GM and non GM supply chains: Their CO-EXistence and TRAceability

Outcomes of Co-Extra

New multiplexing tools for reliable analysis of GMOs

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To enforce the existing regulations on commercialisation and environmental release of genetically modified organisms (GMO), adequate tools for its detection, identification and quantification are required. The most accepted GMO detection methods are based on specific DNA sequence detection by means of polymerase chain reaction (PCR) techniques, able to detect even small amounts of transgene sequences in raw materials and processed foods. PCR assays can be used for screening purposes (e.g. targeting transgenic elements commonly used in GMOs), to detect junctions of contiguous transgenic elements, and to identify a GMO event (by targeting the junction regions between the insert and recipient plant genomic DNA or event-specific rearrangements). Additional amplification of a plant species specific gene is necessary as control. A number of these methods (including quantitative assays) are available that have been validated by official bodies or reference laboratories e.g. the EU Joint Research Laboratory.

The presence of GMO material on the market is increasing; and so is the number of GMOs approved worldwide (including stacked events) and in the pipeline. At the same time, the genetic elements introduced into new GMOs and the host plant species are becoming more diverse. This increases the cost and working power required for GMO analysis. In this context, the widely used single-target detection methods are not considered sufficient to fulfil the current and envisaged need for analysis. Consequently it is necessary to introduce new analytical technologies for reliable, low cost, high throughput, standardised GMO analysis.

The development of analytical methods and strategies for multiplex detection, identification and/or quantification of GMO has been a major priority within the Co-Extra project. The combination of two or more PCR assays in one single reaction (multiplexing) is not an easy strategy due to the interaction and competition between the reaction components and products; and the combination of high numbers of reactions is at the expense of the sensitivity and uniform amplification of the different targets. Numerous duplex reactions –often targeting the transgenic sequence and a control or two major screening elements- are available, and so are oligoplex PCRs targeting a limited number of sequences. Above a certain degree of multiplexing, novel strategies (as compared to agarose gels and real-time PCR chemistries) are required to identify the reaction products. Examples are capillary gel electrophoresis (CGE) based and hybridization in array format technologies. These approaches can allow simultaneous detection of the products of a number of oligoplex PCRs performed in parallel, resulting in higher multiplexing level, throughput and lower cost.

The use of oligoplex (and multiplex) PCR assays is foreseen as a first analysis that allows qualitative detection of GMO(s) in a sample. It can be then complemented with singleplex, validated (if possible), specific real-time PCR assays for GMO quantification when required. However, some oligoplex approaches incorporate special adaptations to achieve (semi)quantitative results, such as quantitative competitive PCR or the use of bipartite primers.

The limitations of PCR for achieving high-grade multiplexing are one of the reasons that prompted the study of alternative, non PCR-based approaches that could potentially allow multiplexing. Examples are the loop-mediated isothermal amplification (LAMP) strategy coupled to bioluminescent assay for real-time (BART) detection system; and the NASBA (nucleic acids based amplification) implemented microarray analysis (NAIMA). Near infrared (NIR) spectra of individual kernels can allow GMO detection by comparison to pre-defined patterns.

New multiplex approaches have recently been designed for simultaneous detection of very high numbers of target sequences: these can be considered as high-grade multiplex approaches. Some of them include a first ligation step that is dependent upon hybridization of two oligonucleotide sequences to the target, subsequent amplification (with universal primers) and detection by hybridization on array support. Examples are a SNPlex method (for single nucleotide polymorphisms detection) designed to identify GMO targets; and a system based on padlock probes (circularizable probes). In a very different approach, a whole genome amplification (WGA) technique can allow producing large amounts of genomic DNA of the sample that are then hybridized to special probes in microtiter plates or microarrays to detect GMO targets (e.g. high density tilling microarray).

This presentation will give an overview of the new technologies for multiplex analysis of GMO developed within the Co-Extra project; and will also discuss on aspects such as the need and problems of validation of multiplex methods; or the difficulties in coupling a high level of multiplexing with cost-effectiveness (including the devices required) and simplicity of the method.