Detection of genetically modified foods Past and future

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Résumé **Détection des aliments génétiquement modifiés**

Le soja de Monsanto et quelques variétés de maïs transgéniques sont les plus connus des plantes génétiquement modifiées en Europe. On les trouve sous diverses formes dans environ 30 000 denrées alimentaires. Les associations de consommateurs ont poussé la Communauté Européenne à proposer de réglementations obligeant à préciser la présence de produits transgéniques dans les aliments. Dans les pays hors de l'Union Européenne, ces réglementations sur les aliments contenant des produits génétiquement modifiés dépendent du pays considéré et cela va de la non obligation d'étiquettage jusqu'à l'interdiction d'importation.

Les produits génétiquement modifiés tels que maïs et soja ne diffèrent ni en structure macroscopique ni en goût des produits originaux, et il est nécessaire pour pouvoir les identifier de développer des méthodes de détection qui peuvent reconnaître les variétés contenant des séquences transgéniques. Plusieurs méthodes ont été développées et validées. On trouve des méthodes physico-chimiques (tel le MALDI-TOF), des méthodes basées sur les protéines (ELISA), jusqu'à la technique la plus usuelle basée sur l'utilisation de l'ADN (PCR et PCR en temps réel). Les principes de ces méthodes sont discutés et les limites de chacune d'elles sont expliquées dans l'article.

Summary The best-known examples for genetically modified crops in Europe are the Roundup Ready soybean from Monsanto and the various transgenic maize varieties. These can be found in various forms in an estimated 30.000 food-products. Consumer concern prompted the European Commission to issue labelling regulations for food-products which require the presence of transgenic material to be indicated. In countries outside of the European Union, these regulations tend to differ from country to country, ranging from no labelling requirements to import bans on products containing genetically modified material.

Most genetically modified materials (maize kernels, soy beans) do not differ morphologically or in taste from their conventional counterparts, and in order to enforce the labelling regulations, detection methods are required which can distinguish the varieties containing the transgenic sequences. Several methods for the detection of transgenic material have been developed and validated. These range from physico-chemical (MALDI-TOF) and protein based methods (ELISA) to the most frequently used DNA-based methods (PCR, real-time PCR). The basics of these methods are discussed and the problems and limits of each method explained.

Mots-clés **Aliments, plantes transgéniques, modifications génétiques, détection, analyse quantitative, réaction en chaîne des polymerases.**

Key-words **Food, transgenic plants, genetic modifications, detection, quantitative analysis, polymerase chain reaction.**

1996 the first large-scale production of genetically modified (GM) crops was launched: cotton, soybeans, maize, canola (rapeseed), tomatoes, potatoes and squash. A summary of crops approved for food-use in Europe can be found in *table I*. Transgenic plants were grown on about 3 million hectares in Canada and 30 million hectares in the US in 2000 [1]. It is estimated that 0.5 million hectares of transgenic crops were grown in China. The major producers of transgenic crops are the US (approximately 70% in 2000), Argentina (20%), and Canada (7%). The projected global market for transgenic plants in the year 2005 is 6 billion dollars. In Europe the first GM food product, tomato puree, was introduced to the UK in February 1996 by Sainsbury's and Safeway.

GM crops have been created for two main reasons: to increase the yield of a crop and to improve the food quality. The yield of a crop can be increased by introducing resistances/tolerances to herbicides, insecticides and certain diseases, by enhancing the productivity or by improving storage, transport and harvest characteristics. As an example for the latter, a genetically modified tomato has been created which skin is more resistant to pressure so that tomatoes at the bottom of a container don't get squashed that easily. Food quality, on the other hand, can be improved by increasing the synthesis of desired and decreasing the synthesis of undesired compounds (e.g. fatty acid composition, anti-nutritional compounds). In addition, by alteration of metabolic pathways, new substances can be synthesised. As an example for the latter, genetically modified oilseed rape has been created with a high content of oleic acids which are thought to be beneficial to human diet.

Of course, one must not forget the third reason for creating genetically modified crops: the economical advantage to biotechnology industry and the farmer and, potentially, the consumer.

The best-known examples for genetically modified crops in Europe are the Roundup Ready soybean from Monsanto and the Novartis Bt-176 corn which got their approval for food-use in Europe in 1996 and 1997, respectively (covered by EC Decision 96/281/EC and EC Decision 97/98/EC). The Monsanto Roundup Ready soybean has a tolerance to the herbicide glyphosate. This is done by expressing a protein in the plant which is only little sensitive to this herbicide. To achieve this, a gene from *Agrobacterium tumefaciens*, strain CP4 is

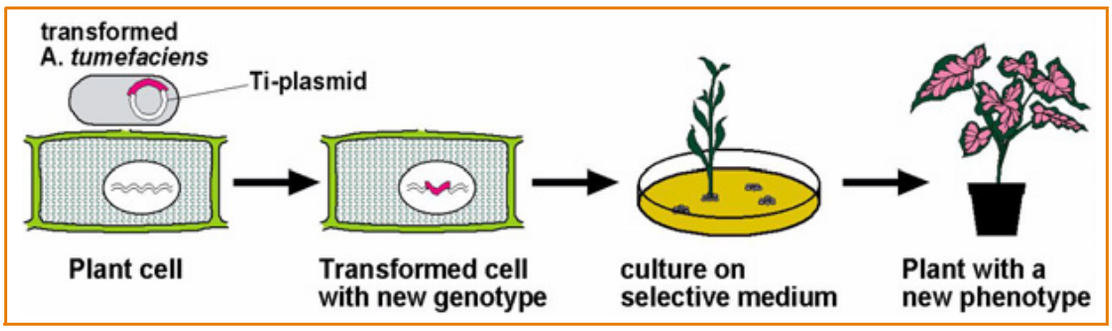

Figure 1 - **Plant transformation**. Genetically modified DNA is inserted into *Agrobacterium tumefaciens*, typically on the Ti plasmid. Plant cells are then transfected with the genetically modified DNA and a plant is re-grown from these cells. This new plant contains the genetically modified DNA in each cell and carris new properties (e.g. new phenotype).

introduced into that plant which encodes a protein very similar to the plants own protein, with the difference that the bacterial protein is only marginally affected by glyphosate. The way genes are introduced and proteins are expressed is summarised in *figure 1*.

The Novartis Bt-176 corn, also known under the trade-names « Maximizer » and « Knockout », was created by introducing a gene from *Bacillus thuringiensis* (Bt) into the corn which expresses the Bt toxin protein (also called *cryIA*). The protein is lethal to certain insects (*Lepidoptera,* e.g. European Corn Borer) when feeding on the plant but is not toxic to other species because their cells do not have receptors for this protein (i.e. the protein is simply ignored).

Why has the detection of genetically modified food become necessary? There is only one reason: to allow the final consumers to make an informed choice whether a food is produced from a GMO or not. The food safety assessment is in all cases the prerequisite for the placing on the market of Novel Foods.

Current labelling regulations

The central regulation for labelling of food or food ingredients consists of or contains genetically modified organisms (GMO) or produced from is the EC Novel Food Regulation [2]. In article 8 of this regulation, it states that labelling is mandatory if « *any characteristic or food property such as composition, nutritional value or nutritional effects or intended use of food* […] *renders a novel food or food ingredient no longer equivalent to an existing food or food ingredient* ». This regulation did not apply to Monsanto Roundup Ready® soya and Novartis Bt-176® maize since these products were already on the market. In September 1998, the EC Regulation 1139/98 [3] came into force. This regulation applies the general principles of Regulation 258/97 specifically to those two products. Labelling is triggered by detectable recombinant DNA or the resulting novel proteins and defines the wording for specific cases. Products exempt from labelling under these regulations are additives, flavourings and extraction solvents.

As published in the *Official Journal of the European Communities* on the 10th of January 2000, the EC Regulation 1139/98 is amended by implementing a tolerance level for adventitious contamination with GMO's. A specific labelling is not required when material derived from GMO is present in a food no higher than 1% of the food ingredients individually considered and the presence of this material is adventitious (EC Regulation 49/2000). Because additives and flavourings are excluded from the scope of the EC Regulation 258/97, specific labelling was not required. EC Regulation 50/2000 laid down that additives and flavourings derived from GM material must be subject to labelling requirements. This is the case where the specified additives or flavourings contain protein and/or DNA resulting from genetic modification. These regulations (49/2000/EC and 50/2000/EC) came into force 90 days after publication in the *Official Journal of the European Commission.*

Outside the European Community several countries have implemented or plan to implement regulations concerning products obtained through genetic modification for placing into the market. A summary can be found in *table I.*

Negative list

EC Regulation 1139/98 also establishes the principle of a negative list for « […] *specific foodstuffs in which neither protein nor DNA*

Table I – **GMO labelling regulations in different countries**.

Country	Labelling	Status	Language	Special provisions / Thresholds
Japan	Proposed to mandate labelling released 29/11/99	Effective 1/4/2001	« GM » and « non-GM »	« Main ingredients » – top 3 or over 5%
Korea	Released labelling proposal 11/99	Effective 1/3/2001 for commodities, 12/7/2001 for processed foods	« Recombinant food », food using recombinant « X »	Labelling for top 5 ingredients for 27 corn, soy or bean sprouts
Malaysia	Biosafety legislation under consideration	Advisory committee formed 5/99		Possible registration and product labelling requirements
Mexico	Labelling under consideration	4/00 Senate passes bill to mandate labelling – house action on hold	« Transgenic food »	Would require labelling for trait
Norway	Mandatory labelling	Implemented 1998	« Genetically modified X » or produced from…	2.0% threshold for finished products or ingredients
Poland	Registration and labelling required	Effective 11/99		
Russia	Mandatory labelling	Became effective 1/7/2000	Genetically modified « soy » or « produced on the basis of… », no « GMO-free »	Provides for a negative list
Saudi Arabia	Mandatory labelling and certification	Effective 1/12/01	Triangle containing « Contains genetically modified product » (in English and Arabic)	Import certification required, safety approval in country of origin required
Sweden	Mandatory labelling	Effective	EU regulations	Does not provide for GM-free claims
Switzerland	Mandatory labelling	Effective	Produced from genetically engineered « X » or GVO product	1.0% threshold for labelling, 0.2% threshold « GVO-free »
Taiwan	Leaning toward voluntary labelling	No legislation drafted	Unknown	
Thailand	Mandatory labelling under consideration	No legislation darfted yet	Unknown	
Turkey	To require GMO-free certification for imports	Effective 1/2001		
USA	For safety, nutrition or allergen reasons – not substantially equivalent	FDA policy adopted 5/92		Labelling of characteristic – not of process or GM origin. Developing voluntary labelling requirements

resulting from genetic modification is present […]. *A list of products not subject to the additional specific labelling requirements shall be drawn up under the procedure laid down in Article 17 of Directive 79/112/EEC, taking account of technical developments, the Scientific Committee on Food and any other relevant scientific advice* » [4]. At present, a number of food ingredients and final products are considered as possible inclusions on such a negative list: starch derivatives (maltodextrin, glucose etc.), protein hydrolysates highly heat-treated finished products, refined oils, purified enzymatic preparations, sugar and soy sauce [5].

Detection of GM foods

The detection of genetically modified plants can have several targets: newly synthesised compounds like fatty acids, a newly expressed protein or nucleic acids (DNA or RNA).

As explained in *figure 1*, DNA is the blueprint of each cell, that is transcribed into the less stable RNA which itself is then translated into a protein.

Depending on the processing, a food containing GM material undergoes one or several processing steps (e.g. heating, pressure, etc.) inducing degradation of the above mentioned compounds. As RNA is a highly unstable molecule which usually does not survive any processing, it is not considered a target for detection. Furthermore, in future genetic modification can be carried out by introducing regulatory sequences, which will not be transcribed into RNA.

Analysis of samples without genetically modified DNA present

For GM food where genetically modified DNA is no longer present in the sample, two types of detection are possible:

• If a new chemical compound (e.g. a new fatty acid) is produced by an altered metabolism as consequence of the genetic modification, this could be tested for by chemical analysis (GC-MS, NMR, FPLC, etc.) or by immunological analysis (ELISA, Western blot). This is, provided the compound is not naturally occurring in the food. Anticipated problems using these methods are cross-reactivity of antibodies and the extensive purification procedures required for GC-MS and NMR.

• If the gene product (protein) is tested for, several options are available:

- **Chemical detection** of the transgenic protein using GC-MS, FPLC or CE. In all cases the expression level of the protein will limit the usefulness of these methods: in approved crops, the expression levels of transgene products in parts of plants used for human consumption are below 0.06%, with most in the lower ppm range and some even in the ppb or ppt range [6]. However, for the detection of chymosyn produced from genetically modified organisms, FPLC has been used successfully. Here the production process for chymosyn from bacteria and cattle results in different characteristic FPLC profiles which can be used for identification [7].

- **Immunological detection** of the transgenic protein using Western Blot or ELISA. In the case of a Western blot, the protein is extracted from a food and immobilised on a membrane (e.g. nitrocellulose). The proteins bound to the membrane are then immersed in a solution containing an antibody which specifically recognises the target protein (e.g. the protein from *Bacillus thuringiensis* in the Novatis Bt-176 corn). The antibody is coupled to an enzyme which catalysis a colour reaction. The intensity of the colour developed on the membrane is proportional to the amount of protein detected by the antibody. In case of ELISA, the same underlying principle is used but the protein is bound to a well of a microtiter plate instead of a membrane. These plates can have up to 300 wells and most parts of this method can be automated. Western Blot for routine GM analysis is not suitable because it is very labour intensive but ELISA offers a number of benefits, including quantitative analysis and high throughput. Several companies/organisations have developed ELISA GM detection systems [8] and one of them has been successfully ring-trialled in Europe for raw and partially processed materials [9]. However, a significant drawback of ELISA systems is that the transgenic proteins may not be expressed in the part of the plant that is used in food-production. As an example, the Bt toxin protein is expressed in the green parts of the plant but not in the maize kernels. Therefore, protein detection using antibodies would not be successful in the maize kernels. Consequently, for maize covered by the above decision, a DNA analysis has to be performed. From the statement in the open call for the EC Tender XXIV/98/A3/001 [10] is appears that DNA methods for the detection of GM products are preferred by the EC at present. This is based on preliminary results from research laboratories. To date, no samples have been identified in which protein but no DNA was found, whereas samples containing only DNA and no detectable protein have been identified. This is the reason why this study focuses on DNA detection methods.

CE:	Capillary Electrophoresis
DNA:	**d**eoxyribo**n**ucleic **a**cid, the blueprint of each cell
ELISA:	Enzyme Linked Immuno Sorbent Assay
EtBr:	**et**hidium **br**omide
FPLC:	Fast Protein Liquid Chromatography
GC-MS:	Gas Chromatography – Mass Spectrometry
NMR:	Nuclear Magnetic Resonance
RNA:	**r**ibo**n**ucleic **a**cid: is transcribed from the DNA

Analysis of samples with genetically modified DNA present

In samples where the genetically modified DNA is present, all exogenous DNA present may be suitable for GMO detection (*figure 2*):

(a) promoter sequences,
(b) the introduced gene,
(c) endogenous terminator sequences,
(d) marker genes used for selection of transformed organisms.

The promoter serves as start signal to switch-on gene expression and the production of the protein.

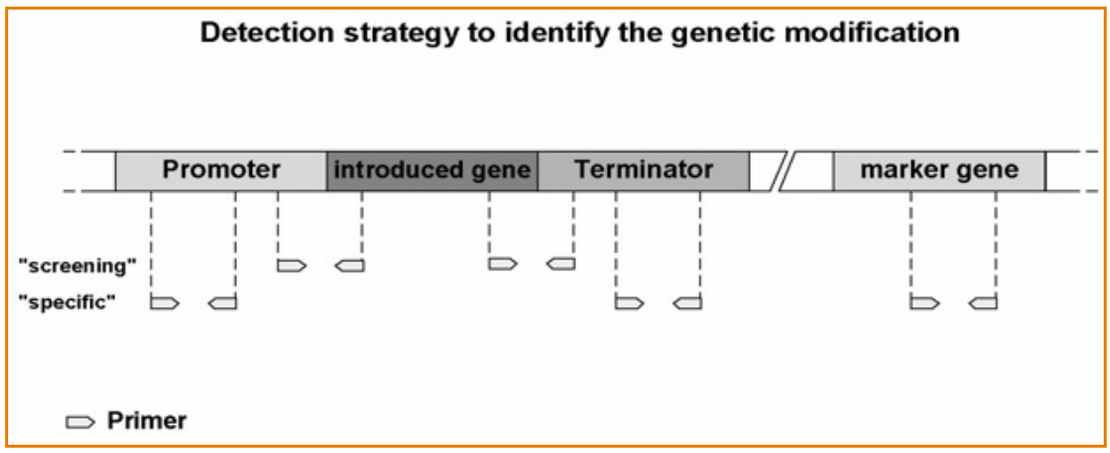

Figure 2 - **The transgenic construct**. This typically consists of the promoter (« on-switch »), the gene coding for the new characteristic of the plant and the terminator (« stop-signal »). In some cases, for easy identification of transgenic plants, a fourth element is introduced: the marker gene. This can be an antibiotic resistance gene which allows the successfully transformed plant cells to grow on medium containing ampicillin while the untransformed cells will die.

In many of the approved plants the 35S promoter derived from the Cauliflower Mosaic Virus (CaMV) is found.

The induced gene encodes the new protein. The gene can either occur naturally or can be fully synthetic, i.e. consist of a sequence of nucleic acids which do not occur naturally and had to be synthesised in a laboratory.

The terminator sequence is the stop-signal. The terminator in many of the approved plants is derived from the nopaline synthase gene (NOS) of *Agrobacterium tumefacience*, a common soil bacterium. This terminator is called NOS or NOS 3'. Although any of the elements mentioned could serve as a target for the detection of genetic modifications, the sequences to be used for this task have to be chosen carefully.

In some cases sequences can be amplified from a food sample that is due to contamination by bacteria or viruses [11]. As both the 35S promoter of CaMV and the NOS terminator of *Agrobacterium tumefaciens* occur naturally, the presence of one of these sequences is not necessarily evidence for the presence of transgenic material. However, the presence of both sequences in a tested sample strongly indicates the presence of genetically modified material. But the optimal strategy to test for sequences not occurring naturally, i.e. to amplify overlapping areas comprising the promoter and gene, i.e. a sequence arrangement which had to be created in a laboratory. This has been done successfully in the case of glyphosate resistant crops (Roundup Ready cotton and soybeans) and the insect resistant maize (Bt maize). A ring-trial organised by the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) with partners from Austria, Switzerland and Germany has been finalised for Roundup Ready soybeans and Bt-176 and Bt-11 maize [12].

Preparation of samples

The extraction procedure for DNA has to be optimised for samples from different foods, but the standard procedure of guanidiniumisothiocyanate, proteinase K and sodium dodecyl sulphate with a subsequent phenol chloroform extraction are applicable to most foods. As an alternative the chaotrophic CTAB is used for DNA extraction in a broad range of different processed food sample. Commercially DNA extraction kits from different suppliers have been tested successfully, too. The subsequent PCR is highly dependend on the successful DNA extraction.

Screening methods

As mentioned above about 80% of the world-wide approved GM crops contain the CaMV 35S promoter and/or the NOS terminator. By using primers in PCR specific for the promoter or the terminator, a large amount of samples can be screened for GM sequences. In the case of a positive result, a subsequent PCR with specific primers for the GM crop can be carried out. Screening PCRs to detect the CaMV 35S promoter or the NOS terminator have been successfully tested by the BgVV in ring trials with GM tomatoes and the Roudup Ready soybeans. On the European level a similar ringtest was organized by the Joint Research Center in Ispra, Italy (Lipp, 1999).

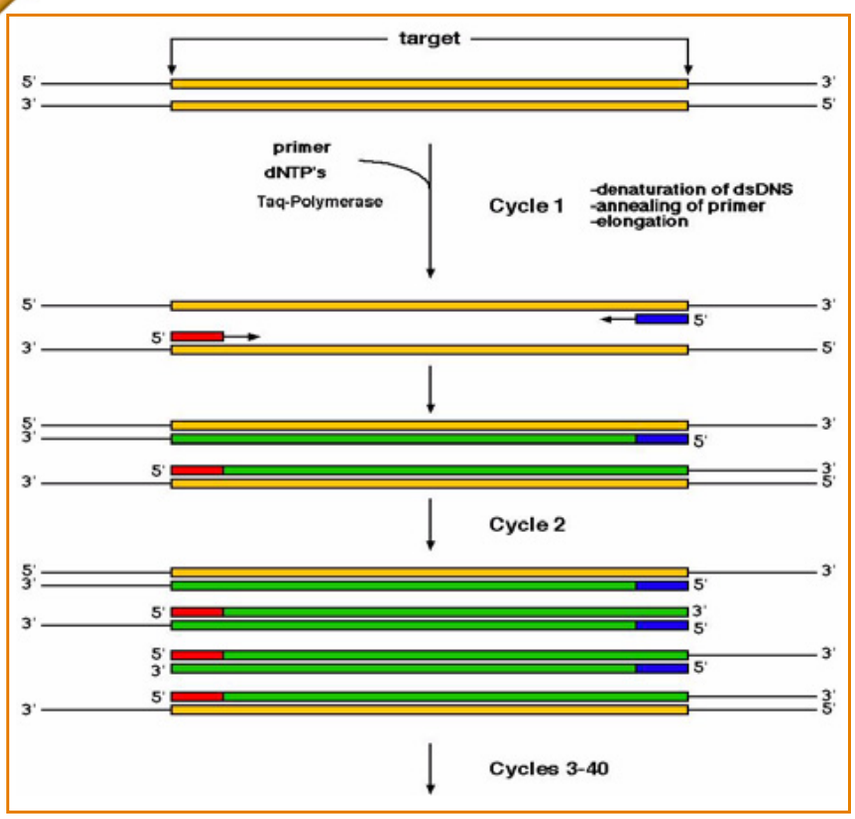

Figure 3 - **Polymerase chain reaction (PCR)**. PCR is an amplification process in which the target DNA (e.g. transgenic DNA sequences) are amplified in amounts that allows visualisation on an agarose gel after staining the DNA with special intercalating colours.

The specific detection

The detection can be performed in several ways:

a) **Selective amplification**: a specific region of the introduced gene is amplified using specific primers which anneal to the sequence and the product is then amplified by standard PCR (*figure 3*). The products are then separated on an agarose gel or polyacrylamide gel and visualised by staining with EtBr (*figure 4*). Problems can arise from non-specifically amplified products that could give rise to false-positives. These can be excluded by using a nested PCR where after amplification of a longer sequence with specific primers an internal part of the amplified sequence is re-amplified using a different set of primers which specifically anneal to internal sites. To exclude possible food contaminants as mentioned above, primers are usually designed to detect the construct which does not occur naturally, rather than sequences which can also occur in certain viruses or bacteria (e.g. for Roundup Ready soya and Bt maize products). Problems occur if polymerase chain reaction (PCR) inhibitors are present in the food matrix. In particular Ca^{2+} ions compete in the PCR with the essential Mg^{2+} ions and cause inhibition of enzymatic activities. The effect can be reversed by addition of excess Mg^{2+} ions. Other inhibitory substances present in some food matrices are high levels of hydrolysed proteins, presumably due to coagulation during PCR. Also specific plant polysaccharides (dextrane sulfate and gum ghatti) strongly inhibit PCR. In the latter case, inhibition can be reversed by additions of non-ionic detergents. In addition, causes for false negative results can be the presence of DNA-degrading nucleases. On the other hand, most salts, oils, carbohydrates and amino acids do not cause inhibitory effects on the PCR. Some detergents like CTAB and SDS also reduce the efficiency of the PCR at low concentrations (> 0.001%). Another possible cause of PCR inhibition is the presence of

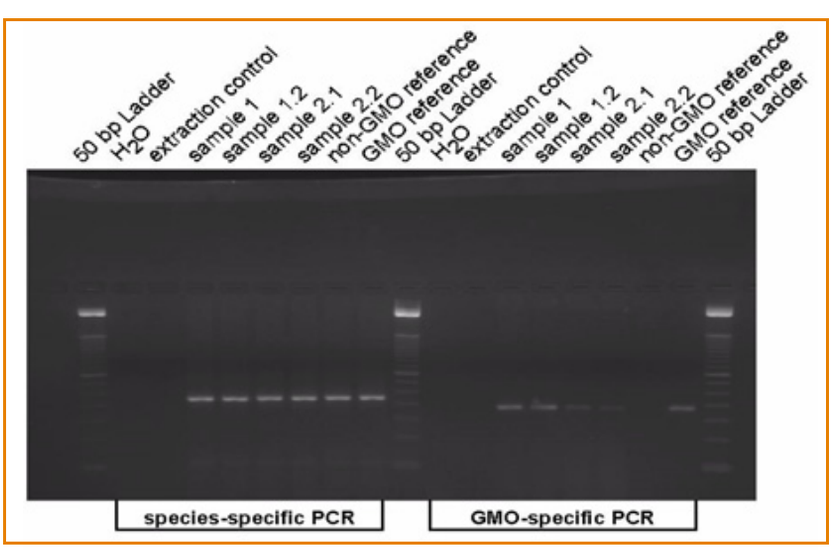

Figure 4 - **Agarose Gel** showing control and GMO-specific PCR. All samples contain the control DNA sequence (« species-specific PCR ») but only samples containing transgenic material also contain the GMO-specific DNA. The horizontal bands in sample 1 and 1.2 show the transgenic DNA being present.

polyamines (spermine and spermidine), which are known to inhibit restriction enzymes and Taq polymerases at certain concentrations [13]. These polyamines can easily be carried over in the purification steps.

b) **Hybridization**: a sequence complementary to the target sequence is designed and labelled (either « cold-labelled », i.e. with Digoxygenin (DIG), biotin or « hot-labelled » with radionuclides, i.e. ^{32}Phosporous). The sequence is then hybridized to the target sequence and detected by exposure of x-ray film or Phosphoimager (^{32}P) or by monoclonal antibodies (DIG, biotin). The target sequence is usually transferred onto a membrane (e.g. nitrocellulose). These techniques are not considered useful for routine application. However, two commercially available systems in conjunction with PCR, ABI Prism 7700™ [14] and Lightcycler™ [15], based on the hybridization technique as detection method, appear to have a number of advantages over conventional hybridization techniques and allow high throughput of samples as well as quantitation.

c) **ELISA-PCR**: several variations of this technique are known to perform well. The first step is the PCR where primers are used which have been labelled with molecules which can be recognised by commercially available antibodies (e.g. biotin, DIG). The labelled primers anneal to the exogenous gene or construct and amplify a fragment of it. The next stage differs depending on the type of ELISA. In one variation, the ELISA plates are coated with antibodies recognising the substance one or both primers are labelled with. The amplicons, i.e. the fragments amplified during the PCR, will therefore be bound to those molecules in the ELISA well (trap). In the following step, a specific sequence which itself is labelled with a different molecule recognisable by a commercially available enzyme-coupled antibody is hybridised to the trapped amplicon and detected by either a colour- or chemiluminescent reaction. In a variant, a sequence complementary to a region of the amplicon is already covalently bound to a CovaLink™ plate, an ELISA plate made from a plastic which preferentially binds nucleic acids. The amplicon from the PCR reaction is hybridised to this sequence and in a subsequent step another probe which is labelled with a molecule recognisable by a commercially available enzyme-coupled monoclonal antibody is hybridized to another part of the amplicon and detected by either a colour- or chemiluminescent reaction (*figure 5*). The systems are more time consuming than standard PCR but have a higher specificity. If the systems have a distinct advantage over real-time analysis (Taqman, Light Cycler) is questionable.

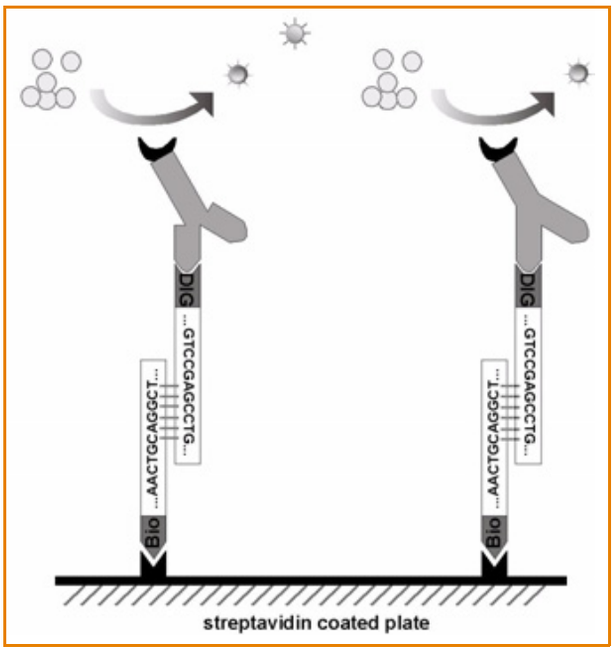

Figure 5 - **PCR ELISA**. The DNA is amplified using specific biotinylated primers which are bound by the streptavidin-coated plate. Complementary « detection » sequences containing a digoxygenin (DIG) label hybridise to the DNA and are recognized by an anti-DIG antibody which is coupled to an enzyme catalysing a colour reaction. The colour-intensity is proportional to the number of target sequences hybridised to the target DNA.

Common problems of the different methods

DNA quality

Very common detection problems arise with highly degraded samples. DNA exhibits variable stability depending on the environmental, the physical and enzymatic conditions. All assays require products that contain intact DNA sequences in the region where the primers for the detection should anneal. Primers should be designed to amplify target sequences which are not longer than 100-200 bp in order to allow detection of genetically modified sequences in processed food. In the case of bread [16], canned corn [17] and soybean protein preparations [18], it was shown that the typical fragment size is smaller than 300 bp.

PCR analysis

One problem of PCR analysis is to design primers that clearly discriminate between transgenic and native organisms. This means the amplified sequence has to be unique to the transgenic organism. The detection of a fusion of a viral

promoter (35S) and the synthetic sequence from *Bacillus thuringiensis* (cryIA) has not been found to occur naturally. And although this is no proof for absence, it gives a more reliable basis for the identification of transgenic plants. The detection of marker genes in transgenic plants again poses the question of ambiguity since plants are hosts for many different micro-organisms and viruses and many marker genes used for plant transformation are of bacterial or viral origin. A positive result from a PCR could be due to bacterial contamination of the plant DNA preparation. It is currently impossible to exclude all uncertainty from the PCR analysis. However, in the absence of other, at least equally efficient and sensitive detection methods, standard PCR is used in most laboratories for the detection of genetically modified material.

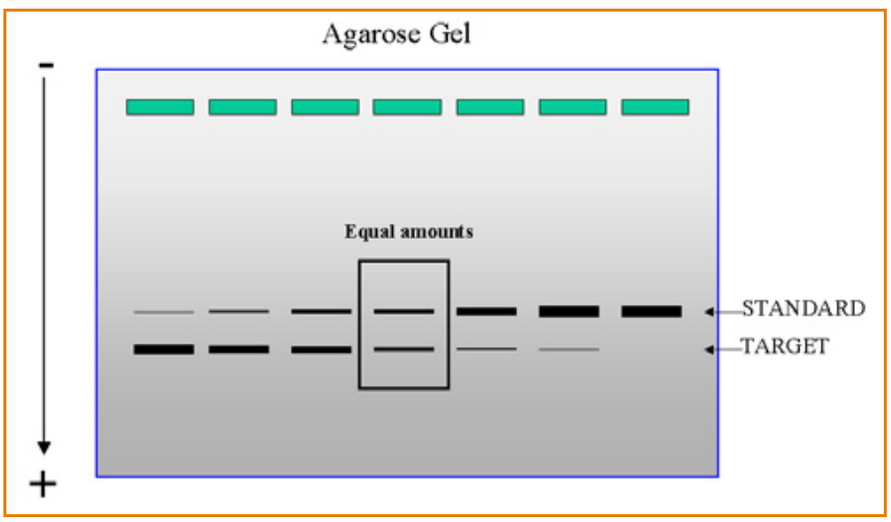

Figure 6 - **Competitive PCR**. The target DNA is titrated against a known standard and then amplified by PCR. When both reactions (in the same reaction vial) show the same band intensity, the amounts of target DNA are equal. Since the amount of standard DNA is known, the amount of target DNA can be calculated from the dilution steps performed.

Quantification

Threshold limit

EC Regulation 49/2000 introducing a 1% threshold limit above labelling is required. Consequently quantitative methods have to be applied to food sample for compliance with the 1% threshold. The Swiss national ring-trial using competitive PCR (see also « Validation of Detection systems and Projects for the Development of Detection Methods for GM materials ») was based on a 2% threshold level.

Apart from the European Community, some other countries have already introduced a threshold level: Norway has introduced the 2% threshold level several years ago and Switzerland has, on the 1st July 1999, introduced the 1% threshold level [19]. Australia and New Zealand have also introduced a 1% threshold level.

PCR is an extremely sensitive method which can detect 0.01% of GM DNA in an assay. But this technology is qualitative and not quantitative. Therefore, even products with very low but detectable levels of GM material (e.g. by adventitious co-mingling) would have to be labelled. However, as it is the opinion of the EC Economic and Social Committee on « Genetically modified organisms in agriculture – Impact on the Common Agricultural Policy » that the concept of a threshold limit should be introduced, one has to identify the problems associated with it: a threshold level for protein is not equal to a threshold level for DNA. For the quantification of protein, the percentage of GM protein reflects the ratio of GM protein to total protein in the sample. For the quantification of GM-DNA the percentage could either reflect total DNA present in the sample or total plant DNA in which the GM occurs (the so-called « genome-equivalent »). In the latter case it will be difficult to calculate the percentage when genomes are mulitploid (e.g. hexaploid wheat) and/or a mixture of different varieties of the same plant is present. The former will be difficult to establish since there are no primers amplifying all DNA which could be present (eukaryotic and prokaryotic). A further problem generally is the quantification of processed food samples since the DNA is at least partially degraded during the production process in most cases. Therefore, no absolute quantitation is possible.

Presently, three approaches to quantitation are being assessed:

a) The protein-based ELISA assay by SDI [20]. This assay has been successfully validated for Roundup Ready GM flour [21].

b) The DNA-based competitive and double competitive PCR system (*figure 6*). This system has been successfully tested in Switzerland in a recent national ring-trial by the governmental Kanton laboratories [22]. A ring-trail with Bt-176 maize organised by the BgVV with different partners from the EU is currently under investigation. This method however is very time consuming. The

advantage is that this method – once established – can be used in any molecular biological laboratory with standard equipment. No purchase of expensive systems is required.

c) The DNA-based PCR/hybridization systems Taqman™ and Lightcycler™ (also called real-time analysis). The systems are based on measuring the emission of light depending on the amount of product amplified by PCR. The amount of product amplified in the linear-exponential phase of the PCR is directly correlated to the amount of target sequences present in the sample. Thereby the amount of target material can be calculated. The Taqman™ and Lightcycler™ systems are relatively expensive equipment but available to several laboratories. Within a national working group according to the the german food act a first ring-trial has been started to quantify Roundup Ready soybean with the Taqman™ as well as with the Lightcycler™ and the ABI systems. Results will be achieved until the November.

Validation of detection systems and projects for the development of detection methods for GM materials

At present the DG JRC [23] has validated [24] a single PCR-based method for the detection of genetically modified products and an ELISA method by SDI. The aim of these validation trial was to analyse flours of soya beans and maize for the presence of the 35S promoter, NOS terminator and the Roundup Ready protein. In addition qualitative ring trials have been co-ordinated by the Federal Institute for Health protection of Consumers and Veterinary Medicine (BgVV), Germany, for Roundup Ready™ soybeans, Bt-176, Bt-11, T-25 and MON-810 as well as real time based methods for the quantification of Roundup Ready™ soybeans and Bt-176 [25].

Present and future of GMO detection

At present, most laboratories analyse products for the presence of GM material by PCR. However, since several countries have already introduced a threshold level above which GM material requires labelling, quantitative methods are more and more in the focus of attention. Namely the real-time analysis methods (ABI Prism 7700 and Light Cycler) are favoured which allow to exactly calculate the amount of genetically modified material present in a sample. But even though, one problem remains: how to cope with the ever increasing number of different modification being approved? If for each modification a PCR assay has to be set up, the analysis will soon become too costly and time-consuming. Even if several PCR's are combined within one assay (mulitplex PCR), where the primer sequences of several different traits are in one reaction tube, the number of different sequences which can simultaneously be amplified in such an assay is usually limited to two or three.

The next development are so-called gene-chips which are already in use in the medical sector. These chips or multi-array assays have a huge number of different DNA sequences bound to defined positions on the chip. The sequences, in the case of GM testing, will bind the complementary target GM sequences from the sample and these can then be amplified using primers which have a fluorescent tag. After PCR the chip is analysed by position for fluorescence, indicating the different GM sequences present in a sample. Such a chip is expected to enter the market in 2001.

Is it thinkable to have, in a few years time, a dipstick assay which allows consumers to test their goods for GM sequences directly in the supermarket without having to rely of testing laboratories? Thinkable it is, but will GM foods still be an issue in a few years time?

This all will depend whether detrimental effects to environment or health can be shown from GM crops.

Notes and references

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