Table 17.1 Plant cell DNA-delivery methods

Method	Comment
Ti plasmid-mediated gene transfer	An excellent and highly effective system that is limited to a few kinds of plants
Microprojectile bombardment	Used with a wide range of plants and tissues; easy and inexpensive
Viral vectors	Not an effective way to deliver DNA to plant cells
Direct gene transfer into plant protoplasts	Can be used only with plant cell protoplasts that can be regenerated into viable plants
Microinjection	Has limited usefulness because only one cell can be injected at a time; requires the services of a highly skilled individual
Electroporation	Generally limited to plant cell protoplasts that can be regenerated into viable plants
Liposome fusion	Can be used only with plant cell protoplasts that can be regenerated into viable plants

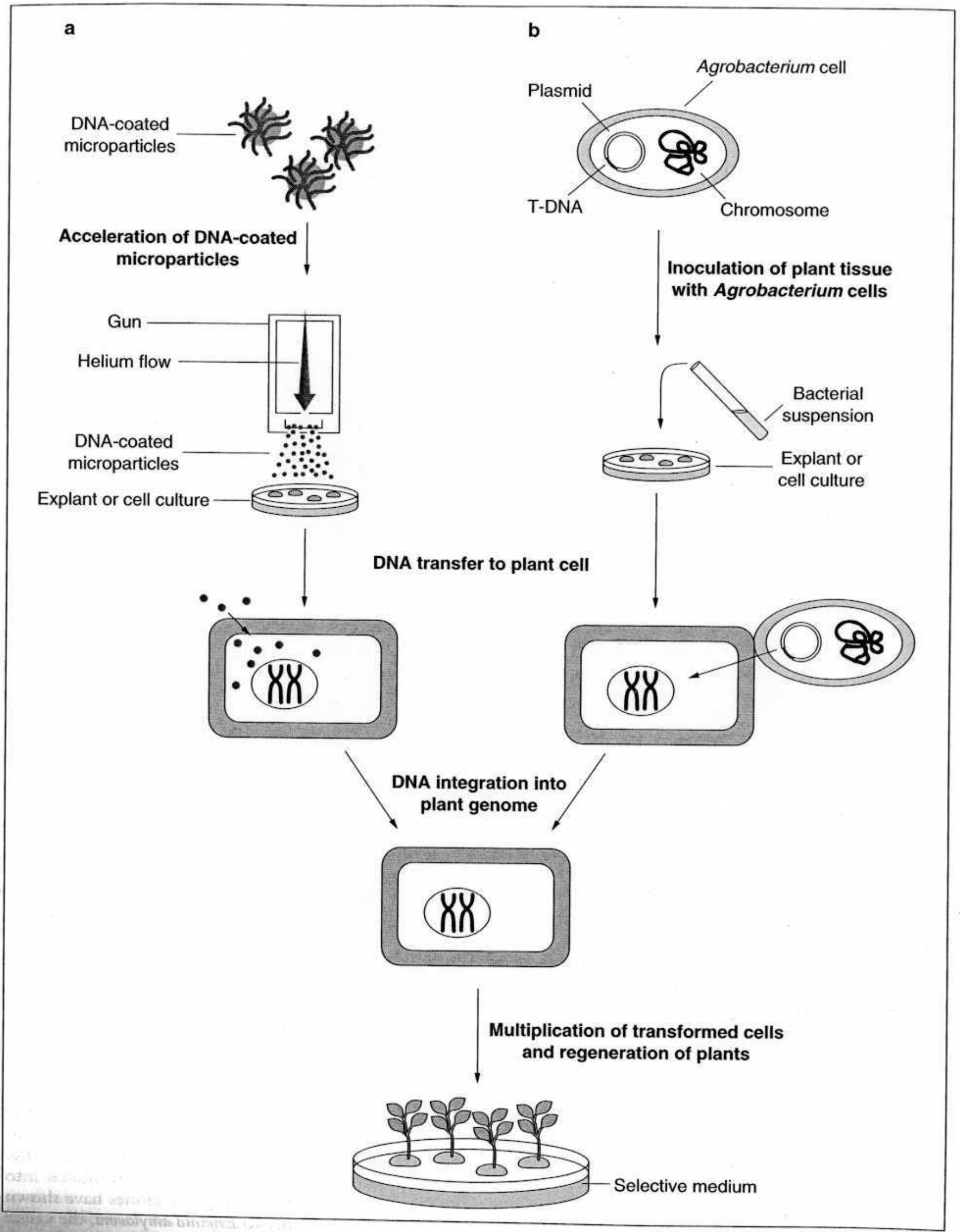


Figure 1

There are two main approaches for transferring genes into plant cells. (**a**) Direct gene transfer delivers DNA directly to the nuclear or plastid genome of the plant cell through

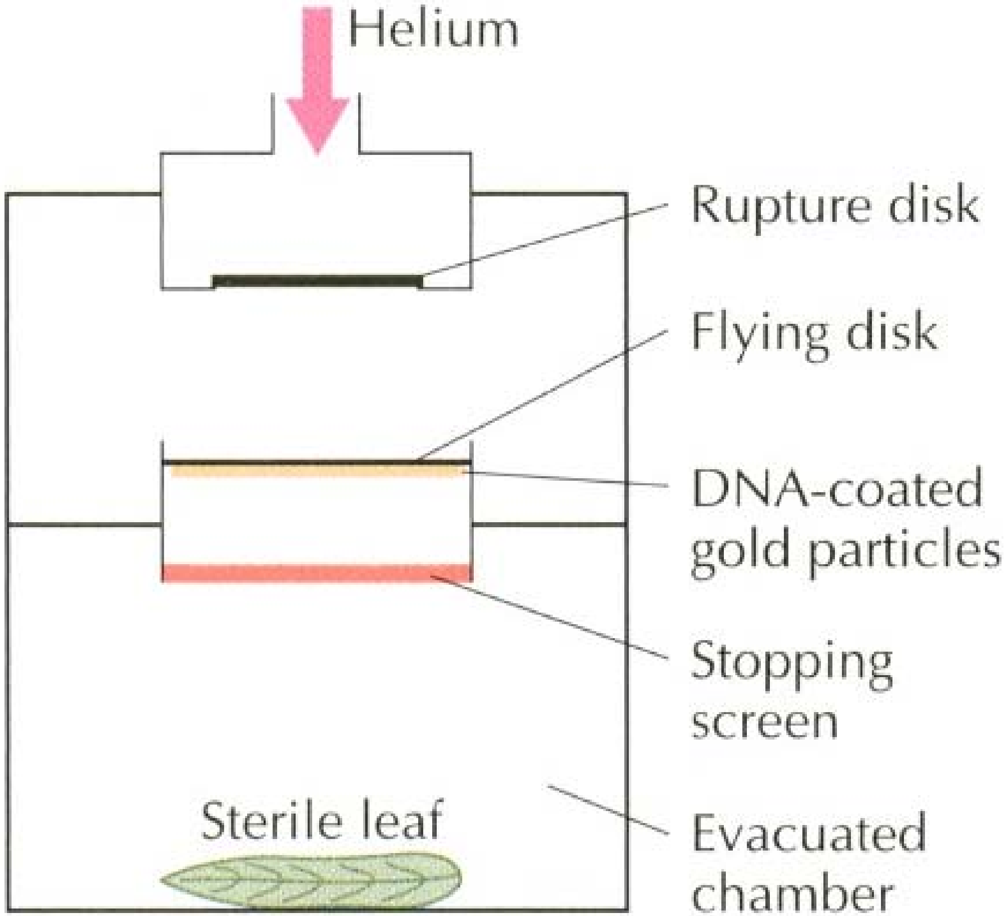


Figure 17.9 Schematic representation of a microprojectile bombardment apparatus. When the helium pressure builds up to a certain point, the plastic rupture disk bursts, and the released gas accelerates the flying disk with the DNA-coated gold particles on its lower side. The gold particles pass the stopping screen, which holds back the flying disk, and penetrate the cells of the sterile leaf.

Table 17.2 Plants that have been genetically transformed

Alfalfa	Cucumber	Orchid	Rye
Apple	Eggplant	Papaya	Sorghum
Arabidopsis	Flax	Pea	Soybean
Asparagus	Grape	Peanut	Strawberry
Banana	Kiwi	Pearl millet	Sugar beet
Barley	Lettuce	Peony	Sugarcane
Bean	Licorice	Pear	Sunflower
Cabbage	Lily	Petunia	Sweet potato
Canola	Lotus	Plantain	Tall fescue
Carnation	Corn	Poplar	Tobacco
Carrot	Norway spruce	Potato	Tomato
Cotton	Oat	Red fescue	Wheat
Cranberry	Orchard grass	Rice	White spruce

Table 17.4 Plant cell reporter and selectable marker gene systems

Enzyme activity	**Selectable marker**	**Reporter gene**
Neomycin phosphotransferase	Yes	Yes
Hygromycin phosphotransferase	Yes	Yes
Dihydrofolate reductase	Yes	Yes
Chloramphenicol acetyltransferase	Yes	Yes
Gentamycin acetyltransferase	Yes	Yes
Nopaline synthase	No	Yes
Octopine synthase	No	Yes
β-Glucuronidase	No	Yes
Streptomycin phosphotransferase	Yes	Yes
Bleomycin resistance	Yes	No
Firefly luciferase	No	Yes
Bacterial luciferase	No	Yes
Threonine dehydratase	Yes	Yes
Metallothionein II	Yes	Yes
enol-Pyruvylshikimate-3-phosphate synthase	Yes	No
Phosphinothricin acetyltransferase	Yes	Yes
β-Galactosidase	No	Yes
Blasticidin S deaminase	Yes	Yes
Acetolactate synthase	Yes	No
Bromoxynil nitrilase	Yes	No

Adapted from Walden and Schell, *Eur. J. Biochem.* **192:**563–576, 1990, and Gruber and Crosby, p. 89–119, *in* B. R. Glick and J. E. Thompson (ed.), *Methods in Plant Molecular Biology and Biotechnology*, CRC Press, Boca Raton, Fla.

Agrobacterium tumefaciens
Ti Plasmid based
DNA Transfer System

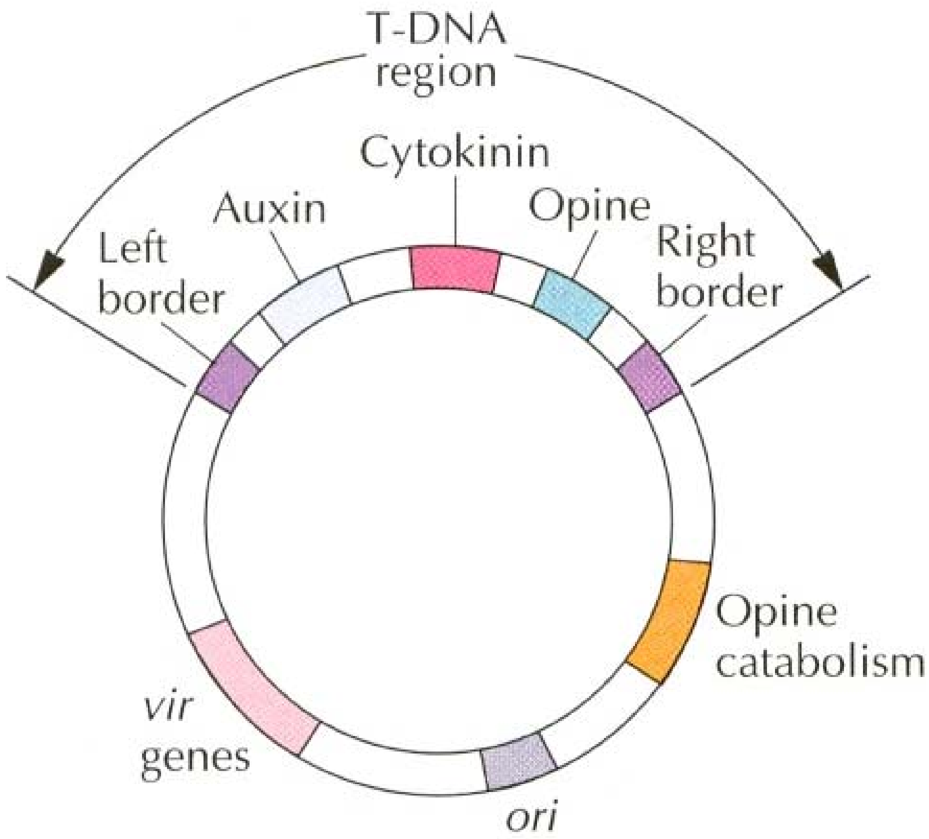
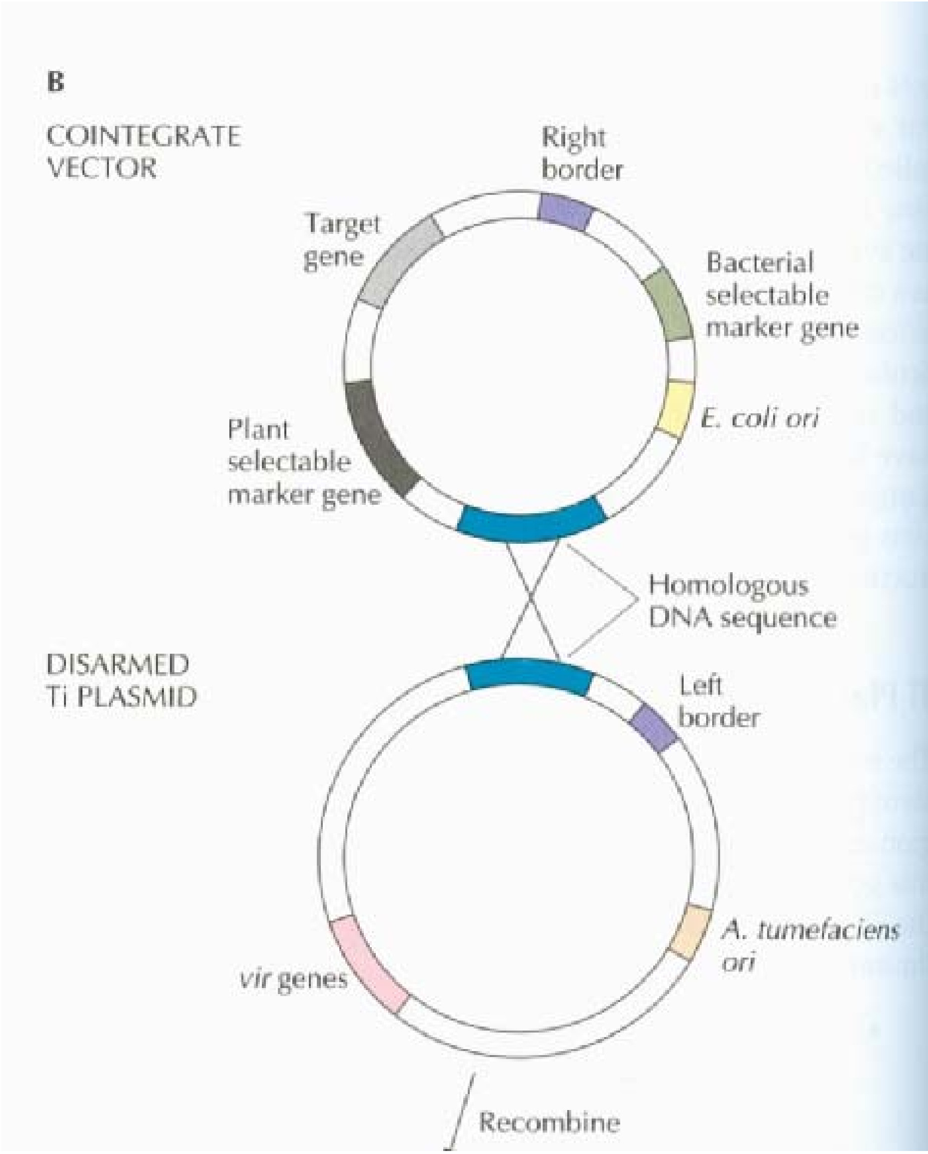
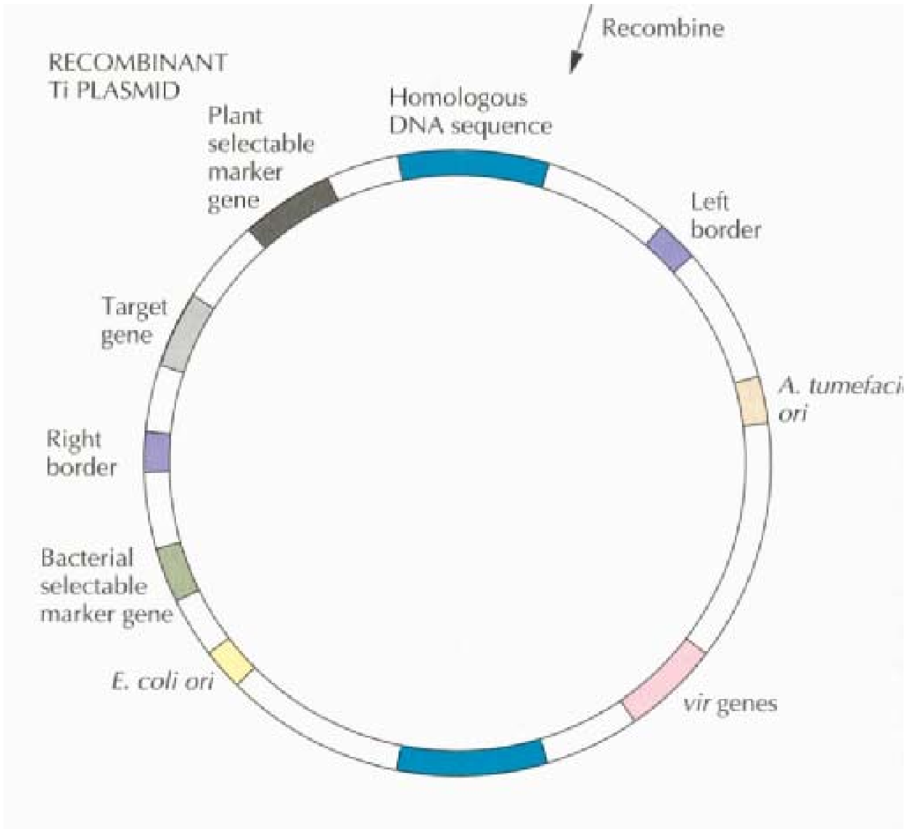


Figure 17.3 Schematic representation of a Ti plasmid. The T-DNA is defined by its left and right borders and includes genes for the biosynthesis of auxin, cytokinin, and an opine; these genes are transcribed and translated only in plant cells. Outside of the T-DNA region, there is a cluster of *vir* genes, a gene(s) that encodes an enzyme(s) for opine catabolism, and an origin of DNA replication (*ori*) which permits the plasmid to be stably maintained in *A. tumefaciens*. None of these features is drawn to scale.

Agrobacterium tumefaciens Ti Plasmid based DNA Transfer System





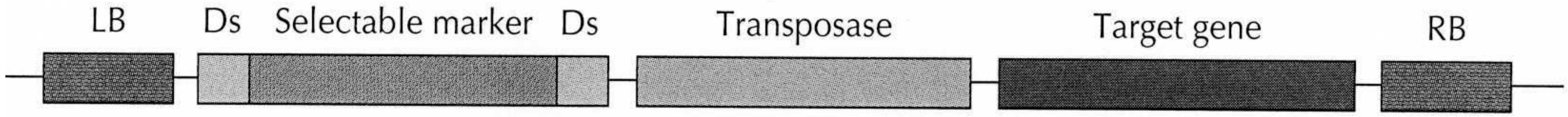
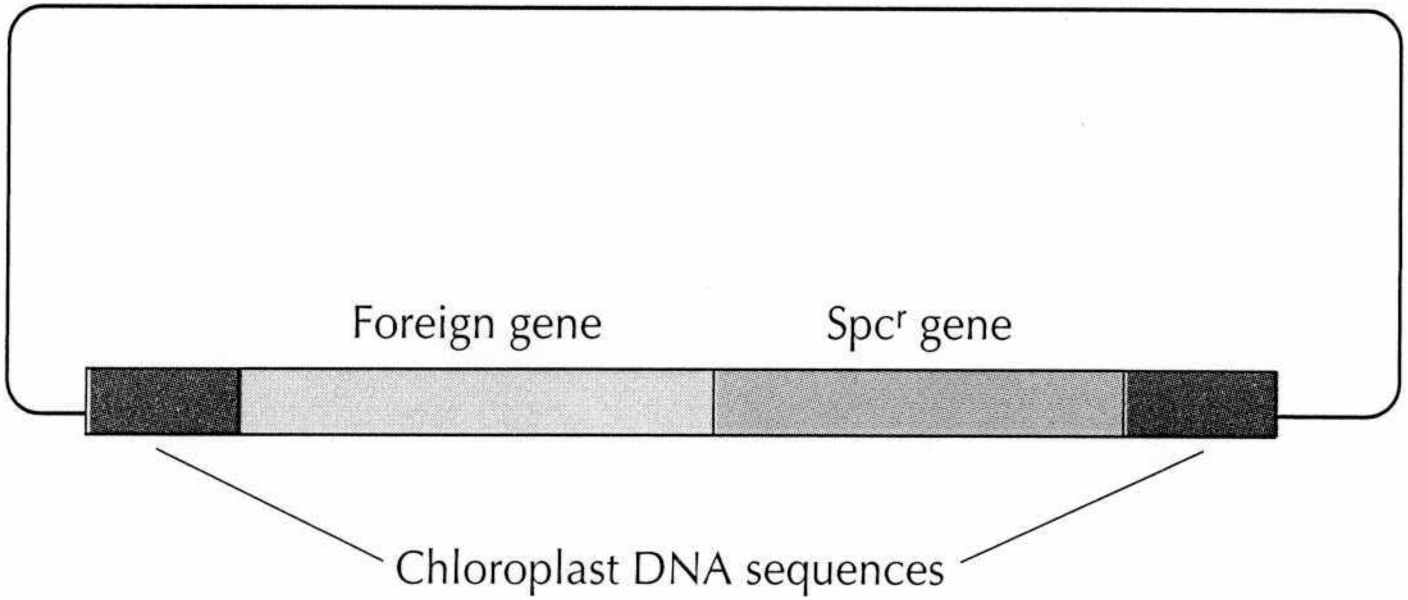


Figure 17.9 Schematic representation of the T-DNA of a vector. Following integration of the T-DNA into the plant chromosomal DNA, the transposase can excise the selectable marker gene and insert it into a different chromosomal location within the plant. Abbreviations: LB, the T-DNA left border; RB, the T-DNA right border; Ds, a plant transposable element. Note that the promoter and transcription termination sequences for the transposase gene, the target gene, and the selectable marker gene are not shown.

Figure 17.7 Plasmid vector used for integrating tandem genes into the chloroplast genome. Abbreviation: Spc^r, spectinomycin resistance.



FREISETZUNGSANTRÄGE IN DER EU

ORGANISMUS	ANZAHL ANTRÄGE	ORGANISMUS	ANZAHL ANTRÄGE
Mais	350	Mais, Raps, Zuckerrübe	4
Raps	272	Petunie	4
Zuckerrübe	214	Kirsche	3
Kartoffel	148	Kiwi	3
Tomate	71	Wein	3
Tabak	49	Apfel	2
Bakterium	39	Blumenkohl, Broccoli	2
Chicorée	37	Hefe	2
Baumwolle	20	Luzerne	2
Sojabohne	12	Olive	2
Weizen	11	Reis	2
Melone	10	Waldbäume	2
Sonnenblume	10	Chrysantheme	1
Pappel	9	Futterrübe	1
Ringelblume	9	Karotte	1
Nelke	8	Kohl	1
Virus	8	Orange	1
Mais, Raps	7	Pelargonie	1
Aubergine	5	Pflaume	1
Erdbeere	5	Rübsen	1
Kopfsalat	5	Steckrübe	1
Kürbis	5	Usambara-Veilchen	1
Blumenkohl	4	Zucchini	1
Eukalyptus	4		
Gerste	4	**Gesamt-Summe**	**1358**

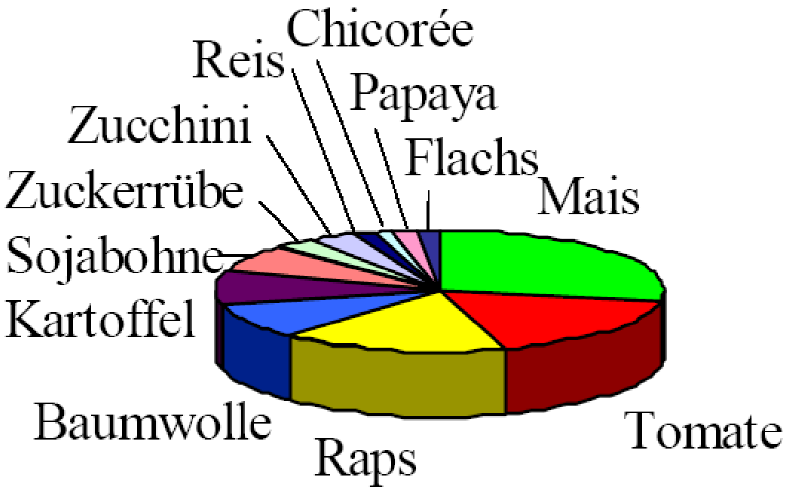
Quelle: RKI, Stand 2/99

FREISETZUNGSANTRÄGE IN DER EU

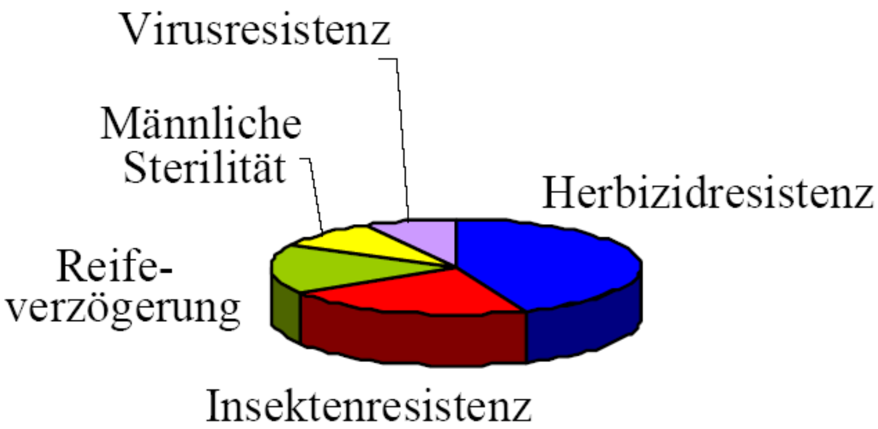
MERKMAL	ANZAHL ANTRÄGE
Herbizidtoleranz	444
Herbizidtoleranz; männlich steril	140
Herbizidtoleranz; Insektenresistenz	117
sonstige	107
Kohlenhydratstoffwechsel	94
Virusresistenz	90
Insektenresistenz	75
Inhaltsstoffe	65
Markierung	60
Herbizidtoleranz; weitere Kombinationen	48
Pilzresistenz	42
Fettsäuremuster	30
Herbizidtoleranz; Virusresistenz	17
Nitratstoffwechsel	10
Bewurzelung	8
Bakterienresistenz	7
Blütenfarbe	5
Summe	**1358**

Quelle: RKI, Stand 2/99

Zulassungen transgener Pflanzen in den USA



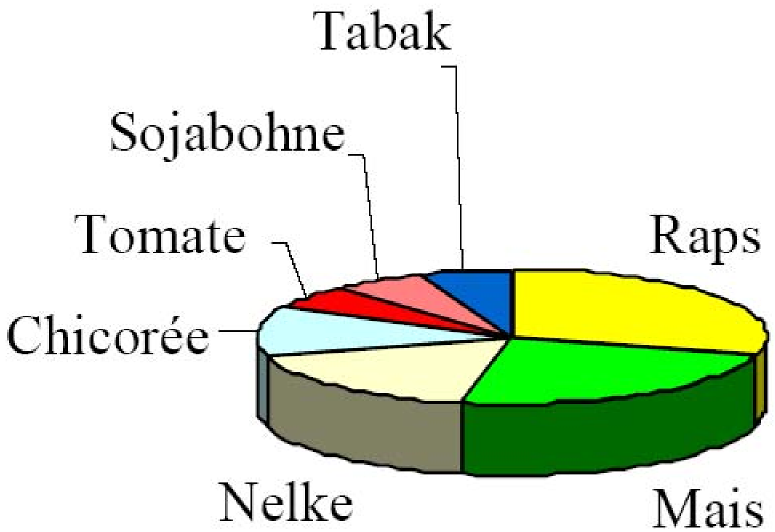
Merkmale der in den USA zugelassenen transgenen Pflanzen



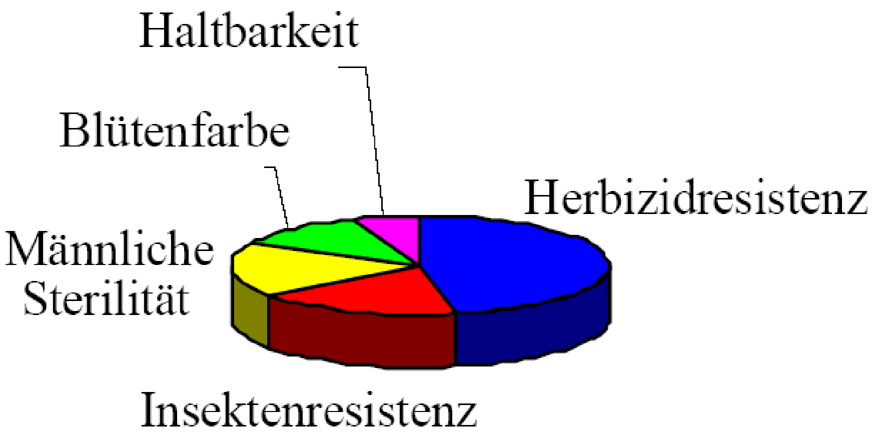
Stand 6/99

Stand 1999

Zulassungen transgener Pflanzen in der EU



Merkmale der in der EU zugelassenen transgenen Pflanzen



Stand 1999

EU-ZULASSUNGEN TRANSGENER PFLANZEN

Pflanze	Merkmal	Firma	Zulassung
Mais	Insektenresistenz (BT), Herbizidtoleranz	Novartis	1/97*[1]
	Herbizidresistenz	AgrEvo	4/98
	Insektenresistenz (BT)	Monsanto	4/98*[2]
	Insektenresistenz (BT), Herbizidtoleranz	Novartis	4/98
Raps	Herbizidresistenz (BASTA)	Plant Genetic Systems	2/96
	Herbizidresistenz	Plant Genetic Systems	6/97
	Herbizidresistenz	Plant Genetic Systems	6/97
	Herbizidresistenz	AgrEvo	4/98
Soja	Herbizidresistenz (ROUND UP)	Monsanto	4/96
Chicorée	Herbizidresistenz (BASTA)	Bejo-Zaden	5/96
Nelke	Modifizierte Blütenfarbe	Florigene	12/97
	Längere Haltbarkeit	Florigene	10/98
	Modifizierte Blütenfarbe	Florigene	10/98
Tabak	Herbizidresistenz	Seita	6/94

*[1] ... Importverbot in Österreich seit 2/97

*[2] ... Importverbot in Österreich seit 5/99

Stand 6/99

MERKMALE TRANSGENER PFLANZEN

PFLANZE	MERKMAL	EU Antrag	EU Zulassung	USA Antrag	USA Zulassung
Mais	Insektenresistenz	1	2+1#		7
	Herbizidresistenz		2		7
	Männliche Sterilität				3
Baumwolle	Insektenresistenz	1			2
	Herbizidresistenz	1			4
Raps	Herbizidresistenz	2	2+1#		3+5#
	Männliche Sterilität	1	2		1+1#
	Fettsäurezusammensetzung			1	
Tomate	Insektenresistenz				1
	Reifeverzögerung	1			10
Kartoffel	Insektenresistenz				4
	Virusresistenz				2
	Stärkezusammensetzung	1			

MERKMALE TRANSGENER PFLANZEN

PFLANZE	MERKMAL	EU Antrag	EU Zulassung	USA Antrag	USA Zulassung
Sojabohne	Herbizidresistenz		1		4
	Fettsäurezusammensetzung			1	
Tabak	Herbizidresistenz		1		
Chicoree	Herbizidresistenz	1	1*		
	Männliche Sterilität	1	1*		1
Futterrrübe	Herbizidresistenz	1			
Zuckerrüben	Herbizidresistenz				2
Flachs	Herbizidresistenz				1
Reis	Herbizidresistenz				1
Zucchini	Virusresistenz				2
Papaya	Virusresistenz				1
Zuckermelone	Virusresistenz			1	
	Reifeverzögerung			1	
Nelke	Haltbarkeit		1		
	Blütenfarbe		2		

* nur als Saatgut   # nur Import, Lagerung, Verarbeitung, nicht Anbau   Stand vom 01.05.99

Herbizide resistance

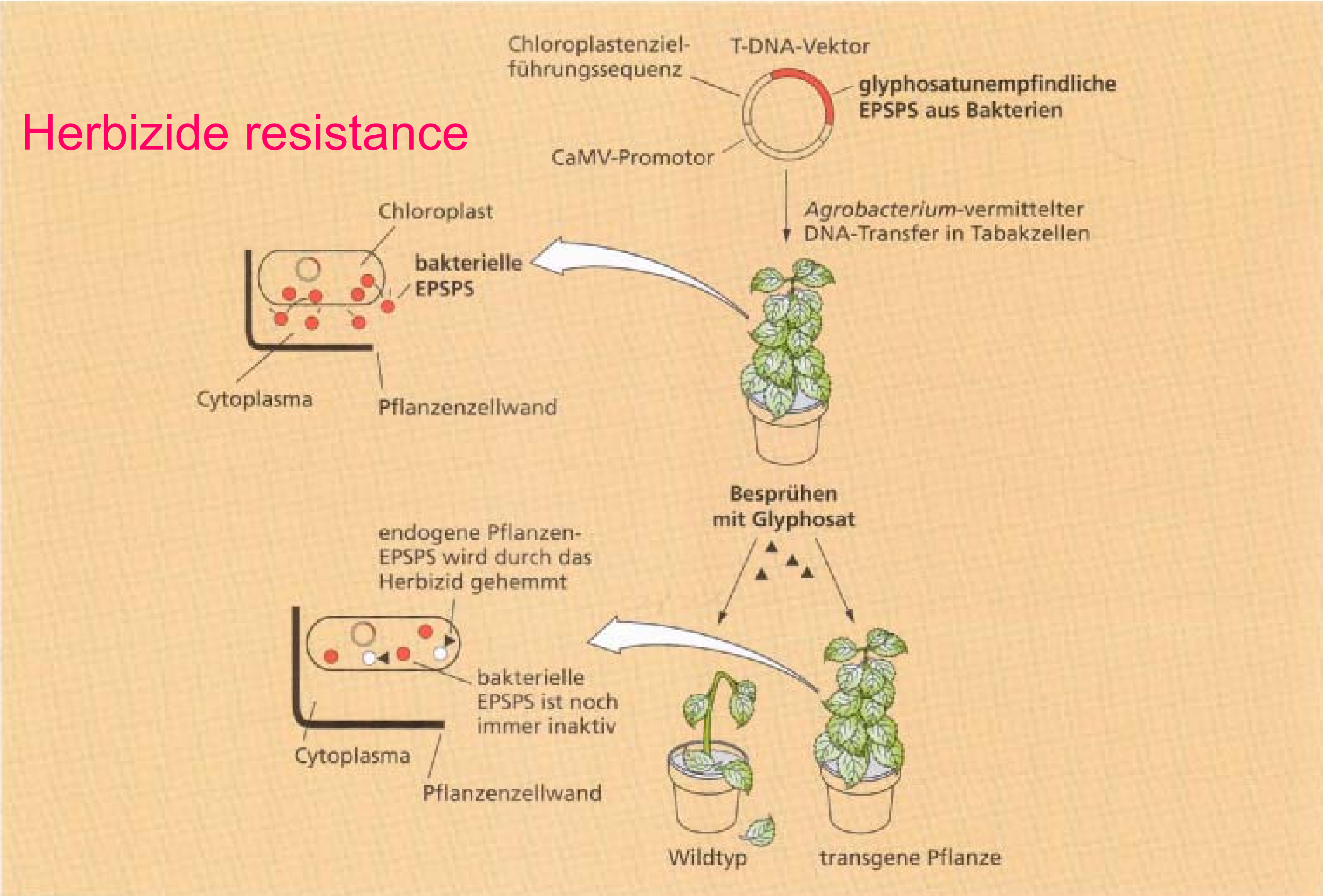


Abbildung 6.10: Herstellung herbizidresistenter Pflanzen. Falls die T-DNA von *Agrobacterium* bakterielle DNA enthält, die für das Photosyntheseenzym EPSPS mit einer Chloroplastenzielsequenz codiert, kann sie in eine Pflanze übertragen werden. Wenn der CaMV-Promotor aktiviert wird, kann die Pflanze (hier Tabak) das bakterielle EPSPS-Enzym herstellen und hohen Konzentrationen Glyphosat widerstehen, die sonst das natürlicherweise in den Pflanzen enthaltene Enzym hemmen würden. Auf diese Weise können sowohl Pflanzen wie Unkräuter mit Glyphosat besprüht werden, und nur die nichttransformierten Pflanzen werden davon betroffen.

Herbizidresistenz

Table 18.4 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.

Herbizidresistenz

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.

Herbizidresistenz

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Imidazolinones	Strains with resistant versions of the enzyme acetolactate synthetase have been selected in tissue culture.
Aryloxyphenoxypropionates, 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.
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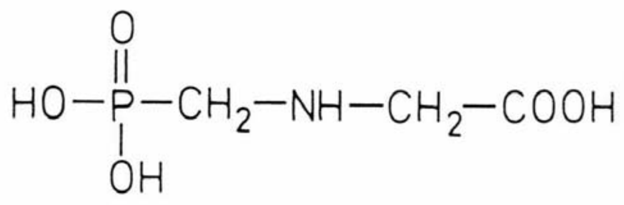
Herbizidresistenz

Abb. 164. Glyphosat.



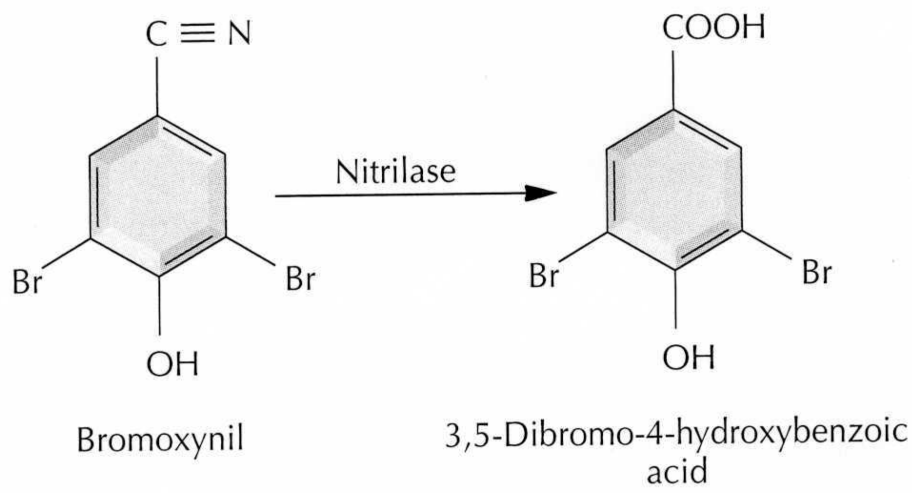
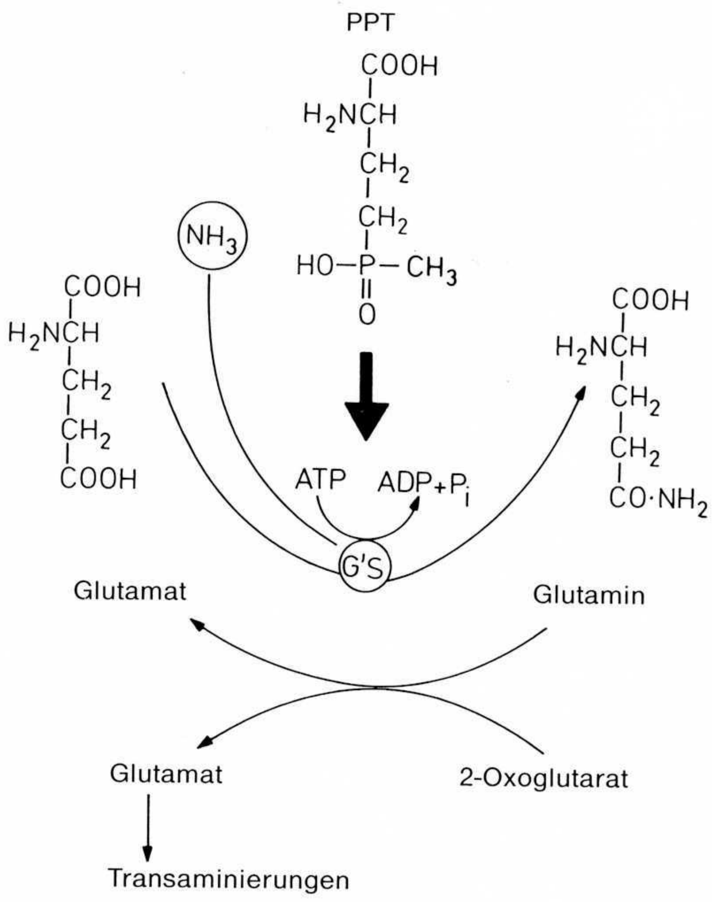


Figure 18.10 Detoxification of the herbicide bromoxynil by the enzyme nitrilase from *K. ozaenae*.

Abb. 165. Phosphinothricin (PPT) als Hemmstoff der Glutaminsynthetase (GS).



SOJABOHNE

Herbizidresistenz

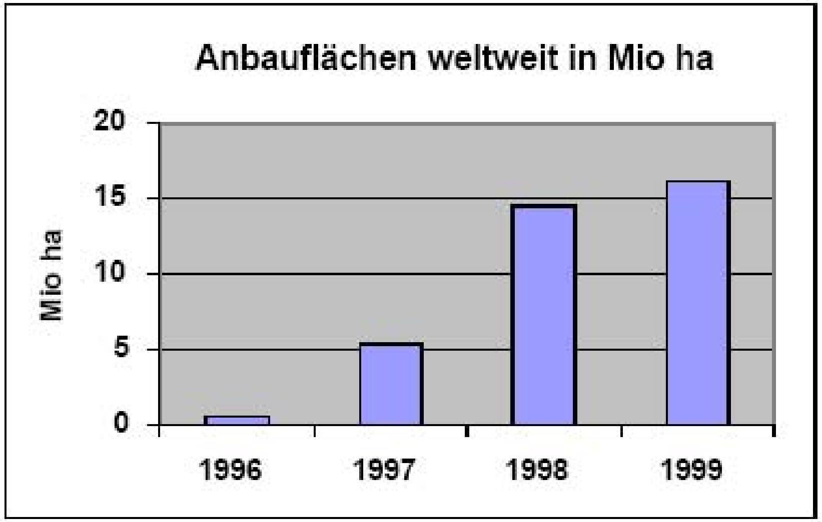
Fettzusammensetzung

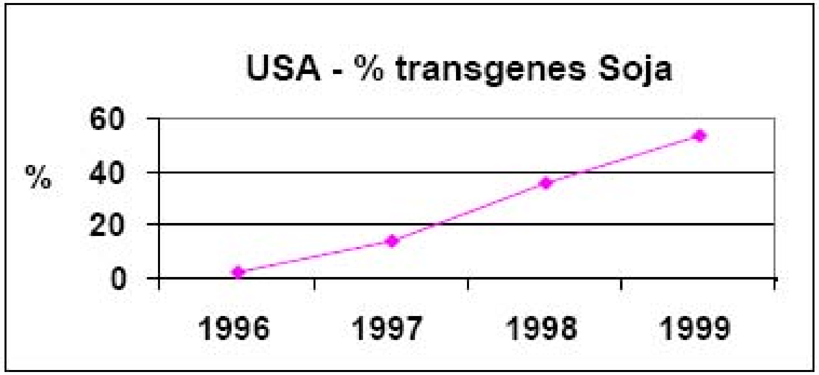
Zulassungen:
EU: 1
USA: 5

Verwendung:
Lebensmittel (v.a. Zutat)
Futtermittel

weitere Züchtungsziele:
veränderte Inhaltsstoffe (Fettsäuren, Aminosäuren),
Insektenresistenz

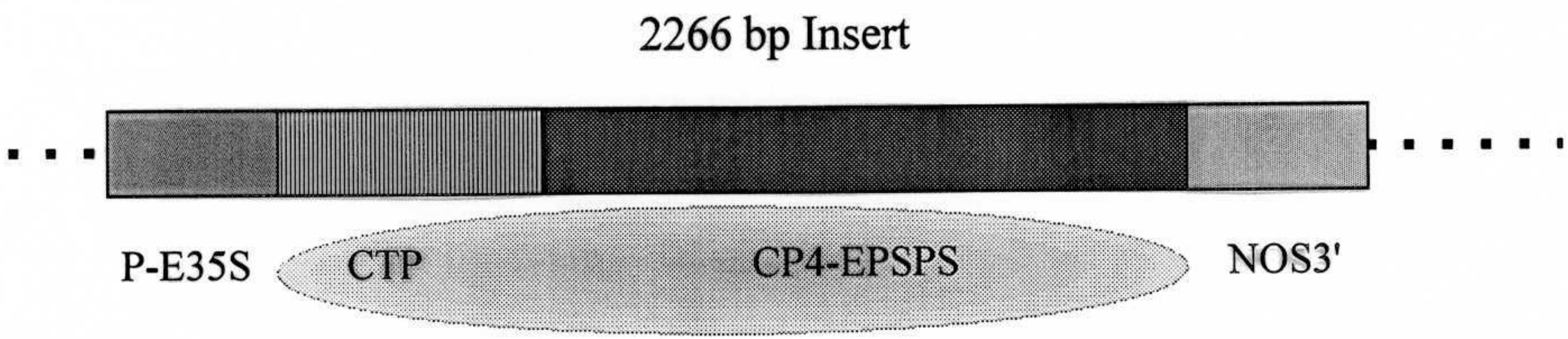
Tendenz:
steigend
Anbau auch in Brasilien





Stand 6/99

Roundup-Ready Sojabohne



P-E35S	E35S Promoter
CTP	5-Enolpyruvylshikimat-3-Phosphat Synthase
CP4-EPSPS	Chloroplasten Transpeptid
NOS3'	Terminator der Nopalinsynthase

Bt-Toxine

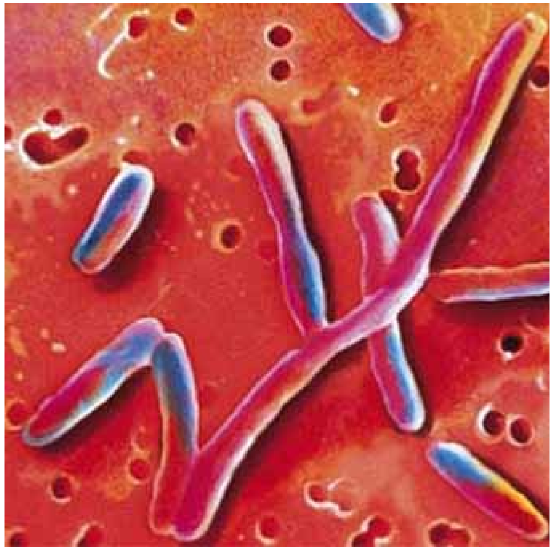
Spezifisch gegen Insekten wirksame Proteine aus Bakterien

Wirken im Darmtrakt der Insekten

Keine Toxizität auf höhere Organismen

Lange Verwendung, auch im Biolandbau

***Bacillus Thuringiensis* Toxin**



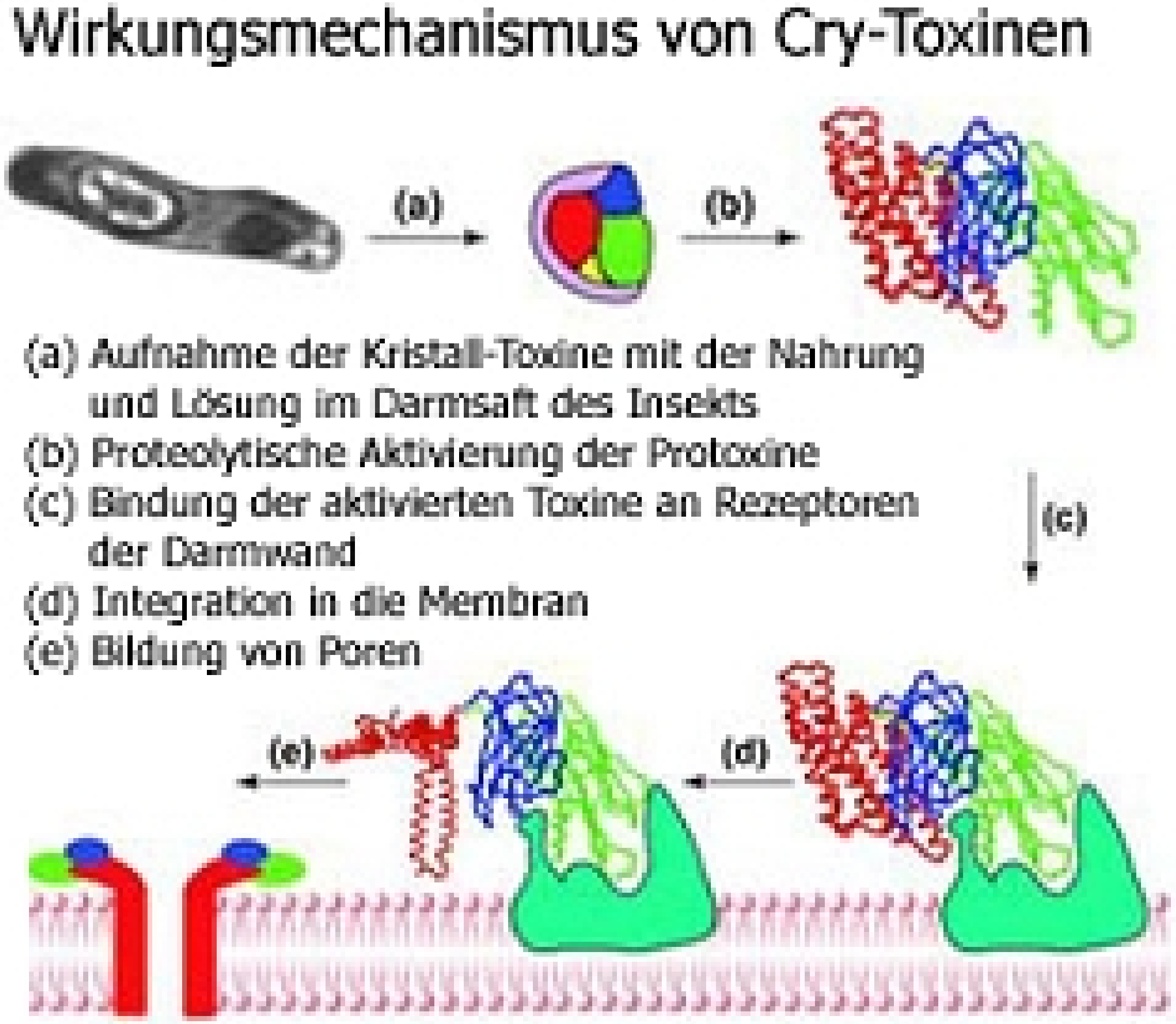
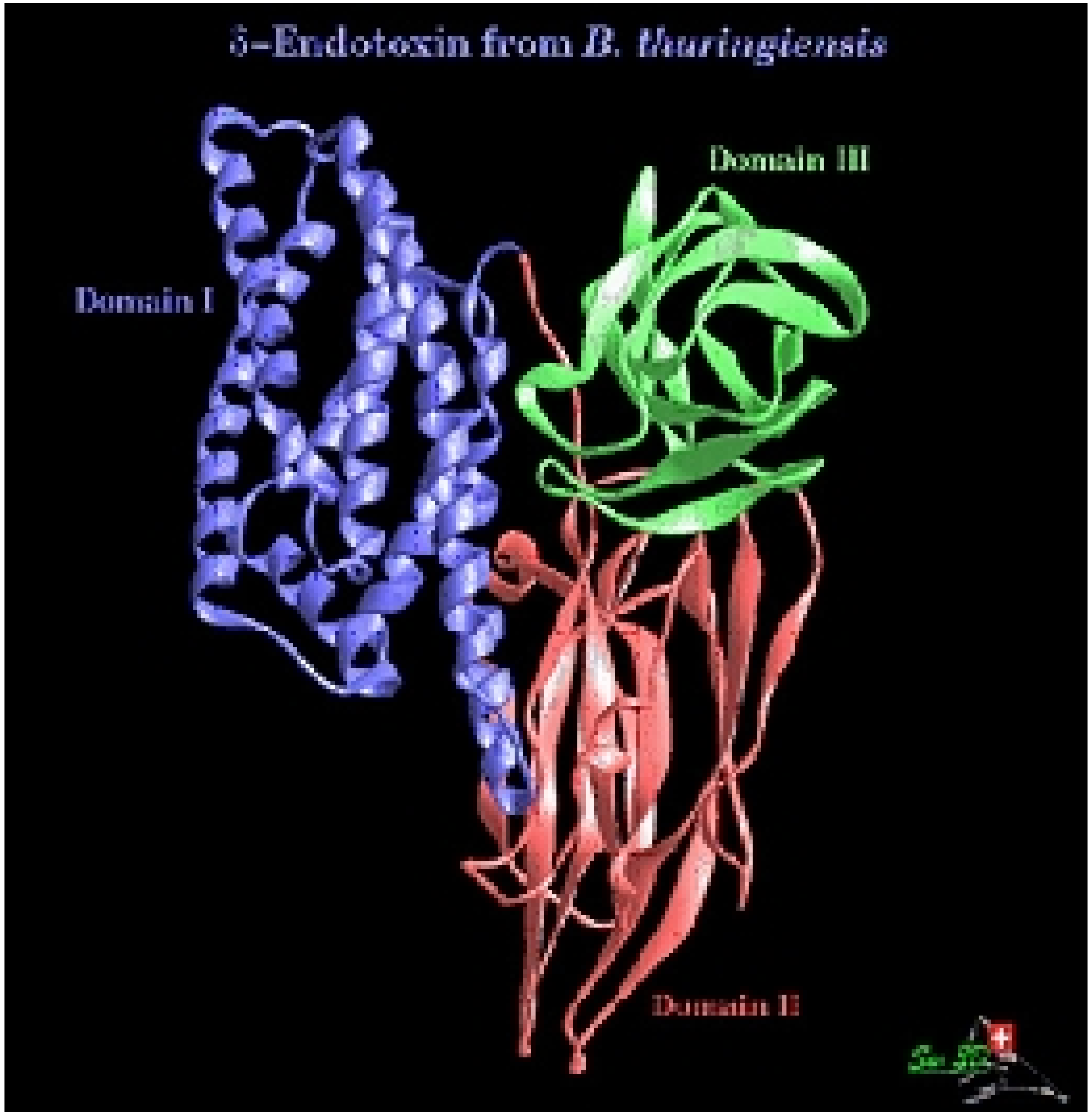


Abb.9: *Wirkungsmechanismus von Cry-Toxinen [Quelle: modifiziert nach de Maagd, Bravo & Crickmore (2001): Trends in Genetics 17 (4), 193-199.]*

http://www.jki.bund.de

Bacillus Thuringiensis Toxin



Wirkungsspektrum - Tabellarische Übersicht

Pathotyp A
- *B.t.* sv. *kurstaki* (*B.t.k.*) gegen Larven bestimmter Lepidopteren, nicht gegen Noctuidae (Eulen)
- *B.t.* sv. *aizawai* (*B.t.a.*) gegen Larven bestimmter Lepidopteren, auch gegen Noctuidae (Eulen)

Pathotyp B
- *B.t.* sv. *israelensis* (*B.t.i.*) gegen Larven bestimmter Dipteren

Pathotyp C
- *B.t.* v. *tenebrionis* (*B.t.t.*) gegen Larven bestimmter Chrysomeliden

http://www.jki.bund.de

Table 18.1 Expression of some *Bacillus thuringiensis* insecticidal toxin genes in transgenic plants

Plant(s)	Gene	% Expression	Insecticidal
Tobacco	*cry1Ab*, full	0.0001–0.0005	No
Tobacco	*cry1Ab*, truncated	0.003–0.012	Yes
Tobacco	*cry1Aa*, full	Not detected	No
Tobacco	*cry1Aa*, truncated	0.00125	Yes
Tobacco	*cry1Ac*, truncated	<0.014	Yes
Tomato	*cry1Ab*, truncated	0.0001	Yes
Cotton	*cry1Ab*, truncated, WT	<0.002	No
Cotton	*cry1Ab*, truncated, PM	0.05–0.1	Yes
Tomato, tobacco	*cry1Ab*, truncated, WT	0.002	Yes
Tomato, tobacco	*cry1Ab*, truncated, PM	0.002–0.2	Yes
Tomato, tobacco	*cry1Ab*, truncated, FM	0.3	Yes

Adapted from Ely, p. 105–124, *in* Entwistle et al. (ed)., Bacillus thuringiensis, *an Environmental Biopesticide: Theory and Practice*, 1993.

Terms and abbreviations: full, the complete protoxin gene; truncated, a shortened version of the protoxin gene; WT, wild-type codons; PM, partially modified codons; FM, fully modified codons.

Bacillus Thuringiensis Toxin

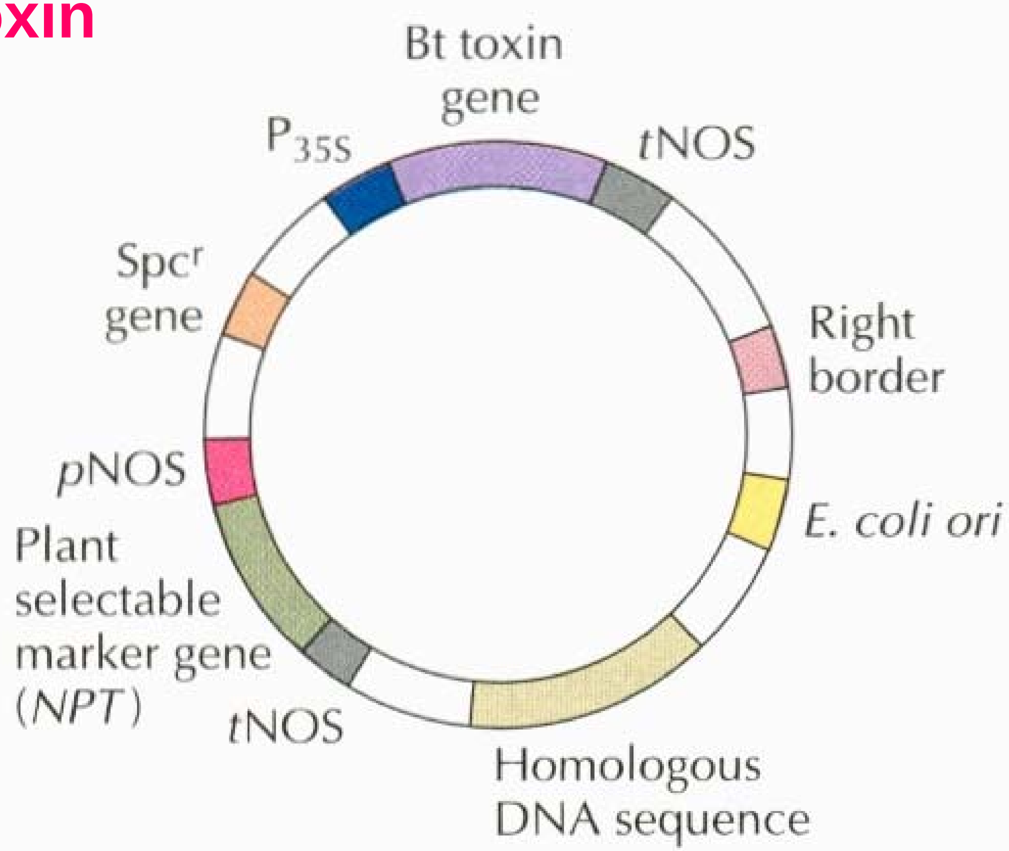


Figure 18.1 Cointegrate cloning vector carrying a *B. thuringiensis* (Bt) insecticidal toxin gene. The toxin gene is under the control of the strong, constitutive 35S promoter (P_{35S}) from cauliflower mosaic virus and the nopaline synthase transcription terminator–polyadenylation site (*t*NOS). The vector has an *E. coli* origin of DNA replication (*ori*); a spectinomycin resistance (Spc^r) gene, which allows the vector to be maintained and selected in *E. coli* cells; a T-DNA right border; a plant selectable marker gene; and a region of DNA that is homologous to DNA in the disarmed Ti plasmid, for integrating the two plasmids. The neomycin phosphotransferase gene (*NPT*), which acts as a plant reporter gene, is under the transcriptional control of nopaline synthase gene sequences (*p*NOS and *t*NOS) and is used to select for kanamycin-resistant transformed plant cells.

Bacillus Thuringiensis Toxin

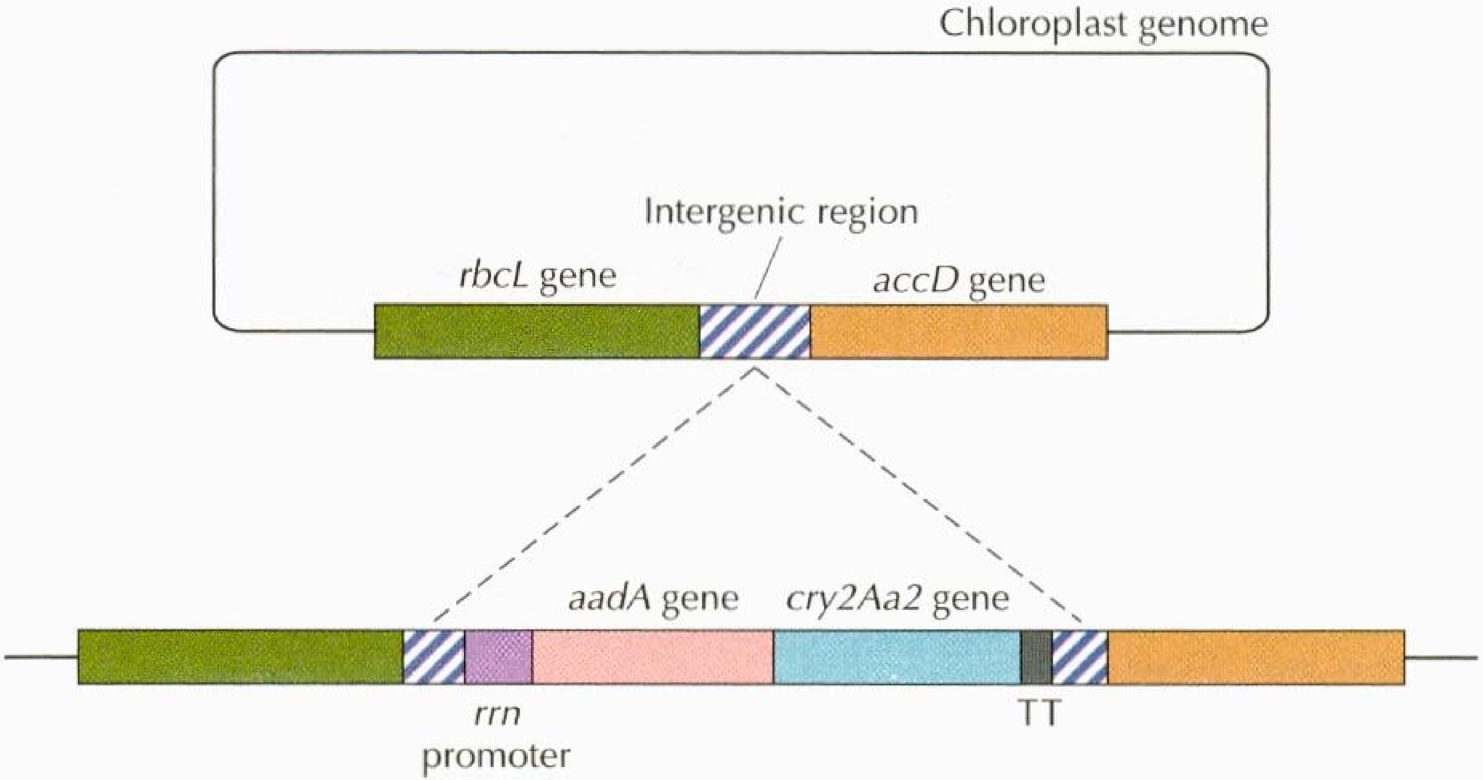


Figure 18.2 Site on the chloroplast genome where a foreign gene encoding the *B. thuringiensis* Cry2Aa2 protoxin is integrated by homologous recombination. The genes *rbcL* and *accD* are both present in a single copy per chloroplast genome. The intergenic region between these two genes, which is the site of insertion of the foreign genes, is smaller than it appears in this representation. The *aadA* gene (encoding spectinomycin and streptomycin resistance) and the *cry2Aa2* gene are both under the transcriptional control of the constitutive chloroplast *rrn* promoter and transcription terminator (TT), and both contain their own ribosomal binding site. Integration of foreign DNA into the intergenic spacer region prevents insertion of a foreign gene from interfering with the expression of any endogenous chloroplast genes. Adapted from Kota et al., *Proc. Natl. Acad. Sci. USA* **96:**1840–1845, 1999.

MAIS

Insektenresistenz (Bt Toxin)

Herbizidresistenz

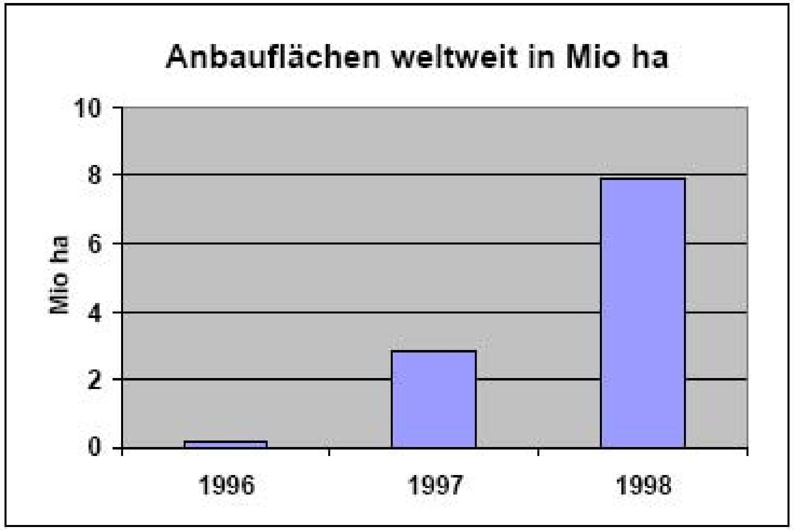
Männliche Sterilität

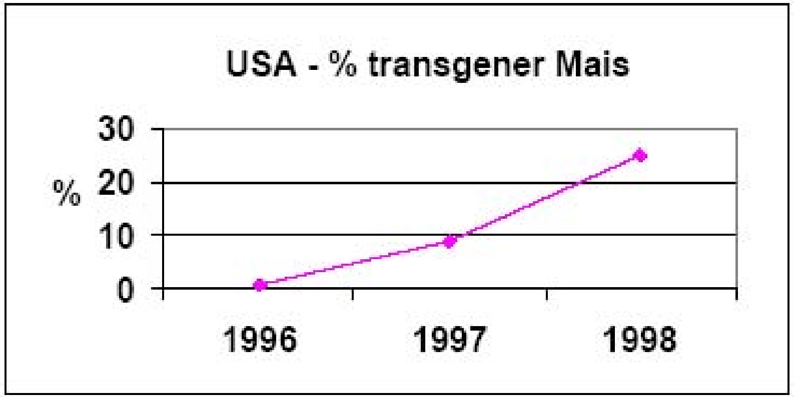
Zulassungen:
EU: 4 (1 Antrag) – in Österreich Importverbot für 2 Sorten
USA: 13

Verwendung:
Lebensmittel (v.a. Zutaten), Futtermittel

weitere Züchtungsziele:
Anpassung an andere Standorte,
verbesserte Protein-, Fettsäurezusammensetzung

Tendenz:
ausserhalb Europas steigend,
in Europa starke Ablehnung (Importverbote!)
Insektenresistenzmanagement gegen Resistenzauftreten,
Studien zeigen Umwelt-Risiken





Stand 6/99

Bt Mais - Vorteile

Anbauverluste durch Maiszünsler

- In USA: bis 20 % oder, 15 Millionen Tonnen pro Jahr
  Das ist vergleichbar mit:
  200% der Maisproduktion von Kanada
  oder 25% des gesamten Maisexportes der USA.

Geschätzte Einsparungen durch Bt-Mais

- 2,5 Millionen Hektar Land
- 100.000 Tonnen Dünger.
- 100 Millionen Liter Treibstoff
- Pflanzenschutzmittel

Angaben von Novartis

Biological Insect Control

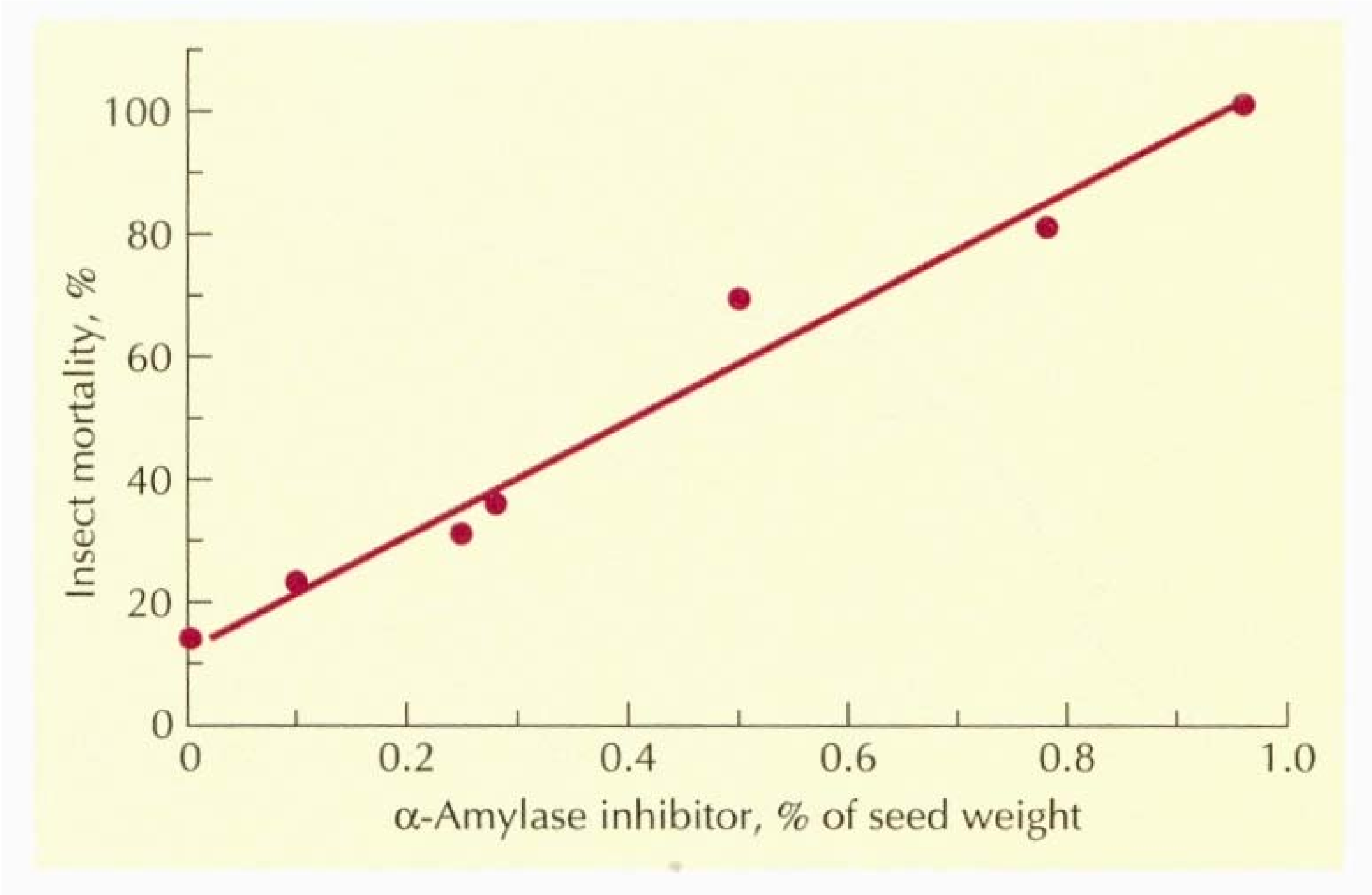
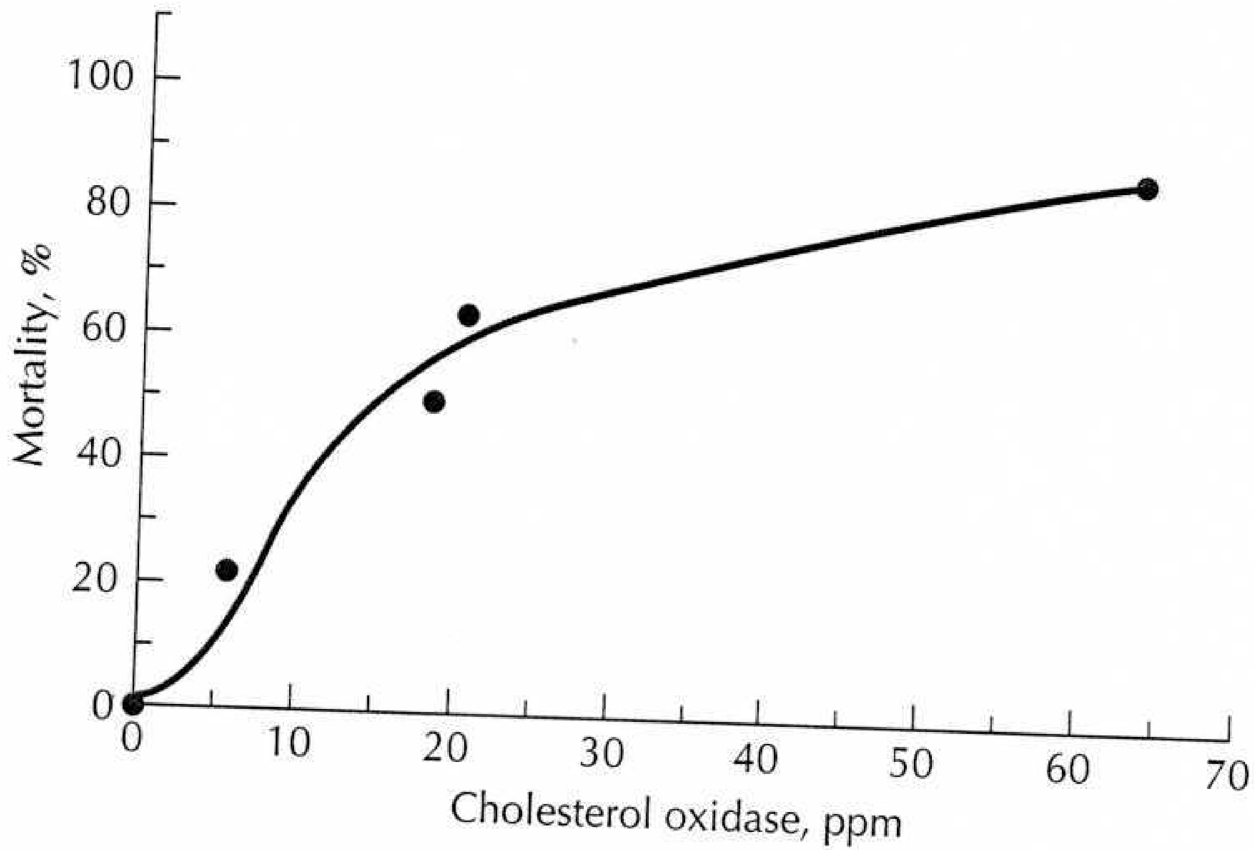


Figure 18.5 Mortality of cowpea weevil larvae reared on transgenic pea plants that produce different amounts of alpha-amylase inhibitor.

Biological Insect Control

Figure 18.5 Effect of increasing amounts of cholesterol oxidase on the mortality of boll weevil larvae. ppm, parts per million. Adapted from Corbin et al., *Appl. Environ. Microbiol.* **60**:4239–4244, 1994.



Biological Insect Control

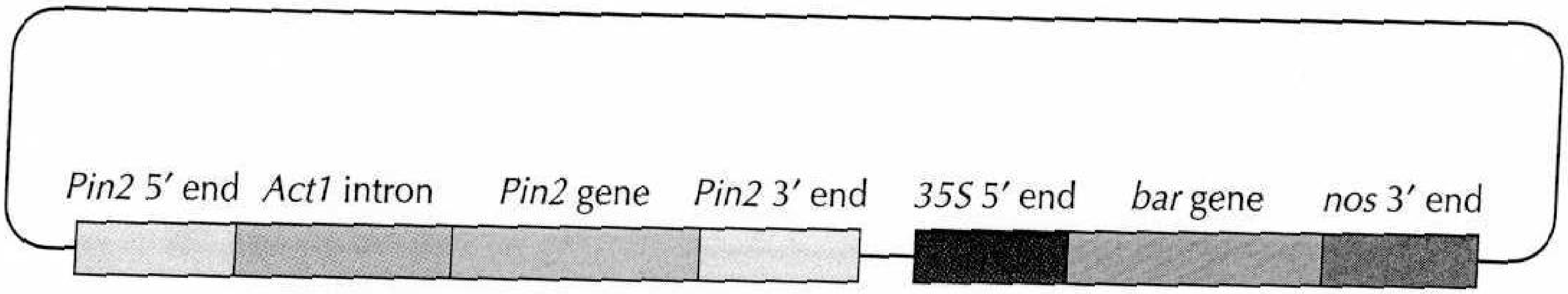


Figure 18.3 Plasmid vector carrying the potato proteinase inhibitor II gene. Designations: *Pin2* gene, the potato proteinase inhibitor II gene; 5′ end, the region of DNA preceding the gene; 3′ end, the region of DNA following the gene; *Act1* intron, the first intron from the rice actin 1 gene; *35S* 5′ end, the 35S promoter from cauliflower mosaic virus; *bar* gene, the bacterial phosphinothricin acetyltransferase gene; *nos* 3′ end, the region of DNA following the nopaline synthase gene. The *bar* gene serves as a selectable marker for transgenic plants, conferring resistance to the herbicide Basta (ammonium glufosinate).

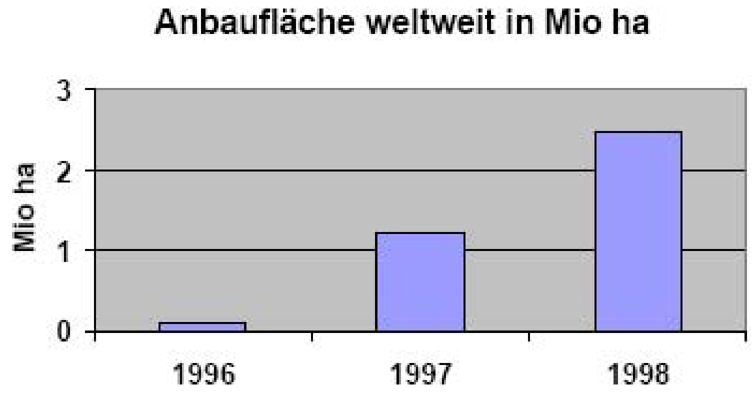
RAPS

Herbizidresistenz

Männliche Sterilität

Fettzusammensetzung

Pilzresistenz

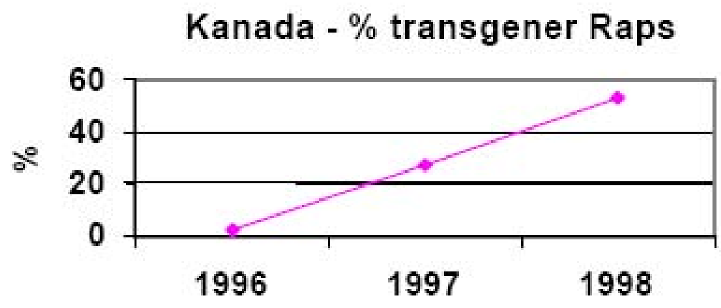


Zulassungen:
EU: 3 (2 Anträge)
USA: 9

Verwendung:
Lebensmittel (Öl)

weitere Züchtungsziele:
veränderte Inhaltsstoffe (Aminosäuren, β-Carotin)

Tendenz:
steigend



Modification of Products

Table 18.6 Transgenic canola varieties with modified seed lipid contents

Seed product	Commercial use(s)
40% Stearic	Margarine, cocoa butter
40% Lauric	Detergents
60% Lauric	Detergents
80% Oleic	Food, lubricants, inks
Petroselinic	Polymers, detergents
"Jojoba" wax	Cosmetics, lubricants
40% Myristate	Detergents, soaps, personal care items
90% Erucic	Polymers, cosmetics, inks, pharmaceuticals
Ricinoleic	Lubricants, plasticizers, cosmetics, pharmaceuticals

Adapted from Murphy, *Trends Biotechnol.* 14:206–213, 1996.

KARTOFFEL

Insektenresistenz

Virusresistenz

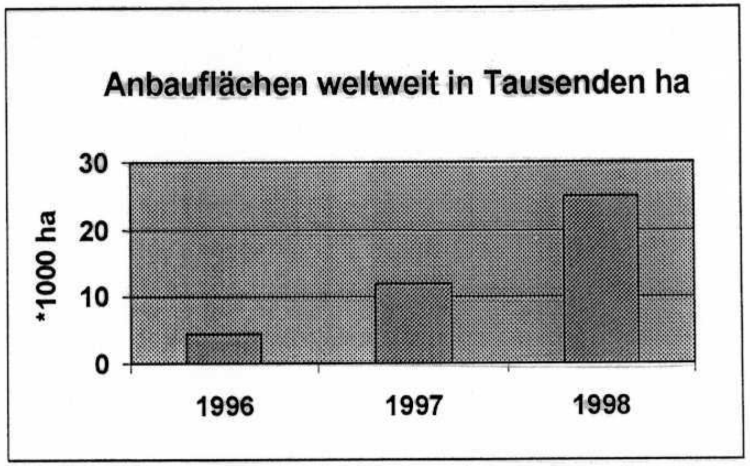
Pilzresistenz

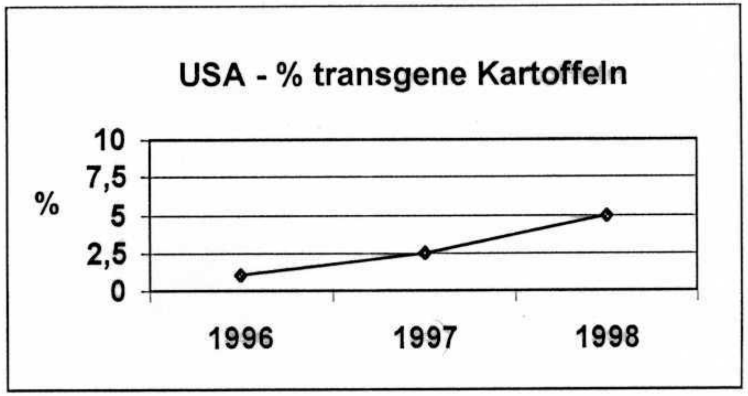
Zulassungen:
EU: (1 Antrag)
USA: 4

Verwendung:
Lebensmittel (auch Zutat),
Industriestärke

weitere Züchtungsziele:
veränderte Inhaltsstoffe (Stärke, Zucker)

Tendenz:
stark steigend





Stand 6/99

BAUMWOLLE

Insektenresistenz

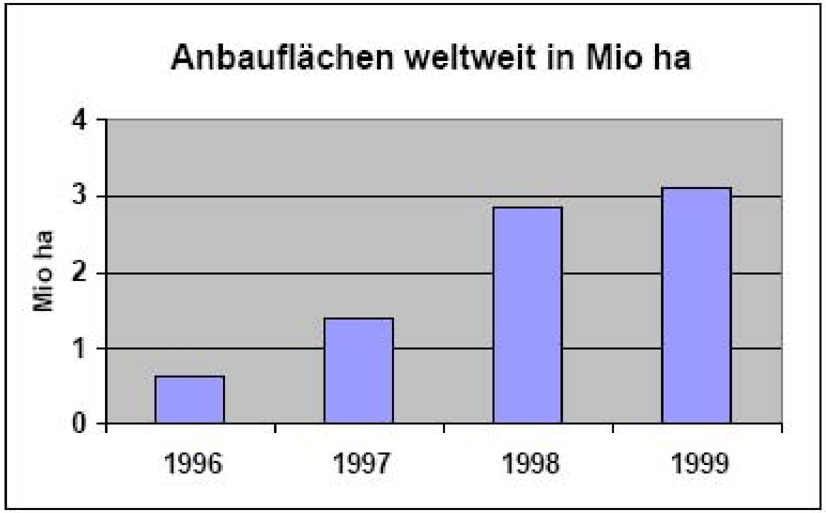
Herbizidresistenz

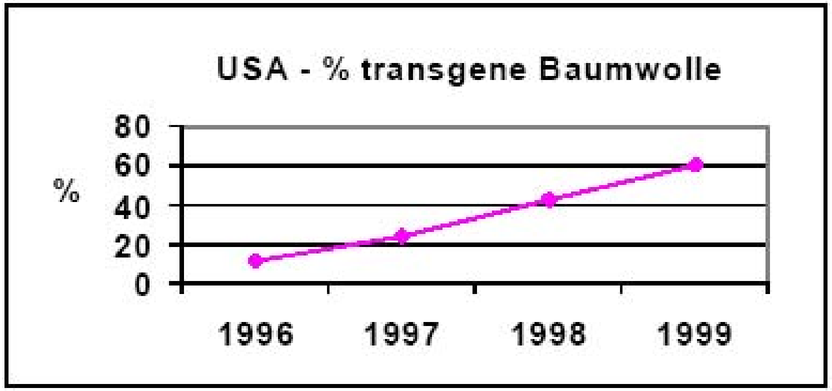
Zulassungen:
EU: (2 Anträge)
USA: 5

Verwendung:
Textilfasern,
Lebensmittelzutaten (Öl, Eiweiß),
Tierfutter

weitere Züchtungsziele:
Faserqualität,
Farbstoffe,
Kälte-, Hitzetoleranz

Tendenz:
steigend
Anbau auch in Afrika, Asien





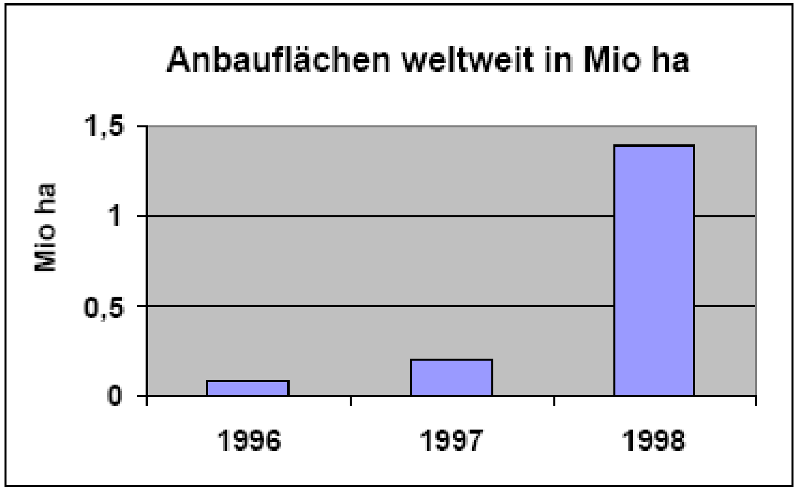
Stand 6/99

TOMATE

Reifeverzögerung

Haltbarkeit

Insektenresistenz



Zulassungen:
EU: (1 Antrag)
USA: 11

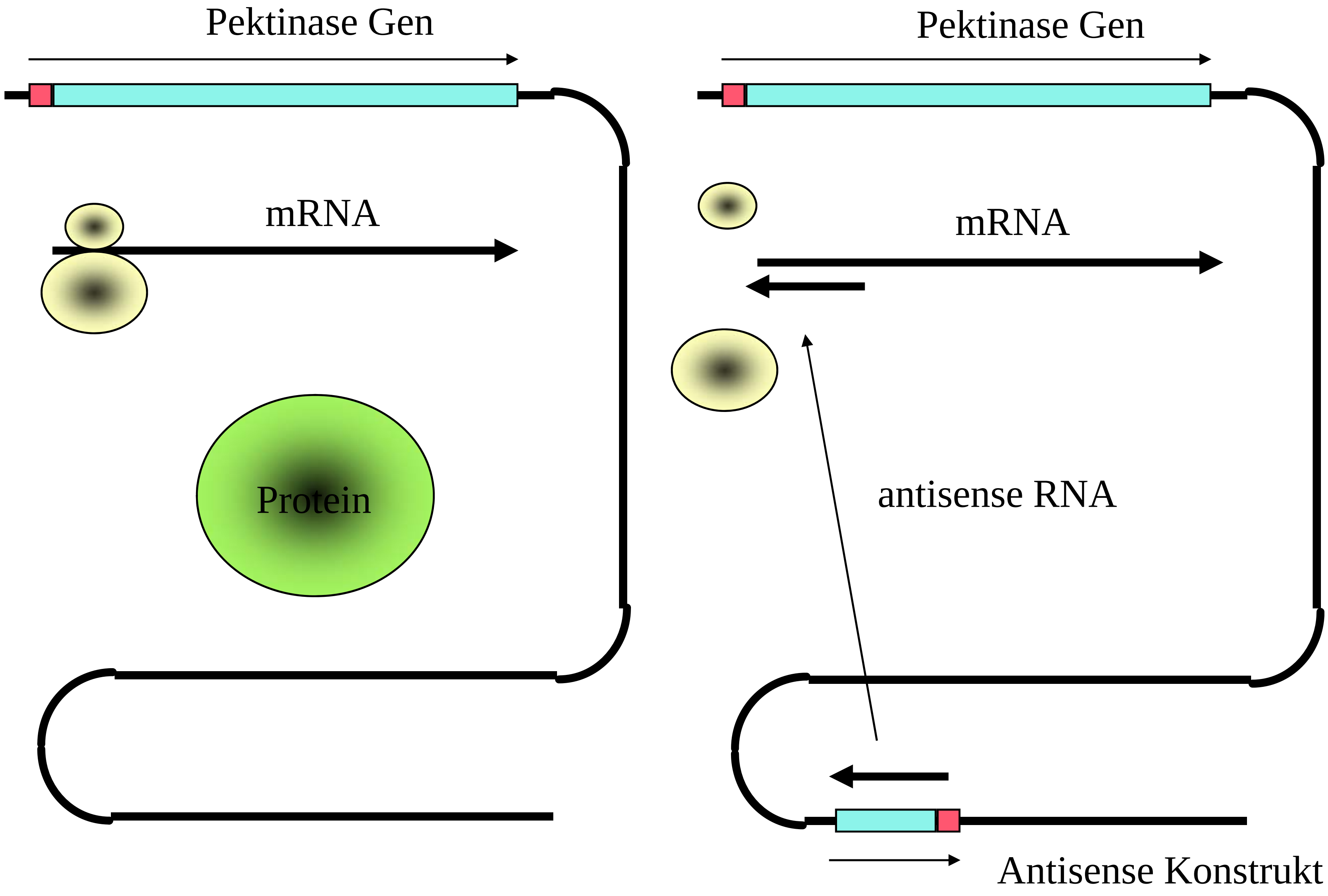
Verwendung:
Lebensmittel (auch Zutat)

weitere Züchtungsziele:
Virusresistenz
veränderte Inhaltsstoffe

Tendenz:
steigend mit der Einführung von Insektenresistenz

Stand 6/99

Antisense-Technik



Virus Resistance

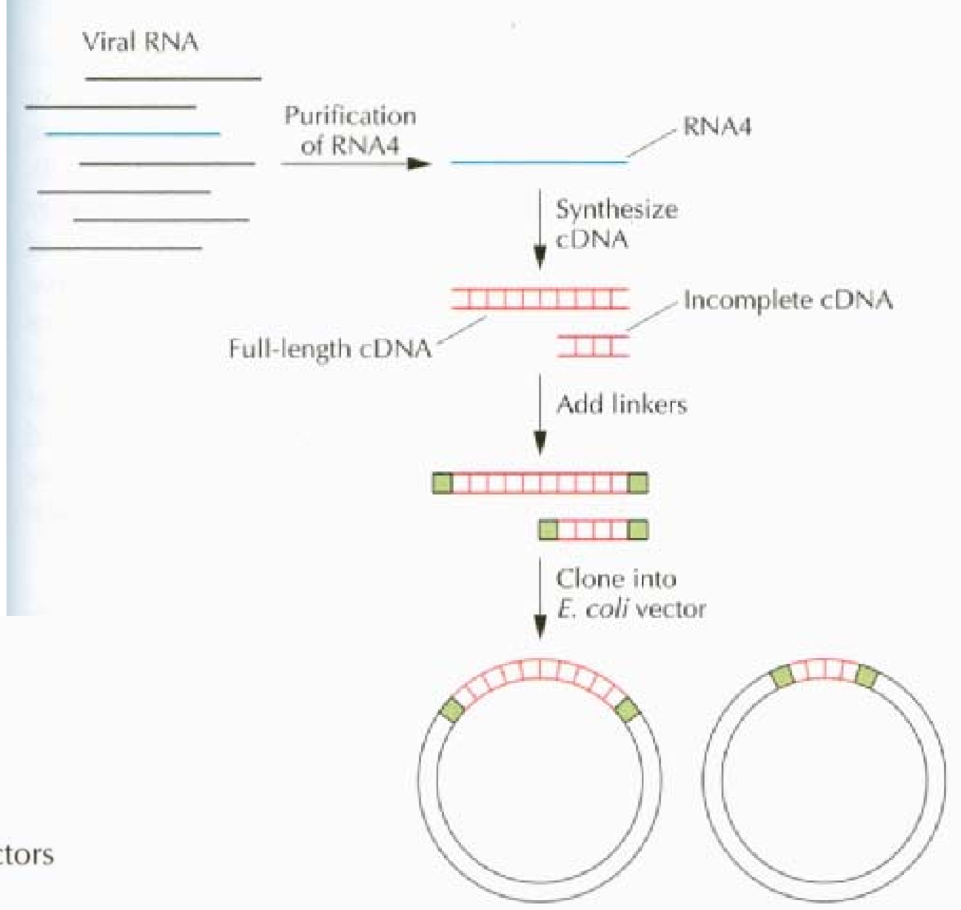
Table 18.3 Some virus-resistant transgenic plants that contain cloned viral coat proteins

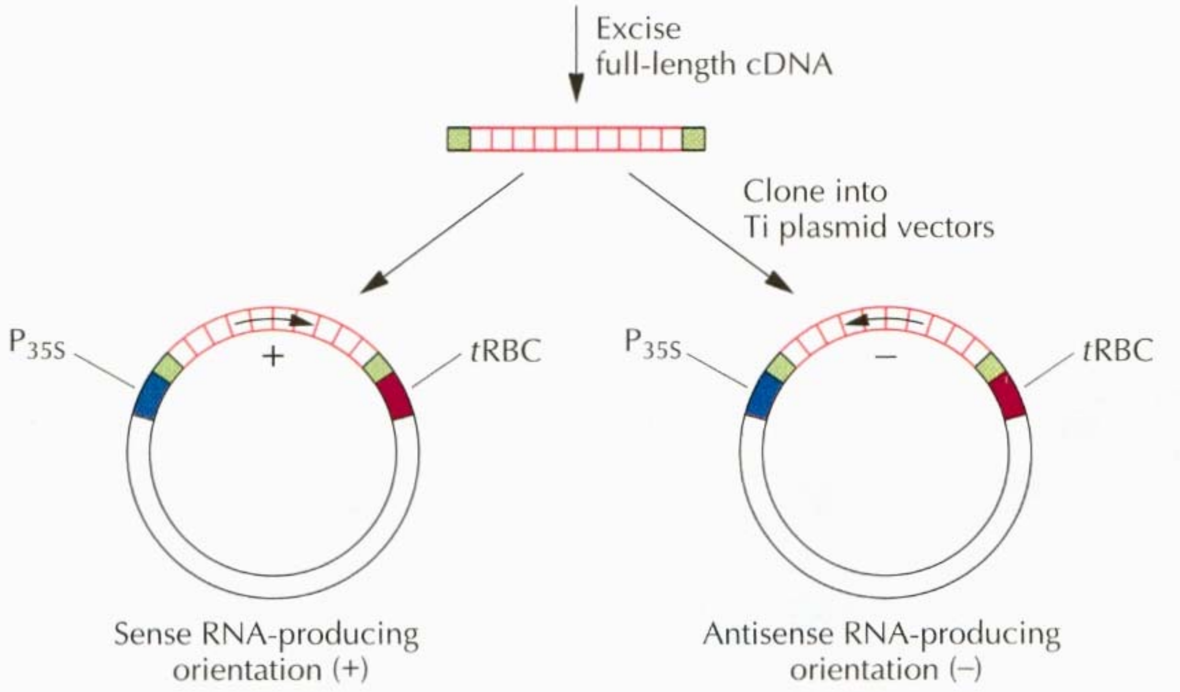
Plant(s)	**Virus that provided the coat protein gene**
Nicotiana benthamiana, N. clevelandii	Plum pox virus
N. benthamiana, squash	Watermelon mosaic virus 2
N. benthamiana, squash	Zucchini yellow mosaic virus
Papaya, tobacco	Papaya ringspot virus
Potato	Potato leafroll virus
Potato	Potato virus Y
Potato, *Nicotiana debneyii*	Potato virus S
Potato, tobacco	Potato virus X
Rice	Rice stripe virus
Tobacco	Arabis mosaic virus
Tobacco	Soybean mosaic virus
Tobacco	Tobacco etch virus
Tobacco	Tobacco streak virus
Tobacco	Tomato spotted wilt virus
Tobacco, alfalfa, tomato	Alfalfa mosaic virus
Tobacco, cucumber	Cucumber mosaic virus
Tobacco, *N. benthamiana*	Tobacco rattle virus
Tobacco, tomato	Tobacco mosaic virus
Tomato	Tomato mosaic virus

Adapted from Fitchen and Beachy, *Annu. Rev. Microbiol.* **47**:739–763, 1993.

Virus Resistance

Figure 18.7 Procedure for introducing cucumber mosaic virus coat protein cDNA into plant cells. RNA4, which encodes the coat protein, is fractionated from a viral RNA preparation and used as the template for the synthesis of double-stranded cDNA. Linkers are added to the cDNA preparation, and the cDNAs are cloned into an *E. coli* plasmid vector. A full-length cDNA clone is identified, excised from the *E. coli* vector, and subcloned into a Ti plasmid cloning vector between the 35S promoter from cauliflower mosaic virus (P_{35S}) and the transcription terminator from the gene for the small subunit of ribulose bisphosphate carboxylase (*t*RBC). This cloning step creates two orientations for the RNA4 cDNA. In one case, the RNA that is transcribed is translated into coat protein (sense RNA), and in the other case, the transcribed RNA is complementary to the mRNA for the coat protein (antisense RNA).





Virus Resistance

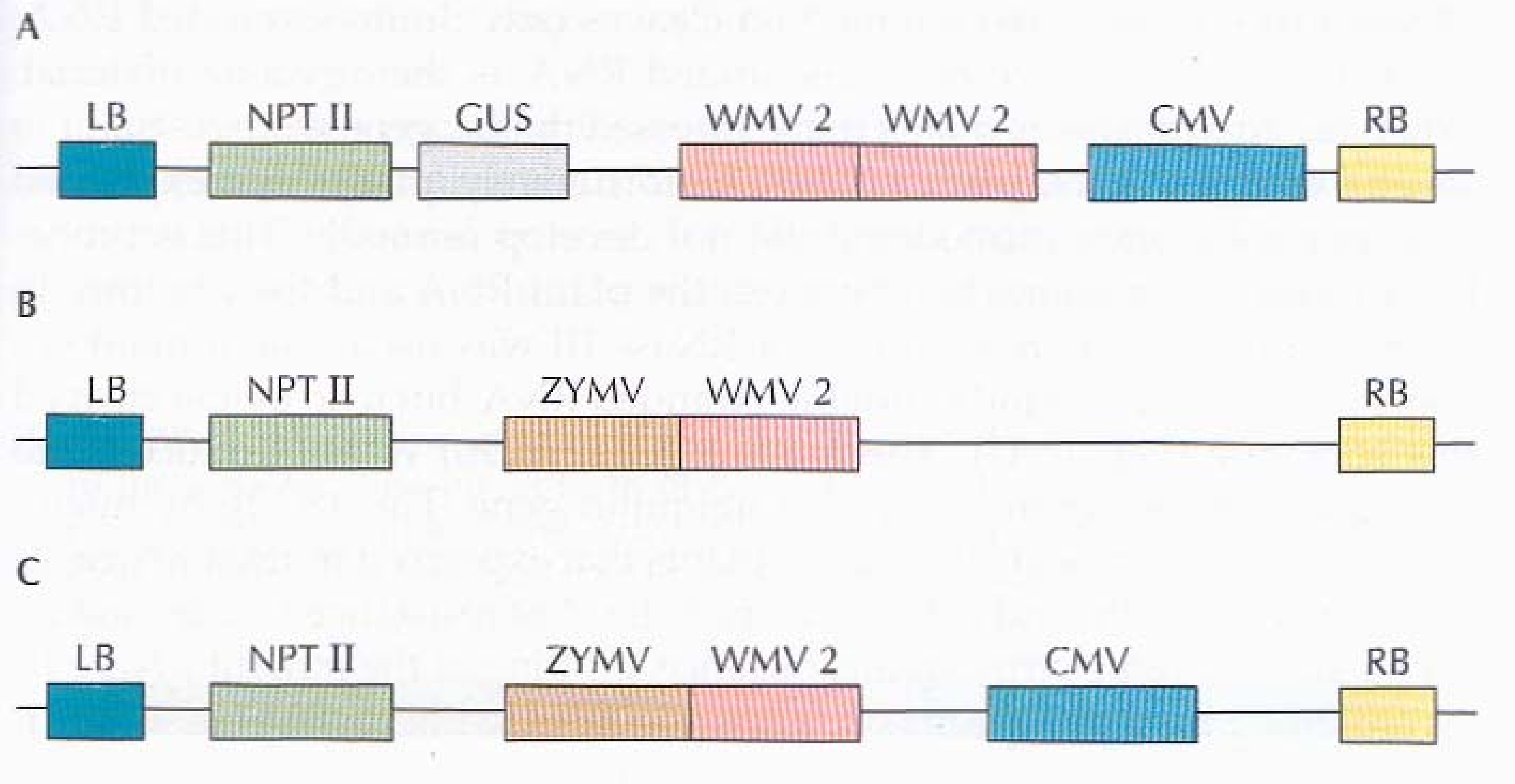


Figure 18.9 **A.** A T-DNA construct with a neomycin phosphotransferase gene (NPT II) as a selectable marker, a β-glucuronidase gene (GUS) as a reporter gene, two copies of the coat protein gene from watermelon mosaic virus 2 (WMV 2), and the coat protein gene from cucumber mosaic virus (CMV). The left and right borders of the T-DNA are indicated by LB and RB, respectively. **B.** Similar to panel A without CMV and GUS, with one copy of WMV 2, and with the coat protein gene from zucchini yellow mosaic virus (ZYMV). **C.** Same as panel B with the addition of CMV. All of the genes in these constructs include both promoters and transcription terminator regions.

Virus Resistance

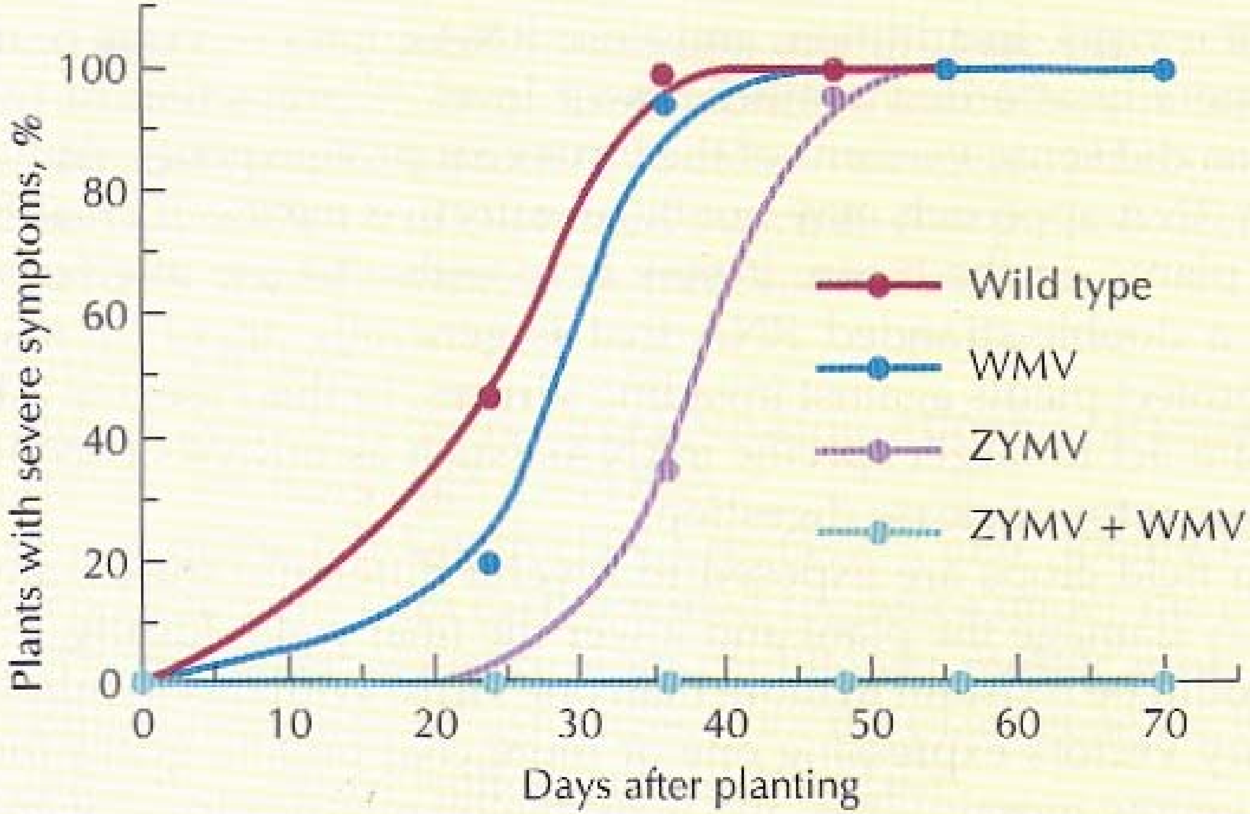
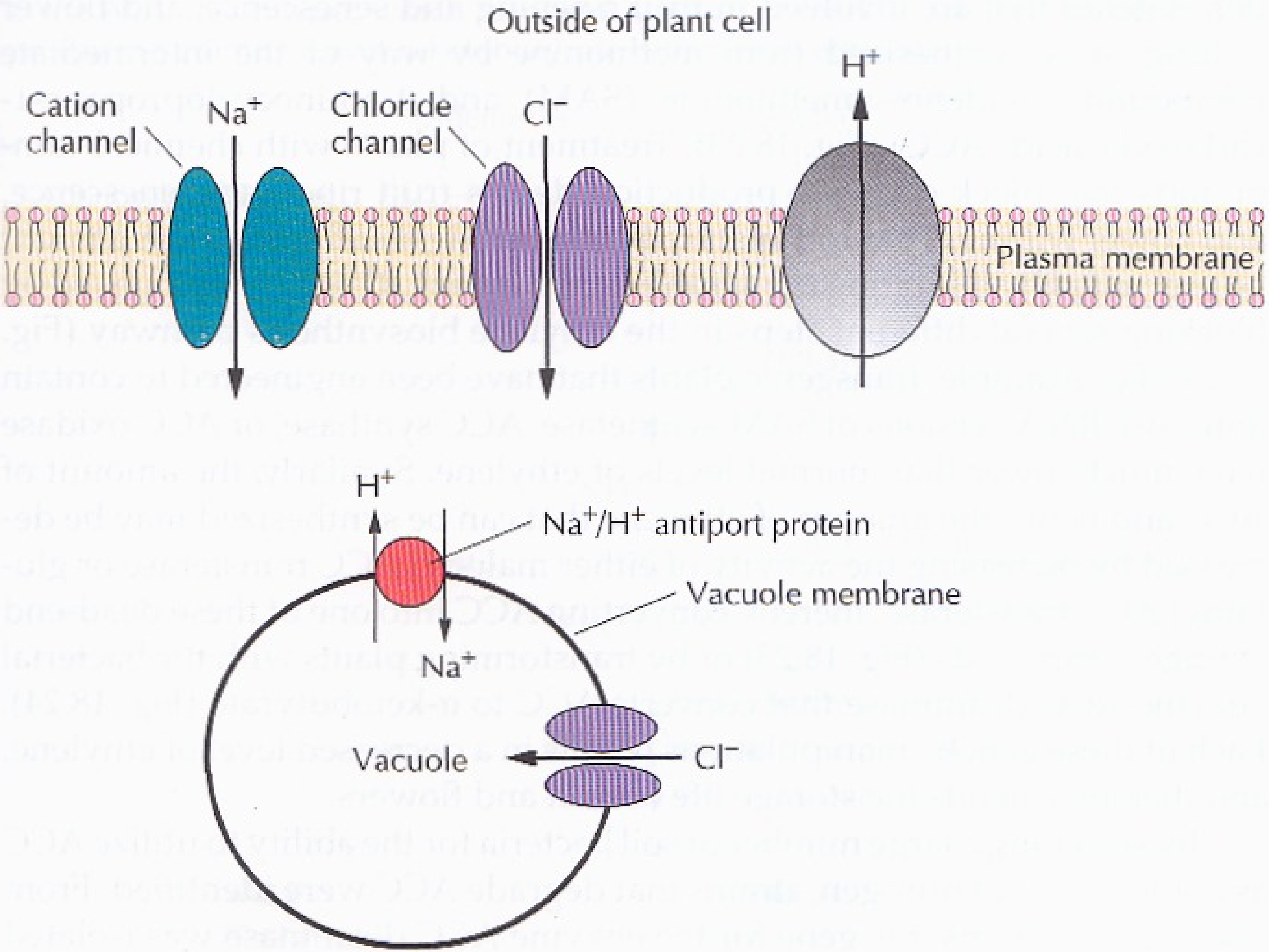


Figure 18.10 Disease frequency in transgenic and nontransformed (wild-type) yel-low crookneck squash in the field. Aphids were used to transmit a mixture of zucchin yellow mosaic virus (ZYMV) and watermelon mosaic virus 2 (WMV) to the squash plants. Adapted from Fuchs and Gonsalves, *Bio/Technology* **13:**1466–1473, 1995.

Dry Resistance

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



Plant Colour

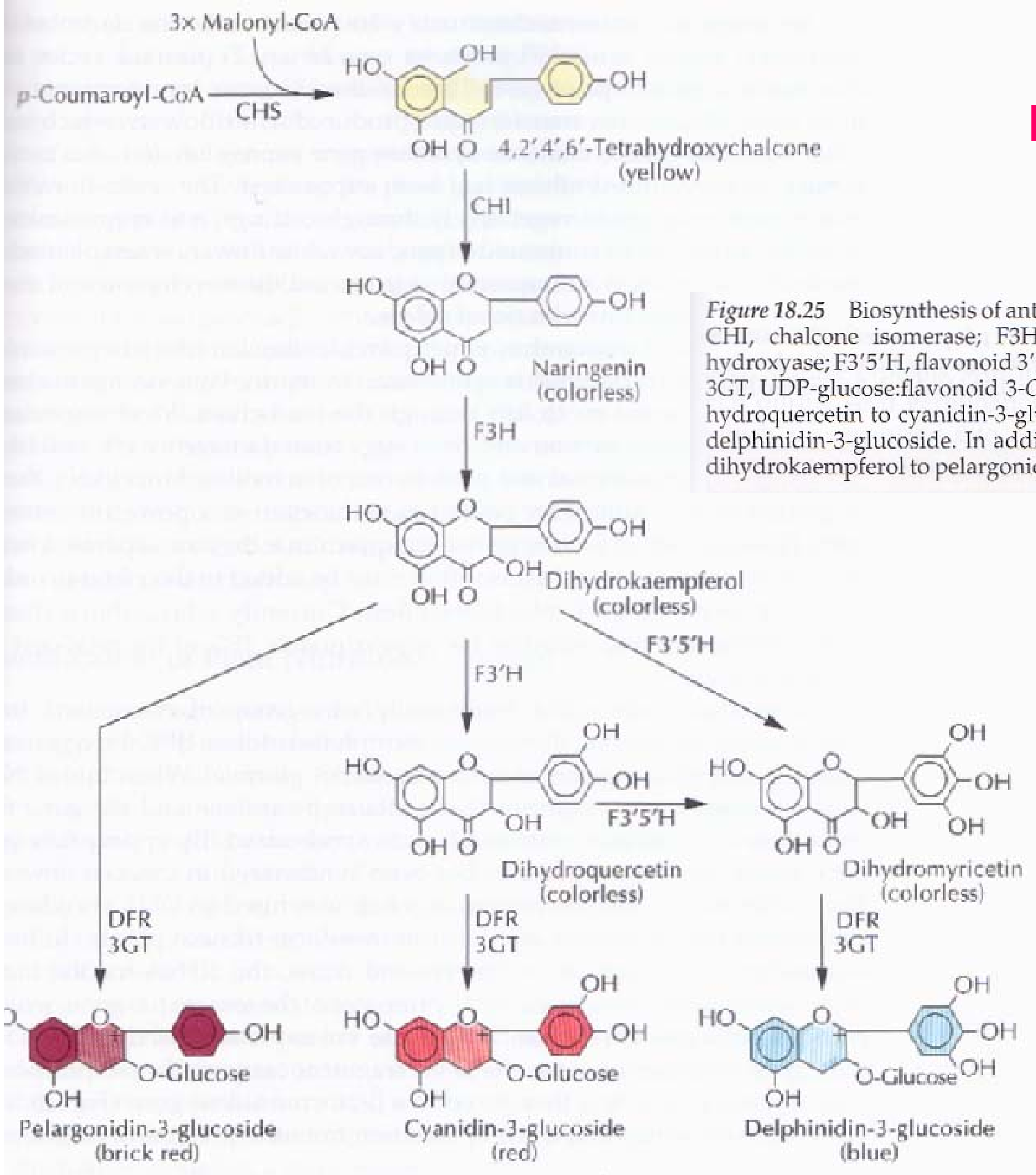


Figure 18.25 Biosynthesis of anthocyanins. Abbreviations: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavonone 3-hydroxylase; F3′H, flavonoid 3′-hydroxyase; F3′5′H, flavonoid 3′,5′-hydroxylase; DFR, dihydroflavonol 4-reductase; 3GT, UDP-glucose:flavonoid 3-*O*-glucosyltransferase. Petunia DFR can convert dihydroquercetin to cyanidin-3-glucoside and can convert dihydromyricetin to blue delphinidin-3-glucoside. In addition to these conversions, maize DFR can convert dihydrokaempferol to pelargonidin-3-glucoside.

Impfbanane ??!!

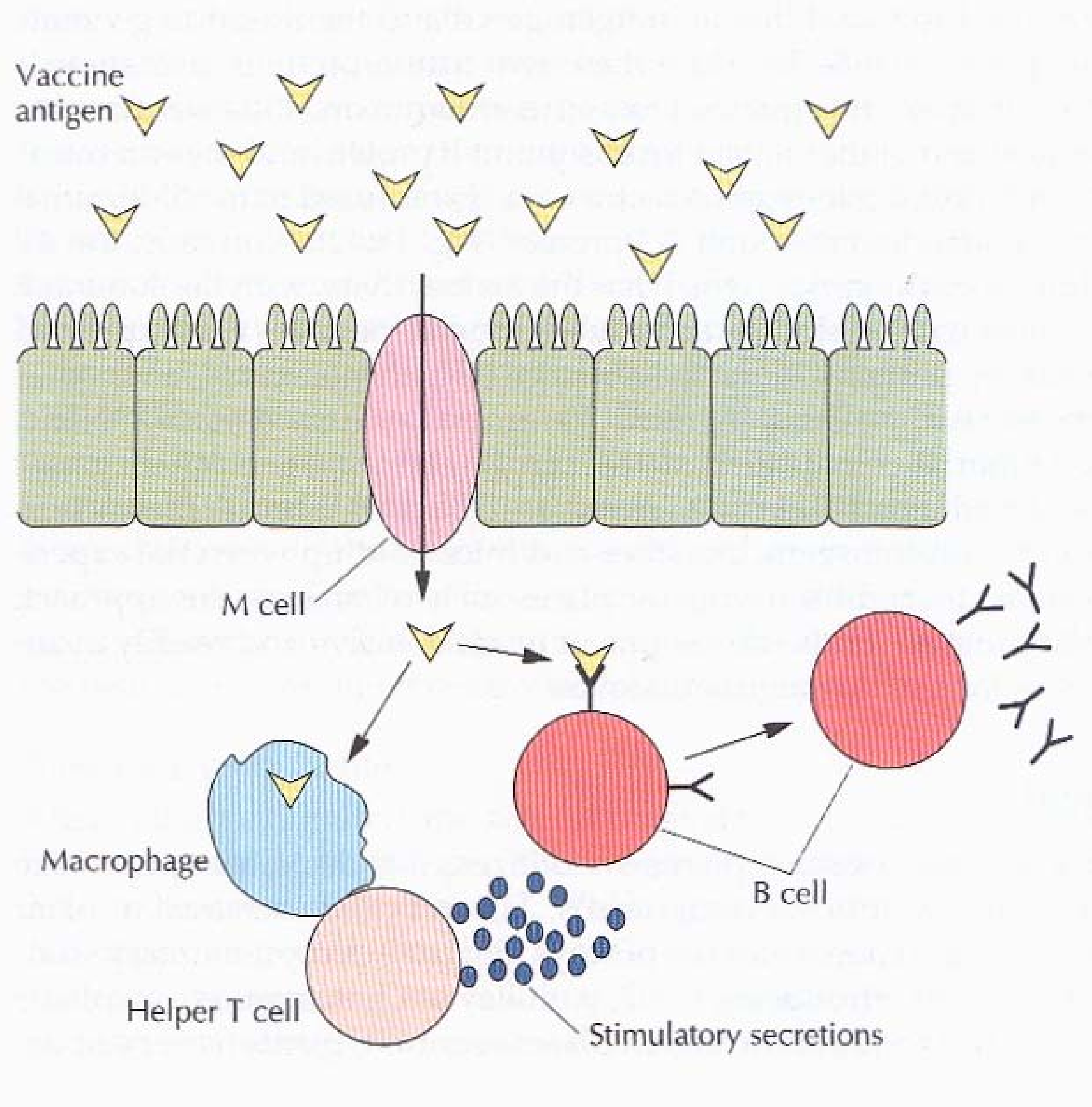


Figure 18.41 Schematic representation of how an edible vaccine generates an immune response against an antigen from an infectious agent. The antigen, which is expressed as part of a plant, binds to and is taken up by M cells present in the lining of the intestine and is then passed to other cells in the immune system, including macrophages and B cells. The macrophages display portions of the antigen to the helper T cells, which in turn respond by secreting small molecules that activate B cells to synthesize and release antibodies that can neutralize the antigen.

Impfstoffe und verwendete Pflanzen

Impfstoff gegen Cholera
Verwendete Pflanze: Kartoffel, Tomate, Tabak
Direkt essbar: Ja

Impfstoff gegen Norwalk-Virus
Verwendete Pflanze: Kartoffel, Tomate, Tabak
Direkt essbar: Ja

Impfstoff gegen Papilloma-Virus (HPV)
Verwendete Pflanze: Kartoffel, Tomate, Tabak
Direkt essbar: Ja

Impfstoff gegen Tollwut
Verwendete Pflanze: Spinat
Direkt essbar: Ja

Impfstoff gegen Hepatitis B (HVB)
Verwendete Pflanze: Kartoffel, Lupine
Direkt essbar: Ja

Impfstoff gegen E. Coli Enterotoxin (ETEC)
Verwendete Pflanze: Kartoffel, Tabak, Mais
Direkt essbar: Ja

Impfstoff gegen Transmissible Gastroenteritis Virus,
Eine Schweinekrankheit
Verwendete Pflanze: Mais
Direkt essbar: Ja

Impfstoff gegen Non Hodgkins Lymphom
Verwendete Pflanze: Tabak (Tabakmosaikvirus)
Direkt essbar: Nein

Impfstoff gegen Grippe (Rhino RX)
Verwendete Pflanze: Tabak
Direkt essbar: Nein

www.gen-ethisches-netzwerk.de/

Golden Rice

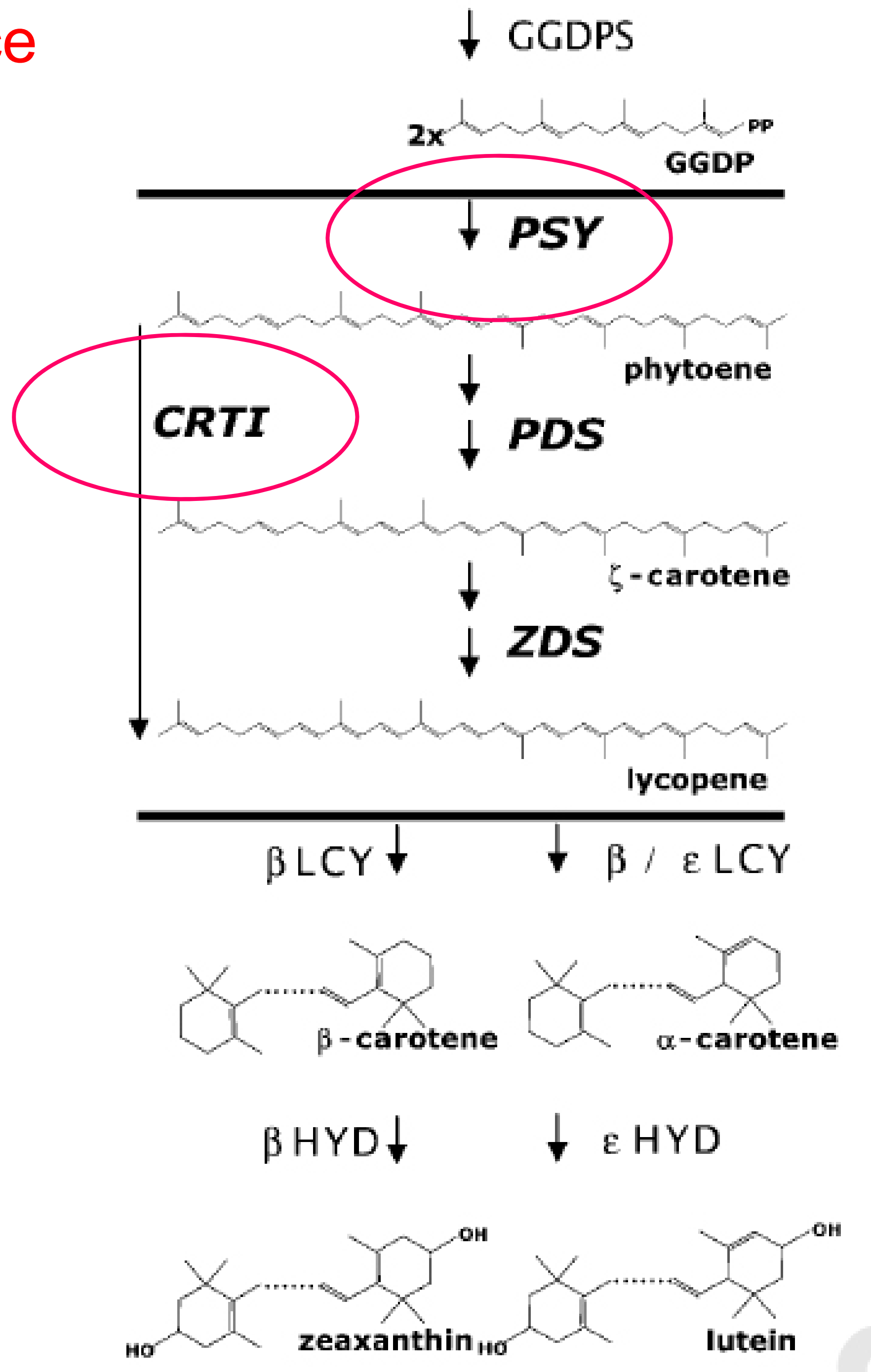
Vitamin A Mangel: Asien

500.000 Kinder pro Jahr erblinden

Abbildung 6.11: „Goldener Reis" ist nur eine der genetisch modifizierten Nutzpflanzen, die Entwicklungsländern nutzen. Entwicklungsländer sind maßgeblich für die Erhöhung des Weltnahrungsmittelbedarfs.

Expression von ß-Carotin
in Reis

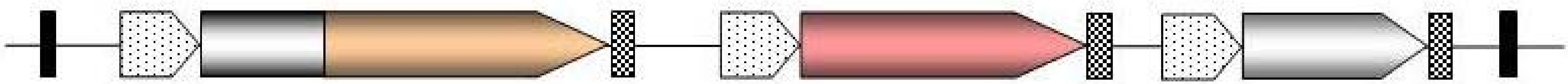
Golden Rice



The precursor molecule for carotenoid biosynthesis is geranylgeranyl diphosphate (GGDP). Horizontal bars delimit the steps of the carotenoid biosynthetic pathway that were overcome using the two transgenes phytoene synthase (PSY) and the multifunctional bacterial carotene desaturase (CRTI), rather than the two plant desaturases PDS and ZDS.

http://www.goldenrice.org/

Golden Rice



Gene construct used to generate *Golden Rice*. RB, T-DNA right border sequence; Glu, rice endosperm-specific glutelin promoter; tpSSU, pea ribulose bis-phosphate carboxylase small subunit transit peptide for chloroplast localisation; nos, nopaline synthase terminator; Psy, phytoene synthase gene from *Narcissus pseudonarcissus* (GR1) or Zea mays (GR2); Ubi1, maize polyubiquitin promoter; Pmi, phosphomannose isomerase gene from *E. coli* for positive selection (GR2); LB, T-DNA left border sequence.

The image clearly shows the progress made since the proof-of-concept stage of *Golden Rice*. The new generation, also known as GR2 contains β-carotene levels that will allow to provide an adequate amount of pro- vitamin A in normal children's diets in SE Asia.

http://www.goldenrice.org/

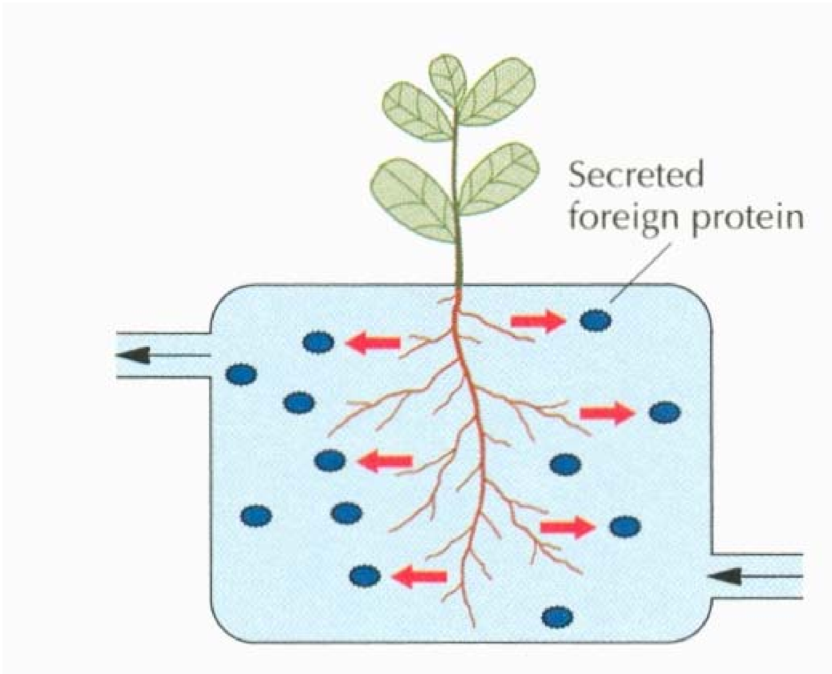


Figure 17.16 Schematic representation of a plant in hydroponic culture secreting proteins and small molecules (red arrows) to the medium. The arrows at the inlet and outlet ports indicate direction of flow of added nutrient solutions. The proteins secreted by the roots arc concentrated and harvested from the hydroponic medium and then purified.

- 20.11.14