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Qualitative and Quantitative Event-Specific PCR Detection Methods for Oxy-235 Canola Based on the 3′ Integration Flanking Sequence

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As more genetically modified plant events are approved for commercialization worldwide, the event-specific PCR method has become the key method for genetically modified organism (GMO) identification and quantification. This study reveals the 3′ flanking sequence of the exogenous integration of Oxy-235 canola employing thermal asymmetric interlaced PCR (TAIL-PCR). On the basis of the revealed 3′ flanking sequence, PCR primers and TaqMan probe were designed and qualitative and quantitative PCR assays were established for Oxy-235 canola. The specificity and limits of detection (LOD) and quantification (LOQ) of these two PCR assays were validated to as low as 0.1% for the relative LOD of qualitative PCR assay; the absolute LOD and LOQ were low to 10 and 20 copies of canola genomic DNA in quantitative PCR assay, respectively. Furthermore, ideal quantified results were obtained in the practical canola sample detection. All of the results indicate that the developed qualitative and quantitative PCR methods based on the revealed 3′ integration flanking sequence are suitable for GM canola Oxy-235 identification and quantification.

KEYWORDS: Genetically modified organisms; event-specific; Oxy-235 canola; TAIL-PCR; real-time PCR

INTRODUCTION

In the past two decades, modern biotechniques have been widely developed and applied in agriculture, and more than 100 genetically modified (GM) plant events have been approved for commercialization worldwide. The global planted area of GMOs has reached 102 million hectares in 2006, and more than 22 countries planted GM crops (*1*). To strengthen the regulation of GMOs and protect the consumers' authority, more than 40 countries and areas have published a series of laws and statutes for GMO regulation and labeling. In the European Union (EU), the authorization and use of GM foods and feed are stipulated by the provisions in regulations (EC) 1829/2003 and (EC) 1830/2003, and the GM foods must be labeled at the threshold of 0.9% (*2*). China has also published a GMO regulated law and four matched statutes since 2001, and any GM plants and their derivates must apply for the authorization and labeling before import or sale in China (*3*).

For the purpose of GMO labeling, molecular detection techniques have been developed and used for GMO detection, such as the immunity methods for protein (ELISA, later flower strips) and the nucleic acids detection methods (qualitative and quantitative PCR). To date, the PCR technique for nucleic acids analysis has been the key method for GMO detection; four PCR detection systems were developed for GMO identification and quantification based on the four different kinds of target DNA fragments of exogenous integration, respectively, such as screen-, gene-, construct, and event-specific DNA fragments (*4, 5*), and several screen-, gene-, construct, and event-specific PCR methods for GMOs were established (*6–10*).

Canola is one of the most important oil crops, and it is planted worldwide, especially in China, India, and Canada. With the purpose of improving the output and decreasing the herbicide dosage in canola plants, more than 10 genetically modified herbicide-tolerant canola events, such as GT73, GT200, T45, and Oxy-235, have been developed and commercialized worldwide (*11*). In 2006, the planted area of GM canola occupied 5% of the global biotech crop area with 4.8 million hectares (*1*). In 2004, seven GM canola events (Ms1Rf1, Ms1Rf2, Ms8Rf3, T45, GT73, Topas 19/2, and Oxy-235) were approved for being imported as raw and processed materials in China (*12*), resulting in the requirement of developing these GM canola detection methods for the execution of GMO labeling. To date, several methods and Chinese national standards for GM canola detection have been published, such as the event-specific PCR detection of GT73and T45 canola, the national standards for Ms1Rf2, Ms1Rf1, and Ms8Rf3 detection (*13–16*). However, no paper for GM canola Oxy-235 detection has been reported. The GM bromoxynil-tolerant canola Oxy-235 event was developed by transforming the Westar canola variety (*Brassica napus* L.) with plasmid pRPABL-150a using the *Agrobacterium tumefaciens*-mediated transformed method, and a single copy

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Table 1. Primers and Probes Used in This Study

PCR system	name	sequence (5′−3′)	amplicon length (bp)	ref
TAIL-PCR	NOS-1F	CTGTTGAATTACGTTAAGCATG		this work
	NOS-2F	GCATGACGTTATTTATGAGATGGGTT		this work
	NOS-3F	ACATTTAATACGCGATAGAAAAC		this work
	AD2	NGTCGASWGANAWGAA		*19*
qualitativePCR	HMG-1F	GGTCGTCCTCCTAAGGCGAAAG	219	*13*
	HMG-2R	GCAACCAACAGGCACCATC		
	Oxy-1F	GTGGACCCTTGAGGAAACTG	324	this work
	Oxy-2R	CTGACGACAGTTCTGCTCCAT		
real-time PCR	HMG-3F	GGTCGTCCTCCTAAGGCGAAAG	99	*20*
	HMG-4R	CTTCTTCGGCGGTCGTCCAC		
	HMG-p	HEX-CGGAGCCACTCGGTGCCGCAACTT-TAMRA		
	Oxy-3F	CTAACTTTTGGTGTGATGATGCTGA	124	this work
	Oxy-4R	CGATAGATGGTGGTGTGAGTCTTG		
	Oxy-p	FAM AGCTGATGGCAAGTTAATCTCCCCGAAGTCG Dabcyl		

of T-DNA, containing the *bxn* gene, was integrated at a single site in canola event Oxy-235 (*17*).

In this study, we revealed the 3′-integration flanking sequence of Oxy-235 canola by thermal asymmetric interlaced PCR (TAIL-PCR) and established qualitative and quantitative PCR methods based on the revealed event-specific sequence.

MATERIALS AND METHODS

Materials and DNA Extraction. Genetically modified canola events (Ms8Rf3 and T45) were developed and supplied by Bayer Cropscience, GM canola events (Ms1Rf2 and Ms1Rf1) were developed by Aventis CropScience, and GM canola GT73 seeds were developed and supplied by Monsanto Co. Nontransgenic canola seeds were purchased from local markets in Shanghai, China. Plant genomic DNA was extracted and purified using a Plant DNA Mini-prep Kit (Shanghai Ruifeng Agrotech Co., Ltd., Shanghai, China) according to the manufacturer's manual. The quantity of DNA in the samples was calculated using absorbance measurements at 260 nm wavelength, and its copy number was calculated from the quantity of DNA and canola genomic DNA average size (*18*).

Oligonucleotide Primers and Probes. The random primer AD2 suitable for plant genome reported by Liu et al. (*19*) was used for the flanking sequence amplification in TAIL-PCR. The target-specific primers (NOS-1F, 2F, and 3F) of TAIL-PCR were designed on the basis of the NOS terminator sequence of Oxy-235 exogenous integration. The primers and probes of the qualitative and quantitative PCR assays for Oxy-235 were designed with Primer Express software version 2.0 (Applied Biosystems, Foster City, CA) according to the 3′-integration flanking sequence revealed from TAIL-PCR. The primer pair Oxy-1F/2R was used for qualitative detection of Oxy-235 canola. The primer pair Oxy-3F/4R and TaqMan probe Oxy-p were employed for the quantification of Oxy-235 event. The canola *HMG I/Y* gene was selected as endogenous reference gene; its primers (HMG-1F/2R,

Figure 1. Schematic diagram of the integrated heterologous DNA in GM canola event Oxy-235.

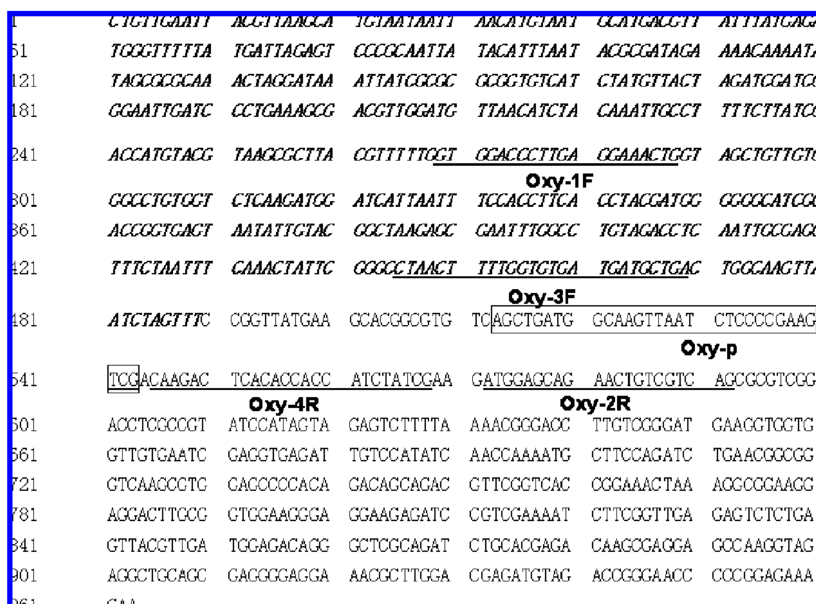

Figure 2. Revealed sequence between the 3′ exogenous integration and the canola genome of Oxy-235 canola. Italic boldface letters represent the sequence of partial NOS terminator and T-DNA right region, and normal letters show the flanking canola genomic sequence. Primers and probe used for qualitative and quantitative PCR amplification in **Table 1** are underlined and boxed.

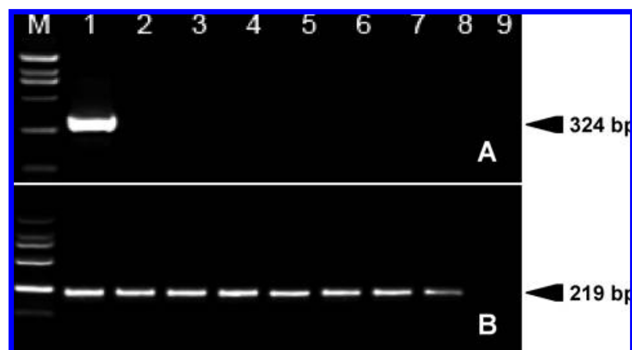

Figure 3. Three percent agarose gel electrophoresis of PCR products amplified with the Oxy-235 event-specific primers OXY-1F/2R and *HMG I/Y* gene primers HMG-1F/2R: (**A**) Oxy-235 event-specific PCR amplified results; (**B**) *HMG I/Y*-specific PCR amplified results. Lanes: 1–8, amplification of Oxy-235 canola, T45 canola, Ms8Rf3 canola, Ms1Rf1 canola, Ms1Rf2 canola, RT73 canola, Topas 19/2 canola, and non-GM canola, respectively; 9, NTC (no template control); M, DL2000 DNA marker with six different sizes of DNA fragments (2000, 1000, 750, 500, 250, and 100 bp).

Figure 4. Sensitivity test of Oxy-235 event-specific assay. PCR products were amplified from mixed Oxy-235 canola DNAs with different GM contents. Lanes: 1, NTC; 2–8, amplification of mixed GM maize DNAs with 10.0, 5.0, 3.0, 1.0, 0.1, 0.05, and 0.01% GM contents, respectively; M, DL2000 DNA marker with six different sizes of DNA fragments (2000, 1000, 750, 500, 250, and 100 bp).

HMG-3F/4R) and TaqMan probe (HMG-p) were used in this study (*20*). All of the primers and probes were synthesized by Invitrogen Co., Ltd. (Shanghai, China), and are listed in **Table 1**.

Determination of the 3′-Integration Flanking DNA Sequence Using TAIL-PCR. The molecular character of exogenous integration of Oxy-235 canola has been reported, and a single copy of T-DNA, containing the *bxn* gene, was integrated at a single site in Oxy-235 without any rearrangements (**Figure 1**) (*21*). For cloning the flanking sequence of exogenous integration, TAIL-PCR was used, and three nested target-specific primers were designed on the basis of the NOS terminator. TAIL-PCR reactions were performed with the following protocol. Briefly, the first TAIL-PCR was performed in a total volume of 50 μL containing 1× PCR buffer with $MgCl_2$ (TaKaRa Biotechnology Co., Ltd., Dalian, China), 400 μM dNTPs, 0.4 μM primer NOS-1F, 4 μM primer AD2, 2.5 units of *TaKaRa Ex Taq* HS DNA polymerase (TaKaRa Biotechnology Co., Ltd.), and 100 ng of Oxy-235 canola genomic DNA. The PCR program for the primary TAIL-PCR consisted of a denaturation step of 1 min at 94 °C followed by 1 min at 98 °C and the following amplification cycles: 5 cycles of 30 s at 94 °C, 1 min at 65 °C, and 2 min at 72 °C; 1 cycle of 30 s at 94 °C, 3 min at 25 °C, and 2 min at 72 °C, 15 cycles of 30 s at 94 °C, 1 min at 65 °C, 2 min at 72 °C, 30 s at 94 °C, 1 min at 65 °C, 2 min at 72 °C, 30 s at 94 °C, 1 min at 44 °C, and 2 min at 72 °C; and a final step of 10 min at 72 °C. Secondary TAIL-PCR amplification was carried out in a total volume of 50 μL containing 1× PCR buffer with $MgCl_2$, 400 μM dNTPs, 0.4 μM primer NOS-2F, 4 μM primer AD2, 2.5 units of *TaKaRa Ex Taq* HS DNA polymerase, and 1 μL of primary PCR products. The secondary TAIL-PCR program was 15 cycles of 30 s at 94 °C, 1 min at 65 °C, 2 min at 72 °C, 30 s at 94 °C, 1 min at 65 °C, 2 min at 72 °C, 30 s at 94 °C, 1 min at 44 °C, 2 min at 72 °C, and a final step of 10 min at 72 °C. Tertiary TAIL-PCR amplification was carried out in a total volume of 50 μL containing 1× PCR buffer with $MgCl_2$, 400 μM dNTPs, 0.4 μM primer NOS-3F, 4 μM primer AD2, 2.5 Uunits of *TaKaRa Ex Taq* HS DNA polymerase, and 1 μL of secondary PCR products. The tertiary TAIL-PCR program was 15 cycles of 30 s at 94 °C, 1 min at 65 °C, 2 min at 72 °C, 30 s at 94 °C, 1 min at 65 °C, 2 min at 72 °C, 30 s at 94 °C, 1 min at 44 °C, 2 min at 72 °C, and the last step of 10 min at 72 °C. All PCR reactions were carried out in a PTC-100 thermocycler (MJ Research, Waltham, MA). Parallel amplifications with nontransgenic canola genomic DNAs were carried out to identify Oxy-235 specific PCR products. The second and tertiary PCR amplification products were analyzed by 2% agarose gel electrophoresis with ethidium bromide staining. The amplified fragments of similar size in these two PCR amplifications were reclaimed and purified with a Gel Extraction Mini Kit (Watson Biotechnologies, Inc., Shanghai, China) and ligated into the pMD18-T vector (TaKaRa Biotechnology Co., Ltd.). The cloned DNA sequence was analyzed using the ABI PRISM 3730 Genetic Analyzer by Shanghai Invitrogen Co., Ltd. (Shanghai, China). Then, the sequencing DNA fragments were confirmed by the BLASTN program in the GenBank database.

Qualitative PCR Assay. In qualitative PCR assays, all reactions were carried out in 30 μL volume reactions, with 100 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 μM of each dNTP, 0.8 μM of each primer, and 1.5 units of *Taq* DNA polymerase (TaKaRa Biotechnology Co., Ltd.). The PCR amplifications were performed in a PTC-100 Thermocycler (MJ Research) with the following program: 1 step of 7 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 58 °C and 30 s at 72 °C, and the final step of 7 min at 72 °C. PCR amplified products were electrophoresed in 3% agarose gels for approximately 30 min at 100 V and stained with ethidium bromide for visualization. Each reaction of one test was repeated three times, each time with triple-parallels.

TaqMan Real-Time PCR Assay. Real-time PCR reactions were carried out with a fluorometric thermal cycler Rotor-Gene 3000 (Corbett Research) in final volumes of 25 μL. Fluorescence was monitored during every PCR cycle at the annealing step. Reactions contained the following ingredients: 1× PCR buffer, 400 μM each of dATP, dGTP, dCTP, and dUTP, 300 nM Oxy-235 event-specific primers or 600 nM *HMG I/Y* gene primers, 150 nM Oxy-235 event-specific and *HMG I/Y* gene probes, 1.5 units of *Taq* DNA polymerase, 6.5 mM $MgCl_2$, and 5 μL of template DNA samples. Real-time PCR reactions were carried out with the following program: 5 min at 95 °C, 45 cycles of 30 s at 95 °C, and 60 s at 60 °C. Data were analyzed with Rotor gene 3000 software version 6.0 (Corbett Research). All of the PCR reagents were purchased from Biocolor Biotechnology Co., Ltd. (Shanghai, China) except for primers and probes. All of the real-time PCRs of each test were performed with three replicates and three parallels for each replicate.

Validation of Oxy-235 Event-Specific Real-Time PCR Assay. To evaluate the Oxy-235 event-specific real-time PCR assay and GM Oxy-235 canola sample quantification, a series of Oxy-235 canola genomic DNA dilutions were used for the test of reproducibility and repeatability, determination of the limits of detection and quantification (LOD and LOQ), and construction of standard curves. Genomic DNA isolated from 100% Oxy-235 canola was serially diluted with 0.1× TE buffer to final concentrations equivalent to 20000, 2000, 200, 20, 4, and 2 copies of haploid genome/μL, considering 1.3 pg per haploid genome in the case of canola according to the description of Weng et al. (*20*). In each reaction, 5 μL of diluted DNA sample was added, and all reactions were repeated three times, each time with triple parallels for each template DNA.

RESULTS AND DISCUSSION

Cloning and Confirming the 3′-Integration Flanking Sequence of Oxy-235 Canola. Three nested target-specific primers were designed according to the NOS terminator sequence of the T-DNA inserted in Oxy-235 canola, and these three primers were employed with AD2 arbitrary primer for the 3′ flanking sequences by means of TAIL-PCR. The combination of the arbitrary primer AD2 and target-specific primer NOS-3F in tertiary amplification reactions amplified a unique fragment about 1000 bp in size. In the negative control using the

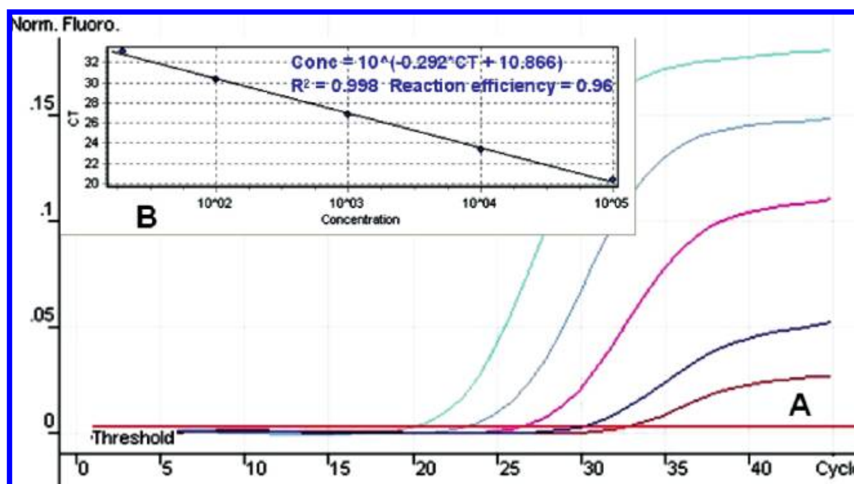

Figure 5. Amplification plots and standard curve for Oxy-235 event-specific real-time PCR assay: (**A**) amplification curves (serial DNA dilutions corresponding to 100000, 10000, 1000, 100, and 20 copies of Oxy-235 haploid genome per reaction) were generated for Oxy-235 canola quantification; (**B**) parameters of the regression line through data points are indicated within the standard curve.

Table 2. Repeatability and Reproducibility of the Developed Oxy-235 Event-Specific PCR Assays

target copies	Ct values			mean	mean copy no.	SD^r	SD^R	RSD^r (%)	RSD^R (%)
	1	2	3						
100000	20.17	20.12	20.15	20.15	96199.61	0.12	0.03	0.60	0.12
10000	23.41	23.43	23.32	23.39	10891.64	0.09	0.06	0.38	0.25
1000	26.78	26.74	26.83	26.78	1109.86	0.14	0.05	0.52	0.17
100	30.25	30.15	30.36	30.25	107.65	0.13	0.11	0.43	0.35
20	33.08	33.17	32.88	33.04	16.49	0.18	0.15	0.54	0.45

Table 3. Amplification Data Used To Determine the Absolute LOD and LOQ

template copies	signal rate (positive signals)	mean Ct value	SD	RSD (%)
100000	9/9	20.17	0.11	0.55
10000	9/9	23.36	0.07	0.30
1000	9/9	26.68	0.13	0.49
100	9/9	30.32	0.09	0.30
20	9/9	33.08	0.19	0.57
10	4/9	ND	ND	ND

nontransgenic canola genomic DNA as the template, no amplified fragment was observed in the parallel experiments. The unique TAIL-PCR amplicon about 1000 bp in size was cloned into the pMD18-T vector, and three individual clones were selected and sequenced. The result of sequence analysis showed that one 963 bp DNA fragment was obtained in TAIL-PCR. The nucleotide–nucleotide BLAST (BLASTN) results of the obtained 963 bp DNA sequence indicated that the 963 bp DNA sequence included two parts, one was a 489 bp sequence of NOS terminator and exogenous T-DNA right border region, and the other was a 474 bp *Brassica rapa* subsp. *pekinensis* BAC clone KBrB071P24 from 48524 to 48977 bp (GenBank accession no. AC189451), which demonstrated that the cloned 963 bp DNA fragment contained the conjunction of the exogenous T-DNA and canola genome sequence (**Figure 2**).

Oxy-235 Event-Specific Qualitative PCR Assay. When the 3′-integration flanking sequence of Oxy-235 canola was revealed, the qualitative specific primer pair OXY-1F/2R was designed and employed to develop the Oxy-235 event-specific qualitative PCR assay. The primers OXY-1F and OXY-2R were located at the NOS terminator and *Brassica napus* genome, respectively. The canola *HMG I/Y* gene was selected as endogenous reference gene, and the primer pair HMG-1F/2R was employed for canola identification. As expected, in the established Oxy-235 event-specific PCR assay, only a single 324 bp DNA fragment was obtained from Oxy-235 canola, and no fragment was detected in other GM canola events (Ms8Rf3, Ms1Rf2, Ms1Rf1, T45, Topas 19/2, and GT73), nontransgenic canola, and no template control (NTC) (**Figure 3A**). The target fragment of the canola *HMG I/Y* gene (219 bp) was detected in all GM canola and nontransgenic canola samples except NTC (**Figure 3B**). The above data confirmed that the obtained DNA fragment is unique for Oxy-235 canola and from the Oxy-235 event-specific region between the 3′ flanking sequence of exogenous integration and canola genomic DNA.

For one ideal qualitative PCR assay, high testing sensitivity is important and necessary, especially for the PCR assays of GMO detection. To test the LOD of the established Oxy-235 PCR assay, the mixed DNA samples were prepared from Oxy-235 canola event and non-GM canola at various levels, such as 0.01, 0.05, 0.1, 1.0, 3.0, 5.0, and 10.0%. The LOD test results showed that the 324 bp target DNA fragment was detected from all of the levels tested except the 0.01 and 0.05% levels, indicating that the LOD of the Oxy-235 PCR assay is 0.1% in 100 ng genomic DNA (**Figure 4**).

Oxy-235 Event-Specific Quantitative Real-Time PCR Assay. The TaqMan primers (OXY-3F/4R) and probe (OXY-p) based on the revealed 3′ flanking sequence were designed and employed to develop the event-specific real-time PCR assay for Oxy-235 canola. The primers OXY-3F and OXY-4R were located at the T-DNA right border region and *B. napus* genome, respectively. Probe OXY-p was located at the canola genomic DNA. For the total canola genome quantification, the *HMG I/Y* real-time PCR assay employing primers (HMG-1F/2R) and probe HMG-p was used according to the previous paper (*20*). For development of the Oxy-235 event-specific real-time PCR assay, the quantitative standard curve, repeatability and reproducibility, and limits of detection and quantitation (LOD and LOQ) were performed using a series of Oxy-235 canola genomic DNA dilutions as templates in real-time PCR assay.

Construction of Standard Curves. When the real-time PCR conditions were optimized, the quantitative standard curves of Oxy-235 event-specific real-time PCR assay was constructed by employing the Oxy-235 genomic DNAs of 100000, 10000, 1000, 100, and 20 copy haploid genomes. The real-time PCR amplified results showed that the PCR reaction efficiency was

Table 4. Quantitative Analysis of the Mixed Oxy-235 Canola Samples

sample	Ct mean 1	Ct mean 2	Ct mean 3	mean of all Ct values	SD	RSD (%)	calcd DNA amount (pg)	GM content (%)	bias (%)
				*HMG I/Y*PCR Assay					
S1 (5.0%)	21.54	21.48	21.52	21.51	0.03	0.15	99387.65		
S2 (3.0%)	21.55	21.53	21.59	21.56	0.03	0.14	96216.99		
S3 (1.0%)	21.61	21.49	21.46	21.52	0.08	0.37	98869.99		
				Oxy-235 Event-Specific PCR Assay					
S1 (5.0%)	25.11	25.12	25.15	25.13	0.02	0.08	4500.54	4.53	9.44
S2 (3.0%)	25.96	26.06	25.99	26.00	0.05	0.20	2498.06	2.60	13.46
S3 (1.0%)	27.17	27.19	27.11	27.16	0.04	0.15	1151.48	1.16	16.46

0.96, and the squared regression coefficient (R^2) of the standard curve was 0.998 (**Figure 5**). The high PCR reaction efficiency indicated that this real-time PCR assay was suitable for Oxy-235 genomic DNA quantification. The good linearity between DNA quantities and fluorescence values (Ct) indicated that these assays were well suitable for quantitative measurements.

Repeatability and Reproducibility. Repeatability and reproducibility of Oxy-235 real-time PCR assay were determined and calculated using the described Oxy-235 genomic DNA dilutions. The standard deviation (SD^r) and relative standard deviation (RSD^r) of repeatability were calculated according to the nine Ct values from three parallels and three replications; standard deviation (SD^R) and relative standard deviation (RSD^R) of reproducibility were calculated from the mean Ct values of each replication. In the test of repeatability and reproducibility of Oxy-235 real-time PCR assay, the SD^r values ranged from 0.09 to 0.18, and the RSD^r values ranged from 0.38 to 0.60%, The SD^R values ranged from 0.03 to 0.15, and the RSD^R values ranged from 0.12 to 0.45% (**Table 2**). The results of the repeatability and reproducibility tests indicated that the Oxy-235 event-specific quantitative PCR assay is stable and reliable in GM Oxy-235 canola quantification.

Limits of Detection and Quantification (LOD and LOQ). In real-time PCR, the LOD and LOQ are the most important parameters, referring to the lowest quantity of the target that can be reliably detected and quantified with a probability of ≥95% (*22, 23*). The absolute limit is the lowest number of initial template copies that can be detected and quantified. Relative limit refers to the lowest percentage of GMO relative to the species that can be detected and quantified, and practical limit is the functional limit of the sample during the practical analysis. To test the LOD and LOQ of the established Oxy-235 event-specific real-time PCR assay, a series of Oxy-235 genomic DNA dilutions (20000, 2000, 200, 20, 4 and 2 copies/μL) were prepared and tested three replicated times and three parallel reactions each time (**Table 3**). As expected, the ability to detect Oxy-235 canola decreased with decreasing genomic DNA copy numbers, and 10 copies of Oxy-235 genomic DNA have been detected four times in a total of nine repeated reactions. The results indicated that the LOD value was about 10 copies. The data also showed that the SD values of the nine reactions with the same template concentration increased with decreasing copy number. To obtain reliable quantification results under ideal conditions, approximately 20 initial template copies were required, and we concluded that the LOQ of the event specific real-time PCR assay was 20 copies of haploid genome.

GM Canola Oxy-235 Sample Quantification. To test the applicability of Oxy-235 real-time PCR assay for practical detection, three mixed Oxy-235 canola DNA samples, that is, S1, S2, and S3 with 5, 3, and 1%, respectively, were artificially prepared by mixing the pure Oxy-235 DNA with non-GM canola genomic DNA on a genome/genome basis and were used for quantification in developed Oxy-235 event-specific PCR assay. As shown in **Table 4**, the mean quantitative results of these three DNA samples (S1, S2, and S3) were 4.53, 2.60, and 1.16%, respectively. The quantified biases from true values of these three samples were 9.44, 13.46, and 16.46%, respectively. These results showed that the bias values of practical samples were lower than the acceptance threshold of 25% of one GMO detection method (*24*), indicating that the developed Oxy-235 real-time PCR assay is creditable and suitable for the quantification of GM Oxy-235 canola and its derivates.

Conclusion. In summary, we cloned the 3′ flanking sequence of exogenous integration of Oxy-235 canola and established the event-specific qualitative and quantitative PCR assays for the identification and quantification of Oxy-235 canola and its derivates. The LOD of qualitative PCR was 0.1% for Oxy-235 canola, and the LOD and LOQ of quantitative PCR assays were 10 and 20 copies of canola haploid genome, respectively. Furthermore, lower bias values were observed in practical sample quantification. These results demonstrated that the developed event-specific qualitative and quantitative PCR assays for Oxy-235 canola are easily and reliably applied to GM Oxy-235 canola and its derived products.

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