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ORIGINAL PAPER

A practical approach to screen for authorised and unauthorised genetically modified plants

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Abstract In routine analysis, screening methods based on real-time PCR are most commonly used for the detection of genetically modified (GM) plant material in food and feed. In this paper, it is shown that the combination of five DNA target sequences can be used as a universal screening approach for at least 81 GM plant events authorised or unauthorised for placing on the market and described in publicly available databases. Except for maize event LY038, soybean events DP-305423 and BPS-CV127-9 and cotton event 281-24-236×3006-210-23, at least one of the five genetic elements has been inserted in these GM plants and is targeted by this screening approach. For the detection of these sequences, fully validated real-time PCR methods have been selected. A screening table is presented that describes the presence or absence of the target sequences for most of the listed GM plants. These data have been verified either theoretically according to available databases or experimentally using available reference materials. The screening table will be updated regularly by a network of German enforcement laboratories.

Keywords Genetically modified organism (GMO) · Screening table · Real-time PCR · Unauthorised GMO · GMO matrix · Placing on the market · Genetically modified food · Genetically modified feed

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Introduction

In routine analysis of food, feed and seeds, screening methods based on polymerase chain reaction (PCR) are generally applied for the detection of the presence of genetic modifications in the meaning of European legislation [1]. For this purpose, DNA-sequences are targeted, that are frequently inserted into GM plants, e.g. specific promoters, terminators or sequences of genes conferring tolerances to herbicides or protection against certain insects [2, 3]. In addition, targeting of transforming DNA constructs with two or more different genes or genetic elements including their junctions can also be used for screening. Screening for presence of the Cauliflower Mosaic Virus (CaMV) 35S promoter (P-35S) and the *nos* terminator sequences derived from *Agrobacterium tumefaciens* (T-nos) are commonly used [1]. However, due to the lack of specificity for the identification of the particular GM plant event, positive screening results have to be verified using construct- and/or event-specific methods, if available [1, 4].

Before quantification using event-specific methods, the application of real-time PCR-based screening methods in certain cases allows the semi-quantitative estimation of the GM plant fraction present in a screening-positive sample. In addition, real-time PCR has become state of the art in routine analysis because of the reduced risk of sample contamination during sample processing and the increased specificity of detection by using target-specific hybridisation probes. For example, the development and interlaboratory validation of simplex and duplex real-time PCR methods for the separate or simultaneous detection and semi-quantitative estimation of the P-35S and the T-nos sequence have been described [5–8]. However, with the increasing worldwide commercialisation of GM plants, efficient strategies for screening

analysis require the combination of more genetic elements targeted by real-time PCR methods.

The use of screening methods that are validated and standardised in interlaboratory trials screening methods contributes to a uniform level of performance as well as to a comparability of the results. The proof that screening methods detect the targeted GM plant events should be deduced not only according to the sequence information but should also be demonstrated practically by the use of reference materials, particularly to define the specificity and sensitivity of the method. This is important not only for the GM plants already authorised for placing on the market, but also for upcoming GM crops that might be commercialised in the near future [9].

Recently, several laboratories have started to establish a so-called GMO matrix which basically is a table in which the rows represent the content of genetic elements of the respective GMO, while the columns define the targeted sequence elements analysed in the matrix [10–12]. This way, each GMO can be described by its specific combination of the respective target sequences. This matrix allows an easy identification of the potential GM event(s) by comparison of the screening results obtained with the analysed sample.

The present publication describes the development and proposed maintenance of a screening table as a practical tool for the routine testing for the presence of authorised and unauthorised GM plants. The general layout of the screening table has recently been published as a short note in a German journal [13]. The table gives a practical example of a 'GMO matrix' in the format of an Excel spreadsheet. Targets for screening are only included, if collaborative trial validated real-time PCR methods already exist. In addition to available theoretical data indicating the presence of the target sequences, information about the experimentally verified presence or absence of the targeted sequence in reference materials of the individual GM plants is given.

Materials and methods

Reference materials

Reference materials (flours) of the following GM lines were purchased from IRMM (Geel, B): GM maize lines Bt11, Bt176, MIR604, MON810, MON863, MON88017, NK603, GA 21, TC1507, 3272, 59122; sugar beet GM line H7-1; cotton GM lines 3006-210-23x281-24-236; soybean GM lines GTS40-3-2, DP305423 and DP356043.

Reference materials (flours) from maize GM lines MON88017, potato GM line EH92-527-1, rape seed GM line GT73, cotton GM lines LL25, MON1445, MON531, MON15985 and soybean GM lines MON89788, A2704-12 and A5547-127 were obtained from AOCS (Urbana, USA). Reference materials (genomic DNA extracted from leaves) for rice GM line LL62, rape seed GM lines MS8, RF3, T45, TOPAS19/2 and maize GM line T25 were purchased from Bayer CropScience (Gent, B). Genomic DNA from maize CBH351 and rapeseed OXY235 was purchased as DNA solution containing about 1% GM DNA in background of non-GM plant DNA (Fluka, Buchs, Switzerland).

Plant materials (seeds or leaves) from sugar beet GM line GTSB77, rape seed GM lines MS1xRF1, Falcon GS40/90, Liberator pHoe6/Ac, LPAAT/Trierucin, Laurat pCGN3828 and from maize GM line T14 were obtained from deliberate field trials. Bt63 rice reference material and DNA from LL601 rice was obtained from the CRL-GMFF (Ispra, I). DNA from GM rapeseed line 23–198 was available from deliberated field trials and used in a German collaborative trial; reference material for GM papaya SunUp originated from a positive sample from local food market (origin Hawaii).

Detailed descriptions of commercial reference materials (catalogue numbers, GM content, status of the materials) are given in a publicly available list [14].

DNA extraction

DNA was extracted from seeds, leaves and flours with the Qiagen Plant Mini Kit (Qiagen, Hilden, D) starting with a CTAB extraction [15].

The concentration of DNA was estimated photometrically at 260 nm [15] in a TrayCell (Hellma, Müllheim, Germany).

Quality control of DNA and preparation of dilutions

Amplifiability of DNA preparations was checked by target taxon-specific real-time PCR methods [6, 16]. For sensitivity testing, additional dilutions of DNA with 0.2× TE [2 mM Tris–HCl and 0.2 mM ethylenediaminetetraacetic acid (EDTA), adjusted to pH 8.0] were prepared to obtain concentrations of about ten to 50 copies per PCR. Copy numbers were calculated on basis of the genome sizes [17], taking the zygosity status of the GM plant materials and the number of integrated copies of the target sequence (if information is available) into account.

Real-time PCR

DNA solutions were adjusted to about 20 ng/µl with 0.2× TE and 5 µl of this solution was used for real-time PCR. The following real-time PCR methods were applied according to the procedures described in the references:

Detection of sequences from CaMV P-35S [6] and *A. tumefaciens* T-nos [7], detection of a sequence from the ctp2-cp4epsps junction of the chloroplast-transitpeptide

(CTP2) from *Arabidopsis thaliana* and the *epsps* gene from *A. tumefaciens strain CP4* [18, 19], detection of a sequence from the *bar* gene from *Streptomyces hygroscopicus* [20] as well as a sequence from the P35S-pat junction of the CaMV P-35S promoter and the synthetic *pat* gene [18, 21]. Details on the primers and probes, the amplicon sequences and sizes as well as the corresponding Genbank accession numbers are given in Table 1.

Results and discussion

Selection of target sequences for screening

Screening methods are an important and in practise the most used tool for the detection of GM plants. Due to the lack of event-specific methods, usually, there is no alternative to screening methods concerning the detection of GM plants not authorised as food and feed in accordance with legislation. Positive screening results, e.g. for the P-35S sequence, may be an indication of the presence of GM plants, if natural contamination, e.g. by CaMV DNA, can be excluded.

In order to detect as many different GM plant events as possible, screening for P-35S and T-nos alone is not always sufficient. In addition, results from screening for more than these two elements can give additional information about the possible presence or absence of different GM plant events. Therefore, we combined the P-35S and T-nos screening methods with three additional targets frequently occurring in GM plants [6]. Target sequences are the two constructs ctp2-cp4epsps and P35S-pat and the bar gene from *S. hygroscopicus* (Table 1).

For the P-35S and T-nos as well as the three additional real-time PCR methods, full validations in collaborative trials were performed within Swiss and German standardisation networks in the last years. The results of these studies have been published [6–8, 21, 22] and the method protocols are publicly available in international or national official standards [6, 19–21, 23].

The screening table

Table 2 presents the current screening table. It is an Excel spreadsheet that is based on a set of the five real-time PCR methods described above. These targets are tabulated along with a list of 81 different GM plant events comprising nine food- and feed-related crops (potato, maize, soya, rapeseed, rice, sugarbeet, papaya, tomato, cotton). Information about the presence or absence of the target sequences is either obtained from public databases [2, 3] or by alignments with available sequence database entries [24, 25]. In cases where the transformation experiment led to a single transformation event and in cases where the insert of a given transformation event is characterised in detail, the lines of the spreadsheet contain single GM plant events. In several cases, more than one GM event is given per line. These GM events result from individual transformation experiments using identical constructs.

An illustration of the screening table and explanations of the details are given in Fig. 1. It is foreseen that the functional Excel spreadsheet is continuously updated by the German network of enforcement laboratories and can be downloaded from the internet [22, 26].

Experimental verification of presence or absence of screening elements

DNAs extracted from available reference materials were used to experimentally verify that the target sequence in the given GM plant is detected with the respective real-time PCR screening method. The genomic DNAs were tested in at least two laboratories. For the verification of negative

Table 1 Amplicon sequences and sizes

Method	Amplicon sequence (5′–3′)	Amplicon size (bp)	Accession number	Reference
P-35S	**gcctctgccgacagtggt**cccaaagatggacccccacccacgaggagcatcgtggaaaaa **gaagacgttccaaccacgtctt**	82	FN550389FB727463 (soybean event DP-356043)	(6, 8)
T-nos	**catgtaatgcatgacgttatttatg**agatgggtttttatgattagagtcccgcaattat **acatttaatacgcgatagaaaacaa**	84	FN550390EU880444 (rice event TT51-1)	(7, 8)
bar	**acaagcacggtcaacttcc**gtaccgagccgcaggaaccgcag**gagtggacggacgacctc**	60	FN550386BD242717 (glufosinate tolerant rice)	(20, 22)
ctp2-cp4epsps	**gggatgacgttaattggctctg**agcttcgtcctcttaaggtcatgtcttctgtttccacggcgtg catg**cttcacggtgcaagcagcc**	88	FN550387 DJ05815 (maize event MON88017)	(18, 19)
P35S – pat	**aagttcatttcatttggagaggaca**gggtacccggggatccaccatgtct ccggagaggagaccagttgagattaggccag**ctacagcagctgatatggccg**	102[a]	FN550388DL476427 (maize event DAS-59122-7)	(18, 21)

Bold letters indicate the oligonucleotide forward and reverse primer sequence, whereas the underlined letters indicate the probe sequence

[a] The size may vary depending on the P35S-pat construct present in the specific event (e.g. T45=110 bp; Bt11=402 bp)

Table 2 Screening table for the detection of genetically modified plants

Name of Event	Plant	Authorisation EU	P35S		T-nos		CTP2-CP4EPSPS		bar		35S-pat	
			S	R	S	R	S	R	S	R	S	R
ATBT04-X (New Leaf)	potato	-	+		-		-		-		-	
BT6, BT10, Bt12, BT16, BT17, BT18, BT23 (New Leaf)	potato	-	+		+		-		-		-	
RBMT15-101, SEMT15-02, SEMT15-15, HLMT15-46 (New Leaf)	potato	-	-		+		-		-		-	
RBMT21-129, RBMT21-152, RBMT21-350 (New Leaf)	potato	-	-		+		-		-		-	
RBMT22-082, RBMT22-186, RBMT22-238, RBMT22-262(New Leaf)	potato	-	-		+		+		-		-	
SPBT02-5, SPBT02-7	potato	-	+		+		-		-		-	
EH92-527-1	potato	P	-	wp	+	+	-	-	-	-	-	-
AV43-6-GT	potato	P	-		(+)		-		-		-	
3272	maize	P	-	wp	+	+	-	-	-	-	-	-
676, 678, 680	maize	-	+		-		-		-		+	
59122	maize	A	+	+	-	-	-	-	-	-	+	+
Bt11	maize	A	+	+	+	+	-	-	-	-	+	+
B16 (DLL25)	maize	-	+		-		-		+		-	
Bt176 (176; Maximizer)	maize	A	+	+	-	-	-	-	+	+	-	-
CBH-351 (StarLink)	maize	-	+	+	+	+	-	-	+	+	-	-
DAS-06275-8	maize	-	+		-		-		+		-	
DBT418 (Bt-Xtra)	maize	-	+		-		-		+		-	
GA 21 (Roundup Ready)	maize	A	-	-	+	+	-	-	-	wp	-	-
LY038	maize	P	-		-		-		-		-	
MIR 162	maize	P	-		+		-		-		-	
MIR604	maize	P	-	-	+	+	-	-	-	-	-	-
MON 80100	maize	-	+		+		+		-		-	
MON 802	maize	-	+		+		+		-		-	
MON 809	maize	-	+	+	+	+	+	+	-	-	-	-
MON 810	maize	A	+	+	-	-	-	-	-	-	-	-
MON 832	maize	-	+		+		+		-		-	
MON 863 (YieldGard)	maize	A	+	+	+	+	-	-	-	-	-	-
MON 88017	maize	P	+	+	+	+	+	+	-	-	-	-
MON89034	maize	P	+		+		-		-		-	
MON 87460	maize	P	+		+							
MS3 (SeedLink)	maize	-	+		+		-		+		-	
MS6 (SeedLink)	maize	-	+		+		-		+		-	
NK 603 (Roundup Ready)	maize	A	+	+	+	+	+	+	-	wp	-	-
T14	maize	-	+	+	-	-	-	-	-	-	+	+
T25	maize	A	+	+	-	-	-	-	-	-	+	+
TC1507 (DAS 1507)	maize	A	+	+	-	-	-	-	-	-	+	+
TC-6275 (DAS-06275-8)	maize	-	+		-		-		+		-	
DP 098140-6	maize	-	+	+	-		-		-		-	

amplification results (indicated as "–"), a high amount of amplifiable non-target DNA, e.g. 2.500 copies, should be used and the analyses have to be negative in at least two replicates. For the verification of positive amplification results (indicated as "+"), a low amount of amplifiable DNA of the event to be tested in the range of the limit of detection (ten to 50 copies per reaction) is recommended. All tests have to be positive in at least five replicates.

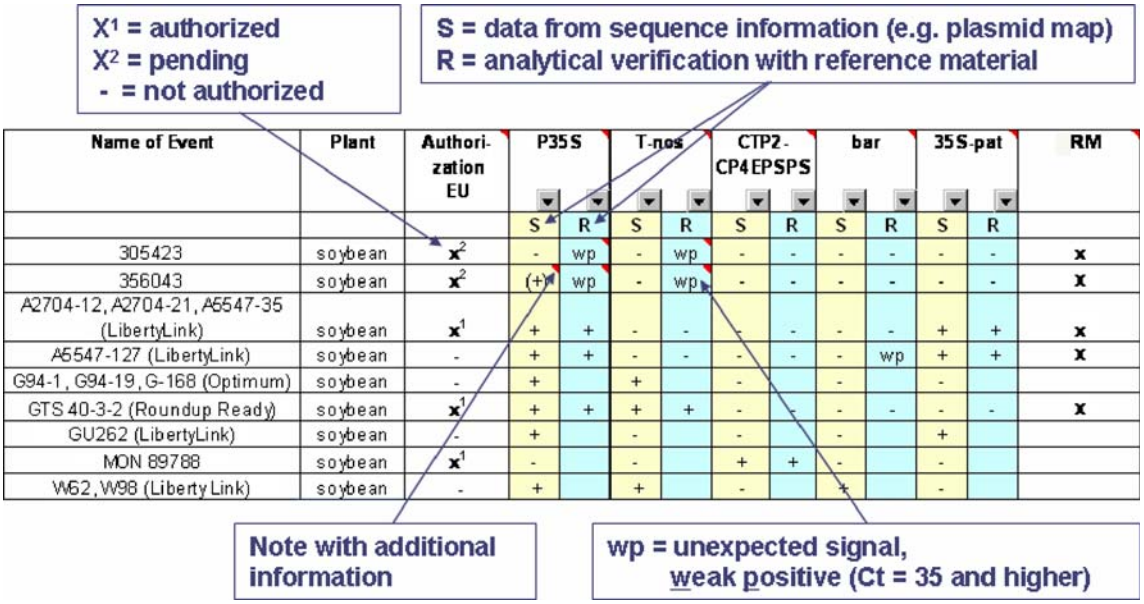
As far as reference material is available (see also Materials and methods), the database information was

Table 2 (continued)

Name of Event	Plant	Authorisation EU	P35S		T-nos		CTP2-CP4EPSPS		bar		35S-pat	
			S	R	S	R	S	R	S	R	S	R
55-1, 63-1 (Sunup)	papaya	-	+	+	+	+	-	-	-	-	-	-
X17-2	papaya	-	+		+		-		-		-	
23-198, 23-18-27 (Laurical)	rape	-	+	+	-	-	-	-	-	-	-	-
Falcon GS40/90	rape	A	+	+	-	-	-	-	-	-	+	+
GT200	rape	-	-		-		+		-		-	
GT73	rape	A	-	-	-	wp	+	+	-	wp	-	-
HCN 10, HCN 92, Topas19/2 (LibertyLink)	rape	A	+	+	-	wp	-	-	-	-	+	+
Liberator L62 (pHoe6/AC)	rape	A	+		-		-		-		+	
MS1, RF1, RF2; MS1xRF1 (PGS1), MS1xRF2 (PGS2) (SeedLink)	rape	A	-	-	+	+	-	-	+	+	-	-
MS8, RF3, MS8xRF3 (SeedLink)	rape	A	-	-	+	+	-	-	+	+	-	-
OXY 235	rape	-	+	+	+	+	-	-	-	-	-	
Phy 23	rape	-							+		-	
PHY36	rape	-	-		-		-		+		-	
T45, HCN 28 (LibertyLink)	rape	A	+	+	-	-	-	-	-	-	+	+
LL62 (LibertyLink)	rice	P	+	+	-	-	-	-	+	+	-	-
LL06	rce	-	+		-		-		+		-	
LL601	rice	-	+	+	-	-	-	-	+	+	-	-
Bt63 (TT51-1)	rice	-	-	-	+	+	-		-	-	-	
KMD1	rice	-	+		+		-		-		-	
305423	soybean	P	-	wp	-	wp	-	-	-	-	-	-
356043	soybean	P	+	+	-	wp	-	-	-	-	-	-
A2704-12, A2704-21, A5547-35 (LibertyLink)	soybean	A	+	+	-	-	-	-	-	-	+	+
A5547-127 (LibertyLink)	soybean	-	+	+	-	-	-	-	-	wp	+	+
G94-1, G94-19, G-168 (Optimum)	soybean	-	+		+		-		-		-	
GTS 40-3-2 (Roundup Ready)	soybean	A	+	+	+	+	-	-	-	-	-	-
GU262 (LibertyLink)	soybean	-	+		-		-		-		+	
MON 89788	soybean	A	-		-		+	+	-		-	
BPS-CV127-9	soybean	P	-		-		-		-		-	
W62, W98 (Liberty Link)	soybean	-	+		+		-		+		-	
1345-4 (Endless summer)	tomato	-	+		+		-		-		-	
35 1 N	tomato	-	-		+		-		-		-	
5345	tomato	-	+		+		-		-		-	
8338	tomato	-	+		+		-		-		-	
B, Da. F (Vegadura)	tomato	-	+		+		-		-		-	
FlavrSavr	tomato	-	+		-		-		-		-	
GTSB77 (Roundup Ready)	sugar beet	P	+	+	-	wp	+	+	-	-	-	-
T120-7 (LibertyLink)	sugar beet	-	+		-		-		-		+	
H7-1 (Roundup Ready)	sugar beet	A	-	wp	-	wp	+	+	-	-	-	-
MON 1445	cotton	A	+	+	+	+	-		-	-	-	
MON 531	cotton	A	+	+	+	+	-	wp	-	-	-	
MON 15985	cotton	A	+	+	+	+	-		-	-	-	
LL25	cotton	P	+	+	+	+	-	-	+	+	-	
281-24-236 x 3006-210-23	cotton	P	-	-	-	-	-	-	-	-	-	

Legend:

S = theoretical (e.g. database) ; R = verified by means of reference materials or other control samples; wp = weak amplification, Ct value > 35. Underlined: Reference material/event, that was used for experimental verification (R). Orange: event not detected by the proposed screening. A=EU authorised; P=EU authorisation pending; "-"=EU unauthorised



Name of Event	Plant	Authorization EU	P35S		T-nos		CTP2-CP4EPSPS		bar		35S-pat		RM
			S	R	S	R	S	R	S	R	S	R	
305423	soybean	x²	-	wp	-	wp	-	-	-	-	-	-	x
356043	soybean	x²	(+)	wp	-	wp	-	-	-	-	-	-	x
A2704-12, A2704-21, A5547-35 (LibertyLink)	soybean	x¹	+	+	-	-	-	-	-	-	+	+	x
A5547-127 (LibertyLink)	soybean	-	+	+	-	-	-	-	-	wp	+	+	x
G94-1, G94-19, G-168 (Optimum)	soybean	-	+		+		-		-		-		
GTS 40-3-2 (Roundup Ready)	soybean	x¹	+	+	+	+	-	-	-	-	-	-	x
GU262 (LibertyLink)	soybean	-	+		-		-		-		+		
MON 89788	soybean	x¹	-		-		+	+	-		-		
W62, W98 (Liberty Link)	soybean	-	+		+		-		+		-		

Fig. 1 Screenshot of the screening table (for explanation see boxes)

confirmed. Some events could not be tested due to the lack of reference material or other positive control samples. In such cases, no information is given in the "R" rows of Table 2. If more than one GM plant event is given per line, only material of the underlined event was used.

In most cases, target sequences were detectable with sufficient sensitivity (Table 2). In cases where the sensitivity for some events was above the LOD of ten to 50 copies, the Excel spreadsheet provides information in the "comment" field. For example, P35S-pat PCR detection of maize event Bt11 is not sensitive enough to comply with the requirements of routine analysis of enforcement laboratories.

This result can be explained by the rather large amplicon of 402 bp generated with genomic Bt11 DNA, because in this event the P35S-pat construct differs from those present in other GM maize events (TC1507, T25, 59122, etc.) (see also Table 1).

In several cases, weak positive amplifications with Ct values of ≥35 were observed when using DNA extracted from reference materials of non-target GM plants. In all these cases, flour materials had been used. Such unexpected amplifications did not occur, when reference DNA from leaves were used. It is assumed that contaminations of the flour-based reference materials by GM materials/plants carrying the targeted sequences are causing these weak positive results. For example, in reference materials of the soybean events DP-305423 and DP-356043, traces of soybean event GTS 40-3-2 (Roundup Ready soy) were detected.

Thus, further verification of these results by other materials (e.g. DNA from leaves) should be considered, if available. It is remarked that the reference materials are certified only for the absence of the GM event of interest (e.g. <0.04% for NK603 maize material BF415a of IRMM), but not for the absence of other GM events and derived sequences. Therefore, these reference materials should generally not be used in non-target GM specificity tests and, more importantly, not as negative DNA target controls.

The limiting factor to experimentally verify the presence of the target sequence for all listed GM plants is the lack of adequate reference material. Therefore, experimental verification data for several GM plants will be added as soon as a respective new reference material is available.

Practical use of the screening table

Screening for GM plants

As shown in the screening table (Table 2), the combination of these PCR methods can be used as a practical GMO matrix for the screening for all relevant GM crops, either authorised or so far not authorised for placing on the market in the European Union. Except for maize event LY038, soybean events DP-305423 and BPS-CV127-9 and cotton event 281-24-236×3006-210-23, at least one of the five sequences targeted by this screening approach is present in any GM plant described.

The screening approach can either be done step-by-step or by simultaneously targeting the genetic elements and/or constructs using the P-35S, T-nos, bar, ctp2-cp4epsps and P35S-pat simplex or duplex real-time PCR assays. After a first series of PCR tests, the results are systematically compared by application of the Excel sorting and filtering functionalities. Based on this filtering, it is possible to conclude which GM plant events may be present and which

additional screening PCR test(s) have to be conducted to discriminate among the putatively remaining GMOs. In addition, the analysis could be combined with plant taxon-specific methods, either as initial analytical step or after the first or second screening round, in order to identify the crop species composition present in the sample.

It has to be mentioned that—depending on the material analysed—possible contaminations by material of other species (so-called botanical impurities, e.g. soy flour in maize flour) have to be considered. As was observed also by other researchers in the field of GMO analysis, GM maize, soybean and rapeseed reference materials from IRMM or AOCS sometimes contain traces of other GM materials. Screening methods with a broad specificity for several GMOs will therefore detect such impurities resulting in weak positive signals caused by cross-contaminated GM reference materials.

Example for the identification of GM plant events

The results obtained in the screening for the target sequences described here may reduce the number of possible events that have to be considered for a final event-specific identification after initial screening. On the basis of the experimental results, the integrated Excel-based filtering functions of the screening table are useful to condense the number of candidate event-specific tests to be performed. For example, if a maize flour sample has to be screened for the presence of GM material, it is advisable first to use the P-35S and T-nos screening tests, either by the simplex or the duplex methods [6–8]. If the sample is positive for both targets, but negative for bar, ctp2-cp4epsps and P35S-pat, the filter functions of the spreadsheet will indicate the presence of eight different events (Fig. 2).

A significant Ct-value difference obtained in the P-35S and the T-nos amplifications (e.g. more than 3.3) indicate the presence of more than one event. As already mentioned, for event LY038 an additional (event-specific) test will be required to detect also this GM maize. For maize samples that are P35S and T-nos positive, it may be advisable to verify that only maize DNA is present in the sample (e.g. to exclude that the result is caused by traces of GTS-40-3-2 soy DNA). If no other plant species can be identified, the filter functions of the spreadsheet indicate MON863 and MON89034 as candidates (Fig. 2). If similar P-35S and T-nos Ct-values are observed, the presence of stacked events should also be considered.

In case of maize flour, contaminations with CaMV and/or *Agrobacterium* ssp. are very rare. However, in certain situations (strong positive results for P-35S and/or T-nos and the presence of unauthorised GM plants is suspected), appropriate control PCRs are recommended. In cases where the analyses of samples resulted P-35S and T-nos positive and no GM plant event was identified in the event-specific tests, sample contamination with *Agrobacterium* and/or CaMV should be excluded before further analysing the data. Appropriate real-time PCR-based detection methods for control of CaMV or *Agrobacterium* DNA have been described [26, 27].

Conclusion and further work

In conclusion, a GMO matrix in the format of the screening table as presented here can be a helpful tool in routine analysis of GM plants. Updated GMO databases and a network of collaborating laboratories are required to further develop and maintain this screening tool, e.g. by constantly including all upcoming GM plant events and by the addition of further targets which are frequently present in GM plants. Furthermore, it is necessary to verify theoretical assumptions as soon as an additional reference material (plant material and/or genomic DNA) becomes available. The network of the official German enforcement laboratories are progressing in the collaborative trial validation of further methods for screening (e.g. P-FMV and P-nos-nptII).

Name of Event	Plant	Authorisation EU	P35S		T-nos		CTP2-CP4EPSPS		bar		35S-pat	
			S	R	S	R	S	R	S	R	S	R
3272	maize	P	-	wp	+	+	-	-	-	-	-	-
GA 21 (Roundup Ready)	maize	A	-	-	+	+	-	-	-	wp	-	-
MIR 162	maize	P	-		+		-		-		-	
MIR604	maize	P	-	-	+	+	-	-	-	-	-	-
MON 810	maize	A	+	+	-	-	-	-	-	-	-	-
MON 863 (YieldGard)	maize	A	+	+	+	+	-	-	-	-	-	-
MON89034	maize	P	+		+		-		-		-	
DP 098140-6	maize	-	+	+	-		-		-		-	

Fig. 2 Example of a list of GM maize events that are displayed by the Excel spreadsheet after using filter functions (filters: plant = maize; P-35S and T-nos +; *bar* ctp2-cp4epsps and P35S-pat −)

The combination of such methods in multiplex real-time PCR methods in the near future will reduce costs and will increase the efficiency of GMO screening approaches. As soon as additional simplex or multiplex PCR methods are officially established, additional columns will be inserted into the screening table. It is planned, that an updated version of the screening table and a list of available reference materials is published at least every 6 months. Within the network of German laboratories, an extended version of the screening table is foreseen, that provide additional information, particularly with additional columns comprising screening targets which are "in the pipeline" as well as detailed information (e.g. lot numbers) about the reference material used for the experimental verification. In addition, we propose that a so-called Reference GMO matrix is established to enhance transparency and harmonisation of the GMO screening approach, e.g. within the European Network of GMO Laboratories (ENGL).

References

1. Holst-Jensen A, Ronning S, Lovseth A, Berdal K (2003) PCR technology for screening and quantification of genetically modified organisms. Anal Bioanal Chem 375:985–993
2. Bruderer S, Leitner KE (2003). Genetically Modified (GM) Crops: molecular and regulatory details. Version 2 (30/06/2003). BATS, Centre for Biosafety Assessment, Technology and Sustainability. http://www.bats.ch/gmo-watch/GVO-report140703.pdf
3. AGBIOS (2009) http://www.agbios.com/dbase.php. Cited 30 June 2009
4. International Organization of Standardization. ISO 24276:2006 Foodstuffs—methods of analysis for the detection of genetically modified organisms and derived products—general requirements and definitions
5. Feinberg M, Fernandez S, Cassard S, Bertheau Y (2005) Quantitation of 35S promoter in maize DNA extracts from genetically modified organisms using real-time polymerase chain reaction, part 2: interlaboratory study. J AOAC Int 88:558–573
6. International Organization of Standardization. 21570: 2005 Methods of analysis for the detection of genetically modified organisms and derived products—quantitative nucleic acid based methods. Annex B1
7. Reiting R, Broll H, Waiblinger HU, Grohmann L (2007) Collaborative study of a T-nos real-time PCR method for screening of genetically modified organisms in food products. J Verbr Lebensm 2:116–121
8. Waiblinger HU, Ernst B, Anderson A, Pietsch K (2007) Validation and collaboration study of a P35S and T-nos duplex real-time PCR screening method to detect genetically modified organisms in food products. Eur Food Res Technol 226:1221–1228
9. Stein AJ, Rodriguez-Cerezo E (2009) The global pipeline of new GM crops—implications of asynchronous approval for international trade. JRC Sci Tech Rep. doi:10.2791/12087
10. Leimanis S, Hamels S, Naze F, Mbella G, Sneyers M, Hochegger R, Broll H, Roth L, Dallmann K, Micsinai A, La Paz J, Pla M, Brunen-Nieweler C, Papazova N, Taverniers I, Hess N, Kirscheit B, Bertheau Y, Audeon C, Laval V, Busch U, Pecoraro S, Neumann K, Rösel S, Van Dijk J, Kok E, Bellocchi G, Foti N, Mazzara M, Moens W, Remacle J, Van den Eede G (2008) Validation of the performance of a GMO multiplex screening assay based on microarray detection. Eur Food Res Technol 227:1621–1632
11. Mano J, Shigemitsu N, Futo S, Akiyama H, Teshima R, Hino A, Furui S, Kitta K (2009) Real-time PCR array as a universal platform for the detection of genetically modified crops and its application in identifying unapproved genetically modified crops in Japan. J Agric Food Chem 57:26–37
12. Van den Bulcke (2008). Development of an Integrated Platform for the Detection of Materials derived from Geneteically Modified Crops in Food and Feed Products. http://gmoglobalconference.jrc.ec.europa.eu/2008/Presentations/VAN DN BULCKE.pdf Cited 17 July 2009
13. Waiblinger HU, Boernsen B, Pietsch K (2008) Praktische Anwendung für die Routineanalytik—Screening-Tabelle für den Nachweis zugelassener und nicht zugelassener gentechnisch veränderter Pflanzen. Deut Lebensm-Rundschau 104:261–264
14. Federal Office of Consumer Protection and Food Safety. Detection and Control. http://www.bvl.bund.de/cln_007/DE/06__Gentechnik/00__doks__downloads/Referenzmaterialien,templateId=raw,property=publicationFile.pdf/Referenzmaterialien.pdf. Cited 16 July 2009
15. International Organization of Standardization. ISO 21571:2005 Methods of analysis for the detection of genetically modified organisms and derived products—nucleic acid extraction.
16. Community Reference Laboratory, GM food and feed. Status of dossiers. http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm. Cited 16 July 2009
17. Arumuganathan K, Earle ED (1991) Nuclear content of some important plant species. Plant Mol Biol Rep 9:208–218
18. Waiblinger HU, Ohmenschläger M, Pietsch K, Ritter W, Steegmüller J, Krech A, Horn P, Schroeder A (2005) Die Untersuchung von transgenen Rapspollen in Honigen mittels Real-time-PCR. Deut Lebensm-Rundschau 101:543–549
19. Official Collection of Test Methods (2008) Detection of the CTP2-CP4-EPSPS gene sequence for screening of materials derived from genetically modified organisms (GMO) in foodstuffs – construct-specific method. German food and feed law – food analysis, article 64, L 00.00-125. Beuth, Berlin
20. Official Collection of Test Methods (2008) Specific detection of a frequently used DNA sequence from genetically modified organisms (GMO) derived from the bar-gene of Streptomyces hygroscopicus in foodstuffs—screening method. German food and feed law—food analysis, article 64, L 00.00-124. Beuth, Berlin
21. Real-time PCR for the quantification of genetically modified rapeseed lines using the 35S/pat-construct (2006). J Verbr Lebensm 3: 111-114 and website of the ‚Länderarbeitsgemeinschaft Gentechnik'. http://www.lag-gentechnik.de/dokumente/SOP_UAM_pat_quant_28032006.pdf (german language)
22. Grohmann L, Brünen-Nieweler C, Nemeth A, Waiblinger HU (2009) Collaborative trial validation studies of real-time PCR based screening methods for detection of the bar gene and the ctp2-cp4epsps construct. J Agric Food Chem. doi:10.1021/jf901598r
23. Official Collection of Test Methods (2008) Detection of DNA sequences from CaMV 35S promoter and T-nos for screening of materials derived from genetically modified organisms (GMO) in foodstuffs—screening method. German food and feed law—food analysis, article 64, L 00.00-122. Beuth, Berlin
24. Nucleotide sequence database GenBank of the National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/mapview/. Cited 17 July 2009
25. GMO Detection Method Database (GMDD). http://gmdd.shgmo.org/ Cited 17 July 2009
26. http://www.gdch.de/strukturen/fg/lm/ag/screening.xls
27. Chaouachi M, Fortabat MN, Geldreich A, Yot P, Kerlan C, Kebdani N, Audeon C, Romaniuk M, Bertheau Y (2008) An accurate real-time PCR test for the detection and quantification of cauliflower mosaic virus (CaMV) applicable in GMO screening. Eur Food Res Technol 227:789–798