Foods Derived from Genetically Modified Organisms and Detection Methods

by Wolfram Hemmer

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Table of Contents

Summary

This report reviews currently approved genetically modified food crops, products from modified microorganisms for food use, and molecular methods currently applicable or under development for detecting foods derived from genetically modified organisms (GMOs).

Up-to-date reviews on approvals of genetically engineered organisms or food products (in the United States, the United Kingdom, the Netherlands, Australia, Canada, Japan, the European Community, and Switzerland), including a comprehensive compilation of genetic and biochemical information on the respective products are presented. >From a survey of the genetically engineered agricultural crops it was found that there were 28 approved, genetically distinct plant products (Minor genetic differences between several lines of certain products derived from independent transformation events of the same or virtually the same host plants were not considered here.). These products were chicory (1), corn (6), cotton (4), papaya (1), potato (2), rapeseed (4), soybean (2), squash (2), tobacco (1) and tomato (5). The survey on the genetic elements (promoters, structural genes, terminators) introduced into the approved modified agricultural crops along with other pertinent data presented in this report, could provide the basis for the development of efficient screening methods and product-specific techniques for detecting genetically engineered food products.

The other main objective of this paper is to review published analytical methods developed for identifying genetically engineered foods. Relevant methods which may be useful for designing identification techniques are also given. Included are: validated, official methods for the detection of GMOs; methods developed for the identification of GMOs in food stuffs published in specialised scientific journals or in reports unavailable in databases; other scientific articles describing the identification of DNA sequences that have also been used for generating GMOs; and publications from relevant, related fields such as authenticity testing or the detection of pathogens. Most of the techniques currently available use the polymerase chain reaction (PCR) for the amplification of DNA-sequences introduced into the plants by genetic engineering. The applicability of other nucleotide-based and protein-based detection techniques for the analysis of food stuffs is also discussed.

With the increasing availability of genetically engineered plant products, it will become a necessity to have the proper techniques for the identification of such foods as a means for controlling adherence to labelling requirements and other regulations. A two-step approach might be most adequate and effective. First, widely applicable PCR-based screening methods should be used which target sequences present in genetic elements most commonly found in transgenic crops, such as the aminoglycoside-3'-phosphotransferase (nptII) marker gene, the cauliflower mosaic virus 35S promoter (P-35S) or the nos-terminator (nos 3'). Second, the product could then be conclusively identified through a product-specific technique. The accessibility to comprehensive databases containing relevant genetic information on genetically engineered products may be an important factor in promoting and coordinating the development of identification techniques.

1 Introduction

Biotechnological procedures have been employed over millennia to produce human food stuffs such as bread, yoghurt, beer, wine or cheese. Ancient peoples made use of microorganisms like yeast and bacteria without even knowing of their existence. Today, we know that there are innumerable distinct yeast and bacterial strains, some of which are exploited in commercial fermentation processes after having being selected for certain characteristics to optimise product quality or production processes (Hui and Khachatourians, 1995). Only limited information is available about the genetic background of the specific traits of most of the microorganisms we employ today in fermentation processes. Modern techniques of biotechnology make it possible to introduce distinct genes or groups of genes into a variety of organisms. The application of genetic engineering has become essential for biotechnology and many other modern biological and medical sciences.

Apart from amylases which have been used for starch processing since the early 1980s, chymosin was the first commercial biotechnology product to be used in human food stuffs. It serves as a substitute for the calf stomach preparations, traditionally used as the natural source of chymosin, in the manufacture of cheese (Teuber, 1993). Although approved for use in cheese production by Swiss authorities as early as 1988, recombinantly produced chymosin has never been commercially used in this country due to a voluntary renunciation by the cheese manufacturers. It has since been approved in more than 20 countries (Teuber, 1993); more than 60 % of the hard cheese in the United States is produced by means of recombinantly produced chymosin. Germany, on the other hand, represents one of the more

prominent countries that have not (yet) approved this product (Krohn and Pfleger, 1994).

In some countries other recombinantly expressed enzymes and organic molecules produced through genetic engineering have also been approved. However, regulations with respect to enzymes and other products produced by genetically modified microorganisms differ significantly from nation to nation.

1.1 Field trials

Genetic engineering of agricultural crops has become a main activity of the research departments in the agro-industry. GMOs comprising at least 27 distinct plant species have been tested in field trials in the European Community (EC) (Table 1).

Table 1: Field tests in the European Community and in the United States

Plant	*EC**	*USA***
Alfalfa	2	18
Amelanchier laevis		<6
Apple	1	5
Arabidopsis		<6
Barley		<6
Belladonna		<6
Broccoli		<6
Carnation	3	
Carrot	1	<6
Cauliflower	5	
Chicory	32	<6
Chrysanthemum	1	<6
Cotton	1	191
Cranberry		<6
Creeping Bentgrass		7

Plant	*EC**	*USA***
Papaya		<6
Pea		<6
Peanut		<6
Pepper		<6
Petunia	1	<6
Plum		<4
Poplar	6	<6
Potato	86	261
Rape / oilseed rape	188	57
Rice		13
Silver birch	1	
Soybean	6	278
Spruce		<6
Squash	2	106***

Cucumber		12
Eggplant		<6
Eucalyptus	3	
Gladiolus		<6
Grapevine	2	<6
Lettuce	4	6
Maize (corn)	192	1019
Marigold	8	
Melon	4	106***
Onion		<6
Subtotal	295	-

Strawberry	1	5
Sugarbeet	109	23
Sugarcane		<6
Sunflower	6	8
Sweet Potato		<6
Sweetgum		<6
Tobacco	30	98
Tomato	45	321
Walnut		<6
Watermelon		<6
Wheat	6	14
Total	746	ca. 2450

* Source: SNIFS (1996) as of 31 October 1996
** Source: APHIS ('Field Test Permits' and 'Notifications' 1987-1996, as of 31 October 1996);
the numbers marked with *** represent the sum of melon and squash field releases in the US.

Most of the field tests within the EC were performed in Belgium, France, Italy, the Netherlands and in the United Kingdom (81 %; >70 field tests per country); only 19 % of the releases took place in Austria, Denmark, Germany, Finland, Portugal, Spain and Sweden (SNIFS, 1996). Until October 1996, only 2 field tests had been conducted in Austria and 2 in Switzerland, and 60 in Germany. In contrast, the number of field tests in France totalled 228 and in the United States with more than 2,000[1]. This uneven distribution is only partially accountable by regulatory and climatic differences of the countries cited; differences in the general public acceptance of gene technology in each country apparently plays an important role. In particular, the German-speaking populations in Europe appear more sceptical than others towards the application of this technology in the food industry. The public attitude towards gene technology should not be overlooked; in 1995, more than half of the field sites in Germany for testing transgenic plants were deliberately destroyed (Abbott, 1996; Hobom, 1996).

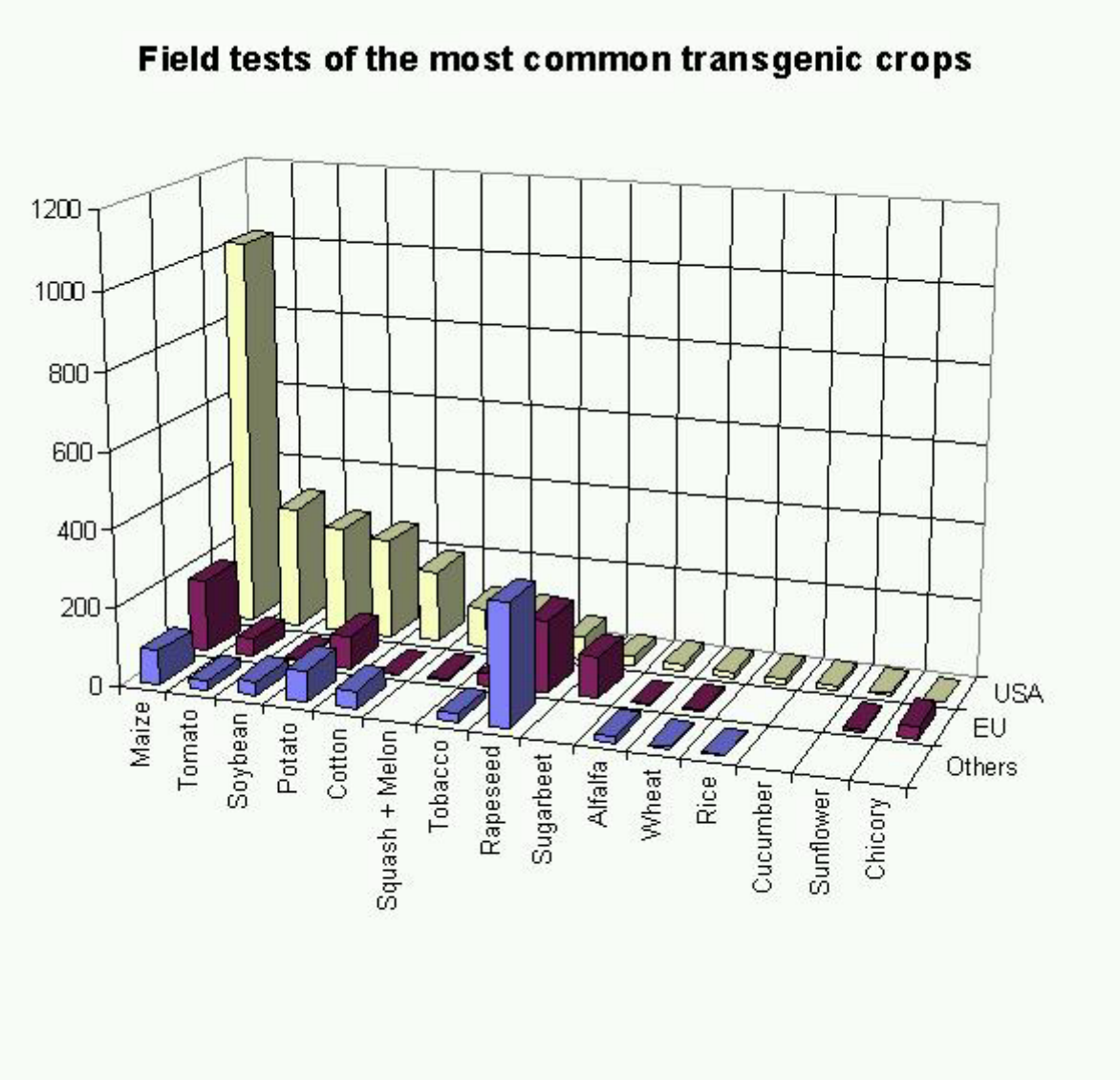

Figure 1. Field tests of the most common transgenic crops. Sources: (i) USA: APHIS ('Field Test Permits' and 'Notifications' 1987-1996, as of 31 October 1996; (ii) EU: SNIFS (1996) as of 31 October 1996; (iii) Others (Australia, Bulgaria, Canada, Japan, New Zealand, Switzerland, South Africa and several developing countries): OECD-database on field trials as of 24 October 1996; DeKathen, 1996.

The list of transgenic plants which have been field tested in the US is far more extensive than the one for the EC (Table 1, Figure 1). In Canada1, several hundred field tests have taken place. Approximately 150 releases have been reported in developing countries (De Kathen, 1996)1. Until 31 December 1995, reportedly 11 and 22 field tests took place in Russia and Hungary, whereas the figure of 60 field tests was reported for China (James and Krattiger, 1996). A single approval for a field test can include several field sites. This may in part be accountable for the fact that figures for the field tests in certain countries given by James and Krattiger (1996) are somewhat higher than e.g. the numbers derived from the 'summary notifications' (SNIFS) in the EC.

Most of the research in the application of gene technology on food crops has sought to improve product quality and agronomic traits and develop resistance to pests (Table 2). Background literature about techniques and research goals in the area of

transgenic plants can be found in recently published reviews (Lupi, 1995; Bendiek et al., 1996; James and Krattiger, 1996; Niederhauser et al., 1996; Estruch et al., 1997; Gaede, 1997), in special issues of journals in German (Biologie in unserer Zeit 4/1995: 'Gentechnik und Lebensmittel') or English (Trends in Biotechnology: 'Plant-product and crop biotechnology', Vol. 13 [9], 1995) and in books (Brandt, 1995; Potrykus and Spangenberg, 1995).

Table 2: Research objectives

I Product quality:	• Carbohydrate metabolism • Colour • durability • Fatty acid metabolism • Firmness • Fruit ripening delay • Processing value
II Pest resistance:	• Bacterial resistance • Fungal resistance • Insect resistance • Nematode resistance • Viral resistance
III Agronomic trait:	• Drought resistance • Herbicide tolerance • Hybrid system • Nitrate reduction • Salt tolerance • Temperature resistance
IV Others:	• Heavy metal tolerance • Monitoring

[1] Data from various sources on field trials in the US, the EC as a whole and individual EC countries (Austria, Belgium, Denmark, Finland, France, Germany, Italy, the Netherlands, Portugal, Spain, United Kingdom), Switzerland, Bulgaria, Canada, Australia, New Zealand, Japan and some developing countries (Argentina, Belize, Bolivia, Chile, Costa Rica, Cuba, Dominican Republic, Egypt, Guatemala, India, Mexico, Peru, South Africa, Thailand and the Commonwealth of Puerto Rico) are continuously being compiled in a database at the agency BATS.

1.2 Transformation methods and genetic elements introduced into transgenic plants

Numerous methods have been developed that are used to introduce and integrate 'foreign' DNA into plant cells, leading to transformed plant phenotypes. Only those methods used in the transformation of approved agricultural crops will be briefly described below. These are methodologies based on (i) biological vectors, (ii) physical or (iii) chemical methods (occasionally used in combination with

electroporation). For a more detailed description of the methodology, including protocols, the reader is referred to Potrykus and Spangenberg (1995).

A transformation system should allow for (Niederhauser et al., 1996):

- Stable integration into the host genome without structural alterations of the foreign DNA.
- Integration of a distinct number of copies of the transforming DNA (usually 1).
- Stability of the new phenotype over several generations.
- Eventual tissue- and development-specific regulation of the introduced gene.

Points one and three are affected primarily by the choice within the first generation of transformants and by long-term selection for a transgenic marker. The fourth feature depends on the choice of the promoter regulating the transcription of the transgene, but possibly also on the presence of targeting sequences that are directing the gene product to certain organelles (e.g. chloroplast transit peptide sequences). The currently used transformation methods do not allow for a precise prediction of the number of copies of the transforming DNA that will be integrated into the plant cell genome. Conventional back-crossing of the transformants with the untransformed phenotype is frequently one technique for reducing the number of copies of the transforming DNA (per haploid genome) to one or a few. Structural integrity of the introduced DNA and the precision with which the boundaries of the sequences which will ultimately integrate into the host genome can be predicted is partially dependent on the choice (if there is one) of the transformation system (see below).

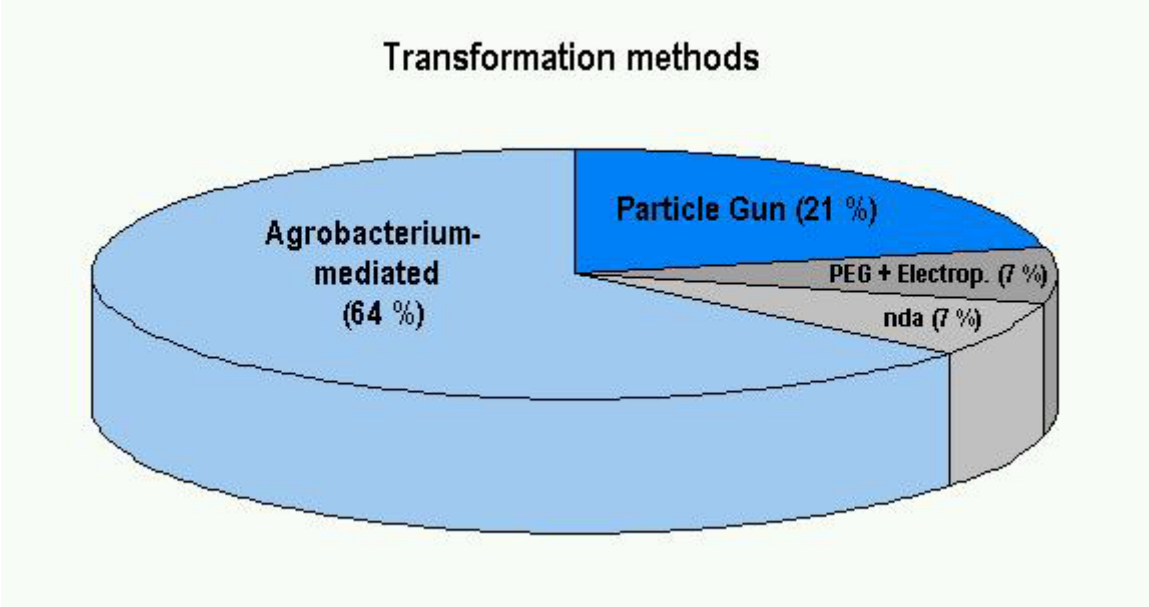

Figure 2. Prevalence of transformation methods used for approved genetically engineered agricultural crops (28 genetically distinct products in total; see later sections). At least 18 out of the 28 products (64 %) have been transformed with *Agrobacterium*, 6 products with physical methods (e.g. particle gun) and 2 with either a chemical method (e. g. using polyethyleneglycol, PEG) or by electroporation. No data were available (nda) on the transformation system used for 2 of the 28 genetically distinct products.

Among the array of genetically engineered plants which have currently been approved, the transformation method of choice has been the use of modified plasmids of *Agrobacterium* (Figure 2). This is most often a binary vector system derived from *Agrobacterium tumefaciens*, where one vector contains the genes to be transferred and the other harbours genes (*vir* genes, not transferred) encoding the necessary functions for transfer to occur (McBride and Summerfelt, 1990). The system is based on a 'disarmed' Ti-plasmid with genes responsible for the crown gall disease being removed. The foreign DNA is confined by the right and left border sequences (25 basepairs each); these are the only elements from *Agrobacterium* transferred together with the T-DNA. This method ensures that a defined region of the presented DNA is precisely transferred to the new host genome. As mentioned earlier, several copies of this DNA may integrate at the same or at distinct sites in the plant chromosome.

Other transformation methods are based on physical and chemical principles. According to one method, DNA fragments are bound to the surface of minute metal particles and shot into plant cells using specially developed devices. The chemical methods make use of polyethyleneglycol (PEG) or CaCl2 to facilitate the entrance of foreign DNA through the plant cell wall. Electroporation represents another transformation method. Plants transformed using electroporation, chemical or physical methods generally carry copies of the entire DNA fragments presented. These plants may thus contain copies of the antibiotic resistance genes used for the propagation of the respective constructs in bacteria, if such has not been prevented by removing the respective genes through restriction enzymes prior to transformation. Frequently, some sections of the presented plasmid sequences are not transferred using these methods. Therefore, the boundaries of the transferred DNA will be predicted with less precision using these methods than with *Agrobacterium*-mediated transformation methods.

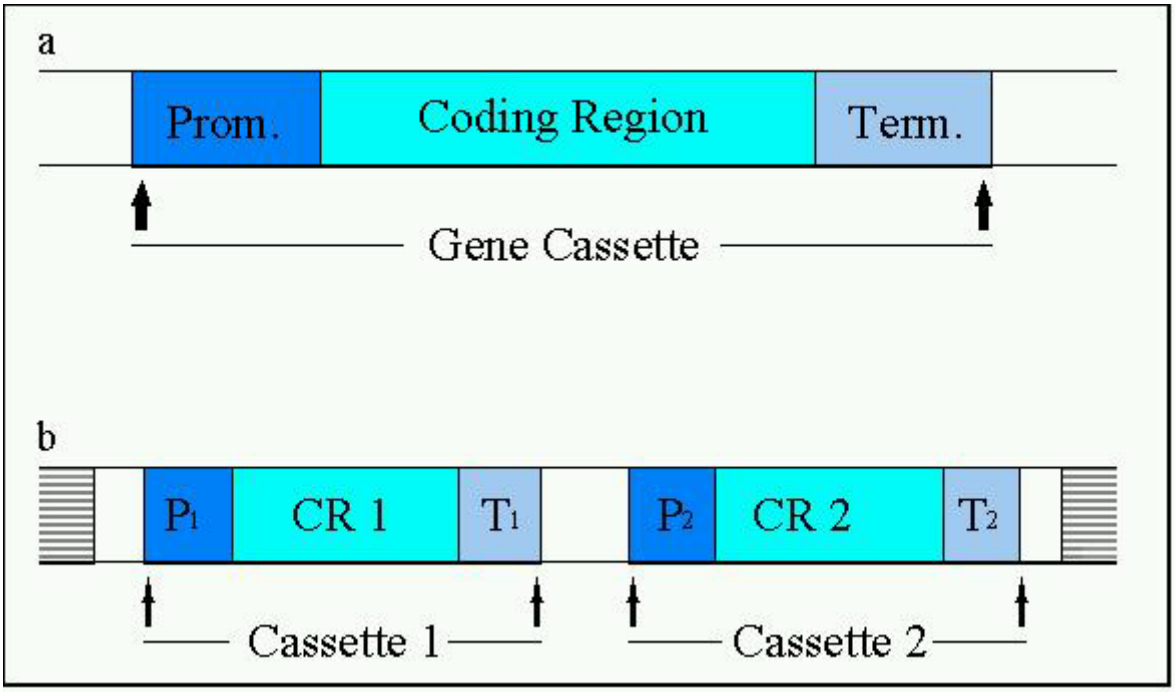

Figure 3. (a) Schematic representation of gene cassettes, consisting of a promoter (P), a structural gene ('coding region') and a terminator (T); (b) frequently, two (or

more) cassettes are transferred together and integrated into the host genome (horizontally bars) at one or several sites.

Enzymes are the products of the majority of transgenes introduced into the currently approved genetically engineered agricultural crops. The expression of these enzymes has conferred novel traits to the respective plants. Proteins without an enzymatic activity, such as viral coat proteins or the Bt-toxin (-endotoxin from *Bacillus thuringiensis*), or antisense constructs have also been expressed. Efficient expression of structural genes is assured only when they are controlled by plant-derived promoters or by other promoters that are active in plant cells such as the cauliflower mosaic virus 35S promoter. Terminator sequences also have to originate from plant sources or from plant pests such as the cauliflower mosaic virus or *Agrobacterium*.

Direct selection for the many of the actual trait genes (e.g. those conferring delayed fruit ripening) is not possible. Therefore selectable marker genes, such as genes allowing growth in the presence of antibiotics or herbicides, are often co-transformed with the actual trait gene(s) together with appropriate regulatory sequences (Figure 3b). The final number of 'foreign' gene cassettes that are present in a transgenic crop may be as high as 4 or 5 due to the presence of multiple trait genes and marker gene(s) (Table 3).

1.3 Detection methods

The new regulation on food stuffs ('Lebensmittelgesetzgebung') that became effective in Switzerland stipulates that all food stuffs, food additives and processing aids that are derived from or which contain GMOs require premarket approval (Article 15, LMV) and must be labelled as 'GVO-Erzeugnis' (GMO-product) according to Articles 22k and 23, 'Lebensmittelverordnung' (LMV). Products exempt from the labelling requirement need to be free of the GMO itself and must have been purified from the (transgenic) DNA (e.g. chemically defined substances like sugar). The novel food regulations under discussion within the EC commission will basically require labelling of GMO products if they are distinguishable from conventional products by scientific methods.

All GMOs that are used in food stuffs in the United Kingdom have to be approved by the ACNFP (Advisory Committee on Novel Foods and Processes). The British FAC (Food Advisory Committee) has developed a classification system that should be helpful in deciding whether labelling of a product is necessary or desirable (Atkins et al., 1992; Teuber, 1993):

- Nature-identical food products of genetically modified organisms: foods that are the product of, or which contain products of a GMO (but not the organism itself, its cells or DNA) and are identical to products from conventional organisms traditionally consumed (e.g. chymosin). The FAC concluded that labelling of chymosin or cheese produced with it is not required.
- Foods containing recombinant DNA which were produced by introducing genes only from the gene pool of its own species (self-cloning); e.g. bakers' and brewers' yeast that have been approved in the United Kingdom (Table 4, page 17). Again, the committee considered labelling not to be absolutely necessary, but recommended a case-by-case approach.

- Novel foods derived from GMOs but which contain neither the GMO itself nor its cells nor DNA and which differ from products conventionally consumed in Western Europe. Labelling of such products is recommended.
- Foods containing recombinant DNA (or the GMO or its cells) which were produced by introducing genes from the gene pool of a different species. Labelling of such products may be required, but the committee favoured a case-by-case examination.

The ongoing globalisation of trade is also affecting the food sector. It is plausible that genetically engineered crops approved only in foreign countries will make their way to the local market, especially when the respective country is a major producer of the crop. Current examples are the herbicide-tolerant soybean (Roundup Ready™, RR) from Monsanto and products from it or insect-resistant corn from CIBA-GEIGY that arrived in Switzerland and the EC, respectively, before approval of these products was granted (Butler, 1996). The soybean has by now been approved for food use in the United States, the European Community, Canada, the United Kingdom, the Netherlands, Japan, Switzerland, Mexico and Argentina. In the US, which is the main export country for soybeans worldwide, no special labelling of the genetically engineered soybean or products derived from it is required. Within the US for the year 1996, RR soybeans have mostly been processed indiscriminately from conventional soybean varieties. Some processing companies such as Central Soya Co. (Fort Wayne, Indiana) apparently intended to separate their products according to the soybean source (Wadman, 1996), but the bulk of the 1996 soybean harvest was not separated and may contain up to 1-2 % genetically modified soybeans.

The observed relative ease with which genetically modified products cross borders should be an added impetus for the EC and Swiss authorities to develop adequate methodology for identifying GMOs in food stuffs. This will facilitate controlling the adherence to the respective regulatory guidelines. Accurate labelling would also be an information service to consumers who want to exercise their freedom of choice in the market place. In the past months control authorities, trade and consumer organisations, as well as groups such as Greenpeace, have shown increasing interest in the development and increased availability of specific identification methods for GMO-products. A recently founded company in Iowa, USA provides analyses of predominantly raw, unprocessed food for approximately $ 450 per sample (Sept. 1996). It was reported that there has been tremendous interest in the analyses of the company (Wadman, 1996).

In contrast, there is only a very limited number of published detection methods designed to identify approved genetically modified food products such as the Flavr Savr™ tomato (Meyer, 1995a,b). Development of proper product identification methods is made difficult by the lack of specific information on the precise genetic changes differentiating genetically engineered products from their conventional counterparts. Therefore, it is not surprising that reviews published within the last few years mainly discuss theoretical aspects dealing with the identification of genetically engineered food products (Bähler, 1994; Schulze, 1994; Hammes and Hertel, 1995; Engel et al., 1995) or focus on the state-of-the-art in transgenic plant research (Niederhauser et al., 1996).

One central objective of this paper is to review published methods that have been designed for identifying genetically engineered foods and methods which may be relevant for the design of new methods. Information has been gathered from:

- Methods which have been (or soon will be) validated and published in official collections of methods for the identification of products in food derived from or consisting of GMOs.
- Methods which have been (or soon will be) published in scientific journals for the identification of food products consisting of or derived from GMOs.
- Publications in scientific journals describing the detection of GMOs (mostly microorganisms) in the environment.
- Articles in scientific journals describing the identification of DNA sequences or gene products that are also present as transgenes or respective translation products in approved GMOs.
- Publications from the area of food science (e.g. authenticity testing) containing information on the applicability of DNA-based methods for the analysis of processed foods.
- Methods for detection of pathogens in food; methods in clinical or veterinary diagnostics and other relevant areas.
- Highly specialised reports (e.g. annual reports from food control authorities) covering the detection of GMOs in food that are unavailable in common databases.
- Ongoing research projects concerning the detection or monitoring of GMOs.

The following compilation includes methods based on the detection of proteins as well as RNA- (NASBA) and DNA-based amplification techniques such as polymerase chain reaction (PCR), ligase chain reaction (LCR), Q-beta-replicase. The main focus is on DNA-based methods, in particular PCR, which represents the state-of-the-art technique for GMO detection in food. PCR combines high specificity with wide applicability with respect to the nature of the sample and suitability for laboratory diagnostics. Therefore, this report will also consider several articles dealing with specific problems which may arise when applying PCR for the analysis of food stuffs, and several approaches to prevent or counteract these problems.

2 GMO Products

The following sections will present an overview of the currently approved food products which either are or have been derived from GMOs. Special focus centres on genetically engineered agricultural crops as well as the genes and regulatory elements used to generate these transgenic plants.

2.1 Regulations

Since the end of the 1980s GMO regulatory processes have been developed in the United States and Western Europe. By now many countries have introduced legislation regulating the release and approval of GMOs (OECD, 1995; Screen Newsletter, 1995). In August 1996, the first gene regulatory body in Eastern Europe was established in Bulgaria, regulating the release of genetically modified higher plants for both, research and commercial purposes. The sections below briefly

summarise existing regulations and name the competent authorities for Switzerland, the European Community, Germany and the United States dealing with GMOs.

2.1.1 Switzerland

The new ordinance on food stuffs ('Lebensmittelverordnung', LMV) that became effective in Switzerland on 1 July 1995, requires that all food stuffs, food additives and processing aids consisting of or derived from GMOs have premarket approval (Article 15, LMV) and that they have to be labelled as 'GVO-Erzeugnis' (Articles 22k and 23, LMV) (Pauli and Schwab, 1996). Only those products free of the GMO itself and which were purified from the (transgenic) DNA (e.g. chemically defined substances like sugar) may be exempt from the labelling requirement. The ordinance for the authorisation procedure for GMO food stuffs ('Verordnung über das Bewilligungsverfahren für GVO-Lebensmitel, GVO-Zusatzstoffe und GVO-Verarbeitungshilfsstoffe', VBGVO) has just recently been published (19 November 1996) and was enacted on 1 December 1996.

2.1.2 The European Community

The regulatory framework with respect to GMOs in the European Community is currently based on the European Commission Directives for deliberate release (90/220/EEC) and for contained use (90/219/EEC), each of which was published in 1990 (for a review on the regulations see: Schauzu [1996; 1997]). A new regulation on novel foods and food ingredients was adopted by the Council in December 1996 and by the European Parliament in January 1997. It has been decided that the novel food regulation will become effective ninety days after its publication; i.e. by end of April, 1997.

Directive 90/220/EEC of 23 April 1990 for deliberate release regulates the release of GMOs (both for research and commercial purposes) into the environment. It was amended by directive 94/15/EC. A simplified procedure for multi-site and repeat releases has been introduced for the most common types of genetically engineered plants (decisions 93/584/EEC and 94/730/EC). Several decisions related to directive 90/220/EEC are 91/596/EEC, 92/146/EEC, 93/572/EEC and 94/211/EC. For the commercialisation of GMO products in the EC member states Denmark, the United Kingdom and the Netherlands, apart from approval according 90/220/EEC, additional approval of GMO crops according to national legislation are currently required. Such national legislation is likely to be superseded by the novel food directive when this enters into force.

The novel food regulation that has been discussed within the EC commission will probably require labelling of GMO products if they can be distinguished from respective conventional products by scientific, analytical methods. In contrast to Swiss regulations, the novel food regulations will not include enzymes, vitamins or processing aids derived by GMOs (Pauli, 1997).

In addition to the directive 90/219/EEC of 23 April 1990 and 94/51/EC of 7 November 1994, regulating the contained use of genetically modified microorganisms, further EC-regulations may be relevant: (i) directives 90/679/EEC and 93/88/EEC on the protection of workers from risks related to exposure to biological agents at work; (ii) directive 93/41/EEC on the approximation of national measures relating to the

placing on the market of high-technology medicinal products, particularly those of biotechnology; (iii) directive 93/114/EEC on additives in feeding stuffs, including additives containing or consisting of GMOs.

The use of herbicides (including the use on herbicide-tolerant plants) is regulated by directive 91/414/EEC of 15 July 1991 and several amendments. These regulations are currently still being implemented into national legislation of EC member states (e.g. in Germany). According to directive 91/414/EEC, only those applications of the respective herbicide that have been tested and registered will be approved. Directive 70/457/EEC (including several amendments) is relevant for the registration of food crop varieties. Varieties registered in any EC member state (e.g. in Germany by the 'Biologische Bundesanstalt für Land- und Forstwirtschaft' in Braunschweig) are combined in a common EC list of varieties for agricultural crops. Seeds of any variety registered in that list may be sold in any of the member states. So far, this list has not contained any transgenic varieties (Source: Bundessortenamt, Hannover; 30th of January 1997).

2.1.3 USA

In the United States three independent authorities are involved in the regulation of the release of genetically engineered plants and their use as food stuffs. In contrast to the European authorities, who use a process-oriented approach, the responsible federal agencies in the United States prefer a product-oriented approach for the regulation of genetically engineered products. This latter system does not categorise genetically engineered products on the basis of the technique by which they were developed, but solely on the actual plant characteristics.

1. APHIS (Animal and Plant Health Inspection Service) of the USDA (US Department of Agriculture). The APHIS authorises experimental field releases by issuing 'Field Test Permits'. Since 1993 a simplified procedure has been applicable under certain conditions for the approval of releases ('Notifications'). APHIS regulations under 7 CFR Part 340 pertain to the import, interstate movement or release of certain genetically engineered plants, including the deregulation for commercial release. The list of deregulated products can be accessed on the World Wide Web site of the Biotechnology, Biologics and Environmental Protection division (BBEP) of USDA-APHIS under: 'http://www.aphis.usda.gov/bbep/bp/'.

2. FDA (Food and Drug Administration). The FDA has the authority under the 'Federal Food, Drug and Cosmetic Act' to ensure the safety and wholesomeness of most food stuffs (except meat and poultry, which are regulated by the USDA, and agents with pesticidal characteristics falling under the jurisdiction of EPA [see below]). Although premarket approval of a product by the FDA is not formally required, all companies that applied for a new transgenic crop so far completed their consultations with FDA prior to the market introduction of the product. The FDA's policy statement on foods developed by biotechnology (including transgenic plants) can be accessed on the World Wide Web site 'http://www.fda.gov'. The FDA does not require labelling of food consisting of or derived by genetically engineered organisms. Special labelling would be obligatory if the composition of a food developed through genetic engineering differed significantly from its conventional counterpart (e.g. if a product contained substances that were not constituents in the human diet before, or if the product contained an allergen that the consumer would not expect in that food).

3. EPA (Environmental Protection Agency). Only some GMOs require approval by the EPA. This federal agency regulates transgenic plants that contain pesticidal components, such as genes or gene products that confer resistance against insects (e.g. -endotoxins from *Bacillus thuringiensis*), and thus are considered pesticides themselves. Regulation of such crops by EPA is required only when field testing becomes large scale or the determination of a tolerance level or exemption from a tolerance is required. Aspects of plant health and environmental risks of such plants are assessed by the EPA. In addition, the EPA approves changes in the registration for herbicide use on (transgenic) crops but does not assess aspects of plant health or potential environmental risks of herbicide-tolerant crops.

2.2 Commercialisation of genetically modified products

The first approval of a genetically engineered plant for human consumption was given in the United States. The Flavr Savr tomato from Calgene received approval from USDA/APHIS and FDA in 1992 and 1994, respectively. Since then, 25 additional transgenic plants have been approved by US authorities. Several of these products have also been approved in other countries, and two further ones in the European Community. These and others are summarised in tables in the following sections.

The new traits introduced into currently approved genetically engineered plants can be categorised as follows (see also Table 2):

I Improved product quality (durability, firmness, fruit ripening delayed, processing value)
II Pest resistance (insects, nematodes, viruses)
III Agronomic benefits (herbicide tolerance, hybrid system)

The Flavr Savr tomato belongs to the first category. Constitutive expression of the Flavr Savr gene (antisense-construct derived from the polygalacturonase [PG] gene from tomato) results in a dramatically decreased PG-activity in the transgenic tomatoes. The enzyme PG degrades pectin, a major constituent of the cell wall of the fruit. Thus the Flavr Savr tomatoes can ripen on the vine longer and be harvested long after they turned red, without the risk of excessive softening after harvest. In contrast, more than 80 % of the conventional tomatoes sold in the US are picked while green and exposed to external sources of ethylene in industrial pants to develop red colour.

The majority of the approved genetically modified plants can be categorised under groups II and III. Several of the pest-resistant transgenic crops carry genes also conferring herbicide resistance for selection purposes (Tables 3-10). The most frequently used selection marker is the nptII gene also termed kanr or neor, coding for the enzyme aminoglycoside 3'-phosphotransferase II (APH(3')II). Expression of this enzyme allows for selection on media containing kanamycin (kan), and neomycin (neo, G418) (see also section 4).

2.3 Approval of genetically engineered products

Examples of the data derived from national approvals of GMOs or GMO-products are presented below. Part of the available information about a product and its approval in

a certain country are summarised in a database[1]. An example is given in Box 1 which displays information available on the approval(s) of the Flavr Savr tomato in the US (ID 1). All sets of information are sampled in an extensive database at the agency BATS. The data are structured in such a way that each approval in any of the countries surveyed is represented by a single entry with a unique identification number (ID) assigned to it (approval-based database)[1].

If more than one country approved the same or virtually the same product from a given company, a corresponding number of IDs was assigned to the product. Tables 4-10 contain some information taken over from applications or approvals describing the respective product in more detail (e.g. those summarised in Tables 3 or 4) if it was obvious that the respective approvals related to the same transformation event(s). Such information is presented in *italics*.

2.3.1 Differences in national approvals of the 'same' products

The grounds on which approvals are garnted in different countries for aparently the same products can differ significantly (with respect to information relevant for identification methods), although these differences become obvious only upon closer examination. For example, the approval of processing products of the genetically engineered tomato from Zeneca in the United Kingdom relates to transformation events involving the plasmid pJR16S, whereas the approval in the US covers lines transformed with either pJR16S or pJR16A. In the former vector the transgene, a truncated version of the tomato polygalacturonase gene, is oriented in the sense orientation (S), while in the latter it represents the respective antisense (A) construct. These different lines obviously do not display different phenotypes; however, understanding the differences described is essential for the design of reliable PCR-based detection methods and for the evaluation of existing nucleotide-based methods.

Another example are differences in the number of distinct approved lines of a new transgenic variety, which were derived by independent transformation events using the same plasmid; the assessment of the Flavr Savr tomato from Calgene by the ACNFP (United Kingdom) includes only 10 lines, whereas in the United States the USDA-APHIS has approved already more than 40 distinct lines of this transgenic tomato (Box 1).

Box 1. Datasheet ID 1 (Flavr Savr™ Tomato)

ID	1
Product	Tomato
Further specification	GM lines from approx. 40 different transformation events, using 2 slightly different plasmids and crosses with traditional varieties (501-1436-1001, 501-1436-1035, 502-1436-2021, 7B-1436-92, 22B-1436-215, 28B-1436-419, 28B-1436-425, 28B-1436-498, N73-1436-111, 114F-4109a-26, 141F-4109a-81, 105F-1436-2018, 105F-1436-2035, 105F-1436-2049, 35F-4109a-3023, 84F-4109a-148, 88F-4109a-2797, 121F-4109a-333, 121F-4109a-1071, 121F-4109a-1120, 137F-4109a-71, 138F-4109a-164, 519A-4109a-4527, 519A-4109a-4621, 519A-4109a-4676, 531A-4109a-2105, 531A-4109a-2270, 532A-4109a-5097, 540A-4109a-1739, 585A-4109a-3530, 585A-4109a-3604, 519A-4109a-4645, 540A-4109a-1823 and 7 further lines in document 94-125-1)

Scientific name	*Lycopersicon esculentum* Mill
Host organism	*Lycopersicon esculentum* Mill, tomato lines 501, 502, 7B, 22B, 28B, N73, 114F, 141F, 105F, 35F, 84F, 88F, 121F, 137F, 138F, 519F, 531A, 532A, 540A and 585A)
Product name	Flavr Savr™ Tomato (MacGregor's)
Company	Calgene Inc.
Contact	Keith Redenbaugh, Ph.D.; Regulatory Manager; Calgene Inc.; 1920 Fifth Street; Davis, CA 95616, USA
Altered trait	Fruit ripening delayed
Classification	PQ
Purpose	Enhanced fresh market value
Plasmid	pCGN1436 (driving nptII by mas 5' and mas 3') or pCGN4019a (driving nptII by P-35S and tml3')
Inserted genes	Flavr Savr™ gene (= antisense polygalacturonase) (1-3 copies), nptII (1-3 copies), partial LB and RB, at a single site (haploid)
Transfection method	*Agrobacterium tumefaciens*
Transgene 1	Flavr Savr™ gene (polygalacturonase (PG) antisense gene)
Source of tg 1	Tomato
Protein product 1	None
Expression 1	No; level of native PG mRNA is >90 % reduced; residual enzyme activity of native PG is < 1 % of control lines
Mechanism 1	Antisense RNA complexes endogenous sense mRNA for PG (transcription for native mRNA might also be downregulated), thus reducing the levels of PG which normally degrades pectin, a major component of the cell wall in tomato fruit
Promoter 1	(double-) CaMV 35S
Terminator 1	tml 3'
Transgene 2	nptII (=kan r, neo r = neomycin phosphotransferase II gene)
Source of tg 2	Transposon Tn5 (*E. coli* K12)
Protein product 2	APH(3')II (Aminoglycoside-3'-phosphotransferase II)
Expression 2	< 0.08 % of total protein
Mechanism 2	Allows for selection during plant tissue culture. APH(3')II inactivates neomycin, kana-mycin and genticin/G418) by ATP-dependent phosphorylation of the 3'-hydroxyl group of the aminohexose moiety of these aminoglycoside antibiotics. This phosphorylation interferes with uptake and binding of the aminoglycoside to the bacterial ribosome
Promoter 2	mas 5' (mannopine synthase) or CaMV35S promoter (different plasmid)
Terminator 2	mas 3' (polyA region from mannopine synthase gene of pTiA6) or tml 3' (see plasmid)

Transgene 3	parts of lacZ
Source of tg 3	*E. coli*
Protein product 3	None
Expression 3	No
Mechanism 3	
Promoter 3	-
Terminator 3	-
Gene sources	Tomato, bacteria
Detailed sequences	pCGN1436 sequence from LB to RB
References for	pg, nptII
Approved by 1	APHIS docket-no 92-087-1, 94-096-1, 94-125-1, 95-015-1, 95-056-1
Approved for 1	USA
Restrictions 1	
Requirements 1	
Labelling 1	
Date 1	10/92, 10/94, 11/94, 3/95, 7/95
Approved by 2	FDA approved
Approved for 2	USA
Restrictions 2	
Requirements 2	
Labelling 2	not required
Date 2	5/94
Approved by 3	EPA approval not required
Approved for 3	
Restrictions 3	
Requirements 3	

Date 3	-
References	USDA/APHIS; Safety assessment (Redenbaugh et al., 1992)
Safety remarks	Data on potential toxins, tomatine level, acute toxicity tests in rats
Qualities checked	Increased fungal resistance, stable inserted, taste, horticultural traits, Ca, Mg, Fe, Na; vitamins A, B1, B2, B6 and C

The data were derived from official documents (e.g. the Official Journal of the European Communities, Federal Register), approving and consulting authorities (e.g. EC, USDA/APHIS, FDA, EPA, Health Canada, MAFF, GMAC, RIKILT-DLO, RKI and the Federal Offices for Public Health of Denmark, Japan and Switzerland), petitions, company information and scientific publications.

In addition, certain information that is relevant for the design of identification methods is mentioned in one approval, but was not mentioned in the available documentation concerning the approval of the same product in another country. For example, the executive summary of the approval of the genetically engineered oilseed rape from Plant Genetic Systems in Great Britain did not mention that the sequence of the gox-gene was 'optimised for plant expression' as was noted in documents of the FDA, indicating that the codon usage of this gene (and thus the DNA sequence) has been specifically changed (Communication, Monsanto). The latter is, again, important for the application of nucleotide-based detection systems employing specific oligonucleotide primer that should bind to the respective gene.

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[1] All entries in the tables 3-10 with assigned identification numbers are included in the database.

2.3.2 Summaries of national and multi-national approvals of genetically engineered products

Genetically engineered products have been recently approved in a many countries. Tables 3-10 present thorough compilations of information pertaining to approved genetically modified crops and other products in the United States (Table 3), the United Kingdom (Table 4) , the European Community (Table 5) , Switzerland (Table 6) , the Netherlands (Table 7) , Canada (Table 8) , Japan (Table 9) and Australia (Table 10) . The authorisations of transgenic food crops are listed comprehensively. Information is provided which is relevant to the design and evaluation of nucleotide-based detection methods such as the transgenes used, the sources of the transgenes, the regulatory sequences used (promoters, terminators), and data on the actual expression levels of the transgenes in the approved products. More extensive information is available in the respective datasheets1 (see also Box 1). Data on genetically modified microorganisms was not available for all countries and thus are not always described.

During preparation of this report several genetically modified products have received approval in the EC as well as in Switzerland and in the United Kingdom. Several

applications for transgenic plants are pending in the EC. A summary of approvals is given in Table 11. This table also indicates applications that may be pending; however, a definitive survey on future products is not possible for all countries since many regulatory authorities do not release specific information on pending applications.

2.3.3 Restrictions

An approval in one of the tables shown does not necessarily imply that the product in question is already commercially available, as there are often several hurdles to overcome before a product may enter the market. Although the Ministry of Agriculture, Fisheries and Food (MAFF) approved the Flavr Savr tomato for food use, it may still not be imported or grown in the UK since this would require approval according to the EC directive 90/220/EEC. Other genetically engineered products have received restricted approvals by the EC. For example, the modified soybean from Monsanto may only be imported but not grown, whereas genetically modified chicory (Bejo-Zaden) and oilseed rape (PGS) may only be grown for seed production in limited areas.

In the US, the BXN-cotton from Calgene (ID 7) was approved for planting on a limited acreage (50,000 acres or 20,000 hectare) per year for the first three years of commercial cultivation. There are also examples of restricted authorisations that limit crop cultivation within geographical boundaries (various US approvals; insect-resistant cotton in Australia). Further information on restrictions of specific approvals is compiled in the BATS database.

2.3.4 Commercially available products

Even though a genetically engineered product has been approved by a given responsible authority, it may not necessarily be grown on a large scale or be available commercially. Sometimes there may be a delay in the exploitation of an approved product, for reasons which are not always apparent. For example the herbicide-tolerant tobacco plant from Seita which received unrestricted approval according to directive 90/220/EEC in 1994 has not yet been grown on large scale. There has not even been an application for the registration of the use of the respective herbicide on this variety according to the EC directive 91/414/EEC. Another example is the genetically engineered tomato from DNA Plant Technology. This was test-marketed in 1995 but since then it has been withdrawn from the market due to a patent licence dispute.

Company information from Calgene stated that the following genetically engineered crops were commercially available in the United States for the year 1995: squash (virus-resistant) from Seminis (formerly Asgrow), tomato (delayed fruit ripening) from DNA Plant Technology (test marketing), cotton (bromoxinyl-tolerant), rapeseed with increased laurate content (Calgene) and the Flavr Savr tomato (Calgene). According to a 1996 report, insect-resistant corn from CIBA-GEIGY (and Mycogen), as well as insect-resistant cotton and potatoes (both from Monsanto) had also entered the commercial market. Other additions to the 1996 list are herbicide-tolerant soybean from Monsanto (grown in the US) and herbicide-tolerant rapeseed from AgrEvo/Hoechst, grown in Canada.

Some data are summarised in Table 12 on the absolute acreage of genetically engineered crops and on how these numbers compare to the total acreage of the conventional and modified crops. In 1996, the genetically engineered soybean from Monsanto, grown on approximately 1,000,000 acres in the US, occupied 1-2 % of the total area devoted to soybean growth in North Amerika (within North America, soybean is predominantly grown in the US). Another some 375,000 acres were reportedly planted with the Roundup Ready soybean™ from Monsanto in Argentina (James and Krattiger, 1996) in 1996. In contrast, the 2,000,000 acres (approx. 800,000 hectare) used for growing genetically engineered cotton in 1996, represents almost 14 % of the total US cotton production. On the opposite end, the EC approved herbicide-tolerant rapeseed from Plant Genetic Systems is reportedly grown on only 25-50 acres (10-20 hectares) for seed production (Communication AgrEvo, Germany).

Tomato paste, produced from genetically engineered tomatoes from Zeneca in the US, has been commercially available in the United Kingdom since 1996, distributed by Sainburry's and Safeway supermarkets. Approximately 900,000 cans were sold in 1996 (Communication from Zeneca, United Kingdom), implying that a considerable percentage of the British population has bought (and probably consumed) this tomato paste. Further products produced from these genetically engineered tomatoes from Zeneca have only been available in small quantities as samples (e.g. ketchup) and have not been commercially sold. For 1997, Zeneca plans to launch this tomato (or products thereof) on the US market.

2.3.5 Transgenic organisms in China

European experts who have visited release sites of genetically modified plants in China have concluded that China has gained a 'leading' position in the large-scale release of virus-resistant transgenic crops. The most frequently used plants were tobacco and tomatoes, but experiments were also performed with pepper, potato and rice crops. In 1996, virus-resistant tomatoes and tobacco were grown on 50,000 (Braunschweiger and Conzelmann, 1997) and 2,000,000 acres (James and Krattiger, 1996), respectively in China. These figures are comparable to the acreage figures for the most abundantly grown transgenic crops in the US. Other experiments to increase the nitrogen-fixation capacity of barley, rice, wheat and watermelons by the use of genetically modified bacteria (*Alcaligenes faecalis, Enterobacter cloacae, Klebsiella oxytoca*) are currently being performed (BINASNews, 1996). No information was available at the time of this writing concerning the existence of regulatory processes or legislation governing genetically modified organisms in China.

2.4 Enzymes derived by recombinant technology

Applications of gene technology for the production of processing aids are mainly focused on the use of recombinantly expressed enzymes (Braunschweiger and Conzelmann, 1997). The regulations on enzymes, including those produced by genetic engineering, are not standardised in Europe. The EC directive 90/220/EEC applies only to the release of GMOs, or to products containing GMOs, and therefore does not concern enzymes deriving from recombinant technology. Nor it is likely that the planned novel food legislation of the EC will include enzymes. The Netherlands is one of the few countries providing a complete list of enzymes (or other processing

aids and food additives) used in the food industry (Table 7). In Switzerland and the UK, several enzymes have also been listed (Tables 4 and 6), whereas no such lists were available from most of the other countries. Regulations on enzymes, if present at all, vary greatly among different countries.

In Germany, the use of enzymes (and microorganisms) in food does not generally require specific approval according to the LMBG ('Lebensmittel- und Bedarfsgegenständegesetz'). For certain applications of enzymes, such as in cheese production, specific registrations may be required; the German regulation for the manufacture of cheese discriminates between natural rennet and its substitutes (e.g. chymosin), requiring approval for the latter.

For the reasons given above, data on the approval of enzymes or other processing aids or food additives derived by genetically modified microorganisms has not been systematically summarised here. Approved enzymes are listed only for the Netherlands, Switzerland and the United Kingdom. The use of chymosin, expressed either in *Kluyveromyces lactis*, *E. coli* K12 or *Aspergillus niger* ssp. *awamori*, reportedly has been approved until 1993 in at least 17 countries, including Belgium, Chile, Denmark, Finland, Hungary, Ireland, Israel, New Zealand, Norway, Poland, Portugal, South Africa, Sweden, Switzerland, the United Kingdom, the United States, and in the former Yugoslavia (Teuber, 1993).

In contrast to the regulatory situation, the picture of commercial availability of recombinantly expressed enzymes is much clearer. At least 19 different enzymes, most of which can be applied in the food industry, have been produced by heterologous expression in various genetically modified microorganisms (Table 13). In addition, some food additives are also produced by means of genetically engineered microorganisms. Vitamin B12 manufactured from Rhône-Poulenc has been recently approved for food use in Switzerland (Table 6), apparently using genetically modified *Agrobacterium radiobacter* (Braunschweiger and Conzelmann, 1997). Efforts to produce Vitamin B2 (riboflavin) using a recombinant *Bacillus subtilis* strain have also been reported (van Loon et al., 1996). Very recently (on 17 February, 1997), riboflavin from Hoffmann-La Roche has been granted food approval by the British Ministry of Agriculture, Fisheries and Food (Table 4).

2.5 Genetically modified animals

Genetically modified animals (predominantly mice) have become essential for many areas of biomedical research. In clinical research, there is great interest in genetically engineered pigs as donors of organs suitable for xenotransplantation. Another promising domain of pharmaceutical research is the expression of therapeutically valuable proteins in plants and in the mammary glands of mammals ('molecular farming').

Applying gene technology to produce animal feed stock for human consumption is still considered to be very time-consuming and expensive, despite the availability of techniques to introduce genes into a variety of species (Sachse, 1996). Long generation times, low transformation efficiency, laborious methodology as well as the fact that knowledge about the genetics of relevant species remaines scant greatly has limited the scientific advances in this area. Furthermore, some early experiments using growth-promoting genes had negative side effects on animal health (Sachse,

1996). Transgenic fish, in particular salmon and trout species with additional copies of growth-promoting genes (Devlin et al., 1995), may be the first commercialised transgenic animals. As early as 1992, transgenic salmons carrying additional growth hormone genes have been shown to grow significantly faster than control salmons (Du et al., 1992).

Gene technology has already found some application in food production through bacterially-expressed BST (bovine somatotropin), which is used in the United States and some countries in Eastern Europe to increase the milk production of (non-transgenic) cows. However, in the European Community and in Switzerland the use of BST is prohibited.

3 Methods for identifying genetically engineered foods

The use of recombinantly produced chymosin in cheese production since the end of the 1980s represents one of the first applications of genetic engineering in the food industry. The Flavr Savr tomato was the first genetically modified product entering commerce that was itself a GMO; it thus brought the consumer into close contact with new plant technology. Since then, at least 27 other genetically engineered agricultural crops have been approved.

The ever-increasing number of approvals granted spurred strong interest in developing methods for identifying GMOs in food. The sceptical attitude prevalent in the populations of certain European countries (especially the German-speaking populations) towards gene technology have reinforced interest in detection methods. The availability of suitable identification procedures is necessary also for various food control activities, such as the observance of regulations on the labelling on GMOs and of regulations with respect to seed certification. The requirements on the specificity of detection methods will increase significantly with the number of distinct products available, the appearance of mixtures of distinct GMO products and increased processing of such products or complex mixtures.

Almost the entire first generation of detection methods is based on techniques applying the polymerase chain reaction (PCR) (Saiki et al., 1985; Mullis and Falloona, 1987). Although other methods may be suitable for certain tasks, their range of application tends to be much more limited than the PCR. This gap may decrease with more research and development in the respective areas. This report concentrates on PCR-based methods and certain experiences made with this technique in general and with special regard to the analysis of food stuffs. Alternative methods worth considering for special applications are also discussed. Numerous schemes for nucleotide-based amplification methods as depicted in Wolcott (1992), Carrino and Lee (1995) or current scientific text books are mentioned as useful background on the various techniques.

3.1 PCR-based methods

Although the principles of PCR had been conceptually described already in 1971 (Kleppe et al., 1971), experimental data were first published in the mid 1980s (Saiki et al., 1985). Since then, this technique has revolutionised molecular biology and many other areas in the bio-medical sciences. The number of references to PCR in the scientific literature has been estimated to be more than 40,000 (White, 1996).

The high chemical and thermal stability of DNA, the high sensitivity of the method, its technical simplicity, the vast amount of experience already accumulated with it, along with the apparent potential for automation (Abramowitz, 1996; White, 1996) are main advantages of this method, establishing the current prevalence of PCR-based detection methods. This preference is likely to continue in the foreseeable future.

3.1.1 Officially validated identification methods

This section discusses methods which have been specifically developed to detect GMOs or products derived from GMOs in food stuffs and that have been included in a collection of official methods. Methods currently in the process of being validated will also be discussed.

The only official, validated methods that have been published so far, were developed by the BgVV-working group ('Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin', Berlin) for the 'development of methods to identify foods produced by means of genetic engineering' ('Entwicklung von Methoden zum Nachweis mit Hilfe gentechnischer Verfahren hergestellter Lebensmittel'). These methods have been included in the listing of official methods ('Amtliche Sammlung von Untersuchungsverfahren') according to Article 35 of the German Food Act (LMBG, Lebensmitel- und Bedarfsgegenständegesetz). Included among these methods were the results of inter-laboratory studies with participants from academic research institutes, private laboratories and food control authorities.

Three methods have been developed by this group, two of which have already been included in the list of official methods according to §35 LMBG. The two detection methods describe the PCR-based identification of a genetically engineered potato and a genetically modified microorganism in fermented raw sausages. A third assay, describing the detection of a genetically modified microorganism used as a starter culture in yoghurt, was tested in an inter-laboratory study in the end of 1996. All these methods have a model character since none of the utilized GMOs has been approved yet in any country and the use of any of these organisms in their current form in food is not intended. The methods for the detection of genetically engineered potatoes and genetically modified microorgansims in fermented raw sausages have recently been reviewed in the *Bundesgesetzblatt* (Schulze et al., 1996).

3.1.1.1 Genetically modified potatoes

PCR amplification of the altered DNA sequence and validation via DNA probe hybridisation has been used to identify a genetically engineered potato (LMBG-Methodensammlung, 1996; Schulze et al., 1996). The potatoes tested carried an introduced invertase gene from *Saccharomyces cerevisiae* and a transgene coding for a hygromycin phosphotransferase. The method is well documented; DNA extraction and DNA amplification by PCR were followed by separating the amplification product using agarose gel electrophoresis and controlling the length of the amplified product by size controls. After electrophoresis, the DNA was transferred onto membranes and analysed for the presence of the respective DNA sequence using DNA-DNA hybridisation (Southern-Blot). The specificity of the method was confirmed in inter-laboratory studies which yielded a reliability of more than 97 %. The amplicon (amplified DNA fragment) was 837 basepairs in length and contained sequences of the hygromycin phosphotransferase gene (Table 14). The specificity of

the method is somewhat limited since any organism carrying this gene could be detected by the method.

3.1.1.2 Genetically engineered *Lactobacillus* in raw sausages

An analogous method developed by the BgVV working group was designed to identify genetically engineered *Lactobacillus curvatus* in raw sausages by PCR-based DNA amplification and hybridisation (LMBG-Methodensammlung, in press; Schulze et al., 1996). The *Lactobacillus curvatus* strain carried a plasmid-encoded catalase gene (katA) derived from *Lactobacillus sake* (Hertel et al., 1995a; Hammes and Hertel, 1996). The plasmid also carried a gene coding for chloramphenicol acetyl transferase (cat). Apart from its somewhat modified DNA extraction procedure, the method is similar to what was described for the potato. The reliability of the technique was determined in inter-laboratory studies to be more than 95 %. The primers used were complementary to sequences in the plasmid (in the cat gene) and to sequences in the katA gene, respectively, resulting in an amplicon of 1321 basepairs (Table 14). Since the amplicon contains the interface between plasmid sequences (cat) and the transgene (katA) this method should be highly specific for the genetically engineered microorganism that was used. Because the sequence of interest was located on a plasmid, the copy number of the sequence (and thus the sensitivity of the method) may be higher as compared to sequences that are integrated into the bacterial genome. The applicability of the method for strongly heat- or acid-treated samples may be limited due to the comparably large size of the amplicon chosen. (This subject will be dealt with in subsequent sections.)

3.1.1.3 Genetically engineered *Streptococcus* used a starter culture in yoghurt

Another model system for detecting a genetically modified microorganism was elaborated for *Streptococcus thermophilus,* a bacterial strain used as a starter culture in yoghurt (LMBG-Methodensammlung, in preparation). The method is analogous to the methods described before; again, the DNA extraction procedure was somewhat optimised (Lick et al., 1996a). Since certain results of the inter-laboratory studies were still unavailable as of January 1997; a precise assessment on its reliability can not yet be provided. The primers recognise sequences of the homologous lacZ gene and the (heterologous) chloramphenicol acetyl transferase (cat), which represents the transgene in this model-GMO (Heller, 1995). The amplicon used was 623 basepairs in size (Table 14) and contains an interface between homologous and heterologous sequences, ensuring high specificity of the method. As a positive control, species-specific PCR amplification of the lacZ sequences from *Streptococcus thermophilus* was used.

3.1.2 Methods developed to detect GMOs and published in scientific journals

The first method for GMO identification in food stuffs was specifically developed to identify the Flavr Savr tomato (Meyer, 1995a). Relatively few articles have been written on the detection of approved genetically engineered plant products and published in specialised peer-reviewed journals. The PCR-based method developed for the Flavr Savr tomato has been applied already in food control laboratories in Germany, such as the 'Chemische Landesuntersuchungsanstalt' in Freiburg (Annual report CLUA, 1995; Pietsch and Waiblinger, 1996; Pietsch et al., 1997; Waiblinger et al., 1997), and in Switzerland by the 'Kantonales Laboratorium' in Basel-Stadt

(personal communications, P. Brodmann, Kantonales Laboratorium Basel; Waiblinger et al., 1997). The amplified DNA fragment is 427 basepairs in size (Table 14) and contains the interface between one of the transgenes (antisense polygalacturonase gene construct) and the promoter used to regulate this gene (P-35S promoter from cauliflower mosaic virus). For verification of the amplification product described in this method, agarose gel electrophoresis in combination with restriction enzyme analysis was employed.

For many of the genetically modified plants that have been developed (see Table 1, Figure 1), PCR assays have been used to confirm or control the success of plant transformation and thus can be found in many articles describing the generation of a transgenic plant. However, due to the large number of transformed plants it is almost impossible to provide a comprehensive compilation of these publications. A selection of articles describing PCR assays employing primers specific for genetic elements which have been used for the generation of currently approved genetically engineered agricultural crops is mentioned below. Some experimental details of the PCR assays such as primer length and sequence, location of primer binding sites, amplicon length and whether cycling parameters were described, are listed in Table 14. The table contains references to publications on alfalfa (Blake et al., 1991), corn (Golovkin et al., 1993), papaya (Yang et al., 1996), potato (Jongedijk et al., 1992) and soybean (Padgette et al., 1995).

The following genetic elements (in general, only promoters, structural genes and terminators are mentioned) are described in the publications cited in Table 14: P-35S promoter from cauliflower mosaic virus (Jongedijk et al., 1992; Golovkin et al., 1993; Padgette et al., 1995), the gene coding for CP4 epsps (5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp., strain CP4) (Padgette et al., 1995), the gus (beta-glucuronidase) gene (Blake et al., 1991; Yang et al., 1996), the nptII (aminoglycoside-3'-phosphotransferase gene from transposon 5) gene (Blake et al., 1991) and the nos-terminator (Padgette et al., 1995), derived from the 3'-region of the nopaline synthase gene from *Agrobacterium tumefaciens*. With the exception of transgenic cotton from DuPont (ID 22) and rapeseed from Monsanto (ID 65), all of the approved genetically engineered agricultural crops have been transformed with constructs containing either the cauliflower mosaic virus 35S-promoter (P-35S) or its derivatives, the nos-terminator (nos 3') or both of these elements. These elements were derived from either a plant virus or from *Agrobacterium,* respectively.

3.1.3 Highly specialised reports on the detection of GMOs in food unavailable in databases

This section concerns articles or methods mentioned in special reports (e.g. reports commissioned by national authorities), annual reports from food research institutes, petition documents from companies or information presented as posters at conferences. References to these works cannot be easily found by literature searches in commonly available databases; dissemination of these references occurs mainly through personal communications.

Specialised reports of this nature have described detection methods for genetic elements used in the generation of transgenic corn (Waiblinger et al., 1997; Pietsch and Waiblinger, 1996; Pietsch et al., 1997; PGS-petition, 1995), cotton (DuPont-petition), potato (Pietsch et al., 1997; Waiblinger et al., 1997), sugar beet (Pietsch et

al., 1997; Waiblinger et al., 1997), soybean (Pietsch et al., 1997; Waiblinger et al., 1997; Wurz and Willmund, 1997), tobacco (Kriete et al., 1996) and tomato (Pietsch et al., 1997; Waiblinger et al., 1997; Pietsch and Waiblinger, 1996; Zeneca-petition, 1994). Almost all of these methods were PCR-based and were applied to approved genetically engineered products or to genetic elements that have been frequently used for the generation of the approved transgenic plants (Pietsch et al., 1997; Waiblinger et al., 1997). Experimental details such as primer sequences, amplicon length and cycling parameter are summarised in Table 14.

An identification procedure for tomato paste manufactured from genetically engineered tomatoes from Zeneca and sold in the UK in 1996 has been reported (press release No. 057/29.5.96 of the University of Bremen, Germany). The method is based on PCR amplification of a 506 basepair fragment from the nptII gene (personal communication G. Meyer, Hanse Analytik, Bremen). That a DNA fragment of this size could be successfully amplified from a heat-treated sample with low pH (approximately pH 3) may be surprising at first glance. Other sources, however, have also reported that DNA has been amplified from similar samples, even when the length of the chosen amplicons was considerably shorter, using 137 basepair (Personal communication H.U. Waiblinger, Chemische Landesuntersuchungsanstalt, Freiburg; Allmann et al., 1993) and 226 basepair fragments (Ford et al., 1996; Barallon et al., 1996).

There have also been reports of attempts to identify artificially-introduced DNA in bread (Annual Report BFE, 1995). In a model detection system, flour from rye was spiked with *E. coli* cells or DNA, containing a phytase gene. *E. coli* DNA could neither be detected in fermented dough nor in the final bread product. When large quantities of bacterial cells (more than 1010 cfu/g) were added, the presence of foreign DNA was detectable. Such quantities, however, were considered to be highly unlikely for 'realistic' applications. Whereas no commercial approval of any cereal variety exists at present, a genetically modified bakers' yeast developed for bread making has been approved in the UK, although it is reputedly not in use.

Several publications have focused on the detection of DNA derived from decomposing transgenic plant material in the soil. The PCR systems described were specific for genetic elements that had been introduced in genetically engineered corn (synthetic pat gene) and rapeseed (pat, P-35S) (Ernst et al., 1996; Feldmann et al., 1996; Kirchhof et al., 1996), or derived from the so-called 'Changins-potato' (PVY-cp, nptII) field tested in Switzerland (Stax et al., 1994). In addition, the primers used for the detection of the nptII gene in soil bacteria or other environmental sources using genuine (Smalla et al., 1993) or nested PCR (Tsushima et al., 1995) may also be applicable for the detection of this frequently used transgene in foods.

3.1.4 PCR diagnostics - problems and possible solutions in application

This section will briefly review some general considerations important to the design and execution of PCR. It will also discuss certain problems that may arise when applying PCR for the analysis of food stuffs with special regard to problems due to the nature of the food matrix and the applicability to processed food.

3.1.4.1 Choice of primers and general methodological parameters

The main criterion determining the specificity of a PCR assay is the choice of the primers. To ensure uniqueness of a sequence the primer should be at least 20 bases in length for statistical reasons (Berry and Peter, 1984). On the other hand, the primers should not exceed this size too much, since this would result in unspecific annealing of the primers during the extension phase generally performed at 72 °C. Given a 50 % A/T-content of a primer, this would limit the primer length to approximately 24 bases or to 29 bases assuming an A/T-content of 76 %. The average length of the primers listed in Table 14 is 21 bases. The primary choice and optimisation of primer sequences for PCR can be facilitated by the use of software programmes (Meyer, 1995b). Although a single mismatch between primer and target-DNA can influence the hybridisation under certain experimental conditions (Ikuta et al., 1987), the efficiency of PCR under conditions used in routine diagnostics is only expected to be affected if the mismatch is located in one of the extreme 3' nucleotide positions of a primer. Exchanges on 4 positions in a primer sequence, however, can be specifically detected and even be used for monitoring purposes of genetically altered microorganisms (Hertel et al., 1992; Ludwig et al., 1995). Other important methodological parameters for the development of a PCR test are the optimisation of the amplification reaction and the choice of a positive control (Wolcott, 1992; Mullis et al., 1994; Karch et al., 1995). Furthermore, the use of standardised reagents and protocols is essential for the reproducibility of such tests (Mahony et al., 1994).

3.1.4.2 Avoiding false-positive and false-negative results

Avoiding false-positive as well as false-negative results is very important for the reliability of a PCR test. False-positive results can arise from carry-over contamination (in particular, from previous PCR-assays). Several techniques have been published for avoiding carry-over effects (for a review, see: Carrino and Lee, 1995; Wolcott, 1992). The most frequently used method for 'preamplification sterilisation' employs the enzyme uracil-DNA-glycosylase, which removes all uracil bases from the DNA sugar-phosphate backbone (Longo et al., 1990, Müller et al., 1996). Unspecific primers or insufficiently restrictive conditions during the amplification reaction should also be avoided to prevent false-positive results.

False-negative results can be assessed by the parallel processing of a second PCR designed as positive control, such as a PCR-specific eucaryotic DNA (Allmann et al., 1993; Meyer, 1995a) or plant-specific sequences (Pietsch et al., 1997). These sequences are generally present in many copies within a single cell. When the target sequence is expected to be present only in a very low concentration, indicating that sensitivity will be an issue, it may be advantageous to include also a positive control targeted to a sequence which will be present in similarly low concentrations. If necessary, the positive control can be processed together with the target sequence by using multiplex-PCR (Feldmann et al., 1996; Cha and Tilly, 1993).

One very important and effective means of optimising the specificity of a PCR assay for the detection of GMOs is to choose the primers in such a way that they are located on different genetic elements (e.g. promoter, structural gene, terminator, vector-sequence). The primers should be specific for target sequences which do not occur naturally (at least not in that specific combination) in the respective crops (e.g. when the genetic elements originate from different phyla) (Meyer, 1995a), thus ensuring a high specificity of the test. In order to develop a truly specific method for a given GMO product, it is highly effective to choose a unique combination of elements

(eventually by including the criteria of the length of the amplicon) that occurs neither in conventional products nor in other genetically engineered organisms that have been generated or approved. The interface between inserted DNA (T-DNA) and host-DNA may offer another unique nucleotide sequence providing an ideal target sequence for a highly specific PCR test. Such nucleotide sequences from interfaces between host DNA and transforming DNA have been described for several approved products in their respective petition documents.

3.1.4.3 Sensitivity

The sensitivity of a PCR test can be significantly improved by increasing the number of cycles (Candrian, 1994; Meyer et al., 1994). The application of 'magnetic capture-hybridisation-technique' has also been shown to augment the sensitivity of an assay by two orders of magnitude (Kirchhof et al., 1996; Jacobsen, 1995). Using 'hemi-nested PCR' or 'nested PCR' (Brockmann et al., 1996; Meyer, 1995b; Lunel et al., 1995) instead of conventional PCR represents another way of increasing assay sensitivity. Sensitivity may be assessed through a positive control which targets a sequence of similar length expected to be present in similar quantity as the actual target sequence.

3.1.4.4 DNA quality

The 'quality' of the DNA present in the samples is of particular significance in food diagnostics. The average length of DNA fragments present in the test sample is the main determinant of DNA 'quality'. It is essential that the average size of the DNA fragments in the probe not be significantly smaller than the target sequence (amplicon length) in the assay. Damage within the DNA fragments caused by chemical, physical or enzymatic processes (e.g. depurination, UV-damage) is also relevant.

Various factors may contribute to the degradation of DNA in food stuffs: (i) hydrolysis of the DNA due to prolonged heat treatment (Table 15); (ii) enzymatic degradation by nucleases; and (iii) increased depurination and hydrolysis of DNA at low pH.

Therefore, the quality of the DNA in processed foods, heat-treated in conditions of low pH, such as tomato ketchup or soy sauce (Meyer, 1995b), is much diminished and represents a particular challenge to performing nucleotide-based amplification and detection methods. Table 15 provides an overview of the average DNA length that can be expected from fresh and processed food stuffs.

3.1.4.5 PCR methods applied to processed foods

It can be concluded from data presented in Table 15 that PCR assays in routine diagnostics should certainly not target sequence stretches longer than 500 basepairs. Instead, it may be rather favourable to restrict amplicon length to below 300 basepairs, when the assay is to be used with processed foods.

It may, therefore, be appropriate to test the average length of DNA fragments present in a given probe. For this purpose, the probe may be tested for the detectability of a sequence that must be present in the actual (non-degraded) probe using an amplicon with a size similar to the actual target sequence. In a project evaluating the possibility

of detecting honey containing genetically engineered pollen, DNA fragments from 226 up to more than 1,300 basepairs have been amplified out of honey (Du Prat et al., 1996) using plant universal primers (Chaw et al., 1993). These fragments could also be amplified in tomato puree from conventional and genetically modified tomatoes, tomato soup, passata, ketchup, sun-dried tomatoes, genetically engineered potatoes, mashed potatoes, potato salad, canned potatoes, frozen chips, and oil-fried chips produced from genetically engineered potatoes (Ballaron et al., 1996; Ford et al., 1996). In addition, the identification of a 150 basepair fragment by PCR analysis of lecithin probes has been reported (Personal communication D. Bobbink, Greenpeace e. V., Hamburg, and A. Wurz, Hydrotox GmbH, Freiburg).

Further aid for the development of PCR assays applicable to processed foods can be obtained from articles on authenticity testing in food diagnostics (for a review, see: Meyer and Candrian, 1996; Candrian, 1994).

3.1.4.6 Inhibition of PCR and DNA extraction procedures

PCR can be inhibited by various compounds present in food stuffs. Hemoglobin (Ruano et al., 1992), nitrite salts used in sausages (Hertel et al., 1995b) and diary products (Bickley et al., 1996) have been shown to be potent inhibitors of the PCR reaction. A long list of salts, carbohydrates and other compounds frequently used in buffer solutions also decrease the performance of PCR (Rossen et al., 1992; Hammes and Hertel, 1995). The choice and optimisation of the DNA extraction procedures which eliminate potential inhibitory components may thus be of pivotal importance for the success of a given PCR method (Du Prat et al., 1996; Ford et al., 1996; Meyer and Candrian, 1996). Overly high concentrations of DNA itself may also inhibit PCR (Candrian, 1994).

Apart from optimising the DNA extraction there are other ways of counteracting the inhibitory effects on PCR. If the DNA content is not limiting, the simplest and possibly most effective way to avoid inhibition of PCR is the dilution of the sample. Application of 'nested PCR' appears to be particularly advantageous for the analysis of highly processed tomato products (Personal communication, H. Parkes, Laboratory of the Government Chemist, Middlesex, UK). Repeated freeze-thawing (Stary et al., 1996) and the addition of single-strand DNA-binding proteins (Vahjen and Tebbe, 1994; Kreder, 1996) have also been reported as effective methods for minimising PCR inhibition effects. A detailed discussion on compounds that inhibit PCR and on methods for removing inhibitors has recently been published by Gasch et al. (1997).

3.1.4.7 Verification of PCR results

There are several methods for verifying PCR results; they vary in reliability, precision and cost. In almost all methods used, PCR products are separated using gel electrophoresis and checked for the expected size. Parallel to that separation or subsequent to it, there are various verification techniques applicable. Specific cleavage of the amplification product by the use of restriction enzyme(s) followed by an additional separation of the fragments by electrophoresis represents one method (Meyer, 1995a, Pietsch et al., 1997). More time-consuming but also somewhat more specific is the transfer of the separated amplification products onto membranes (Southern Blot) followed by hybridisation with a DNA probe specific for the target sequence (LMBG-Methodensammlung, 1996; Schulze et al., 1996). Also worth

considering is the application of a special electrophoresis technique that separates DNA fragments not only by size but by the relative composition of bases (Wawer et al., 1995). Verification of PCR products may be done by direct sequencing (Kocher, 1992; Feldmann et al., 1996). Other elegant techniques which can be performed on microtitre plates analogous to an ELISA test are also available: one technique is using DNA double strand-specific antibodies (DNA-Hybridisation Immuno-Assay, DIA), as described by Müller et al. (1996); another method employs biotinylated and digoxigenin-labelled primers (Börchers et al., 1997).

3.1.4.8 Reviews on the application of PCR in other areas

Apart from authenticity testing, mentioned already earlier, PCR has been used for several years in other fields of diagnostic applications, such as the detection of pathogens in food (Olsen et al., 1995), in parasitology (Felleisen et al., 1996) and veterinary (Pfeffer et al., 1995) and clinical diagnostics (Karch et al., 1995; Ronai and Yakubovskaya, 1995). In addition, PCR has been employed for monitoring of genetically engineered microorganisms in the environment (Jansson, 1995).

3.2 Various nucleotide-based amplification methods and their applicability

Most of the methods mentioned in this section have generally not yet been used widely for the identification of genetically engineered food or food stuffs. This survey, therefore, very much restricts itself to survey review articles that may simplify access to additional readings. Some of the techniques may, under certain circumstances, be appropriate for food analyses. Ongoing research projects (see later sections) include the evaluation of the applicability of some of these methods for the detection of genetically engineered food.

3.2.1 Ligase Chain Reaction (LCR)

The ligase chain reaction is a DNA amplification method based on repeated cycles of oligonucleotide hybridisation and ligation (Backman and Young, 1989; Carrino and Lee, 1995). The method employs sets of oligonucleotides specific to stretches of the target sequence that are in close proximity to each other, as well as another set of oligonucleotides that is complementary to the first set. The protocol is very similar to PCR, except that LCR uses a heat-stable ligase. Polymerase activity is not needed since the primers basically constitute virtually the entire length of the target sequence. Therefore, the length of the amplicon will generally be limited by the availability of longer oligonucleotides. Although known for years now, LCR or variations of this technique (e.g. Gap-LCR) is by far not as significant in routine diagnostics as is PCR (Carrino and Lee, 1995; Pfeffer et al., 1995).

3.2.2 Nucleic Acid Sequence-Based Amplification (NASBA)

This technique mimics the process of retroviral replication (Compton, 1991) and has been used until now primarily for the amplification of RNA molecules (Carrino and Lee, 1995). The method might be applicable for the detection of expressed transgenes and/or viable microorganisms (Blais et al., 1997). Because RNA molecules are present in much higher copy numbers than the respective gene (provided the gene is expressed), NASBA may demonstrate a greater degree of sensitivity compared to PCR for certain applications (Lunel et al., 1995). However,

RNA is much more sensitive to degradation than DNA; therefore, the probe material must necessarily be very fresh and appropriately handled. For heat-treated and other processed foods the applicability of NASBA seems very limited. As PCR assays of fresh foods are normally sufficiently sensitive, it seems unlikely that NASBA will find broad application in food analysis.

3.2.3 'Self-sustained sequence replication' (3SR) and 'Q replicase amplification'

Methods for the identification of pathogenic microorganisms have already been developed based on the isothermal 3SR and Q replicase amplification techniques (Carrino and Lee, 1995; Pfeffer et al., 1995). Despite a high amplification rate, these techniques are of less significance in diagnostics as compared to PCR (Pfeffer et al., 1995). Moreover, the alleged technical advantage of an isothermal reaction (Pfeffer et al., 1995), with fast amplification that is not limited by defined temperature and time-cycles and requiring less special equipment, can actually be a disadvantage when compared to methods such as PCR and LCR, which employ pre-set cycles: discrete obligatory temperature cycles have been considered to be a main cause for the relatively minor tendency of PCR for certain experimental artefacts ('in vitro evolution', i.e. amplifying artificially small DNA fragments), whereas isothermal techniques favour fast replicators (Bull and Pease, 1995) and thus short amplicons.

3.2.4 Fingerprinting techniques (RFLP, AFLP, RAPD, etc.)

Fingerprinting techniques such as RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism) or RAPD (Random Amplified Polymorphic DNA) are used in forensic analysis and for the classification of organisms. They have been successfully used in combination with PCR amplification to classify microorganisms (Tichy and Simon, 1994) and for other applications (Welsh et al., 1995). Fingerprint techniques are applicable for the analysis of complex mixtures of microorganisms used as starter cultures. In this context, fingerprinting may allow to confirm, if a given genetic modification is indeed present in the expected genetic background of a given microorganism. These techniques are based on the comparison of the genomes of related organisms but they may not be sensitive enough to resolve the difference between the DNA of transgenic organisms and their conventional counterparts. The genetic differences among varieties of the same crop are by far greater than differences between a genetically engineered crop and its conventional counterpart. Therefore, with fingerprinting methods it is essential that the DNA compared be derived from exactly the same crop variety before and after transformation. If more than one transgenic product of a certain species (e.g. corn) exists, the DNA of all the respective hosts will be required. Such conditions are difficult to satisfy. Furthermore, fingerprint techniques apparently cannot be used for analysing complex food mixtures or processed foods.

3.2.5 Probe hybridisation

Hybridisations using DNA probes have been frequently used for the detection of pathogens in food (Jones, 1991). One model system for the detection of genetically modified bacteria in milk has been published (Casey et al., 1993). The degree of sensitivity and specificity of probe hybridisation is significantly lower than that achieved through the previously described amplification techniques. Since plants

have particularly large genomes but transgenes are present only one or a few copies (thus the relative concentration of target sequence to total DNA is low), the application of probe hybridisation for detecting GMO crops does not seem very promising. However, provided that the target sequence is present in sufficient concentrations (multiple copies of the transgenes, small genome size [e.g. bacteria]), probe quantity is not significantly limited, and highly specific oligonucleotide probes are available, probe hybridisation may provide a simple technique worth considering for screening purposes.

3.3 Protein-based methods

Detection methods based on the immunological detection of proteins or on the comparison of protein patterns (e.g. one- or two-dimensional gel electrophoresis) require that the sample or the protein of interest are not significantly degraded. Thus, the application of protein-based detection methods for the identification of genetically engineered food products is generally restricted to fresh (or frozen) and unprocessed foods.

Protein samples obtained from GMOs can be resolved with one-dimensional SDS-gel electrophoresis. Unfortunately, the resolution is not sufficient to clearly distinguish the protein pattern of a GMO from the protein pattern of its conventional counterpart. Two-dimensional gel electrophoresis provides better resolution, but still may generally not be able to provide unequivocal identification of a (trans-) gene product unless combined with immunological methods. The expression level of transgene products in plants were reported to constitute 0 to 2 % of the total soluble protein even when strong constitutive promoters were used to drive expression (Longstaff et al., 1995). Expression levels found in approved genetically engineered crops are generally lower (Table 3) than the reported upper figure of 2 %. Provided that specific antibodies against the proteins encoded by the transgenes are available, one-dimensional (Padgette et al., 1995; Wood et al., 1995; Yang et al., 1996) and certainly also two-dimensional gel electrophoresis, in combination with Western-blot analysis are suitable detection methods. ELISA can also be an inexpensive but powerful technique (Padgette et al., 1995; Wood et al., 1995). Recently developed techniques using immunosensors have up to now mainly been used for the analysis of serum and blood samples (Morgan et al., 1996). All immunological methods described above, depend on the availability of highly specific antibodies. The latter are commercially available only for a small number of proteins that are the products of transgenes used in approved genetically engineered crops. To our knowledge, these are antibodies against the nptII-gene product, NPTII, or APH(3')II, and against the product of the *gus* gene. Since the nptII gene is present in 17 of the 28 approved genetically engineered agricultural crops (see section 4.1.1) and is under the control of a eucaryotic promoter in 16 of these crops (Tables 3 and 5), the development of a screening method based on the immunological detection of NPTII (ELISA-, Dot-Blot-, or Western analysis) may represent an interesting and rather inexpensive possibility.

3.4 Detection of enzymatic activities

The detection methods based on measuring enzymatic activities are limited to the detection of transgenes that represent enzymes. Enzymatic function of a protein depends on the structural preservation of the protein molecule even more than the recognition of the protein by antibodies. Therefore, an important restriction of

enzymatic methods is the requirement that the sample must be fresh enough to contain enzymatic activity. With this in mind, it seems highly unlikely that detection of certain enzymatic activities, e.g. by measuring the enzymatic EPSPS activity (Padgette et al., 1987; 1988), will find broad application in the detection of genetically engineered food.

4 Genetic elements used in approved genetically modified agricultural crops - implications for screening and product-specific detection methods

Almost all approved genetically engineered crops (Tables 3-10) were first approved in the United States. Exceptions are the genetically modified chicory from Bejo-Zaden (ID 35), genetically engineered tobacco from Seita (ID 32) and the genetically modified carnations approved in Australia. An analysis of the genetic elements used for the generation of the 28 distinct genetically engineered agricultural crops approved in the United States and the European Community therefore represents a comprehensive basis for the development of nucleotide-based detection methods. These 28 approved products include the following crops: chicory (1), corn (6, products derived from the same transformation events such as ID 14 and ID 61 are treated as a single product in this inventory), cotton (4), papaya (1), potato (2), rapeseed (4), soybean (2), squash (2), tobacco (1) and tomato (5). For several products, plants (lines) originating from several distinct transformation events have been approved. This has resulted in minor differences in the content of certain genetic elements present in these lines. These differences are indicated by brackets (Tables 3-5) or by asterisks in the relevant Tables (Tables 16-19).

4.1. Survey of genetic elements introduced into approved transgenic crops

The structural genes and the respective regulatory sequences (promoters and terminators) which have been used as transgenes are summarised in the following sections. Depending on their frequency of use in approved genetically modified crops and the probability of a 'natural' occurrence of these sequences in food, sequences within single genetic elements are applicable for the screening for GMO-food. Genetic elements that have been used in isolated cases may allow specific detection of the given product for as long as the respective element is not employed in further approved products. Thus, methods based on the detection of sequences within a single genetic element will in the long run be better suited for screening purposes.

4.1.1 Survey of the structural genes used

Almost 35 distinct structural genes (including variants) have been used for the generation of the currently approved transgenic crops (Table 16). Some of these genes such as accd, accS, sam-k and some genes coding viral coat proteins occur only in a single genetically engineered product. Therefore, the identification of sequences of one of these genes in food would represent a product-specific detection method provided the actual sample did not contain the natural sources of these sequences (e.g. from bacteriophages or plant viruses).

The most frequently used transgene is the nptII gene, originally isolated from the bacterial transposon 5. The nptII gene has been introduced into 17 out of the 28 approved agricultural crops. In 16 products it functioned as a marker gene under the control of a eucaryotic promoter; thus, nptII sequences seem to be well suited for

screening purposes. It should be noted, however, that nptII occurs frequently in bacteria found in the environment (Smalla et al., 1993; Redenbaugh et al., 1994). The presence of these naturally occurring bacteria in a sample may, therefore, lead to a false-positive result. Future transgenic crops are expected to contain fewer or no marker genes in the final products since marker-free insertion techniques or methods to eliminate marker genes from transgenic plants (for review see Yolder and Goldbrough, 1994 and references in Niederhauser et al., 1996) and microorganisms (Sanchis et al., 1997) are already available.

Other structural genes have been employed less frequently. From the 28 approved crops, variants of the -endotoxin gene from *Bacillus thuringiensis* or of the bar gene originally isolated from *Streptomyces hygroscopicus* are found in 6 products each. Variants of the CP4 epsps gene from *Agrobacterium,* the -lactamase gene and of the polygalacturonase gene have been introduced in 3 to 5 products each (Figure 4). In these cases various factors may have to be assessed to judge the applicability of DNA-based detection methods: (i) the presence of the transgene in the respective transformation event (line); (ii) the 'completeness' of the respective sequence (incompletely transferred, or 'truncated' or 'altered' versions of genes may be present); (iii) the use of 'synthetic' versions of genes that have an altered codon usage in order to optimise gene expression in the host organism.

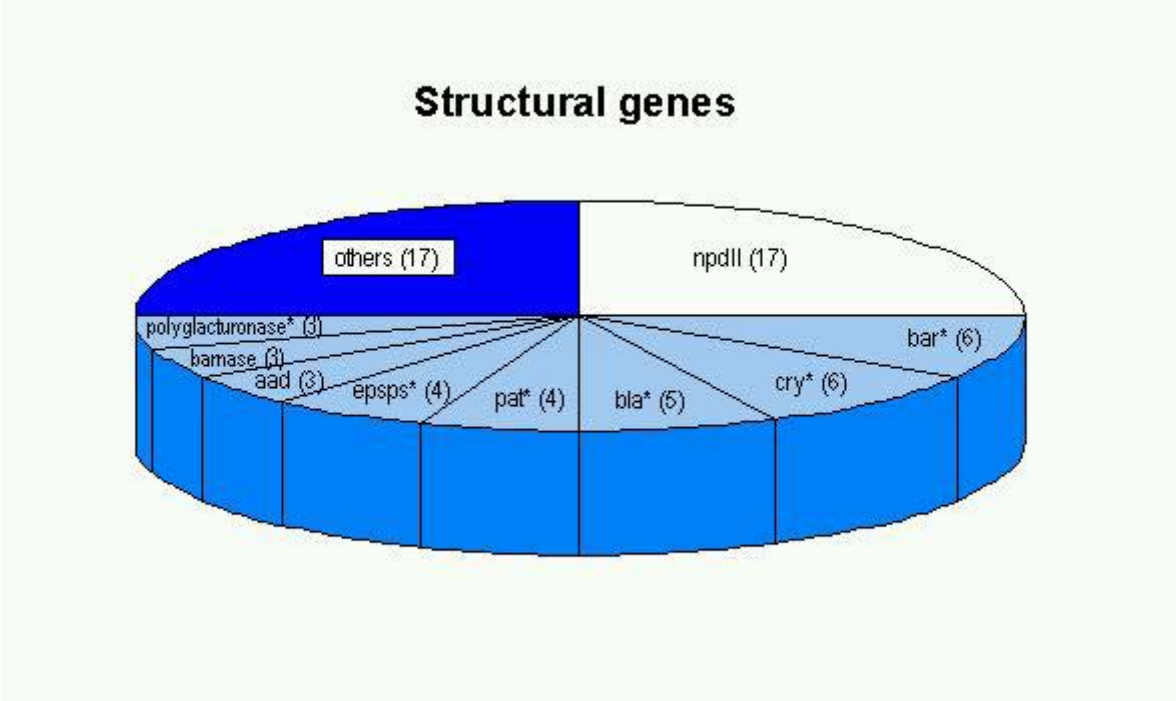

Figure 4: Number of occurrences of the most frequently used transgenes introduced into the currently approved genetically engineered agricultural crops (in total 28 distinct products were approved; see text). In some cases (indicated by asterisks) distinct variants of genes or 'synthetic' versions were used. (See also Table 16.)

Both the gene encoding for barnase and the aad gene are present in three products each, whereas a synthetic version of the pat gene can be found in four products. Seventeen other genes are present in one or two of 17 different products.

Sequences from structural genes originating from homologous sources (up to now mostly antisense constructs in tomatoes) are suitable for the detection of GMOs only if certain prerequisites are fulfilled. When the coding sequences occur in the (copies of the) transgene and also in the naturally occurring copies of the gene, a clever choice of primers for a PCR assay may allow discrimination of the amplification products of the native gene and the transgene by the length of the amplified fragment. This can be achieved if the two primers bind to sequences on the chromosomal gene that are situated on different (normally adjacent) exons. Whereas analysis of conventional products would result in the amplification of a single long fragment that includes the sequence of the intron between the respective primer binding sites, analysis of the corresponding genetically modified product would result in the appearance of an additional, shorter amplification product lacking the intron sequence (since transgenes originate from c-DNA sequences). A description of this methodology was contained in the petition for the genetically engineered tomato from Zeneca (Petition from Zeneca for genetically modified tomatoes, 1995). However, such a strategy requires not only the knowledge of the c-DNA sequence (of the transgene) but also precise information about the intron-exon boundaries of the chromosomal gene, which is not always available.

4.1.2 Survey of the promoters used

The expression of a new phenotype is dependent on various factors, including the site of integration of the T-DNA whose location may have an impact on processes such as gene silencing (P. Meyer, 1995). Alteration of the codon usage is a method frequently used to optimise bacteria-derived transgenes for plant expression, based on the redundancy of the genetic code and the distinct prevalence of various codons and their respective t-RNAs in different phyla.

One of the most important factors for achieving the desired expression levels of a transgene is the choice of the promoter that regulates the transcription of the respective transgene. Many of the transgenes that are present in 22 out of these approved genetically modified products are regulated by the constitutive 35S promoter from the cauliflower mosaic virus (P-35S = P-CaMV 35S) or by derivatives of this promoter (e.g. with enhancer or duplicated). PCR-assays detecting the presence of these promoter sequences appear to be well suited for the development of detection methods (Figure 5, Tables 17 and 19).

The promoter from the nopaline synthase gene of *Agrobacterium tumefaciens* (P-nos) has been used in 7 genetically engineered products, whereas 4 products carry transgenes that are regulated by variants from the ribulose-1,5-bisphosphate carboxylase promoter derived from various plants. P-TA29 represents a tissue- and development- specific promoter isolated from tobacco that drives expression of one or several transgenes in 3 products. Seven genetically modified crops contain still other promoters, while ten harbour genes with bacterial promoters (Figure 5).

Some promoters until now have only been employed in single products; however, the detection of internal sequences of these promoters is generally not appropriate for detecting GMOs since many of these, such as the tissue-specific promoters P-PEPC and P-CDPK, originate from agricultural crops.

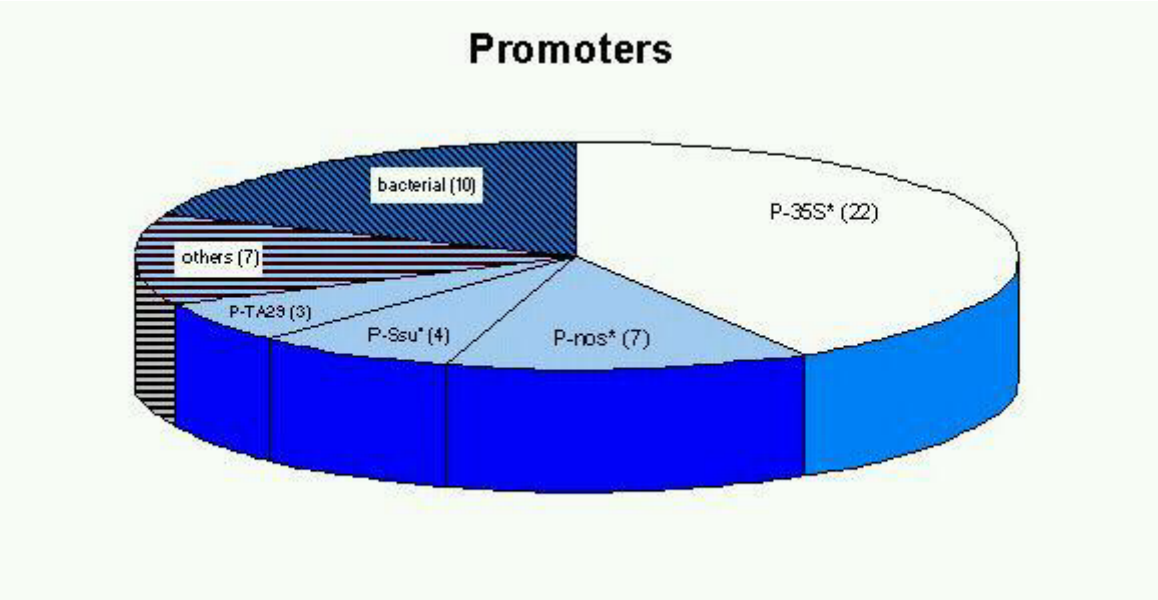

Figure 5: Number of occurrences of the most frequently used promoters introduced into the currently approved genetically engineered agricultural crops (in total 28 distinct products were approved; see text). In some cases (indicated by asterisks) distinct variants of the respective promoter were used (see Table 17).

4.1.3 Survey of the terminators used

In the approved transgenic crops the terminator that is used most frequently to terminate transgene transcription is nos 3', isolated form the nopaline synthase gene from *Agrobacterium tumefaciens*. It has been used in at least 16 of the 28 products. No data were available on the terminators of three transgenes used in two different crops (Papaya [ID 26] and rapeseed [ID 60]).

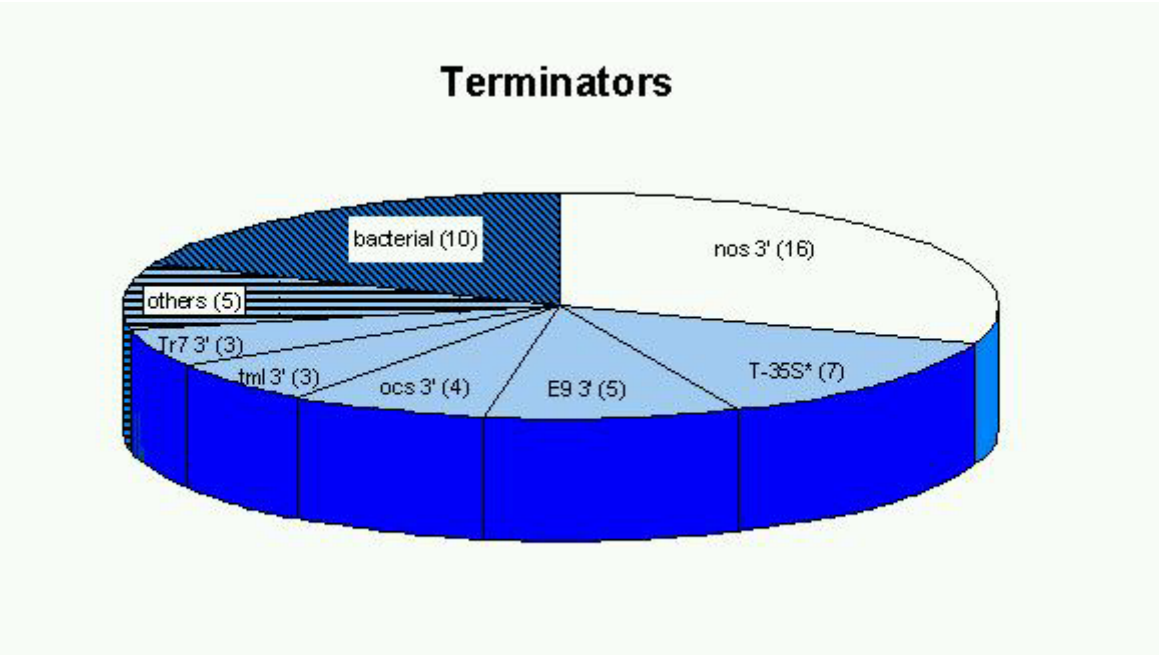

Figure 6. Number of occurrences of the most frequently used terminators introduced into the currently approved genetically engineered agricultural crops (in total 28

distinct products were approved; see text). In some cases (indicated by asterisks) distinct variants of the respective terminator were used (see Table 18).

In at least seven products the terminator of the cauliflower mosaic virus (T-35S) or derivatives thereof was used, while the plant-derived terminator E9 3' (from pea) was employed in five crops and ocs 3', Tr7 3' and tml 3' (all from *Agrobacterium tumefaciens)* in at least four and three products, respectively (Tables 3, 5 and 18, Figure 6). Another five terminators were used in a single crop each.

4.2 Development of screening methods

The promoter from cauliflower mosaic virus is the most abundantly used transgenic element in approved genetically engineered crops; it is present in 22 out of 28 plants in its original version or as one of its variants (see Table 17). At least six different publications with respect to the origin of the respective P-35S derivative used in the various crops have been cited in publicly available petition documents (Franck et al., 1980; Gardner et al., 1981; Odell et al., 1985; Kay et al., 1985; Pietrzak et al., 1986; Sanders et al., 1987). Some of the versions mentioned display only minor differences or vary by the fusion to distinct 5'-untranslated regulatory regions (Table 17). It will be necessary to take these differences into account for the development of broadly applicable screening methods. A PCR-assay based on P-35S sequences that have been shown to be present in various transgenic crops has already been developed (Pietsch et al., 1997; Waiblinger et al., 1997).

Nos 3', originally derived from *Agrobacterium tumefaciens* (Hernalsteens et al., 1980; Depicker et al., 1982; Bevan et al., 1983; Bevan, 1984; An, 1986), is the most frequently used terminator in approved transgenic crops, present in at least 16 out of 28 approved products (Tables 3, 5 and 18). A PCR-assay based on nos 3' sequences has already been developed and tested (Pietsch et al., 1997; Waiblinger et al., 1997). At least four of the six genetically engineered crops that are not detectable on the basis of P-35S sequences should be detectable by a PCR assay using nos 3' sequences (Tables 3 and 5). The two products that can be assayed neither by a PCR test based on P-35S nor on nos 3' sequences are genetically modified oilseed rape from Monsanto (ID 65) and cotton developed by DuPont (ID 22). A PCR assay for the gene for acetolactate synthase (als) that is present in this cotton has been described already (Petition from DuPont, 1995). Detection methods for the oilseed rape from Monsanto may be developed on the basis of the described genetic elements (Table 3).

For a PCR screening method to be widely applicable, the following criteria should be met:

- Primers should be selected that are specific for genetic elements present in a large number of genetically engineered agricultural crops.
- The genetic elements on which the assay is based should not occur naturally in the respective plants.
- The assay should not rely on genetic elements that occur in organisms that may appear frequently as contaminants of the food stuff under analysis.
- Within the chosen elements, sequences should be selected that allow specific identification of as many variants as possible of the respective element. In

addition, primers with complementary sequences or primers with a secondary structure should be avoided.

- The designed amplicon should be relatively small to warrant broad applicability of the test also to heat-treated samples or materials with low pH and/or highly degraded DNA. In this respect, amplified fragments should not exceed 500 basepairs; a length between 150 and 300 basepairs seems ideal. An even smaller amplicon length (below approximately 100 basepairs) is possible; however, it may require the separation of the amplification products in special type of gels in order to distinguish frequently appearing artificial PCR-products of twice the length of the primers (in general 40-50 (2 x 20-25) basepairs) from the actual target sequence.

Table 19: How many PCR systems are needed?

Genetic Elements	*Number*	*Identifiable Products (total 28)*
P-35S*	1	22
nos 3'	1	16
P-35S*, nos 3'	2	26 (or 27)
P-35S*, nos 3', E9 3', als	4	28
nptII	1	17
P-35S*, nptII	2	25
P-35S*, nptII, nos 3'	3	26 (or 27)
P-nos*	1	7
P-35S*, P-nos*	2	25

P-35S* = P-35S including derivatives

Apart from the combination P-35S*/nos 3' there are a number of other combinations that would permit screening for the presence of most approved genetically engineered crops, some of which are described in Table 19. Other combinations are possible by including data from the Tables 3, 5, 16, 17 and 18 such that the identification of products from all approved transgenic crops is achievable.

Screening methods using P-35S and nos 3' sequences evidently are the most favourable candidates for broad method applicability. Assays based on nptII sequences may also be promising, although it has to be taken into consideration that

nptII is frequently found in bacteria (Smalla et al., 1993; Redenbaugh et al., 1994) and therefore might lead to false-positive results. In order to judge the reliability of positive results from screening methods based on P-35S and nos 3' sequences, it might also be worthwhile to assess the probability of naturally occurring contamination of foods by plant pest organisms, such as cauliflower mosaic virus or *Agrobacterium*.

4.3 Product-specific detection methods and available sequence information

Product-specific detection methods based on PCR technology ideally employ target sequences uniquely found in the respective genetically engineered organism. This can be accomplished by choosing primers that bind to two different adjacent genetic elements combined in the respective product but which are found neither naturally in that combination nor in any other known approved transgenic product. These primers result in amplification products containing interfaces between unique[1] combinations of:

- regulatory sequences and structural genes,
- leader sequences (e.g. chloroplast transit peptide sequences) and structural genes,
- different regulatory elements, or
- the interface between the genomic sequence of the host plant and the DNA that was introduced.

Specifically altered sequences of structural genes to allow plant-specific codon usage (here, termed 'synthetic' genes) or chimeric constructs may also allow specific identification of the respective products.

The availability of precise and comprehensive sequence information is an important prerequisite for the development of such product-specific DNA-based detection methods. If products have been approved that may have originated from various transformation events, the data should also assess differences in the content of genetic elements between these lines. Ideally, the data should include complete sequences of the vectors used for plant transformation, knowledge as to which parts of the plasmid are stably integrated into the host genome as well as the sequences of the sites of integration, when possible. Minimal requirements are specific sequence information on a unique expression cassette (promoter-structural gene-terminator) or the sequence of a transgene with altered codon usage.

Some of the sequence information that has been disclosed on the currently approved genetically modified agricultural crops is presented in Table 20.

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[1] In order to distinguish different genetically modified products containing, for example, the same (trans-) gene and promoter, but divergent untranslated sequences in between, the length of the amplicon may also be considered as a factor to achieve a 'unique' combination.

5 National research projects devoted to the detection of GMOs

Several countries and communities are currently funding research projects with the objective of developing or optimising methods for the detection of genetically modified organisms. Most of the research efforts focus on the identification of food stuffs, consisting of or containing GMOs. Other activities are aiming at the detection of GMOs in the environment.

5.1 The European Community

In October 1996, a three year EC project was started on the 'development of methods to identify foods by means of genetic engineering' (DMIF-GEN). Approximately 20 European laboratories are currently participating in this project under the coordination of Dr. G. Schreiber, 'Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin' (BgVV) (Schreiber, 1997). The Swiss Federal Office of Public Health is one of the partners in that project and the agency BATS will also take part in the near future. The main objectives of the EC project include the optimisation of existing detection methods, as well as the development of new identification methods, taking into special consideration the problems derived from the nature of the food matrix and the respective organism used. The methods to be studied include multiplex-PCR, PCR ELISA, DNA-Biosensors, direct hybridisation, 3SR, NASBA, AFLP and protein diagnostic approaches.

5.1.1 Germany

During the last few years a working group for the development of methods to detect foods produced by means of genetically engineering ('Entwicklung von Methoden zum Nachweis mit Hilfe gentechnischer Verfahren hergestellter Lebensmittel') consisting of predominantly German participants and headed by the BgVV has developed three PCR-based detection methods using model organisms. Two of these methods, one for the detection of a genetically modified potato and the other for a genetically engineered *Lactobacillus* in raw sausages have been completed and published (Schulze et al., 1996; LMBG-Methodensammlung, 1996 and 'in press') (see section 3.1.1). The third method, which was developed for the detection of *Streptococcus* in yoghurt, will soon be finalised. Several of the partners that participated in this working group now are taking part in the EC project described under 5.1.

5.1.2 The United Kingdom

The Institute for Food Research in Norwich started a project in 1994 on 'tagging genetically engineered organisms'. In addition, several projects are being pursued at the Laboratory of the Government Chemist in Middlesex (project titles are given):

- 'Development and validation of DNA probe and PCR technology and the introduction of reporter genes to detect very low levels of GMOs in the environment' (MTS-programme, 1992-1997).
- 'Detection of genetically modified organisms in foods' (MAFF-funded, 1995-1998).
- 'Honey from GM plants: integrity of pollen DNA, and expression of promoters in floral organs' (MAFF-funded, 1995-1997).
- 'Monitoring releases of GM crop plants: herbicide-tolerant transgenic oilseed rape' (DOE -funded, 1994-97).

- 'Development of methods to identify foods by means of genetic engineering' (EC-funded, 1996-1999).

5.1.3 The Netherlands

A project in the Netherlands was recently initiated for the 'development of a method to screen for the presence of transgenic soybean.' The contributing partners are RIKILT-DLO and TNO-Voeding. The project is funded by the Ministry of Agriculture, Nature Conservation and Fisheries and the Ministry of Health, Welfare and Sports.

5.2 Switzerland

Research with the objective to develop detection methods, to test such methods in inter-laboratory studies and to apply them in the laboratories that are finally responsible for the control of actual food samples are carried out by the Swiss Federal Office of Public Health (BAG), a federal sub-commission (SK 29A), academic laboratories and by food control laboratories ('Kantonale Laboratorien') of the federal states ('Kantone').

A screening method applicable to the detection of the approved transgenic soybeans (Pietsch et al., 1997; Waiblinger et al., 1997) is being tested in an inter-laboratory study. A product-specific method developed in collaboration with R. Meyer, Nestlé, is also being tested. Furthermore, a highly sensitive product-specific method for the detection of transgenic soybeans from Monsanto using nested PCR (University of Berne), and a specific method for detecting the presence of transgenic corn from CIBA-GEIGY (now Novartis) in food (SK29A, BAG, Kantonale Laboratorien Basel and Bern) are currently being developed.

5.3 Canada

The Research Division of the Bureau of Microbial Hazards in 'Health Canada' is collaborating with several academic laboratories on the development of methods to detect GMOs; the projects include the detection of GMOs in food stuffs using DNA probe hybridisation and conventional as well as in situ PCR. Another project involves the use of bioluminescence technology in order to monitor GMOs in food; further projects focus on monitoring of GMOs in the environment.

6 Laboratories for food control in Switzerland

In order to better assess the current activities, personnel structures and technical facilities of the food control laboratories in the Swiss federal states (cantonal laboratories) and in Liechtenstein with regard to the molecular techniques they are applying to the detection of GMOs in food, a brief questionnaire was sent to individual laboratories in summer, 1996.

The laboratories of Basel and Bern indicated that they were already engaged in developing such molecular techniques. These and another four out of the 21 laboratories (Kantonale Laboratorien Aargau, St. Gallen, Solothurn and Thurgau) are presently using PCR-based methods; in some cases, PCR diagnostics are routinely employed on a daily basis. DNA probe hybridisation is less frequently used. In some of the laboratories not yet using the described methods, interest was expressed in

establishing molecular techniques such as PCR in the near future. Laboratories from large retailers tend to collaborate with experienced academic or official laboratories.

The questionnaire revealed strong interest on the part of the surveyed laboratories to develop methods for detecting GMO-derived foods. On the other hand, the questionnaire revealed a substantial lack of specific information about GMOs, in particular with regard to precise sequence information on approved genetically modified organisms. The situation is evidently similar in Germany, according to sources from German food control laboratories. A major stumbling block hampering the elaboration of identification methods is the dearth of reliable genetic information. Comprehensive databases in this field may considerably facilitate research efforts in this field.

7 Prospects

Currently available methods for detecting GMOs are almost exclusively based on PCR. The predominance of this technique is likely to persist due to its broad applicability (mainly based on the chemical stability of DNA), the tremendous experience which has already been gained with it and the high potential for automation. However, other techniques are currently being tested in projects which seek to develop detection procedures for GMOs. New results may recommend the use of some of these techniques for specialised applications.

In order to prevent food control expenses from escalating, it is desirable that inexpensive and widely applicable screening methods for the detection of food derived from genetic engineering are developed. With the increasing number of commercially sold GMO products these screening methods are likely to be complemented by product-specific detection methods.

National and international coordination of the development of methods to identify GMOs would be highly appreciated. A comprehensive database containing all national approvals of GMOs with the respective genetic and sequence information is bound to promote greater research efficiency in this area. In this respect, it should be noted that the database on which the tables and statistics shown in this report are based will be developed further and partially integrated into the database of an ongoing EC project (DMIF-GEN). The expansion of the database on subjects such as environmental and consumer safety information is planned on a modular basis. This information should provide a basis for the efficient development of identification methods by expert groups, help to assess the safety of genetically engineered food and facilitate information transfer to the consumer and the general public on genetically modified products.

8 GLOSSARY

3SR	self-sustained sequence replication (trade name)
7S 3'	terminator sequence of the alpha-subunit of the soybean beta-conglycinin gene
12:0 ACP	acyl-carrier protein (ACP) binding to laurate (12:0)
aad	3''(9)-O-aminoglycoside adenylyltransferase; conveys (bacterial) resistance to streptomycin and spectinomycin

accd	aminocyclopropane carboxylate deaminase
AccS	aminocyclopropane carboxylate (ACC) synthase (e.g. Acc2)
ACNFP	Advisory Committee on Novel Foods and Processes
ACP	acyl-carrier protein, see 12:0 ACP
AFLP	Amplified Fragment Length Polymorphism
als	acetolactate synthase (sulfonylurea tolerance)
'altered' gene	changes in the gene result in changes in amino acid sequence
AMFEP	Association of Manufacturers of Fermentation Enzyme Products
ampR	ampicillin resistance gene (see bla)
AMV-5'-utr	5' leader sequence of the RNA4 transcript of alfalfa mosaic virus
A. niger	*Aspergillus niger*
APH(3')II	aminoglycoside-3'-phosphotransferase (NptII)
aphIV	hygromycin-phosphotransferase
APHIS	Animal and Plant Health Inspection Service
ArabSSU1A	small subunit gene of ribulose-1,5-bisphosphate carboxylase from *Arabidopsis thaliana*
BAG	Bundesamt für Gesundheit (Switzerland)
bar	gene coding for a phosphinothricin acetyltransferase from *Streptomyces hygroscopicus;* homologoue to pat
barnase	ribonuclease from *Bacillus amyloliquefaciens*
barstar	specific inhibitor of the barnase from *Bacillus amyloliquefaciens*
BATS	Agency for Biosafety Research and Assessment of Technology Impacts of the Swiss Priority Programme Biotechnology of the Swiss National Science Foundation
BgVV	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin
bla	beta-lactamase gene; conveys resistance to beta-lactam antibiotics (e.g. penicillin, ampicillin); from Tn3 (see also ampR)
Bt	Bacillus thuringiensis
BXN	bromoxinyl-resistance gene (nitrilase) from *Klebsiella*
Cab22R 5' utr	5' untranslated region from the Cab22R gene from *Petunia hybrida*
CaMV 35S promoter	see P-35S
cat	chloramphenicol transacetylase (e.g. from *Staphylococcus aureus*)
CMV	cucumber mosaic virus
CMV-5' utr	5'-untranslated region from CMV RNA3
CMV/WMV2 CP	chimaeric CP consisting mainly of WMV2 CP sequences but with the N-terminus of CMV CP

CoPB	Colorado potato beetle (Coleoptera)
CP	viral coat protein
CP4 epsps	5-enolpyruvylshikimate-3-phosphate synthase, here from *Agrobacterium* sp. (strain CP4)
cry	&lambda-endotoxin from *Bacillus thuringiensis*; class of genes providing insect resistance to certain specific insects; the active form of the toxin binds to cells in the insect gut, ultimately leading to cell lysis and insect death
ctp	sequence coding for a chloroplast transit peptide (CTP)
CUP1	yeast gene involved in copper resistance
d-P-35S	duplicated version of P-35S
d-P-nos	duplicated version of P-nos
DMIF-GEN	development of methods to identify foods produced by means of genetic engineering (EC project)
docket-#	document number (according to www-site: <http://www.aphis.usda.gov/bbep/bp/petday.html>)
DOE	Department Of The Environment (UK)
dr	developmentally regulated
E9 3'	3' sequence of small subunit of rbcS (ribulose-1,5-bisphosphate carboxylase) E9 gene (from pea)
e-P-35S	enhanced version of P-35S
ECB	European corn borer (Lepidoptera)
EPA	(US) Environmental Protection Agency
epsps	see CP4 epsps
FAC	Food Advisory Committee (UK)
FDA	(US) Food and Drug Administration
FDHC	Food Directorate Health Canada
fwt	fresh weight
gent	gene isolated from *E. coli* which conveys resistance to the antibiotic gentamycin
GIBiP	Green Industry Biotechnology Platform
Gly, gly	Glyphosate (active compound in the herbicide Roundup™); inhibits plant 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS), which is essential for the synthesis of aromatic amino acids in plant cells
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
gox	glyphosate oxidoreductase
gus	beta-glucuronidase, marker gene
HC	Health Canada

HT	herbicide tolerant
I9	intron 9 from corn PEPC gene
ID	identification number (designated to the approval of a GMO product in a single country)
IR	insect resistant
IVS2/IVS6	intron(s) from maize alcohol dehydrogenase 1S gene
K. lactis	Kluyveromyces lactis
lacZ	beta-galactosidase gene
LCR	Ligase Chain Reaction
LMBG	Lebensmittel- und Bedarfsgegenständegsetz
LMV	'Lebensmittelverordnung' (ordinance on food stuffs)
M13 gene III	gene III of bacteriophage *M13*
MAFF	Ministry of Agriculture, Fisheries and Food (UK)
m-HSP70-utr	untranslated leader sequence of HSP70 gene from maize
mas 3'	terminator from mannopine synthase gene from *Agrobacterium*
mas 5'	promoter from mannopine synthase gene from *Agrobacterium*
MHW	Ministry of Health and Welfare (Japan)
n.d.	not detected
nar	no approval required
NASBA	Nucleic Acid Sequence-Based Amplification
nda	no data available
NFA	National Food Agency (of Denmark)
nptII	(neomycin-3'-phosphotransferase) aminoglycoside-3'-phosphotransferase gene from TN5
nos 3'	terminator of nopaline synthase gene (from *Agrobacterium tumefaciens*)
nqda	no quantitative data available
ocd	ornithine cyclodeaminase gene (*Agrobacterium* Ti plasmid C58)
ocs 3'	octopine synthase gene terminator (*Agrobacterium tumefaciens*)
P-35S	(= CaMV 35S promoter) promoter from the cauliflower mosaic virus
P-adh1	promoter from yeast alcohol dehydrogenase
P-BcNa	promoter from a napin storage protein gene (BcNa1) from *Brassica rapa*
P-CDPK	pollen-specific CDPK (calcium-dependent protein kinase) promoter (from corn)
P-CMoVb	see P-FMV
PCR	Polymerase Chain Reaction
P-E8	modified E8 gene promoter from tomatoes; tissue-specific, developmentally

	regulated
P-EFiαA	promoter from elongation factor αA
P-FMV	35S promoter from a modified figwort mosaic virus (caulimovirus group)
P-HelSsu	Rubisco small subunit promoter (from *Helianthus annuus*)
P-nos	promoter of nopaline synthase gene (from *Agrobacterium tumefaciens*)
P-PEPC	green tissue-specific phosphoenolpyruvate carboxylase (PEPC) promoter (from corn)
P-SsuAra	ribulose-1,5-bisphosphate carboxylase (= Rubisco) small subunit ats1A promoter from *Arabidopsis thaliana*, (P-SSU1A)
P-TA29	developmentally regulated promoter from anther-specific TA29 gene from *Nicotiana tabacum*
P-HSP70-utr	untranslated leader sequence of *Petunia* HSP70 gene
pat	gene coding for a phosphinothricin acetyltransferase from *Streptomyces viridochromogenes;* homologoue to bar
pg	gene coding for polygalacturonase (PG)
PQ	product quality
PRV	papaya ringspot virus
Pt, pt	phosphinothricin also known as glufosinate ammonium (active compound in the herbicides Basta®, Ignite®, Liberty®, Finale™ and others); inhibits glutamine synthase which is essential for ammonia detoxification in plant cells
PVX-cp	potato virus X coat protein gene
Rikilt-dlo	State Institute for Quality Control of Agricultural Products (the Netherlands)
RKI	Robert Koch-Institut (Germany)
Rubisco	ribulose-1,5-bisphosphate carboxylase
S. hygrsoc.	*Streptomyces hygroscopicus*
S. virdoc.	*Streptomyces viridochromogenes*
sam-k	S-adenosylmethionine hydrolase
ss	seed-specific
ssp	sub-species
STA2	yeast gene with starch degrading activity
Su, su	sulfonylurea herbicides (are e.g. active compound in the herbicide Staple®) inhibit most variants of aceto lactate synthase (als), a key enzyme in the biosynthesis of the essential amino acids Isoleucine, leucine and valine
'synthetic' gene	modified gene that employs an altered codon usage (not resulting in changes of the amino acid sequence)
T-35S	terminator from the 35S gene from the cauliflower mosaic virus
T-BcNa	terminator from a napin storage protein gene (BcNa1) from *Brassica rapa*
T-SSU1A	3' region of small subunit of ribulose-1,5-bisphosphate carboxylase (here from

	Glycine max)
tet	gene isolated from *E. coli* and *Bacillus cereus* which conveys resistance to the antibiotic tetracycline
tfp	total fruit protein
tg	transgene
Tn	transposon
tp	total protein
Tr7 3'	3' regulatory region from *Agrobacterium tumefaciens* of the T-DNA transcript 7
ts	tissue-specifc
T-TL-DNA gene 7	TL-DNA gene 7 terminator from *A. tumefaciens*; (probably identical to Tr7 3')
USDA	United States Department of Agriculture
uoh	(registration of) use of herbicide (at the EPA); see also nar*
utr	untranslated region (non-coding sequence)
VR	virus resistant
Western	Western analysis, immunoblotting
WMV	watermelon mosaic virus
ZYMV	zucchini yellow mosaic virus

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