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Development and Validation of a P-35S, T-nos, T-35S and P-FMV Tetraplex Real-time PCR Screening Method to Detect Regulatory Genes of Genetically Modified Organisms in Food

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Abstract: In routine analysis screening methods based on real-time PCR (polymerase chain reaction) are most commonly used for the detection of genetically modified (GM) plant material in food and feed. Screening tests are based on sequences frequently used for GM development, allowing the detection of a large number of GMOs (genetically modified organisms). Here, we describe the development and validation of a tetraplex real-time PCR screening assay comprising detection systems for the regulatory genes Cauliflower Mosaic Virus 35S promoter, *Agrobacterium tumefaciens* nos terminator, Cauliflower Mosaic Virus 35S terminator and Figwort Mosaic Virus 34S promoter. Three of the four primer and probe combinations have already been published elsewhere, whereas primers and probe for the 35S terminator have been developed in-house. Adjustment of primer and probe concentrations revealed a high PCR sensitivity with insignificant physical cross-talk between the four detection channels. The sensitivity of each PCR-system is sufficient to detect a GMO concentration as low as 0.05% of the containing respective element. The specificity of the described tetraplex is high when tested on DNA from GM maize, soy, rapeseed and tomato. We also demonstrate the robustness of the system by inter-laboratory tests. In conclusion, this method provides a sensitive and reliable screening procedure for the detection of the most frequently used regulatory elements present in GM crops either authorised or unauthorised for food.

Keywords: GMO detection · P-35S · P-FMV · Screening · T-35S · Tetraplex real-time PCR · T-nos

Introduction

Every year an increasing number of different genetically modified (GM) plants are grown on commercial scale. This growing number of different genetically modified crops comes along with an increase in the diversity of genetic modifications in commercialised GMOs. Therefore, broad and sensitive testing methods are needed. In Europe, the routine analysis of food, feed and seeds are mostly based on PCR screening methods for the detection of authorised and not authorised GMO crops because DNA is more resistant to food processing than proteins. Real-time PCR has become state of the art in routine analysis because of the reduced risk of sample contamination and the possibility to quantify the analyte, at least in a semiquantitative manner. Among the currently proposed real-time PCR chemistry, Taqman™ seems to represent the most appropriate system regarding muliplexing capacities, ease of primer and probe design, as well as cost considerations.

PCR tests are generally divided in four categories: screening, construct-specific, event-specific and taxa reference systems for quantification. Screening tests are based on common sequences frequently used for GMO development such as promoters, terminators and genes of interest like cry encoding insecticidal proteins from *Bacillus thuringiensis* or sequences of genes conferring tolerances to herbicides or antibiotics.

In routine analysis most laboratories prefer to carry out initial screening PCRs targeting regulatory DNA elements such as the 35S Promoter of Cauliflower Mosaic virus or the nos terminator of *Agrobacterium tumefaciens*. In order to detect these two genes, singleplex real-time PCR systems were published.[1,2] The invention of multiplex real-time PCR systems was a remarkable step forward in terms of saving costs, time and template DNA of samples with very low content of DNA.[3–9] A critical aspect linked to multiplex real-time PCR assays is the increased possibility of cross-talk, which is an increase of fluorescence in a detection channel caused by a dye, that is not intended to be measured in the given detection channel. Hence, it is of particular importance to optimise the combination of reporter dyes in a multiplex assay.

This article describes the development and validation of a sensitive tetraplex real-time PCR for the simultaneous detection and semiquantitative estimation of the P-35S and T-nos which is extended for the detection of T-35S and P-FMV sequence, two regulatory elements often incorporated in GM plants. Positive screening results have to be verified using construct- and/or event-specific methods.

Materials and Methods

Materials

During the development of this method, the following reference materials have been used: Roundup Ready™ soya flour (RRS or GTS 40-3-2) from IRMM (Geel, Belgium) was purchased from Sigma-Aldrich, Buchs, Switzerland; T25 maize kernels were a gift from the Federal Food Safety and Veterinary Office, Bern, Switzerland; GT73 rapeseed kernels were

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