JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 7227–7232 7227
DOI:10.1021/jf901172d

The Gene *MT3-B* Can Differentiate Palm Oil from Other Oil Samples

Li Zhang,[‖] Gang Wu,[‖] Yuhua Wu, Yinglong Cao, Ling Xiao, and Changming Lu*

Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, No. 2 Xudong Second Road, Wuhan 430062, China. [‖] These two authors contributed equally to this paper.

The practice of blending cheap palm oil with more expensive oils is currently rampant owing to the increased global price of oil and the price gap between types of oils. This adulteration poses a serious threat to the trade of edible oil and negatively affects consumers. The aim of this study was to identify the presence of palm oil as an additive in more expensive oils using a PCR-based technique. A taxon-specific gene, *MT3-B*, was found by searching the GenBank database. *MT3-B* showed high oil palm (*Elaeis guineensis* Jacq.) specificity, low intraspecies variability, and a low copy number. On the basis of the *MT3-B* sequence, conventional and real-time PCR assays were established to detect palm oil contamination by amplifying an amplicon of 109 bp. The lowest copy number that the conventional PCR method could detect was five haploid copies; the limit of detection (LOD) for the real-time PCR assay was estimated to be five haploid copies. Experimental results demonstrated that the PCR-based methods were specific, sensitive, and reliable and could successfully detect the palm oil component of mixed oil samples.

KEYWORDS: *Elaeis guineensis*; oil palm; taxon-specific gene; *MT3-B*; conventional PCR; real-time PCR; oil detection

INTRODUCTION

Palm oil is an edible plant oil derived from the fruit and kernels (seeds) of the oil palm *Elaeis guineensis*. In recent years, the global production of palm oil has surpassed that of soybean oil, and it has become the world's leading edible oil (*1*). Because the palm oil is readily available and sold at low prices, adulteration of expensive oils, such as peanut oil and soybean oil, with cheap palm oil is currently rampant. This adulteration poses a serious threat to the trade of edible oil and is a health risk to consumers. There is an urgent need for the development of accurate, rapid, reliable, and cost-efficient identification techniques to protect consumers against this food fraud.

The standard qualitative methods for the detection of adulterants have been covered under IS: 548 (Part-II)-1976. Most of these methods are based on the development of a characteristic color or the appearance of turbidity/precipitate to determine the amount of a marker compound or compounds in a suspect material and subsequently compare the value(s) obtained with those established for equivalent material (*2*). Because the macro- and microcomponents of oils are semivolatile, a considerable number of high-performance liquid chromatography (HPLC) and gas chromatography (GC) methods have been developed to determine the composition of various oils (*3*). Although HPLC is the method of choice, it is limited by extensive sample cleanup, use of expensive solvents, and longer periods of column stabilization (*4*). In fact, the natural variability of chemical composition prevents the use of one discriminative marker for each type of oil. Compound-focused methods tend to lack sensitivity, to have unknown limits of detection, or to be tedious or time-consuming (*4*, *5*).

The introduction of Polymerase Chain Reaction (PCR) has provided a vast range of sensitive and reliable techniques for the control of food authenticity. Some have used universal primers to identify the cultivar origin of freshly pressed olive oil using the random amplified polymorphic DNA (RAPD-PCR) method (*6*), differentiate oils from different olive genotypes using simple sequence repeat (SSR) techniques (*7*), and trace the origin of olive oil by an amplified fragment length polymorphism (AFLP) technique (*8*). On the other hand, some researchers have developed species-specific primers for the detection of olive oil (*9*), peanut allergen residue (*10*), and wheat adulteration (*11*). These species-specific primers can distinguish one species from others, providing a valuable tool for adulteration identification. More recently, with the increased commercial cultivation of genetically modified crops, many species-specific genes have been exploited to meet the need of genetically modified organism (GMO) detection as endogenous reference genes in many crops. The species-specific genes reported include *10 kDa zein* (*12*), *invertaseI* (*13*), and *HMG-A* (*14*) for maize, *lectin* (*15*) for soybean, *BnAccg8* (*16*) and *HMG-I/Y* (*17*) for rapeseed, *LAT52* (*18*) for tomato, *RBE4* (*19*) and *SPS* (*20*, *21*) for rice, and *SadI* (*22*) for cotton. Corresponding PCR methods have been established. Previous studies have demonstrated that PCR methods are simple, fast, specific, and sensitive enough to detect product

*Author to whom correspondence should be addressed (telephone +86-27-86728186; fax +862786711573; e-mail changminglu@gmail.com).

Published on Web 07/23/2009

Table 1. Primers and Fluorogenic Probes Used for Conventional and Real-time PCR Systems

PCR system	orientation	name	sequence (5′−3′)	length	position	amplicon size
conventional and real-time	forward primer	*Mt1*	AGGGCAATTCCTAATGTATTGGAC	24	2556−2580	109
	reverse primer	*Mt2*	CGCAGTTAGAGCCGCATTT	19	2646−2664	
	probe	*Mtp*	CGAGGAAGTCGTTGAGGTGGCAGC	24	2603−2626	

fraud. However, no oil palm specific PCR method for distinguishing oil palm from other plant species has been reported yet.

In this study, we identify and validate an oil palm (*E. guineensis*)-specific gene and establish conventional and real-time PCR methods based on the taxon-specific gene sequence. This is the first time that species-specific PCR tests have been used to determine the presence of palm oil in costly oil.

MATERIALS AND METHODS

Materials. Two oil palm (*E. guineensis*) varieties were provided by Hainan University (Sanya, China) and Yunnan Animal Husbandry Bureau (Luxi, China). Leaf samples from different species, such as lady palm (*Rhapis excelsa*), finger palm (*Rhapis multifida*), pygmy date palm (*Phoenix roebelenii*), date palm (*Phoenix dactylifera*), triandra palm (*Areca triandra*), coconut palm (*Cocos nucifera*), areca palm (*Chrysalidocarpus lutescens*), and California fan palm (*Washingtonia filifera*), were kindly provided by Zhuhai Research Institute of Landscape Architecture Science (Zhuhai, China). Rapeseed (*Brassica napus*), soybean (*Glycine max*), peanut (*Arachis hypogaea*), sesame (*Sesamum indicum*), sunflower (*Helianthus annuus*), maize (*Zea mays*), rice (*Oryza sativa*), thale cress (*Arabidopsis thaliana*), and tobacco (*Nicotiana tabacum*) were collected by our own laboratory. Oil samples were purchased from a local grain and oil market in Wuhan, China.

DNA Extraction. Large-scale genomic DNA was isolated from 8 g of young leaves according to the protocol of Saghai-Maroof (*23*).

DNA extraction from oil samples was performed according to the following protocol. Thirty milliliters of petroleum ether was added to 30 mL of oil, and the mixed sample was uniformly suspended with an electromagnetic stirrer for 4 h. Then, 40 mL of PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) plus 80 μg of yeast tRNA (Sigma-Aldrich, St. Louis, MO) was added, and the stirring process was continued for another 3 h. The sample was centrifuged at 12000*g* for 15 min. The lower phase was transferred into a new 100 mL centrifuge tube, and an equal volume of isopropanol was added, together with a $^1/_{10}$ volume of 3 M sodium acetate. The solution was mixed well and incubated without shaking at 22 °C for 6 h. The solution was then centrifuged at 12000*g* for 25 min, the supernatant was discarded, and the precipitate was dissolved in 600 μL of 0.1 M TE (1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, pH 8.0) and transferred into a new 2 mL tube. An equal volume of chloroform/isoamyl alcohol (24:1) was then added. The sample was mixed well and centrifuged at 12000*g* for 10 min. The supernatant was pipetted into a new 2 mL tube, and an equal volume of isopropanol was added, together with a $^1/_{10}$ volume of 3 M sodium acetate and 25 μg of glycogen (Sigma-Aldrich). The sample was well mixed and incubated at −20 °C for 2 h. The sample was then centrifuged at 12000*g* for 25 min, the supernatant was discarded, and the precipitate was soaked in 600 μL of 75% ethanol. Centrifugation was carried out once more at 12000*g* for 10 min, the supernatant was carefully pipetted off, and the precipitate was dried at room temperature and dissolved in 30 μL of 0.1 M TE solution.

DNA Quantification. The purity and concentration of the extracted DNA was quantified using a Lambda 25 UV−vis spectrophotometer (Perkin-Elmer Applied Biosystems, Foster City, CA). Concentrations were further assessed by electrophoresis on a 1% agarose gel in 1× TAE (0.04 M Tris, pH 8.5, 0.001 M EDTA, and 0.02 M acetic acid) and stained with ethidium bromide. The UV-fluorescent emission was recorded and quantified using Quantity One software (Bio-Rad Laboratories Inc., Hercules, CA). One kilobase DNA ladder (Fermentas, Vilnius, Lithuania) was used as a DNA size marker.

Oligonucleotide Primers and Probes. Oligonucleotide primers and TaqMan fluorescent dye-labeled probes between the primers were designed using Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, CA), specifying an optimal melting temperature of about 60 °C for the primers and about 70 °C for probes. The 5′ ends of all TaqMan fluorescent probes were labeled with the fluorescent reporter 6-carboxyfluorescein (FAM), and the 3′ ends were labeled with the fluorescent quencher Black Hole Quencher 1 (BHQ1). All primers and fluorescent probes were synthesized and purified by Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. (Shanghai, China). The primer pair *Mt1*/*Mt2* was used in conventional PCR to amplify one DNA fragment of 109 bp and was also used in combination with the *Mtp* probe in real-time PCR. The sequences of the primers are shown in **Table 1**. The positions of the primers are depicted in **Figure 1**.

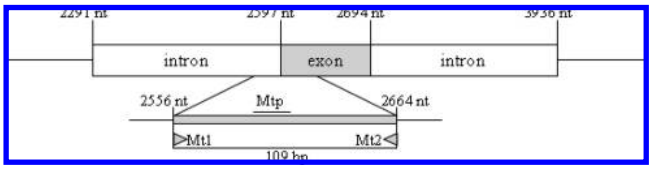

Figure 1. Schematic diagram illustrating the *MT3-B* gene and the positions of the PCR primers. Primers for conventional and real-time PCR analysis are indicated by arrowheads. The TaqMan probe used for the real-time assays is indicated by a solid bar.

PCR Conditions. Conventional PCR detection in this study was run in a GeneAmpTM PCR System 9700 (Applied Biosystems) using the optimized conventional PCR condition of 1× PCR buffer, 2.5 mM $MgCl_2$, 200 μM dNTP, 0.25 μM primers, 0.5 unit of DNA polymerase (Fermentas), and 1 μL of different DNA samples for a final reaction volume of 25 μL. The amplification reaction was run under the following cycle conditions: denaturation for 2 min at 94 °C; 40 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C; and terminal elongation for 2 min at 72 °C. The PCR products were size fractionated using gel electrophoresis on a 2.8% agarose gel in 1× TAE buffer and stained with ethidium bromide. The UV-fluorescent emission was recorded using Quantity One software (Bio-Rad Laboratories Inc.). A low molecular weight DNA ladder (New England BioLabs, Beverly, MA) was used as the DNA size marker.

Real-time PCR assays were carried out on a fluorometric thermal cycler (DNA Engine Opticon 2 Continuous Fluorescence Detector, MJ Research, Waltham, MA) in a final volume of 20 μL. Fluorescence signals were monitored and analyzed at the annealing step during every PCR cycle using MJ Opticon Monitor version 2.02 (Bio-Rad). The PCR reaction mixture for the TaqMan real-time PCR system contained the following reagents: 1× PCR buffer, 3.0 mM $MgCl_2$, 200 μM dNTP, 400 nM primers, 200 nM probe, and 0.75 unit of DNA polymerase (Takara, Shiga, Japan). All real-time PCR reactions were carried out using the same program: denaturation for 2 min at 95 °C, followed by 50 cycles of 15 s at 95 °C, 60 °C annealing and extending for 1 min, and a fluorescence measurement after the annealing and extending step. The PCR reaction mixture for the SYBR Green I real-time PCR system contained 1× SYBR Green I PCR Master Mix (Finnzymes, Espoo, Finland) and 400 nM primers. All SYBR Green I real-time PCR reactions were carried out using the same program: denaturation for 2 min at 95 °C, followed by 50 cycles of 15 s at 95 °C, and 60 °C annealing and extending for 1 min. Melting curves of the PCR products were analyzed after PCR with a linear temperature gradient from 60 to 95 °C.

Selection of a Suitable Target Gene for Specific Conventional and Real-time PCR Detection of Oil Palm. An oil palm-specific gene is necessary for the unequivocal detection of oil palm components in mixed oil samples. The target gene should be species specific, have a low copy number in the genome, and exhibit low heterogeneity among cultivars (*16*). To select such genes, different plant DNA databases such as NCBI were exhaustively searched for DNA sequences. A BLASTn analysis was

performed, and sequences showing high similarity with DNA from other plants were excluded.

According to the candidate gene sequences, three forward primers and three reverse primers for each gene were designed, and a total of nine primer sets were obtained by combining each forward primer with each reverse primer. None of these primers annealed to any plant DNA other than the *E. guineensis MT3-B* gene when analyzed with BLASTn search software against the GenBank database. The nine primer sets for each gene were first subjected to conventional PCR and SYBR Green I real-time PCR amplification using 0.2 ng of oil palm DNA.

Analysis of Mixed Known and Unknown Oil Samples. To test the applicability of the designed *MT3-B* gene-based PCR system, the developed real-time PCR system was used to detect the presence of the oil palm component from various oil samples, including the crude palm oil, crude soybean oil, and a known mixture with equal amounts of crude palm oil and crude soybean oil, as well as an oil sample purchased from a local grain and oil market. In the real-time PCR assays, 20 ng of oil palm leaf DNA was used as a positive control, 20 ng of yeast tRNA as a negative control, and water as a blank control.

RESULTS AND DISCUSSION

Selection of a Suitable Target Gene. After an exhaustive search for DNA sequences followed by a BLASTn analysis, two candidate genes showed very low similarity with DNA from other plants. Those were *MT3-B* and *PATE*, encoding *E. guineensis* metallothionein and palmitoyl-ACP thioesterase, respectively. *MT3-B* is known to bind metals in stable metal−thiolate clusters (*24*), and *PATE* catalyzes the hydrolysis of medium- and long-chain acyl-CoAs (*25*).

Through conventional PCR and SYBR Green I real-time PCR amplification with the nine primer sets for each gene, the most effective and reliable primer sets were selected to develop the oil palm detection method. The primer pair *Mt1*/*Mt2*, amplifying a fragment of 109 bp, was chosen for the *MT3-B* gene, and *PATE1*/*PATE2*, producing a 253 bp amplicon, was chosen for the *PATE* gene. Considering that the PCR assay may be used to detect heavily processed samples, we gave priority to the primer pair *Mt1*/*Mt2* because it yields an amplicon of shorter size (*26*).

Development of Conventional and Real-Time PCR Assays. The conventional PCR conditions were optimized with the annealing temperature between 55 and 65 °C, $MgCl_2$ concentrations between 1.5 and 5 mM, and primer concentrations between 50 and 500 nM in a gradient PCR instrument (DNA Engine Dyad, Bio-Rad Laboratories). The primer pair *Mt1*/*Mt2* was used in conventional PCR under the optimized condition, giving rise to a 109 bp amplification product. Similar sizes have been designed for the maize-specific *Invertase I* gene (122 bp) (*13*) and the cotton-specific *sadI* gene (107 bp) (*22*), as well as other species-specific genes.

To obtain the best amplification for the real-time PCR assay, the concentrations of $MgCl_2$ (from 3 to 6 mM), dNTPs (from 200 to 600 nM), and the primers/probe (from 100 to 800 nM) were optimized by using the primers *Mt1*/*Mt2* and the probe *Mtp*. The optimal PCR conditions were selected and described in detail under Materials and Methods.

Evaluation of the *MT3-B* Gene. To test the species specificity of the *MT3-B* gene, we collected 17 different plant species, including 8 species from Palmaceae (*Rhapis excelsa*, *Rhapis multifida*, *Phoenix robelenii*, *Areca triandra*, *Phoenix dactylifera*, *Cocos nucifera*, *Chrysalidocarpus lutescens*, and *Washingtonia filifera*), 5 species from oil crops (*Brassica napus*, *Glycine max*, *Arachis hypogaea*, *Sesamum indicum*, and *Helianthus annuus*), 2 species from cereal crops (*Zea mays* and *Oryza sativa*), and 2 species from model plants (*Arabidopsis thaliana* and *Nicotiana tabacum*). Both conventional and real-time PCR reactions were run on the whole collection of plant DNA, which was either evolutionarily related to oil palm or frequently found in food and edible oil. No amplification was observed with DNA from any of the tested species other than oil palm, both for conventional (**Figure 2D**) and for real-time PCR (**Figure 3**). These results demonstrate that the *MT3-B* gene is highly specific for oil palm in both conventional and real-time PCR analyses and that we can employ the *MT3-B*-based PCR method to distinguish oil palm from other oil crops.

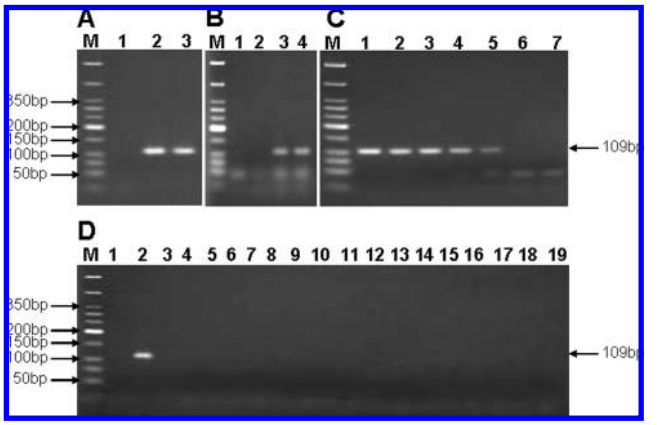

Figure 2. Agarose gel electrophoresis of conventional PCR products amplified with the *Mt1*/*Mt2* primer pair: (**A**) amplification of DNA from two different oil palm cultivars [lane 1 is a water blank control; lane M is a low molecular weight DNA marker (NEB N3233 V)]; (**B**) amplification of DNA from two palm oil samples (lane 1 is a yeast tRNA negative control; lane 2 is a water blank control); (**C**) amplification of serial dilutions of oil palm DNA (lanes 1−6 correspond to 20, 2, 0.2, 0.02, 0.01, and 0.002 ng of oil palm DNA, respectively; lane 7 is a blank control); (**D**) amplification of DNA from 17 different plant species (lanes 3−19 correspond to *Brassica napus*, *Glycine max*, *Arachis hypogaea*, *Sesamum indicum*, *Helianthus annuus*, *Rhapis excelsa*, *Rhapis multifida*, *Phoenix robelenii*, *Areca triandra*, *Phoenix dactylifera*, *Cocos nucifera*, *Chrysalidocarpus lutescens*, *Washingtonia filifera*, *Zea mays*, *Oryza sativa*, *Arabidopsis thaliana*, and *Nicotiana tabacum*, respectively; lane 1 a blank control, and lane 2 is an oil palm positive control).

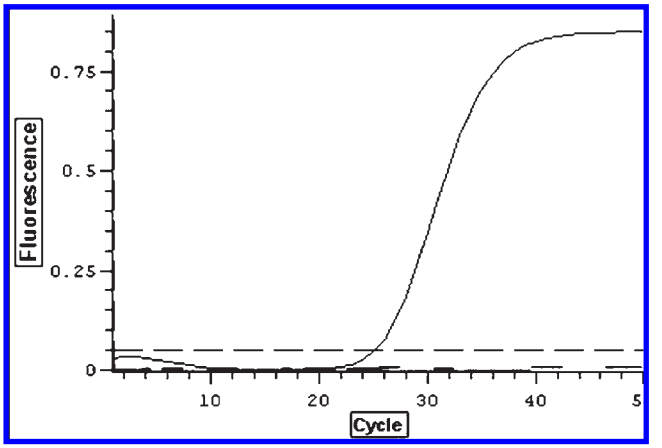

Figure 3. Species specificity analysis of the *MT3-B* gene with the TaqMan real-time PCR assay. The amplification plot was generated from 17 different plant species (*Brassica napus*, *Glycine max*, *Arachis hypogaea*, *Sesamum indicum*, *Helianthus annuus*, *Rhapis excelsa*, *Rhapis multifida*, *Phoenix robelenii*, *Areca triandra*, *Phoenix dactylifera*, *Cocos nucifera*, *Chrysalidocarpus lutescens*, *Washingtonia filifera*, *Zea mays*, *Oryza sativa*, *Arabidopsis thaliana*, and *Nicotiana tabacum*), with 20 ng of oil palm leaf genomic DNA as positive control and water as blank control.

An ideal taxon-specific gene must exhibit high intraspecies similarity while maintaining species specificity. To investigate whether different oil palm cultivars exhibit high similarity within the amplified *MT3-B* sequence, we performed conventional and real-time PCR on a fixed amount of DNA (2 ng) from different oil palm cultivars. The oil palm is a plant with a short history of cultivation and little genetic improvement. Because of the lack of oil palm cultivars, we were only able to collect two different cultivars. Additionally, two palm oil samples imported from other countries were added to this experiment. The two different oil palm cultivars and the two palm oil samples were tested in conventional PCR, and similar amplification bands were obtained (**Figure 2A**,**B**).

Additionally, the *MT3-B* gene should be present in the same copy number in the different cultivars. *MT3-B* is a well-characterized gene. Southern blot analysis demonstrates that the *MT3-B* gene has a single copy in the oil palm genome (*24*). Real-time PCR analysis was performed with DNA from each oil palm cultivar in triplicate, and similar Ct values were obtained (**Figure 4**). The stability of Ct values indicated that both varieties were amplified equally. Moreover, the real-time PCR system can be used to amplify the DNA from different cultivars with the same efficiency. Therefore, we can conclude that the *MT3-B* gene and corresponding primers/probe are suitable for the identification of oil palm.

Sensitivity and Reliability of the Assay. To determine the limit of our assay, a dilution series ranging from 20 to 0.002 ng of DNA/μL was prepared from oil palm genomic DNA. The dilution series was assayed using both conventional and real-time PCR reactions. The lowest amount of DNA that we could detect by conventional PCR was 0.01 ng (**Figure 2C**). On the basis of the oil palm genome size of 1700 Mbp per haploid genome (*27*), the sensitivity of conventional PCR corresponds to an average of five template copies. This sensitivity is similar to that obtained using *lectin* (*15*) or *BnAccg8* (*16*) for conventional GMO detection.

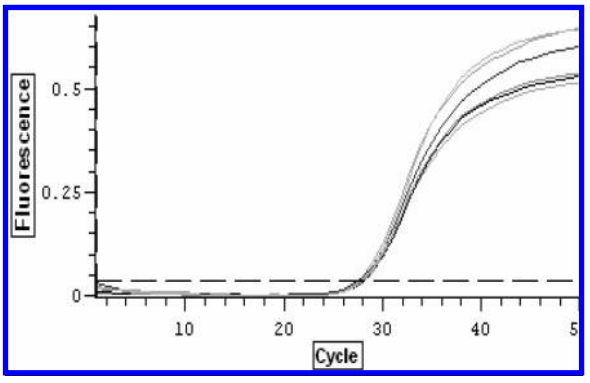

Figure 4. Amplification stability analysis of the *MT3-B* gene with the TaqMan real-time PCR assay. The amplification plot was generated from two different *Elaeis guineensis* cultivars in triplicate amplifications with 2 ng of oil palm genomic DNA.

For the real-time PCR assay, a standard curve was generated by plotting the Ct values obtained in each real-time PCR reaction against the original amount of oil palm DNA (**Figure 5**). The R^2 value was 0.998, and the slope was −3.23. The standard curve shows a good linear relationship between the original amount of template and the Ct values. On the basis of the slope of the standard curve, the amplification efficiency of *MT3-B* was calculated to be 104%, which was in the range of 90−105% recommended by the European Commission (*28*).

Three replicated real-time PCR analyses were performed to assess the sensitivity of the real-time PCR assays. **Table 2** shows the Ct value of each real-time PCR in detail. The data reveal that the lowest copy number that the real-time PCR can detect is five copies. The fluorescent signal could be detected even when the amount of the DNA template was as low as 2 pg, whereas only one or two of the three parallels were negative (data not shown). The LOD is defined as the lowest copy number that exhibits positive results in 95% of the replicates. On the basis of the real-time PCR assay results, the LOD was estimated to be in the range of five copies of genomic DNA.

To test the reliability of the real-time PCR system, the repeatability of the real-time assay was evaluated using the serial dilutions described above. Each serial dilution was assayed in triplicate and repeated three times. The repeatability standard deviation (SD^R) ranged from 0.31 to 0.56 (**Table 2**). We noted that the SD values increase with the concentration decreasing on

Table 2. Repeatability of Oil Palm Real-Time PCR Assays

		Ct values for reaction					
DNA amount (ng)	copy no.	1	2	3	mean	SD^R	RSD^R (%)
20.000	10011	25.58	25.76	25.16	25.50	0.31	1.21
2.000	1001	28.66	29.16	28.40	28.74	0.39	1.34
0.200	100	31.79	32.16	31.25	31.73	0.46	1.44
0.020	10	34.68	35.45	34.35	34.82	0.56	1.62
0.010	5	36.32	36.51	35.79	36.21	0.37	1.03

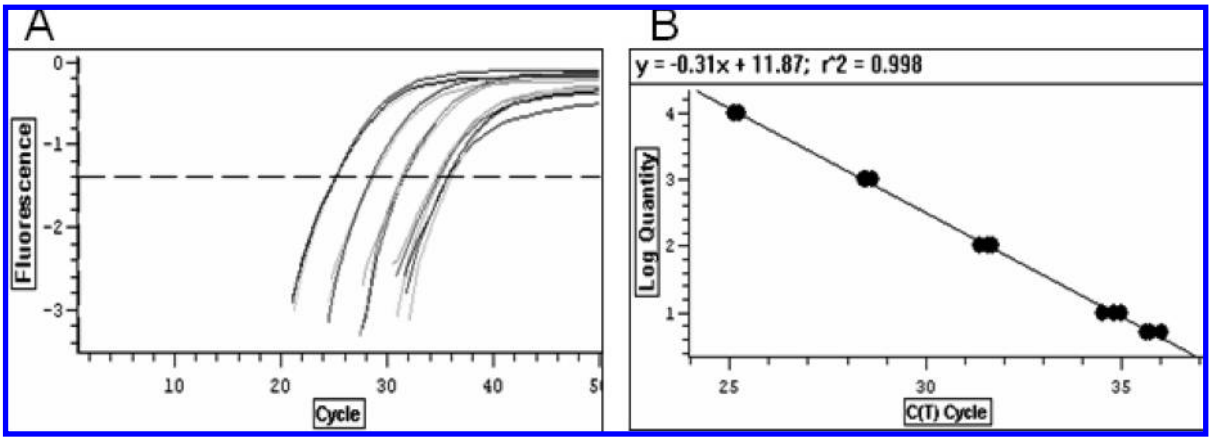

Figure 5. Standard curves for the real-time PCR systems: (**A**) amplification plots generated by the serial dilutions of oil palm DNA correspond to 20, 2, 0.2, 0.02, and 0.01 ng with the *Mt1*/*Mt2* primer pair and the *Mtp* TaqMan probe; (**B**) standard curve generated from the amplification data given in **A**.

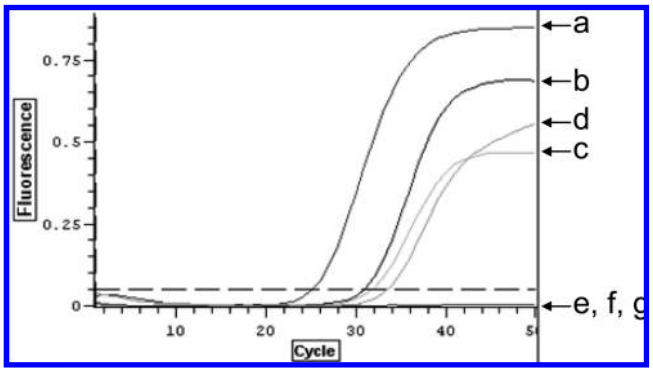

Figure 6. Specific detection of oil palm in real-time PCR: (a) 20 ng of oil palm DNA; (b) oil palm crude oil; (c) known mixture with crude palm oil and crude soybean oil: (d) edible vegetable oil from the local grain and oil market; (e) crude soybean oil; (f) 20 ng of yeast tRNA as negative control; (g) water as blank control. The amplification plot was generated with the *Mt1*/*Mt2* primer pair and the *Mtp* TaqMan probe.

the whole. These small deviations could be explained by the uncertainty in the DNA purity and quantitation after extraction (*29*). The existence of the cytoplasmic DNA, RNA, protein, and some chemicals was thought to be a possible reason for the impurity of genomic DNA and the instability of the PCR results. The molecular fluctuations with low copy numbers of initial template are also thought to contribute to the quantitative uncertainty in PCR reactions (*30*). However, the data in **Table 2** demonstrate that the observed small deviations in this research are acceptable, and the *MT3-B* assay has good repeatability.

In the final analysis, the high efficiency and sensitivity of this assay suggest that it is well suited for use as a reference gene system for the identification of palm oil components and, in the future, that this gene could be used for GM oil palm quantification.

Analysis of Mixed Known and Unknown Oil Samples Using PCR Assays. The established real-time PCR system was used to detect the presence of the oil palm component from various oil samples. As shown in **Figure 6**, an expected amplification plot was generated in DNA from the oil palm leaves, the oil palm crude oil, the mixture with palm oil and soybean oil, and the edible vegetable oil from the local grain and oil market, but not in DNA from the crude soybean oil, the yeast tRNA, and the water. It is concluded that the PCR methods established in this study are suitable for use as an oil palm-specific gene in PCR analysis aimed to detect palm oil components in mixed oil samples. This assay also indicated that palm oil was present in the edible vegetable oil purchased from the local grain and oil market, despite the fact that palm oil was not included as an ingredient on the vegetable oil's label.

In this study the assays were optimized for use of the equipment and chemicals described in this paper, especially for the MJ Research (now Bio-Rad) fluorometric thermal cycler DNA Engine Opticon 2. As far as we know other systems and chemicals may be used, but reaction conditions should be verified and reoptimized according to the manufacturer's advice.

LITERATURE CITED

(1) http://en.wikipedia.org/wiki/Palm_oil.
(2) Shukla, A. K.; Dixit, A. K.; Singh, R. P. Detection of adulteration in edible oils. *J. Oleo Sci.* **2005**, *54*, 317–324.
(3) Aparicio, R.; Aparicio-Ru, R. Authentication of vegetable oils by chromatographic techniques. *J. Chromatogr., A* **2000**, *881*, 93–104.
(4) Kamm, W.; Dionisi, F.; Hischenhuber, C.; Engel, K. H. Authenticity assessment of fats and oils. *Food Rev. Int.* **2001**, *17*, 249–290.
(5) Karoui, R.; De Baerdemaker, J. A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chem.* **2007**, *102*, 621–640.
(6) Cresti, M.; Linskens, H. F.; Mulcahy, D. L.; Bush, S.; Di Stilio, V.; Xu, M. Y.; Vignani, R. Preliminary communication about the identification of DNA in leaves and in olive oil of *Olea europaea*. *Adv. Hortic. Sci.* **1996**, *10*, 105–107.
(7) Breton, C.; Claux, D.; Metton, I.; Skorski, G.; Berville, A. Comparative study of methods for DNA preparation from olive oil samples to identify cultivar SSR alleles in commercial oil samples: possible forensic applications. *J. Agric. Food Chem.* **2004**, *52*, 531–537.
(8) Pafundo, S.; Agrimonti, C.; Marmiroli, N. Traceability of plant contribution in olive oil by amplified fragment length polymorphisms. *J. Agric. Food Chem.* **2005**, *53*, 6995–7002.
(9) Wu, Y.; Chen, Y.; Ge, Y.; Wang, J.; Xu, B.; Huang, W.; Yuan, F. Detection of olive oil using the Evagreen real-time PCR method. *Eur. Food Res. Technol.* **2008**, *227*, 1117–1124.
(10) Scaravelli, E.; Brohee, M.; Marchelli, R.; Hengel, A. J. Development of three real-time PCR assays to detect peanut allergen residue in processed food products. *Eur. Food Res. Technol.* **2008**, *227*, 857–869.
(11) Bryan, G. J.; Dixon, A; Gale, M. D.; Wiseman, G. A PCR-based method for the detection of hexaploid bread wheat adulteration of durum wheat and pasta. *J. Cereal Sci.* **1998**, *28*, 135–145.
(12) Vaitilingom, M.; Pijnenburg, H.; Gendre, F.; Brignon, P. Real-time quantitative PCR detection of genetically modified Maximizer maize and Roundup Ready soybean in some representative foods. *J. Agric. Food Chem.* **1999**, *47*, 5261–5266.
(13) Vollenhofer, S.; Burg, K.; Schmidt, J.; Kroath, H. Genetically modified organism in food-screening and specific detection by Polymerase Chain Reaction. *J. Agric. Food Chem.* **1999**, *47*, 5038–5043.
(14) Hernandez, M.; Duplan, M. N.; Berthier, G.; Vaitilingom, M.; Hauser, W.; Freyer, R.; Pla, M.; Bertheau, Y. Development and comparison of four real-time Polymerase Chain Reaction systems for specific detection and quantification of *Zea mays* L. *J. Agric. Food Chem.* **2004**, *52*, 4632–4637.
(15) Meyer, R.; Chardonnens, F.; Hubner, P.; Luthy, J. Polymerase Chain Reaction (PCR) in the quality and safety assurance of food: detection of soya in processed meat products. *Z. Lebensm. Unters. Forsch.* **1996**, *203*, 339–344.
(16) Hernandez, M.; Rio, A.; Esteve, T.; Prat, S.; Pla, M. A rapeseed-specific gene, *Acetyl-CoA Carboxylase*, can be used as a reference for qualitative and real-time quantitative PCR detection of transgenes from mixed food samples. *J. Agric. Food Chem.* **2001**, *49*, 3622–3627.
(17) Weng, H.; Yang, L.; Liu, Z.; Ding, J.; Pan, A.; Zhang, D. Novel reference gene, high-mobility-group protein I/Y, used in qualitative and real-time quantitative Polymerase Chain Reaction detection of transgenic rapeseed cultivars. *J. AOAC Int.* **2005**, *88*, 577–584.
(18) Yang, L.; Pan, A.; Jia, J.; Ding, J.; Chen, J.; Cheng, H.; Zhang, C.; Zhang, D. Validation of a tomato-specific gene, LAT52, used as an endogenous reference gene in qualitative and real-time quantitative PCR detection of transgenic tomatoes. *J. Agric. Food Chem.* **2005**, *53*, 183–190.
(19) Jeong, S.; Pack, I.; Cho, E.; Youk, E.; Park, S.; Yoon, W.; Kim, C.; Choi, Y.; Kim, J.; Kim, H. Molecular analysis and quantitative detection of a transgenic rice line expressing a bifunctional fusion TPSP. *Food Control* **2007**, *18*, 1434–1442.
(20) Ding, J.; Jia, J.; Yang, L.; Wen, H.; Zhang, C.; Liu, W.; Zhang, D. Validation of a rice specific gene, sucrose phosphate synthase, used as the endogenous reference gene for qualitative and real-time quantitative PCR detection of transgenes. *J. Agric. Food Chem.* **2004**, *52*, 3372–3377.
(21) Yang, L.; Ding, J.; Zhang, C.; Jia, J.; Weng, H.; Liu, W.; Zhang, D. Estimating the copy number of transgenes in transformed rice by real-time quantitative PCR. *Plant Cell Rep.* **2005**, *23*, 759–763.
(22) Yang, L.; Chen, J.; Huang, C.; Liu, Y.; Jia, S.; Pan, L.; Zhang, D. Validation of a cotton-specific gene, *Sad1*, used as an endogenous

reference gene in qualitative and real-time quantitative PCR detection of transgenic cottons. *Plant Cell Rep*. **2005**, *24*, 237–245.
(23) Saghai-Maroof, M. A.; Soliman, K.; Jorgensen, R. A.; Allard, R. W. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. U.S.A*. **1984**, *81*, 8014–8018.
(24) Abdullah, S. N. A.; Cheah, S. C.; Murphy, D. J. Isolation and characterization of two divergent *type3 metallothioneins* from oil palm *Elaeis guineensis*. *Plant Physiol. Biochem*. **2002**, *40*, 255–263.
(25) Othman, A.; Lazarus, C.; Fraser, T.; Stobart, K. Cloning of a palmitoyl-acyl carrier protein thioesterase from oil palm. *Biochem. Soc. Trans*. **2000**, *28*, 619–622.
(26) *ISO 21569; Foodstuffs—Methods of Analysis for the Detection of Genetically Modified Organisms and Derived Products—Qualitative Nucleic Acid Based Methods*; International Standardization Organization: Geneva, Switzerland, 2005.
(27) Singh, R.; Zaki, N.; Ting, N. C.; Rosli, R.; Tan, S. G.; Low, E. T.; Ithnin, M.; Cheah, S. C. Exploiting an oil palm EST database for the development of gene-derived SSR markers and their exploitation for assessment of genetic diversity. *Biologia* **2008**, *63*, 227–235.
(28) Community Reference Laboratory for GM Food and Feed. Event-specific Method for the Quantification of the Oilseed Rape Line RT73 Using Real-time PCR, **2007**; http://gmo-crl.jrc.ec.europa.eu/summaries/RT73_validated_Method.pdf.
(29) Corbisier, P.; Trapmann, S.; Gancberg, D.; Hannes, L.; Van, I. P.; Berben, G.; Schimmel, H.; Emons, H. Quantitative determination of Roundup Ready soybean (*Glycine max*) extracted from highly processed flour. *Anal. Bioanal. Chem*. **2005**, *383*, 282–290.
(30) Peccoud, J.; Jacob, C. Theoretical uncertainty of measurements using quantitative polymerase chain reaction. *Biophys. J*. **1996**, *71*, 101–108.

Received April 8, 2009. Revised manuscript received July 4, 2009. Accepted July 04, 2009. This study is supported by a project of the National Natural Science Foundation of China (30700666).