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Journal of Cereal Science 43 (2006) 250–257

Journal of CEREAL SCIENCE

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Event-specific qualitative and quantitative PCR detection of MON863 maize based upon the 3′-transgene integration sequence

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Received 21 August 2005; received in revised form 16 October 2005; accepted 22 October 2005

Abstract

An event-specific detection method was developed based on the flanking sequence of an exogenous integrant in the transgenic maize MON863 which contains *cry3Bb1* gene expressing a *Bacillus thuringiensis* Cry3Bb1 protein that is selectively toxic to a maize root worm pathogen. The 3′-integration junction between host plant DNA and integrated DNA of transgenic MON863 maize was isolated using thermal asymmetric interlaced (TAIL)-PCR. The event-specific primers and TaqMan probe were designed based upon the isolated 3′-integration junction sequence, and qualitative and quantitative PCR systems were established employing these designed primers and probe. In this system, the limit of detection of the qualitative PCR assay was estimated to be 40 initial haploid copies. The limit of quantitation of the quantitative PCR assay in authentic MON863 maize seeds was estimated to be approximately 80 haploid copies. GM MON863 contents were also quantified relative to endogenous maize starch synthase IIb (*zSSIIb*) gene DNA, and the results were expressed as the percentage of genetically modified MON863 maize DNA relative to the total content of maize DNA. All the results indicated that the established MON863 event-specific qualitative and quantitative PCR detection system based on the 3′-integration junction was reliable, sensitive and accurate.

Keywords: Qualitative and quantitative PCR; GMOs; Event-specific detection; MON863; TAIL-PCR; Transgene integration

1. Introduction

Several genetically modified plants (GMPs) have been approved for cultivation and commercialization in different countries in recent years. In the past nine years from 1996 to 2004, the global area of biotech crops increased more than 47-fold, from 1.7 million hectares in 1996 to 81.0 million hectares in 2004 (James, 2005). In 2004, there were 140 million hectares of maize grown globally, and 14% were genetically modified (GM) (James, 2005). Since, consumers are concerned about the use of genetically modified plants in food production, many food producers and food suppliers offer foods whose identity shows them to be from crops that are essentially free of GMOs (Holck et al., 2002). In this situation, labeling of GM foods has become more important and necessary and GMO labeling regulations are in force. Thus the European Union (EU) has set the maximum threshold for GM contamination of final food and feed products at 0.9% per ingredient (European Commission, 2003a,b). In Korea, the threshold is 3% (Ministry of Agriculture and Forestry, 2000) and in Japan, 5% (Matsuoka, 2001). In China, 17 kinds of GM products derived from five different plants are required to be labeled, these include maize seeds and oil, ketchup, soybean sauce and oil, rape and cotton seeds (Yang et al., 2005a). GMO have been successfully labeled to allow their detection using four novel endogenous reference genes: *SPS,* rice, (Ding et al., 2004); *LAT52,* tomato (Yang et al., 2005b); *Sad1,* cotton (Yang et al., 2005a) and *HMG I/Y*, rapeseed (Weng et al., 2005). Qualitative and quantitative real-

Abbreviations: GMOs, genetically modified organisms; GMPs, genetically modified plants; LOD, limit of detection; LOQ, limit of quantitation; PCR, polymerase chain reaction; SD, standard deviation; *zSSIIb*, maize starch synthase IIb.

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doi:10.1016/j.jcs.2005.10.003

time PCR detection methods for transgenic Huafan No. 1 tomato, GK19 cotton, SGK321 cotton and Mon531 cotton are now well-established in China (Yang et al., 2005c,d).

Four categories of PCR strategies have been developed for the detection of GM plants and their derived products: screening, gene-specific, construct-specific and event-specific PCR detection, but their ability to discriminate between GM- and non-GM derived DNA is variable (Anklam et al., 2002). Some screening methods risk yielding false positives due to contamination or infection by natural viruses (Wolf et al., 2000). Gene-specific and construct-specific methods may yield false positives when the modified gene and construct is used in several GMO and with variable numbers of copies. Moreover, these methods cannot distinguish between different GMO if the same construct has been integrated (Rønning et al., 2003). To overcome these specificity problems, a line or transformation event-specific PCR should be used. Already, some event-specific, quantitative methods have been introduced for GM crops, such as, Roundup Ready soybean (Berdal and Holst-Jensen, 2001; Taverniers et al., 2001; Terry and Harris, 2001), MON810 (Hernandez et al., 2003; Holck et al., 2002), Bt11 (Rønning et al., 2003), Starlink (Windels et al., 2003), NK603 maize (Huang and Pan, 2004), Mon1445 and Mon531 cotton (Yang et al., 2005e). At present no event-specific PCR detection methods for MON863 maize have been reported.

The transgenic MON863 maize developed by Monsanto Company was produced by biolistic transformation of the inbred line A634. The introduced DNA (Fig. 1A) contained the modified *cry3Bb1* gene from *B. thuringiensis* subsp. *kumamotoensis* under the control of the 4-*AS1* promoter (*CaMV 35S* promoter with four repeats of an activating sequence), plus the 5′ untranslated leader sequence of the wheat chlorophyll a/b binding protein (wt CAB leader) and the rice *actin* intron, and the transcription termination sequence was provided from the 3′ untranslated region of the wheat 17.3 kD heat shock protein (*tahsp17*). The *cry3Bb1* gene codes for a delta-endotoxin which binds to specific sites located on the lining of the midgut of susceptible coleopteran insect species and interferes with midgut function, thus acting as a potent and highly specific insecticide. The introduced DNA also contained a copy of the neomycin phosphotransferase II gene (*NPTII*) under the control of the *CaMV 35S* promoter and the 3′ untranslated termination sequence from the *Agrobacterium tumefaciens* nopaline synthase gene (*NOS*) (www.agbios.com).

In the spring of 2003, event MON863 for corn rootworm control was fully approved for food and feed use in the US, Canada and Japan. Its use would eliminate the use of

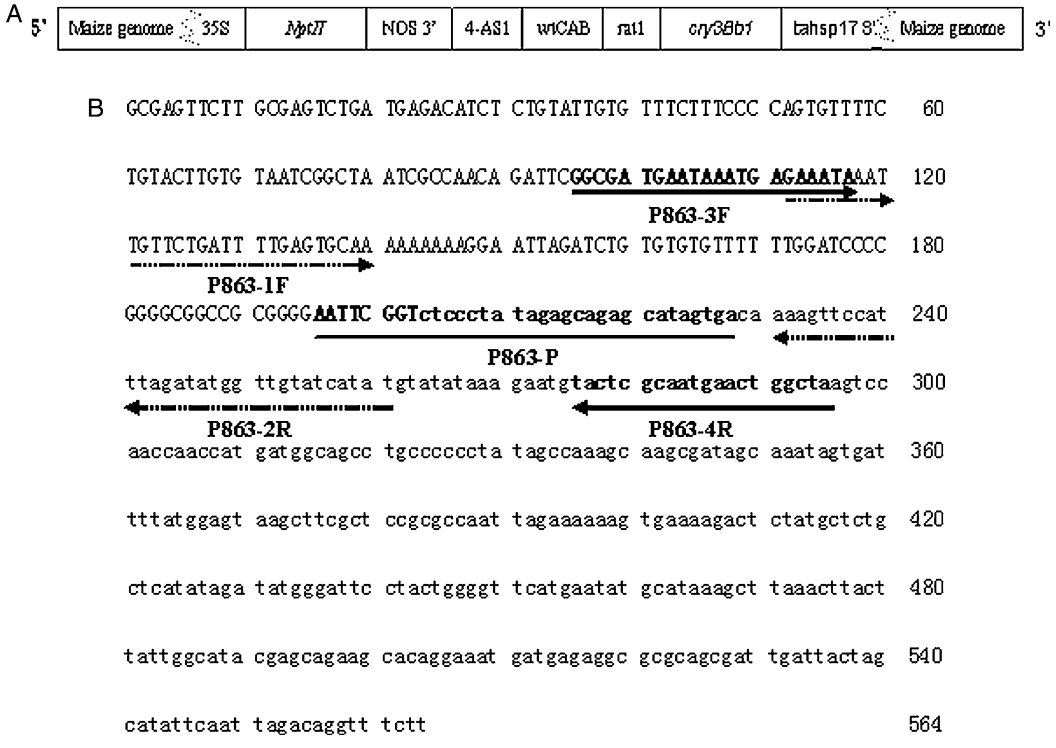

Fig. 1. Genome organization of event MON863 in transgenic maize. (A) Schematic representation of the construct PV-ZMIR13 introduced into MON863 maize. (B) The TAIL-PCR amplified 3′-junction sequence was cloned and sequenced. Capital letters represent the sequence from PV-ZMIR13 including the *tahsp17*, and lowercase letters show the flanking genomic sequence. The position of the P863-3F and P863-4R sense and antisense primers are highlighted in bold and indicated by a*rrows*. The position of the probe P863-P is *underlined*. *Dotted arrows* indicate the positions of primers (P863-1F/2R) used for quantitative detection. *35S* (the *CaMV 35S* promoter); *NPTII* (the neomycin phosphotransferase II encoding gene); *NOS* 3′ (the 3′ untranslated termination sequence from the *Agrobacterium tumefaciens* nopaline synthase gene); *4-AS1* (*CaMV 35S* promoter with four repeats of an activating sequence); wt CAB (the 5′ untranslated leader sequence of the wheat chlorophyll a/b binding protein); rat1 (rice *actin* intron); *cry3Bb1*(gene from *B. thuringiensis* subsp. *Kumamotoensis,* codes for a delta-endotoxin); *tahsp17* 3′ (3′untranslated region of the wheat 17.3 kD heat shock protein).