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ORIGINAL PAPER

Validation and collaborative study of a P35S and T-nos duplex real-time PCR screening method to detect genetically modified organisms in food products

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Abstract In this work the intra and inter-laboratory validation of a duplex real-time PCR screening method for the detection of genetically modified (gm) plants is described. Target DNA sequences from Cauliflower Mosaic Virus 35S promoter (P35S) and nos-terminator from *Agrobacterium tumefaciens* (T-nos) are amplified. The duplex real-time PCR method is using primer and probe sequences that have already been published for the individual ("single") detection of both target sequences. The validation showed sensitivity comparable to the single PCR standard methods. In addition, combined with a reference gene and using reference standard material, the method can be used to semi-quantitatively estimate the amount of gm plants in an unknown sample.

Keywords Duplex real-time PCR · Screening · Ring trial · Genetically modified plant · Validation study

Introduction

For the detection and quantification of genetically modified (gm) organisms, especially gm plant material in foods, mostly real-time polymerase chain reaction (PCR) methods are used [1–8].

For screening purposes, DNA sequences present in different gm plants are amplified. Due to the lack of specificity, positive screening results have to be verified using construct- and/or event-specific methods, if available [4, 8].

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Already 10 years ago, first PCR methods for screening of gm plants using the sequences from cauliflower mosaic virus (CaMV) 35S promoter (P35S) and nos-terminator from *Agrobacterium tumefaciens* (T-nos) have been published [1].

Until now, in routine analysis PCR detection methods mostly targeting these two sequences are used. Since then, also real-time PCR methods to screen for the P35S and T-nos sequence have been established and validated in ring-trials [7, 9].

By the use of real-time PCR screening methods, a semi-quantitative estimation of gm plant in the sample is possible.

So far, in routine analysis DNA extracts of samples are analysed separately for each target DNA sequence by real time PCR (single PCR). With the increasing number of gm plant events on the global market, efficient strategies for screening analysis require the use of several target sequences (e.g. up to five). Therefore, a fast and economic approach can be the simultaneous amplification of two or more target DNA sequences in one analysis by multiplex real-time PCR.

In the past years, first approaches using multiplex real-time PCR for gm plant analysis have been reported [11]. One of the main challenges of multiplex real-time PCR methods is to ensure sensitivity and reproducibility. In order to avoid false negative results in gm plant screening analysis, a high sensitivity of the method is very important and the establishment and optimisation of such methods may be more complex compared with single PCR methods.

This work describes the optimisation and validation of a duplex real-time PCR for the simultaneous detection and semiquantitative estimation of the P35S and the T-nos sequence in transgenic maize reference samples.

Materials and method

Materials

Reference materials (flours) from gm maize with defined amounts (% w/w) of the transformation events Bt11, MON810 and GA21, each in conventional maize were used (IRMM, Geel, Belgium). In addition, maize samples with negative results for P35S and T-nos in previous analyses were used for preparing DNA mixtures. Further standard materials (flours) of the transgenic maize events Bt176, MIR604, MON 810, MON863, NK603, T25, TC 1507, 59122 and the soybean GTS 40-3-2 were purchased from IRMM. Materials from transgenic potato event EH92-527-1 and transgenic rape GT73 were purchased from AOCS (Urbana, IL, USA). DNA extracts from transgenic rapeseed falcon GS40/90, Topas 19/2, T45, MS8, RF3 and transgenic rice LL62 were purchased from Bayer Crop Science (Gent, B). Materials (seeds) from transgenic sugar beet GTSB77 and from maize events T14 and MON809 were obtained from field release trials; DNA from LL601 rice was obtained from CRL (Ispra, I); further DNA from 23-198 rapeseed and sunup papaya was available from previous German ring trials.

DNA extraction

DNA was extracted from seeds and flours with the Qiagen Plant Mini Kit (Qiagen, Hilden, Germany) starting with a CTAB extraction [5].

DNA calibration standards

DNA, extracted from reference material containing 5% (w/w) Bt11 (BF 412F, IRMM) maize in non-gm maize was used for calibration and quantification by real-time PCR. The concentration of DNA was estimated using photometry (OD_{260}). DNA-standards were defined by copy number (cp) of haploid genome equivalents per microlitre. For the calculation, a haploid genome weight for maize of 2.72 pg was assumed [12]. Dilution series were prepared to yield about 2,500, 500, 250, 50 and 10 cp per 5 µl each.

Maize DNA mixtures

Maize DNA from reference standards (w/w each) 0.1% Bt11, 1% Bt11, 0.1% MON810, 5% MON 810, 0.1% GA21, 5% GA21 and non-gm maize was adjusted to 136 ng each (= about 50,000 cp) per 5 µl. Mixtures (1 + 1) of these DNA extracts were prepared to yield about: 2.5% MON810 respectively 2.5% GA21; 0.05% MON810 respectively 0.05% GA21; 0.05% MON810 with 2.5% GA21 and 0.05% GA21 with 2.5% MON 810. The concentration "0.02% Bt11" was prepared by a mixture of 0.1% and non-gm maize DNA (1 + 4). Five microlitres of adjusted DNA and DNA-mixtures were used for real-time PCR amplification.

Real-time PCR

Real time PCR was performed with ABI 7500 system (Applied Biosystems, Darmstadt, Germany). The PCR reaction mix in a final volume of 25 µl with optimized primer and probe concentrations is listed in Table 1. Sequences of primers and probes are named in Table 2. Master mix for real-time RCR was the TaqMan® universal PCR Master Mix (Applied Biosystems), primers and probes were synthesised by Eurogentec (Seraing, Belgium). The

Table 1 Reaction mix for real-time PCR (ABI 7500)

Reagent	Final concentration	Volume per reaction (µl)
TaqMan® universal PCR Master Mix (2×)	1×	12.5
Primer 35S-F (2 µM)	0.1 µmol/l	1.25
Primer 35S-R (2 µM)	0.1 µmol/l	1.25
Probe 35S-TMP FAM (2 µM)	0.1 µmol/l	1.25
Primer 180-F (20 µM)	1.0 µmol/l	1.25
Primer 180-R (20 µM)	1.0 µmol/l	1.25
Probe TM-180 YY (4 µM)	0.2 µmol/l	1.25
DNA-extract (samples or standards)	Samples: about 50,000 cp maize DNA per reaction	5
Total reaction volume	–/–	25

Table 2 Sequences of primers and probes

Name	Sequence	Reference
35S-FTM	5′-gCCTCTgCCgACAgTggT-3′	[7]
35S-RTM	5′-AAgACgTggTTggAACgTCTTC-3′	
35S-TMP-FAM	5′-FAM-CAAAgATggACCCCCACCCACg-BHQ1-3′	
180-F	5′-CATgTAATgCATgACgTTATTTATg-3′	
180-R	5′-TTgTTTTCTATCgCgTATTAAATgT-3′	[9]
TM-180YY	5′-YY-ATgggTTTTTATgATTAgAgTCCCGCAA-BHQ1-3′	

FAM 6-Carboxylfluorescein, *YY* Yakima yellow, *BHQ* black hole quencher

following real-time PCR program was used: two initial steps, the first one with 120 s at 50 °C (activation of UNG for decontamination), the second one with 600 s at 95 °C (activation of polymerase), then 45 cycles with 15 s at 95 °C and 60 s at 60 °C.

Ring trial

A ring trial was carried out by ten members of the working group, "Development of methods for the identification of food produced with genetic engineering", within the scope of the official method collection according to § 64 of the German food and feed code (LFGB). Encoded samples from the nine different maize DNA mixtures mentioned above (containing the transformation events Bt11, MON 810 and/or GA21) plus one negative maize DNA sample were sent to the participants. In addition, for the quantification of the P35S and T-nos-sequence, each lab received standard DNA extracted from Bt11 reference material (see "DNA calibration standards"). Furthermore, aliquots of primers, probes and TaqMan® universal PCR master mix were sent to the labs. Each DNA sample had to be analysed in five replicas resulting in total 50 results per DNA sample. Eight labs used the ABI 7500 real-time PCR system, two labs used the ABI 7900 system and the Stratagene MX3005 cycler, respectively.

Discussion and results

Establishment and optimisation of duplex real-time PCR

General remarks

One of the primary goals of the optimisation of multiplex real-time PCR assays is a good PCR efficiency for all target sequences, comparable to those of the single PCR systems. According to the ENGL performance requirements for analytical methods of GMO testing [13], slope of the standard curve should be in the range of −3.1 to −3.6, theoretically equivalent to efficiencies between 110 and 90%.

Especially if there is an excess of one target sequence in the duplex PCR, amplification of the sequence present in minor amounts can be suppressed by competitive effects. To ensure optimal amplification conditions for all targets a lot of parametres have to be considered, e.g. concentration of polymerase and nucleotides and buffer composition including magnesium salt concentration. Due to the fact that currently an increasing number of commercial master mixes for multiplex real-time PCR are offered and that a broad palette of such optimised reagent kits can be expected in the near future, method optimisation did not focus on these reagents.

In this work, primer and probe systems already established and validated in single format should also be tested for their suitability to work under duplex real-time PCR conditions. For this purpose, ring-trial tested detection systems for the P35S [7] and the T-nos sequence [9] were chosen.

Optimisation of duplex real-time PCR assays was performed by variation of primer probe concentration and—if possible—maintaining PCR master mix reagents and cycling conditions of single real-time PCR methods. Main approaches were

- minimising competitive effects by limiting the primer concentration for the system with better PCR efficiency (when analysed with primer concentrations of single PCR methods)
- optimizing probe concentration (between 50 and 300 nM). Increase in probe concentration may lead to stronger fluorescence signals (ΔRn value) and therefore better sensitivities at low target concentrations [14].

For gm plant screening purposes, high sensitivity of the method is most important to exclude false-negative results in samples. Therefore, method optimization aimed at a limit of detection (LOD) in the range of about ten genome copies. Usually, ring-trial tested screening-methods exhibit such LODs [7, 9]. For low processed food ingredients of plant origin (e.g. soy flour) this is equivalent to a relative LOD of about 0.02 to 0.05% [3].

Signal parametres for optimisation of the sensitivity were in first optimisation steps: successful amplification of low copy numbers (10 cp) at lowest possible Ct values with highest possible increase of fluorescence (ΔRn).

Optimisation of P35S/T-nos-duplex real-time PCR-system

Primer concentration varied for both systems from 100 to 1,000 nM and probe concentration from 100 to 300 nM. First experiments showed that limitation of primers and probe of the more efficient P35S-system and then optimisation of primer/probe concentrations of the T-nos system was most promising (data not shown). In Fig. 1, amplification curves of the T-nos system at the 10 cp level under variation of primer and probe concentrations are shown (exemplarily with some of the primer and probe concentrations). Data showed, that low primer concentration for the P35S-system (100 nM) and high primer concentrations for the T-nos-system (1,000 nM) yielded the best results (low Ct values, high ΔRn). Influence of probe concentration was low for the P35S-system, therefore low probe concentration was chosen to minimise competitive and crosstalk effects.

In the final experiments, P35S primer and probe concentration was fixed at 100 nM each, while T-nos primer and probe concentration was varied at concentrations from 800

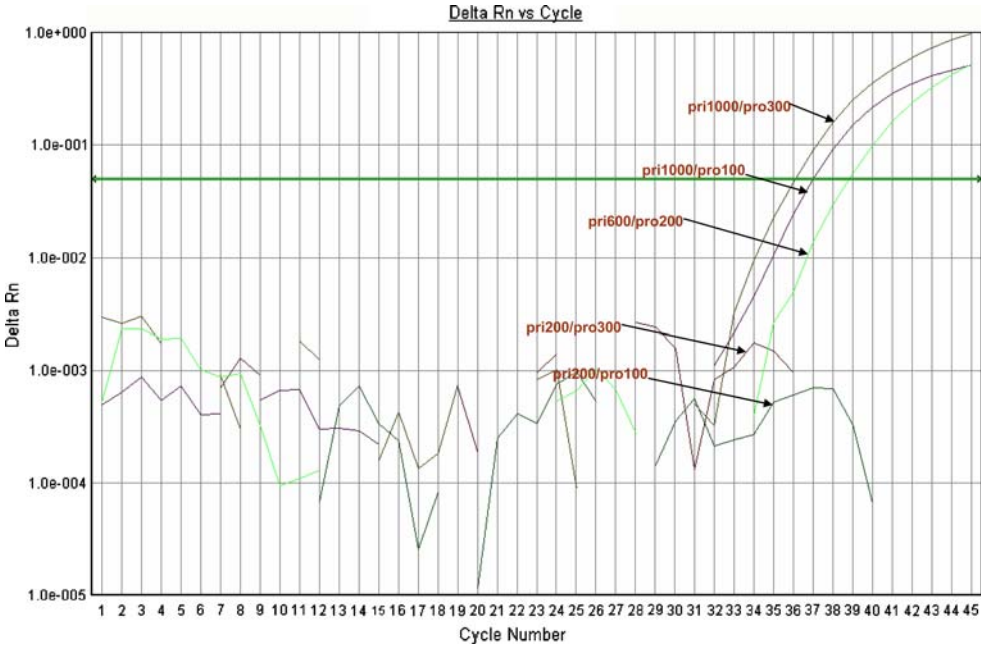

Fig. 1 Optimisation of T-nos system, examples of some amplification curves of about ten copies of the T-nos sequences when tested with different combinations of primer (*pri*) and probe (*pro*) concentrations

to 1,000 nM and 100 to 300 nM, respectively. The best results, which were chosen for further validation experiments, were obtained with the concentrations listed in Table 1 (data not shown).

In-house validation

Dilution series: working range, precision and PCR-efficiency

Bt11 maize (5%) reference material, which contains both the P35S and the T-nos sequence, was used for the validation experiments. In Table 3, results of a dilution series of genomic DNA, extracted from 5% Bt11-maize reference material is shown; each level was analysed in four replicas.

The results demonstrate good precision and sensitivity data for both systems even for low copy number dilutions. RSD_r values fulfill ENGL performance requirements of <30% [13] in the whole range except that from the 10 cp dilution level. This level was estimated to be near the LOD (see ring-trial data below) [3]. Assuming 50,000 cp of maize DNA in a sample, this would correspond to an LOD of 0.02%.

It has to be remarked, that the calculation of copy numbers (see "Materials and methods") is based on assumptions, e.g. Bt11 reference maize powder is made from material that is heterozygous related to the transgene [15],

Table 3 In-house validation of P35S/T-nos duplex real-time PCR system, dilution series of Bt11 genomic DNA

Expected concentration (cp number/reaction)	Mean measured concentration (cp number/reaction)	Mean Ct values	RSD_r % (cp numbers)	CI 95%
P35S-system				
2,500	2,468	28.6	17.1	27.2
500	470	31.3	10.6	16.9
250	249	32.3	17.3	27.5
50	56	34.7	18.0	25.4
10	16	36.7	50.0	47.8
T-nos system				
2,500	2,806	28.5	10.6	14.9
500	614	30.7	17.2	22.3
250	323	31.7	28.0	34.5
50	64	34.1	11.1	23.3
10	18	35.9	10.0	35.7

Precision data [RSD*r* and confidence interval (CI 95%)] were calculated from results of four PCR reactions per dilution level

and T-nos and P35S sequences are each present in two copies in the transgenic event. We recognise that copy numbers cannot be calculated exactly using weight based ERM reference material [15] and point out that copy numbers based results are only given on an estimation basis.

Compared with in-house validation data of the single PCR systems, PCR-efficiency and precision of the duplex system did not deteriorate and is similar to those of the single method (Table 4).

Quantification of genetically modified maize: sensitivity, trueness and precision

As a next step, trueness and precision were tested with Bt11 maize reference materials and mixtures thereof, containing defined amounts (w/w) of gm maize. Each DNA solution was adjusted to about 50,000 genome copies of maize. For the relative quantification of amplifiable DNA in the samples, a sequence of the species-specific invertase gene was used as reference gene [15]. Each concentration level (% Bt11) was analysed in five replicas. Additionally, reference materials of MON810 maize (P35S positive, T-nos negative) and GA21 maize (P35S negative, T-nos positive) were analysed. For calibration, DNA dilutions from the 5% Bt11 maize reference material were used.

The sensitivity of about 0.02%, estimated from dilution series described above, could be confirmed by analysis of reference samples (DNA-dilution of 0.1% Bt11 reference material with non-GMO maize DNA). The samples containing 0.05% MON810 (P35S positive) and 0.05% GA21 (T-nos positive) as well as the mixtures of GA21 and MON810 gave unambiguous positive results. Mixtures of a low level of MON810 respectively GA21 with a high amount of the GMO containing the competing sequence (0.05 vs. 2.5%) were chosen to check for losses in sensitivity and for competitive effects.

Even at low levels of about 0.05% gm maize, precision data fulfilled ENGL precision criteria for $RSD_r < 30\%$ [13], except from 2.5% GA21 (T-nos, $RSD_r = 30\%$) (see Table 5).

Specificity

The P35S- and/or T-nos sequence could be detected in reference materials as expected from database information or previous single analyses (data not shown). In addition,

Table 4 Comparison between P35S/T-nos single and duplex PCR systems

	P35S (single)[a]	P35S (duplex)	T-nos (single)[a]	T-nos (duplex)
Working range	10 to 2,500 copies per PCR			
Slope/efficiency	−3.88/81%	−3.41/97%	−3.17/106%	−3.42/96%
R^2	>0.995	0.99	0.98	0.96
Estimated limit of detection	10 copies	10 copies	10 copies	10 copies

[a] In-house validation data of CVUA Freiburg

Table 5 Trueness and precision of P35S/T-nos duplex real time PCR-system, determined using gm maize DNA from Bt11, MON810 and GA21 maize

Reference material: percent gm-maize (wt%), expected value	Mean (%) ($n = 5$)		Standard deviation (% Bt11)		RSD_r/CI 95% (%)	
	P35S	T-nos	P35S	T-nos	P35S	T-nos
0.02% Bt11 (P35S pos; T-nos pos)	0.02	0.01	0.01	0.002	42/53	30/38
0.1% Bt11 (P35S pos; T-nos pos)	0.07	0.05	0.02	0.01	24/29	23/28
1.0% Bt11 (P35S pos; T-nos pos)	0.82	0.79	0.10	0.10	12/15	12/15
2.5% MON810 (P35S pos; T-nos neg)	1.72	0	0.13	–	8/10	–
0.05% MON810 (P35S pos; T-nos neg)	(0.06)	0	0.01	–	19/24	–
2.5% GA21 (P35S neg; T-nos pos)	0[a]	(5.86)	–	0.97	–	17/20
0.05% GA21 (P35S neg; T-nos pos)	0	(0.06)	–	0.01	–	15/19
0.05% MON810 + 2.5% GA21 (P35S pos; T-nos pos)	(0.09)	(8.85)	0.02	2.64	27/34	30/37
2.5% MON810 + 0.05% GA21 (P35S pos; T-nos pos)	(1.93)	(0.06)	0.21	0.01	10/13	18/22

[a] One of five replica positive; <10 copies in brackets: data for information only, no assessment of trueness possible (Bt11 quantification standard was used)

materials theoretically not containing at least one of the both sequences (MON810: T-nos negative; GA21: P35S negative) gave the expected results.

However in the analysis of 2.5% GA21 maize for the P35S sequence, one of five reactions gave weak positive signals (below 10 cp). In comparison, the analysis with the single methods also yielded a weak amplification. We have to remark that all the commercially available "0%" reference materials (e.g. 0% MON 810, 0% GA21) gave more or less weak positive signals in P35S and/or T-nos PCR. The materials are certified only to be negative regarding the event, but not regarding contaminations by other gm plant ingredients [16]. Even in own mixtures of maize flours, that gave negative results in P35S and T-nos PCR in previous analyses, sporadic and very weak signals were detected when analysed in higher numbers of replica (see also ring-trial).

Thus, it can be suggested that these signals are not caused by method (i.e. false positive results), but by the materials available.

Ring-trial validation

Slope of calibration standards, efficiency

Before evaluation of the results, PCR efficiencies of the Bt11 calibration curve of the individual labs were compared. Overall, PCR efficiency, calculated from the slopes, was satisfactory. The lowest efficiencies with 66% for the T-nos system and 83% for the P35S system were obtained within one laboratory. However, data of all laboratories were included, outliers were not eliminated.

Sensitivity and precision (RSD_R)

In Tables 6 and 7 the proportions of positive results for P35S and T-nos and the precision data are specified for the different DNA samples. The level 0.02% Bt11 was detected unambiguously with both systems (50 of 50 respectively 49 of 50 reactions). This also applies to the levels 0.05% GA21 and 0.05% MON810, including mixtures with high amounts of the competing sequence.

Regarding precision (RSD_R), except from 1% Bt11 level, the data for the T-nos system (RSD_R = 25–61%) did not quite meet all the requirements of ISO 24276 [8] and ENGL [13] for quantification. According to these requirements, RSD_R should be <25% (ISO 24276) respectively 33% (ENGL) over the majority of the dynamic range and below 33% (ISO 24276) respectively 50% at the limit of quantification (LOQ).

However, for the P35S system all the quantification requirements were met at the levels 1% and higher (RSD_R = 10–15%), and at least the LOQ requirements of ENGL for all other samples with lower levels between 0.02 and 0.1% (RSD_R = 27–42%).

Specificity

As already observed within the in-house validation, weak amplifications in the "P35S negative" samples were observed with the P35S system (in one case Ct 36, other-

Table 6 Ring-trial validation of the P35S/T-nos duplex real time PCR, results of P35S system

P35S-system		Mean efficiency: 93% (83–99%)						
Reference material: percent gm-maize (wt%), expected value	Number of positive results/number of PCR reactions[a]	Ct values		Copy number P35S			% P35S (related to copy numbers of maize)[c]	
		Mean	Stdev[b]	Mean	Stdev	RSD_R[d] (%)	Mean	Stdev
0.02% Bt11 (P35S pos; T-nos pos)	50/50	36.4	1.0	13	4.8	38	0.03	0.01
0.1% Bt11 (P35S pos ; T-nos pos)	50/50	34.1	0.5	56	15	27	0.11	0.03
1.0% Bt11 (P35S pos; T-nos pos)	50/50	30.8	0.6	470	72	15	0.94	0.14
2.5% MON810 (P35S pos; T-nos neg)	50/50	29.4	0.6	(1,170)	(116)	10	(2.3)	(0.23)
0.05% MON810 (P35S pos; T-nos neg)	50/50	34.4	0.8	(29)	9.4	32	(0.06)	(0.02)
2.5% GA21 (P35S neg; T-nos pos)	7/50[e]	−Ct min = 36)	–	–	–	–	–	–
0.05% GA21 (P35S neg; T-nos pos)	10/50	−Ct min = 38)	–	–	–	–	–	–
0.05% MON810 + 2.5% GA21 (P35S pos; T-nos pos)	50/50	34.5	5.0	(43)	(18)	42	(0.09)	(0.04)
2.5% MON810 + 0.05% GA21 (P35S pos; T-nos pos)	50/50	29.4	0.6	(1,192)	(152)	13	(2.4)	(0.3)
"0%" gm maize	10/50[e]	−(Ct min = 38)	–	–	–	–	–	–

For legend see Table 7

Table 7 Ring-trial validation of the P35S/T-nos duplex real time PCR, results of T-nos system

T-nos-system		Mean efficiency: 102% (66–120%)						
Reference material: percent gm-maize (wt%), expected value	Number of positive results/number of PCR reactions[a]	Ct values		Copy number T-nos			% P35S (related to copy numbers of maize)[c]	
		Mean	Stdev[b]	Mean	Stdev	RSD_R[d] (%)	Mean	Stdev
0.02% Bt11 (P35S pos; T-nos pos)	49/50	37.6	1.4	5.7	3.5	61	0.01	0.01
0.1% Bt11 (P35S pos; T-nos pos)	50/50	34.7	0.9	37	13	35	0.07	0.03
1.0% Bt11 (P35S pos; T-nos pos)	50/50	31.2	0.7	404	101	25	0.81	0.20
2.5% MON810 (P35S pos; T-nos neg)	0/50	–	–	–	–	–	–	–
0.05% MON810 (P35S pos; T-nos neg)	0/50	–	–	–	–	–	–	–
2.5% GA21 (P35S neg; T-nos pos)	50/50	27.9	4.2	(4,500)	(1,722)	38	(9.0)	(3.5)
0.05% GA21 (P35S neg; T-nos pos)	50/50	33.6	0.9	(81)	(23)	28	(0.16)	(0.05)
0.05% MON810 + 2.5% GA21 (P35S pos; T-nos pos)	50/50	27.8	4.1	(4,650)	(1,850)	40	(9.3)	(3.7)
2.5% MON810 + 0.05% GA21 (P35S pos; T-nos pos)	50/50	33.9	1.0	(69)	(27)	40	(0.1)	(0.06)
"0%" gm maize	5/50[d]	−(Ct min = 39)	–	–	–	–	–	–

Values in brackets: data for information only, assessment of trueness not possible (Bt11-maize standard used for calibration)

[a] Results with Ct > 40 were evaluated as negative

[b] Standard deviation

[c] 50,000 copies of maize-DNA

[d] Reproducibility standard deviation (inter-laboratory variation)

[e] Weak amplifications observed in several labs in single PCR reactions (e.g. 1 or 2 positives of 5 reactions)

wise Ct 38 and higher), corresponding to <10 copies (Table 6).

These findings are supposed to originate from very low contaminations of the non-gm maize sample by P35S- and T-nos-containing materials—instead of method-inherent false-positive findings (see above). Detailed re-analysis after ring-trial with the single PCR showed, that in the materials "0% gm maize", "0.05% GA21" and "2.5% GA21", a low contamination by P35S-containing components, in the sample "0% gm maize" additionally by T-nos containing components were detectable.

Besides it should be mentioned, that amplifications only occurred within one or two of five reactions per sample. In such cases, results are reported as negative [8].

Trueness

Laboratories participating in the collaborative study received DNA-extracts that were adjusted to about 50,000 copies of the maize reference gene per reaction. The proportions of the copy numbers, of the P35S- and the T-nos-sequence in relation to 50,000 copies of the maize reference gene, are compiled in the two last columns of Tables 6 and 7 (mean and standard deviation).

Good recoveries of about 80% and more were achieved at the levels 0.1% Bt11 and 1% Bt11 for the P35S system and at the level 1% Bt11 for the T-nos system (ENGL-requirement: ±25% of the reference value [13]).

The quantification of MON810 and GA21 lead to divergent results, when Bt11 calibration standard is used. These effects are caused by different integration frequencies of the P35S and T-nos sequence in Bt11 in relation to GA21 and MON810 and by different zygote or ploidy of the plant materials.

Conclusion of validation experiments and outlook

In conclusion, validation data show that the duplex real-time PCR method can serve the purpose of semiquantitative screening. Most of the validation data fulfil the ENGL minimum performance requirements for quantitative analytical methods for GMO testing. Positive screening results—especially if results indicate gm proportions higher than about 0.05–0.1% [10] and also if non-authorised gm plant material may be present—should be verified and quantified using construct or event-specific methods employing the identified event as quantification standard (if available).

At the moment, such screening methods are the only tool for the detection of gm plants that are not authorised in the European Union. For this purpose, we have combined this duplex real-time PCR methods with further real-time PCR

systems targeting sequences frequently occurring in gm plants. This work is foreseen to be published in the near future.

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