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Report on the Verification of the Performance of Bt11, MIR162, 1507 and GA21 Event-specific Methods on the Bt11 x MIR162 x 1507 x GA21 Maize Using Real-time PCR

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Report on the Verification of the Performance of Bt11, MIR162, 1507 and GA21 Event-specific Methods on the Bt11 x MIR162 x 1507 x GA21 Maize Using Real-time PCR

26 September 2013

European Union Reference Laboratory for GM Food and Feed

Executive Summary

An application was submitted by Syngenta Crop Protection AG to request the authorisation of genetically modified Bt11 x MIR162 x 1507 x GA21 maize (tolerant to herbicides containing glufosinate ammonium and glyphosate and resistant to important lepidoptera maize pests) and all sub-combinations of the individual events as present in the segregating progeny, for food and feed uses, and import and processing, in accordance with articles 5 and 17 of Regulation (EC) N° 1829/2003 GM Food and GM Feed [(1)]. The unique identifier assigned to Bt11 x MIR162 x 1507 x GA21 maize is SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØØ21-9.

Bt11 x MIR162 x 1507 x GA21 maize has been obtained by conventional crossing between four genetically modified maize events: Bt11, MIR162, 1507 and GA21. No new genetic modification was used for the development of Bt11 x MIR162 x 1507 x GA21 maize.

The EU-RL GMFF has previously validated individually, and declared fit for purpose, the detection methods for the single events Bt11, MIR162, 1507, GA21 and has published the corresponding reports http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx; therefore, in line with the approach defined by the ENGL (Annex 1, http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf) the EU-RL GMFF has carried out only an *in-house* verification of the performance of each validated method when applied to DNA extracted from Bt11 x MIR162 x 1507 x GA21.

The results of the *in-house* verification study were evaluated with reference to ENGL requirements and to the validation results on the individual events; as a result, the EU-RL GMFF concludes that the individual methods meet the ENGL criteria and can also be applied to Bt11 x MIR162 x 1507 x GA21 maize.

This report is published at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx.

Quality assurance

The EU-RL GMFF is ISO 17025:2005 accredited [certificate number: ACCREDIA 1172 (Flexible Scope for DNA extraction and qualitative/quantitative PCR) - Accredited tests are available at http://www.accredia.it/accredia_labsearch.jsp?ID_LINK=293&area=7].

The original version of the document containing evidence of internal checks and authorisation for publication is archived within the EU-RL GMFF quality system.

The EU-RL GMFF is also ISO 17043:2010 accredited (proficiency test provider) and applies the corresponding procedures and processes for the management of ring trials during the method validation.

The EU-RL GMFF conducts its activities under the certification ISO 9001:2008 of the Institute for Health and Consumer Protection (IHCP) provided by CERMET.

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Content

1. Legal context

The European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF) is established by Regulation (EC) No 1829/2003" [(1)] on genetically modified food and feed".

The Regulation provides the basis for ensuring a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed.

According to the Regulation, no person shall place on the market a GMO for food or feed use unless it is covered by an authorisation granted in accordance with the conditions set out in the Regulation.

According to Articles 5 and 17, the application for authorisation shall be accompanied, among others, by methods for detection, sampling and identification of the transformation event, and by samples of the food and feed and their control samples. The EU-RL GMFF shall test and validate the method of detection and identification proposed by the applicant.

As Regulation (EC) No 1829/2003 establishes a threshold (0.9%) under which labeling of food or feed is not required (provided that the presence of a GMO is adventitious or technically unavoidable), the method of detection proposed by the applicant shall be quantitative.

The applicant shall include a description of the methods of detection, in accordance to Annex I of Regulation (EC) No 641/2004 of 6 April 2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 [(2)]. This Annex provides detailed guidance on method validation, on testing needed to be carried out by the applicant and relative information to be provided to the EU-RL GMFF, as well as on submission of food and feed samples and their controls.
The EU-RL GMFF evaluates the information submitted for its completeness and fitness for purpose, assisted in this task by the European Network of GMO Laboratories (ENGL).

The overall method's performance is evaluated against criteria defined by the ENGL and compiled in the document entitled "Definition of minimum performance requirements for analytical methods for GMO testing", (http://gmo-crl.jrc.ec.europa.eu/doc/Method%20requirements.pdf) [(3)]. The document details criteria to be fulfilled by the method prior to validation by the EU-RL GMFF and that should be demonstrated upon completion of a validation study.

The inter-laboratory studies are carried out by the EU-RL GMFF in accordance to the following internationally accepted guidelines, e.g. ISO 5725: 1994 and the IUPAC "Protocol for the design, conduct and interpretation of method-performance studies" [(4, 5)].

2. Introduction

The EU legislative system [1, 2] for genetically modified food and feed provides that any GMO for food and feed use shall undergo the authorisation process before it can be placed on the market. This holds true also for a GMO obtained by conventional breeding of two or more single genetically modified events.

Consequently, the application for authorisation of a GMO composed of two or more events shall be accompanied, among others, by an event-specific method of detection and by samples of the food and feed and their control samples. The EU-RL GMFF shall test and validate the method of detection proposed by the applicant and shall report to the European Food Safety Authority, who will include the EU-RL GMFF reports in the overall Opinion.

As so far no specific detection method exists that would uniquely identify a GMO composed by two or more events combined by conventional breeding, the EU-RL GMFF follows a realistic approach when assessing this type of applications. According to this approach, the EU-RL GMFF carries out an in-house verification of the performance of each method, previously validated on the respective single lines, when applied to the GMO combining the single events.

This approach takes motivation from the assumption that the previously validated detection methods targeting specific sequences of the single events should perform equally well when applied to the same events combined in one genome by conventional breeding.

Although infrequent, possible reasons for underperformance of the methods when applied to the GMO combining the single events could be DNA sequence changes in the plant genome if, depending on the position of such change(s), they would affect an event-specific PCR method.

The analytical approach followed by the EU-RL GMFF is therefore proportionate, as the in-house verification allows ensuring that underperformance of the methods is ruled out while avoiding the repetition of costly and time consuming inter-laboratory validation studies.

Upon reception of methods, samples and related data (step 1), the EU-RL GMFF carried out the assessment of the documentation (step 2) and the in-house verification of the methods (step 3) according to the requirements of Regulation (EC) No 641/2004 (Annex I).

The results of the in-house verification study were evaluated with reference to ENGL method performance requirements and to the validation results on the individual events.

3. Step 1 (dossier reception and acceptance)

Syngenta Crop Protection AG submitted the detection methods and the corresponding control samples of Bt11 x MIR162 x 1507 x GA21 maize.

The dossier was found to be complete in all the requested parts and thus was moved to step 2.

4. Step 2 (dossier scientific assessment)

During step 2, the EU-RL GMFF carried out the scientific assessment of documentation and data. The data provided by the applicant were assessed against the method acceptance criteria set out by the ENGL.

Two requests of complementary information were addressed to the applicant. The first request concerned the full sequence of the inserts in electronic ASCII text files and the second request concerned the zygosity of GM events.

The EU-RL GMFF verified the data and the complementary information received and concluded that the methods meet the ENGL method verification criteria [(3)]. Based on the considerations above, the dossier was moved to step 3.

5. Step 3 (EU-RL GMFF experimental testing)

In step 3 the EU-RL GMFF performed a verification of the detection methods in order to assess their performance when applied to DNA extracted from Bt11 x MIR162 x 1507 x GA21 maize, as if applied to DNA extracted from the original single event plants.

Details on step 3 activities and results are provided below.

5.1 Materials

For the verification of the quantitative event-specific methods, the following control samples were provided by the applicant:

- genomic DNA extracted from seeds of Bt11 x MIR162 x 1507 x GA21 maize
- genomic DNA extracted from seeds of non-transgenic maize.

Using the control samples provided, samples containing mixtures of Bt11 x MIR162 x 1507 x GA21 and non-GM maize genomic DNA at different GM concentrations were prepared by the EU-RL GMFF in a constant amount of total maize DNA. These samples were used for the *in-house* verification of the methods.

The protocols (reagents, concentrations, primer/probe sequences) followed in the *in-house* verification were those already published as validated methods for the individual Bt11, MIR162, 1507, GA21 events (available at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx).

Table 1 shows the five GM levels used in the verification of the Bt11, MIR162, 1507, GA21 methods.

Table 1. Percentage of Bt11, MIR162, 1507, GA21 in Bt11 x MIR162 x 1507 x GA21 in the verification samples.

Bt11 GM% (GM DNA / Non-GM DNA x 100)	**MIR162 GM%** (GM DNA / Non-GM DNA x 100)	**1507 GM%** (GM DNA / Non-GM DNA x 100)	**GA21 GM%** (GM DNA / Non-GM DNA x 100)
0.09	0.1	0.1	0.09
0.4	0.4	0.5	0.5
0.9	0.9	0.9	0.9
5.0	2.0	2.0	5.0
8.0	5.0	5.0	8.0

5.2 DNA extraction

Methods for DNA extraction from maize seeds and grains were previously evaluated by the EU-RL GMFF in order to confirm their performance characteristics and were considered fit for the purpose. The protocols for the DNA extraction methods are available at http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11field_maize_DNAExtr.pdf and at http://gmo-crl.jrc.ec.europa.eu/summaries/GA21%20Syng_DNAExtr_report.pdf.
Consequently, the EU-RL GMFF did not verify the DNA extraction methods previously found suitable for DNA extraction of PCR-grade quality from maize seeds and grains.

5.3 Experimental design

Eight runs for each method were carried out. In each run, samples were analysed in parallel with both the GM-specific system and the reference system *Adh1* (*alcohol dehydrogenase 1*) for GM events Bt11, MIR162 and GA21 and *hmga (high mobility group protein a*) for GM event 1507. Five GM levels per run were examined and two replicates for each GM level were analysed. PCR analysis was performed in triplicate for all samples. In total, for each method (Bt11, MIR162, 1507, GA21), the quantification of the five GM levels was performed as an average of sixteen replicates per GM level. An Excel spreadsheet was used for determination of GM%.

5.4 PCR methods

During the verification study, the EU-RL GMFF carried out parallel tests on DNA extracted from Bt11 x MIR162 x 1507 x GA21 maize using the single detection methods previously validated on events Bt11, MIR162, 1507, GA21 separately.

For the detection of events Bt11, MIR162, 1507, GA21 in Bt11 x MIR162 x 1507 x GA21 maize, DNA fragments of 68-bp, 92-bp, 58-bp and 101-bp respectively are amplified using specific primers. PCR products are measured during each cycle (real-time) by means of target-specific oligonucleotide probes labelled with two fluorescent dyes: FAM (6-carboxyfluorescein) is used as reporter dye at its 5′-end and TAMRA (6-carboxytetramethylrhodamine) as a quencher dye at its 3′-end.

For quantification of events Bt11, MIR162 and GA21, a taxon-specific reference system amplifies a 135-bp fragment of maize alcohol dehydrogenase 1 (*Adh1*) endogenous gene (GenBank N. X04050), using two *Adh1* gene-specific primers and an *Adh1* gene-specific probe labelled with VIC and TAMRA. For quantification of event 1507, a taxon-specific reference system amplifies a 79-bp fragment of maize high mobility group protein a (*hmga*) endogenous gene (GenBank N AJ131373), using two *hmga* gene-specific primers and an *hmga* gene-specific probe labelled with FAM and TAMRA.

For quantification of events Bt11, MIR162 and GA21 in a DNA test sample, the normalised ΔCt values of the calibration samples are used to calculate, by linear regression, a standard curve (plotting ΔCt values against the logarithm of the amounts of Bt11, MIR162 and GA21 events DNA, respectively). The normalised ΔCt values of the unknown samples are measured and, by means of the regression formula, the relative amount of Bt11, MIR162 and GA21 events, respectively, are estimated. For 1507, standard curves are generated both for the 1507 and the *hmga* specific systems by plotting the Ct values of the calibration standards against the logarithm of the DNA copy numbers and by fitting a linear regression into these data. Thereafter, the standard curves are used to estimate the DNA copy numbers in the unknown samples. The percentage value of event 1507 in the unknown samples is estimated by dividing the GM copy number by the copy number of the maize reference gene *hmga* and by multiplying by 100 (GM%=1507/*hmga* x 100).

For detailed information on the preparation of the respective standard curve calibration samples please refer to the protocols of the validated methods at http://gmo-crl.jrc.ec.europa.eu/.

5.5 Deviations from the validated methods

The applicant doubled the sulforhodamine concentration specified in GA21 validated method in order to get passive reference fluorescence values above the background for the ABI 7900 HT system used in the testing. Moreover, in the method for 1507 the applicant replaced ROX produced by Applied Biosystems with ROX produced by Invitrogen although a comparable final concentration was maintained.

5.6 Results

The verifications performed by the EU-RL GMFF for the Bt11, MIR162, 1507 and GA21 methods are presented in Tables 2, 3, 4 and 5 respectively.
The values of the slopes of the standard curves, from which the PCR efficiency is calculated using the formula $[10^{(-1/slope)} - 1] \times 100$, and of the R^2 (expressing the linearity of the regression) are reported for all PCR systems in the eight runs.

Table 2. Values of standard curve slope, PCR efficiency and linearity (R^2) for the Bt11 method on Bt11 x MIR162 x 1507 x GA21.

Run	Bt11		
	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.60	90	1.00
2	-3.29	101	1.00
3	-3.51	93	1.00
4	-3.36	99	1.00
5	-3.30	101	1.00
6	-3.33	100	1.00
7	-3.50	93	1.00
8	-3.40	97	1.00
Mean	-3.41	97	1.00

Table 3. Values of standard curve slope, PCR efficiency and linearity (R^2) for the MIR162 method on Bt11 x MIR162 x 1507 x GA21.

Run	MIR162		
	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.40	97	1.00
2	-3.37	98	1.00
3	-3.24	104	1.00
4	-3.17	107	0.99
5	-3.42	96	1.00
6	-3.27	102	0.99
7	-3.19	106	0.99
8	-3.30	101	1.00
Mean	-3.30	101	1.00

Table 4. Values of standard curve slope, PCR efficiency and linearity (R^2) for the 1507 method on Bt11 x MIR162 x 1507 x GA21.

Run	1507			*hmga*		
	Slope	PCR Efficiency (%)	Linearity (R^2)	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.18	106	1.00	-3.22	105	0.99
2	-3.08	111	1.00	-3.26	103	1.00
3	-3.28	102	0.98	-3.32	100	1.00
4	-3.01	115	0.98	-3.21	105	1.00
5	-3.22	104	0.98	-3.15	108	1.00
6	-3.16	107	0.99	-3.21	105	1.00
7	-3.15	108	0.99	-3.27	102	1.00
8	-3.08	111	0.99	-3.30	101	1.00
Mean	-3.15	108	0.99	-3.24	103	1.00

Table 5. Values of standard curve slope, PCR efficiency and linearity (R^2) for the GA21 method on Bt11 x MIR162 x 1507 x GA21.

Run	GA21		
	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.54	91	1.00
2	-3.50	93	1.00
3	-3.58	90	1.00
4	-3.40	97	1.00
5	-3.56	91	1.00
6	-3.41	96	1.00
7	-3.49	93	1.00
8	-3.46	95	1.00
Mean	-3.49	93	1.00

The mean PCR efficiencies of the calibration curves for each of the four event-specific methods were above 90% (97% for Bt11, 101% for MIR162, 108% for 1507 and 93% for GA21, respectively). The linearity of the methods (R^2) was between 0.99 and 1.00 for all four methods. The data presented in Tables 2, 3, 4 and 5 confirm the appropriate performance characteristics of the four methods when tested on Bt11 x MIR162 x 1507 x GA21 in terms of PCR efficiency and linearity.

The EU-RL GMFF also assessed the values of trueness and precision expressed as relative repeatability standard deviation (RSDr %) of the four methods applied to samples of DNA extracted from Bt11 x MIR162 x 1507 x GA21.

Tables 6 to 9 report the trueness and precision for each GM level for each of the four methods.

Table 6. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the Bt11 method applied to Bt11 x MIR162 x 1507 x GA21 maize DNA.

Bt11					
Unknown sample GM%	**Expected value (GMO%)**				
	0.09	**0.4**	**0.9**	**5.0**	**8.0**
Mean	0.09	0.40	0.87	4.9	8.0
SD	0.02	0.04	0.11	0.62	1.24
RSD_r (%)	19	9.8	12	13	16
Bias (%)	3.6	0.5	-3.7	-1.4	-0.03

Table 7. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the MIR162 method applied to Bt11 x MIR162 x 1507 x GA21 maize DNA.

MIR162					
Unknown sample GM%	**Expected value (GMO%)**				
	0.1	**0.4**	**0.9**	**2.0**	**5.0**
Mean	0.09	0.36	0.83	2.0	4.7
SD	0.01	0.04	0.12	0.45	0.46
RSD_r (%)	11	9.9	15	23	9.7
Bias (%)	-8.8	-11	-7.3	0.03	-5.2

Table 8. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the 1507 method applied to Bt11 x MIR162 x 1507 x GA21 maize DNA.

1507					
Unknown sample GM%	**Expected value (GMO%)**				
	0.1	**0.5**	**0.9**	**2.0**	**5.0**
Mean	0.09	0.42	0.87	1.7	5.4
SD	0.01	0.07	0.08	0.39	0.50
RSD_r (%)	9.7	17	9.6	23	9.2
Bias (%)	-6.9	-16	-2.8	-15	8.7

Table 9. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the GA21 method applied to Bt11 x MIR162 x 1507 x GA21 maize DNA.

GA21					
Unknown sample GM%	**Expected value (GMO%)**				
	0.09	**0.5**	**0.9**	**5.0**	**8.0**
Mean	0.09	0.47	0.83	4.5	7.5
SD	0.01	0.02	0.04	0.16	0.21
RSD_r (%)	6.8	4.6	4.8	3.6	2.8
Bias (%)	-1.9	-5.6	-8.2	-9.6	-6.4

The trueness of the method is estimated using the measurements of the method bias for each GM level. According to the ENGL acceptance criteria and method performance requirements, the trueness of the method, measured as bias from the accepted value, should be ± 25% across the entire dynamic range. As shown in Tables 6, 7, 8 and 9, the values range from -3.7% to 3.6% for Bt11, from -11% to 0.03% for MIR162, from -16% to 8.7% for 1507 and from -9.6% to -1.9% for GA21. Therefore, the four methods satisfy the above mentioned requirement throughout their respective dynamic ranges.

Tables 6, 7, 8 and 9 further document the relative repeatability standard deviation (RSD_r) as estimated for each GM level. As indicated by the ENGL, the EU-RL GMFF requires RSD_r values to be below 25%. As it can be observed from Tables 6 to 9, the values range between 9.8% and 19% for Bt11, between 9.7% and 23% for MIR162, between 9.2% and 23% for 1507 and between 2.8% and 6.8% for GA21. Therefore, the four methods satisfy this requirement throughout their respective dynamic ranges.

6. Comparison of method performance on Bt11 x MIR162 x 1507 x GA21 and on the single events

An indicative comparison of the performance of the four methods applied to Bt11 x MIR162 x 1507 x GA21 maize and on the single events is shown in Tables 10, 11, 12 and 13. The performance of the methods on the single lines was previously validated through international collaborative trials (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx).

The comparison of data generated in different testing conditions and different times is intended to be only of qualitative nature; differences in the figures reported are not necessarily statistically significant.

Table 10. Trueness (bias %) and relative repeatability standard deviation (RSD_r %) of the Bt11 detection method applied to Bt11 x MIR162 x 1507 x GA21 and to event Bt11.

Trueness and repeatability of Bt11 quantification on Bt11 x MIR162 x 1507 x GA21			**Trueness and repeatability of Bt11 quantification on single event Bt11***		
GM%	**Bias (%)**	**RSD_r (%)**	**GM%**	**Bias (%)**	**RSD_r (%)**
0.09	3.6	19	**0.09**	2.2	17
0.4	0.5	9.8	**0.4**	-1.9	13
0.9	-3.7	12	**0.9**	1.8	11
5.0	-1.4	13	**5.0**	-5.2	13
8.0	-0.03	16	**8.0**	-1.2	9.0

*method validated in inter-laboratory study (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx)

Table 11. Trueness (bias %) and relative repeatability standard deviation (RSDr %) of the MIR162 detection method applied to Bt11 x MIR162 x 1507 x GA21 and to event MIR162.

Trueness and repeatability of MIR162 quantification on Bt11 x MIR162 x 1507 x GA21			**Trueness and repeatability of MIR162 quantification on single event MIR162***		
GM%	**Bias (%)**	**RSD_r (%)**	**GM%**	**Bias (%)**	**RSD_r (%)**
0.1	-8.8	11	**0.1**	0.2	13
0.4	-11	9.9	**0.4**	2.9	12
0.9	-7.3	15	**0.9**	-1.7	12
2.0	0.03	23	**2.0**	1.4	10
5.0	-5.2	9.7	**5.0**	4.3	8

*method validated in inter-laboratory study (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx)

Table 12. Trueness (bias %) and relative repeatability standard deviation (RSD_r %) of the 1507 detection method applied to Bt11 x MIR162 x 1507 x GA21 and to event 1507.

Trueness and repeatability of 1507 quantification on Bt11 x MIR162 x 1507 x GA21			**Trueness and repeatability of 1507 quantification on single event 1507***		
GM%	**Bias (%)**	**RSD_r (%)**	**GM%**	**Bias (%)**	**RSD_r (%)**
0.1	-6.9	9.7	**0.1**	6.0	18
0.5	-16	17	**0.5**	-4.0	12
0.9	-2.8	9.6	**0.9**	3.7	7.7
2.0	-15	23	**2.0**	-1.7	8.5
5.0	8.7	9.2	**5.0**	8.4	14

*method validated in inter-laboratory study (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx)

Table 13. Trueness (bias %) and relative repeatability standard deviation (RSD_r %) of the GA21 detection method applied to Bt11 x MIR162 x 1507 x GA21 and to event GA21.

Trueness and repeatability of GA21 quantification on Bt11 x MIR162 x 1507 x GA21			**Trueness and repeatability of GA21 quantification on single event GA21***		
GM%	**Bias (%)**	**RSD_r (%)**	**GM%**	**Bias (%)**	**RSD_r (%)**
0.09	-1.9	6.8	**0.09**	-8.7	23
0.5	-5.6	4.6	**0.5**	0.8	17
0.9	-8.2	4.8	**0.9**	1.6	20
5.0	-9.6	3.6	**5.0**	-5.6	20
8.0	-6.4	2.8	**8.0**	-8.5	17

*method validated in inter-laboratory study (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx)

The trueness of the four event-specific methods when applied to Bt11 x MIR162 x 1507 x GA21 maize is within the acceptance range set by ENGL (± 25%) for the whole dynamic ranges studied.

The relative repeatability standard deviation (RSD_r %) of the four event-specific methods when applied to Bt11 x MIR162 x 1507 x GA21 maize are below the ENGL acceptance level established at maximum 25%.

The data presented in Tables 10, 11, 12 and 13 show that the methods perform according to the ENGL performance criteria when applied to DNA extracted from the maize single events Bt11, MIR162, 1507 and GA21 and to the DNA extracted from Bt11 x MIR162 x 1507 x GA21 maize.

7. Conclusions

The performance of the four event-specific methods for the detection and quantification of events Bt11, MIR162, 1507, GA21, when applied to DNA extracted from Bt11 x MIR162 x 1507 x GA21 maize, meets the ENGL performance criteria (ENGL), as assessed on the control samples provided by the applicant.

Indeed, the EU-RL GMFF verification has demonstrated that the PCR efficiency, linearity, trueness and repeatability of the four methods were within the limits established by the ENGL.

In conclusion, the verification study confirmed that the four methods are capable to detect, identify and quantify each of the GM events when applied to genomic DNA of suitable quality, extracted from Bt11 x MIR162 x 1507 x GA21 maize.

Therefore these methods, developed and validated to detect and quantify the single events, can be equally applied to the quantification of the respective events combined in Bt11 x MIR162 x 1507 x GA21 maize.

8. References

1. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance). OJ L 268, 18.10.2003, p. 1–23.

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Title: Report on the Verification of the Performance of Bt11, MIR162, 1507 and GA21 Event-specific Methods on the Bt11 x MIR162 x 1507 x GA21 Maize Using Real-time PCR

Author(s): Elena Nardini, Maria Grazia Sacco, Marco Mazzara, Joachim Kreysa

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Abstract

An application was submitted by Syngenta Crop Protection AG to request the authorisation of genetically modified Bt11 x MIR162 x 1507 x GA21 maize (tolerant to herbicides containing glufosinate ammonium and glyphosate and resistant to important lepidoptera maize pests) and all sub-combinations of the individual events as present in the segregating progeny, for food and feed uses, and import and processing, in accordance with articles 5 and 17 of Regulation (EC) N° 1829/2003 GM Food and GM Feed (1). The unique identifier assigned to Bt11 x MIR162 x 1507 x GA21 maize is SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØØ21-9.

Bt11 x MIR162 x 1507 x GA21 maize has been obtained by conventional crossing between four genetically modified maize events: Bt11, MIR162, 1507 and GA21. No new genetic modification was used for the development of Bt11 x MIR162 x 1507 x GA21 maize.

The EU-RL GMFF has previously validated individually, and declared fit for purpose, the detection methods for the single events Bt11, MIR162, 1507, GA21 and has published the corresponding reports http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx; therefore, in line with the approach defined by the ENGL (Annex 1, http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf) the EU-RL GMFF has carried out only an in-house verification of the performance of each validated method when applied to DNA extracted from Bt11 x MIR162 x 1507 x GA21.

The results of the in-house verification study were evaluated with reference to ENGL requirements and to the validation results on the individual events; as a result, the EU-RL GMFF concludes that the individual methods meet the ENGL criteria and can also be applied to Bt11 x MIR162 x 1507 x GA21 maize.

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