PART VII
TRANSGENIC PLANTS: THIRD GENERATION

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Plant Biotechnology

Vietnam OpenCourseWare

April 2009

Third generation (1998-): Non-traditional products

Plants are a reservoir of valuable pharmacological compounds, and people have utilized plants as therapeutic products for thousands of years.

In the third generation, scientists apply *Molecular farming* to produce foreign proteins in plants may fill a more direct role as producers of antibodies, vaccines or protein-based signal molecule therapeutics, biodegradable thermoplastics such as polyhydroxybutyrate (PHB). Plants can be transform to improve resistance to abiotic stress. If transporters are known, foreign proteins can also be used to engineer plants to phytoremediate toxic soils…. One of the earliest examples of the production of pharmaceutical polypeptides in plants exploited the natural high level expression of seed storage proteins. The neuropeptide enkephalin was produced in *Brassica napus* as part of the seed storage protein 2S albumin.

Transgenic Plant As A Factory for Many Industries

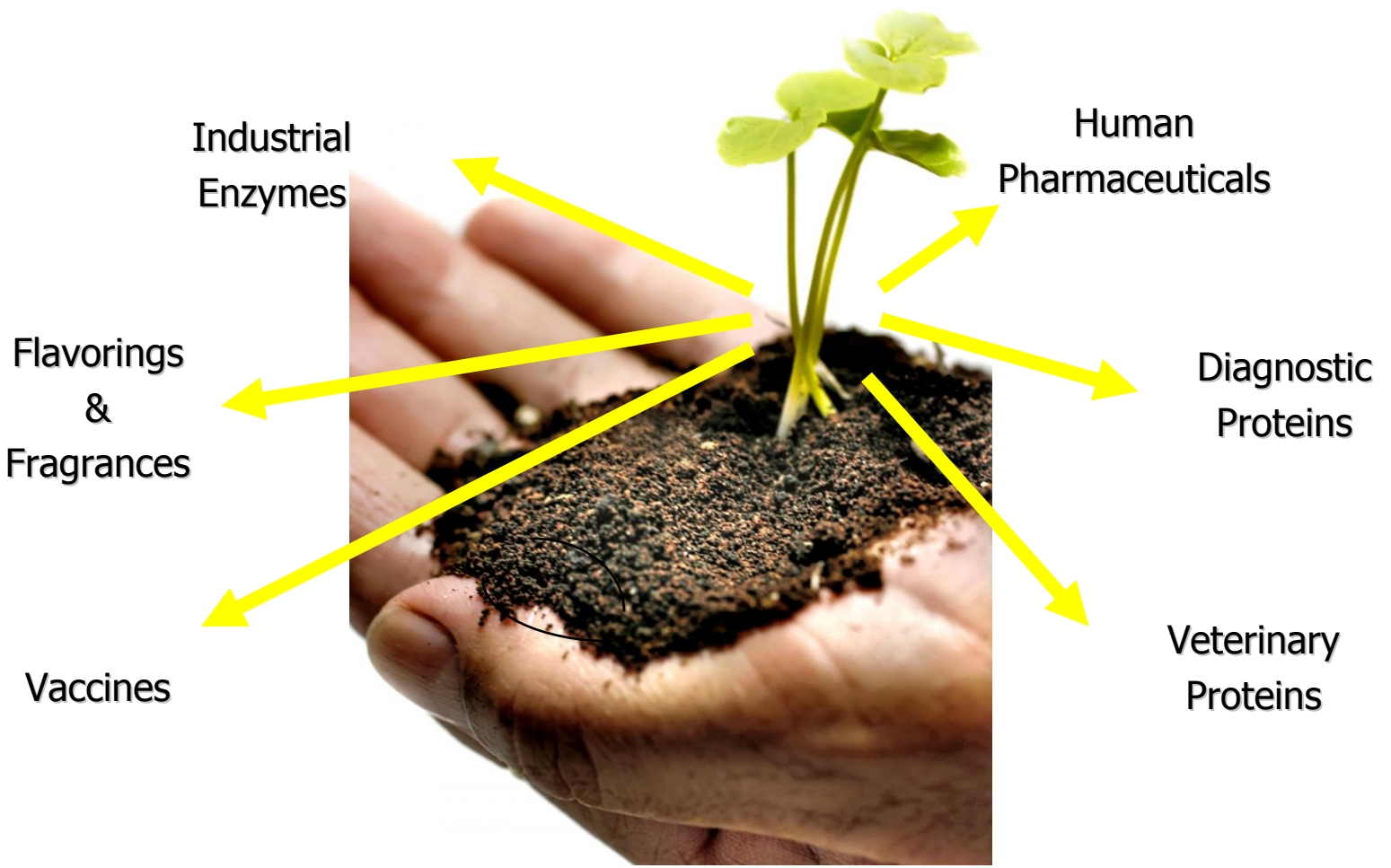


Photo source: *www.central-bible.org*

7.1. Molecular farming in plants

Production of recombinant plant proteins offers a series of potential benefits such as cheap production and storage, large-scale manufacture of biopharmaceuticals, higher health safety level in comparison with bacteria or animal origin.

A further advantage of plant biopharmaceuticals is the potential elimination of a purification process, in case plant tissues are used as food. Last but not least, it is noteworthy that the target protein may be inserted into a particular cell compartment (chloroplast); its stability is consequently increased or this protein may even be expressed in this compartment .

Three general approaches have been followed to produce foreign proteins in plants:

✓ *The target protein can be expressed from a transgene stably introduced into the genome of a plant. Sites of integration into host DNA vary among transformed lines, and expressed sequences are subject to gene silencing. Thus, expression levels are often low and can vary greatly.*

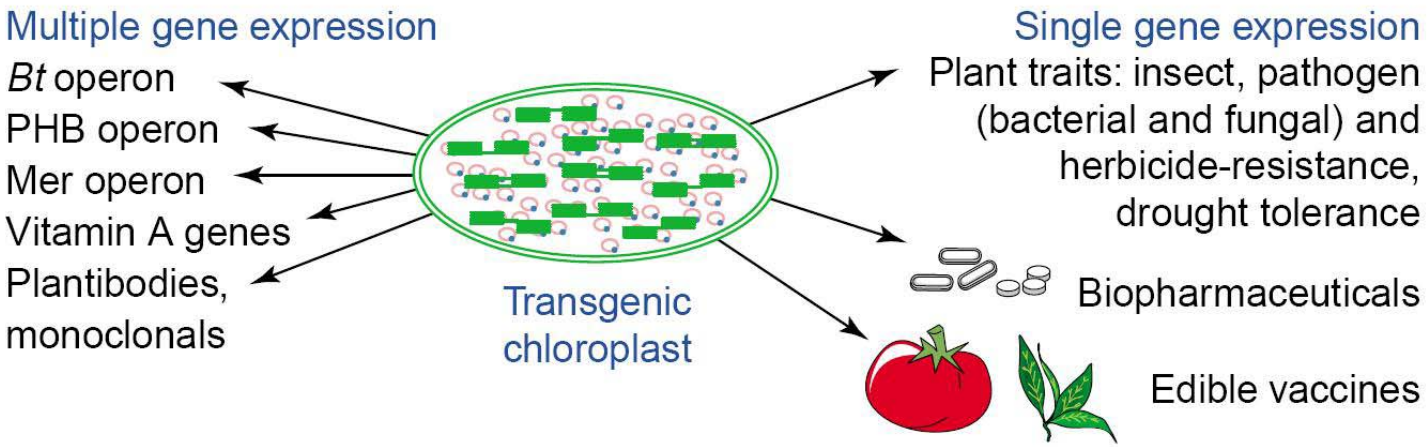
✓ *A second alternative for plant expression is to transiently express foreign proteins from recombinant plant viral sequences inoculated onto plant tissues infected with recombinant. Several species have been tested but recombinant protein accumulation generally occurs in leaves.*

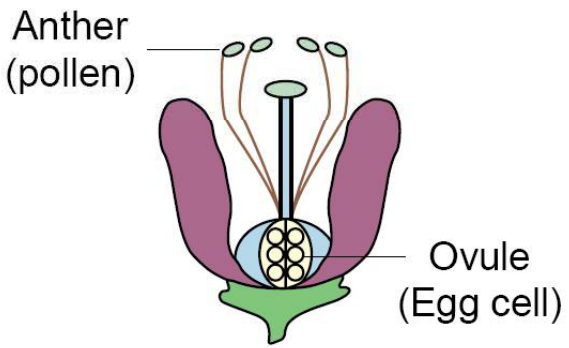
Post-translational modifications such as glycosylation can also be accomplished.

✓ *The third alternative for plant-based expression is to integrate transgenes into plastid genomes.*

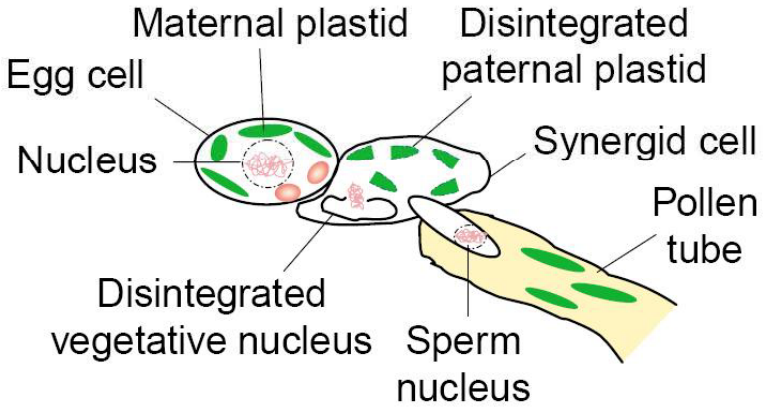
✓ *Transplastomic plants do not appear to be subject to silencing and, because the transgene is targeted to a specific locus, expression levels tend to be uniform*.

✓ *Plastids are maternally inherited; there are no containment concerns regarding transgenic pollen.*

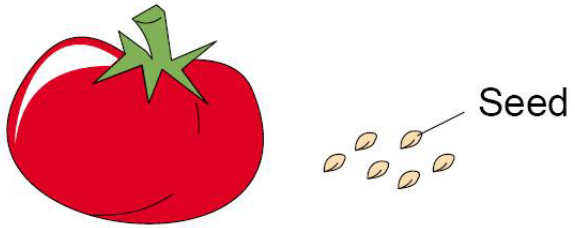




T_0 reproductive organs



Disintegration of paternal plastids



Maternal inheritance of transgenic traits

Source fromTRENDS in Plant Science Vol.7 (2). 2002

However, only a few species are subject to plastid transformation and most research has focused on tobacco leaves, currently limiting options for long-term storage, processing and delivery. Plastid expression also offers fewer alternatives for post-translational modification, although foreign proteins can still form disulphide bridges, and a recent report shows that lipid modification can also be achieved.

Photo source: *www.electricfreeze.com*

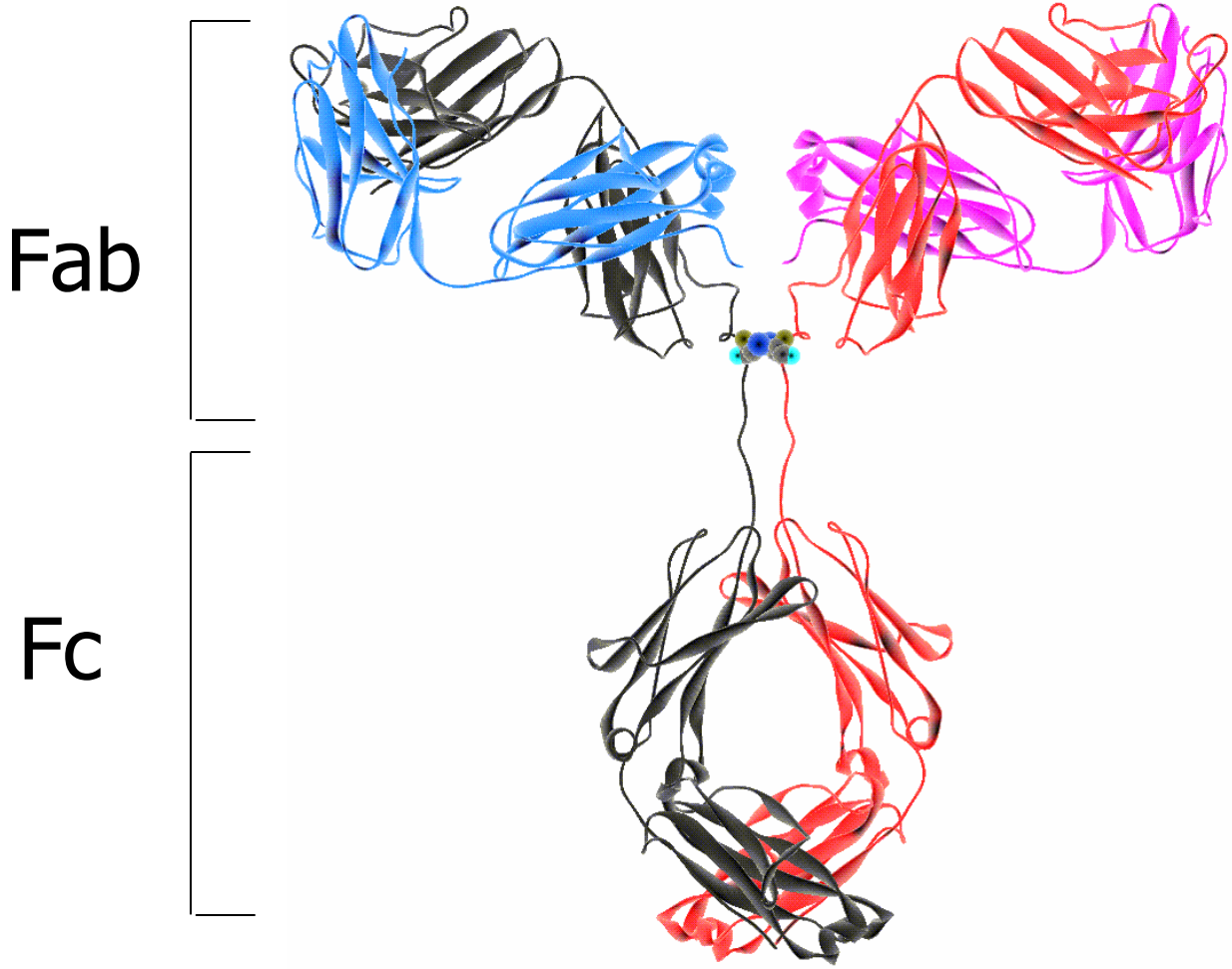
7.2. Molecular farming of antibodies to disease

Antibodies and their derivatives account for more than 20% of all the biopharmaceuticals under current development. Part of the reason for the prevalence of antibodies is their manifold uses. Their specificity for particular antigens allows them to be used as diagnostic agents, therapeutic drugs and even as novel industrial enzymes (abzymes).

An estimated 1000 therapeutic antibodies are being developed by biopharmaceutical companies around the world, over 200 of which are already in clinical trials

An antibody molecule comprises two heavy chains and two light chains joined by disulphide bonds . The C-terminal region of the heavy chain forms the Fc portion of the antibody, performing particular effector functions. The N-terminal regions are variable and, along with the light chains, are involved in antigen binding. The Fc region contains a conserved asparagine residue at position 297 to which glycan chains are added during the processing of immunoglobulin chains.

Antibody structure



Molecular farming of antibodies in plants involves five essential steps:

1) *obtaining the relevant cDNA encoding the antibody of interest;*

2) inserting the cDNA into a plant expression construct;

3) transferring the expression construct into a suitable heterologous expression host and producing a functional recombinant protein;

4) scaling up production to commercial levels;

5) downstream processing and quality control.

As well as full-size antibodies, derivatives with alternative structures can also be produced . These include Fab and F(ab*')2 fragments (which contain only the sequences* distal to the hinge region of a full-length antibody); single-chain Fv fragments (scFvs, which contain the variable regions of the heavy and light chains joined by a flexible peptide linker); chimeric antibodies containing components of different classes (e.g. chimeric IgG/A); fusion proteins with additional functionality(e.g. interleukin-scFv fusions) and bispecific scFvs.

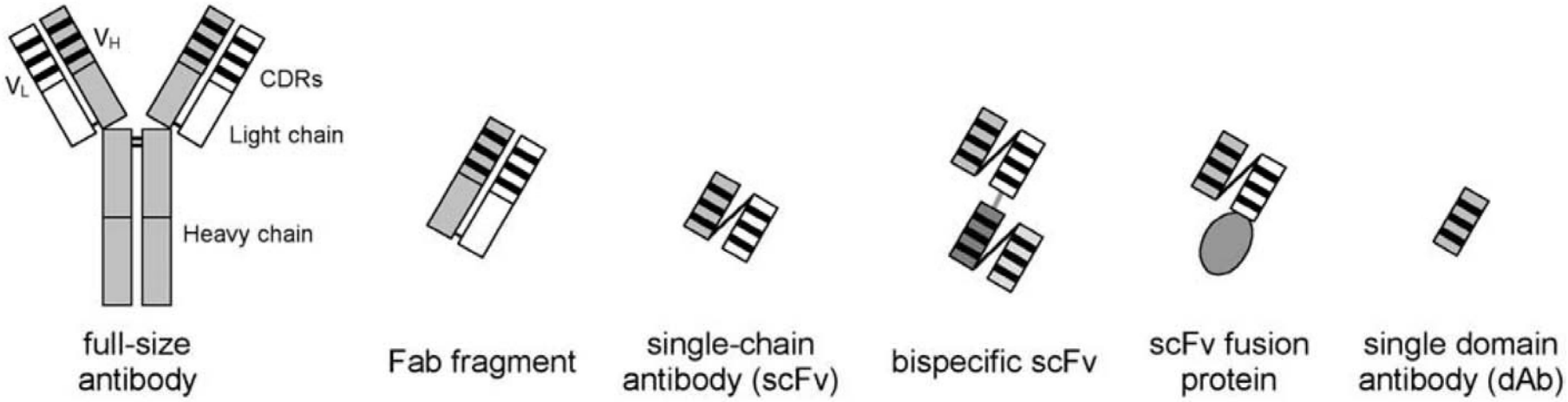


Figure 1. Different types of recombinant antibody produced in plants. V_H and V_L, antigen-binding domains of heavy and light chain; CDRs, complementarity-determining regions.

Source from CMLS, Cell. Mol. Life Sci. 60, 2003

Table 2. Diagnostic and therapeutic antibodies produced in plants. *Source from CMLS, Cell. Mol. Life Sci. 60, 2003*

Antibody format	Antigen	Cellular location	Transformed	Maximum species
dAb	substance P (neuropeptide)	apoplast	*Nicotiana benthamiana*	1% TSP leaves
IgG1, Fab	human creatine kinase	apoplast	*N. tabacum, Arabidopsis thaliana*	0.044% TSP leaves, 1.3% TSP leaves
SIgA	streptococcal surface antigen (I/II)	apoplast	*N. tabacum*	500 µg/g FW leaves
scFv	human creatine kinase	Cytosol, apoplast	*N. tabacum*	0.01% TSP leaves
scFv-IT	CD-40	apoplast	*N. tabacum* cell culture	not reported
IgG1	herpes simplex virus 2	apoplast	*Glycine max*	not reported
IgG	colon cancer antigen	ER	*N. benthamiana*	not reported
IgG1	human IgG	apoplast	*Medicago sativa*	1% TSP
scFv, IgG1	carcinoembryonic antigen	apoplast, ER	*N. tabacum* (transient expression)	5 µg scFv/g leaves, 1 µg IgG/g leaves
scFv	38C13 mouse B cell lymphoma	apoplast	*N. benthamiana*	30.2 µg/g leaves
scFv	carcinoembryonic antigen	apoplast, ER	*Oryza sativa*	3.8 µg/g callus, 29 µg/geaves, l 32 µg/g seed
scFv	carcinoembryonic antigen	apoplast, ER	*Triticum aestivum*	900 ng/g leaves, 1.5 µg/g seed
scFv	Carcinoembryonic antigen	ER	*Pisum sativum*	9 µg/g seed
IgG1	streptococcal surface antigen (I/II)	plasma membrane	*N. tabacum*	1.1% TSP leaves

dAb, single-domain antibody; FW, fresh weight; scFv-IT, scFv-bryodin-immunotoxin; SIgA, secretory IgA; ER, endoplasmic reticulum; TSP, total soluble protein

A number of potential therapeutic antibodies have been produced in plants and four case studies are described briefly below.

1)A chimeric secretory IgG-IgA antibody produced in transgenic tobacco plants has been developed to prevent the oral bacterial infection that contributes to dental caries.

2)2) Antibodies and antibody fragments specific for the human CEA have been produced in tobacco, rice, wheat and pea. Plant-produced anti- CEA antibodies are likely to allow the development of an inexpensive method for tumour detection and antibody- based cancer therapy.

3) A humanized antibody against herpes simplex virus 2 (HSV-2) has been produced in transgenic soybean and shown to be efficient in preventing vaginal HSV-2 transmission in mice.

4) A plant virus transient expression system has been used to produce a tumour-specific vaccine for the treatment of malignancies. Tobacco plants were infected with a modified tobacco mosaic virus vector encoding an idiotype-specific scFv corresponding to the immunoglobulin from the 38C13 mouse B cell lymphoma. These plants secreted high levels of scFv protein to the apoplast.

These case studies demonstrate that most therapeutic antibodies currently produced in alternative systems could be produced inexpensively in plants. This would reduce the cost of treatment and increase the number of patients with access to such medicines. This emphasizes the fact that molecular farming in plants can remove financial barriers to the wider use of antibodies in medicine and research. Thus, in the future, antibodies may find uses in the kinds of therapy where cost makes their current use prohibitive.

7.3. Molecular farming of Anti cancers

Post-translational modifications (i.e. glycosylation, folding and multimeric assembly) usually render active proteins (compared with the often insoluble and non-functional proteins rendered by microorganisms), and at the same time they have a low risk of containing human pathogens and endotoxins. It is therefore not surprising that anti-cancer recombinant proteins have been the subject of many reports concerning Plant-Made Pharmaceuticals (PMPs). Although recombinant expression of anti-cancer drugs in plants has been the subject of recent investigation, plants have long been recognized as a traditional source of anti-cancer compounds.

Antibodies for several types of cancer have been expressed in plant leaves or seeds . It is significant that these antibodies were shown to display antitumor activity comparable to that of their mammalian cell-produced counterparts when assayed in mouse models.

Biologically active Cetuximab (Erbitux), an anti-EGFreceptor monoclonal antibody from Imclone, was expressed in corn; Nimotuzumab (TheraCIM), another anti-EGF-receptor antibody from CIMAB–YM Biosciences , has been expressed in Nicotiana.

Recombinant anti-cancer pharmaceuticals expressed in plants

Molecule	Host and/or system	Cancer target
Antibodies		
IgG	Transgenic tobacco	EpCAM colorectal cancer
IgG	Transgenic tobacco	anti-Lewis Y
Murine 38C13, scFv	Tobacco leaves; virus vector	B cell lymphoma
IgG	Transgenic corn	Epidermal Growth Factor Receptor
IgG	Tobacco leaves; agroinfiltration	Epidermal Growth Factor Receptor
IgG1, scFv	Tobacco leaves; agroinfiltration	CEA
Diabody	Tobacco leaves; agroinfiltration; transgenic plants	CEA
scFv	Rice cell culture	CEA
scFv	Transgenic wheat	CEA
scFv	Transgenic pea	CEA
IgG	Transgenic corn	EpCAM colorectal cancer

Source from TRENDS in Biotechnology Vol.25 (10), 2007

7.4. Edible vaccin

Edible vaccines are sub-unit vaccines (antigen) that introduce selected genes into the plants and facilitate the production of the encoded protein. Edible vaccines are mucosal-targeted vaccines that stimulate both the systematic and mucosal immune network. Successful expression of antigens in plants was achieved for *E. coli heat-labile enterotoxin B subunit (LT-B)* , Hepatitis B virus surface antigen , Rabies virus G-protein, Norwalk virus capsid protein and Cholera toxin B subunit (CT-B) etc.

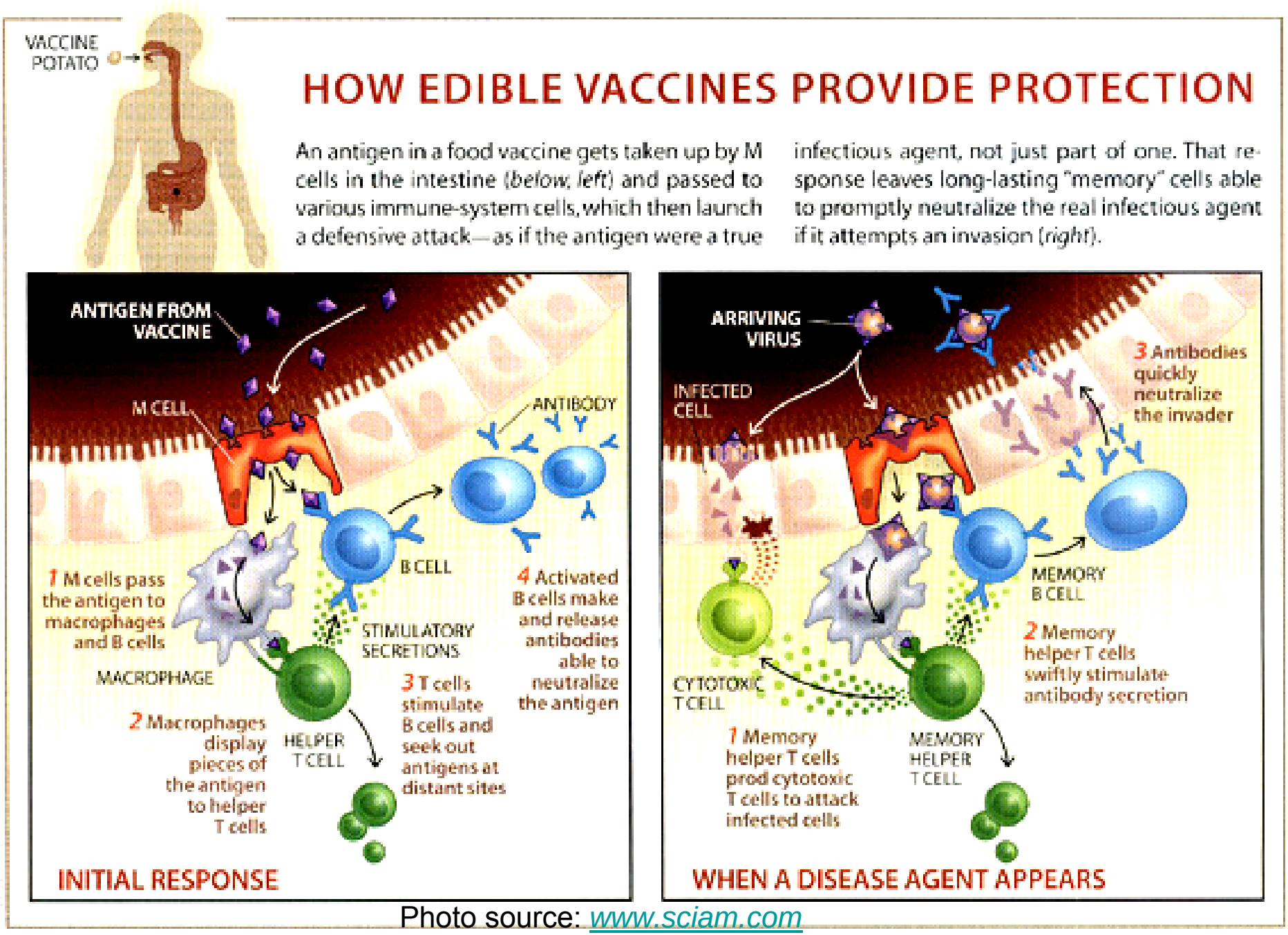


Photo source: www.sciam.com

A number of edible plants have been genetically modified to express a variety of vaccine targets.

Table 1. Edible transgenic plant vaccines

Vaccine	Edible plant
Norwalk virus particle	Potato Tomato
Heat-labile enterotoxin B subunit	Potato Maize Soybean
Cholera toxin B subunit	Rice Potato
Enterotoxigenic *Escherichia coli* fimbrial subunit	Soybean
Japanese cedar pollen peptide	Rice

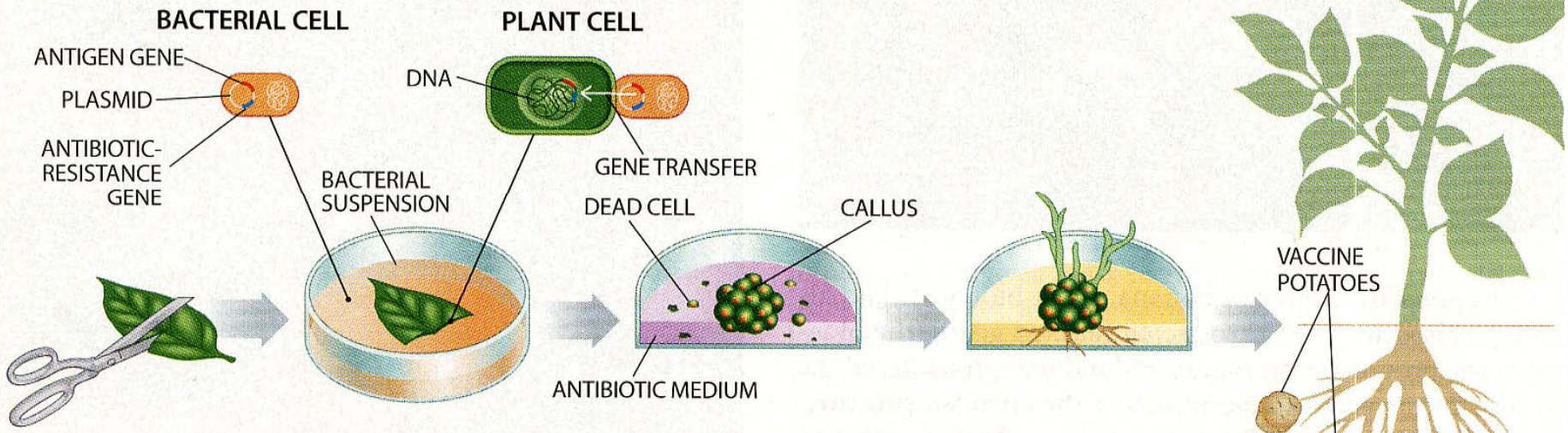
Potatoes are a good system in which to test the idea of edible vaccines. Bananas is a good candidate for edible vaccines since they were eaten raw, appealing to children, inexpensive to produce, native to many developing countries.

1) Toxins produced by *E. coli and V. cholerae can cause* acute watery diarrhoea. The synthetic genes of *E.coli heat-labile enterotoxin B* subunit (LT-B) and cholera toxin B subunit (CT-B) are related proteins with structure, function and immunochemistry similar to those of the actual toxins found in each species. The production of these recombinant proteins in yeast or bacteria is expensive compared to the production of a recombinant potato that produces modified LT-B. It has been shown that mice which eat the transgenic potato raise antibodies in response to the potato LT-B that were effective in inhibiting LT activity on mammalian cells.

Synthetic LT-B coding sequence (sLT-B) was modified for cloning in plants. The potato plant cells were transformed by leaf disc co-cultivation. The transformants were selected and are regenerated as plantlets on a selective media. Tubers from a mature plant were used as seed for the next production cycle. Later the mice were fed with transformed potato, and it was found that the mice had shown resistance to the pathogen. The transgenic potato is useful as a vaccine component or as a booster vaccine.

HOW TO MAKE AN EDIBLE VACCINE

One way of generating edible vaccines relies on the bacterium *Agrobacterium tumefaciens* to deliver into plant cells the genetic blueprints for viral or bacterial "antigens"—proteins that elicit a targeted immune response in the recipient. The diagram illustrates the production of vaccine potatoes.



1 Cut leaf.

2 Expose leaf to bacteria carrying an antigen gene and an antibiotic-resistance gene. Allow bacteria to deliver the genes into leaf cells.

3 Expose leaf to an antibiotic to kill cells that lack the new genes. Wait for surviving (gene-altered) cells to multiply and form a clump (callus).

4 Allow callus to sprout shoots and roots.

5 Put in soil. Within three months, the plantlets will grow into plants bearing antigen-laden vaccine potatoes.

Photo source: www.sciam.com

2) The hepatitis B virus is estimated to have infected 400 million people throughout the globe, making it one of the most common human pathogens. The hepatitis B surface antigen (HbsAg) subtype was cloned into CaMv plasmid and the regenerated plants from the transformed cells were shown to produce HbsAg. Furthermore, expression of the antigen was found to be higher in roots of the transgenic potato than in leaf tissues. However the expression of HbsAg in transgenic potatoes is not sufficient for using as oral vaccine. Further studies are underway to increase the level of the HbsAg by using different promoters such as the patatin promoter, and different transcription regulating elements.

3) Transgenic potatoes carrying a gene for the capsid protein of Norwalk virus – NVCP (causative agent of epidemic gastroenteritis of humans) have been prepared. Capsid protein was expressed in potato tubers, in the amount of approximately 0.37% of total protein. Immunogenicity of transgenic potato plants was tested in mice; IgG antibodies against recombinant Norwalk virus were detected in them. Capability of this "edible vaccine" to activate the immune system was tested also in human volunteers; immune response was activated in the majority (95%) of the people.

4) Measles is a highly contagious viral disease caused by the Paramyxovirus. Spread by air, it includes symptoms such as high fever, skin rash and spots, and it can lead to many different complications which can be even more severe than the disease itself. Each year, almost one million children die from measles, and many of the survivors are weakened by pneumonia or encephalitis or become deaf. Recent studies report expression of the Paramyxovirus surface protein hemagglutinin in tobacco, potato, rice and lettuce with satisfying results. Serum samples from healthy experimental animals, fed with transgenic banana, were analyzed for the presence of anti-hemagglutinin-specific antibodies. The results are highly significant and demonstrate that the banana plant can produce the antigenic hemagglutinin protein of the measles virus and elicit immune responses in experimental animals.

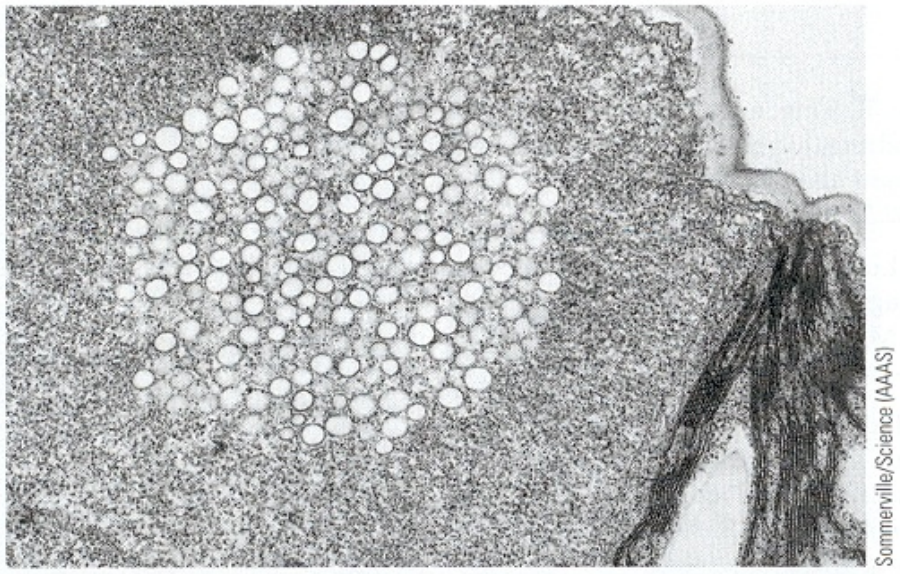
5) In the past 15 years, investigators have identified several beta cell proteins that can elicit autoimmunity in people predisposed to Type I diabetes. The development of plant based diabetes vaccines in potatoes and tobacco containing insulin or GAD linked to the innocuous B subunit of the *V. cholerae* toxin (to enhance uptake of the antigens by M cells) was attempted. The developed transgenic potato and tobacco plants when fed to nonobese diabetic mice, showed increased levels of IgG1, an antibody associated with cytokines that suppress harmful immune responses. "Molecular Pharming" to produce autoantigens in plants targeting other autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus and even transplant rejection is under way.

The other approach, to enhance the expression in plastids, is advocated by some. One such approach is making use of bacterial entertoxins such as CT-B or LT-B. Integration of an unmodified CTB gene into the tobacco chloroplast genome results in accumulation of up to 4.1% of total soluble leaf protein as functional CTB oligomers (410-fold higher than the unmodified CTB gene expressed via the nuclear genome). In addition, binding assays confirm that Chloroplast-synthesized CTB binds to the intestinal membrane GM1- ganglioside receptor, indicating correct folding and disulfide bond formation of the plant-derived CTB pentamers. Increased production of an efficient transmucosal carrier molecule and delivery system, such as CTB, in transgenic chloroplasts makes plant-based oral pharmaceuticals commercially feasible. Because the quaternary structure of many proteins is essential for their function, this investigation shows the potential for other foreign multimeric proteins to be properly expressed and assembled in transgenic chloroplasts.

Recently, Nochi *et al. (2007) describe a rice-based oral vaccine* that potentially addresses many of these topics. At hand is the need for vaccine development and strategies to aid underserved nations with the ability to produce vaccines locally in a cost effective manner. Because rice is produced in many such areas, this current work shows the feasibility of propagating rice-based vaccines that are truly edible vaccines, unlike the earlier work with tobacco that ultimately provided the mechanisms for edible vaccine development. In addition, this approach breaks the cold-chain barrier that for many conventional vaccines drives up cost and creates storage problems. In fact, it is estimated that removal of this barrier could give an added benefit of as much as $300 million per year, which could provide vaccines for an additional 10 million children.

7.5. Biodegradable plastics (PHB)

Biodegradable plastics are an environmentally friendly alternative to synthetic petrochemical polymers and could be used for many purposes in industry and in daily life, and there is a need to develop renewable resources for the future economy. Poly (3-hydroxybutyrate) (PHB) was the first plastic-like compound to be synthesized in transgenic plants. Genes coding for the three key enzymes of the PHB biosynthetic pathway were transferred into plant genomes. Targeting these three enzymes to the chloroplast led to high levels of PHB accumulating in the leaves (up to 40% of leaf dry weight) .



Poly-(3-hydroxybutyrate)

(R)-3-Hydroxybutyryl-CoA

Source from Current Opinion in Plant Biology 8:188–196, 2005

Unfortunately, PHB accumulation and plant growth showed a strong negative correlation.This was probably because the acetyl-CoA pools in the plastids were exhausted. Introducing the PHB-biosynthetic pathway into the plastid genome also caused significant growth defects in transplastomic tobacco plants.

Plastic-like biomaterials can also be achieved by polymerizing amino acids such as poly-γ-glutamate, poly-γ-lysine , poly-aspartate, and cyanophycin a possible source for poly-aspartate of starch production from potatoes (Katrin Neumann et al., 2005).

The ultimate goal of scientists developing biodegradable plastics is agricultural production for industrial applications. Therefore, economic considerations such as the cost of the production systems and the price of plastics play an important role in the development of biodegradable plastics and have to be taken into account from the beginning of such developments. This work opens the way to the future production of biodegradable plastics using a plant based production system. Cyanophycin as a co-product of starch production from potatoes seems to be a possible way to succeed in economic terms.

The economics of molecular farming in plants

From the point of view of industry, the key advantage of molecular farming in plants is the capacity for virtually limitless scale up with minimal associated costs. This will allow transgenic plants to be cultivated over large areas and the potential for profit will increase with scale. There is a long-term demand for many proteins which is barely met by current production systems. Human serum albumin, for example, has an annual worldwide demand of over 500 tons. Currently, the protein is isolated at great expense from its natural source, blood. Transgenic plants could be used as an alternative, cheaper and safer production system limited only by the amount of plant biomass that could be harvested.

High-intensity agriculture can produce surprisingly large amounts of biomass. For example, intensive cultivation of tobacco plants can yield 170 tonnes per hectare. Assuming that the levels of production seen at the laboratory scale could be maintained in the field and that for every 170 tonnes harvested, 100 tonnes are harvested leaves, a single hectare of tobacco could yield 50 kg of a secretory IgA per harvest. Production costs of only US $ 40 per gram have been estimated and with optimization of downstream processing methods this could be reduced to US $ 20. This compares favourably with animal culture systems which are more expensive by two orders of magnitude.

7.6. Phytoremediation

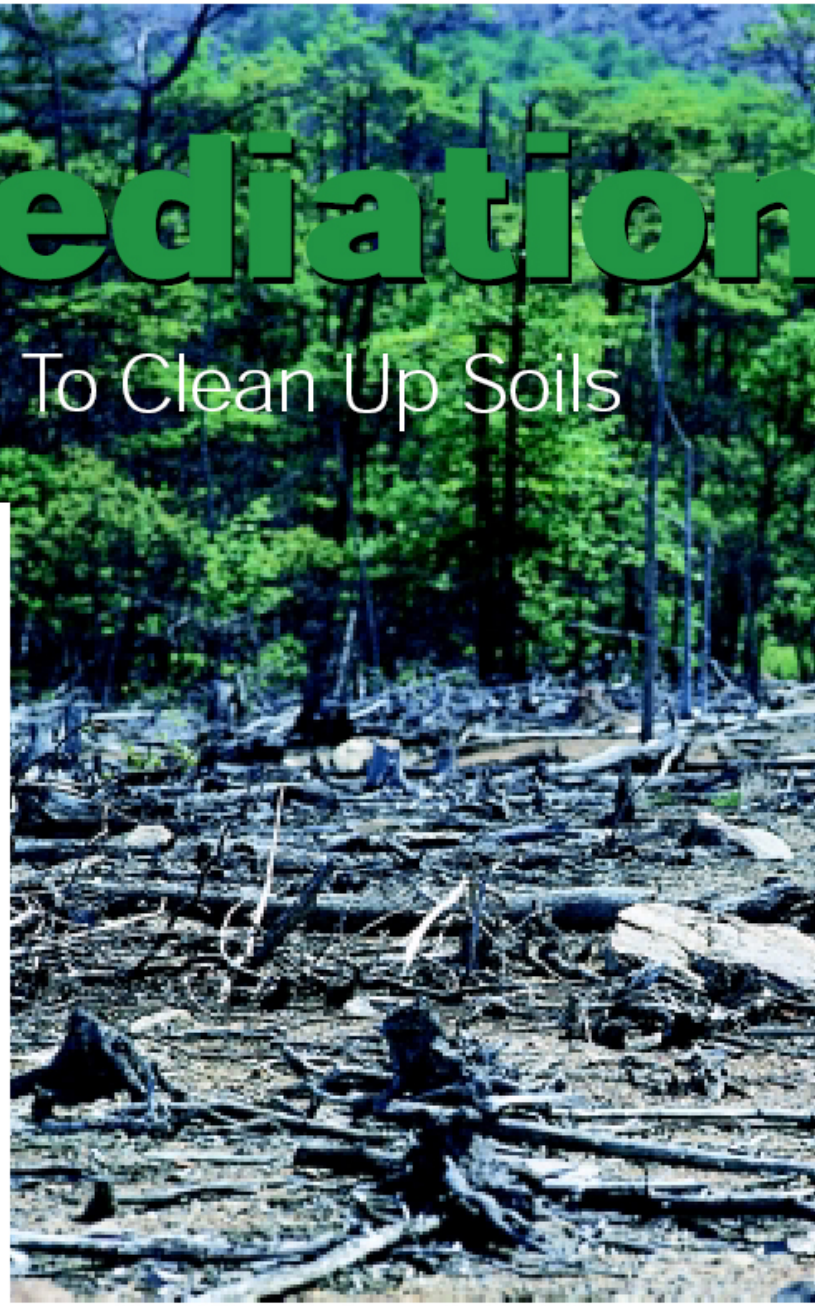
Phytoremediation

Using Plants To Clean Up Soils

Photo source: dels.nas.edu

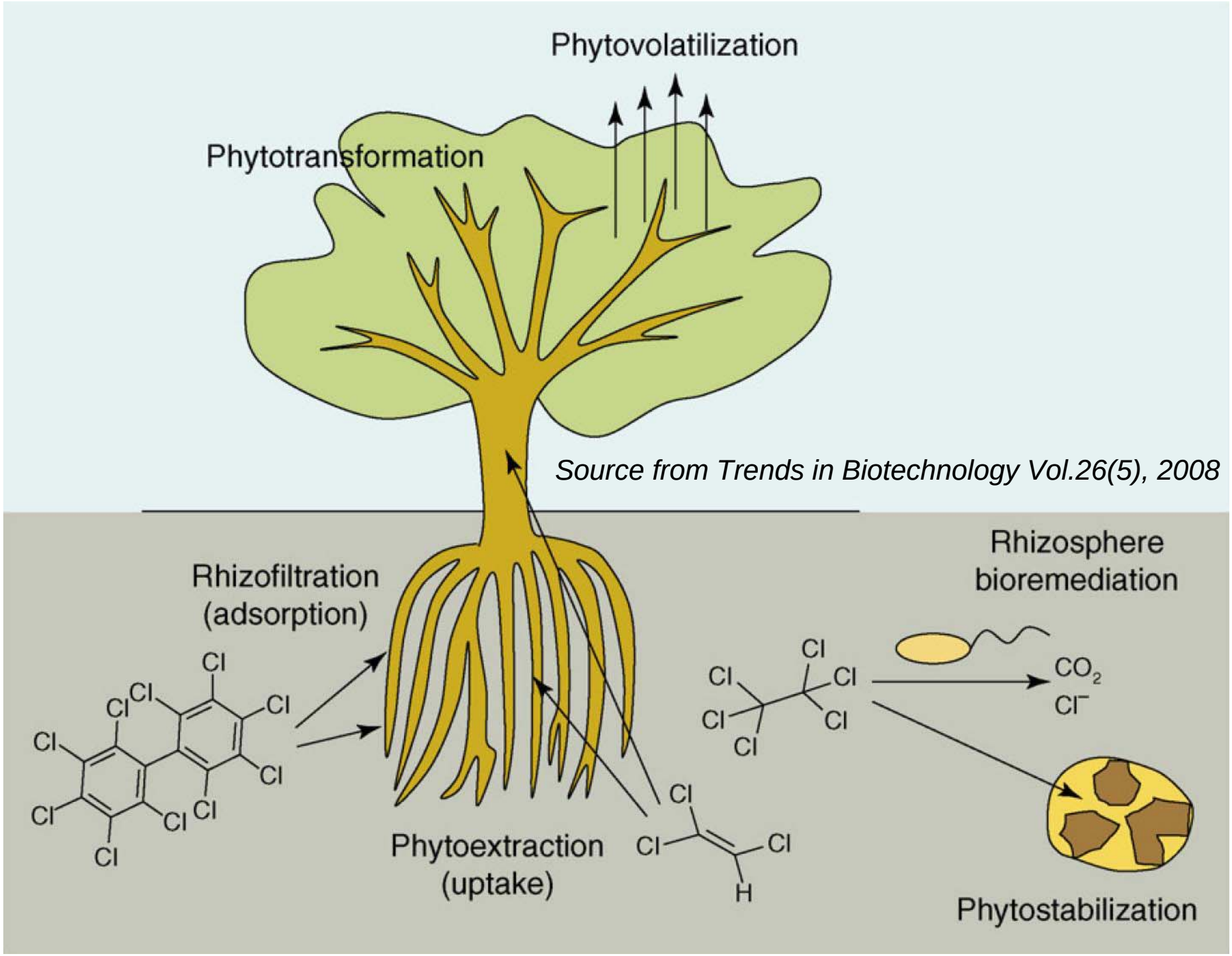
Leaves accumulate metals and are harvested to prevent soil recontamination.

Roots take up metals from contaminated soils and transport the metals to stems and leaves.



Could this be a job for soil-cleaning superplants? The lack of vegetation in the barren area above is a result of the soil's high zinc content and low pH. This site in Palmerton, Pennsylvania, was contaminated by a zinc smeltery operated from 1890 to 1980.

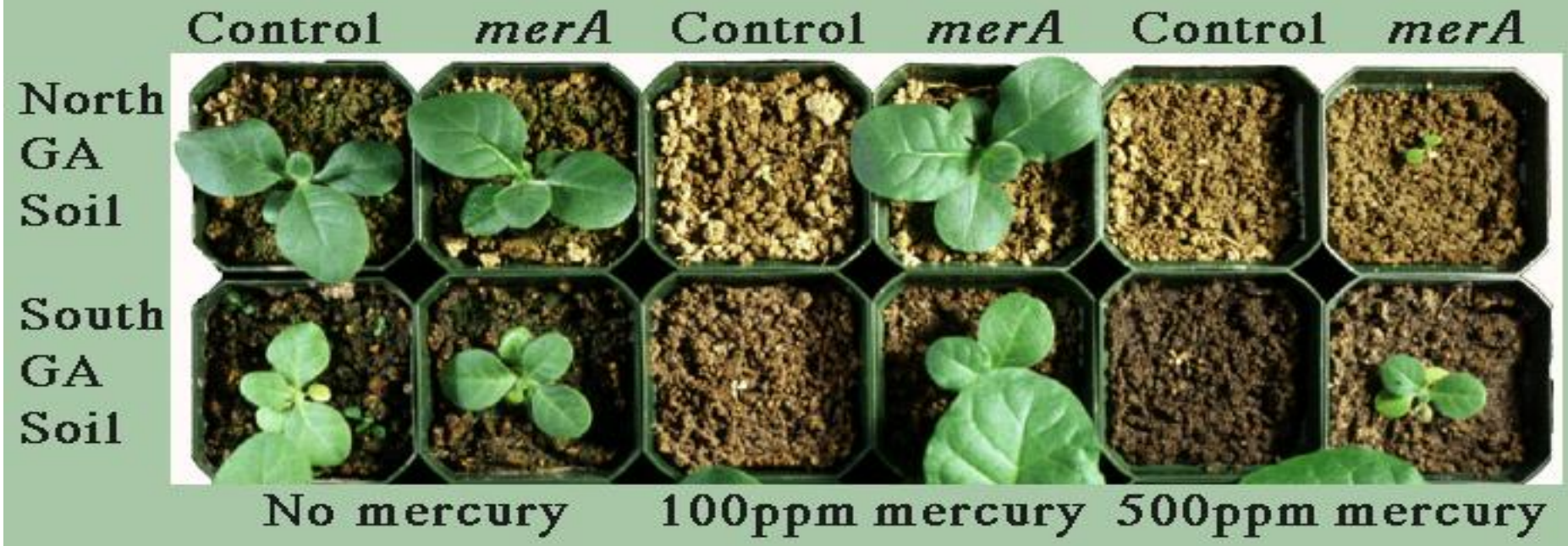
Phytoremediation is the use of plants to clean up environmental pollution. Phytoremediation involves several processes: pollutants in soil and groundwater can be taken up inside plant tissues (phytoextraction) or adsorbed to the roots (rhizofiltration); pollutants inside plant tissues can be transformed by plant enzymes (phytotransformation) or can volatilize into the atmosphere (phytovolatilization); pollutants in soil can be degraded by microbes in the root zone (rhizosphere bioremediation) or incorporated in soil material (phytostabilization).



Source from Trends in Biotechnology Vol.26(5), 2008

Historically, transgenic plants for phytoremediation were first developed in an effort to improve heavy metal tolerance; for example, tobacco plants expressing a yeast metallothionein gene for higher tolerance to cadmium, or *Arabidopsis thaliana* overexpressing a mercuric ion reductase gene for higher tolerance to mercury. The first attempts to transform plants for phytoremediation of organic compounds targeted explosives and halogenated organic compounds in tobacco plants. Although tobacco and *A. thaliana* are good laboratory models, their small stature might not be suitable for field applications. Because detoxification of organic pollutants by plants is often slow, lead to the accumulation of toxic compounds that could be later released into the environment.

Arabidopsis plant with Mer A gene inserted is able to grow on mercurium contaminated soils



www.appliedphytogenetics.com/ apgen/

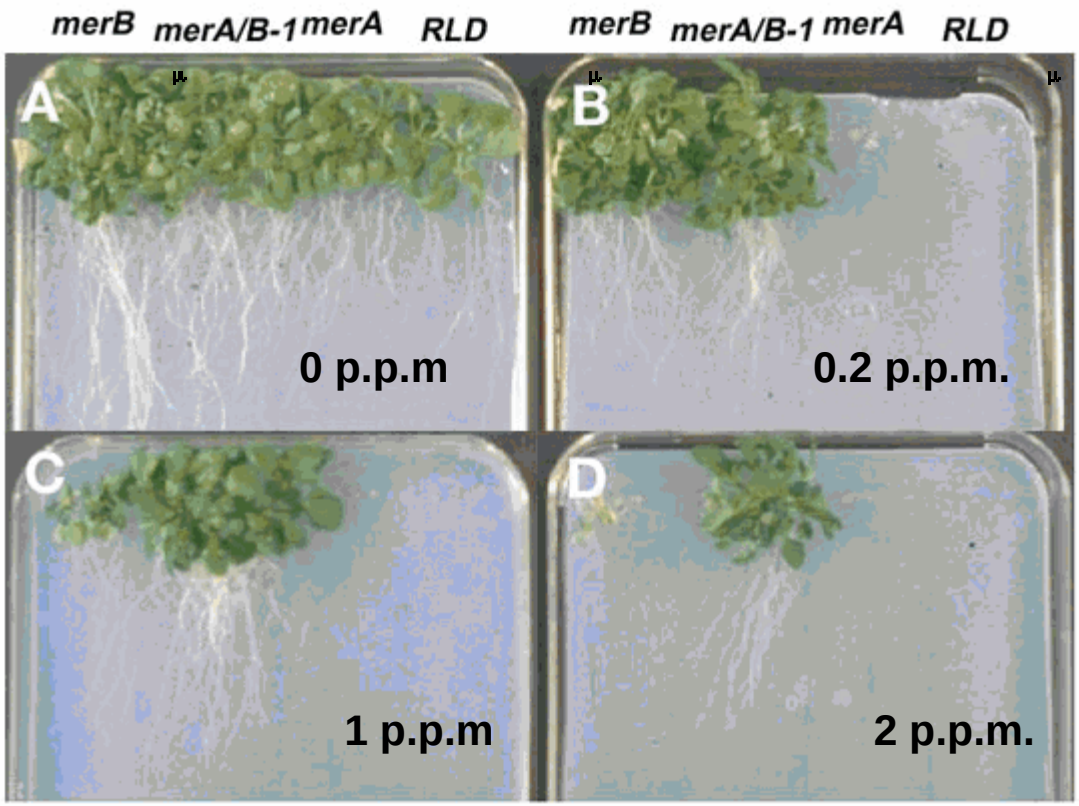
Both merA and merB in Arabidopsis

***merA* for mercuric reductase**

***merB* for organomercurial lyase**

50 times more tolerant to Hg than wt plants

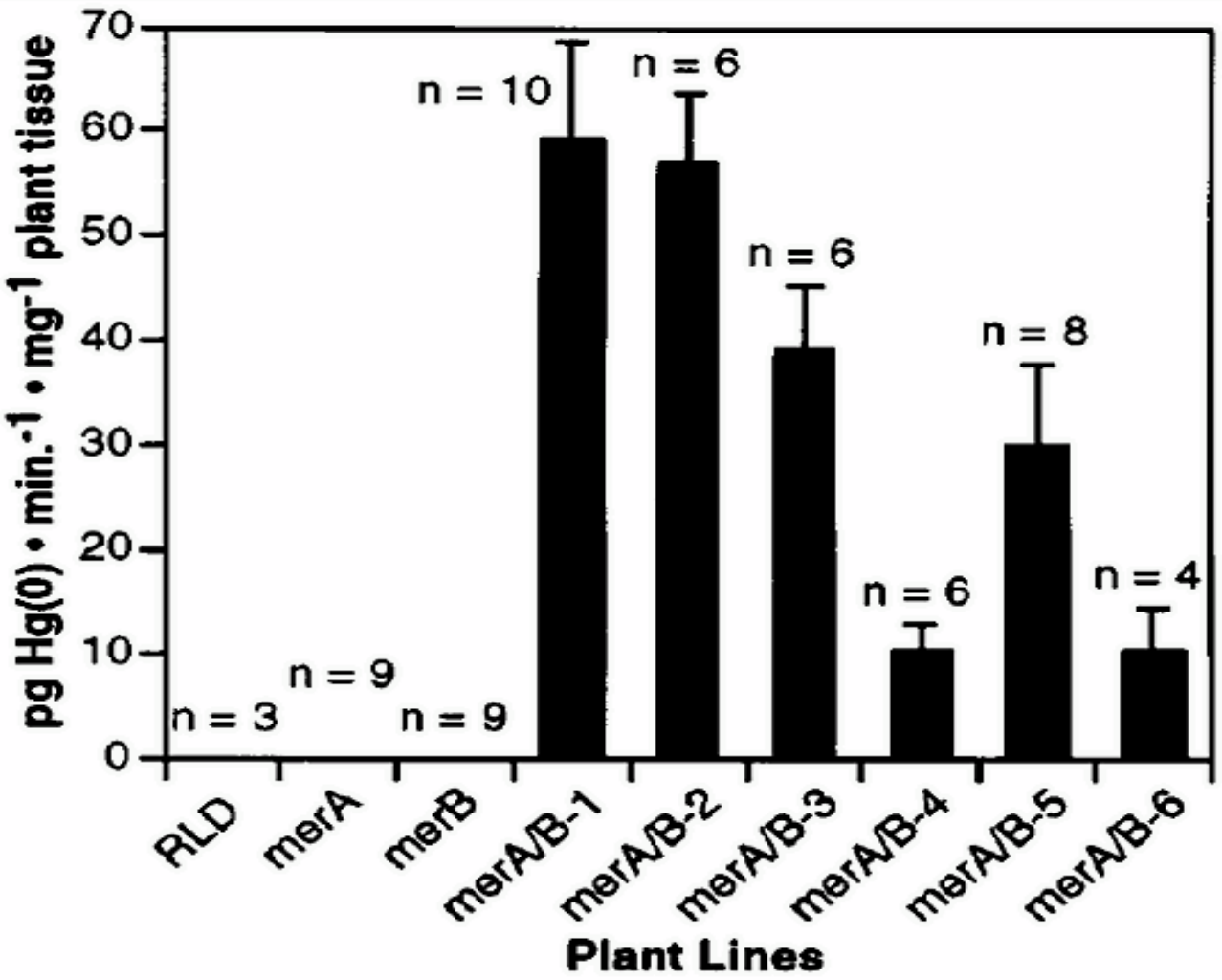
10 times more tolerant to Hg than merA plants



CH_3HgCl

Scott P. Bizily et al., 2000

Only MerA/MerB plants are producing gaseous Hg in a safe (non-bioaccumulative) form

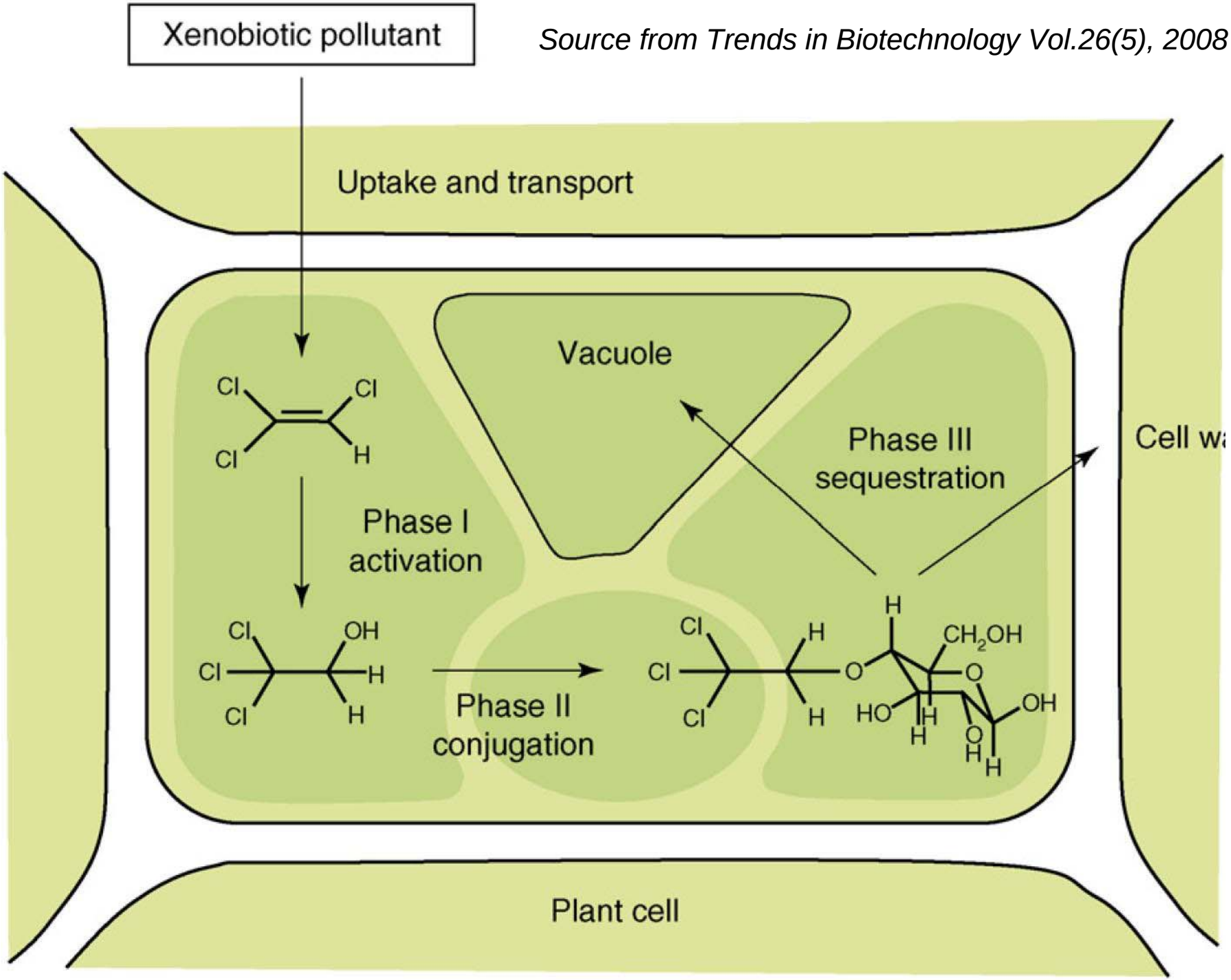


6 different strains of MerA/B arabidopsis vs. wt, MerA and MerB

Scott P. Bizily et al., 2000

A recent publication by Doty and colleagues (2007) describes the development of transgenic poplars (Populus) overexpressing a mammalian cytochrome P450, a family of enzymes commonly involved in the metabolism of toxic compounds. The engineered plants showed enhanced performance with regards to the metabolism of trichloroethylene and the removal of a range of other toxic volatile organic pollutants, including vinyl chloride, carbon tetrachloride, chloroform and benzene. This work suggests that transgenic plants might be able to contribute to the wider and safer application of phytoremediation.

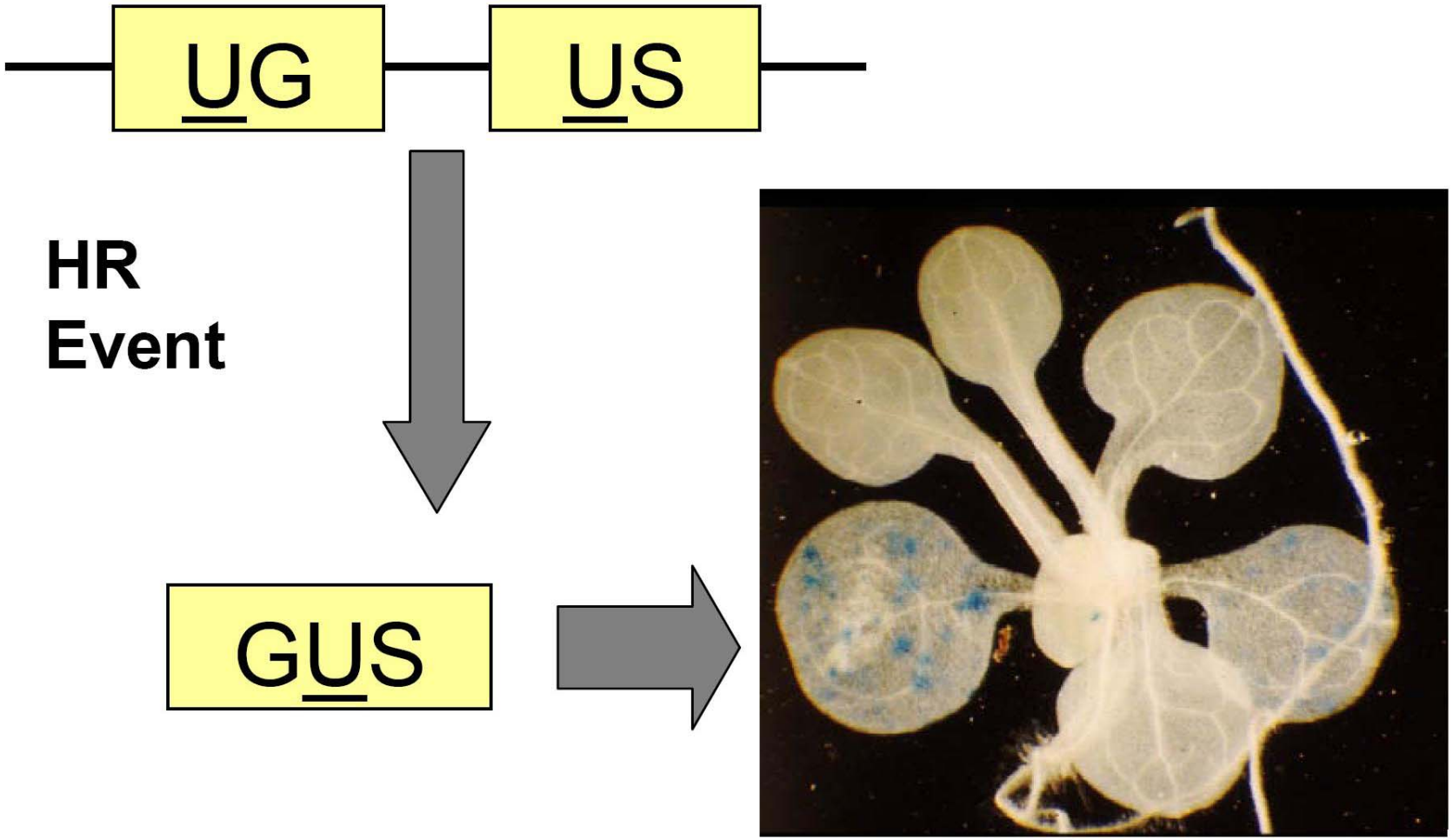
Source from Trends in Biotechnology Vol.26(5), 2008



7.7. Phytosensors

Transgenic plant biosensors of Environmental Pollution Genotoxicity

Transgenic plant biomonitors used for the evaluation of genotoxicity are relatively cheap and simple in use. The assays used in the past decade were based on the restoration of the transgenes β-glucuronidase (uidA or GUS) and luciferase activity in Arabidopsis thaliana or *Nicotiana tabacum* plants transformed with non-active forms of these marker genes. A system for the detection of HR events in transgenic Arabidopsis and tobacco plants utilized the overlapping and truncated versions of a β-glucuronidase. Recombination events at a transgene locus lead to restoration of the uidA (GUS) transgene and synthesis of the β-glucuronidase enzyme.



Transgenic "recombination" system for the detection of environmental mutagens. Transgenic plants carry in the genome two non-functional truncated copies of the GUS gene, depicted as “UG” and “US”. The two parts of the truncated, overlapping GUS gene can be in either orientation with respect to each other. Activation of the β- glucuronidase (GUS) gene via homologous recombination (HR) restores the gene activity and is visualized as blue spots after histochemical staining (**Source from *Sensors , 8, 1539-1558. 2008)***

Upon histochemical staining, the enzyme cleaves the substrate X-gluc which results in the formation of a blue precipitate. Upon ethanol treatment and chlorophyll removal, cells in which recombination events occurred can be precisely localized as blue sectors on the transparent plants. This enables developing a quantitative assay . These plants responded strongly to various mutagens such as UV-C, X-rays and methyl methanesulfonate (MMS) by increasing the mutation frequency in a transgene. Transgenic biomonitoring of HR frequency permits the detection of even minor portions of all possible changes in DNA.

7.8. Salt tolerance

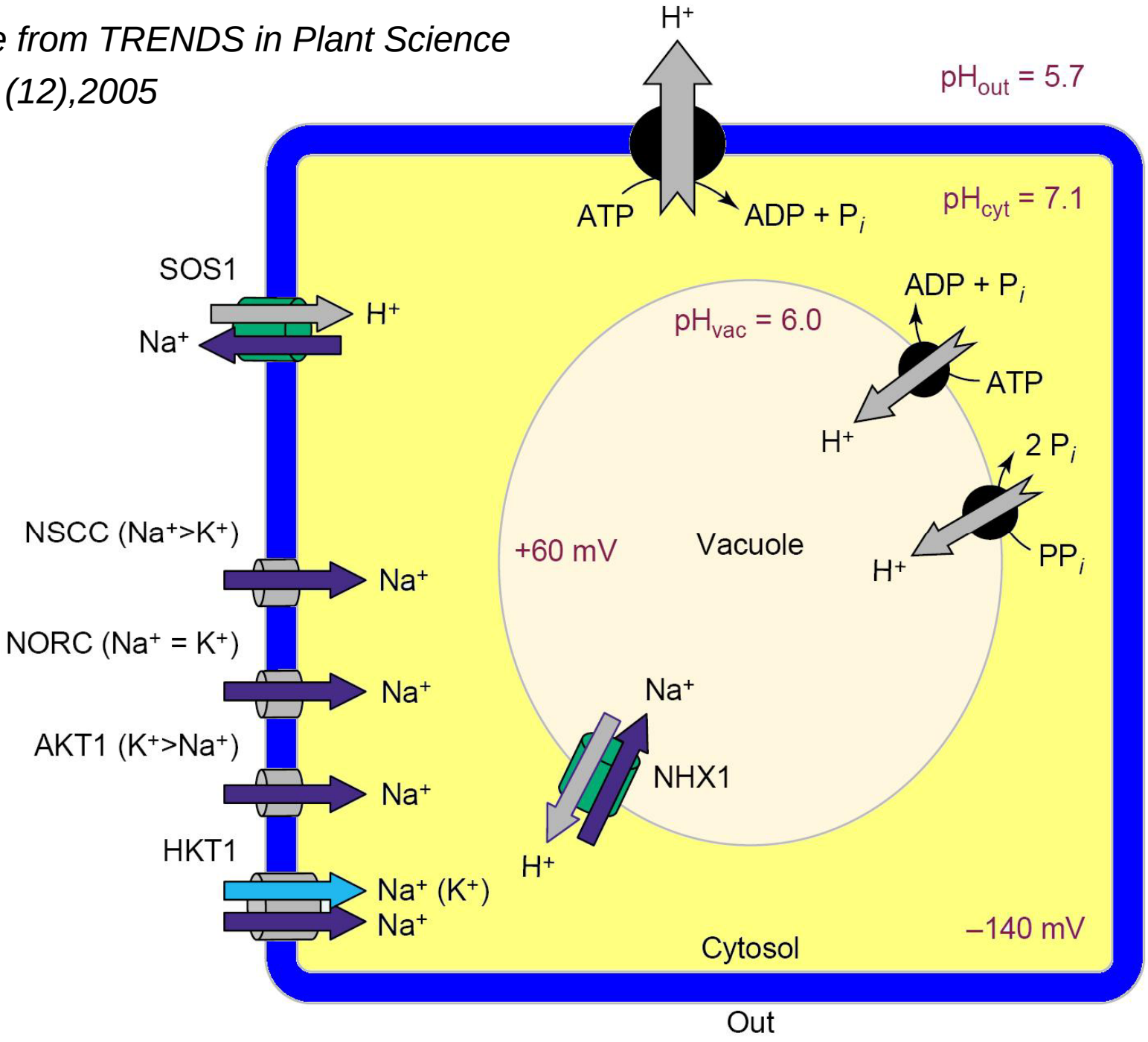
Salt tolerance using transgenic approaches

Soil salinity, one of the major abiotic stresses reducing agricultural productivity, affects large terrestrial areas of the world; the need to produce salt-tolerant crops is evident. Plants respond to salinity using two different types of responses. Salt-sensitive plants restrict the uptake of salt and adjust their osmotic pressure by the synthesis of compatible solutes (e.g. proline, glycinebetaine and sugars). Salt-tolerant plants sequester and accumulate salt into the cell vacuoles, controlling the salt concentrations in the cytosol and maintaining a high cytosolic K+/Na+ ratio in their cells.

Two main approaches are being used to improve salt tolerance: (I) the exploitation of natural genetic variations, either through direct selection in stressful environments or through mapping quantitative trait loci and subsequent marker-assisted selection; and (II) the generation of transgenic plants to introduce novel genes or to alter expression levels of the existing genes to affect the degree of salt stress tolerance.

The overexpression of AtNHX1, a vacuolar Na+ /H+ antiporter, in Arabidopsis resulted in transgenic plants that were able to grow in high concentrations of salt .

Source from TRENDS in Plant Science
Vol.10 (12),2005



The paramount role of Na+compartmentation in plant salt tolerance has been further demonstrated in transgenic tomato plants overexpressing AtNHX1. The transgenic tomato plants grown in the presence of 200 mM NaCl were able to grow, flower and set fruit. Similar results were obtained with transgenic *Brassica napus* (canola) overexpressing AtNHX1.,rice vacuolar Na+/H+ antiporter (OsNHX1). The overexpression of AtNHX1 resulted in enhanced salt tolerance in transgenic maize and wheat. The overexpression of BnNHX1 (*Brassica napus*), HbNHX1 (barley) and GhNHX1 (cotton) resulted in enhanced salt tolerance in transgenic tobacco..

a

b

c

Control tomatoes at 200 mM NaCl

Transformed tomatoes at 200 mM NaCl

Barnum, "Biotechnology: An introduction", 2nd edn

Table 1 Mechanisms, genes and genetically modified plant species implicated in plant responses to abiotic stress

Mechanism	Genes	Species	Reference
Transcription control	CBF1	*Arabidopsis thaliana*	Jaglo-Ottosen et al. 1998
	DREB1A	*A. thaliana*	Kasuga et al. 1999
	CBF3	*A. thaliana*	Gilmour et al. 2000
	CBFs	*Brassica napus*	Jaglo et al. 2001
	CBF1	*Lycopersicon esculentum*	Hsieh et al. 2002
	CBF4	*A. thaliana*	Haake et al. 2002
	AtMYC2 and AtMYB2	*A. thaliana*	Abe et al. 2003
	ABF3 or ABF4	*A. thaliana*	Kang et al. 2002
	HSF1 and HSF3	*A. thaliana*	JH Lee et al. 1995; Prändl et al. 1998
	HsfA1	*L. esculentum*	Mishra et al. 2002
	spl7	*Oryza sativa*	Yamanouchi et al. 2002
Compatible solute			
Proline	P5CS	*Nicotiana tabacum*	Kishor et al. 1995; Konstantinova et al. 2002; Hong et al. 2000
	ProDH	*A. thaliana*	Nanjo et al. 1999
Myo-inositol	IMT1	*N. tabacum*	Sheveleva et al. 1997
Sorbitol	*stpd1*	*N. tabacum*	Sheveleva et al. 1998
Antioxidants and	CuZn-SOD	*N. tabacum*	Gupta et al. 1993a, 1993b; Pitcher and Zilinskas 1996
detoxification	Mn-SOD or Fe-SOD	*Medicago sativa*, *N. tabacum*	McKersie et al. 1996, 1999, 2000; Van Camp et al. 1996
	GST and GPX	*N. tabacum*	Roxas et al. 1997
	*chy*B	*A. thaliana*	Davison et al. 2002
	Aldose-aldehyde reductase	*N. tabacum*	Oberschall et al. 2000
Ion transport	*AtNHX1*	*A. thaliana*	Apse et al. 1999
		B. napus	Zhang et al. 2001
		L. esculentum	Zhang and Blumwald 2001
	SOS1	*A. thaliana*	Shi et al. (2003)
	HAL1	*Cucurbita melo*	Bordas et al. 1997
			Rus et al. 2001
	AVP1	*A. thaliana*	Gaxiola et al. 2001
Hsps and molecular	Hsp17.7	*Daucus carota*	Malik et al. 1999
chaperones	Hsp21	*A. thaliana*	Härndahl et al. 1999
	AtHSP17.6A	*A. thaliana*	Sun et al. 2001
	DnaK1	*N. tabacum*	Sugino et al. 1999
	SP1	*Populus tremula*	Wang et al. 2003
LEA-type proteins	COR15a	*A. thaliana*	Artus et al. 1996; Steponkus et al. 1998; Jaglo-Ottosen et al. 1998
	HVA1	*O. sativa*	Xu et al. 1996
		Triticum aestivum	Sivamani et al. 2000
	WCS19	*A. thaliana*	Ndong et al. 2002

Source from Planta 218: 1–14, 2003

7.9. The future of GM plants

Public acceptance of genetically altered crops is one of the biggest obstacles to commercial application. First generation GM plants may only slowly gain public favor because of concern about unintended environmental and dietary effects. A feasible solution may be to include comprehensive metabolite analysis by metabolomics. Metabolomics is defined as "the technology geared towards providing an essentially unbiased, comprehensive qualitative and quantitative overview of the metabolites present in an organism".

Metabolomics is now rapidly developing by a combination of a variety of techniques such as gas chromatography mass spectrometry (GC–MS), liquid chromatography mass spectrometry (LC–MS), Fourier transform mass spectrometry (FT–MS), quadrupole time-of flight mass spectrometry (QTOF–MS) and nuclear magnetic resonance (NMR). Metabolomics is being applied not only to functional identification of novel genes but also to the investigation of compositional similarities between GM and conventional crops. Consumers may yet be skeptical about GM plants, despite the accumulation of scientific data, due to the inherent sociological or psychological factors associated with the risks of new technology.[70]