The bacterium *Agrobacterium tumefaciens* is a pathogen of plants that causes crown gall tumors.

The components of T DNA transfer

The T-DNA is transferred to the plant cell.

- 25 bp repeats flanking T DNA mediate the transfer of DNA to plant cell.

T-DNA contains two types of genes:

1. oncogenic genes (responsible for tumor formation).
2. genes encoding enzymes that synthesize opines, compounds synthesized and excreted by crown gall cells and consumed by *A. tumefaciens* as carbon and nitrogen sources.

*vir* genes required for the transfer of the T-DNA from the bacterium to the plant cell.

The generation of a GM plant

Bacterium contains two plasmids:

- *Ti* plasmid that lacks T DNA, and thus can’t cause crown gall disease.
- Recombinant plasmid that has a selectable trait (here the gene that confers kanamycin resistance) and the gene of interest flanked by the T DNA repeats.

The *vir* genes of the *Ti* plasmid now cause the gene of interest to be transferred into plant cells.
*Agrobacterium* mediated transfer works for dicots, like cotton.

BUT, not for monocots, like corn or wheat.

There are other ways to get DNA into monocots.

One way is to shoot genes in with a “gene gun” using gold particles coated with DNA. This approach is sometimes referred to as biolistic transformation.

OK, now what genes are put into the plants?

- Genes that act as pesticides.
- Genes for herbicide resistance.
- Genes to increase nutritional value.
- Genes for pharmaceuticals.

**Bacillus thuringiensis** (Bt)

Bacterium kills insects.

Discovered in 1911 as pathogen of flour moths.

Used in the US in the 1950s.

Replaced by more effective insecticides in the 1960s.

With resistance to synthetic pesticides, Bt research funded in the 1980s.

Bt strains produce a complex mixture of Cry toxins that kill Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), and Coleoptera (beetles).

The end of the insect

Cry toxins are a large proteins that must be activated before it has any effect.

Forms insoluble crystals under normal conditions.

Protein made soluble in the conditions found in the gut of insect larvae and hence is highly specific.

Once soluble, the protoxin is cleaved by a gut protease to produce an active toxin, delta-endotoxin, that creates holes in cell membranes. As a result, gut cells die, and the larva stops feeding.

Trangenic plants that express delta endotoxin are resistant to insect pathogens

Bt corn: European and Southwestern corn borers, and corn rootworm

Bt cotton: tobacco budworm and cotton bollworm

Bt potato: Colorado potato beetle (off the market in 2001)
Bt transgenic plants

Pros
Less insecticides used
In US, insecticide applications for cotton
- 4.6 1992-1995
- 0.8 1996-2001
Cheaper?

Cons
Health concerns: allergenic proteins
Environmental concerns:
- Effects on other organisms (pest parasites or predators; beneficial insects)
- Development of resistance
- Transfer to wild species

Herbicide resistance
Plants make aromatic amino acids like phenylalanine. Glyphosate, the active ingredient in the herbicide Roundup, inhibits the enzyme EPSP synthetase, which is required for the synthesis of these amino acids. Plants, especially weeds, will die when treated with glyphosate.

With use, weeds resistant to Roundup are selected. Need more Roundup, but at higher concentrations crops are affected.

The gene for a glyphosate-resistant form of EPSP synthetase has been introduced into commercial plants. Now Monsanto can sell both the highly resistant plants and Roundup to farmers.

Transgenic plants for nutrition
Vitamin A deficiency causes symptoms ranging from night blindness to those of xerophthalmia and keratomalacia, leading to total blindness. In Southeast Asia, it is estimated that a quarter of a million children go blind each year because of this nutritional deficiency (1). Furthermore, vitamin A deficiency exacerbates afflictions such as diarrhea, respiratory diseases, and childhood diseases such as measles (2, 3). It is estimated that 124 million children worldwide are deficient in vitamin A (4) and that improved nutrition could prevent 1 million to 2 million deaths annually among children (3).

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Modifying the nutritional content of food.

“At first, the grains of rice that Ingo Potrykus sifted through his fingers did not seem at all special, but that was because they were still encased in their dark, crinkly husks. Once those drab coverings were stripped away and the interiors polished to a glossy sheen, Potrykus and his colleagues would behold the seeds’ golden secret. At their core, these grains were not pearly white, as ordinary rice is, but a very pale yellow—courtesy of beta-carotene, the nutrient that serves as a building block for vitamin A.” J. MADELEINE NASH/ZURICH, TIME Magazine July 31, 2000

Engineering the Provitamin A (β-Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm

Xudong Ye,1,4 Salim Al-Babili,2,6 Andreas Klöti,1,5 Jing Zhang,1 Paola Lucca,1 Peter Beyer,1,5 Ingo Potrykus1,5

Rice (Oryza sativa), a major staple food, is usually milled to remove the oil-rich aleurone layer that turns rancid upon storage, especially in tropical areas. The remaining edible part of rice grains, the endosperm, lacks several essential nutrients, such as provitamin A. Thus, predominant rice consumption promotes vitamin A deficiency, a serious public health problem in at least 26 countries, including highly populated areas of Asia, Africa, and Latin America. Recombinant DNA technology was used to improve its nutritional value in this respect. A combination of transgenes enabled biosynthesis of provitamin A in the endosperm.

In other words, golden rice is transgenic rice with genes for the synthesis of β-carotene, the precursor of Vitamin A. The genes are from the daffodil and a bacterium.

A NAC Gene Regulating Senescence Improves Grain Protein, Zinc, and Iron Content in Wheat

Cristobal Uauy,1,2 Assaf Distelfeld,3,4 Tdun Fahima,1 Ann Blechl,3 Jorge Dubcovsky5

Enhancing the nutritional value of food crops is a means of improving human nutrition and health. We report here the positional cloning of Gpr-ß2, a wheat quantitative trait locus associated with increased grain protein, zinc, and iron content. The ancestral wild wheat allele encodes a NAC transcription factor (WAM-ß2) that accelerates senescence and increases nutrient remobilization from leaves to developing grains, whereas modern wheat varieties carry a nonfunctional WAM-ß2 allele. Reduction in mRNA levels of the multiple WAM homologs by RNA interference delayed senescence by more than 3 weeks and reduced wheat grain protein, zinc, and iron content by more than 30%.


"Malnutrition is a problem of poverty, not technology." Day-cha Siripat of Thailand’s Alternative Agriculture Network

Pharma crops
Plants engineered to produce:
- Drugs
- Antibodies
- Vaccines
- Industrial and research chemicals


So who oversees all these Biotech Products?

FDA regulates novel substances in foods and feeds on the basis of dietary risk

USDA (APHIS) regulates new plant varieties on the basis of ecological risk

EPA regulates pesticides and new microbes on the basis of human and ecological risk
Since 1991, the USDA has approved ~400 field tests of transgenic crops

Use of GM crops on the rise?