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Results of an interlaboratory assessment of a screening method of genetically modified organisms in soy beans and maize

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Abstract

The preliminary results on an interlaboratory trial on the detection of genetically modified organisms (GMO) are presented. The method applied is based on the detection of modified DNA using the polymerase chain reaction (PCR) for amplification. The amplified fragments analysed are derived from the 35S promotor and the NOS terminator used for modification and are present in 26 from the 28 GMOs currently already approved or under approval by the competent authorities. This method fits as a screening method indicating the presence of GMO in food. However, it does not allow an identification of the kind of GMO present in the samples. Samples of soybean and maize flour containing 0%, 0.1%, 0.5% and 2% GMO had been prepared for this study and are also already commercially available. In this paper the combined results from 27 laboratories are presented, indicating that on average the probability of false positive or false negative results is only about 1% for soybeans and below 5% for maize.

1. Introduction

Today 28 transgenic plants are already approved or under approval by the competent authorities. This poses a problem for control authorities to verify the compliance with labelling, as required by EU Directives and Regulations (1813/97, 97/258 EEC, 97/35 EEC). Until now, there is no internationally validated method available for the detection of transgenic plants in food. As the variety of transgenic plants is increasing, e.g. several different varieties of maize are already existing, a screening method that allows the detection of the presence of transgenic plants in food irrespective of the individual variety would be very welcome for control authorities. A screening method suitable for the detection of 26 out of the 28 transgenic plans of interest in 1997 was already published by Pietsch, Waiblinger, Brodmann and Wurz (1997) and has been already established as an official method in the German and Swiss Food Law. This method is based on the detection of two regulatory sequences existing in these 26 transgenic plants. These sequences contain fragments of the CAMV 35S promotor originating from the cauliflower mosaic virus and the NOS terminator originating from the nopalin synthase gene originating from the *Agrobacterium turmefacens* (Hemmer, 1997, Niederhauser, Gilgen & Meyer, 1996). This screening method is based on the detection of modified DNA by using the polymerase chain reaction (PCR) technique.

The sensitivity of the PCR technique generally depends on the chemicals and apparatus used. Each combination, namely of the enzyme polymerase and the thermocycler used, needs its own optimisation. In order to perform this tasks, standardised and well-characterised samples have to be available. For the time being this material was not commercially available. Hence, reference material was prepared at the Institute of Reference Material and Measurements at the Joint Research Center in Geel, Belgium. Two sets of material had been prepared for this study and are presently commercially available. The matrix chosen for its importance on the market had been the RoundupReady soybean from Monsanto and the BT-176 maize from Novartis. Each matrix is available in concentrations of 0%, 0.1%, 0.5% and 2% transgenic plants in a mixture with conventional

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(non-GMO) maize or soybeans, respectively. For each matrix the grains had been ground and sieved separately in a manner that avoided the contamination of the not genetically modified plants by the flour of transgenic plants. The flours were subsequently re-suspended in water in the appropriate quantities, dried, ground and filled in one batch in vials under inert gas atmosphere. Thus homogeneity could be insured, most of all because all vials produced for each matrix have been produced in one single batch for each concentration of each matrix.

2. Method

The method is based on the publication of Pietsch et al. (1997). No details will be given here. For this interlaboratory study, participants were requested to optimise the working conditions of the thermocycler (temperature program) according to their local needs.

Sample preparation required an extraction method for the DNA. Here as well, no specific method was asked, however detailed description of two DNA extraction methods, the CTAB method (Taberlet, Gielly, Pautou & Bouvet, 1991) and a method based on the WIZARD Kit (Promega, USA) was given. Participants were allowed to modify all aspects of the method (with the exception of the primers). However, detailed documentation was required to be sent to the co-ordinator of the study.

The design had foreseen a control PCR for the amplifiability of the extracted DNA. The method given in the method description as distributed to the participants was based on the detection of chloroplast DNA. However, here as well participants were free to apply a system of their choice, providing the fact that it gave comparable results.

The verification of the results had to be done by the digestion of the amplified fragments with restriction endonucleases. For the fragment of the 35S promoter the restriction endonuclease Xmn 1 was chosen and for the fragment form the NOS terminator the restriction endonuclease Nsi 1, respectively. The 195 bp fragment from the 35S promotor was digested in two fragments, 80 bp and 115 bp, while the 180 bp fragment from the NOR terminator resulted in two fragments of 84 bp and 96 bp, respectively.

Moreover, participants were required to include the complete results (inclusive documentation of the electrophoresis gels) for the following steps:

- quality of extracted DNA,
- control PCR for amplifiability of the extracted DNA, and
- results of the digestion of the fragments by the corresponding restriction endonucleases.

Each PCR had to include the following controls:

- a blank,
- negative control,
- a positive control,
- and on each gel a length standard was required.

In this paper the first results comprising 27 laboratories are given. The following laboratories have already sent their results:

- INRA, Montpellier, France,
- Laboratoire Interregional de Strasbourg, Illkirchen Gräfenstaden, France,
- Umweltbundesamt, Vienna, Austria,
- Central Science Laboratory, Norwich, UK,
- Danish Veterinary and Food Administration, Søbørg, Denmark,
- Nestle Research Center, Lausanne, Switzerland,
- Migros-Genossenschaft-Bund, Courtepin, Switzerland,
- Österreichisches Forschungszentrum Seibersdorf, Seibersdorf, Austria,
- Staatl. Lebensmitteluntersuchungsamt Braunschweig, Germany,
- Kantonales Labor Basel, Basel, Switzerland,
- Universitaet Hohenheim, Stuttgart, Germany,
- Unilever Research Laboratory, Vlaardingen, The Netherlands,
- Bundesanstalt fuer Lebensmitteluntersuchung undforschung, Hoertner,
- Istituto Superiore di Sanita, Rome, Italy,
- Gene-scan, Freiburg, Germany,
- Chemische Landesuntersuchungsanstalt Freiburg, Germany,
- Landesunersuchungsamt für das Gesundheitswesen, Südbayern, Germany,
- BgVV, Jena, Germany,
- Laboratory of Government Chemists, Middlesex, UK,
- TNO, Food and Nutrition Research Institute, Zeist, The Netherlands,
- genFOR, Grafschaft-Gelsdorf, Germany,
- Hanse Analytik, Bremen, Germany,
- State Laboratory, Dublin, Ireland,
- IBET, Oeiras, Portugal,
- Versuchsstation Schweizerischer Brauereien, Zürich, Switzerland, and
- Department of Food Technology and Nutrition, Gent, Belgium.

Three laboratories were excluded from data evaluation. They did not obtain conclusive results. One laboratory adjusted the sensitivity of its analysis in such a way, that samples containing 0.1% transgenic material gave a negative result. Consequently, these results are not included in the data evaluation. Some laboratories applied different PCR methods (based on a different set of primers) for several samples. As these results are not comparable to the others, they were excluded as well.

All participants received a labelled sample containing 0% and 2% of flour from transgenic plants for each

matrix. For each concentration and each matrix the participants received on average 4 samples. This means, the number of samples was varied between 3 and 5 and the total number of samples for each participant was 36, that is 32 unknown and four known samples.

As the method applied here does not result in a quantitative result, the results obtained were classified as correct if a positive signal was obtained for samples containing transgenic material or if a negative result was obtained for the 0% samples. All other combinations were regarded as wrong.

Table 1 presents the nomenclature used in this paper, where as

specificity = IV/(III + IV) is a measure for false positive results,
sensitivity = I/(I + II) is a measure for false negative results,
predictive value = I/(I + III) is the ratio for correct positive results and false positive results,

As the sample set distributed for analysis contains 25% of the samples without transgenic plants, a prevalence of about 75% is to be expected by the design of this study. The optimum value for specificity, sensitivity, predictive value and concordance is 100%.

3. Results and discussion

The determination of the 35S promotor or of the NOS terminator and the confirmation of positive signals by restriction endonuclease digestion was requested for both, soybeans and maize. In the case of the maize, that does not contain the NOS terminator, results were used to check for irregular amplification products and for possible contamination. However, not all laboratories performed this analysis. Only one laboratory had problems with an amplification product of about 200 bp for maize, however, it could not be digested by Nsi 1. Thus, no confirmed false positive results were obtained for the NOS terminator in maize.

Until now, in total 1898 results were obtained on 635 samples. The analysis of soybeans has shown less problems compared to the analysis of maize. This is both reflected in the following tables and confirmed by comments from participants.

Table 1

Reality	Test results	
	Positive	Negative
Positive	I	II False negative
Negative	III False positive	IV

3.1. Evaluation of results from the analysis of soybeans

In Table 2 there are only six false negative and five false positive results. Four of the six false negative results had been observed from one laboratory only, suggesting that this laboratory did not adjust its sensitivity appropriately.

In general the determination of the NOS terminator was observed to be somewhat more insensitive, this can

Table 2
Analysis of the 35S promotor in soybeans (348 total number of analysis – 97.72% sensitivity, 94.12% specificity, 98.09% predictive value)

Reality	Test results	
	Positive	Negative
Positive	257	6
Negative	5	80

Table 3
Analysis of NOS terminator in soybeans (348 total number of analysis – 95.06% sensitivity, 98.82% specificity, 99.60% predictive value)

Reality	Test results	
	Positive	Negative
Positive	250	13
Negative	1	84

Table 4
Combined analysis of 35S promoter and NOS terminator in soybeans (329 total number of analysis – 99.16% sensitivity, 98.77% specificity, 99.60% predictive value)

Reality	Test results	
	Positive	Negative
Positive	246	2
Negative	1	80

Table 5
Analysis of the 35S promotor in maize (329 total number of analysis – 89.02% sensitivity, 95.18% specificity, 98.21% predictive value)

Reality	Test results	
	Positive	Negative
Positive	219	27
Negative	4	79

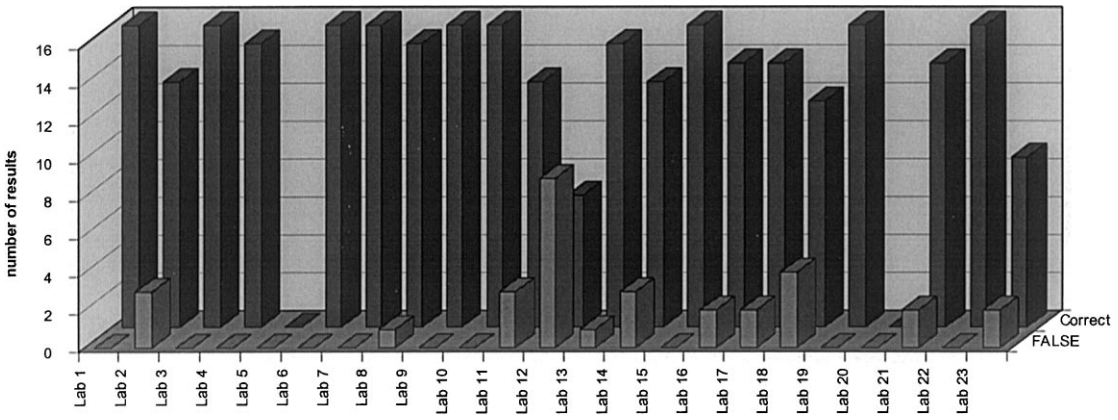

Fig. 1. Results from the determination of the 35S promotor in maize. False results are combining false negative and false positive results.

also be seen in Table 3. However, it seems as well, that the analysis of the NOS terminator is less prone to false positive results compared to the analysis of the 35S promotor.

The results from the combined analysis of the 35S promotor and the NOS terminator are given in Table 4. This table indicates that for 329 out of the 348 samples an unequivocal result is obtained. This means that both, the determination of the 35S and the NOS is either both positive or negative. The probability of a false classification in this case is below 1%. All 19 samples not given in Table 4 do not present a conclusive result. That is, these 19 samples show either a positive result for the determination of the 35S promotor or a negative results for the NOS terminator or vice versa.

3.2. Evaluation of results from the analysis of maize

According to the participant it was more difficult to obtain concise results for maize. This is certainly related to the fact the genom in maize is considerably larger than the one in soybeans, thus the number of copies of DNA obtained by extraction is far lower.

This is reflected in the results given in Table 5. There is clearly a rather large number of false negative results. All four false positive results are originating from one lab only, and they could not be confirmed by digestion with restriction endonucleases.

In Fig. 1 the results for the determination of the 35S promotor in maize are given for each laboratory individually. More than half of the participating laboratories could perform the analysis with none or only one or two false positive or false negative results. Laboratory number 2 observed four false positive results for the 0% sample. However, these results were not confirmed by the digestion with restriction endonuclease.

The majority of the 27 false negative samples are found on a level of 0.1% transgenic maize. If this concentration level is excluded from the evaluation, the overall performance improves dramatically, see Table 5.

4. Conclusions

The analysis of only the 35S promotor or only the NOS terminator in soybeans leads to a probability for false positive or false negative results of about 4%. However, combining these two analysis and evaluating concise results lowers this error to about 1%. The analysis of maize products seems to be more difficult, probably due to the larger genome of maize and subsequently the lower number of gene copies extracted from the matrix. The overall probability of a false positive or a false negative result is below 10%. The majority of errors are false negative errors for the sample containing 0.1% GMO. Discarding these samples leads to an error probability of below 5%. There are always a few false positive results regarding the analysis of the 35S promotor or the NOS terminator alone. In the majority of cases these false negative results could not be confirmed be the corresponding digestion with restriction endonucleases.

The errors in the results were not homogeneously distributed over the participating laboratories. In all analyses, more than half of the laboratories did perform the analysis without any errors.

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