Flavr Savr Tomato, Golden Rice, Bt Cotton

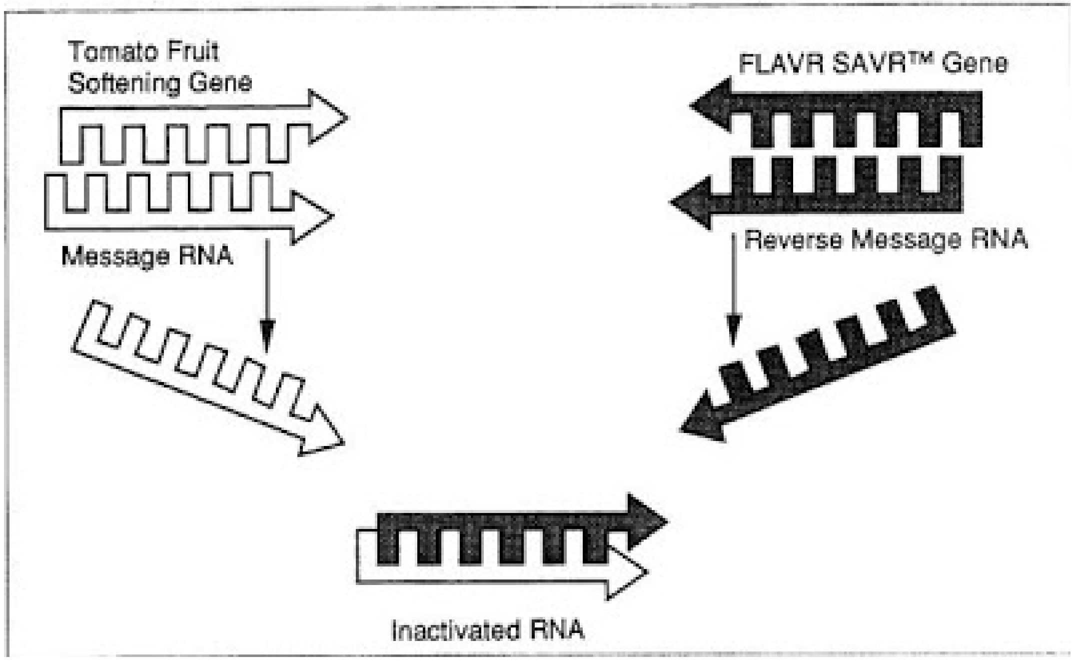
Mitesh Shrestha

Flavr Savr Tomato

- The first FDA approved genetically modified food
- Licensed in 1994
- Ripening causes production of an enzyme Polygalacturonase in a gradual increasing level, which is responsible for softening of the tomato and which becomes the cause of rottening
- Calgene introduced a gene in plant which synthesize a complementary mRNA to PG gene and inhibiting the synthesis of PG enzyme.

Antisense RNA

Antisense RNA (asRNA) is a single-stranded RNA that is complementary to a messenger RNA (mRNA) strand transcribed within a cell.



Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8805–8809, December 1988
Biochemistry

Reduction of polygalacturonase activity in tomato fruit by antisense RNA

(plant transformation/cauliflower mosaic virus 35S promoter/lycopene accumulation)

RAYMOND E. SHEEHY, MATTHEW KRAMER, AND WILLIAM R. HIATT*
Calgene, Inc., 1920 Fifth Street, Davis, CA 95616

Communicated by E. Peter Geiduschek, August 4, 1988

Put PG gene sequences in the reverse orientation into the vector

↓

Transformation

↓

Isolate the nuclei used for in vitro transcription

↓

Isolate the RNA

↓

Hybridize RNA

↓

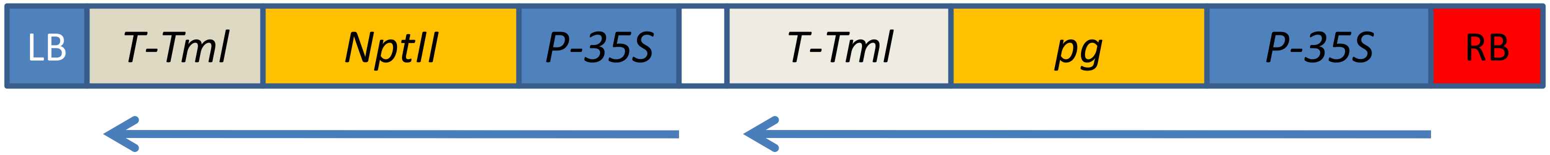
Measure PG activity

↓

Lycopene determination

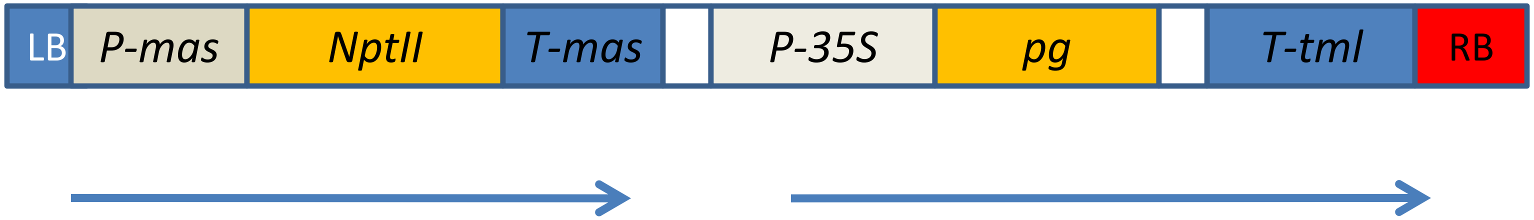
Flavr Savr Tomatoes

- **Constructs used:** pCGN1436, pCGN1547, pCGN1548, pCGN1549, pCGN1557, pCGN1558, pCGN1559, pCGN1578 or pCGN4109

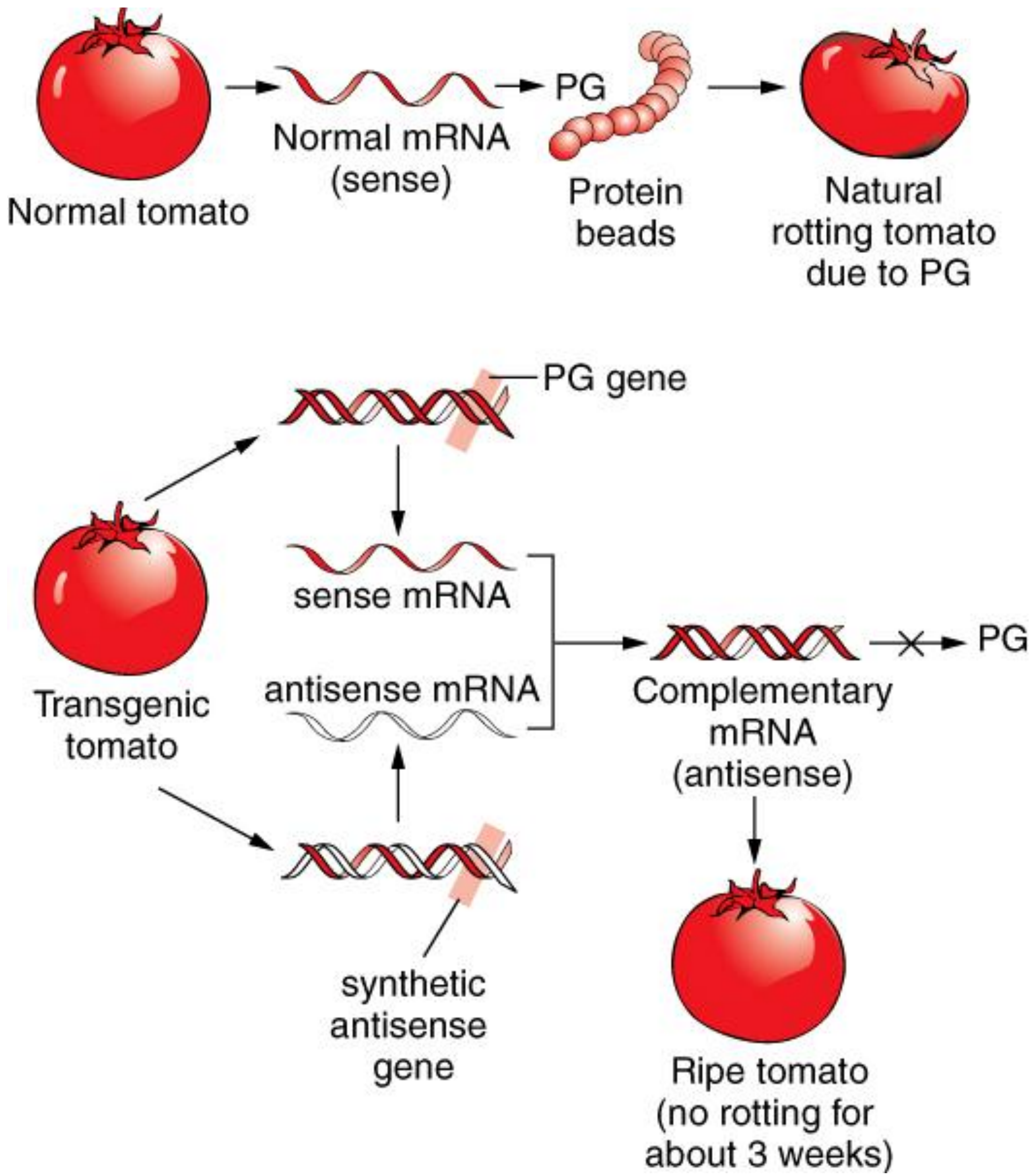


Components of construct pCGN4109: tml-termination sequences from plasmid pTiA6 plasmid, *npt-neomycin phosphotransferase; pg-polygalacturonase, 35S- CaMV 35S promoter, LB- left border, RB-right border*

Flavr Savr Tomatoes



Components of construct *pCGN14369*:*mas-mannopine synthase gene from A. tumefaciens*, tml-termination sequences from plasmid pTiA6 plasmid, *npt-neomycin phosphotransferase; pg-polygalacturonase, 35S- CaMV 35S promoter, LB- left border, RB-right border*


Normal tomato
Normal mRNA
(sense)
PG
Protein
beads
Natural
rotting tomato
due to PG
PG gene
sense mRNA
Transgenic
tomato
antisense mRNA
Complementary
mRNA
(antisense)
PG
synthetic
antisense
gene
Ripe tomato
(no rotting for
about 3 weeks)

Effects of Malnutrition

- Symptoms of vitamin A deficiency (VAD) include; night blindness, increased susceptibility to infection and cancer, anemia (lack of red blood cells or hemoglobin), deterioration of the eye tissue, and cardiovascular disease
- Nearly 9 million children die from malnutrition each year. A large proportion of those children die from common illnesses that could have been avoided through adequate nutrition
- The reduced immune competence increases the morbidity and mortality rates of children

Who Began the Golden Rice Project?

- Started in 1982 by Ingo Potrykus-Professor emeritus of the Institute for Plant Sciences
- Peter Beyer-Professor of Centre for Applied Biosciences, Uni. Of Freiburg, Germany
- Funded by the Rockefeller Foundation, the Swiss Federal Institute of Technology, and Syngenta, a crop protection company.

- *Golden Rice* Humanitarian Board-responsible for the global development, introduction and free distribution of Golden Rice to target countries.

Why Rice?

- Global staple food. Cultivated for over 10,000 years
- Rice provides as much as 80 percent or more of the daily caloric intake of 3 billion people, which is half the world's population

- Other plants, such as sweet potatoes have varieties that are either rich (orange-fleshed) or poor (white fleshed) in pro-vitamin A
- Carrots were originally white or purple in the 1600's. A Dutch horticulturist mutated the carrot to produce carotenes to symbolize the color of the Dutch Royal House of Orange

Golden rice

- **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 of rice.
- **Biofortification** - The creation of plants that make or accumulate micronutrients
- The research was conducted with the goal of producing a fortified food to be grown and consumed in areas with a shortage of dietary vitamin A, which is estimated to kill 670,000 children under 5 each year.
- Golden rice differs from its parental strain by the addition of **three beta-carotene biosynthesis gene**

Golden Rice

- Orange/golden colour due to synthesis of proVitamin A in the kernels
- Developed at the Swiss Federal Institute of Technology (ETH) Zurich
- Provitamin A pathway is an extension of lycopene pathway
- Rice embryos can produce Geranyl Geranyl pyrophosphate, but are not capable of synthesis of either lycopene or β carotene as they lack the required enzymes

- Golden rice was designed to produce beta-carotene, a precursor of vitamin A, in the edible part of rice, the endosperm.
- The rice plant can naturally produce beta-carotene in its leaves, where it is involved in photosynthesis.
- However, the plant does not normally produce the pigment in the endosperm, where photosynthesis does not occur.
- Golden rice was created by transforming rice with two beta-carotene biosynthesis genes:

  1.psy (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*)

  2.crtI from the soil bacterium *Erwinia uredovora*

- The *psy* and *crtI* genes were transformed into the rice nuclear genome and placed under the control of an endosperm-specific promoter, so they are only expressed in the endosperm.
- The exogenous *lyc* gene has a transit peptide sequence attached so it is targeted to the plastid, where geranylgeranyl diphosphate formation occurs.
- The bacterial *crtI* gene was an important inclusion to complete the pathway, since it can catalyze multiple steps in the synthesis of carotenoids up to lycopene, while these steps require more than one enzyme in plants.
-] The end product of the engineered pathway is lycopene, but if the plant accumulated lycopene, the rice would be red.
- Recent analysis has shown the plant's endogenous enzymes process the lycopene to beta-carotene in the endosperm, giving the rice the distinctive yellow color for which it is named.

Golden Rice

- Conversion of phytoene to β carotene requires four enzymes: Phytoene synthase, phytoene desaturase, carotene desaturase and lycopene cyclase to convert phytoene to β carotene
- Early transformation experiments with *psy* (phytoene synthase) from daffodil fused to a rice endosperm specific promoter resulted in accumulation of phytoene in rice endosperm – opened new possibilities for manipulation of pro-vitamin A synthesis in rice grains

Golden Rice

- But the rice plant still needed three more enzymes to convert phytoene to β carotene: phytoene desaturase, carotene desaturase and lycopene cyclase
- The first two desaturases introduce double bonds in phytoene to produce lycopene and the lycopene cyclase forms rings in to β carotene
- A carotene desaturase from a bacteria *Erwinina uredovora* can substitute for these two desaturases

Carotenoid biosynthesis pathway in golden rice

Geranyl geranyl pp

↓ Phyotene synthase

Phytoene + pyrophosphate

↓ Crt1

Zeta carotene

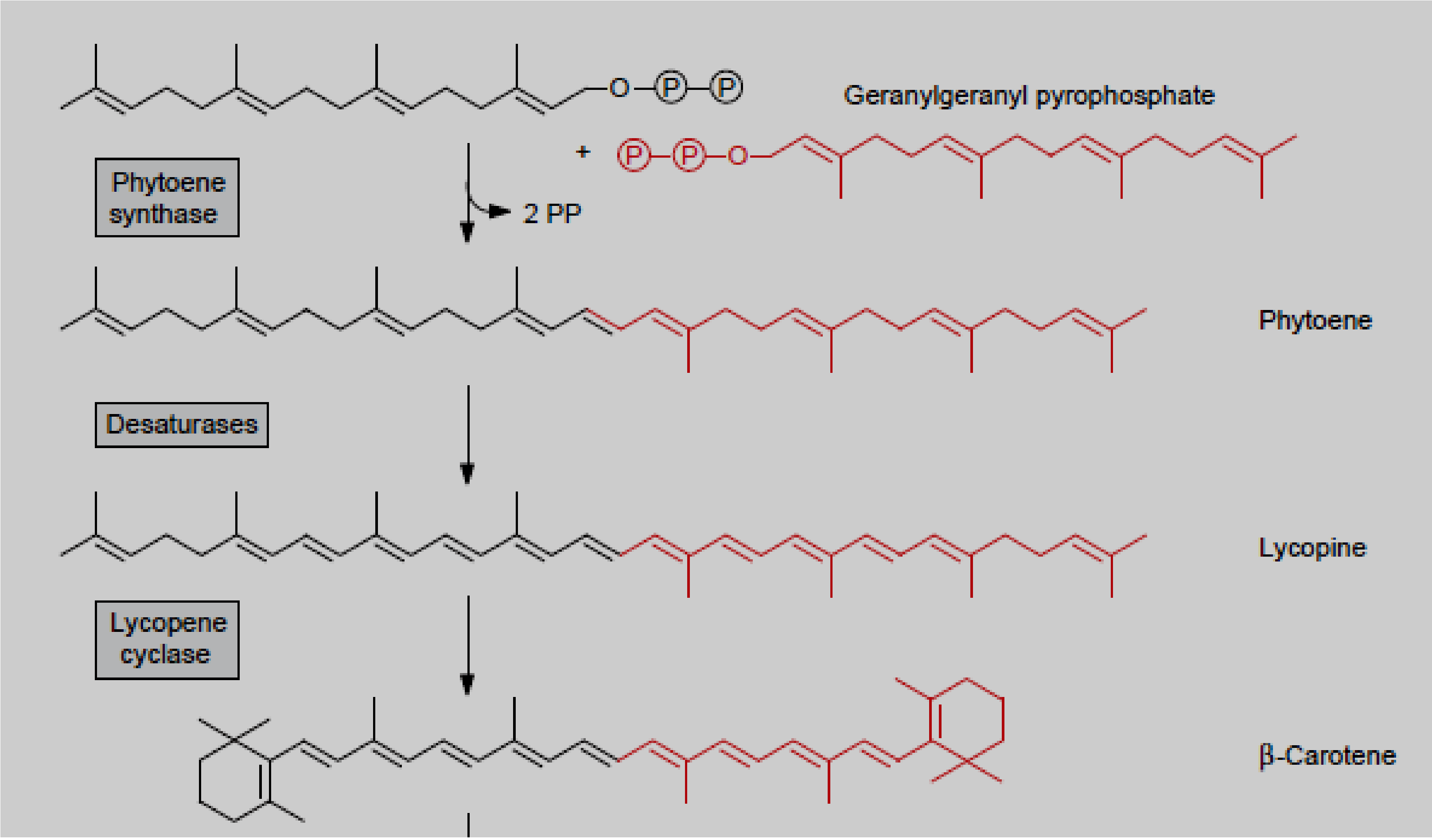
↓ Crt2

Lycopene

↙ ↘ Lycopene cyclase

Alpha carotene   beta carotene

Golden Rice

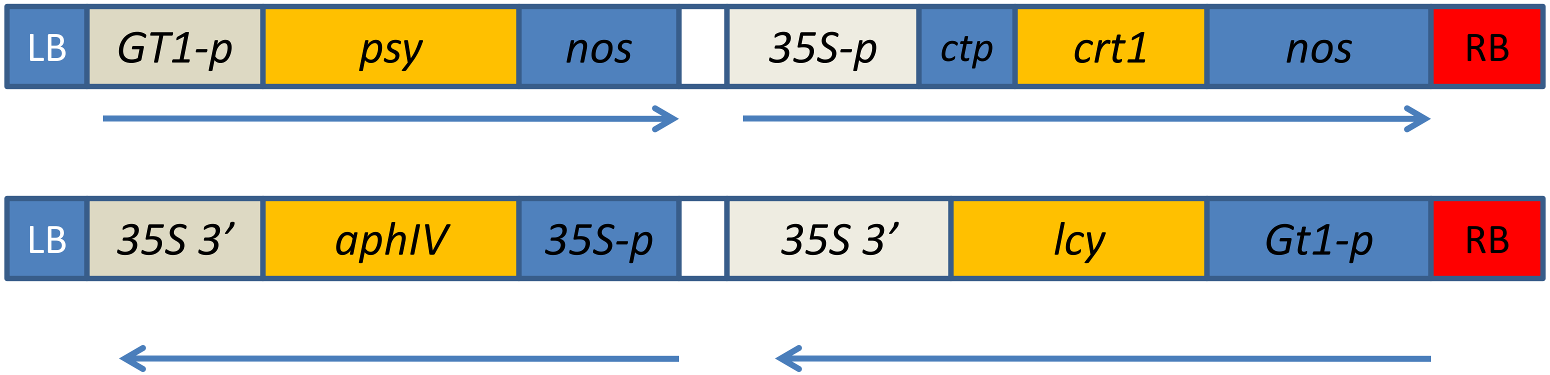


O
P
P
Geranylgeranyl pyrophosphate
Phytoene synthase
+
P
P
O
2 PP
Phytoene
Desaturases
Lycopine
Lycopene cyclase
β-Carotene

Golden Rice

Comparison of Rice (left), Golden rice1 (middle) and Golden rice 2 (right). Source: Paine et al., 2005

Golden Rice



Constructs for Golden rice1: Initially the rice plant was co-transformed with two constructs. The first construct pZPsC consists of phytoene synthase (*psy*) gene from daffodil (*Narcissus pseudonarcissus*) driven by the rice glutelin promoter (*Gt1*) in tandem with a carotene desaturase gene (*crt1*) from *Erwinina uredovora* driven by the 35S promoter. The second construct pZLcyH contains a daffodil lycopene cyclase (*lcy*) gene driven by the rice glutelin promoter in tandem with the hygromycin-resistace selectable marker gene (*aphIV*) driven by CaMV 35S promoter. **Source: Slater et al, 2008. p252.**

Golden Rice

DNA construct present in Golden Rice 2 (Paine and others 2005). Key to source of DNA elements: *Glu: rice* glutelin Glut01 (Glu) promoter (nucleotides 1568--2406) *SSUcrtl: functional fusion of the pea RUBISCO small subunit* plastid transit peptide with *Erwinia uredovora crtI (D90087; Misawa and others 1993) Terminator regions of A. tumefaciens* nos (nucleotides 1848–2100, V00087). *Zea mays phytoene synthase (psy) Zea Mays polyubiquitin Ubi–1 promoter* with intron *E. coli phospho-mannose isomerase (PMI) selectable marker.*

http://onlinelibrary.wiley.com/doi/10.1111/j.1541-4337.2007.00029_7.x/pdf

Differences between Golden rice 1 and 2

- 35 µg of carotinoids per gram of dry Golden rice 2 instead of 1.6 µg of carotinoids per gram of dry Golden rice 1.
- More efficient *phy* gene introduced.
- Removal of CaMV 35S by polyubiquitin gene.
- Incorporation of phosphomannose-isomerase sugar-based selection system instead of antibiotic selection system.

Controversy Against “Fool’s Gold”

- Health
  - May cause allergies or fail to perform desired effect
  - Supply does not provide a substantial quantity as the recommended daily intake
- Environment
  - Loss of Biodiversity. May become a gregarious weed and endanger the existence of natural rice plants
  - Genetic contamination of natural, global staple foods
- Culture
  - Some people prefer to cultivate and eat only white rice based on traditional values and spiritual beliefs

What is *Bt* cotton?

- Genetically modified to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis*.
- Toxins are crystalline proteins (Cry-proteins) that target specific pests.

Bt crop	Country
Cotton	Argentina, Australia, Brazil, Burkina Faso, Canada, China, Colombia, Costa Rica, European Union (EU), India, Japan, Mexico, Myanmar, New Zealand, Pakistan, Paraguay, Philippines, Singapore, South Africa, South Korea, United States of America (USA)
Eggplant	Bangladesh
Maize	Argentina, Australia, Brazil, Canada, Chile, China, Colombia, Egypt, El Salvador, EU, Honduras, Indonesia, Japan, Malaysia, Mexico, New Zealand, Panama, Paraguay, Philippines, Russian Federation, Singapore, South Africa, South Korea, Switzerland, Taiwan, Thailand, Turkey, USA, Uruguay
Poplar	China
Potato	Australia, Canada, Japan, Mexico, New Zealand, Philippines, Russian Federation, South Korea, USA
Rice	China, Iran
Soybean	Argentina, Australia, Brazil, Canada, China, Colombia, EU, Japan, Mexico, New Zealand, Paraguay, South Korea, Taiwan, Thailand, USA, Uruguay
Tomato	Canada, Chile, USA

Source: ISAAA's GM Approval Database. http://www.isaaa.org/gmapprovaldatabase/.

How is it produced?

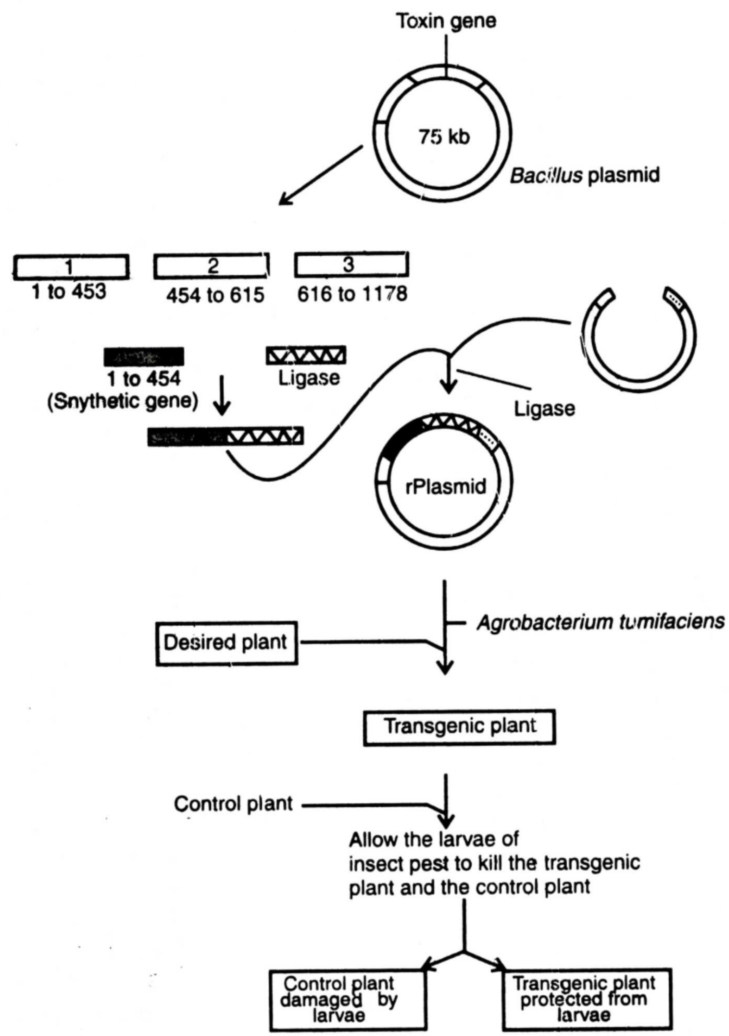


Table: Various *cry* genes encoding for the proteins with varying degree of specificity to different insect groups

Cry protein	Protein size (kDa)	Susceptible insect class
Cry1A(a-i)	133	Lepidoptera
Cry1B(a-g)	140	Lepidoptera
Cry1C(a, b)	133-134	Lepidoptera
Cry1D(a, b)	131-132	Lepidoptera
Cry1E(a, b)	133-134	Lepidoptera
Cry1F(a, b)	132-134	Lepidoptera
Cry1G(a-c)	132-133	Lepidoptera
Cry1H(a, b)	133	Lepidoptera
Cry1I(a-d)	81	Lepidoptera
Cry1J(a-d)	133	Lepidoptera
Cry1Ka	137	Lepidoptera
Cry1La	133	Lepidoptera
Cry2A(a-e)	71	Lepidoptera
Cry3Aa, Cry3B(a, b), Cry3Ca	73-75	Coleoptera
Cry4Aa, Cry4Ba	135,128	Diptera
Cry5A(a, b)	142-152	Nematodes
Cry5Ac	135	Hymenoptera
Cry5Ba	140	Hymenoptera
Cry6Aa, Cry6Ba	143	Nematodes
Cry7A(a, b)	129-130	Coleopteran
Cry8Aa, Cry8B(a-c), Cry8(Ca-Ha)	131	Coleopteran
Cry9Aa, Cry9B(a, b), Cry9Ca, Cry9D(a, b), Cry9E(a, d)	130	Lepidoptera
Cry10Aa	78	Diptera
Cry11Aa	72	Diptera
Cry11B(a, b)	81	Diptera

Source: Karthikiyen et al., 2012

Mechanism Action of Bt toxin

Toxin Protein

- with three domain(Domain I, II and III)
- I- involved in pore formation
- II- Receptor binding (toxin selectivity)
- III- protection to the toxin from proteases

Solubilization in the mid gut after ingestion by insect larva

↓

converted to protoxins (133-138 Kda)

↓

Protoxins then cleaved by midgut proteases into two halves, with N- terminal half having toxic property

↓

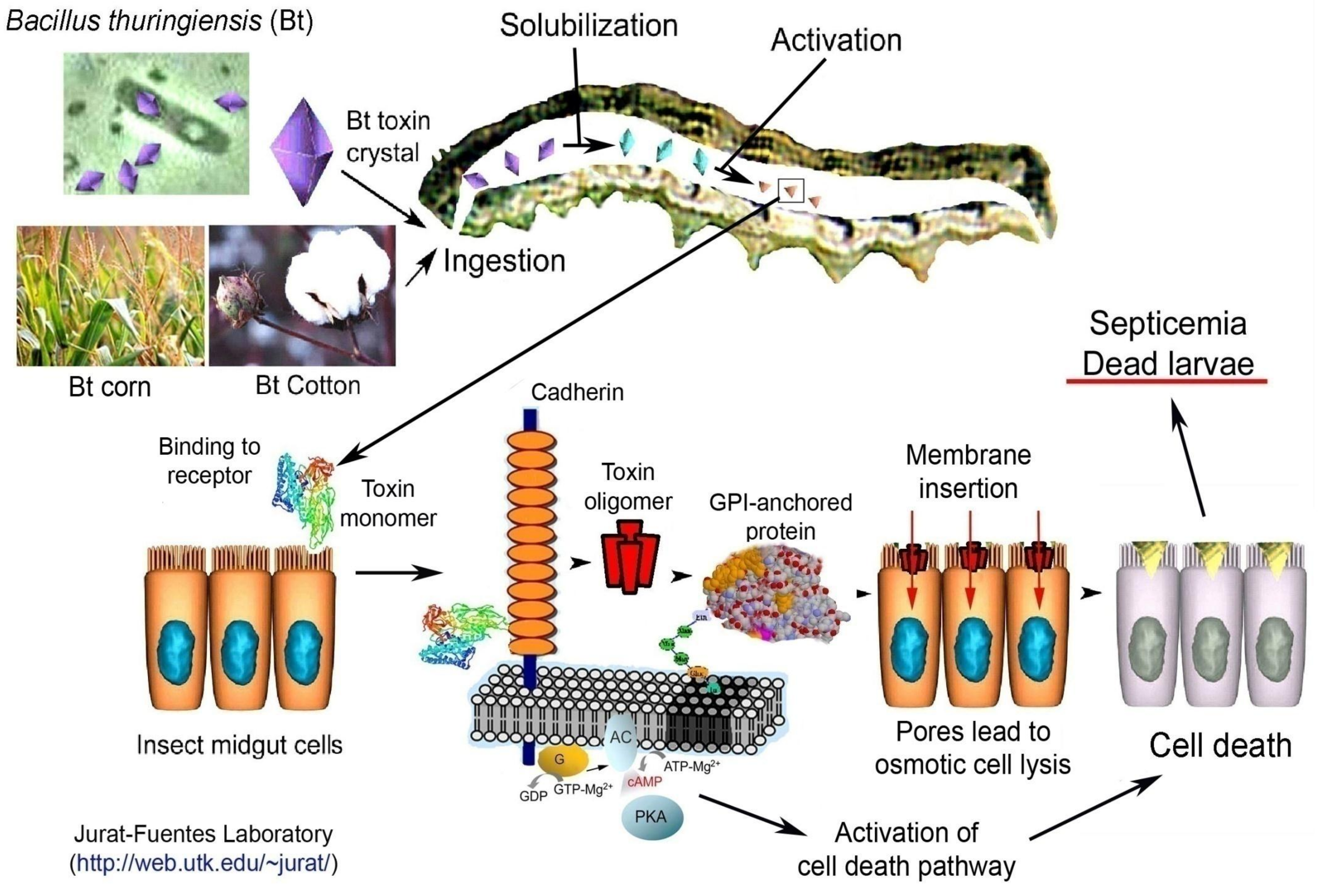
Such fragment bind to midgut epithelial membrane (Receptor Binding)

↓

Domain I insert into the membrane leading to pore formation

↓

The disturbance in osmotic equilibrium and cell lysis lead to insect paralysis and death


Bacillus thuringiensis (Bt)
Bt toxin crystal
Solubilization
Activation
Ingestion
Bt corn
Bt Cotton
Septicemia
Dead larvae
Cadherin
Binding to receptor
Toxin monomer
Toxin oligomer
GPI-anchored protein
Membrane insertion
Insect midgut cells
AC
G
GDP
GTP-Mg2+
ATP-Mg2+
cAMP
PKA
Pores lead to osmotic cell lysis
Cell death
Activation of cell death pathway
Jurat-Fuentes Laboratory
(http://web.utk.edu/~jurat/)

Advantages & Limitations

- Advantages
  - High insect specificity
    - Control crop damage and disease vectors
  - Nontoxic to non-target species
  - Biodegradable
  - Reduction of other insecticides
    - 94.5 million kg (19.4%) from 1996 to 2005 for cotton
  - Yield increases

- Limitations
  - Susceptible to resistance
  - High seed cost

Other insecticidal proteins:

1. Proteinase inhibitor (PIs)

- Plant defence protein present mainly in seeds and tubers
- Example : CowpeaTrypsin inhibitor (*cpTi*), showing resistance against Brunchid Beetle.
- Such *cpTi* have expressed in transgenic plant and showed resistance to tobacco Bud worm (Hilder et al, 1987)
- Following Cowpea Ti, several PIs (including **potato serine PI (*PPI-II*), tomato serine PI (*TI-II*), rice cystein PI (*OC-1*), Mustard trypsin inhibitor (*MTI-2*)**) have been expressed in transgenic plant.

Transgenic plant expressing PIs, α- amylase inhibitor & lectins

Crop	Gene	Target pest
Tobacco	*Cowpea serine PI*	Tobacco bud worm
Tobacco	*Potato serine PI*	Tobacco hornworm
Rice	*Cowpea serine PI*	Stemborer
Potato	*Cowpea serine PI*	*Lacanobia*
Potato	*Oryzacystatin*	Potato beetle
Tobacco	*Hornworm PI*	Whitefly
Pea	*Bean α-AI*	Bruchids
Potato	*Snowdrop lectin*	Potato aphid
Rice	*Snowdrop lectin*	Brown plant hopper

Mode of action:

Inhibit gut proteinase of insect

↓

No protein digestion

↓

Deficiency of essential amino acids

↓

Exert physiological stress on the insect

↓

Growth Retardation

2. Plant lectins

- Proteins having affinity for specific carbohydrates (Carbohydrates binding protein).

- Bind to glycoprotein in the peritrophic matrix lining of the insect midgut to disrupt digestion process and nutrient assimilation. Examples includes:

  - ✓ transgenic tobacco and potato expressing a lectins from snowdrop (*Galanthus nivalis*) found toxic to aphids.

  - ✓ Transgenic rice (engineered with lectins) showed resistance against Brown plant hopper and Green leaf hopper.

  - ✓ Wheat germ agglutinin, peas lectins, jacalins & rice lectins have been expressed in tobacco, maize and potato mainly against aphids.

Some lectins are toxic or allergenic to mammals

3. α- amylase inhibitors (α-AI)

- **α-AI** forms complex with certain insect amylases & is supposed to play a role in plant defence against insect. For example
  - ✓ Expression of bean α-AI gene in pea confer resistance to the Burchid beetles

4. Chitinases

- **Chitin:** forms Insoluble polysaccharide, found in exoskeleton & gut lining of insects and protect them from water loss and abrasive agents .
- Because of critical functions chitin is potential target.
- Expression of protein that interfere with chitin metabolism is likely to have serious effect on the growth of insect
- Use if chitinases in conjugation with Bt toxin would enhance more effective control of insect pests