Identification and quantification of three genetically modified insect resistant cotton lines using conventional and TaqMan real-time polymerase chain reaction methods.

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Abstract

As the genetically modified organisms (GMOs) labeling policies are issued in many countries, qualitative and quantitative polymerase chain reaction (PCR) techniques are increasingly used for the detection of genetically modified (GM) crops in foods. Qualitative PCR and TaqMan real-time quantitative PCR methods to detect and identify three varieties of insect resistant cottons, i.e., Mon531 cotton (Monsanto Co.) and GK19 and SGK321 cottons (Chinese Academy of Agricultural Sciences), which were approved for commercialization in China, were developed in this paper. Primer pairs specific to inserted DNAs, such as Cowpea trypsin inhibitor (CpTI) gene of SGK321 cotton and the specific junction DNA sequences containing partial Cry1A(c) gene and NOS terminator of Mon531, GK19, and SGK321 cotton varieties were designed to conduct the identified PCR assays. In conventional specific identified PCR assays, the limit of detection (LOD) was 0.05% for Mon531, GK19, or SGK321 in 100 ng of cotton genomic DNA for one reaction. Also, the multiplex PCR method for screening the three GM cottons was established, which could save time and cost in practical detection. Furthermore, a real-time quantitative PCR assay based on TaqMan chemistry for detection of insect resistant gene, Cry1A(c), was developed. This assay also featured the use of a standard plasmid as a reference molecule, which contained a transgene specific region of a reference molecule. When compared with a specific region of the transgene in cotton, GM cotton samples were detected with 10% accuracy, in terms of the detection limit and the relative standard deviation (RSD) of the error. The LOD of the assay was 1.0% in 100 ng of cotton genomic DNA. The results indicated that our established conventional and TaqMan real-time PCR assays were applicable to detect the three insect resistant cottons qualitatively and quantitatively.