Chapter 19 & 20-Genetic Engineering of Plants: Applications

- Insect-, pathogen-, and herbicide-resistant plants
- Stress- and senescence-tolerant plants
- Genetic manipulation of flower pigmentation
- Modification of plant nutritional content
- Modification of plant food taste and appearance
- Plant as bioreactors
- Edible vaccines
- Renewable energy crops
- Plant yield

Are we eating genetically engineered plants now?

You bettcha!

- 91 genetically engineered plants approved in the US

Your query has returned 91 records. For further information on a particular event, click on the appropriate links under the Event column in the following table.

Creeping Bentgrass Sugar Beet Argentine Canola Papaya Chicory Melon Squash Soybean Cotton Flax, Linseed Tomato Alfalfa Tobacco Rice Plum Potato Wheat Maize

- 152 genetically engineered plants approved in the world

Your query has returned 152 records. For further information on a particular event, click on the appropriate links under the Event column in the following table.

Creeping Bentgrass Sugar Beet Argentine Canola Polish Canola Papaya Chicory Melon Squash Carnation Soybean Cotton Sunflower Lentil Flax, Linseed Tomato Alfalfa Tobacco Rice Plum Potato Wheat Maize

-See http://www.cera-gmc.org/?action=gm_crop_database for details

Genetically engineered crops/foods allowed in the US food supply

Product	Institution(s)	Engineered Trait(s)	Sources of New Genes	Name
Canola	Bayer	Resist glufosinate herbicide to control weeds	Bacteria, virus	LibertyLink-2000
Canola	Monsanto	Resist glyphosate herbicide to control weeds	Arabidopsis, bacteria, virus	Roundup Ready-1999
Canola	Monsanto	Altered oil (high lauric acid) for soap and food products	Calif bay, turnip rape, bacteria, virus	Laurical-1995
Canola	Bayer	Male sterile to facilitate hybridization; resist glufosinate herbicide to control weeds	Bacteria	SeedLink 2000
Chicory (radicchio)	Bejo Zaden	Male sterile to facilitate hybridization	Bacteria	SeedLink-1997
Corn	Bayer	Resist glufosinate herbicide to control weeds/male sterile to facilitate hybridization	Bacteria, virus	SeedLink-?
Corn	Bayer	Resist glufosinate herbicide to control weeds	Bacteria, virus	LibertyLink-?
Corn	Bayer	Resist glufosinate herbicide to control weeds/Bt toxin to control insect pests (European corn borer)	Bacteria, virus	StarLink-1998 (animals only)
Corn	Dow/Mycogen	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	NatureGard-1995
Corn	Dow/Mycogen DuPont/Pioneer	Resist glufosinate herbicide to control weeds/Bt toxin to control insect pests (Lepidopteran)	Corn, bacteria, virus	Herculex I-2001
Corn	DuPont/Pioneer	Male sterile to facilitate hybridization	Potato, corn, bacteria, virus	?-1998
Corn	Monsanto/DeKalb	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt-Xtra-1997
Corn	Monsanto/DeKalb	Resist glufosinate herbicide to control weeds	Bacteria, virus	?-?
Corn	Monsanto	Bt toxin to control insect pests (European corn borer)	Bacteria	YieldGard-1996
Corn	Monsanto	Resist glyphosate herbicide to control weeds/Bt toxin to control insect pests (European corn borer)	Arabidopsis, bacteria, virus	?-1998
Corn	Monsanto	Resist glyphosate herbicide to control weeds	Arabidopsis, bacteria, virus	Roundup Ready-1998
Corn	Syngenta	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt11-1996
Corn	Syngenta	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	Knock Out-1995
Corn (pop)	Syngenta	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	Knock Out-1998
Corn (sweet)	Syngenta	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt11-1998
Cotton	Monsanto/Bayer	Resist bromoxynil herbicide to control weeds/Bt toxin to control insect pests (cotton bollworms and tobacco budworm)	Bacteria	?-1998
Cotton	Monsanto/Bayer	Resist bromoxynil herbicide to control weeds	Bacteria, virus	BXN Cotton-1995
Cotton	Monsanto	Bt toxin to control insect pests (cotton bollworms and tobacco budworm)	Bacteria	Bollgard-1995
Cotton	Monsanto	Resist glyphosate herbicide to control weeds	Arabidopsis, bacteria, virus	Roundup Ready-1996
Flax	Univ Saskatchewan	Resist sulfonylurea herbicide to grow in soils with herbicide residues	Arabidopsis, bacteria	CDC Triffid-1999
Papaya	Cornell Univ/Univ Hawaii	Resist papaya ringspot virus	Bacteria, virus	Sunup, Rainbow-1997
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)	Bacteria	NewLeaf-1995
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)/resist potato virus Y	Bacteria, virus	NewLeaf Y-1999
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)/resist potato leafroll virus	Bacteria, virus	NewLeaf Plus-1998
Soybean	Bayer	Resist glufosinate herbicide to control weeds	Bacteria, virus	?-1998
Soybean	DuPont	Altered oil (high oleic acid) to increase stability, reduce polyunsaturated fatty acids	Soybean, bean, bacteria, virus	?-1997
Soybean	Monsanto	Resist glyphosate herbicide to control weeds	Petunia, soybean, bacteria, virus	Roundup Ready-1995
Squash	Seminis Vegetable Seed	Resist watermelon mosaic 2 and zucchini yellow mosaic viruses	Bacteria, virus	Freedom II-1995
Squash	Seminis Vegetable Seed	Resist watermelon mosaic 2, zucchini yellow mosaic, cucumber mosaic viruses	Bacteria, virus	?-1997
Sugarbeet	Bayer	Resist glufosinate herbicide to control weeds	Bacteria, virus	?-2000
Sugarbeet	Monsanto/Syngenta	Resist glyphosate herbicide to control weeds	Bacteria, virus	?-1999
Tomato (cherry)	Agritope	Altered ripening to enhance fresh market value	Bacteria	?-1996
Tomato	DNA Plant Technology	Altered ripening to enhance fresh market value	Tomato, bacteria, virus	Endless Summer-1995
Tomato	Monsanto/Calgene	Altered ripening to enhance fresh market value	Tomato, bacteria, virus	FlavrSavr-1994
Tomato	Monsanto	Altered ripening to enhance fresh market value	Bacteria	?-1995
Tomato	Zeneca/PetoSeed	Thicker skin and altered pectin to enhance processing value	Tomato, bacteria, virus	?-1995

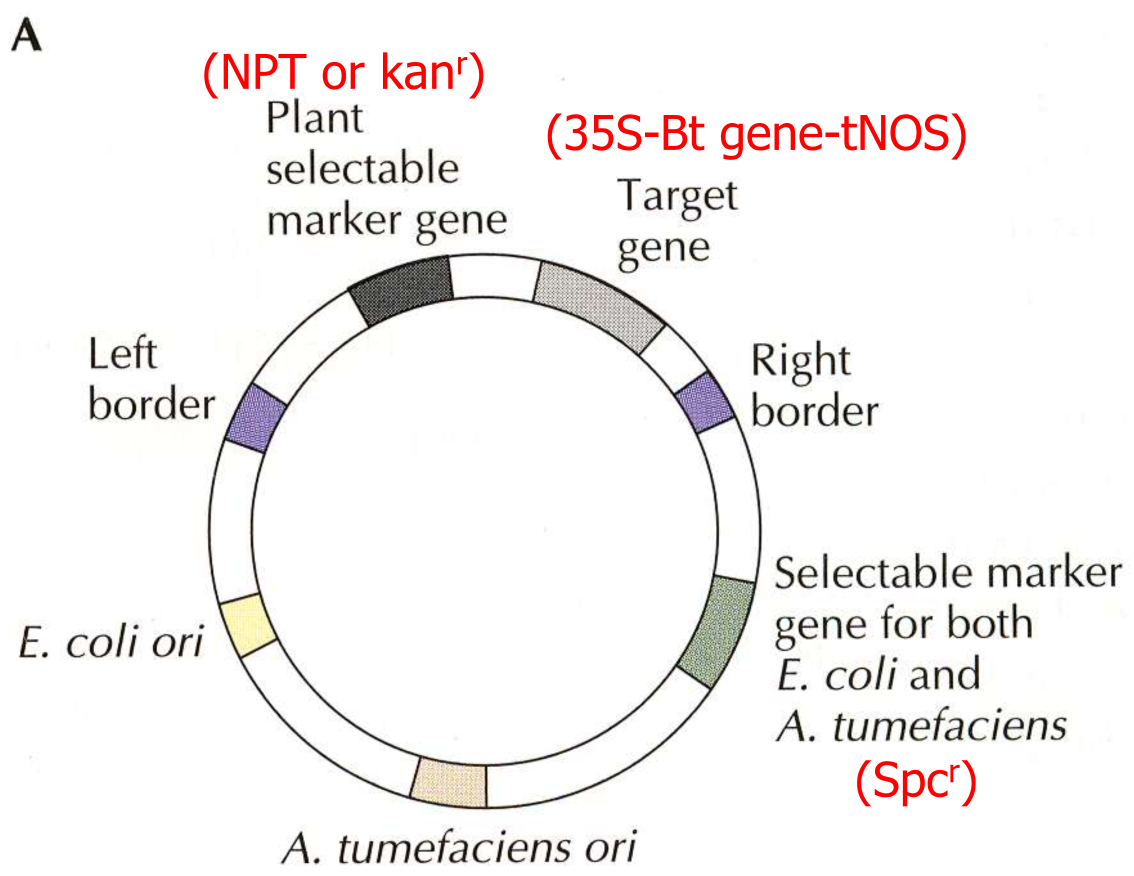
Insect-resistant plants

- Bt toxin
- Cowpea trypsin inhibitor
- Proteinase inhibitor II
- α-amylase inhibitor
- Bacterial cholesterol oxidase
- Combinations of the above (e.g., Bt toxin and proteinase inhibitor II)

Genetic engineering of Bt-plants

- Expression of truncated Bt genes encoding the N-terminal portion of Bt increase effectiveness
- Effectiveness enhanced by site-directed mutagenesis increasing transcription/translation
- Effectiveness further enhanced by making codon bias changes (bacterial to plant)
- 35S CaMV and rbcS promoters used
- Integration and expression of the Bt gene directly in chloroplasts
- Note that Lepidopteran insects like corn rootworm, cotton bollworm, tobacco budworm, etc., cause combined damages of over $7 Billion dollars yearly in the US

Fig. 18.7/19.3 A binary T-DNA plasmid for delivering the Bt gene to plants (not a cointegrate vector)



Effectiveness of insecticide and Bt-tomato plants in resisting insect damage

% of plants or fruits damaged

Insect	wt tomato -insecticide	wt tomato +insecticide	Bt-tomato -insecticide	Bt-tomato +insecticide
Tobacco hornworm	48	4	1	0
Tomato fruitworm	20	nd	6	nd
Tomato pinworm	100	95	94	80

nd, not determined

For a visual look at the effectiveness of Bt-plants:

- You can download a quicktime movie clip on "Insect resistance with Bt" from Dr. Goldberg's web site http://www.mcdb.ucla.edu/Research/Goldberg/research/movie_trailers-index.htm

Strategies to avoid Bt resistant insects

- Use of inducible promoters (that can be turned on only when there is an insect problem)
- Construction of hybrid Bt toxins
- Introducing more than one Bt gene (“stacking”)
- Introduction of the Bt gene in combination with another insecticidal gene
- Spraying low levels of insecticide on Bt plants
- Use of spatial refuge strategies

Genetically engineered Bt-plants in the field

Product	Institution(s)	Engineered Trait(s)	Sources of New Genes	Name
Corn	Bayer	Resist glufosinate herbicide to control weeds/Bt toxin to control insect pests (European corn borer)	Bacteria, virus	StarLink-1998 (animals only)
Corn	Dow/Mycogen	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	NatureGard-1995
Corn	Dow/Mycogen DuPont/Pioneer	Resist glufosinate herbicide to control weeds/Bt toxin to control insect pests (Lepidopteran)	Corn, bacteria, virus	Herculex I-2001
Corn	Monsanto/DeKalb	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt-Xtra-1997
Corn	Monsanto	Bt toxin to control insect pests (European corn borer)	Bacteria	YieldGard-1996
Corn	Monsanto	Resist glyphosate herbicide to control weeds/Bt toxin to control insect pests (European corn borer)	Arabidopsis, bacteria, virus	?-1998
Corn	Syngenta	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt11-1996
Corn	Syngenta	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	Knock Out-1995
Corn (pop)	Syngenta	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	Knock Out-1998
Corn (sweet)	Syngenta	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt11-1998
Cotton	Monsanto/Bayer	Resist bromoxynil herbicide to control weeds/Bt toxin to control insect pests (cotton bollworms	Bacteria	?-1998
		and tobacco budworm)		
Cotton	Monsanto	Bt toxin to control insect pests (cotton bollworms and tobacco budworm)	Bacteria	Bollgard-1995
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)	Bacteria	NewLeaf-1995
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)/resist potato virus Y	Bacteria, virus	NewLeaf Y-1999
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)/resist potato leafroll virus	Bacteria, virus	NewLeaf Plus-1998

Figure 19.3

Binary cloning vector carrying a cowpea trypsin inhibitor gene

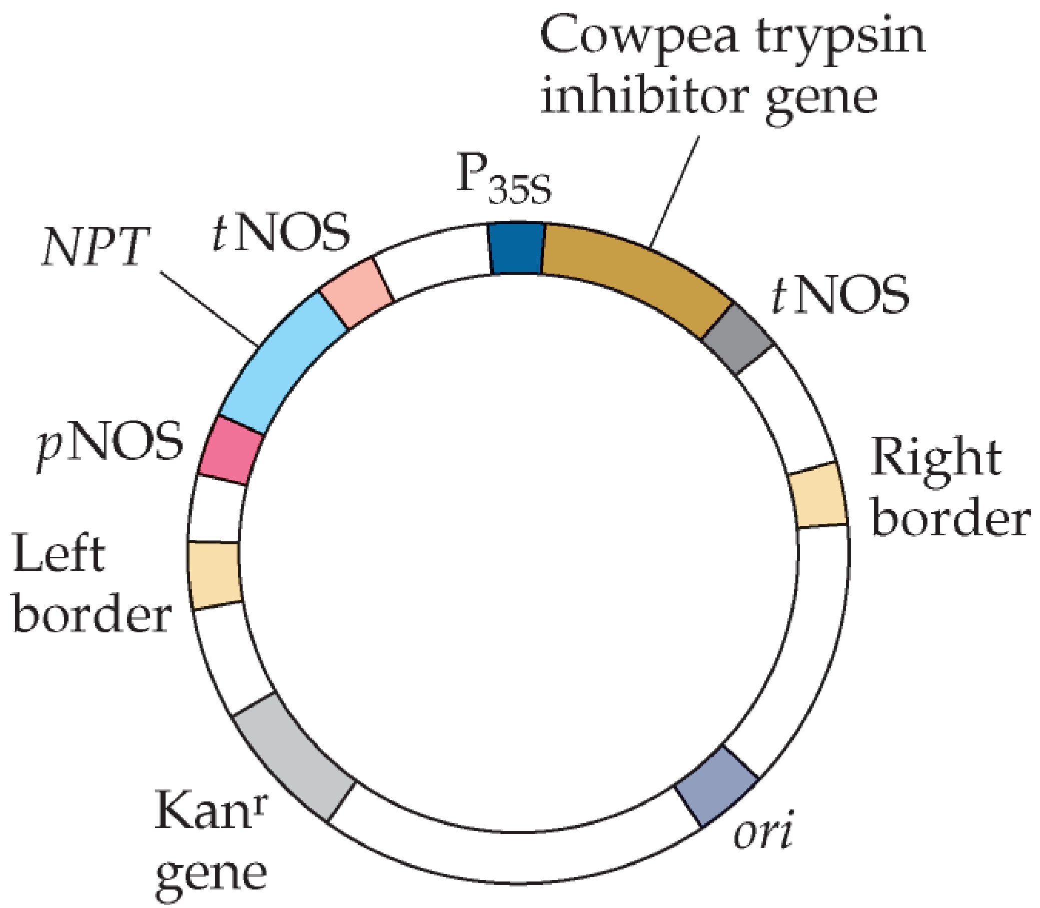
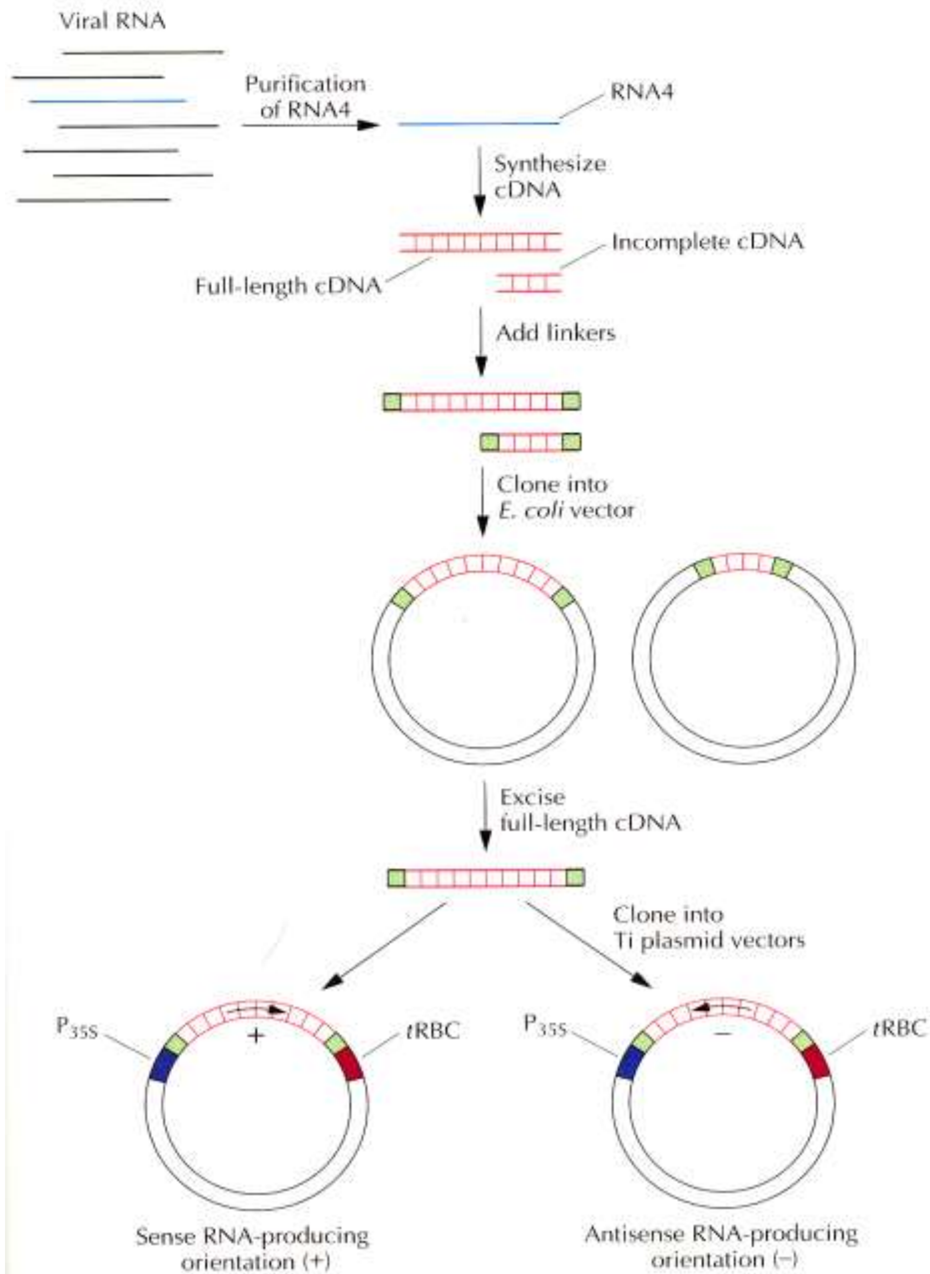


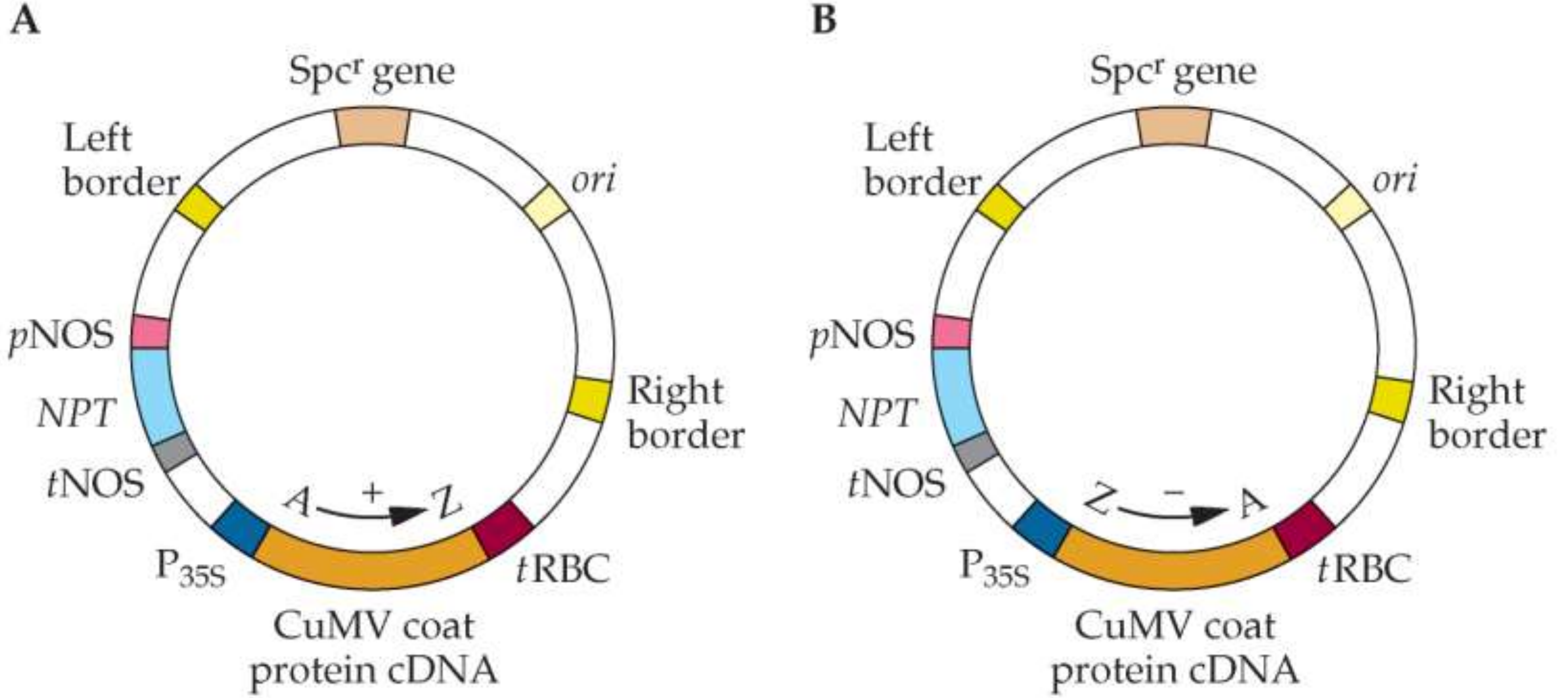
Fig. 18.7 Procedure for putting CuMV coat protein into plants



Virus-resistant plants

- Overexpression of the virus coat protein (e.g. cucumber mosaic virus in cucumber and tobacco, papaya ringspot virus in papaya and tobacco, tobacco mosiac virus in tobacco and tomato, etc.)
- Expression of a dsRNase (RNaseIII)
- Expression of antiviral proteins (pokeweed)

Figure 19.12



Binary cloning vector carrying the protein-producing sense (A) or antisense RNA-producing (B) orientation of the cucumber mosaic virus coat protein (CuMV) cDNA

Genetically engineered Papaya to resist the Papaya Ringspot-Virus by overexpression of the virus coat protein

Herbicides and herbicide-resistant plants

- Herbicides are generally non-selective (killing both weeds and crop plants) and must be applied before the crop plants germinate
- Four potential ways to engineer herbicide resistant plants
  1. Inhibit uptake of the herbicide
  2. Overproduce the herbicide-sensitive target protein
  3. Reduce the ability of the herbicide-sensitive target to bind to the herbicide
  4. Give plants the ability to inactivate the herbicide

Table 19.3

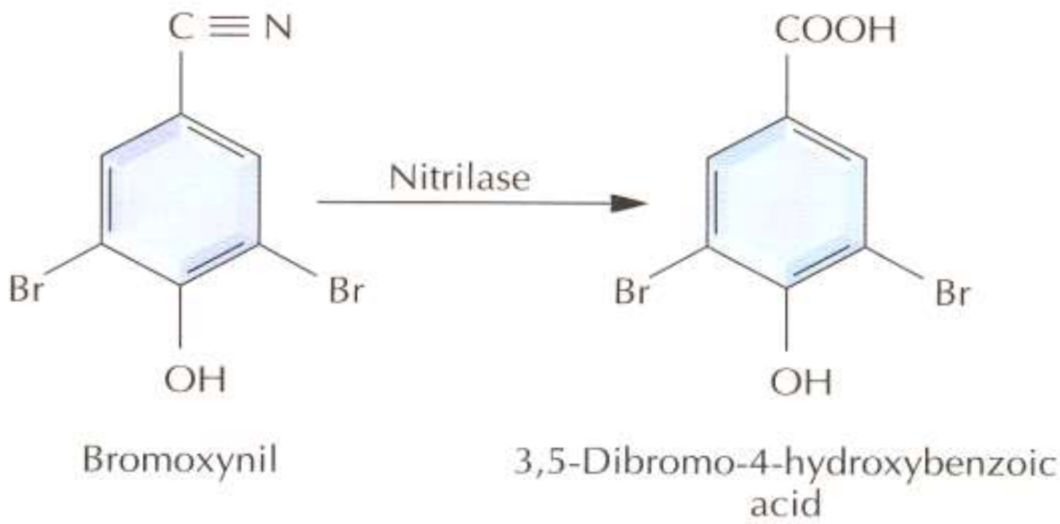
TABLE 19.3 Some examples of gene-based herbicide resistance

Herbicide(s)	Mode of development of herbicide resistance
Triazines	Resistance is due to an alteration in the *psbA* gene, which codes for the target of this herbicide, chloroplast protein D-1.
Sulfonylureas	Genes encoding resistant versions of the enzyme acetolactate synthetase have been introduced into poplar, canola, flax, and rice.
Imidazolinones	Strains with resistant versions of the enzyme acetolactate synthetase have been selected in tissue culture.
Aryloxphenoxypropionates, cyclohexanediones	These herbicides inhibit the enzyme acetyl coenzyme A carboxylase. Resistance, selected in tissue culture, is due either to an altered enzyme that is not herbicide sensitive or to the degradation of the herbicide.
Glyphosate	Resistance is from overproduction of EPSPS, the target of this herbicide. Resistance has been engineered by transforming soybean with the gene for a glyphosate-resistant EPSPS and tobacco with a glyphosate oxidoreductase gene, which encodes an enzyme that degrades glyphosate.
Bromoxynil	Resistance to this photosystem II inhibitor has been created by transforming tobacco and cotton plants with a bacterial nitrilase gene, which encodes an enzyme that degrades this herbicide.
Phenoxycarboxylic acids (e.g., 2,4-D and 2,4,5-T)	Resistant cotton and tobacco plants have been created by transformation with the *tfdA* gene from *Alcaligenes*, which encodes a dioxygenase that degrades this herbicide.
Glufosinate (phosphinothricin)	Over 20 different plants have been transformed with either the *bar* gene from *Streptomyces hygroscopicus* or the *pat* gene from *S. viridochromogenes*. The phosphinothricin acetyltransferase that these genes encode detoxifies this herbicide.
Cyanamide	Resistant tobacco plants were produced when a cyananide hydratase gene from the fungus *Myrothecium verrucaria* was introduced. The enzyme encoded by this gene converts cyanamide to urea.
Dalapon	Tobacco plants transformed with a dehalogenase gene from *Pseudomonas putida* can detoxify this herbicide.

Herbicide-resistant plants:

Giving plants the ability to inactivate the herbicide

- Herbicide: Bromoxynil
- Resistance to bromoxynil (a photosytem II inhibitor) was obtained by expressing a bacterial (*Klebsiella ozaenae*) nitrilase gene that encodes an enzyme that degrades this herbicide

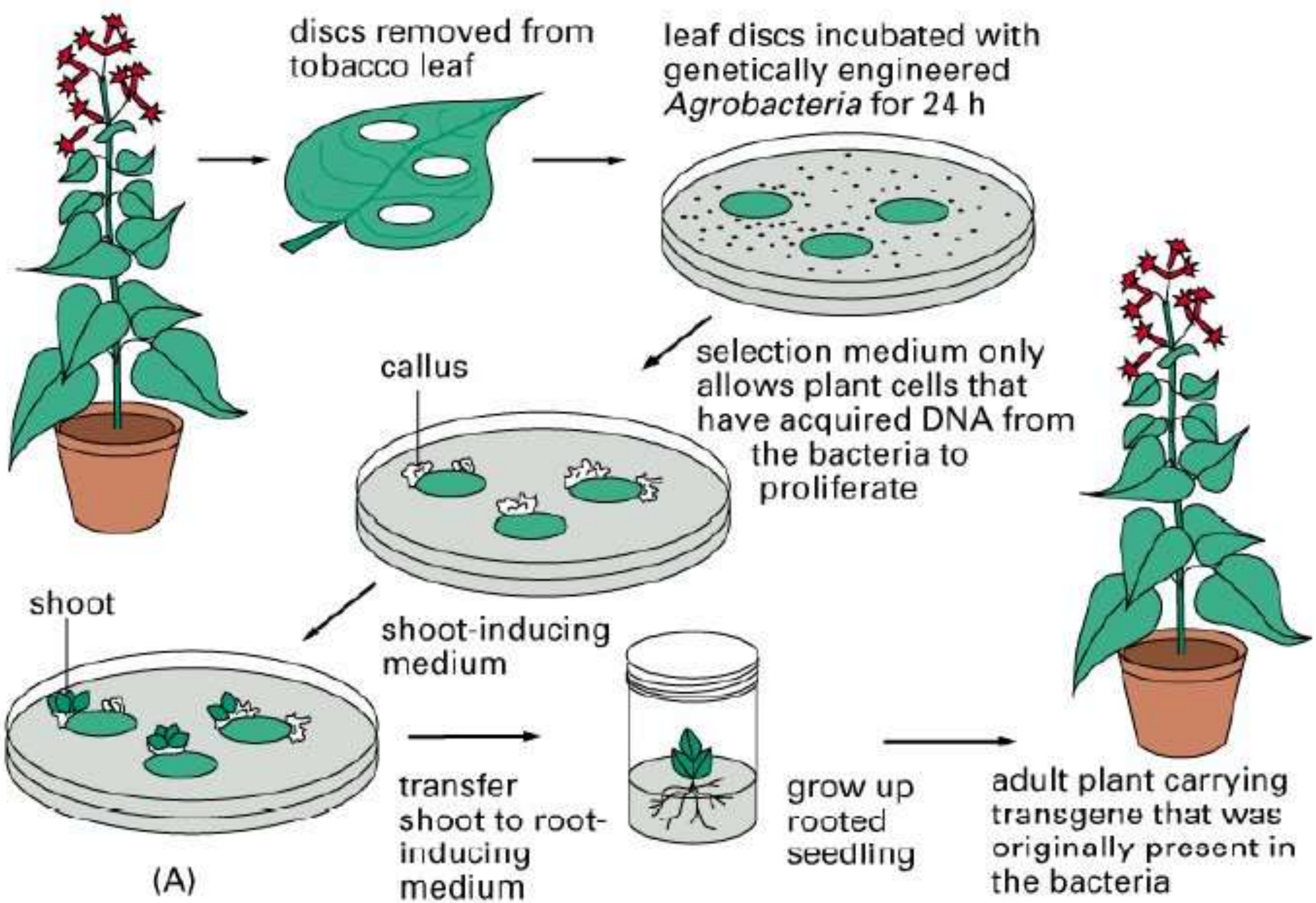


Herbicide-resistant plants:

Reducing the ability of the herbicide-sensitive target to bind to the herbicide

- Herbicide: Glyphosate (better known as Roundup)
- Resistance to Roundup (an inhibitor of the enzyme EPSP involved in aromatic amino acid biosynthesis) was obtained by finding a mutant version of EPSP from *E. coli* that does not bind Roundup and expressing it in plants (soybean, tobacco, petunia, tomato, potato, and cotton)
- 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) is a chloroplast enzyme in the shikimate pathway and plays a key role in the synthesis of aromatic amino acids such as tyrosine and phenylalanine
- This is a big money maker for Monsanto!

How to make a Roundup Ready Plant



Alberts et al. (2002) Molecular Biology of the Cell 4/e

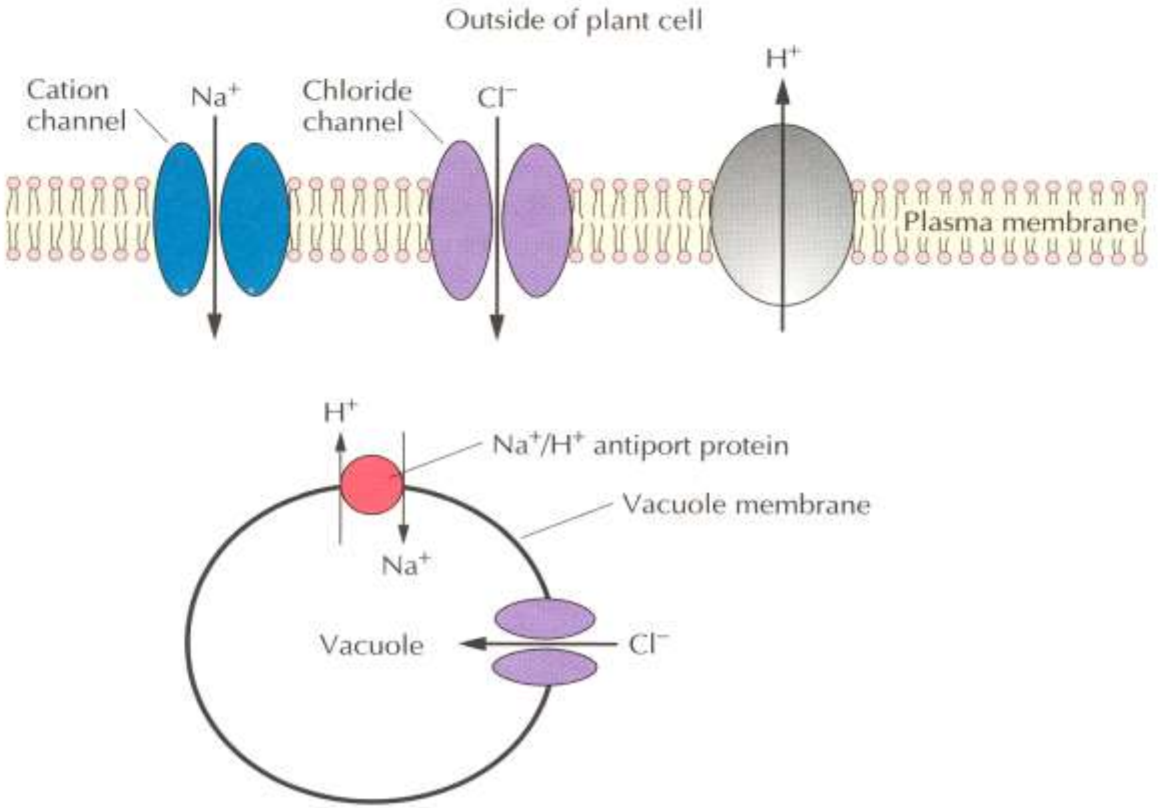
Fungus- and bacterium-resistant plants

- Genetic engineering here is more challenging; however, some strategies are possible:
- Individually or in combination express pathogenesis-related (PR) proteins, which include β1,3-glucanases, chitinases, thaumatin-like proteins, and protease inhibitors
- Overexpression of the NPR1 gene which encodes the “master” regulatory protein for turning on the PR protein genes
- Overproducing salicylic acid in plants by the addition of two bacterial genes; SA activates the NPR1 gene and thus results in production of PR proteins

Development of stress- and senescence-tolerant plants: genetic engineering of salt-resistant plants

- Overexpression of the gene encoding a Na^+/H^+ antiport protein which transports Na^+ into the plant cell vacuole
- This has been done in *Arabidopsis* and tomato plants allowing them to survive on 200 mM salt (NaCl)

Figure 18.22 Schematic representation of ion transport in the plant *A. thaliana* showing the Na^+ ions being sequestered in the large vacuole.



Development of stress- and senescence-tolerant plants: genetic engineering of flavorful tomatoes

- Fruit ripening is a natural aging or senescence process that involves two independent pathways, flavor development and fruit softening.
- Typically, tomatoes are picked when they are not very ripe (i.e., hard and green) to allow for safe shipping of the fruit.
- Polygalacturonase is a plant enzyme that degrades pectins in plant cell walls and contribute to fruit softening.
- In order to allow tomatoes to ripen on the vine and still be hard enough for safe shipping of the fruit, polygalacturonase gene expression was inhibited by introduction of an antisense polygalacturonase gene and created the first commercial genetically engineered plant called the FLAVR SAVR tomato.

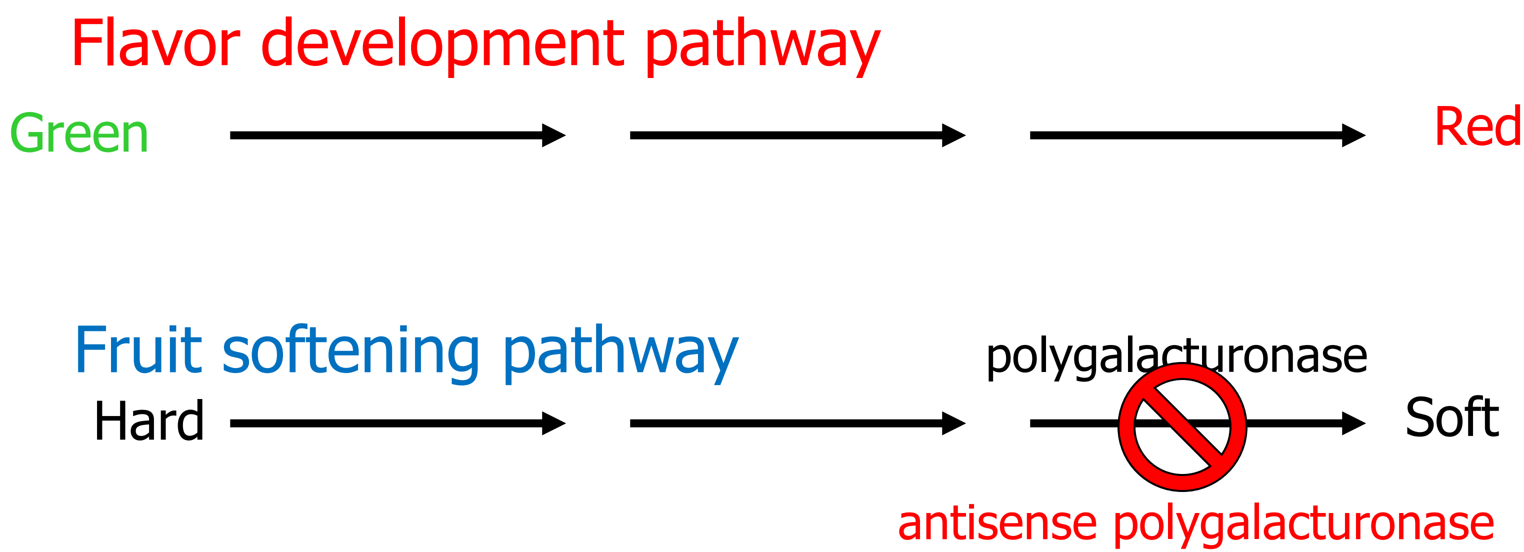
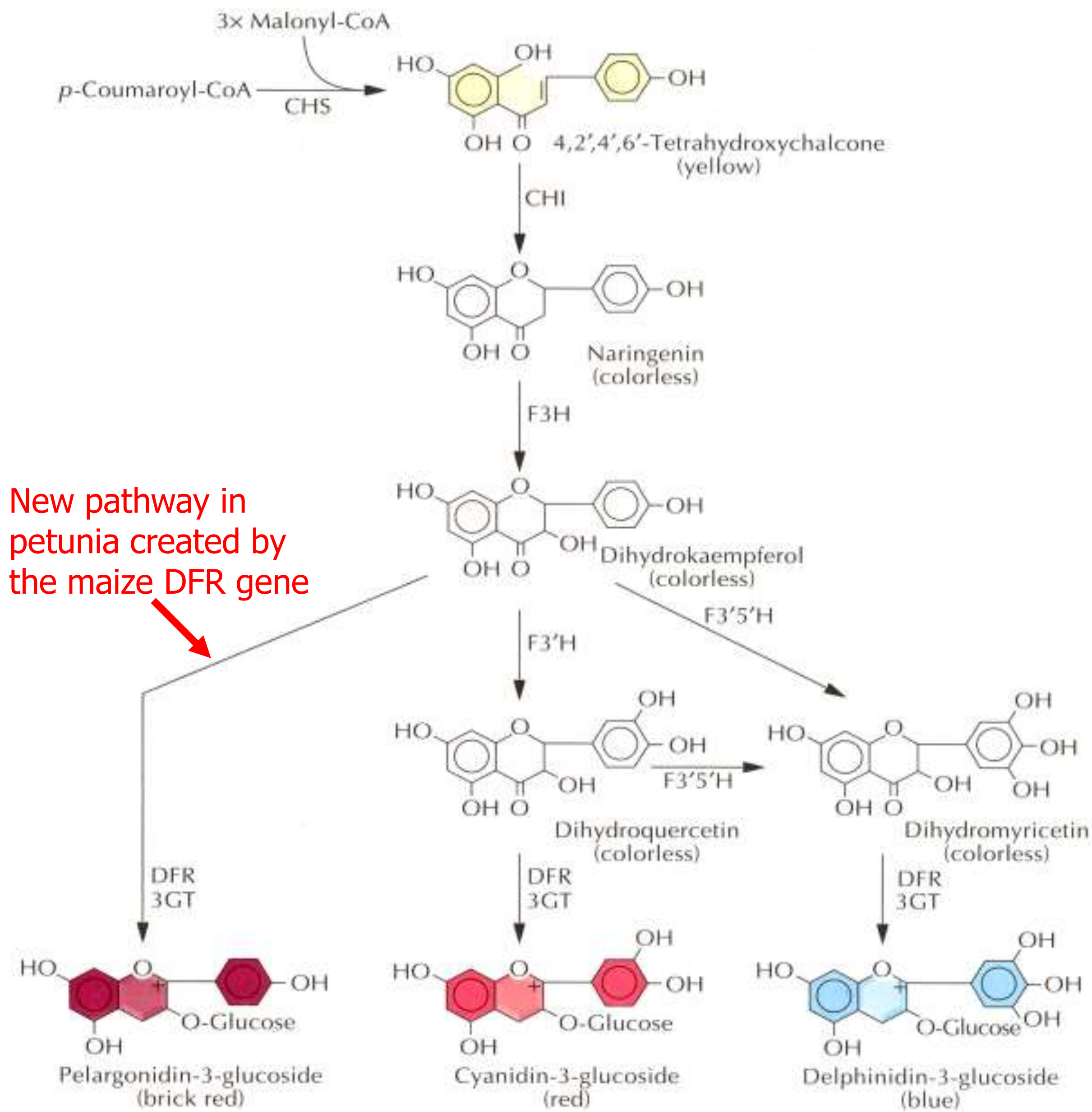


Fig. 20.18 Genetic manipulation of flower pigmentation

- Manipulation of the anthocyanin biosynthesis pathway
- Introduction of maize dihydroflavonol 4-reductase (DFR) into petunia produces a brick red-orange transgenic petunia
- Novel flower colors in the horticultural industrial are big money makers!
- Note a blue rose would make millions!

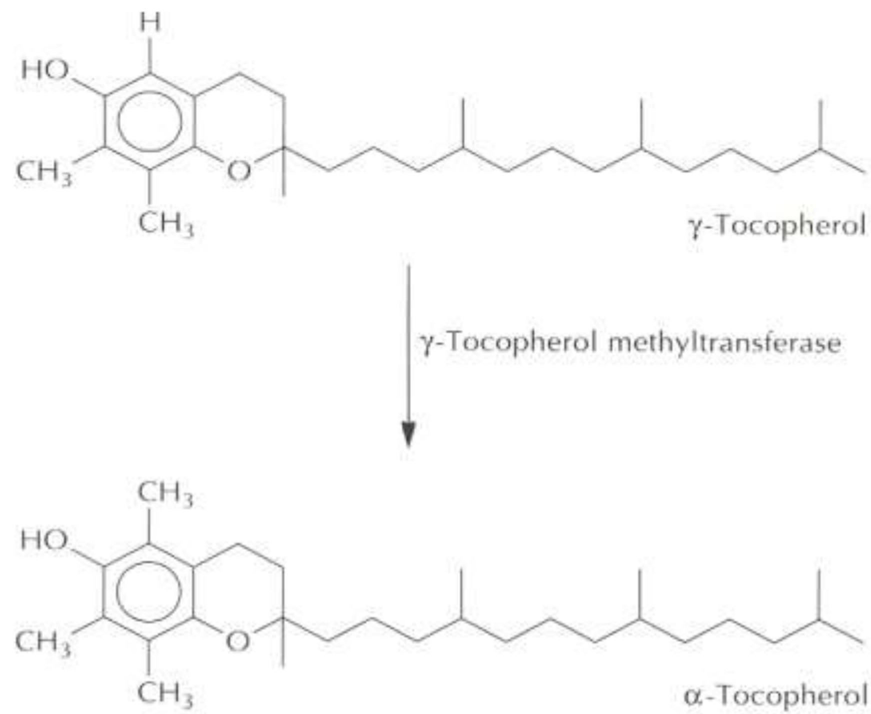


Modification of plant nutritional content

- Amino acids (corn is deficient in lysine, while legumes are deficient in methionine and cysteine)
- Lipids (altering the chain length and degree of unsaturation is now possible since the genes for such enzymes are known)
- Increasing the vitamin E (α-tocopherol) content of plants (Arabidopsis)
- Increasing the vitamin A content of plants (rice)

Modification of plant nutritional content: increasing the vitamin E (α-tocopherol) content of plants

- Plants make very little α-tocopherol but do make γ-tocopherol; they do not produce enough of the methyltransferase (MT)
- The MT gene was identified and cloned in *Synechocystis* and then in *Arabidopsis*
- The *Arabidopsis* MT gene was expressed under the control of a seed-specific carrot promoter and found to produce 80 times more vitamin E in the seeds



Dean DellaPenna, Michigan State Univ. Professor
B.S. 1984, Ohio University

Modification of plant nutritional content: increasing the vitamin A content of plants (Fig. 20.7)

- 124 million children worldwide are deficient in vitamin A, which leads to death and blindness
- Mammals make vitamin A from β-carotene, a common carotenoid pigment normally found in plant photosynthetic membranes
- Here, the idea was to engineer the β-carotene pathway into rice
- The transgenic rice is yellow or golden in color and is called "golden rice"

GGPP

↓ Daffodil phytoene synthase gene

Phytoene

↓ Bacterial phytoene desaturase gene

Lycopene

↓ Daffodil lycopene β-cyclase gene

β-carotene

↓ Endogenous human gene

Vitamin A

Plants as bioreactors

- Production of therapeutic agents (proteins)
- Production of recombinant vaccines or edible vaccines
- Production of antibodies

Table 20.6

TABLE 20.6 Comparison of recombinant protein production in plants and other systems

Parameter	Bacteria	Yeast	Mammalian cell culture	Transgenic plants
Glycosylation	None	Incorrect	Correct	Generally correct
Assembles multimeric proteins	Limited	Limited	Limited	Yes
Production costs	Medium	Medium	High	Low
Protein-folding accuracy	Low	Medium	High	High
Protein yield	High	High	Medium	Medium
Scale-up costs	High	High	High	Low
Time required	Low	Low	High	Medium
Skill level required for growth	Medium	Medium	High	Low

Table 20.7

TABLE 20.7 Some of the therapeutic agents produced in transgenic plants

Protein	Plant(s)	Application(s)
Human protein C	Tobacco	Anticoagulant
Human hirudin variant 2	Tobacco, canola, Ethiopian mustard	Anticoagulant
Human granulocyte–macrophage colony-stimulating factor	Tobacco	Neutropenia
Human erythropoietin	Tobacco	Anemia
Human enkephalins	Thale cress, canola	Antihyperanalgesic by opiate activity
Human epidermal growth factor	Tobacco	Wound repair, control of cell proliferation
Human α-interferon	Rice, turnip	Hepatitis C and B
Human serum albumin	Potato, tobacco	Liver cirrhosis
Human hemoglobin	Tobacco	Blood substitute
Human homotrimeric collagen I	Tobacco	Collagen synthesis
Human α1-antitrypsin	Rice	Cystic fibrosis, liver disease, hemorrhage
Human growth hormone	Tobacco	Dwarfism, wound healing
Human aprotinin	Corn	Trypsin inhibitor for transplantation surgery
Angiotensin-1-converting enzyme	Tobacco, tomato	Hypertension
α-Tricosanthin	Tobacco	HIV therapy
Glucocerebrosidase	Tobacco	Gaucher disease
Human muscarinic cholinergic receptors	Tobacco	Central and peripheral nervous system
Human interleukin-2 and interleukin-4	Tobacco	Immunotherapy
Human placental alkaline phosphatase	Tobacco	Children with achonodroplasia or cretinism
Human insulin	Safflower	Diabetes
Trout growth factor	Tobacco	Fish growth
Lipase	Corn	Cystic fibrosis
Lactoferrin	Rice	Diarrhea

HIV, human immunodeficiency virus.

Table 20.8

TABLE 20.8 Some recombinant vaccine antigens expressed in plants

Vaccine antigen	Plant(s) or vector
Hepatitis virus B surface proteins	Tobacco, potato, yellow lupin, lettuce
Malaria parasite antigen	Virus
Rabies virus glycoprotein	Tomato
Human rhinovirus 14 and human immunodeficiency virus (HIV) epitopes	Virus
E. coli heat-labile enterotoxin	Tobacco, potato
Norwalk virus capsid protein	Tobacco, potato
Diabetes-associated autoantigen	Tobacco, potato, carrot
Mink enteritis virus epitope	Virus
Rabies and HIV epitopes	Virus
Foot and mouth disease VP1 structural protein	*Arabidopsis*, alfalfa
Cholera toxin B subunit	Potato
Human insulin–cholera toxin B subunit fusion protein	Potato
Human cytomegalovirus glycoprotein B	Tobacco
Dental caries (*S. mutans*)	Tobacco
Respiratory syncytial virus	Tomato

Note that in some cases the antigen was cloned into a transient-expression system, such as a plant virus (usually tobacco mosaic virus), that could be sprayed onto the leaves of a variety of different plants and begin producing protein within 2 weeks.

Table 20.9

TABLE 20.9 Some antibodies and antibody fragments that have been produced in plants

Host plant	Antigen
Tobacco	Phosphonate ester
Tobacco	(4-Hydroxy-3-nitrophenyl)acetyl
Tobacco	Phytochrome
Tobacco	Artichoke mottled crinkle virus
Tobacco	Human creatine kinase
Tobacco	*Streptococcus mutans* cell surface antigen SA I/II
Tobacco	Fungal cutinase
Tobacco	Oxazolone
Tobacco	Abscisic acid
Tobacco	Cell surface protein from mouse B-cell lymphoma
Tobacco	Human carcinoembryonic antigen
Tobacco	Tobacco mosaic virus
Tobacco	Gibberellin
Tobacco	Beet necrotic yellow vein virus coat protein
Tobacco	Stolbur phytoplasma membrane protein
Tobacco	Root rot nematode surface glycoprotein
Petunia	Dihydrofolate reductase
Soybean	Herpes simplex virus
Pea	Abscisic acid
Pea	Human cancer cell surface antigen
Tobacco	substance P (neuropeptide)
Tobacco	CD40 (cell surface protein)
Tobacco	38C13 mouse B-cell lymphoma
Alfalfa	Human IgG

Plants are also being genetically engineered for:

- Biofuel production (e.g., lower lignin, lower recalcitrance)
- Phytoremediation (i.e., bioremediation using plants)
- Biopolymers (i.e., biodegradable plastics)