

3rd ISTA Proficiency Test on GMO Testing on Zea mays (MON810+T25)

Summary of Results

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1. AIM

The aim of this 3rd ISTA Proficiency Test on GMO Testing is to check the ability of individual laboratories to detect and, on a voluntary basis, to quantify the presence of GM seeds in samples of conventional seed of *Zea mays* L.

The object of data analysis is not to identify deviating laboratories but to compile the performances in the laboratories and to provide data for the laboratories' internal performance data base.

2. EXPERIMENTAL DESIGN

For this proficiency test three different maize seed lots were provided: a non GM, a MON810 GM and a T25 GM seed lot.

Each participating laboratory received a set of 12 maize samples. Samples in each set were given a random number so that, for example, positive samples due to MON810 and T25 sent to laboratory 1 could have the same or a different number as compared to those sent to laboratory 2. Each sample contained about 1500 seeds (determined by weight) and was labelled only with a random sample number and a laboratory number. Three samples were negative (no GM seeds added) and 9 samples were positive. The positive samples were made positive by adding seeds from the MON810 and T25 seed lot to negative seeds. The negative seeds were determined by weight whereas the positive seeds were counted and weighed. For three samples, 1 seed from the MON810 and 2 seeds from the T25 seed lot were added to approximately 1497 (362.3g) negative seeds so that the expected value for the GM content in these positive samples is 0.2% (Table 1). For three samples, 10 seed from the MON810 and 20 seeds from the T25 seed lot were added to approximately 1470 (355.7g) negative seeds so that the expected value for the GM content in these

positive samples is 2.0%. For three samples, 20 seed from the MON810 and 40 seeds from the T25 seed lot were added to 1440 (348.5g) negative seeds so that the expected value for the GM content in these positive samples is 4.0%.

To avoid cross contamination, the negative samples were prepared first, whereas the positive samples were prepared after sealing all negative bags.

2.1 Qualitative Test

Laboratories could use the method they thought appropriate for this test. The results for the qualitative test, i.e. a sample is positive or negative, had been submitted for each sample along with the sample identification number provided by ISTA. Participants were not expected to identify the events in the positive samples.

2.2 Quantification of GM Content in Positive Samples

Laboratories could do a quantification of the GM content in the positive samples by either a sub-sampling quantification or by a quantitative test (real time PCR).

2.2.1 Sub-sampling Quantification (Semi-quantitative Test)

The participants should report as a result of this test whether the GM content in the test sample was below-equal or above the 1%, the % of GM seeds and the testing plan, i.e. the number of sub-samples tested, the size of sub-samples (number of seeds), the number

of positive sub-samples per sample, the false positive and false negative rate which was used for calculation of results, and the decision rule (maximum number of positive samples to accept = 1%). The laboratories could use the method they thought appropriate for this test. The SEEDCALC programme was recommended to use for designing the testing plan (available on the ISTA Website).

2.2.2 Quantitative Test

This quantitative test is for checking the ability of the laboratories to quantify the GM content in a sample. The participants should report the quantitative estimated value of the GM content of the test sample (mean % of GM seeds) and classified whether the sample was negative or positive with a GM content below-equal or above 1%. The laboratories could use the method they thought appropriate.

3. RESULTS

Forty-eight laboratories out of 50 received the samples and 40 submitted their results (Figure 1). All 40 laboratories reported qualitative results that could be evaluated. Seven laboratories performed the sub-sampling testing strategy. Twenty-two laboratories reported quantitative results. Twenty laboratories reported values for the GM content of the seed samples whereas 2 laboratories did not submit values but performed the classification.

The identity of the individual laboratories is kept confidential.

Table 1: Overview of the spiking levels of the test samples:

| # of samples | A-C | D-F | G-I | K-M |
|---------------------|------|------|------|------|
| Spiking level | 0% | 0.2% | 2.0% | 4.0% |
| # of negative seeds | 1500 | 1497 | 1470 | 1440 |
| # of MON810 seeds | 0 | 1 | 10 | 20 |
| # of T25 seeds | 0 | 2 | 20 | 40 |

3.1 Descriptive Statistics of the Qualitative Results

Each laboratory reported for the individual sample whether this is a negative sample or a positive sample. There was no identification or quantification requested. So, for a given sample, the result reported by the laboratory can be either correct or false (Figure 2 and Table 2).

Out of the 40 laboratories:

- 33 laboratories reported results without any false results, all 12 tested samples were classified correctly. This are 81% of the laboratories.

- 96.1% of the 491¹ samples were reported correctly by the 40 laboratories.

- In total, 8 laboratories reported false results, 1 laboratory reported both, false positive results and false negative results, 5 laboratories only false negatives and 2 laboratories only false positives.

- 3 laboratories reported false positive results (between 1 and 2 out of the 3 negative samples (1/3) and 2/3) with a total number of 4 out of 122¹ negative samples tested. This are 7.5% of the laboratories and 3.3% of the negative samples.

- 6 laboratories reported false negative results (between 2/9 and 3/9) with a total number of 16 out of 369 positive samples tested. This are 15% of the laboratories and 4.3% of the positive samples.

- With respect to the spiking level, 6 laboratories reported false negative results with positive samples of 0.2% GM content. Between 1/3 and 3/3 samples were classified falsely as negative with a total number of 14 samples out of the 123 positive samples of 0.2% GM content. These are 15% of the laboratories and 11.4% of the 0.2% GM content samples.

- With respect to the spiking level, 0 labora-

tories reported false negative results with positive samples of 2% GM content.

- With respect to the spiking level, 2 laboratories reported false negative results with positive samples of 4% GM content. 1/3 sample was classified falsely as negative with a total number of 2 samples out of 123 positive samples of 4% GM content. These are 5% of the laboratories and 1.6% of the 4% GM content samples.

3.2 The Sub-sampling Quantification Results

Five laboratories reported for the individual

sample a value for the GM content of the samples. In some cases, due to the testing plan chosen for the testing, a value could not be calculated for the higher GM spiking level, 2% and 4% (see lab 21 and 24, Table 3).

Further, 5 laboratories reported as a result of this test whether the GM content is below-equal or above 1% GM content of the seed sample (Table 4). Two laboratories out of 5, which used this approach, classified all samples correctly. Three laboratories had difficulties in categorising the samples with a GM content of 2%. Between 1 and 3 samples were classified falsely.

¹ One sample was not analysed by a laboratory because it had been damaged during shipment.

Table 2: Number and percentage of all, negative and positive samples reported as false.

| | # of samples tested | # of samples reported as false | # of samples reported as false |
|------------------|---------------------|--------------------------------|--------------------------------|
| All samples | 491 ¹ | 19 | 3.9% |
| Negative samples | 122 ¹ | 4 | 3.3% |
| Positive samples | all | 369 | 4.3% |
| | 0.2% GM content | 123 | 11.4% |
| | 2% GM content | 123 | 0.0% |
| | 4% GM content | 123 | 1.6% |

Table 3: Percentage of GM content reported by the laboratories for the seed samples are given in the table below. The column letters (A to M) correspond to the column letters in Table 1 showing the sample numbers. All laboratories used sub-sampling quantification.

| | 0% | 0% | 0% | 0.2% | 0.2% | 0.2% | 2% | 2% | 2% | 4% | 4% | 4% |
|-----|----|----|----|------|------|-------|------|-------|-------|-------|-------|-------|
| Lab | A | B | C | D | E | F | G | H | I | K | L | M |
| 7 | 0 | 0 | 0 | 0.07 | 0.07 | 0.007 | 0.7 | 0.88 | 0.55 | 1.97 | 1.42 | 1.42 |
| 21 | 0 | 0 | 0 | 0.74 | 0.54 | 0.65 | >0.4 | >0.4 | >0.4 | >0.4 | >0.4 | >0.4 |
| 24 | 0 | 0 | 0 | 0.14 | 0.22 | 0.22 | 2.32 | >2.59 | >2.32 | >3.04 | >2.59 | >2.32 |
| 41 | 0 | 0 | 0 | 0.1 | 0.2 | 0.2 | 2 | 1.7 | 2 | 3.7 | 4.3 | 3.8 |
| 57 | 0 | 0 | 0 | 0.08 | 0.38 | 0.51 | 0.51 | 0.81 | 0.38 | 1.95 | 1.93 | 2.66 |

Table 4: The table shows the negative samples (column A to C, white), the samples with a GM content of <1% (0.2%, column D to F, light yellow) and of >1% (2% and 4%, column G to M, orange), respectively. If a laboratory reported that a sample with a GM content of 0.2% has a GM content >1% or that a sample with a GM content of 2% or 4% has a content <=1%, the cells are marked red. If a laboratory reported false positive or false negative results the cell is also marked red and pos and neg, respectively.

| Lab | A | B | C | D | E | F | G | H | I | K | L | M |
|-----|---|---|---|-----|---|-----|---|---|---|---|---|---|
| 7 | | | | | | | | | | | | |
| 57 | | | | | | | | | | | | |
| 47 | | | | neg | | neg | | | | | | |
| 38 | | | | | | | | | | | | |
| 41 | | | | | | | | | | | | |

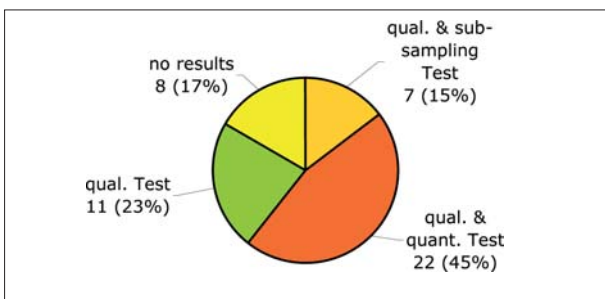


Figure 1: Performed tests by the participating laboratories.

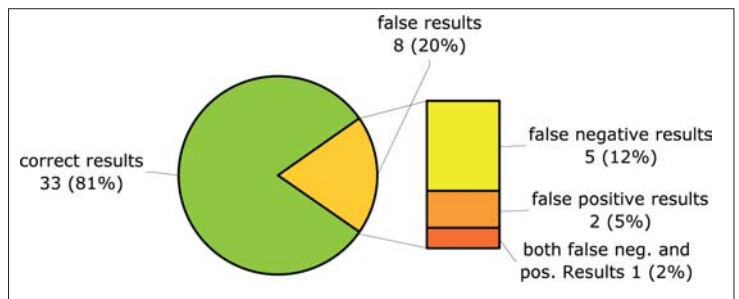


Figure 2: Percentage of laboratories reporting correct and false results.

3.3. The Quantitative Test Results

Twenty laboratories reported for the individual test sample the estimated value of the GM content as the percentage of e.g. haploid genomes, DNA or seed by mass (Table 5). Twenty-one laboratories reported for each sample if the value was below-equal or above 1% (Table 6).

Two laboratories, 15 and 35, did not report values for the GM content of the samples but categorised the samples. Eleven laboratories classified correctly. One laboratory reported one sample with the

0.2% GM content as above 1%. Ten laboratories reported the samples with the 2% GM content as below-equal 1%. Between 1/3 and 3/3 samples were reported falsely. No laboratory classified samples with a 4% GM content as below-equal 1%.

Acknowledgments

I would like to thank S. Zanetti and her staff for the support in preparation of the samples at the Agroscope, FAL Reckenholz, Switzerland. Thanks are also due to Bayer Crop Science for providing T25 GM seeds and Monsanto for MON810 GM seeds for the preparation of positive samples.

Table 5: Percentage of GM content reported by the laboratories for the seed samples are given in the table below. The column letters (A to M) correspond to the column letters in Table 1 showing the sample numbers.

| | 0% | 0% | 0% | 0.2% | 0.2% | 0.2% | 2% | 2% | 2% | 4% | 4% | 4% | | | |
|-----|-------|----|------|------|------|------|------|------|------|------|------|------|-----------------------------|--------------------|-----------------|
| Lab | A | B | C | D | E | F | G | H | I | K | L | M | screening or event-specific | # of flour samples | # of replicates |
| 4 | 0 | 0 | 0 | 0.12 | 0.12 | 0.12 | 0.82 | 0.87 | 1.43 | 1.67 | 1.92 | 2 | sc | 2 | 2 |
| 5 | 0 | 0 | 0 | 0.22 | 0.2 | 0.5 | 1.5 | 2 | 0.88 | 2.9 | 2.7 | 2.9 | sc | ? | ? |
| 11 | 0 | 0 | 0 | 0.2 | 0.3 | 0.1 | 1.7 | 1.6 | 2.3 | 3.4 | 3.1 | 3.6 | sc | 2 | 3 |
| 16 | 0 | 0 | 0 | 0.19 | 0.14 | 0.19 | 0.94 | 1.13 | 1.06 | 1.94 | 3.06 | 3.54 | sc | 2 | 3 |
| 17 | 0 | 0 | 0 | 0.32 | 0.43 | 0.17 | 3.01 | 4.64 | 3.97 | 5.25 | 6.31 | 5.25 | sc | 2 | 3 |
| 22 | <0.01 | <1 | <1 | <1 | <1 | 0.1 | 0.49 | 0.99 | 0.87 | 1.85 | 1.44 | 2.18 | sc | 3 | 2 |
| 23 | 0 | 0 | 0 | 0.3 | 0.3 | 0.3 | 2.9 | 2.9 | 3.2 | 5.7 | 6.5 | 6.4 | ? | 2 | 2 |
| 27 | 0 | 0 | 0 | 0.08 | 0.08 | 0.15 | 1.16 | 1.33 | 1.86 | 3.01 | 3.18 | 1.44 | sc/e | 2 | 2 |
| 28 | 0 | 0 | 0 | 0.27 | <0.1 | 0.23 | 0.97 | 1.47 | 0.72 | 3.87 | 7.88 | 2.46 | e | 2 | 1 |
| 31 | 0 | 0 | 0 | 0.15 | 0.15 | 0.12 | 1.03 | 1.1 | 1.04 | 3.18 | 2.38 | 2.34 | sc | 3 | 3 |
| 32 | 0 | 0 | 0 | 0.09 | 0.09 | 0.06 | 0.7 | 0.8 | 0.7 | 1.8 | 1.4 | 1.4 | sc | 2 | 1 |
| 33 | 0 | 0 | 0 | 0.18 | 0.3 | 0.3 | 3.91 | 3.07 | 2.02 | 6.22 | 5.79 | 5.27 | e | 2 | 1 |
| 36 | 0 | 0 | 0 | 0.3 | 0.2 | 0.2 | 2.4 | 2.3 | 2.7 | 6.2 | 5.2 | 5.1 | sc | 2 | 2 |
| 37 | 0 | 0 | 0 | 0.16 | 0.18 | 0.12 | 1.65 | 1.12 | 1.25 | 3 | 2.53 | 3.11 | sc | 2 | 3 |
| 39 | 0 | 0 | <0.2 | <0.2 | <0.2 | <0.2 | 0.6 | 0.7 | 0.8 | 1.4 | 1.8 | 1.6 | sc | 2 | 2 |
| 40 | 0 | 0 | 0 | 0.05 | 0.07 | 0.05 | 1 | 0.89 | 1.04 | 1.67 | 1.69 | 1.89 | sc | 2 | 4 |
| 44 | 0 | 0 | 0 | 0.14 | 0.13 | 0.14 | 1.57 | 1.34 | 1.59 | 3.27 | 3.01 | 3.17 | ? | 2 | 3 |
| 45 | 0 | 0 | 0 | 0.27 | 0.23 | 0.22 | 2.13 | 2.11 | 2.36 | 5.07 | 4.57 | 4.87 | e | 2 | 3 |
| 46 | 0 | 0 | 0 | 0.4 | 0.1 | 0.1 | 0.9 | 0.7 | 0.8 | 1.3 | 1.5 | 1.6 | e | 3 | 1 |
| 54 | 0 | 0 | 0 | 0.25 | 0.18 | 0.28 | 2.29 | 1.83 | 2.02 | 4.31 | 4.54 | 4.6 | ? | ? | ? |

Table 6: The table shows the negative samples (column A to C, white), the samples with a GM content of <1% (0.2%, column D to F, light yellow) and of >1% (2% and 4%, column G to M, orange), respectively. If a laboratory reported that a sample with a GM content of 0.2% has a GM content >1% or that a sample with a GM content of 2% or 4% has a content <=1%, the cells are marked red. If a laboratory reported false positive or false negative results the cell is also marked red and pos and neg, respectively.

| | 0% | 0% | 0% | 0.2% | 0.2% | 0.2% | 2% | 2% | 2% | 4% | 4% | 4% |
|-----|----|-----|-----|------|------|------|----|----|----|----|----|----|
| Lab | A | B | C | D | E | F | G | H | I | K | L | M |
| 22 | | pos | pos | | | | | | | | | |
| 39 | | | pos | | | | | | | | | |
| 31 | | | | | | | | | | | | |
| 32 | | | | | | | | | | | | |
| 46 | | | | | | | | | | | | |
| 28 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 40 | | | | | | | | | | | | |
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4th ISTA Proficiency Test on GMO Testing on *Glycine max* (L.) Merr. Summary of Results

By Bettina Kahlert, ISTA GMO Proficiency Test Working Group Leader, ISTA GMO Task Force

1. AIM

The aim of the proficiency test is to check the ability of individual laboratories to detect the presence or absence and to quantify the presence of GM seeds in samples of conventional seed of soybean *Glycine max* (L.) Merr.

The results of the proficiency test rounds are intended to be used by the laboratories for their internal performance evaluation. At this stage performance in the tests, based on voluntary participation, will remain without consequences for the participants. Once GMO testing will be included in the ISTA Accreditation Programme and has become part of a laboratory's intended scope of accreditation, the results from voluntary proficiency tests on GMO testing, maybe taken into account. This will in essence speed up the accreditation process for laboratories that did participate on a high performance level.

2. EXPERIMENTAL DESIGN

For this proficiency test two different soybean seed lots were provided: a non GM and a GTS 40-3-2 GM seed lot. For checking genetic purity, 30,000 seeds of the negative seed lot were tested and proved to be negative and 400 seeds from the positive seed lot were individually tested and proved to be positive. These tests were made in the laboratory of Norbert Leist, Staatl. Landw. Untersuchungs- und Forschungsanstalt Augustenberg, Germany. Based on the testing of the negative seed lot, the potential accidental content of positive seed is below 0.01% with 95% confidence.

Each participating laboratory received a set