Real-time PCR array as a Universal Platform for the GM crop detection and its Application in Identifying Unapproved GM crops in Japan

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Introduction

Many kinds of genetically modified organisms (GMOs) are already in practical use and, especially, the number of commercially available genetically modified (GM) crops is increasing rapidly. In Japan, a total of 76 GM events have been approved for open field cultivation or provision as food, feed, or ornamental plants as living modified organisms (LMOs) under the Act on the Conservation and Sustainable Use of Biological Diversity Through Regulations on the Use of LMOs (Cartagena Protocol domestic law) as of July 8, 2009. Additionally, a total of 98 GM events have been approved for feed under the Food Sanitation Act as of April 30, 2009. Under these circumstances, it is desirable to develop GM crop testing methods that are capable of collecting a lot of information at once. Simultaneous detection methods, such as multiple PCR methods, DNA chip analyses, and membrane hybridization methods, have been developed and reported for some GM crops. Although multiple PCR is one of the most efficient and easiest techniques for multiplex detection, the multiplex reaction is difficult to be applied in practical testing, and false-positive amplifications tend to occur more often than in the simplex reaction. In addition, the interaction between individual reactions in the multiplex system causes unstable testing results in cases in which there is a big gap between the copy numbers of the target DNAs. In the development of an analytical method for regulatory use with GMOs, a validation study among participating laboratories is required to evaluate the performance. Validation studies, however, tend to be time- and cost-consuming. Therefore, a single individual reaction into a validated multiplex reaction system may require substantial effort to reevaluate the whole system. This makes it difficult to supply suitable GM testing methods to testing laboratories in a flexible and improvised manner so that they will be ready to deal with the increasing number of approved GM crops.

In this study, we proposed real-time PCR array with TaqMan chemistry, i.e., 96-well PCR plate prepared with TaqMan probes in each well, as a universalplatform for GM crop detection. The high correlation with specificity and sensitivity of the developed system. We also explored the possibility of adapting this real-time PCR array for the control of unapproved GM crops. Additionally, in order to facilitate the assessment of unapproved GM crop contamination, we designed a Microsoft Excel spreadsheet application, Unapproved GMO Checker version 2.01, and make it available on the Internet.

Experimental procedure

Materials

Genomic DNAs were extracted twice in parallel from the GM and non-GM samples by DNeasy plant max kit (Qiagen). The DNA solutions were analyzed three times with the present system. The results summarized in the table below were completely matched with published information. Besides the results, no false positive amplification was observed in the assay results for non-GM wheat, barley, cotton, sugarbeet, and peas. We concluded the present system is applicable also for these crops.

Methods

In the present system, we can obtain the cycle threshold (CT) values dependent on the copy numbers of target DNAs. We evaluated the linearity of amplification for the representative seven reactions (for Bt11, E176, GA21, M810, P35S, TNOS and SSIIb detections) in the real-time PCR array. The high correlation between the copy numbers of target DNAs and CT values was clearly observed in all reactions. In our system, however, it is difficult to calculate the accurate copy number with calibration curves in each component reactions. Therefore, we can conclude we can use the present system for the semiquantitative analyses.

Specificity of detection

Genomic DNAs were extracted twice in parallel from the GM and non-GM samples by DNeasy plant max kit (Qiagen). The DNA solutions were analyzed three times with the present system. The results summarized in the table below were completely matched with published information. Besides the results, no false positive amplification was observed in the assay results for non-GM wheat, barley, cotton, sugarbeet, and peas. We concluded the present system is applicable also for these crops.

Sensitivity of detection

Simulated test samples containing GM samples at several concentrations were prepared. Basically, ground crop samples were mixed in ground non-GM samples at different mass fractions. Two DNA solutions for every simulated sample were analyzed five times each and the results were summarized in the table below. All GM samples at the 0.25% mixing level are detected. The yellow colored table elements are the representative results indicating the sensitivity of the component reactions.

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Assumption of unapproved GM crop contamination

We developed Unapproved GMO Checker version 3.01 as a spreadsheet application for the assumption of unapproved GM crop contamination. In the present investigation, unapproved GM crop events were defined as GM crops that have not been approved for open field cultivation or provision as food, feed, or ornamental plants under Cartagena Protocol domestic law in Japan. In the development of the application, unapproved GM crops were conceptually defined as (Unapproved GM crops = (Approved GM crops) - (Approved GM crops)). Approved GM crops could be selectively detected using GM event detections, while rDNA segment detections detected various kinds of GM crops ranging from approved to unapproved. Therefore, the assumption of unapproved GM crop contamination is achieved by comparing the results of rDNA segment detection with those of GM event detection in the real-time PCR array. The spread sheet application was freely available at http://www.fasmac.or.jp/gmo/index.html.

The present method does not necessarily promise the absolute detection of unapproved GM crops. Because crops constructed of completely unknown rDNA segments or rDNA segments with modified nucleotide sequences cannot be detected. Also, GM event detection does not completely cover all the approved GM crops as present. Furthermore, if approved and unapproved GM crops were mixed in a sample and both crops shared the same rDNA segments, the unapproved GM crop would be masked by the approved GM crop. If unapproved GM crop contamination is suspected, further analysis, such as sequencing of the rDNA flanking regions, may be required.

Reference


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