden Rice

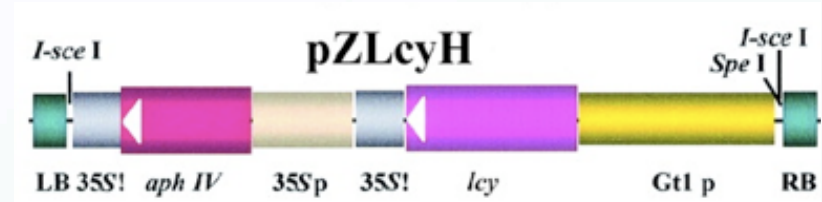
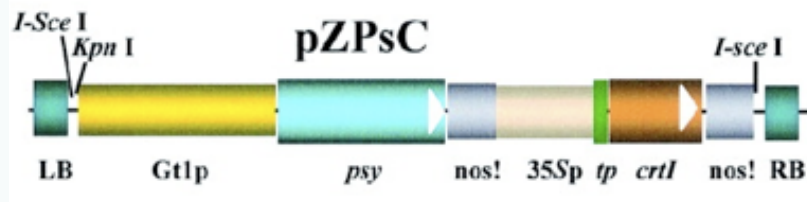
The synthesis of beta-carotene requires plant enzymes: **phytoene synthase**; **phytoene desaturase** and **zeta-carotene desaturase** and **lycopene cyclase**;



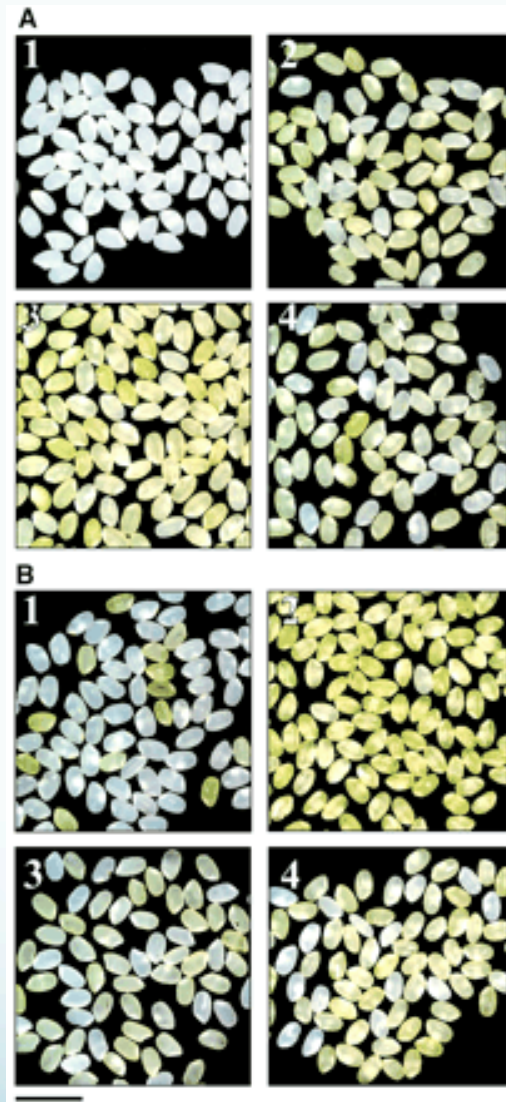
The Original Golden Rice



Structures of the T-DNA region of pB19hpc used in single transformations



pZPsC and pZLcyH used in co-transformations. LB, left border; RB, right border; “!”, polyadenylation signals; p, promoters; psy, phytoene synthase; crtI, bacterial phytoene desaturase; lcy, lycopene beta-cyclase; tp, transit peptide.



- Phenotypes of transgenic rice seeds. Bar, 1 cm. (A) Panel 1, untransformed control; panels 2 through 4, pB19hpc single transformants lines h11a (panel 2), h15b (panel 3), h6 (panel 4). (B) pZPsC/pZLcyH co-transformants lines z5 (panel 1), z11b (panel 2), z4a (panel 3), z18 (panel 4).

- Precultured immature rice embryos were inoculated with *Agrobacterium* LBA4404;
- Golden rice was created by transforming rice with two beta-carotene biosynthesis genes:
 - *psy* (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*)
 - *crt1* from the soil bacterium *Erwinia uredovora*
- The insertion of a *lyc* (lycopene cyclase) gene was thought to be needed but further research showed that it is already being produced in wild type rice endosperm.
- Under greenhouse conditions it produces 1.6 ug/g carotenoids.

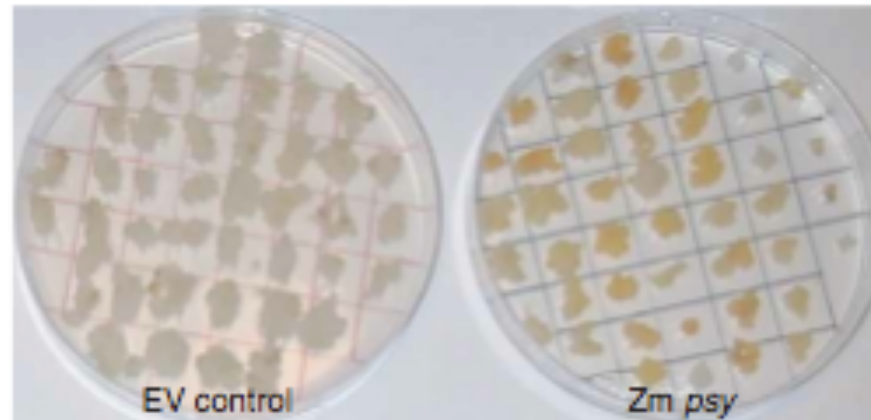
Golden Rice 2

Phytoene synthase is the limiting step for carotenoid biosynthesis and is a major regulatory step;

There was no shortage of the precursor geranyl geranyl diphosphate and no problem with product sequestration;

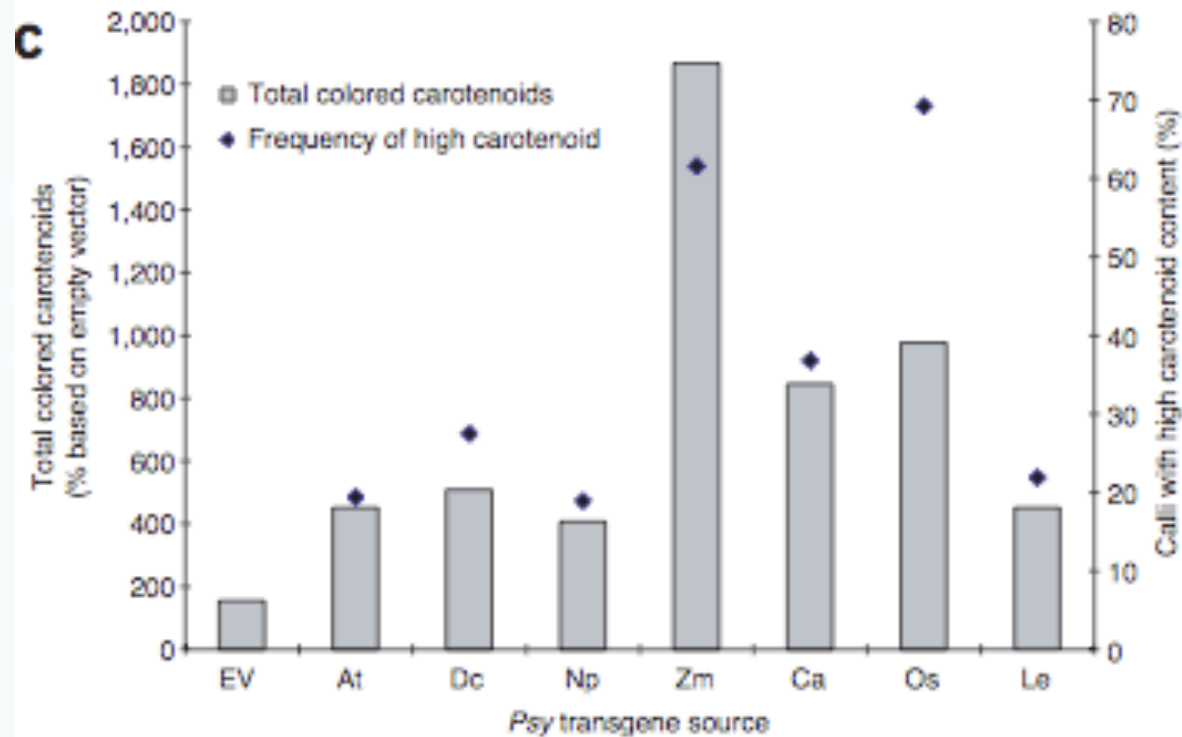
The Golden Rice 2 reported has up to 37 ug/g carotenoid of which 31 ug/g is beta-carotene. Golden Rice 2 produces 23 times more carotenoid than golden rice;

The Golden Rice 2 combines the phytoene synthase gene from maize with *crt1* from the original golden rice.

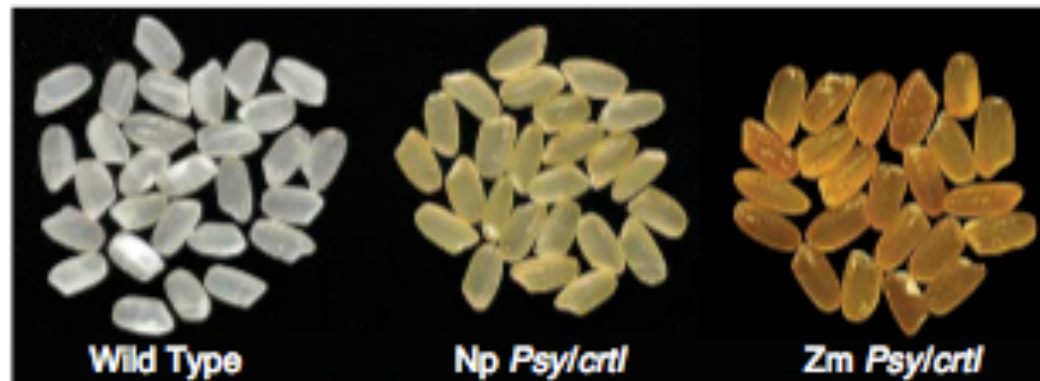


Expression of a *psy* transgene increases the carotenoid content of maize callus.

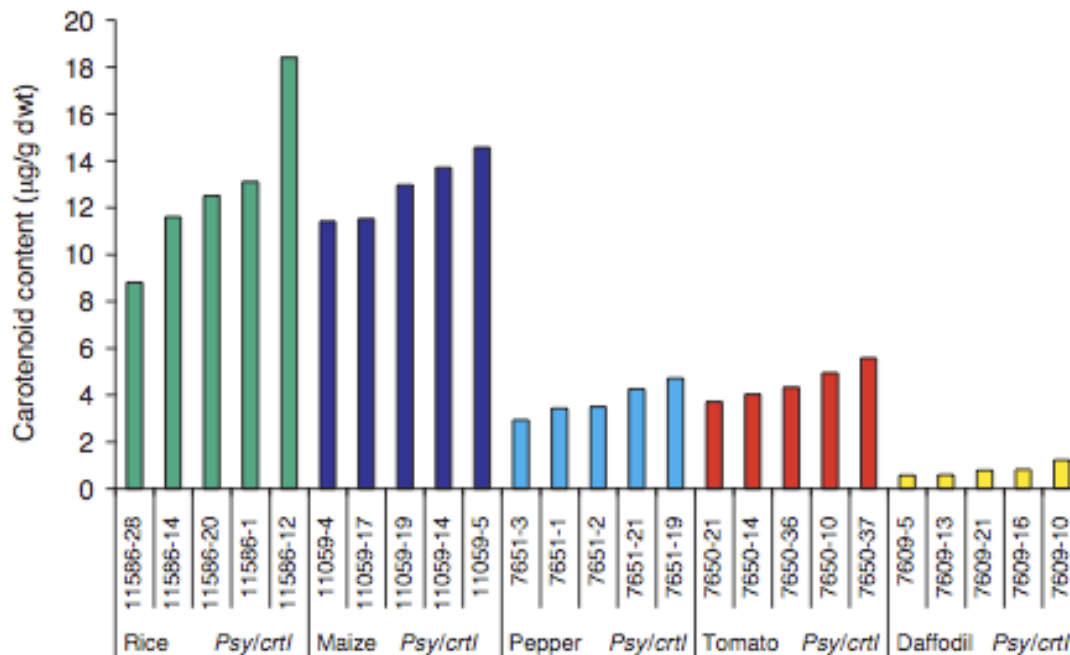
Individual maize calli cotransformed with the plasmid containing the maize *psy* (right, *Zm psy*) and an empty vector (EV) control (left).



- Histogram shows the total colored carotenoid content of maize calli transformed with a given *psy* gene (from *Arabidopsis thaliana* (At), *Daucus carota* (Dc), *Narcissus pseudonarcissus* (Np), *Zea mays* (Zm), *Capsicum annuum* (Ca), *Oryza sativa* (Os) or *Lycopersicon esculentum* (Le)). Data shown represents the 75th percentile for each population of transgenic calli expressed as a percentage of the median empty vector (EV) control value. The second y-axis (diamonds) shows the percentage of calli from each population with a carotenoid content more than fivefold that of the EV median.



- Carotenoid enhancement of the rice endosperm by transformation with *psy* and *crtI*. Photograph of polished wild-type and transgenic rice grains containing the T-DNA with the daffodil *psy* (Np) or maize *psy* (Zm) showing altered color due to carotenoid accumulation.



- The total carotenoid content of T₁ rice seed containing a T-DNA with the *psy* gene from either rice, maize, pepper, tomato or daffodil from the five events with the highest carotenoid content for each T-DNA

Table 1 Carotenoid content and composition of transgenic rice endosperm

psy Source	Event Identity (number of T ₁ transgenic plants analyzed)	Total carotenoid content in T ₁ (T ₂ ^a) seed (μg/g dry weight)	Colored carotenoid composition, % of total in T ₁ (T ₂ ^a) seed				
			β ⁻ carotene	α-carotene	β ⁻ cryptoxanthin	Zeaxanthin	Lutein
Maize	11059-5 (6)	14.5 (14.4)	89.0 (83.3)	9.7 (10.4)	0.6 (2.6)	0.3 (1.9)	0.4 (1.7)
	11059-11 (6)	9.8 (14.2)	85.8 (84.7)	10.4 (9.5)	1.7 (2.9)	1.0 (1.6)	1.0 (1.3)
	11059-14 (5)	13.7 (16.0)	87.1 (86.0)	11.0 (9.3)	1.2 (2.3)	0.3 (1.3)	0.4 (1.1)
	11059-16 (6)	10.1 (11.8)	85.6 (85.8)	10.5 (8.9)	1.7 (2.7)	1.2 (1.5)	1.0 (1.1)
	11059-17 (6)	11.5 (16.5)	86.7 (85.0)	10.4 (9.1)	1.5 (2.6)	0.9 (1.8)	0.5 (1.5)
Pepper	7651-3 (5)	2.9 (2.1)	80.5 (72.7)	9.8 (11.2)	2.7 (4.9)	3.6 (4.9)	3.5 (6.2)
	7651-19 (5)	4.7 (5.2)	77.9 (76.6)	12.4 (11.9)	2.6 (4.5)	4.0 (4.9)	3.1 (2.1)
	7651-21 (5)	4.2 (4.9)	77.8 (78.8)	12.6 (9.9)	2.6 (5.0)	4.1 (4.0)	2.8 (2.2)
Tomato	7650-4 (5)	1.1 (2.2)	64.3 (65.9)	15.5 (9.9)	3.7 (4.7)	5.1 (9.0)	11.4 (10.6)
	7650-8 (4)	0.9 (1.3)	61.5 (58.9)	15.7 (9.8)	4.8 (6.8)	5.6 (12.3)	12.4 (12.2)
	7650-11 (2)	1.2 (2.0)	68.0 (68.4)	13.8 (11.9)	4.9 (6.7)	4.7 (6.8)	8.7 (6.2)
Rice	11586-1	13.1	81.2	13.6	1.7	1.2	2.2
	11586-12	18.4	85.0	12.2	1.0	0.7	1.0
	11586-14	11.6	86.4	9.6	1.9	1.0	1.0
	11586-20	12.5	78.4	16.1	2.2	1.3	1.9
	11586-28	8.8	84.4	10.2	2.5	1.3	1.5
Daffodil	7609-10	1.2	68.5	11.6	6.2	6.8	7.0
	7609-16	0.8	58.5	10.8	4.6	9.4	15.0
	7609-21	0.8	65.8	10.5	4.7	7.8	10.4

^aThe number given represents the average carotenoid content of the homozygous T₂ grain analyzed.

Conclusion

- 'Golden Rice' is a kind of rice engineered to produce beta-carotene (provitamin A).
- Golden rice was created by transforming rice with two beta-carotene biosynthesis genes: *psy* (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*) *crt1* from the soil bacterium *Erwinia uredovora*.
- The Golden Rice 2 combines the phytoene synthase gene from maize with *crt1* from the original golden rice.

Reference

Ye et al. 2000. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287 (5451): 303-305 PMID 10634784

Paine et al. 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology* doi:10.1038/nbt1082