SCREENING OF GENETICALLY MODIFIED PLANTS BY DUPLEX PCR

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Two duplex polymerase chain reactions were developed for screening genetically modified plants. The certified reference materials as dried powders containing 0-5% Roundup Ready soybean and maize MON-810 were used for optimization and validation of PCR methods. The genomic DNAs were extracted by Qiagen DNeasy plant mini kit and analysed using species-specific and GMO-specific uniplex PCR. The PCR products were evaluated by agarose gel electrophoresis. Different combinations of the GMO-specific primers were verified for their suitability for duplex PCR. Two duplex PCRs were developed and optimized, that allow simultaneous detection of two common transgenic elements, such as: cauliflower mosaic virus (CaMV) 35S promoter and Agrobacterium tumefaciens nopaline synthase (NOS) terminator. The developed duplex PCRs are effective tools for rapid and cheap screening of transgenic plants.

INTRODUCTION

Recent developments in plant biotechnology have generated a large number of genetically modified plants [1]. The influence of the transgenic plants on the agriculture, ecology, nature and biodiversity has increased dramatically during last decade, thus causing growing demand for control and detection of genetically modified organisms (GMOs).

The monitoring of GMOs needs reliable GMO detection methods. DNA-based polymerase chain reaction is recognized as the most useful technique for analysis of genetically modified organisms [2, 3]. Qualitative detection aimed at screening purposes is the first important step in GMO analysis. Genetic control elements such as Cauliflower Mosaic virus 35S promoter (CAMV P35S) and the *Agrobacterium tumefaciens* NOS terminator are present in around 95% of currently commercialized GMO plants [4]. Correspondingly, screening methods targeting CAMV 35S promoter and NOS terminator are of great importance. Several PCR methods corresponding to GMO regulatory elements, such as CAMV 35S promoter and the NOS terminator have been developed and validated for screening transgenic products [4-8]. However, there is still growing demand for fast and cheap screening methods to meet challenges of detection increasing numbers of transgenic plants.

In this study simultaneous amplification technique by duplex PCR is described as reliable tool for qualitative screening of GMOs.

OBJECTIVES AND METHODS

Plant material. Soybean and Maize certified reference materials as dried powders were used as GMO standards for development and validation of GMO detection methods, while Roundup Ready soya and MON 810 maize were taken as GM varieties. Certified reference material (ERM-BF-410) of GM Soya bean powder set containing 0, 0.1%, 0.5%, 1%, 2% and 5% Roundup Ready and maize GMO Standard (ERM-BF-413) set for 0, 0.1%, 0.5%, 1%, 2% and 5% MON 810 were purchased commercially (Fluka, Sigma-Aldrich).

Genomic DNA extraction. The certified reference materials were obtained in dried powder form and used directly. DNeasy plant mini kit (Qiagen) was chosen as suitable procedure for DNA extraction based on the results of our previous studies [9-11]. Genomic DNAs were isolated and purified from 100 mg of the powdered samples. The quality and amount of the extracted DNAs were evaluated by agarose gel electrophoresis. The aliquots of 5 µl from each sample were analysed on a 1% agarose gel.

Oligonucleotide Primers. The available literature and GenBank databases were screened in order to identify species-specific and GMO-specific primer sequences. The PCR primer pairs (Table 1) were selected based on the published data [5, 8, 12]. The primers were synthesized and purified by MWG Biotech. They were diluted to a final concentration of 5 µM with bi-distilled water and stored at -20^{0}C until use.