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

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Polymerase chain reaction (PCR) methods are very useful techniques for the detection and quantification of genetically modified organisms (GMOs) in food samples. These methods rely on the amplification of transgenic sequences and quantification of the transgenic DNA by comparison to an amplified reference gene. Reported here is the development of specific primers for the rapeseed (*Brassica napus*) *BnACCg8* gene and PCR cycling conditions suitable for the use of this sequence as an endogenous reference gene in both qualitative and quantitative PCR assays. Both methods were assayed with 20 different rapeseed varieties, and identical amplification products were obtained with all of them. No amplification products were observed when DNA samples from other *Brassica* species, *Arabidopsis thaliana*, maize, and soybean were used as templates, which demonstrates that this system is specific for rapeseed. In real-time quantitative PCR analysis, the detection limit was as low as 1.25 pg of DNA, which indicates that this method is suitable for use in processed food samples which contain very low copies of target DNA.

Keywords: *Brassica napus; rapeseed; canola; oilseed rape; colza; acetyl-coenzyme A carboxylase; Polymerase Chain Reaction; real-time quantitative PCR; GMO detection; reference endogenous gene*

INTRODUCTION

During the past decade, techniques have been developed that have allowed the introduction of candidate genes into plants and their regulated expression in different plant tissues, leading to the production of genetically modified organisms (GMOs) with characteristics of agronomical interest. These organisms have been extensively cultivated, and their derived products were first introduced into the food market in 1994 (*1*). However, acceptance of these GMO-derived products by the public has been controversial, and concerns about their safety persist among consumers. Such negative public response mandates the importance of providing complete information on food composition based on objective scientific studies (*2*).

In Europe, marketing and distribution of GMO-containing foodstuffs is controlled by European Union (EU) regulations 258/97 (*3*), 1139/98 (*4*), 49/2000 (*5*), and 50/2000 (for food additives) (*6*), as well as by National Food Ordinances. These regulations establish that food or food ingredients containing GMOs in concentrations >1% must be labeled to accurately reflect GMO content. Therefore, it is expedient to develop GMO detection and quantification methods that are specific, reliable, sensitive, and suitable to implement on a large number of samples. Polymerase Chain Reaction (PCR) amplification has proven to be one of the most powerful techniques for the detection of specific DNA species. These techniques are especially useful for the identification and quantification of transgenic DNA in GMO food products because of their simplicity, specificity, and sensitivity (*7*−*9*). In addition, the high stability of DNA under the adverse conditions to which some foods are subjected during processing makes PCR-based methods particularly practical.

PCR-based methods to qualitatively detect the presence of the transgene in a range of GMOs are presently available, and some of these methods have been validated by ring trials among European laboratories (*10*). PCR detection is often based on the amplification of transgenic target sequences such as the *CaMV 35S* promoter or the *Agrobacterium nos* terminator, present in most of the GMOs presently approved (*9*). Detection may also be based on the amplification of either the specific transgene introduced into the GMO or the specific insertion event. Examples of these methods are those based on the amplification of the *CP4-EPSP* gene in Roundup Ready soybeans (Monsanto Corp., St. Louis, MO) and the *Bt* genes in Maximizer maize Bt176, Bt-11 maize (Novartis Seeds AG, Basel, Switzerland), Liberty-Link T25 maize (Hoechst Schering AgrEvo GmbH, Berlin, Germany), or Yield Gard maize (Monsanto) [*11*, *12*, *13* (and references therein), *14*].

The use of real-time quantitative PCR detection methods is a very accurate and fast system for the quantitative detection of GMOs in processed food samples. These methods normally rely on the amplification of transgenic specific sequences and their quantification relative to an endogenous reference gene that gives an estimation of the total amount of target DNA in the sample. With this technology, the amount of GMO is calculated as a function of total plant-specific DNA

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Table 1. Primer Pairs and Fluorogenic Probe

PCR system	orientation	name	sequence	length	position
real-time	forward primer	acc1	5′-GGTGAGCTGTATAATCGAGCGA-3′	22	9652−9673
real-time and conventional	reverse primer	acc2	5′-GGCGCAGCATCGGCT-3′	15	9741−9755
conventional	forward primer	acc3	5′-GAGAATGAGGAGGACCAAGCTC-3′	22	9560−9581
real-time	probe	accp	5′-AACACCTCTTCGACATTCGTTCCATTGGTCGA-3′	32	9685−9716

in the food product. PCR primers and fluorogenic probes suitable for use with this technology have been published for genetically modified Roundup Ready soybeans and Maximizer maize Bt176 (*15*). These systems require both the primers specific for the transgene and the species-specific primers complementary to an endogenous reference gene. Amplification of such reference sequences will allow the detection of DNA from the plant species of interest in food samples and at the same time assay for the quality of the extracted DNA, providing a means to quantify the amount of GMO in the processed food sample.

Much effort has been expended to obtain reference genes for the analysis of genetically modified soybean and maize due to the economic importance of these food crops. Eighty-two percent of the transgenic crops grown during 2000 were represented by these two plant species. The next two most important transgenic crops were cotton and rapeseed. Eleven percent of the total cultivated production of rapeseed was transgenic (*16*). Genetically modified rapeseed with tolerance to herbicides such as glyphosate (RT73, Monsanto) or phosphinothricin [Topas 19/2 and MS8xRF3 both from AgrEvo (PGS), the last one also modified to be male-sterile] is already being grown in the United States. Rapeseed lines with improved oil content and composition such as pCGN3828 (Calgene Inc., Davis, CA) are also being introduced into the market (*17*−*20*). Despite the commercial approval of these transgenic varieties, to date no methods have been published that describe their specific identification and quantification in food samples. Here we report qualitative and quantitative PCR-based methods to specifically identify the presence of rapeseed DNA in food. These methods amplify specifically the *acetyl-CoA carboxylase* (ACC) *BnACCg8* gene (*21*), which codifies for an enzyme of fatty acid biosynthesis (*22*, *23*). We show that this system can be used as a species-specific internal control in combination with an appropriate screening or event-specific technique for the identification and/or quantification of GMO rapeseed in a food matrix. We also show that this PCR-based method is suitable for the detection of rapeseed-derived products in processed food such as vegetable oils.

MATERIALS AND METHODS

Materials. DNA samples from seeds of 20 different *Brassica napus* lines were provided by GEVES (France). Fifteen of these lines corresponded to winter-type varieties (European genotypes) and five of them to spring varieties (originated in Canada). All of them exhibited a high degree of variability based on AFLP analysis (Zhang, personal communication). For DNA extraction from soybean and maize, reference material from Fluka (Buchs, Switzerland) was used. Leaves of *Arabidopsis thaliana* ecotype *Columbia*, *Solanum tuberosum* var. Désirée, and *Lycopersicon esculentum* var. *Ailsa Craig* were used for DNA extraction from these plant species. Mixed seeds and rapeseed crude oil were purchased at a local market.

Safety Precautions. Manipulation of potential GMO-containing food was performed following Spanish guidelines (*24*) established according to Directives 90/219/CEE and 90/220/CEE (*25*, *26*).

DNA Extraction. Large-scale genomic DNA was isolated from 30 g of leaves according to the method of Dellaporta et al. (*27*) and purified by using CsCl density gradient centrifugation. Small-scale genomic DNA was isolated from 0.1 g of plant material as described by Meyer and Jaccaud (*11*). Genomic DNA was quantified spectrophotometrically using a GeneQuant RNA/DNA calculator (Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany) and analyzed by 1% agarose gel electrophoresis in 1× TAE (1× TAE is 0.04 M Tris, pH 8.5, 0.001 M EDTA, and 0.02 M acetic acid) with ethidium bromide staining.

Southern Blot. Ten micrograms of maize, 5 μg of soybean and rapeseed, and 2 μg of *A. thaliana* DNA were digested to completion with *Eco*RI or *Hin*dIII as indicated. Digested DNA was resolved in a 0.9% agarose gel electrophoresis as before, blotted onto a Hybond-N^+ nylon membrane (Amersham Pharmacia Biotech Europe GmbH), and hybridized with the *acc2*/*acc3* PCR product probe as described by Sambrook et al. (*28*). The *acc2*/*acc3* PCR product was separated by agarose gel electrophoresis and purified using the GFX PCR DNA and gel band purification kit (Amersham Pharmacia Biotech Europe GmbH). The purified fragment was amplified by PCR in the presence of [^{32}P]-dCTP and further purified through a QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany). Hybridization was performed at 65 °C (*29*), and the filter was washed at 65 °C with 2× SSC 0.1% SDS, 1× SSC 0.1% SDS, and 0.5× SSC 0.1% SDS, for 20 min each. Molecular mass markers (genomic DNA of λ phage digested with *Pst*I) were run on the same gel.

Oligonucleotide Primers and Probe. PCR primers and a fluorogenic probe were purchased from Applied Biosystems. Primer Express 1.5 software (Applied Biosystems Division of Perkin-Elmer Corp., Foster City, CA) was used to design the oligonucleotides. Sequences of the primers and probe used are shown in Table 1. The TaqMan probe was labeled on the 5′ end with the fluorescent VIC reporter dye and with the quencher dye 6-carboxytetramethylrhodamine (TAMRA) attached to its 3′ end. For conventional PCR, we used primers *acc2* and *acc3*. For real-time PCR, primers *acc1* and *acc2* were used in combination with the *accp* probe. In both conventional and real-time PCR the same exon fragment of the *acetyl-coenzyme A carboxylase* gene (*BnACCg8*, accession number X77576) was amplified, giving rise to products of 196 or 104 bp, respectively (PCR conditions were those observed below).

PCR Conditions. PCR reactions were performed with the TaqMan PCR core reagents (Applied Biosystems-Roche Molecular Systems Inc., Branchburg, NJ) in a 20 μL reaction mixture containing 1× PCR buffer II (for conventional PCR) or TaqMan buffer A (includes ROX as a passive reference dye for real-time PCR), 300 nM primers, 200 nM probe, 400 μM each of dATP, dCTP, dGTP, 800 μM dUTP, 1 unit of AmpliTaq Gold DNA polymerase, 0.2 unit of AmpErase uracil *N*-glycosylase (UNG), 3 or 4 mM $MgCl_2$ for conventional and real-time PCR, respectively, and 2.5 μL of the DNA solution. For the generation of a standard curve, the extracted DNA was serially diluted to final concentrations of 50, 5, 0.5, 0.05, and 0.005 ng/μL. The amounts of DNA per reaction tube ranged from 125 ng to 1. 25 pg.

Conventional PCR reactions were run in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus Instruments, Emeryville, CA) using the following cycle conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 63 °C followed by 10 min of elongation at 72 °C. Real-time PCR reactions were run on an ABI PRISM 7700 sequence detection system (Applied Biosystems Division of Perkin-Elmer Corp.) device using the following program: 2 min at 50 °C, 10 min at 95 °C, 50 cycles of 15 s at 95 °C, and 1 min at 60 °C.

The PCR products were analyzed by 3% agarose gel electrophoresis (1× TAE) and stained with ethidium bromide for conventional PCR or by using sequence detection system software 1.7 (Applied Biosystems) for real-time PCR. Quantification was performed by interpolation using a standard regression curve of Ct values generated from DNA samples of known concentrations.

RESULTS AND DISCUSSION

Selection of a Gene Fragment Suitable for Specific Detection and Quantification of Rapeseed DNA. Reliable GMO detection and quantification systems depend on the use of appropriate reference genes, corresponding to endogenous nuclear DNA sequences. Reference genes should be species specific, have a low copy number, and exhibit low heterogeneity among cultivars. Commonly used reference genes are the *10-kDa zein*, *hmg-A* (high mobility group protein A), and *invertase 1* genes for maize (*15*, *30*−*32*) and *lectin* and *hsp* (heat-shock protein) genes for soybean (*15*, *33*, *34*). To select a rapeseed gene suitable for use as an endogenous reference gene for PCR amplification, we searched gene databanks (EMBL) for sequences encoded by a low copy number gene family, with enough interspecific divergence to produce a rapeseed specific product and which have not been subjected to genetic manipulation. After selection of several candidate genes, we chose two sequences encoding the storage protein cruciferin and the acetyl-CoA carboxylase (ACC) enzyme, which catalyzes the formation of malonyl-CoA from acetyl-CoA and bicarbonate during the early steps of fatty acid biosynthesis (*22*, *23*). We designed specific primers on both sequences and tested them for species specificity, in both qualitative and real-time quantitative PCR assays. Initial assays conducted with the *cruciferin* primers showed a lack of specificity for these oligonucleotides, with amplification products obtained from rapeseed, other *Brassica* species, and *A. thaliana* DNA. This result would indicate that the *cruciferin* PCR system is not suitable for use as an endogenous reference gene specific for rapeseed and that PCR primers designed for less conserved sequences are required for these assays. Database searches conducted with the rapeseed *BnACCg* gene revealed that selected nucleotide sequences of this gene are variable enough to allow the synthesis of species-specific primers. This enzyme is encoded in rapeseed by a five-member multigene family from which three members (*BnACCg1*, *BnACCg8*, and *BnACCg10*) have been characterized (*21*). Average overall similarity between these three members is 87.7%, but it decreases toward the C-terminal region of the protein. A complete nucleotide sequence has been reported for the full-length *BnACCg8* gene, which contains an open reading frame of 10747 bp that is interrupted by 31 introns (*35*).

Comparison of the rapeseed BnACCg8 sequence with its *A. thaliana* ortholog showed a high degree of conservation between both genes with selected regions of less similarity. One of these regions in exon 27 (*acc3*/*acc2*, positions 9560−9755) was used to design two forward primers (designated *acc1* and *acc3*), one reverse primer (*acc2*), and the TaqMan probe accp (see Table 1 and Figure 1). Neither these primers nor the TaqMan probe recognized any plant DNA sequence other than the rapeseed *BnACCg8* gene when analyzed using Fasta3 software (EBI European Bioinformatics Institute) against the EMBL GenBank sequences. As shown in Figure 1, primer pair *acc3*/*acc2* amplified a fragment of 196 bp, whereas primer pair *acc1*/*acc2* produced a PCR fragment of 104 bp.

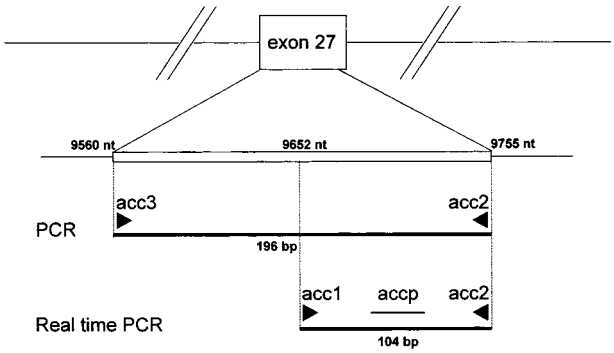

Figure 1. Schematic diagram illustrating the different amplification fragments of exon 27 of the *acetyl-coenzyme A carboxylase* gene. Positions of PCR primers used for qualitative and quantitative PCR analysis are indicated by arrowheads. TaqMan probe used for quantitative assays is indicated by a solid bar.

Qualitative and Real-Time PCR Assays. We have used the primer pair *acc3*/*acc2* in conventional qualitative PCR assays. In this assay system, the primer combination *acc3*/*acc2* was more effective than primer pair acc1/*acc2*. The *acc3*/*acc2* combination amplified a fragment of larger size, which is easier to separate from primers in an analytic agarose gel. This combination of primers was also more specific as it gave rise to an amplification product exclusively with rapeseed DNA. Primer pair *acc1*/*acc2* was used in real-time PCR assays in combination with the *accp* TaqMan probe. This primer pair gives rise to a 104 bp amplification product, which is in a size range considered to be optimal for real-time PCR amplification. Amplification products of similar size have been designed for maize *zein* (69 bp) and soybean *lectin* (81 bp) to be used as reference genes for GMO quantification (*15*). In PCR reactions performed with SYBR Green (Molecular Probes, Inc., Eugene, OR) we detected amplification products not only with rapeseed DNA but also with tomato and maize DNA (data not shown). These latter were unspecific PCR products as they were of a different size and showed a melting temperature (analyzed using the ABI PRISM 7700 dissociation curve software) different from that of the rapeseed product. Furthermore, they did not cross-hybridize with the TaqMan *accp* probe. Sequencing of both maize and tomato fragments verified that they did not correspond to *ACC* genes. These results indicate that this combination of primers is suitable for use in a TaqMan real-time PCR system but not in conventional PCR assays or in SYBR Green based quantitative assays. In the latter two systems the presence of a particular DNA template is detected as an amplified product but does not rely on the specific recognition of the product as occurs with the TaqMan technology. Once the suitability of the different primers was established, we optimized both conventional and real-time PCR for primer and probe concentrations under a range of different $MgCl_2$ concentrations. Optimal conditions were those described under Materials and Methods. Running conditions for real-time PCR were the same as those described in the ABI PRISM 7700 sequence detection system user's manual in order to facilitate multiplexing and routine quantification of several genes in the same PCR run.

Sensitivity of the Assay and Quantification Accuracy. To test the sensitivity of the two assay systems,

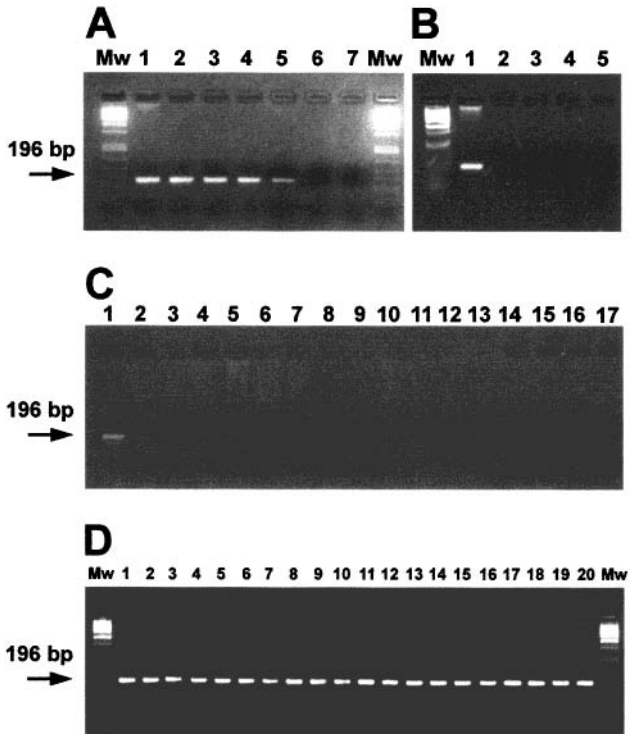

Figure 2. Agarose gel electrophoresis of qualitative PCR products amplified with the *acc2/acc3* primer pair: (A) amplification of serial dilutions of rapeseed DNA (lanes 1−7 correspond to 125 ng, 12.5 ng, 1.25 ng, 0.125 ng, 12.5 pg, 1.25 pg, and 0.125 pg of rapeseed DNA); (B) amplification of DNA from different *Brassica* species (lanes 1−5 correspond to *B. napus*, *B. oleracea* ssp. *botrytis*, *B. oleracea* ssp. *capitata*, *B. oleracea* ssp. *gemmifera*, and *B. oleracea* ssp. *italica*); (C) amplification of DNA from 17 different plant species (lanes 1−17 correspond to *B. napus*, *Z. mays*, *H. annuus*, *S. tuberosum*, *S. bicolor*, *Z. diploperennis*, *L. esculentum*, *G. max*, *O. sativa*, *S. cereale*, *T. aestivum*, *P. miliaceum*, *L. esculenta*, *V. faba*, *P. aureus*, *L. albus*, and *A. thaliana*); (D) amplification of DNA from different rapeseed lines numbered 1−20. Mw: *λPst*I molecular weight markers.

we ran PCR reactions on known amounts of rapeseed genomic DNA ranging from 125 ng to 0.125 pg. Conventional PCR allowed detection in 12.5 pg of genomic DNA (Figure 2A). This sensitivity is similar to that obtained for the soybean *lectin* gene in a single-set primer PCR (*36*) or the maize *10-kDa-zein* gene in nested PCR (*30*). A higher level of sensitivity was attained in real-time PCR assays. We could amplify PCR products with as little as 1.25 pg of rapeseed template DNA with real-time PCR (Figure 3A). On the basis of the rapeseed genome size of 1.2 Mbp per haploid genome (*37*), this corresponds to an average of a single nucleus in the template. This reference system has a sensitivity threshold similar to the one reported for the maize *10-kDa-zein* and soybean *lectin* genes (*15* and references cited therein), currently used as reference genes by different laboratories in routine GMO quantification analysis. Moreover, the rapeseed *BnACCg8* gene (see below) is present in a similar number of copies per haploid genome as the maize *10-kDa-zein* gene (*38*).

To assay the accuracy of the quantification system, we obtained a standard curve by plotting the Ct values obtained in each real-time PCR reaction against the original amount of rapeseed DNA present in the sample. As seen in Figure 3B, the standard curve generated shows an R^2 of 0.992 and a slope of −3.319 (the theoretically optimal value is −3.32). This demonstrates that there is a high correlation between the original amount of DNA in the template and the Ct value obtained after amplification and a high efficiency of the PCR reaction. Only Ct values obtained after amplification of 1.25 pg of template DNA slightly separated from the calculated standard curve, which indicates that at such reduced levels of DNA, quantification is not accurate. These results indicate that in real-time PCR analysis, the rapeseed *BnACCg8* gene exhibits a reliable amplification linearity over 5 orders of magnitude but that quantification is not possible below 12.5 pg of template DNA. Amounts >1.25 μg of rapeseed DNA inhibited the PCR reaction (Figure 3B).

Species Specificity of the Reference Gene. To test the species specificity of the *BnACCg8* reference gene, we ran both conventional and real-time PCR reactions on 125 ng of template DNA from 21 different plant species that were either evolutionarily related to rapeseed or frequently found in food. These included

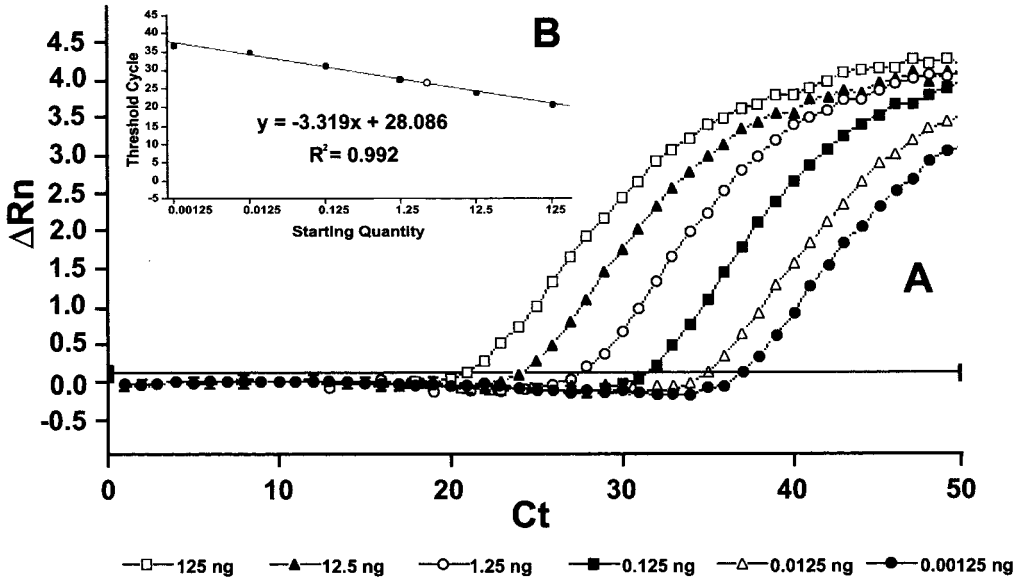

Figure 3. Rapeseed detection and quantification in real-time PCR: (A) amplification plot generated by serial dilutions of rapeseed DNA ranging from 125 ng to 1.25 pg with the *acc1/acc2* primer pair and the *accp* TaqMan probe; (B) standard curve generated from the amplification data given in (A) [the open circle corresponds to an inhibitory concentration (1250 ng) of rapeseed DNA].

Brassica species such as broccoli (*Brassica oleracea* ssp. *italica*), cauliflower (*Brassica oleracea* ssp. *botrytis*), cabbage (*Brassica oleracea* ssp. *capitata*), and Brussels sprouts (*Brassica oleracea* ssp. *gemmifera*), dicot species such as *A. thaliana*, potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), sunflower (*Helianthus annuus*), soybean (*Glycine max*), lentils (*Lens esculenta*), white bean (*Vicia faba*), mung bean (*Phaseolus aureus*), and lupine (*Lupinus albus*), and more distantly related monocot plants such as maize (*Zea mays*), the maize ancestor teosinte (*Zea diploperennis*), sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), rye (*Secale cereale*), wheat (*Triticum aestivum*), and millet (*Panicum miliaceum*). The genome size of these species is not larger than 4-fold the one of rapeseed except for wheat, which is 12-fold (*37*). If the *acc* primers allowed the amplification of any genomic sequence out of these plant DNAs, we should be able to detect it based on the sensitivity of the assay. No amplification was observed with any of the species tested other than rapeseed, both in qualitative (Figure 2B,C) and in real-time PCR (data not shown). Amplification was also not observed when broccoli, cauliflower, cabbage, or Brussels sprout DNA was used as a template. These species are closely related to rapeseed and belong to the same taxonomic genus. These results demonstrate that the *BnACCg8* reference gene is highly specific for rapeseed in both the conventional and real-time PCR systems.

Copy Number of the Rapeseed *BnACCg8* Gene. To analyze the number of copies of the *BnACCg8* gene in rapeseed, we performed Southern blot analysis in which rapeseed genomic DNA was digested with *Eco*RI or *Hin*dIII and hybridized with the 196 bp *acc3/acc2* PCR product. Equivalent amounts of *A. thaliana*, maize, and soybean DNA, digested with *Eco*RI, were also analyzed to further assay the species specificity of the *acc3/acc2* fragment. Two and three hybridizing bands were detected in the lanes corresponding to *Eco*RI- or *Hin*dIII-digested rapeseed DNA (data not shown), which is indicative of the presence of from two to three copies of the *BnACCg8* gene per rapeseed haploid genome. No cross-hybridization was obtained with maize, soybean, or *A. thaliana* DNA, confirming that the rapeseed *acc3/acc2* DNA fragment is specific for rapeseed.

Allelic Variation of the *BnACCg8* Gene among Rapeseed Cultivars. To be useful for the identification and quantification of material derived from a certain plant species in a processed food sample, a PCR system must be able to amplify the DNA from this plant species with the same efficiency independently of the line or cultivar from which it is derived. An ideal reference gene should not exhibit allelic variation among varieties and, at the same time, it should be present in the same number of copies in the different plant cultivars. To investigate whether different rapeseed cultivars exhibit any sort of allelic variation within the *BnACCg8* exon sequence we use as amplification template, we performed both conventional and real-time PCR on a fixed amount of DNA (125 ng) of 20 different rapeseed lines. As shown in Figure 2D, a PCR product of identical size and relative intensity was obtained for all of these lines after conventional PCR. This result indicates that there are no major sequence differences among the different lines in this exon region. Likewise, real-time PCR analysis performed with triplicates of DNA extracted from each rapeseed line exhibited Ct values that varied at most in one cycle between cultivars (Ct values oscillated between 24.32 and 25.38, data not shown). These results are indicative of a similar number of copies of the *BnACCg8* gene among rapeseed lines, with the small variation in Ct values most likely being caused by differences in the quality of the extracted DNA. Allelic variation among different cultivars using real-time PCR has not been investigated for any of the reference genes commonly used.

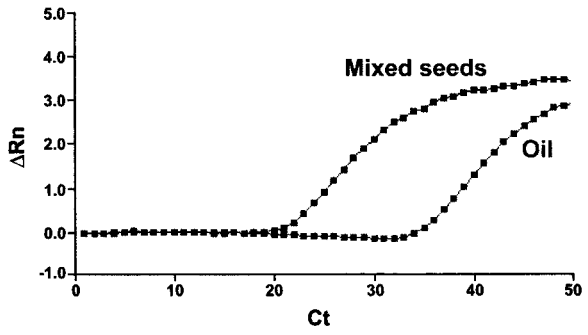

Figure 4. Specific detection of rapeseed in real-time PCR: amplification plot generated from mixed seeds and rapeseed crude oil with the *acc1/acc2* primer pair and the *accp* TaqMan probe.

Rapeseed DNA Detection in Processed Food. We have tested the applicability of the designed *BnACCg8*-based real-time PCR system to specifically detect and quantify the presence of rapeseed DNA in samples of mixed seeds or in rapeseed crude oil. As seen in Figure 4, using the optimal PCR conditions we have developed, we have been able to amplify the *acc1/acc2* PCR product out of DNA preparations extracted from the two samples. Therefore, we can conclude that with the specified conditions the *acc1/acc2* PCR product is suitable for use as a rapeseed endogenous reference gene in PCR analysis aimed to detect and quantify the presence of genetically modified rapeseed in mixed food samples. We have shown that this technique is effective with samples such as vegetable oils that are particularly difficult to analyze at the DNA level.

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