Chapter 25
Vegetables

Evelyn Klocke, Thomas Nothnagel, and Günter Schumann

25.1 Introduction

Vegetables are a target for many transformation purposes. From the first trials for herbicide resistance until now, transformation protocols have been developed for almost all important vegetable crops. *Agrobacterium*-mediated transfer is the base for most transformation protocols for vegetables, as in other crops. Some special method investigations like plastid transformation (see also Chap. 2) and others are outlined below.

With rapidly rising capacities for DNA sequencing, databases for plant genomes are expanding very fast. The abundance of genomic data has an influence on projects for the genetic transformation of various vegetables. The availability of genes is no longer a bottleneck for this work. Increasing knowledge about genomes and a broad public access to DNA data banks boost new possibilities of creating gene constructs for transformation of vegetables. Moreover, the latest RNAi technology (see Chap. 5) will affect the transformation techniques for vegetable crops.

This chapter gives a short overview of GM technology in vegetables. Particularly vegetable crops for the temperate climate in Europe and America are considered (Table 25.1). Special emphasis is placed on the current trends of vegetable transformation, focusing especially on potential practical applications. Some of the investigations belonging to fundamental research are important for an understanding of processes like gene expression, plant development and production of metabolites in vegetables.

E. Klocke, T. Nothnagel and G. Schumann
Julius Kühn-Institute, Institute for Breeding Research on Horticultural and Fruit Crops, Erwin-Baur-Strasse 27, 06484 Quedlinburg, Germany
e-mail: evelyn.klocke@jki.bund.de, thomas.nothnagel@jki.bund.de, guenter.schumann@jki.bund.de

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Table 25.1 Review of genetically engineered vegetables, the aim (or target character) and the description of the transgene. This table contains experiments with established transgenic plants only. Experiments with marker or reporter genes exclusively are only listed as examples.

Character	Transgene	Transgene description	Aim	References
Solanum lycopersicon L.				
Virus resistance	TMV CP	Tobacco mosaic virus (TMV) coat protein	Tolerance to TMV and tomato mosaic virus (ToMV)	Nelson et al. (1988)
	V1	Tomato yellow leaf curl virus (TYLCV) capsid protein	Delayed disease symptoms	Kunik et al. (1994)
	TYLCV C1, -T-Rep	Truncated C1 and T-Rep genes of tomato yellow leaf curl virus (TYLCV)	Resistance to TYLCV	Brunetti et al. (1997), Antignus et al. (2004), Yang et al. (2004), Fuentes et al. (2006)
	TLCV Rep	Replicase – tomato leaf curl virus (TLCV)	Resistance to TLCV	Praveen et al. (2005)
	TLCV CP	TLCV coat protein	Variable resistance to TLCV	Raj et al. (2005)
	TSWV N	Tomato spotted wilt virus (TSWV) nucleo-protein	Resistance to TSWV	Kim et al. (1994), Ultzen et al. (1995)
	N	Gene from *Nicotiana tabacum*	Resistance to TMV and ToMV	Whitham et al. (1996)
	N/Sw-5	Lettuce isolate of TSWV (TSWV-BL)	Resistance to TSWV	Gubba et al. (2002)
	CMV-CP	Cucumber mosaic virus (CMV) coat protein	Resistance to TMV, *Verticillium* and *Phytophthora*	Provvidenti and Gonsalves (1995), Tomassoli et al. (1999)
	CMV	Truncated replicase from CMV	Moderate resistance in T1 progeny to CMV	Nunome et al. (2002)
Fungal resistance	Chi-I,II/Glu-I, II	Class I chitinase and class I β-1,3 glucanase	Resistance to *Fusarium oxysporum* f. sp. *lycopersici*	Jongedijk et al. (1995)
	pcht28	Chitinase	Resistance to *V. dahliae* race 2	Tabaeizadeh et al. (1999)
	tlpD34, M-GLU, Mj-AMP1	Pathogenesis-related protein (PRP)	*Alternaria solani* resistance	Radhajeyalakshmi et al. (2005), Schaefer et al. (2005)
	CABPR1, CAPOA1	PRP	*Phytophthora capsici* enhanced tolerance	Sarowar et al. (2006)
	NPR1	*Arabidopsis* gene; systemic acquired resistance (SAR)	SAR to *F. oxysporum* f. sp. *lycopersici*	Lin et al. (2004)
	pRB7/Thi2.1	*Arabidopsis* thionin; SAR	SAR to *F. oxysporum* f. sp. *lycopersici*	Chan et al. (2005)

Bacterial resistance	bO	Bacterio-opsin	SAR	Rizhsky and Mittler (2001)
	NPR1	*Arabidopsis* gene; SAR	SAR to *Ralstonia solanacearum*	Lin et al. (2004)
	pRB7/Thi2.1	*Arabidopsis* thionin; SAR	SAR to *R. solanacearum*	Chan et al. (2005)
Nematode resistance	Mi-1, a, Mi-1.2	Gene from *Lycopersicon peruvianum*	Resistance to *Meloidogyne incognita*	Vos et al. (1998), Goggin et al. (2004)
Insect resistance	HD-1	*Bt* resistance	*Bt* resistance to *Manduca sexta, Heliothis virescens, H. zea* and *Keiferia lycopersicella*	Fischhoff et al. (1987), Delannay et al. (1989)
	Mi-1, a	Gene from *L. peruvianum*	Resistance to potato aphid (*Macrosiphum euphorbiae*)	Vos et al. (1998)
	StLS1::PI-II/rbcs1A::PCI	Protease inhibitors	Increased resistance to *H. obsoleta* and *Liriomyza trifolii*	Abdeen et al. (2005)
Abiotic stress	codA	Choline oxidase from *Arthrobacter globiformis*	Temperature tolerance (chilling tolerance)	Park et al. (2004a)
	LeGPAT	Glycerol-3-phosphate acyltransferase	Temperature tolerance (chilling tolerance)	Sui et al. (2007)
	cAPX	Cytosolic ascorbate peroxidase	Temperature tolerance (heat stress) and UV-B tolerance	Wang et al. (2006)
	CBF1	*Arabidopsis C* repeat/dehydration-responsive element binding factor 1	Water stress (drought)	Hsieh et al. (2002a)
	ABRC1/CBF1	ABRC1-stress-inducible promotor from barley HAV22 and CBF1	Chilling, drought and salt tolerance	Lee et al. (2003b)
	bspA	Boiling stable protein from *Populus tremula*	Water stress (drought)	Roy et al. (2006)
	HLA1	Gene from *Saccharomyces cerevisae*	Salt tolerance	Gisbert et al. (2000), Rus et al. (2001), Muñoz-Mayor et al. (2008)
	AtNHX1	Gene from *Arabidopsis*	Salt tolerance	Zhang and Blumwald (2001)
	BADH	Gene from *Atriplex hortensis*	Salt tolerance	Jia et al. (2002)
Parthenocarpy	DefH9-iaaM	Genes from *Pseudomonas syringae* pv. *savastanoi* and *Antirrhinum majus*		Ficcadenti et al. 1999
	rolB	*A. rhizogenes*-derived gene		Carmi et al. (2003)

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Table 25.1 (continued)

Character	Transgene	Transgene description	Aim	References
Fruit ripening	CaCel1	Endo-1,4-β-D-glucanase from pepper		Harpster et al. (2002b)
	ACC	RNAi gene silencing	Prolonged shelf life	Xiong et al. (2005)
	GAD	Glutamate decarboxylase	Antisense	Kisaka et al. (2006)
Taste/flavour	E8-monellin	Gene from *Discoreophyllum cumminsii*	Sweetness	Penarrubia et al. (1992)
	Thaumatin	Gene from *Thaumatococcus daniellii*	Sweet taste and liquorice aftertaste	Bartoszewski et al. (2003)
	Δ-9 Desaturase gene	Desaturase gene from *S. cerevisiae*	Changes in the profile of flavour compounds	Wang et al. (1996)
	Adh 2	Alcohol dehydrogenase cDNA	Improved flavour characteristics	Speirs et al. (1998)
Nutritional value	crtI	Phytoene desaturase from *Erwinia uredovora*	Threefold increased β-carotene content	Römer et al. (2000)
	chi	Chalcone isomerase from *Petunia*	Elevated flavanol end-products in the fruit peel	Muir et al. (2001)
	LC/C1	Maize transcription factors	Tenfold higher flavanoid glycoside content	Le Gall et al. (2003)
	HQT	Hydroxycinnamoyl transferase	Increased levels of antioxidant chlorogenic acid (CGA)	Niggeweg et al. (2004)
	DETI	Endogenous photomorphogenesis gene, RNAi gene silencing	Carotenoid and flavonoid content	Davuluri et al. (2005)
	tLcy-b	Lycopene β-cyclase	Conversion of lycopene to β-carotene under field conditions	Giorio et al. (2007)
	Del/Ros1	Transcription factor from *Antirrhinum majus* that regulates anthocyanin production		Butelli et al. (2008)
Processing quality	ipt	Isopentenyl transferase	Higher fruit solids	Martineau et al. (1995)
	LepG, LeExp1	Ripening regulated fruit PG gene and expansin	Increased fruit firmness and juice viscosity	Kalamaki et al. (2003a, b), Powell et al. (2003)
	ySAMdc	Adenosylmethionine decarboxylase from yeast	Increased lycopene content and enhanced fruit quality	Mehta et al. (2002)
Pharmaceuticals	Miraculin	Gene from *Richadella dulcifica*	Twentyfold higher miraculin content, low-calorie sweetener for diabetic	Sun et al. (2007)

	ACEI	Angiotensin-I-converting enzyme inhibitor, TMV-mediated transformation	Antihypertensive tomato fruits	Hamamoto et al. (1993)
	Gp	Rabies glycoprotein, (Agrobacterium tumefaciens) (*A.t.*)-mediated	Vaccine, oral animal immunization, e.g. raccoons	McGarvey et al. (1995)
	P1-2A3C	Polyprotein + protease gene from foot-and-mouth disease virus	Oral immunization, e.g. guinea pigs	Pan et al. (2008)
	RSV-F	Respiratory syncytial virus fusion gene	Vaccine	Sandhu et al. (2000)
	ctxB	Cholera toxin B subunit, *A.t.*-mediated	Vaccine against cholera	Jani et al. (2002), Jiang et al. (2007), Sharma et al. (2008)
	VP1	Coat protein of enterovirus 71 (EV71)	Vaccine against hand-foot-and-mouth disease	Chen et al. (2006a)
	PRS-S1S2S	Synthetic hepatitis B virus (HBV)	Large surface antigen gene	Lou et al. (2007)
	ORF2 (HEV-E2)	Partial gene of hepatitis E virus	Vaccine	Ma et al. (2003)
	Aβ	Human β-amyloid	Vaccine against Alzheimer's disease	Youm et al. (2008)
	sDPT	Synthetic immunoprotective exotoxin epitopes	Vaccine against diphteria–pertussis–tetanus (DPT)	Soria-Guerra et al. (2007)
	AChE	Human acetylcholinesterase	Preventing organophosphate intoxication	Mor et al. (2001)
	IL-12	Mouse interleukin-12	Recombinant protein for mucosal administration	Gutiérrez-Ortega et al. (2005)
	TaxK	Taxadiene from *Taxus baccata*		Kovacs et al. (2007)
	AAT	Modified human α-1-antitrypsin	Therapeutic protein	Agarwal et al. (2008)
	GmIFS2	Isoflavone synthase from *Glycine max*	Isoflavone production for health benefits	Shih et al. (2008)
Antiallergenicity	Lyc e1, Lyc e3	RNAi gene silencing	Low allergenic tomato fruits	Le et al. (2006a, b), Lorenz et al. (2006)
Capsicum annum L.				
Herbicide resistance	pat	Basta resistance		Tsaftaris (1996)

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Table 25.1 (continued)

Character	Transgene	Transgene description	Aim	References
Virus resistance	CMV-CP	Cucumber mosaic virus coat protein (CMV-CP) gene		Zhu et al. (1996)
	CMV	cDNA of CMV satellite RNA	Resistance against cucumber mosaic virus	Kim et al. (1997)
	CMV-CP, ToMV-CP	CMV-CP gene, tomato mosaic virus CP gene		Shin et al. 2002a)
	TMV-CP, PPI1	Tobacco mosaic virus CP gene, pepper PMMV interaction 1 transcription factor gene		Lee et al. (2004)
	CMV-CP, TMV-CP	CMV-CP gene, TMV-CP gene	Field performance	Cai et al. (2003)
Fruit ripening	CaCel1	Supression of endo-1,4-β-D-glucanase from pepper	Influence on cell wall	Harpster et al. (2002a)
Flower development	OsMADS1	Rice OsMADS1 gene	Phenotypic effect	Kim et al. (2001)
Solanum melongena L.				
Insect resistance	cry	*Bt* genes	Resistance against *Leptinotarsa decemlineata*	Arpaia et al. (1997, 2007), Iannacone et al. (1997), Jelenkovic et al. (1998)
	cry	*Bt* gene	Against *Leptinotarsa decemlineata*, field test	Acciarri et al. (2000), Mennella et al. (2005)
	cry	*Bt* gene	Against *Leucinodes orbonalis*	Kumar et al. (1998)
	cry	*Bt* gene	Impact on *Tetranychus urticae* and *Phytoseiulus persimilis*, laboratory test	Rovenská et al. (2005)
	OZC	Oryzacystatin gene	Effect *Myzus persicae* and *Macrosiphum euphorbiae*	Ribeiro et al. (2006)
Fungal resistance	Δ-9 Desaturase	Δ-9 Desaturase gene from yeast	Resistance against *Verticillium dahliae*, changes in fatty acids	Xing and Chin (2000)
	Dm-AMP1	Antimicrobial defensin from *Dahlia merckii*	Against *Botrytis cinera*	Turrini et al. (2004)

	mtlD	*E. coli* mtlD gene	Against *Fusarium oxysporum, Verticillium dahliae* and *Rhizoctonia solani*	Prabhavathi and Rajam (2007)
Nematode resistance	Mi-1.2	Mi-1.2 gene	Resistance against *Meloidogyne javanica*	Goggin et al. (2006)
Abiotic stress	mtlD	Mannitol-1-phosphodehydrogenase gene	Tolerant against osmotic stress by salt, drought and chilling	Prabhavathi et al. (2002)
Embryo development	Atgrp-5	*A. thaliana* glycin-rich gene 5	Controlling embryo development	Magioli et al. (2001)
Parthenocarpy	DefH9-iaaM	*Pseudomonas syringae* gene +regulatory sequences of ovule-specific gene from *Antirrhinum majus*		Rotino et al. (1997), Donzella et al. (2000)
Raphanus sativus L.				
Flower development	GI	Antisense GIGANTEA (GI) gene fragment	Delayed bolting	Curtis et al. (2002), Curtis (2003)
Abiotic stress	LEA	Late embryogenesis abundant gene from *Brassica napus*	Salt tolerance, water deficit	Park et al. (2005a)
Brassica oleracea L.				
Virus resistance	B22IV, B22VI	Capsid gene and antisense gene VI of *Cauliflower mosaic virus* (CaMV)		Passelegue and Kerlan (1996)
	PVY-cr	*Potato virus* Y capsid gene		Radchuk et al. (2000)
Insect resistance	cry	*Bt*	Insect resistance of broccoli against e.g. *Pieris rapae*, *Plutella xylostella*	Metz et al. (1995a, b), Cao et al. (2001, 2002, 2005), Chen et al. (2008b)
	cry	*Bt*	Insect resistance of broccoli against *P. xylostella* using chemically inducible promoter	Bates et al. (2005), Cao et al. (2006a)
	cry	*Bt*	Insect resistance of cabbage against *P. xylostella*	Jin et al. (2000), Bhattacharya et al. (2002)
	cry	*Bt*	Insect resistance of cauliflower against *P. xylostella*	Kuvshinov et al. (2001), Chakrabarty et al. (2002)

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Table 25.1 (continued)

Character	Transgene	Transgene description	Aim	References
	cry	*Bt*	Insect resistance of cabbage against *P. xylostella*, chloroplast transformation	Liu et al. (2008)
	TI	Trypsin inhibitor gene from *Ipomoea batatas*	Tests against *P. xylostella* and *Spodoptera litura*	Ding et al. (1998)
	CpTI	Cowpea trypsin inhibitor	Tests against *Heliothis armigera* and *Pieris rapae*	Hao and Ao (1997), Lv et al. (2005)
	sporamin, spoaMAR	Use of promoter pPspoa/cassette with matrix-attached region (MAR)	Tests against *Helicoverpa armigera*	Chen et al. (2006b)
Fungal resistance	ThEn42	*Trichoderma harzianum* endochitinase gene (cDNA)	*Alternaria* resistance	Mora and Earle (2001)
Bacterial resistance	GO	Glucose oxidase gene from *Aspergillus niger*	*Xanthomonas campestris* resistance	Lee et al. (2002)
Abiotic stress	CUP1	Structural gene of yeast metallothionein gene	Heavy metal tolerance	Hasegawa et al. (1997)
	betA	Bacterial gene for biosynthesis of glycinebetaine	Salt tolerance	Bhattacharya et al. (2004)
	vhb	*Vitreoscilla* haemoglobin overexpression	Tolerance to a prolonged submergence	Li et al. (2005)
Ripening	ACC	Tomato antisense 1-aminocyclopropane-1-carboxylic acid oxidase gene	Ethylene biosynthesis	Henzi et al. (1999, 2000)
	ACO II/IPT	Broccoli antisense ACC oxidase II and isopentenyl transferase gene	Ethylene/cytokinin biosynthesis	Gapper et al. (2002, 2005)
	ACC	ACC oxidase (sense/antisense), ACC synthase (cDNAs)	Ethylene production, delay of chlorophyll loss	Higgins et al. (2006)
	ipt	Retarding effect on post-harvest yellowing	Cytokinin biosynthesis	Chen et al. (2001)
	boers	Mutant broccoli ethylene response sensor gene	Ethylene biosynthesis	Chen et al. (2004)
	BoCP5	Broccoli antisense gene of cystein protease	Influence of post-harvest protease activity	Eason et al. (2005)
	BoINV2	Antisense construct of BoINV2 (soluble acid invertase)	Retarding effect on post-harvest yellowing	Eason et al. (2007)

	BoCLH1	Antisense chlorophylase gene	Retarding effect on post-harvest yellowing and chlorophyll degradation	Chen et al. (2008a)
Self incompatibility	SLG	S locus glycoprotein gene	Self incompatibility	Sato et al. (1991), Toriyama et al. (1991a, b)
	SLR1	Antisense SLR1 glycoprotein gene	Self incompatibility	Franklin et al. (1996)
	SRK, SLG	S locus receptor kinase gene, S locus glycoprotein gene	Self incompatibility	Conner et al. (1997)
Male sterility	DTx-A	Cytotoxic diphtheria toxin A-chain (DTx-A) gene + tapetum-specific promoter	Male sterility	Lee et al. (2003c)
Pharmaceuticals	B5/SARS-CoV	Vaccinia virus glycoprotein B5, human SARS coronavirus glycoprotein	Production of antigens	Pogrebnyak et al. (2006)
Gene function	Ac Tpase	Ds-based two-element transposon system	Transposon activity, insertional mutagenesis	Mckenzie et al. (2002), Mckenzie and Dale (2004)
Brassica rapa L.				
Herbicide resistance	bar/TuMV-NIa	Basta resistance, *Turnip mosaic virus* NIa protease	Method in planta	Qing et al. (2000), Xu et al. (2008)
Virus resistance	TMV-L	*Tobacco mosaic virus*	L coat protein gene	Jun et al. (1995)
	TuMV-NIb	Antisense *Turnip mosaic virus* NIb	TuMV-resistance, method in planta	Yu et al. (2007)
Insect resistance	cry	*Bt*	Insect resistance against *Pieris rapae, Plutella xylostella, Trichoplusia ni*	Cho et al. (2001)
	Cry	*Bt*	Insect resistance, influence on nontarget insects	Kim et al. (2008)
	CpTI	Cowpea trypsin inhibitor + antibacterial peptide gene	Resistance against *P. rapae* and *Erwinia aroidae*	Zhao et al. (2006a, b)
Bacterial resistance	Antibacterial gene	Antibacterial peptide gene	Resistance against *E. aroidae*	Wang et al. 2002
Self incompatibilty	SLG, SRK	S locus glycoprotein gene, S receptor kinase gene	Self incompatibilty	Shiba et al. (1995, 2000), Takasaki et al. (1999, 2000, 2001)
	SP11	S locus protein 11	Self incompatibilty	Shiba et al. (2001), Sato et al. (2003, 2004)

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Table 25.1 (continued)

Character	Transgene	Transgene description	Aim	References
Male sterility	BcMF6	Antisense pollen-expressed polygalacturonase gene BcMF6	Pollen development, A9 promoter	Zhang et al. (2008)
	CYP86MF	Antisense fragment of the CYP86MF gene and the tapetum-specific A9 promoter		Yu et al. (2004), Cao et al. (2006b)
Flower development	OsMADS1	Rice floral development gene (MADS box gene)		Shin et al. (2003)
	BrFLC1, 2, 3	Floral repressor gene	Flowering time	Kim et al. (2007a)
Plant physiology	pRiA4, pRi1855	Genes in pRiA4 and pRi1855	Auxin synthesis, root and plant growth	He et al. (1994, 2000)
Abiotic stress	otsA/LEA	Trehalose-6-phosphate synthase/late embyogenesis abundant protein	Environment stress tolerance	Park et al. (2003), (2005b)
	Cu/ZnSOD, CAT	Maize superoxide dismutase and/or catalase gene	Resistance to SO_2 (chloroplast transformation)	Tseng et al. (2007)
	SOD, CAT	*E. coli* superoxide dismutase and/or catalase gene	Resistance to SO_2	Tseng et al. (2008)
Metabolic engineering	MAM1, CYP79F, CYP83A1	*Arabidopsis* cDNAs	Aliphatic glucosinolate biosynthesis	Zang et al. (2008a)
	CYP79B2, CYP79B3, CYP83B1	*Arabidopsis* cDNAs	Indol glucosinolate metabolism, plant defence	Zang et al. (2008b)
	GLO, JMT	L-Guluno-γ-lactone oxidase (vitamin C metabolism)/jasmonic methyl transferase	Fungal resistance	Min et al. (2007)
Lactuca sativa L.				
Herbicide resistance	bar, glu, EPSPS		Basta and Roundup resistance	McCabe et al. (1999), Mohapatra et al. (1999), Torres et al. (1999), Nagata et al. (2000)
Fungal resistance	glu	β-1,3-Glucanase from *Arthrobacter* spp.	Resistance against *Bremia lactucae*	Dede (1998)
	oxdc	Decarboxylase gene from mushroom	Resistance against *Sclerotinia sclerotiorum*	Dias et al. (2006)

Virus resistance	LMV-0	*Lettuce mosaic virus* (LMV) coat protein gene		Gilbertson (1996), Dinant et al. (1997)
	LBVaV	Coat protein gene of *Lettuce big-vein associated virus* (LBVaV)	Sense and antisense orientation	Kawazu et al. (2006)
	TSWV-BL	Nucleocapsid protein gene of *Tomato spotted wilt virus* (TSWV) and *Lettuce infectious yellow virus* (LIYV)		Falk (1996), Pang et al. (1996)
Insect resistance	SaPIN2a	Proteinase inhibitor II (PIN2) from *Solanum americanum*	Against cabbage looper (*Trichoplusia ni*)	Xu et al. (2004), Chye et al. (2006), Xie et al. (2007)
Abiotic stress and plant physiology	ABF3, ABA	Abscisic acid	Tolerance to drought and cold stress	Vanjildorj et al. (2005)
	LEA	Late embryogenesis abundant protein gene from *Brassica napus*	Tolerance to salt stress and water stress	Park et al. (2005c)
	P5CS	δ-(1)-Pyrroline-5-carboxylate synthetase	Water stress resistance (drought, salt, cold)	Pileggi et al. (2001)
	rolAB	*A. rhizogenes* rolAB genes	Response to auxin	Curtis et al. (1996a)
	GA 20	Overexpression of a pumpkin gibberellin (GA) 20-oxidase gene	Controlling plant stature	Niki et al. (2001)
	etr1-1	Ethylene mutant receptor etr1-1 confers ethylene insensitivity	Effect on the regeneration properties	Kim et al. (2004)
	NR	Post-transcriptional gene silencing	Nitrate content	Curtis et al. (1999), Dubois et al. (2005)
	Ferritin	Iron storage protein	High yield, high iron content and rapid growth rate	Goto et al. (2000)
Metabolic engineering and fuctional food	Monellin	Single-chain monellin gene	Flavour and quality	Penarrubia et al. (1992)
	Miraculin	Synthetic miraculin gene	Taste-modifying proteins, sweetness-inducing activity	Sun et al. (2006)
	STS	Stilbene synthase gene from *Parthenocissus henryana*	Key enzyme in resveratrol biosynthesis	Liu et al. (2006)
	asnA	*E. coli* asparagine synthetase A gene	inulin content increased	Sobolev et al. (2007), Giannino et al. (2008)
	CAX1	*A. thaliana* cation exchanger1 H^+/Ca^{2+}	Increased Ca content	Park et al. (2009)

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Table 25.1 (continued)

Character	Transgene	Transgene description	Aim	References
	R2R3-MYB	Flavonoid biosynthesis factor from *A. thaliana*	Anthocyanin biosynthesis	Park et al. (2008)
	TC/VTE1, γ-TMT	Tocopherol cyclase, γ-tocopherol methyltransferase		Cho et al. (2005), Lee et al. (2007a)
	ipt		Maturity regulation	McCabe et al. 2001
Male sterility	PR-Glu	Pathogenesis-related glucanase gene linked to a tapetum-specific promoter		Curtis et al. (1996b)
Pharmaceuticals	CTB-Pins, sCTB	Cholera toxin B subunit (human proinsulin)	Human therapeutic protein	Kim et al. (2006), Ruhlman et al. (2007)
	sLTB, SARS-CoV	*E. coli* heat-labile enterotoxin B subunit, severe acute respiratory syndrome coronavirus		Li et al. (2006), Kim et al. (2007b)
	MV-H	Measles virus hemagglutinin		Webster et al. (2006)
	E2-CSFV, CP	Glycoprotein of swine fever virus, cystein protease from *Fasciola hepatica*	Oral animal vaccination	Legocki et al. (2005)
	HBsAg	Antigen of hepatitis B virus		Kapusta et al. (1999, 2001), Kawashima et al. (2001)
	hITF	Human intestinal trefoil factor		Zuo et al. (2001)
	ChIFN-α	Chicken α-interferon against vesicular stomatitis virus	Preventing infectious diseases of poultry	Song et al. (2008)
	IFS	Soybean isoflavone genistein	Phytooestrogen	Liu et al. (2007b)
Carrot (*Daucus carota* L.)				
Herbicide resistance	pat		Glufosinate resistance, Liberty resistance	Dröge et al. (1992), Drogelaser et al. (1994), Chen and Punja (2002)
	ALS	Mutant acetolactate synthase gene	Imazapyr resistance	Aviv et al. (2002)
Fungal resistance	Chit	Chitinase genes from tobacco, petunia, bean	Against *Rhizoctonia*, *Alternaria*, *Botrytis*, *Sclerotinia*	Linthorst et al. (1990), Broglie et al. (1991)
	chi-2	Chitinase genes from tobacco, bean, barley	Against *Rhizoctonia*, *Alternaria*, *Botrytis*, *Sclerotinia*	Gilbert et al. (1996), Punja and Raharjo (1996), Jayaraj and Punja (2007)
	CHIT36	Microbial endochitinase *Trichoderma harzianum*	Against *Alternaria*, *Botrytis*	Baranski et al. (2008)

	MF3	Microbial factor from *Pseudomonas fluorescens*	Against *Alternaria*, *Botrytis*	Baranski et al. (2007)
	tlp	Rice thaumatin-like protein		Chen and Punja (2002), Punja (2005)
	ltp	Wheat lipid transfer-protein (PR)		Jayaraj and Punja (2007)
	HLP	Human lysozyme protein	Resistance against *Erysiphe heraclei*	Takaichi and Oeda (2000)
	AP24	Tobacco PR-5 osmotin + chitinase + glucanase	Against *Alternaria, Cercospora, Erisyphe*	Tigelaar et al. (1996), Melchers and Stuiver (2000)
Functional food	CAX1	*A. thaliana* cation exchanger1 H^+/Ca^{2+}	Increase Ca content, fuctional food	Park et al. (2004b)
	bkt	β-carotene ketolase gene from alga *Haematococcus pluvialis*	Fuctional food, neutraceutical	Jayaraj et al. (2008), Jayaraj and Punja (2008)
Pharmaceuticals	LTB	E. coli heat-labile enterotoxin (LTB)	Against cholera and diarrhoea	Rosales-Mendoza et al. (2007, 2008)
	GAD65	Autoantigen in human insulin-dependent diabetes mellitus (IDDM)		Porceddu et al. (1999), Avesani et al. (2003)
	MPT64	*Mycobacterium tuberculosis* gene		Wang et al. (2001)
	HepB	Hepatitis B virus surface protein		Imani et al. (2002)
	tt830-844	Measles-unrelated T cell epitope (tt830-844)		Bouche et al. (2003, 2005)
	MV	Immunodominant antigen of the measles virus		Marquet-Blouin et al. (2003)
Cucumis melo L.				
Virus resistance	CMV-WL	Coat protein-mediated resistance (CP-MR)	Resistance to *Cucumber mosaic virus* (CMV)	Gonsalves et al. (1994)
	ZYMV, WMV	CP-MR	Resistance to *Watermelon virus* 2 (WMV 2) and *Zucchini yellow mosaic virus* (ZYMV)	Fang and Grumet (1993), Clough and Hamm (1995)
	ZYMV, WMV2, CMV	CP-MR	Resistance to WMV 2, ZYMV and CMV	Fuchs et al. (1998)
Abiotic stress	HLA1	Gene from *Saccharomyces cerevisae*	Salt tolerance	Bordas et al. (1997)
Fruit ripening	CmACO1-AS	ACC oxidase antisense	Improved shelf life	Nuñez-Palenius et al. (2006)

(*continued*)

Table 25.1 (continued)

Character	Transgene	Transgene description	Aim	References
	MEL1	Melon ACC oxidase antisense	Extended shelf life	Ayub et al. (1996), Guis et al. (2000)
	ACC	Apple ACC oxidase antisense	Ten days longer shelf life	Silva et al. (2004)
Cucumis pepo L.				
Virus resistance	CMV-CP	Coat protein-mediated resistance (CP-MR)	Resistance to *Cucumber mosaic virus* (CMV)	Tricoli et al. (1995), Fuchs et al. (1998)
	ZYMV, WMV	CP-MR	Resistance to *Watermelon virus* 2 (WMV 2) and *Zucchini yellow mosaic virus* (ZYMV)	Clough and Hamm (1995), Fuchs and Gonsalves (1995), Tricoli et al. (1995)
Cucumis sativus L.				
Virus resistance	CMV-C-CP, CMV-O-CP	CP-MR	Resistance to CMV	Gonsalves et al. (1992), Nishibayashi et al. (1996a)
	pCAMSV	54-kDA replicase gene of CFMMV		Gal-On et al. (2005)
Fungal resistance	RCC2	Rice chitinase cDNA	Resistance to *Botrytis cinerea*	Tabei et al. (1998), Kishimoto et al. (2002, 2003)
	CHI2	Cucumber class III chitinase gene	Resistance strategy to gray mould (*Botrytis cinerea*)	Kishimoto et al. (2004)
Abiotic stress	HLA1	Gene from *Saccharomyces cerevisae*	Salt tolerance under in vitro conditions	Bordas et al. (1997)
	DHN10, DHN24	Dehydrin from *Solanum sogarandium*	Temperature tolerance (increased chilling tolerance)	Yin et al. (2004), Yin et al. (2006b)
Parthenocarpy	DefH9-iaaM	Genes from *Pseudomonas syringae* pv. *savastanoi* and *Antirrhinum majus*		Yin et al. (2006a)
Taste	Thaumatin II	Gene from *Thaumatococcus daniellii*		Szwacka et al. (2002), Gajc-Wolska et al. (2003, 2005)
Pharmaceuticals	mSOD1	Superoxide dismutase (SOD) from cassava		Lee et al. (2003a)
Citrullus lanatus (Thunb.) Matsun. & Nakai.				
Virus resistance	CGMMV-CP	CP-MR	*Cucumber green mottle mosaic virus* (CGMMV)	Park et al. (2005d)
Abiotic stress	HLA1	Gene from *Saccharomyces cerevisae*	Salt tolerance	Ellul et al. (2003)

Pisum sativum L.				
Herbicide resistance	bar		Basta resistance	Schroeder et al. (1993), Shade et al. (1994)
Fungal resistance	Vst1, PGIP	Stilbene synthase gene (Vst1) from grape, polygalacturonase inhibiting protein (PGIP) from raspberry		Richter et al. (2004)
Virus resistance	AMV-CP	Chimeric coat protein	*Alfalfa mosaic virus* (AMV)	Grant et al. (1998), Timmerman-Vaughan et al. (2001)
Insect resistance	AI-1, Al-2	α-Amylase inhibitors 1, 2 from *Phaseolus vulgaris*	Resistance to *Bruchus pisorum* (pea weevil)	Schroeder et al. (1995), Morton et al. (2000), Collins et al. (2006)
Phaseolus vulgaris (L.)				
Herbicide resistance	bar		Glufosinate ammonium resistance	Aragão et al. (2002)
Virus resistance	BGMV-BR, bar	Coat protein from *Bean golden mosaic virus* and Basta resistance		Russell et al. (1993)
	rep-TrAP-Ren, BC1	Antisense of genes from Brasilian isolate *Bean golden mosaic virus* (BGMV-BR)		Aragão et al. (1998)
	AC1, AC2, AC3, BC1	Antisense of AC1, AC2, AC3 and BC1 genes from BGMV		Aragão et al. (1996)
	Rep, bar	Rep gene mutant of BGMV and Basta resistance		Faria et al. (2006)
	ACI-RNAi	Post-transcriptional gene silencing (RNAi) of the ACI gene from BGMV	High-resistance progenies	Bonfim et al. (2007)
Abiotic stress	LEA	Late embryogenesis abundant protein gene from *Brassica napus*	Drought stress resistance	Liu et al. (2005)
Protein content	be2s2	Methionine-rich 2S albumin from the Brazil nut	Increased methionine content	Aragão et al. (1996, 1999)
Cichorium intybus L. & *C. endivia* L.				
Herbicide resistance	csr1-1	Mutant acetolactate synthase gene from *A. thaliana*	Resistance to sulfonylurea herbicides	Vermeulen et al. (1992), Lavigne et al. (1995)
Metabolic engineering	6G-FFT	6G-Fructosyltransferase from onion	Synthesized fructan of the inulin neoseries and linear inulin	Vijn et al. (1997)

(*continued*)

Table 25.1 (continued)

Character	Transgene	Transgene description	Aim	References
	6-SPT	6-Fructosyltransferase from barley	Synthesized branched fructans and tetrasaccharide bifurcose	Sprenger et al. (1997)
Male sterility	barnase and bar	Tapetum-specific promoter and barnase gene from *Bacillus amyloliquefaciens*	Hybrid breeding	Mariani et al. (1992), Williams (1995), www.agbios.com
Spinacea oleracea L.				
Herbicide resistance	pat	Pat gene from *Steptomyces hygroscopicus*	Glufosinate ammonium resistance	Wells (1999), Burgos et al. (2001)
Virus resistance	CMV-CP	Coat protein genes	Against *Cucumber mosaic virus* (CMV)	Yang et al. (1997)
Asparagus officinalis L.				
Herbicide resistance	bar	Phosphinothricin acetyl transferase	Basta resistance	Cabrera-Ponce et al. (1997)
Onion (*Allium cepa* L.)				
Herbicide resistance	bar, CP4		Basta and Roundup resistance	Eady et al. (2003a)
Insect resistance	Cry1Ab, Cry1Ca	*Bt* hybrid genes	Beet armyworm resistance (*Spodoptera exigua*)	Zheng et al. (2005)
Allium tuberosum L. & A. porrum L.				
Herbicide resistance	ALS	Acetolactate synthase (ALS) gene from chlorsulfuron resistant *Arabidopsis mutant*		Park et al. (2002)
Insect resistance	cry	*Bt* hybrid gene which encodes domains I and II of Cry1Ab and domain III of Cry1Ca		Zheng et al. (2004)

25.2 Economically Important Vegetable Families

25.2.1 *Solanaceae*

25.2.1.1 *Solanum lycopersicon* L.

In the family Solanaceae, besides tobacco, tomato has played a key role in genetic engineering techniques in the past years. Among the other vegetable crops, tomato fulfils the basic requirements for gene transfer, which includes its character as a model object for in vitro culture techniques (Bhatia et al. 2004), its moderately sized genome with 950 Mb (Shibata 2005) applicable to recent sequencing technology and its importance as vegetable crop for the fresh market and for processing. Hence, it is not surprising that the first commercialized transgenic food crop ever brought to market was Calgene's 'Flavr Savr' tomato in 1994. It was followed in 1995 by DNA Plant Technology's 'Endless Summer'. 'Flavr Savr' was a success with consumers but failed economically for a variety of reasons (Martineau 2001). In 1996 Zeneca launched a transgenic processing tomato product that was the best selling tomato paste in the United Kingdom during 1999—2000. The paste reduced processing costs and resulted in a 20% lower price (Redenbaugh and McHughen 2004).

Considerable success has been achieved in introducing virus resistance (Kunik et al. 1994; Whitham et al. 1996; Gubba et al. 2002), fungi resistance (Jongedijk et al. 1995; Tabaeizadeh et al. 1999; Radhajeyalakshmi et al. 2005; Sarowar et al. 2006) and bacteria resistance based on systemic acquired resistance (SAR; Rizhsky and Mittler 2001; Lin et al. 2004; Chan et al. 2005). Insect resistance (see also Chap. 10) has been engineered by using bacterial genes derived from *Bacillus thuringiensis* ssp. *kurstaki* (*Bt* genes; Fischhoff et al. 1987; Delannay et al. 1989) or a proteinase inhibitor from potato (Abdeen et al. 2005) which is a part of the plant natural defence mechanism against herbivores. Furthermore *Mi-1*, a *Lycopersicon peruvianum* gene which confers resistance against the three economically important root-knot nematode species (*Meloidogyne incognita*, *M. javanica*, *M. arenaria*; Roberts and Thomason 1986; Goggin et al. 2004), is also active against the potato aphid, *Macrosiphum euphorbiae* (Vos et al. 1998).

Other limiting factors in the horticultural production are abiotic stresses (see Chap. 8), such as extreme temperature, drought and salinity. A transformation system with chloroplast-targeted *codA* gene of *Arthrobacter globiformis* (for method, see Chap. 2), which encodes choline oxidase to catalyse the conversion of choline to glycinebetaine, was successfully established with tomato cv. 'Moneymaker' (Park et al. 2004a). The study demonstrates a better fitness of transgenic plants after chilling at 3 °C for 7 days with regard to their survivability and the fruit set. Other efforts were made to engineer chilling tolerance by ectopic expression of *Arabidopsis CBF1* (Hsieh et al. 2002a, b).

Most commercial tomato cultivars are sensitive to salinity. Considerable genetic knowledge of salt tolerance (Foolad 2004) is the basis for transgenic strategies to overcome this problem (Gisbert et al. 2000; Rus et al. 2001; Jia et al. 2002; Muñoz-Mayor et al. 2008). Due to the complexity of the trait in many cases the increased transgenic salt tolerance was only marginal. However, advancement was the creation of transgenic tomato plants by overexpressing a vacuolar Na^+/H^+ antiport with the *AtNHX1* gene from *Arabidopsis* (Zhang and Blumwald 2001). Transgenic plants grown in the presence of 200 μM sodium chloride flowered and produced fruits.

While most of the above-mentioned traits were agronomical and benefitted primarily the grower and the producer, currently significant efforts are also being made to improve nutrients and consumer qualities. Although technically more difficult and therefore not ideal for the grower, there are many potential opportunities for enhancing nutritional value (Bird et al. 1991; Römer et al. 2000; Muir et al. 2001; Le Gall et al. 2003; Giorio et al. 2007) and organoleptic qualities such as taste (Penarrubia et al. 1992; Bartoszewski et al. 2003) and aroma in the tomato fruits. Important quality parameters of fresh fruits are volatile compounds, which often do not meet the high standards of flavour required by the consumer. For instance the Δ-*9 desaturase* gene from *Saccharomyces cerevisiae* expressed in tomato showed changes in certain flavour compounds (Wang et al. 1996). The overexpression of a non-specific alcohol dehydrogenase gene in tomato fruits (Speirs et al. 1998) altered the levels of aroma determining aldehydes and alcohols. In a preliminary taste trial, the authors identified fruits with elevated alcohol dehydrogenase activity and higher level of alcohols as having a more intense 'ripe fruit' flavour.

Tomato plants have been designed to produce a range of proteins and biomolecules. The cholera toxin B protein has been expressed in tomato plants, and the feasibility to elicit an immune response in mice has been demonstrated (Jiang et al. 2007). Recently Butelli et al. (2008) expressed two transcription factors from *Antirrhinum majus* L. in tomato; the fruit of the plants accumulated anthocyanins at levels substantially higher than previously reported for efforts to engineer anthocyanin accumulation in tomato and at concentrations comparable to the anthocyanin levels found in blackberries and blueberries.

Tomato fruits contain proteins with high allergenic potential (Jäger and Wüthrich 2002). Genetic engineering could be an approach to remove allergens. This was demonstrated in a remarkable way by Le et al. (2006a, b), who designed tomatoes with reduced allergenicity by dsRNAi-mediated inhibition of *ns-LPT* (*Lyc e 1* and *Lyc e 3*, respectively) expression (for details on gene silencing, see Chap. 5). Furthermore it was demonstrated that silencing of the *Lyc* genes by means of RNAi contributes to reducing skin reactivity and is passed on to the next generation of fruits (Lorenz et al. 2006).

25.2.1.2 *Solanum melongena* L.

Eggplant (aubergine) is native to India. Today it is an important crop in tropical and warm parts of the temperate zone. Like other plants of the family Solanaceae it suffers from severe diseases, insect attacks and abiotic stress, leading to high crop loss every year.

In vitro culture methods were used comprehensively to improve the eggplant cultivars (for reviews, see Collonnier et al. 2001; Kashyap et al. 2003). Due to the good response in tissue culture the first attempts at genetic engineering for eggplant were accomplished soon after the first reports on plant transformation of *Arabidopsis* and tomato (Guri and Sink 1988; Rotino and Gleddie 1990). So far, a number of useful genes have been introduced to eggplant. General aspects of genetic modification of plants are discussed in Chap. 1.

Parthenocarpic transgenic eggplants have been successfully achieved by transferring a gene construct consisting of bacterial *iaaM* gene and *DefH9* promotor, specifically to the placenta and ovules (Rotino et al. 1997). Donzella et al. (2000) reported on the field performance of the transgenic pathenocarpic hybrids. They concluded that the transgenic parthenocarpic hybrids allowed an increase in productivity up to 25%.

It was shown that an introduced bacterial mannitol-1-phosphodehydrogenase (*mtlD*) gene evokes a multifactor abiotic stress tolerance (Prabhavathi et al. 2002). Transgenic eggplants featured an improved tolerance to salt, drought and chilling stress. Recently, Prabhavathi and Rajam (2007) described that mannitol-accumulating transgenic eggplants exhibit resistance to fungal wilts. The data suggest that the *mtlD* gene could be useful for both plant biotic and abiotic stress tolerance.

Further efforts are being made to develop eggplant cultivars with resistance against fungal diseases. The fatty acid composition has an impact on resistance to *Verticillium dahliae*. Transfer of yeast Δ-*9 desaturase* gene in eggplant displayed the linkage between plant fatty acid content and the resistance traits (Xing and Chin 2000). After successful transformation with an antimicrobial defensin gene from *Dahlia merckii,* Turrini et al. (2004) found transgenic eggplants had an improved resistance against *Botrytis cinera.*

In tomato the *Mi-1.2* gene confers resistance against nematodes, whiteflies and potato aphids (Nombela et al. 2003). Expression of the tomato *Mi-1.2* gene in eggplants causes resistance against nematodes only, not aphids (Goggin et al. 2006). There is the assumption that the genetic background plays an important role for gene function.

Under the tropical climate eggplant is infested by a number of insect pests. Plant protease inhibitors have a defensive function, targeting leaf-feeding insects like aphids. Transgenic eggplants with an o*ryzacystatin* gene coding for an inhibitor of cystein proteinases have been obtained by *Agrobacterium*-mediated transfer (Ribeiro et al. 2006). In feeding tests the population growth and the survival of *Mycus persicae* Sulzer and *Macrosiphum euphorbiae* Thomas were reduced.

The most destructive insects on eggplants are the Colorado potato beetle (CPB; *Leptinotarsa decemlineata* Say) and the eggplant shoot and fruit borer (ESFB; *Leuconodes orbonalis* Guen.). There are a number of reports about *Bt* transgenic eggplants, describing the transformation procedure. Furthermore, the impact of transgenic *Bt* eggplants on the target insects (CPB or EFSB) as well as on non-target arthropods has been examined thoroughly (Chen et al. 1995; Rovenská et al. 2005;

Arpaia et al. 2007). Connected with current announcements to introduce *Bt* eggplant in commercial use, there is a comprehensive analysis about the potential impacts of *Bt* eggplants on economic surplus in India (Krishna and Qaim 2007, 2008). Safety tests for the *Bt* eggplant have been conducted in India, starting in greenhouses and now moving on to large-scale field trials.

25.2.1.3 *Capsicum annuum* L.

Peppers are cultivated and used around the world as sweet peppers, such as the bell pepper, or as pungent chilli peppers. Pepper originated in the tropics. Today pepper is cultivated also in the subtropics and in temperate climates as a staple vegetable crop. Belonging to the family Solanaceae well known for plants with an excellent tissue culture and transformation capability, pepper is a recalcitrant exception. First, Liu et al. (1990) reported about *Agrobacterium*-mediated transformation of bell pepper. They showed the principal possibility of pepper transformation with foreign genes like *nptII* and *gus*. In 1993, US patent 5262316 (Engler et al. 1993) described the co-cultivation of explant material from the pepper plant with *A. tumefaciens* or *A. rhizogenes* carrying an exogenous DNA sequence. Therefore the invention related to a method for genetically transforming and regenerating pepper plants. Despite a detailed description of the transformation procedure, the patent gives no clearness about the regeneration efficiency. Over the past 15 years a few other groups (e.g. Zhu et al. 1996; Manoharan et al. 1998; Pozueta-Romero et al. 2001; Li et al. 2003; Lee et al. 2004) have been working on the improvement of the transformation system for pepper. In summary it should be stated that the pepper transformation is not a routine method and is highly dependent on genotype and explant source.

Due to the importance of pepper, genetic engineering is (despite the low efficiency of the transformation protocols) a promising tool to improve some cultivars. Pepper yields are endangered every year by severe virus diseases. Kim et al. (1997) induced cDNA of the satellite RNA of the *Cucumber mosaic virus* (CMV) into the pepper genome. The authors described an attenuation of the symptoms in T_1 hot pepper plants. In spite of the positive results there are no more publications with such strategy. Some concerns about the biosafety could be the cause for that.

Another strategy, the virus coat protein mediated protection, was more widely applied (Zhu et al. 1996). Shin et al. (2002a) reported about the testing of transgenic pepper plants expressing the coat proteins of CMV and *Tomato mosaic virus* (ToMV). Cai et al. (2003) gave a detailed report about the development of CMV- and TMV-resistant transgenic chilli pepper, the field performance of some progenies and a biosafety assessment.

It was demonstrated that the expression of tobacco stress-induced gene 1 (*Tsi 1*) in pepper enhanced the resistance of the transgenic pepper plants to various pathogens, including viruses, bacteria and oomycetes (Shin et al. 2002b). Transcriptional regulatory genes may have an impact on the overall disease resistance in pepper.

The risk to overcome such broad resistance should be low, therefore it is a strategy worth further investigation.

The Chinese government approved commercialization of pimientos (Spanish pepper) in the late 1990s, although more detailed information is missing (http://www.chinadaily.com.cn/english/doc/2006-02/14/content_519769.htm).

In India the performance of transgenic bell pepper and chilli with snowdrop lectine gene has been examined in field trials in 2002 (http://www.indiaresource.org/issues/agbiotech/2003/fieldsoftrial.html). The additional lectine gene should evoke resistances against lepidopteran, coleopteran and homopteran pests. Experiments have been performed under the umbrella of Rallis India Ltd and the Bangalore Tata Group. Common knowledge about some results is strictly limited.

Due to its simplicity, herbicide resistance was often the first published genetically engineered trait. Surprisingly that is not correct for pepper. There exists a brief mention by Tsaftaris (1996). A Korean team (Lee et al. 2007b) reported on a conference about the environmental evaluation of herbicide-resistant peppers.

Korean scientists (Kim et al. 2001) introduced rice MADS box genes into pepper, studying the impact of such genes on the plant development.

Harpster et al. (2002a) investigated the function of the *CaCel1* gene by silencing in transgenic pepper. The consequences for fruit ripening process in T_3 plants in a greenhouse were examined. This is the only example that genes isolated from pepper are used for the investigation of their function in pepper. But there are plenty of isolated and notified pepper genes and cDNAs used for further gene expression studies in plants easily accessible for transformation, like *Arabidopsis*, tobacco or tomato; some of the latest of such works were published by e.g. An et al. (2008), Hong et al. (2008), Hwang et al. (2008), Oh et al. (2008).

25.2.2 *Brassicaceae (Brassica oleracea L., B. rapa L., Raphanus sativus L.)*

Substantial work on the elaboration and application of genetic transformation for *Brassica* vegetable crops is in progress throughout the world. *Brassica* vegetables encompass important vegetables, such as cauliflower, broccoli, cabbage and Brussels sprouts. In the Asian cuisine in countries like China, India and Korea *Brassica rapa* L. vegetables play an important role. The high variability of crucifers, their economic impact and their good responsiveness to biotechnological approach are considerable factors so that, from the first possibilities for genetic engineering to date, *Brassica* species are a promising object for such techniques. The development of plants with useful traits is relatively advanced. Despite this only a few field testings with transgenic brassicas have been performed. Commercial cultivars seem to be not in sight.

Early after the first reports of successful transformation of *B. oleracea* using *A. tumefaciens* with marker genes (David and Tempé 1988; Srivastava et al. 1988; De Block et al. 1989) this technique was applied for the investigation of self-incompatibility (Sato et al. 1991; Thorsness et al. 1991; Toriyama et al. 1991a, b). Due to difficulties in transforming *B. rapa*, *s*imilar works for *B. rapa* were published later (Takasaki et al. 1999, 2000, 2001). A valuable trait for breeding purposes, self-incompatibility in Brassicaceae is genetically controlled by some *S* locus genes. Transformation technology has opened up new possibilities to investigate the expression and interaction of the *S* locus genes.

Male sterility is another breeding feature of great worth, enabling F_1 hybrid production on a large scale. In the past decade researchers reported about new approaches concerning the male sterility of *Brassica* species. It should be mentioned that this is a cutting-edge topic with regard to environmental concerns about possible transgene escape. No pollen development could be a solution for safe plant containment. Lee et al. (2003c) obtained several transgenic plants from cabbage, *B. oleracea* ssp. *capitata*, by way of *Agrobacterium*-mediated transformation to test the activity of anther-specific promoter isolated from Chinese cabbage. With that promoter, the expression of the cytotoxic diphtheria toxin A-chain (*DTx-A*) gene resulted in male-sterile cabbages. Using RNA antisense technology (see Chap. 5) and a tapetum-specific promoter (Yu et al. 2004; Zhang et al. 2008) could develop male-sterile Chinese cabbage.

Another possibility to get transgenic plants without dissemination of transgenes via pollen could be chloroplast transformation (Nugent et al. 2006; Liu et al. 2007a, 2008). Liu et al. (2008) reported the acquired insect resistance of cabbage after chloroplast genetic engineering with a *Bt* gene, demonstrating the efficiency of the genetic modification of plastids. They cited Bock (2007) that the plastid transformation is a prerequisite method to produce vaccines or therapeutic proteins in plants. So far, this general statement has not been realized for *Brassica* vegetables. Although the *Brassica* vegetable crops are important, to date only Pogrebnyak et al. (2006) has reported the *Agrobacterium*-mediated transformation of collard and cauliflower with, respectively, a smallpox vaccine candidate gene and a gene coding for SARS coronavirus spike protein.

Every year the yield losses caused by diseases and by insect attacks are high. For the whole complex of engineering disease and pest resistance, many reports are available for both *B. oleracea* and *B. rapa*. Table 25.1 gives a brief overview about the latest publications in that field. Generally, the methods of transformation are well established and a number of scientific teams are performing the transformation of *Brassica* with a high efficacy.

There is a great interest in having a controlled influence on postharvest physiological processes. To gain a deep understanding of the role of ethylene, cytokinin and other factors, broccoli was used as a model species (Henzi et al. 1999, 2000; Chen et al. 2001; Gapper et al. 2005; Higgins et al. 2006). In connection with the improved availability of isolated genes and cDNAs, new studies for postharvest yellowing show the effect of additionally introduced *Brassica* genes in broccoli (Chen et al. 2004, 2008a; Eason et al. 2007). Kim et al. (2007a) transferred floral repressor genes isolated before from *B. rapa* to Chinese cabbage. The results demonstrate that it is feasible to control the flowering time and the undesirable bolting of Chinese cabbage.

Improved access to genes originating from sequencing projects is also reflected in other current works for *Brassica* transformation. For instance, *Arabidopsis* cDNAs were used for metabolic engineering of aliphatic or indole glucosinolates of *B. rapa* (Zang et al. 2008a, b).

Since various factors of abiotic stress seriously impair the growth and development of *Brassica* crops, approaches for improved abiotic stress tolerance are an objective for a number of transformation projects. So far, the investigations have encompassed bacterial, yeast and plant genes. The genetic improvement of heavy metal tolerance in cauliflower by transfer of the yeast metallothionein gene (*CUP1*) was demonstrated by Hasegawa et al. (1997). Li et al. (2005) delivered the gene coding for *Vitreoscilla* haemoglobin (*vhb*) into cabbage. They observed that the overexpression of VHb protein affects the plant's tolerance of submergence stress. The introduction of the bacterial *bet*A gene for the synthesis of glycinebetaine causes a higher salinity tolerance in transgenic cabbage (Bhattacharya et al. 2004). For Chinese cabbage Tseng et al. (2007, 2008) explored the possibility of overcoming the phytotoxic effect of sulfur dioxide and salt stress. They transferred genes coding for superoxide dismutase and catalase from maize and *Escherichia coli*, respectively.

Belonging to the family Brassicaceae, radish (*Raphanus sativus* L.) is a further most common crucifer vegetable consumed worldwide. Radish is greatly recalcitrant in tissue culture. For that reason there are only a few reports about radish transformation. Moreover these reports describe transformation protocols trying to overcome difficulties with tissue culture and regeneration efficiency. Curtis et al. (2002) used the floral-dip method for producing transgenic radish plants with the *GIGANTEA* (*GI*) gene from *Arabidopsis*. Park et al. (2005a) elaborated a transformation protocol via sonification and vacuum infiltration of germinated seeds with *Agrobacterium*, successfully transferring a *LEA* gene (late embryogenesis abundant) from *B. napus*. The accumulation of the foreign protein in radish conferred an increased drought and salt tolerance.

25.2.3 Fabaceae (Pisum sativum L., Phaseolus vulgaris L.)

Whereas most crop species of the Fabaceae are used as protein or oil plants in food industry or animal nutrition, e.g. soybean, chickpea, pea, bean, lentil and others, a few species are also used as vegetables. Two examples are reviewed in this chapter: the garden pea (*Pisum sativum* L.) and the snap bean (*Phaseolus vulgaris* L.). For fresh, frozen or canning purposes, green premature seeds or juvenile pods of the garden pea are harvested and green pods in an early seed development stage of the snap bean are harvested.

After overcoming a number of difficulties during in vitro culture and regeneration, the first transgenic pea plants were reported by de Kathen and Jacobsen (1990) and Puonti-Kaerlas et al. (1990). The transfer of herbicide resistance (*bar)* as a potentially useable trait was reported but not carried through to commercial release

(Schroeder et al. 1993; Shade et al. 1994). Partial resistance to *Alfalfa mosaic virus* (AMV) was observed in transgenic pea engineered with a chimeric virus coat protein (Grant et al. 1998; Timmerman-Vaughan et al. 2001).

Another strategy focused on conferring resistance to pea weevil (*Bruchus pisorum* L.) by expression of an α-amylase inhibitor (*α-A1*) and the phytohemagglutin promoter from *Phaseolus vulgaris* (Shade et al. 1994; Schroeder et al. 1995; Morton et al. 2000; De Sousa-Majer et al. 2004; Collins et al. 2006).

A fungal resistance approach was reported by Richter et al. (2006) who transformed via *Agrobacterium tumefaciens* two antifungal genes coding for a polygalacturonase-inhibiting protein (*PGIP*) from raspberry (*Rubus idaeus* L.) or the stilbene synthase (*Vst1*) from grape.

Analogous to pea, genetic engineering in bean was for a long time limited by the absence of efficient methodologies, from in vitro regeneration systems up to transformation systems. Now, transformation approaches via *Agrobacterium,* electroporation and particle-gun have been achieved (Genga et al. 1991; McClean et al. 1991; Dillen et al. 1995; Kim and Minamikawa 1997).

The first transgenic plant progeny was published by Russell et al. (1993). In a biolistic approach they transferred marker and reporter genes (*pat*, *gus*) and also a coat protein gene isolated from the *Bean golden mosaic virus* (BGMV).

The team of Aragão et al. (1996, 1998) obtained transgenic plants using different genes of BGMV in antisense orientation and showed resistance. Faria et al. (2006) achieved transgenic beans with a vector that contained a mutated virus replication gene (*rep*). Stability of the transgene loci and BGMV resistance were observed in some plant progenies. Bonfim et al. (2007) explored the concept of using an RNA interference construct to silence the ACI viral gene region of BGMV.

The methionine content was significantly increased in transgenic lines engineered via biolistic methods with a gene coding for the methionine-rich storage albumin from the Brazil nut (Aragão et al. 1996, 1999). The same group (Aragão et al. 2002) reported the transfer of herbicide resistance mediated by the *bar* gene to bean.

Transgenic kidney bean with the late embryogenesis abundant (*LEA*) protein gene from *Brassica napus* was produced by using a sonication and vacuum infiltration *Agrobacterium*-mediated transformation approach. Plants expressed a high level of the *LEA* gene showed a high tolerance to salt and water deficit stress (Liu et al. 2005). Whereas a commercial exploitation of GM peas in the medium term is expected especially for dry (seed) pea production (herbicide tolerance, resistance to insects, fungi and virus diseases), a commercial usage of the GM beans is in the long term not expected.

Meanwhile, genetic transformation has been reported in all the major legume crops, like *Cicer arietinum* L., *Cajanus cajan* L., *Vigna*, *Phaseolus*, *Lupinus*, *Vicia* and *Lens* species, but with the exception of soybean, transgenic plants have not yet been commercially released. A translation of knowledge of genomics or functional genomics in the model legumes *Medicago truncatula* and *Lotus japonicus* will open new transgenic approaches in future.

25.2.4 *Cucurbitaceae [Cucumis sativus L., C. melo L., Cucurbita pepo L., Citrullus lanatus (Thunb.) Matsun. & Nakai., and other cucurbit species]*

The cucurbit family (Cucurbitaceae) includes three genera of valuable crop species: *Cucumis, Cucurbita* and *Citrullus*. In the genus *Cucumis*, cucumber (*C. sativus*) and melon (*C. melo*) are the two main crops. Squash, pumpkin and zucchini belong to the genus *Cucurbita,* which includes the cultivated species *C. pepo*, *C. moschata*, *C. maxima*, *C. argyrosperma* and *C. ficifolia*. In the genus *Citrullus*, watermelon is the only species of economic importance (Bates et al. 1990).

Since the first report about successful transformation of cucumber using *A. rhizogenes* (Trulson et al. 1986), a lot of work has been done to establish and improve transformation efficiency not only in *C. sativus* (Schulze et al. 1995; Nishibayashi et al. 1996b; He et al. 2008), but also in *Cucumis melo* (Fang and Gurmet 1990; Valles and Lasa 1994; Galperin et al. 2003; Cürük et al. 2005; Rhimi et al. 2007; Nuñez-Palenius et al. 2007), *Cucurbita pepo* (Katavic et al. 1991; di Toppi et al. 1997), *Citrullus lanatus* (Choi et al. 1994; Cho et al. 2008) and *C. colocynthis* (Dabauza et al. 1997).

The progress made with the application of this technique is reviewed by Yin et al. (2005). The use of viral coat protein genes to confer resistance has been approved for several virus diseases (Gaba et al. 2004). The commercially most successful has been zucchini engineered for resistance to the *Zucchini yellow mosaic virus* and *Watermelon mosaic virus 2* with coat protein genes. The transgenic zucchini traded firstly by Seminis is a cross with Asgrow's transgenic crookneck squash. The Asgrow Company received permission for commercial use in the United States in 1995.

During the past several years, genetic engineering approaches have been employed to develop transgenic cucurbit plants with enhanced tolerance to abiotic stress. In order to induce chilling tolerance in cucumber, the expression pattern of a *Solanum sogarandinum* pGt::*Dhn10* gene encoding a dehydrin DHN10 protein was analysed (Yin et al. 2004). The transgenic lines exhibited a slight enhanced chilling and a freezing tolerance either comparable to or less than the non-transgenic control. Another significant advancement was the transformation of different watermelon [*Citrullus lanatus* (Thunb.)] cultivars expressing the *Saccharomyces cerevisiae HAL1* gene related to salt tolerance (Ellul et al. 2003). The halotolerance observed in T_3 lines confirmed the inheritance of the trait and supports the potential usefulness as a tool for genetic engineering of salt-stress protection.

From a commercial aspect, parthenocarpy is a cost-effective solution to improve fruit set. Moreover, the seedlessness of fruits can increase consumer acceptance. In cucumber the pDefH9::*iaaM* construct was successfully introduced into the genome and 70—90% of the fruits produced by the transgenic lines were parthenocarpic (Yin et al. 2006a).

25.2.5 Asteraceae

25.2.5.1 *Lactuca sativa* L.

Lettuce (*Lactuca sativa* L.) is a major fresh vegetable and is becoming increasingly more important in Europe in the convenience area, e.g. salad mixtures. In Egypt and Asian countries lettuce stems and leaves are consumed in dishes of various kinds, in cooked, raw, pickled or dried form (Ryder 1986). Lettuce belongs to the family Asteraceae, with approximately 100 species of *Lactuca*. Only the four species *L. sativa* L., *L. serriola* L., *L. saligna* L. and *L. virosa* represent the important breeding pool. They are self-fertilized diploids and can be crossed with each other. Modern lettuce breeding is geared towards the areas of disease/insect resistance, improved quality and increased yield.

First, Michelmore et al. (1987) transferred a *npt*II gene for kanamycin resistance using *A. tumefaciens*. Chupeau et al. (1989) transformed lettuce protoplasts with the *npt*II gene using electroporation. Later an iceberg lettuce was successfully transformed with the reporter gene *gus* (Torres et al. 1993). Today transformation using *A. tumefaciens* has become routine in lettuce.

Herbicide-resistant transgenic lettuce was reported by several authors using the *bar* gene (McCabe et al. 1999; Mohapatra et al. 1999) and a glyphosate oxidase gene (*GOX*; Torres et al. 1999; Nagata et al. 2000).

Plants transformed with genes encoding enzymes that hydrolyse fungal cell walls, such as the β-1,3-glucanase from *Arthrobacter* spp. (Dede 1998) or an oxalate decarboxylase gene from edible mushroom (Dias et al. 2006), showed increased resistance against downy mildew (Dede 1998) and *Sclerotinia sclerotiorum* (Dias et al. 2006).

The virus coat protein strategy was successfully applied to enhance resistance to the *Lettuce mosaic virus* (LMV; Dinant et al. 1993, 1997, 1998; Gilbertson 1996) and the *Lettuce big vein associated virus* (LBVaV) and the *Mirafiori lettuce virus* (MLV; Kawazu et al. 2006). A transferred nucleocapsid protein gene of the lettuce isolate of *Tomato spotted wilt virus* (TSWV) increased the resistance to TSWV (Pang et al. 1996) and *Lettuce infectious yellow virus* (LIYV; Falk 1996).

A proteinase inhibitor (*PIN2*) gene from *Solanum americanum* Mill. was used to generate resistance to cabbage looper caterpillars (*Trichoplusia ni* Hübner; Xu et al. 2004; Chye et al. 2006; Xie et al. 2007).

Male sterility (see also Chap. 14) as prerequisite of hybrid breeding could be induced by expressing a *β-1,3-glucanase* gene linked with a tapetum-specific promoter, resulting in the dissolution of the callose wall during the microsporogenesis (Curtis et al. 1996b).

Another research area is designed to influence plant physiology and tolerances to environmental stress. Lettuce engineered with genes coding enzymes of the proline biosynthesis resulted in salt- and temperature-tolerant plants (Curtis et al. 1996a; Pileggi et al. 2001). Overexpression of an *Arabidopsis ABF3* gene (Vanjildorj et al. 2005), or the late embryogenesis abundant protein (*LEA*) gene from *Brassica napus* (Park et al. 2005c) enhanced cold, salt and drought tolerance, too.

A number of examples for the transgenic improvement of horticultural and nutritional quality were reported, especially in the past decade, such as monellin or miraculin synthesis for changes in flavour components (Penarrubia et al. 1992; Sun et al. 2006), increased tocoperol (Cho et al. 2005; Lee et al. 2007a), iron and Ca content (Goto et al. 2000; Park et al. 2009), or the anthocyanin biosynthesis (Park et al. 2008).

Analogous to other crops, pharmaceuticals could be an interesting area for application of genetic engineering in lettuce. Reports so far include the transfer of genes coding the cholera toxin B protein (Kim et al. 2006; Ruhlman et al. 2007), a measles virus hemagglutinin (Webster et al. 2006), an antigen of the hepatitis B virus (Kapusta et al. 1999, 2001; Kawashima et al. 2001) or a human intestinal trefoil factor (Zuo et al. 2001). Further potential applications for oral animal vaccinations were tested, such as against the *Swine fever virus* (Legocki et al. 2005) or the *Vesicular stomatitis virus* of poultry (Song et al. 2008).

Contrary to the high input in transgenic research, transgenic lettuce has not been commercialized so far.

25.2.5.2 *Cichorium intybus* L., *C. spinosum* L., *C. endivia* L.

Cichorium intybus L. (chicory, radicchio) is cultivated as biennial crop widespread in Europe and the world, whereas *C. endivia* L. and *C. spinosum* L. are annuals predominately grown in Europe and North Africa.

First, Sun et al. (1991) reported *A. rhizogenes*-mediated transgenic *C. intybus* which was converted from biennial to annual flowering. Later Genga et al. (1994) and Abid et al. (1995) described the transfer of *gus* gene to radicchio, using *A. tumefaciens*. Herbicide resistance was engineered by an acetolactate synthase gene from *A. thaliana* (Vermeulen et al. 1992; Lavigne et al. 1995). Herbicide resistance is of economic interest because the growth rate of the chicory seedlings in the field is low and fast-developing weeds can suppress them.

A transgenic approach to engineer male sterility as a prerequisite for hybrid breeding was developed and first demonstrated by Mariani et al. (1990, 1992). Next, Bejo Zaden B.V. (The Netherlands) engineered male sterile chicory and radicchio, using a chimeric gene construct of *barnase* gene from *Bacillus amyloliquefaciens*, a tapetum-specific promoter and the selective marker gene *bar*. Bejo received the license to produce F_1 hybrids of chicory and radicchio in 1995; however the licence is not longer valid. Another request for the authorization of salad and GM chicory or radicchio was withdrawn. Today the marketing of these GM vegetables is not allowed in the European Union (EU).

Other approaches focused on metabolic engineering. Transgenic chicory with a 6G-fructosyltransferase from onion (Vijn et al. 1997) or barley (Sprenger et al. 1997) synthesized fructan of the inulin neoseries or branched fructans of the graminan type, respectively. Both may be interesting as potential functional food for diet or in diabetic therapy.

25.2.6 Apiaceae (*Daucus carota* L.)

The family Apiaceae contains approximately 113 cultivated species distributed worldwide. About 21% are used as vegetables, but only carrot, celery and fennel with greater commercial importance (Rubatzky et al. 1999; Pistrick 2002).

Carrot has been extensively studied as a model species for tissue culture, plant somatic embryogenesis and protoplast fusion (Ammirato 1986) and was therefore predestined for transformation approaches. The first transgenic carrots were reported after *A. rhizogenes* infection by Tepfer (1984). Shortly after, Langride et al. (1985) obtained transgenic plants by electroporation of suspension protoplasts with naked DNA. Later, transgenic plants were obtained by *A. tumefaciens* infection of various carrot plant explants and cells (Scott and Draper 1987; Thomas et al. 1989; Wurtele and Bulka 1989).

Herbicide resistance was first introduced into carrot via direct gene transfer of the *pat* gene (Dröge et al. 1992; Drogelaser et al. 1994). Chen and Punja (2002) introduced the *bar* gene and Aviv et al. (2002) a mutant acetolactate gene (*ALS*) from *Arabidopsis thaliana* causing resistance to herbicide Imazapyr.

A number of genes have been introduced to enhance resistance to fungal pathogens, such as chitinases, glucanases, thaumatin-like protein, osmotin and lysozyme. Resistance has been engineered by using chitinases cloned from petunia and tobacco (Linthorst et al. 1990), from beans (Broglie et al. 1991) or from *Trichoderma harzianum* (Baranski et al. 2008). A thaumatin-like protein from rice was expressed in carrot and showed enhanced tolerance to six fungal pathogens (Chen and Punja 2002; Punja 2005). Transgenic carrots with the tobacco osmotin (AP24) in combination with a chitinase and a glucanase gene also expressed broad-spectrum tolerance (Tigelaar et al. 1996; Melchers and Stuiver 2000). Carrot lines which constitutively expressed a human lysozyme showed enhanced resistance to *E. heraclei* and *A. dauci* (Takaichi and Oeda 2000). The microbial factor (MF3) from *Pseudomonas fluorescens* enhanced the resistance to *Alternaria* sp. and *Botrytis cinerea* (Baranski et al. 2007).

An interesting field is the production of biopharmaceuticals. A number of transgenic carrots have been engineered to produce proteins or potential human vaccines, such as enterotoxin (LTB) against cholera and diarrhea (Rosales-Mendoza et al. 2008), the *MPT64* gene of *Mycobacterium tuberculosis* (Wang et al. 2001), the major hepatitis B virus surface protein (Imani et al. 2002), an immunodominant antigen of the measles virus (Bouche et al. 2003, 2005; Marquet-Blouin et al. 2003) and glutamic acid decarboxylase (GAD65) as an autoantigen in autoimmune type 1 diabetes mellitus (Porceddu et al. 1999; Avesani et al. 2003).

Currently two approaches focus on functional foods or nutraceuticals. It was demonstrated that transgenic carrots expressing the *Arabidopsis* H^+/Ca^{2+} transporter *CAX1* increase their calcium content up to 50% compared with the control. Enhancing the concentration of bioavailable calcium in vegetables could prevent calcium malnutrition and reduce the incidence of osteoporosis (Park et al. 2004b). Furthermore, carrots have been engineered into the ketocarotenoid biosynthetic pathway by introducing a β-carotene ketolase gene from the alga *Haematococcus pluvialis*. Transgenic carrots converted up to 70% of total carotenoids to novel ketocarotenoids, showing that carrots are suitable for applications to the functional food, nutraceutical and aquaculture industries (Jayaraj et al. 2008; Jayaraj and Punja 2008).

Transgenic plants have also been obtained in celery (*Apium graveolens* L.; Catlin et al. 1988) and caraway (*Carum carvi* L.; Krens et al. 1997). Both papers describe the establishment of an *Agrobacterium*-mediated transformation protocol, at the moment only of academic value.

At the present time, there are no transgenic carrot cultivars or other Apiaceae commercially available on the market.

25.2.7 Chenopodiaceae (*Spinacia oleracea* L.)

Spinach (*Spinacia oleracea* L.) is one of the most nutritious vegetables, due to a high content of β-carotene and folate; furthermore it is a rich source of vitamin C, calcium, iron, phosphorous sodium and potassium. Current breeding is mainly focused on a number of pests, bacterial and fungal diseases and viruses, as well as on improved nutrition. To increase the resistance level, particular emphasis is given to biotechnological approaches.

The first transformed spinach was reported by Al-Khayri (1995) after introduction of the *gus* gene. Other researchers used these protocols to engineer spinach that carried the coat protein gene for the *Cucumber mosaic virus* (Yang et al. 1997), the *nptII* and *gfp* gene (Zhang and Zeevaart 1999), or the gene for glyphosate tolerance (Wells 1999; Bevitori 2000; Burgos et al. 2001).

No transgenic plants have been commercialized so far.

25.2.8 Liliaceae

25.2.8.1 *Allium cepa* L., *A. porrum* L., *A. sativum* L.

The onion (*Allium cepa*) and its close relatives leek (*A. porrum*) and garlic (*A. sativum*) are very important vegetable crops on a worldwide scale. As monocotyledons, *Allium* species have proven to be recalcitrant to in vitro regeneration and genetic engineering (Eady 1995; Eady et al. 1996; Barandiaran et al. 1998). So it took until 2000, when Eady et al. (2000) published the first repeatable protocol for the production of transgenic *A. cepa* plants, followed by a successful garlic transformation (Kondo et al. 2000). The latter is of particular interest, because garlic breeding has been limited to the clonal selection of wild varieties or mutants, due to the loss of fertile flowers.

Transgenic onion plants tolerant to herbicides (see Chap. 9) containing glyphosate or poshinothricin were recovered by Eady et al. (2003a). The same group

(Eady et al. 2003b) demonstrated that the integration and expression of foreign genes are essentially not different to the Mendelian fashion. The results suggest that the herbicide resistance transformed in elite onion germplasm is expressed and inherited in such a way that it will have a normal agronomic function.

With respect to the beet armyworm (*Spodoptera exigua* Hübner), the most important pest in *Allium* cultivation for (sub)tropical zones, a transgenic pest management strategy seems to be the only way to overcome this problem. Garlic and shallot plants (Zheng et al. 2004, 2005) have been engineered with synthetic *Bt* gene. The produced transgenic *A. cepa* plants grew well in the greenhouse, had a normal phenotype, produced bulbs and were completely resistant to the beet armyworm (Zheng et al. 2005).

25.2.8.2 *Asparagus officinalis* L.

Transgenic asparagus (*Asparagus officinalis* L.) was successfully achieved by *A. tumefaciens*-mediated transformation (Delbreil et al. 1993; Limanton-Grevet and Jullien 2001), microprojectile bombardment (Cabrera-Ponce et al. 1997; Li and Wolyn 1997) and electroporation of protoplasts (Mukhopadhyay and Desjardins 1994). In most experiments the *nptII* marker gene and the *gus* reporter gene were transformed and expressed. Additionally, transgenic asparagus with the *bar* gene was reported by Cabrera-Ponce et al. (1997). A commercial application is not known.

25.3 Conclusions

The commercial applications of genetic engineering technology to vegetables lag far behind those of agricultural crops. As the global acreage of transgenic agricultural crops has expanded dramatically since their introduction in 1996, it is paradoxical that the trend in vegetables is the opposite.

Within the past 15 years alone in the United States and the EU, over 1240 transgenic field trials for vegetables have been documented (Fig. 25.1). Although the number of trials is indicative of who is working on what vegetable, it does not accurately reflect the absolute activity. On the trial number basis, tomato accounts for over half. Transformation technology is potentially an effective tool for vegetable breeding in fields that are not easily accessible by conventional breeding techniques. Nevertheless no more commercial utilization is expected in the near future in Europe or the United States. Only a few GM cultivars are licensed for different countries, such as tomato, zucchini, chicory and eggplant. Despite the transgenic zucchini cultivation in the United States on probably 10 000 ha, no market launch is expected in the EU. In China, GM peppers are supposed to be cultivated. However, reliable information is not yet available, because a lot of the research is being done in the private sector. Commercial utilization of *Bt*-eggplants

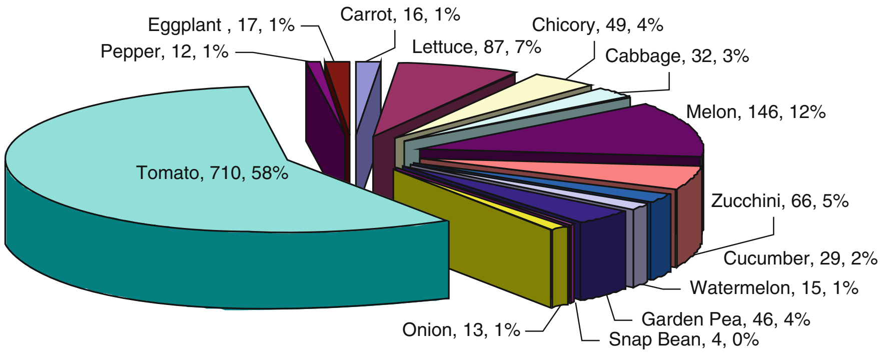

Fig. 25.1 Deliberate releases of GM vegetables into the environment for field trials (1992–2007). Data are presented as: vegetable name; number of field trials worldwide; percentage (sources: http://www.transgen.de, http://www.gmo-compass.org, http://usbiotechreg.nbii.gov/database_pub.asp, http://www.agbios.com)

in India and the Philippines will start in 2009; and the use of GM garden peas is expected in the medium term.

For the whole complex of engineering disease and pest resistance, as well as abiotic stress tolerance, a lot of reports are available. It could be assumed that in the future transgenic methods will be increasingly used for that purpose, due of the growing awareness of the problems connected with the global climate changes.

While the first transgenic vegetables were strongly tailored to the needs of the producers, incentives are needed to share the benefits. Vegetables with clear benefits for the consumers are needed to develop demand. Although technically more difficult, there are many potential opportunities for enhancing the nutritional value or consumer appeal of vegetables through genetic engineering. In addition to modification of flavour, research projects to increase the content of vitamins, minerals or nutraceuticals in vegetables are in progress. Despite the fact that transformation is a powerful approach to plant improvement, the major impediment to genetically engineered vegetables is the reluctance of the consumer and subsequently the market.

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