

Chapter 18-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
 - Plant yield

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

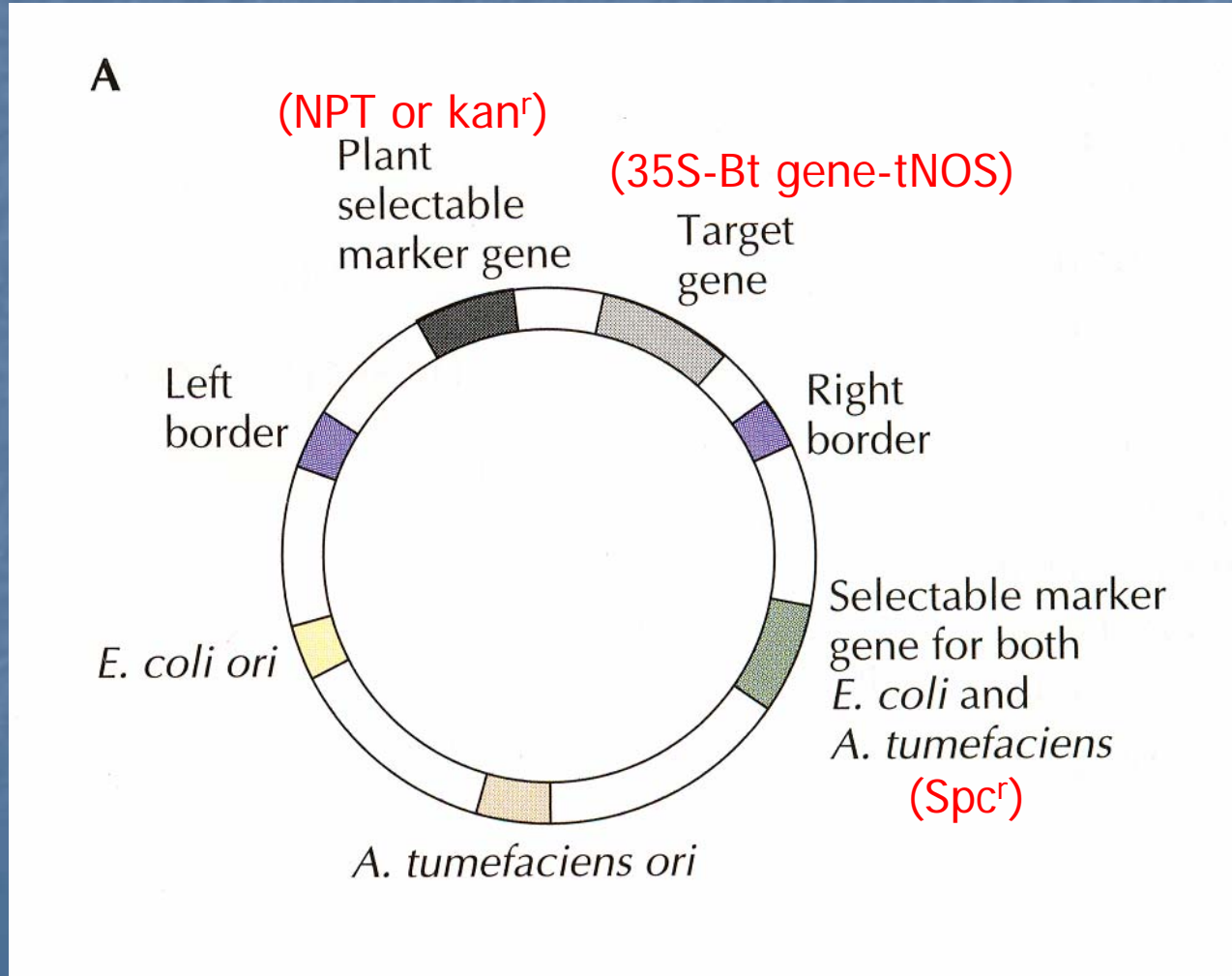


Table 18.2 Effectiveness of insecticide and Bt-tomato plants in resisting insect damage

% 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

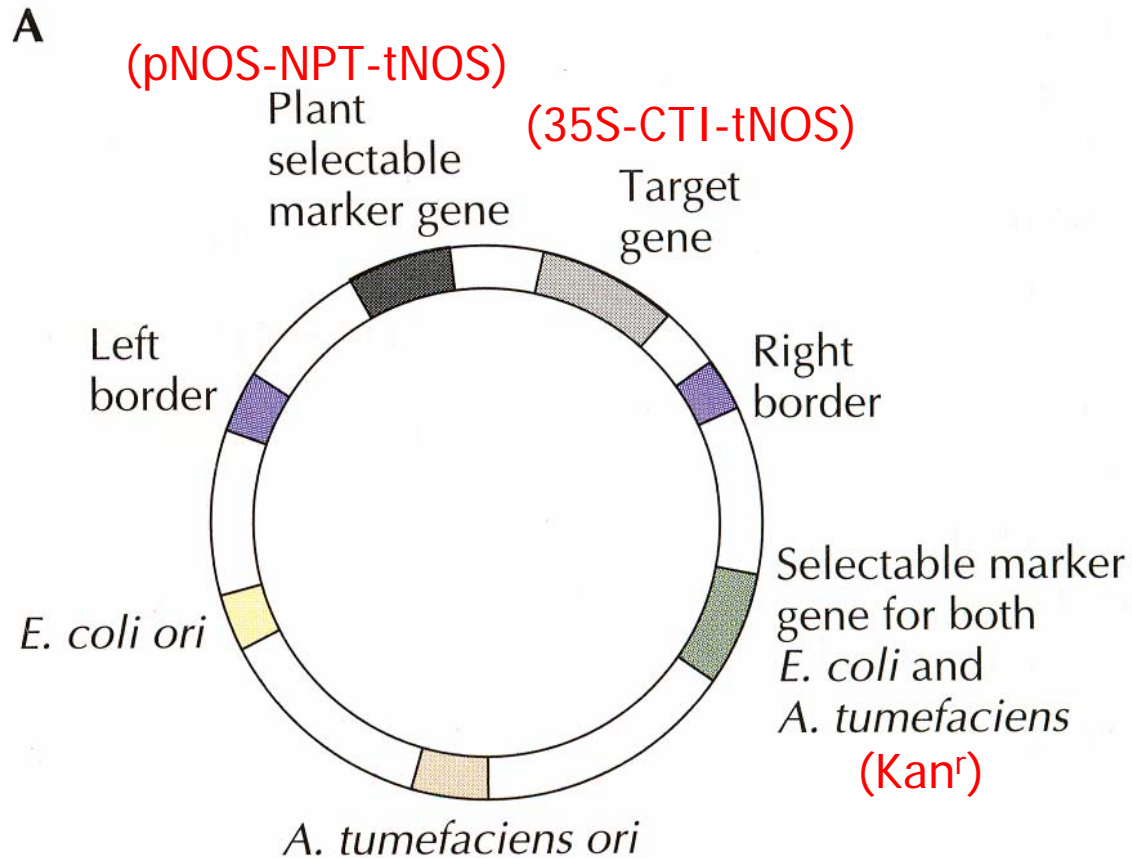
Strategies to avoid Bt resistant insects

- Use of inducible promoters (that can be turned on only when there is an insect problem)
- Construction of hybrid Bt toxins
- 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
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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	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

Fig. 18.3 Binary cloning vector carrying a cowpea trypsin inhibitor (CTI) gene



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 mosaic virus in tobacco and tomato, etc.)
- Expression of a dsRNase (RNaseIII)
- Expression of antiviral proteins (pokeweed)

Fig. 18.7 Procedure for putting CuMV coat protein into plants

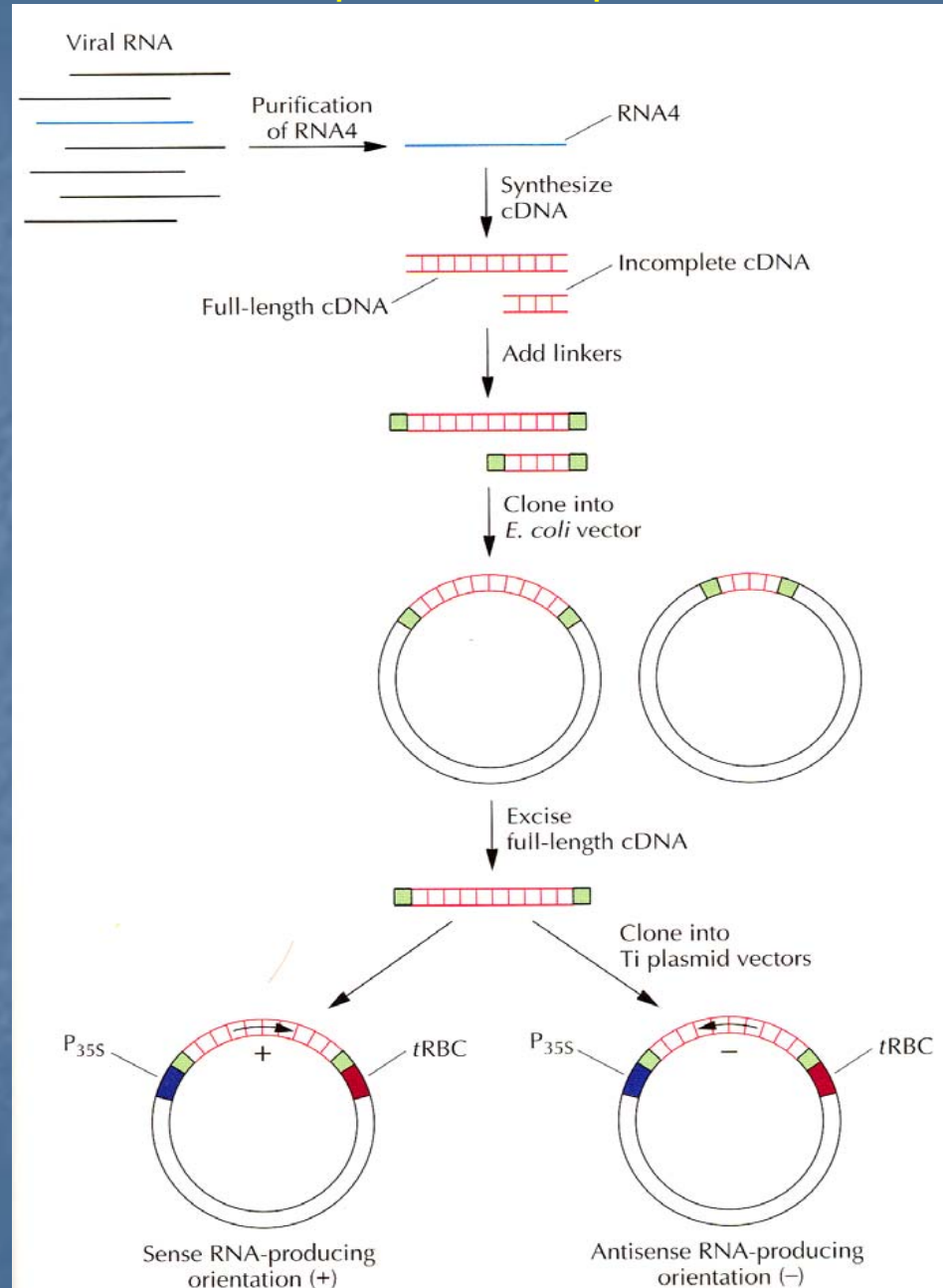
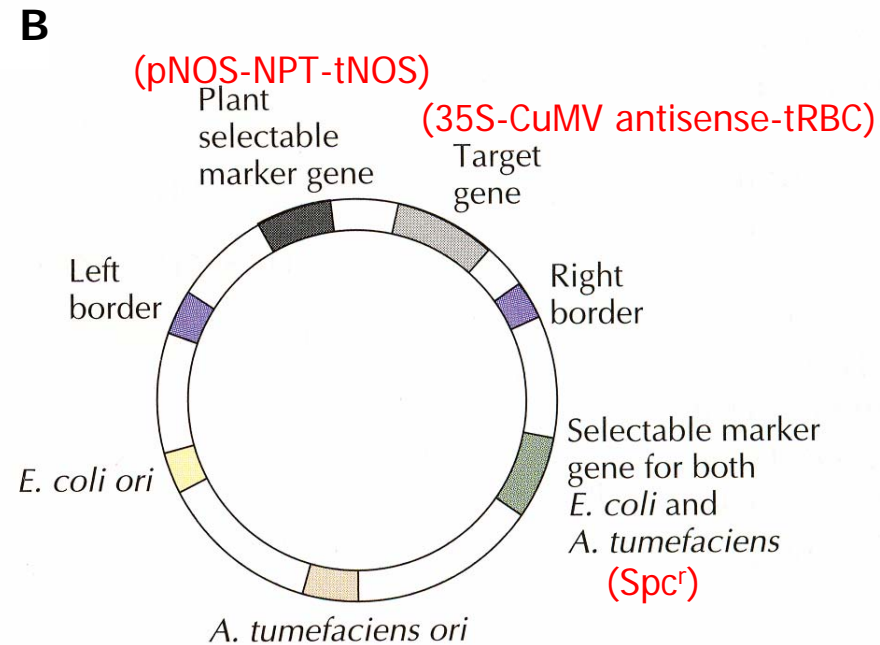
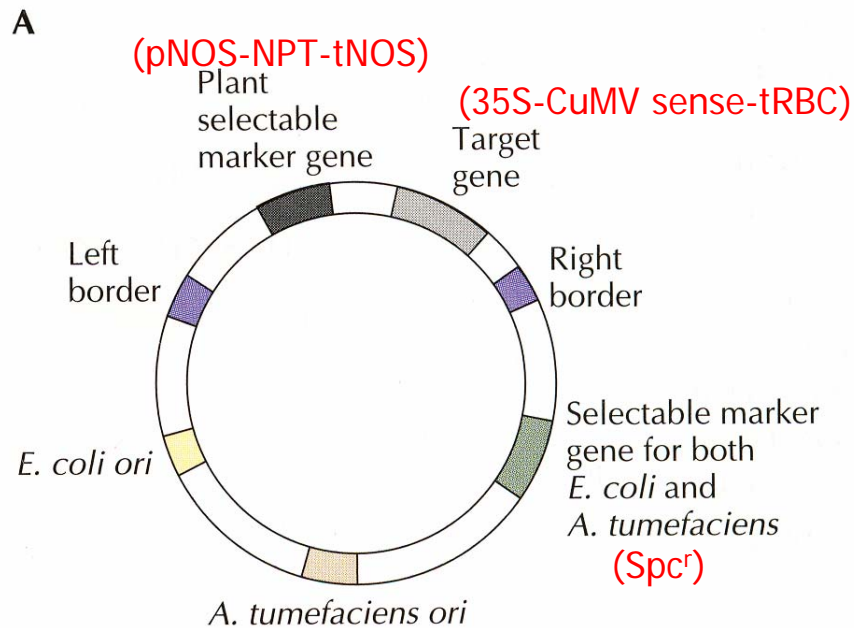


Fig. 18.3 Binary cloning vector carrying the protein-producing sense (A) or antisense RNA-producing (B) orientation of the cucumber mosaic virus coat protein (CuMV) cDNA



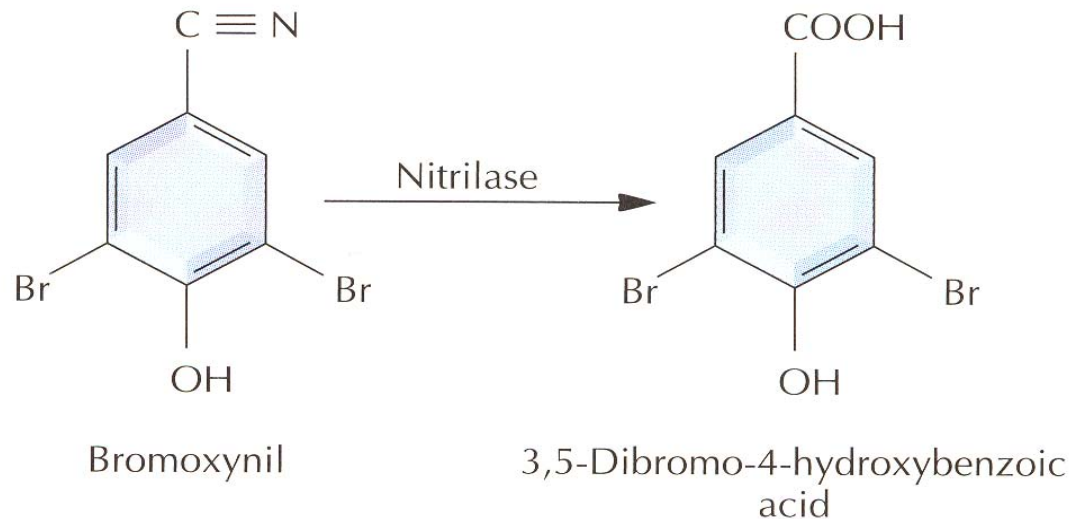
Herbicides and herbicide-resistant plants

- 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

Herbicide-resistant plants:

Giving plants the ability to inactivate the herbicide

- Herbicide: Bromoxynil
- Resistance to bromoxynil (a photosystem 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!

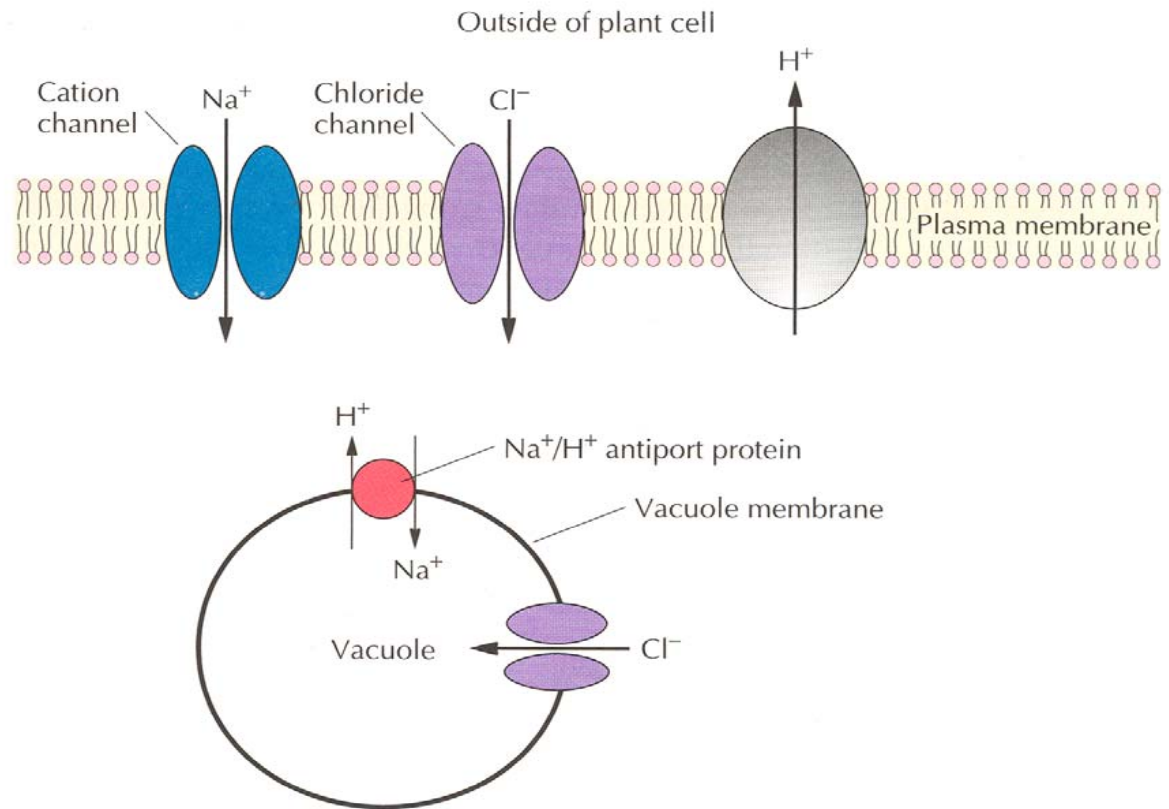
Fungus- and bacterium-resistant plants

- Genetic engineering here is more challenging; however, some strategies are possible:
- Individually or in combination express pathogenesis-related (PR) proteins, which include β 1,3-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**.

Flavor development pathway



Fruit softening pathway

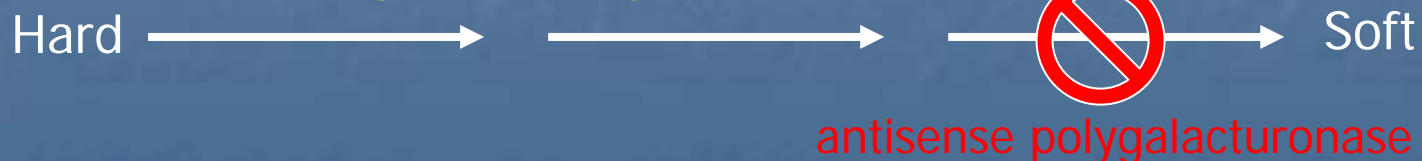
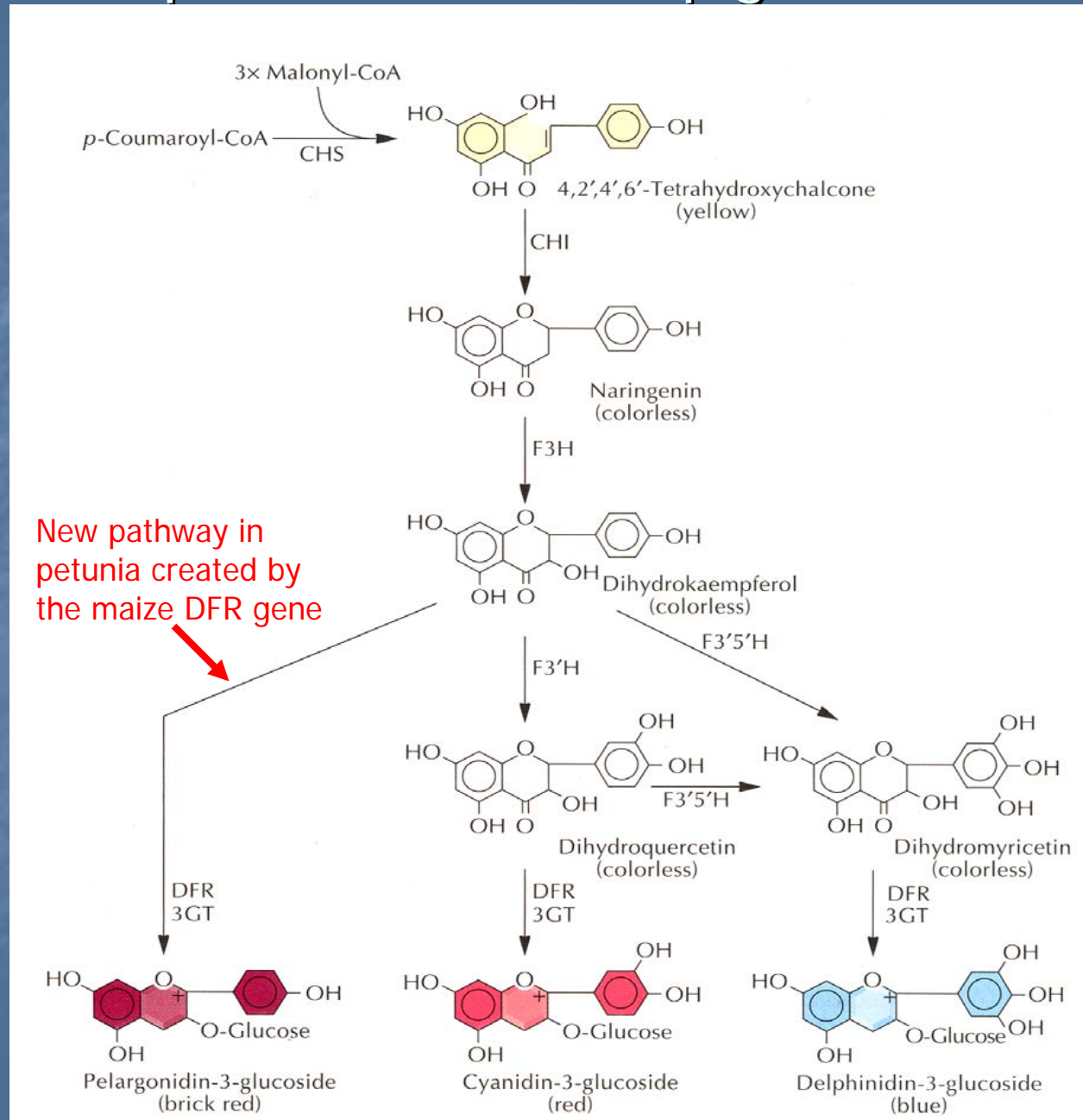


Fig. 18.25 Genetic manipulation of flower pigmentation

- 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!

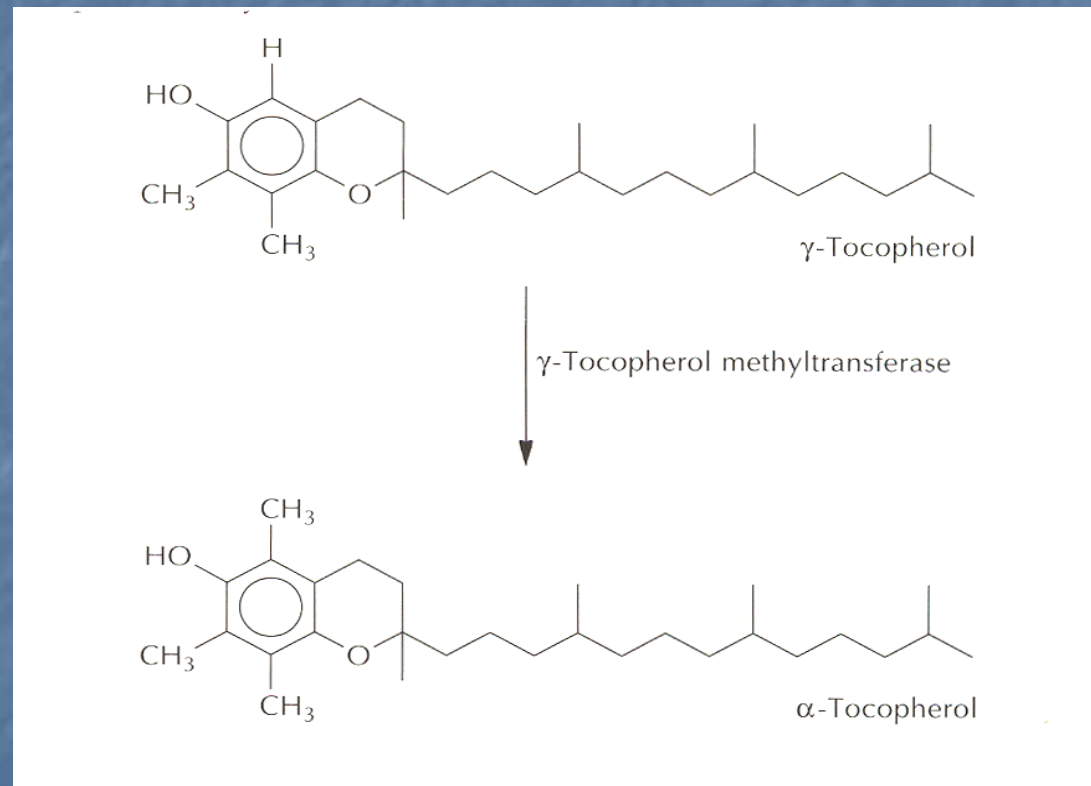


Modification of plant nutritional content

- 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



Modification of plant nutritional content: **increasing the vitamin A content of plants (Fig. 18.32)**

- 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"

