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Position Paper on ISTA's view regarding the units for the reporting of quantitative results on presence of seeds with specified traits in conventional seed lots

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Position Paper on ISTA's view regarding the units for the reporting of quantitative results on presence of seeds with specified traits in conventional seed lots

This position paper is in accordance with the *'Position paper on ISTA's strategy regarding methods for the detection, identification and quantification of genetically modified seeds in conventional seed lots'*.

1. Introduction

Detection and quantification of seeds with specified trait(s) is a challenging area, with continuous technical developments and open questions.

There are different approaches on regulatory, trade and political aspects throughout the world.

ISTA supports a scientifically based approach where different methods are available for the detection, identification and quantification of seeds with specified trait(s) in conventional seed lots, as well as in lots of seeds with the specified trait(s).

Since 2002, ISTA organises worldwide proficiency tests (PTs) on GMO testing to support laboratories in their quality management and for the documentation of laboratory performance, which is a necessary requirement for the ISTA accreditation scheme. In these PTs units for reporting of quantitative results are linked with the analysis-method(s) used; consequently the units accepted for the reporting of quantitative results play a crucial role in the evaluation of laboratory performance.

2. Situation at the end of 2007 and outlook

2.1. Methods used for quantitative analysis

It is widely recognized that, among the various methodologies available, the real-time PCR based quantification is, for the time being, the most appropriate way for the quantification of adventitious presence (AP) of GMO in processed food and feed. However, alternative methods, based on the observation of individual plants/seeds (particularly by means of bioassays), or on the analysis of bulks (sub-sampling approach) and relying on protein-based or end-point PCR methods can also be used for the measurement of AP in seeds and grains.

Sub-sampling, which can be carried out with any kind of qualitative assay, such as endpoint PCR, is robust and efficient when the results are to be expressed in % number of seeds.

2.2. Units of expression of quantitative results

Different units have already been extensively used. They are mainly: % in number of seeds, % in mass of seeds, %DNA copies. In the ISTA PT program on GMO testing, all these three units are acceptable for the reporting of results by the participating laboratories. However, ambiguity appears when reporting in % DNA copies, depending on whether results are expressed per haploid genome or not, and on the zygosity of the certified reference material (CRM) used.

The % DNA copies should be understood as “the percentage of specified trait(s)-DNA copy numbers in relation to target-taxon specific DNA copy numbers calculated in terms of haploid genomes”. The laboratories reporting their results in % DNA copies should prepare the calibration-curves by haploid genome numbers for both GM and taxon PCR targets.

As the real-time PCR based quantification is relative and directly linked to the calibration curve used, the laboratories should report their results by % mass if they prepare their calibration curve using a certificated reference material (CRM) by mass fraction (g/kg) and without taking into account the zygosity of the CRM used. This is true even if the laboratories obtain the copy number by multiplying the DNA quantity by a constant (number of haploid genomes per ng DNA).Most of the certified reference material (CRM) in use for quantification of seeds with specified trait(s) is produced by composing mass fractions [g/kg].

A first CRM for MON810 on the basis of %DNA copies is available since December 2007.

2.3. Impact of biological factors (or genetic structures) of seeds or kernels on real-time PCR based quantification

It is well known that biological factors both of the CRM used for preparing the calibration curve and of the sample to be analysed can impact significantly the final quantitative results (Trifa and Zhang, 2004; Papazova et al., 2006; Holst-Jensen et al., 2006; Zhang et al., 2007).

In maize, for example, seed tissues have different ploidy levels (embryo=2n, endosperm=3n, tegument=2n). This influences the copy number of trait(s) in a seed depending on the trait(s) being carried by the mother (2 transgenic copies for the endosperm) or father (1 transgenic copy for the endosperm) parent of the hybrid. Since the endosperm accounts for 80-90% of total kernel weight, this tissue causes the major impact. Furthermore, between different cultivars, the relative contribution of these tissues is subjected to variations as well. These facts can influence quantification in %DNA copies. This issue is still an open question with research going on.

2.4. Detection and quantification of AP in seeds

Analysis on seed lots has different purposes. The requirements for trade and /or regulatory matters differ from country to country. A single method can not fulfil all needs at reasonable costs and time.

PCR and Bio-assays are seen in some countries as two well accepted techniques for quantification, one or the other being selected depending on the aim of the test and of the level of precision required.

Sub-sampling methods allow quantification based on presence/absence per pools of seeds. They also allow possibilities to detect and quantify the occurrence of single and/or stacked genes in a given seed lot via ISTA methodology developed by the statistics committee.

For the time being the detection and quantification of the occurrence of single and/or stacked genes cannot be achieved by real-time PCR on a single bulked sample.

Acceptance of more than one unit can avoid raising the difficult question of converting factors. A simple mechanical conversion between units is

complex or even impossible, due to the diversity of situations. Buyer/seller/competent authorities can select the appropriate unit and methodology that are best fitted for their particular purpose.

For non-experts it is difficult to interpret results on a seed lot in % DNA copies. For buying/selling seeds that will be grown to become plants, the unit % number of seed can be, in several circumstances, the most appropriate.

Real time PCR is a rather expensive approach: there are situations where simple and fast methods such as strip tests in the field are needed or preferred, rather than sending samples in a laboratory for PCR analysis.

There is a high demand for methods for the detection of unapproved trait(s). This goal can not always be achieved by real-time PCR. In some instances accessibility to the sequence or to an approved method to detect and quantify an unauthorised trait is a problem, even for competent authorities.

Micro-array chip technology might in a near future serve as a screening tool to detect many trait(s) at the same time at reasonable cost and effort.

3. ISTA's position

ISTA is of the opinion that any obligation to the use of a single technique could impair the capability to detect and quantify seeds with specified trait(s). The use of methods that have been proved to be fit for the purpose, repeatable and reproducible, shall be accepted. ISTA is of the opinion that in order to cope with the different aims and situations where quantification of seeds with specified trait(s) is required, more than one unit is acceptable for reporting quantitative results of seeds with specified traits in conventional seed lots. Three units are available and shall be accepted:

% DNA copies

% mass fraction

% number of seeds

When laboratories report their results by %DNA copies, the comments in 2.2 should be taken into account in order to avoid the ambiguity in the comparison of results among laboratories.

There is no generic conversion factor between "% number of seeds" and "% DNA copies". However, in certain cases with appropriate knowledge, specific conversion between the different units of expression is possible.

When testing sample(s) of seeds and expressing results for the seed lot, the lot sampling variability needs to be taken into account.

Different reliable ways (method + testing plan + decision rule) are available to define appropriate tests at reasonable cost and time.