

Comparison of signal enhancement techniques using DNA microarrays for screening GM crops

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Abstract

For the qualification and quantification of genetically modified (GM) crops without PCR, one possible alternative method is the detection of DNA fragments synthesized by random primers by DNA microarrays. Here, we used four signal enhancement techniques adopted in protocols for model target preparation of DNA microarrays and evaluated the detectable copy numbers of the targets. A 100-fold higher detectable copy number of the target was achieved using a fluorescently labeled dendrimer agent with a lower background level than using Cy3-labeled target as the control. This level was estimated to be sufficient for the detection of a single copy gene in GM maize genomic DNA. This model experiment suggests that DNA microarrays will be able to detect introduced genes of GM crops without PCR.

Keywords : DNA microarray, genetically modified organism (GMO), signal enhancement

I Introduction

Statistical data on the worldwide area under cultivation with genetically modified (GM) crops showed it was less than five hectares in 1996, but the area has been increasing, and the acreage of GM crops was over 148,000,000 hectares in 2010¹⁾. The year 1996 was memorable in Japan in that the first three GM crops were introduced with genes for herbicide and harmful insect resistance, traits allowed as safe for food use according to Japanese guidelines for the safety assessment of foods derived from plants containing recombinant DNA. Since then, new GM crops have been commercially developed and more than one hundred GM crops have been authorized through a safety assessment for commercial use in Japan. In particular, in GM maize, two or more individual GM plants having different traits such as herbicide and harmful insect resistance have been hybridized using conventional breeding and many varieties of hybridized GM crops, called stacked GM crops, have been produced. In 2011, 20 GM

maize varieties produced by a single-gene introduction event were authorized as safe; 75 hybrids varieties hybridized in combination with these 20 GM maize varieties have completed safety assessment in Japan. The number of such stacked GM crops having two or more transgenes derived from hybridization of single-event GM varieties has increased²⁾, and the genetic structure of stacked GM crops is getting more complex as a result of multiple rounds of hybridization of single-event GM varieties.

Only GM crops authorized by a food and feed safety assessment can be imported and commercially distributed in most countries. In order to manage the risk regulation of GM crops to accept authorized ones but prohibit non-authorized ones on the market³⁾, detection methods have been required to identify individual GM crops. At the beginning of GM crop history, when the number of GM crop varieties was limited and single-event GM varieties but not hybridized varieties were commercially used, simple features that could be detected as proof of a GM crop were the introduced nucleic