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Method for the Detection of Synthetic *cry3A* in Transgenic Potatoes

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All transgenic cultivars of potatoes registered in Canada and the United States have been modified to express a synthetic *cry3A* gene as a means of conferring resistance against the Colorado potato beetle, an important economic pest of potatoes. A PCR method was developed to amplify a 499 bp region of the synthetic *cry3A* gene. Using this method, synthetic *cry3A* could be detected in six different transgenic cultivars. Positive results could be confirmed with *Pvu*II restriction digestion of the PCR-generated amplicon, which resulted in two fragments that were 283 and 216 bp in size. Of the 52 tuber extracts tested with this method, no false positive or false negative results were obtained, suggesting the method could be used with a high degree of accuracy. The absolute limit of detection was the number of *cry3A* copies present in one or perhaps two haploid copies of the potato genome. The practical limit of detection in tubers on a fresh weight basis was 0.02% for the NL 10-SUP and 0.01% for the remaining cultivars. Synthetic *cry3A* could also be detected in processed food products such as potato chips, shoestring potatoes, and frozen French fries. The method was suitable for screening potato tuber lots and some processed foods for the presence of synthetic *cry3A*.

KEYWORDS: ***Solanum tuberosum*; potato; *cry3A*; GM detection; novel traits; PCR**

INTRODUCTION

In recent years, the commercial production of crops carrying novel traits encoded by recombinant DNA has steadily increased, from less than two million hectares in 1996 to more than 58 million hectares in 2002 (*1*). These novel traits offer numerous agronomic benefits such as herbicide tolerance and pest and disease resistance; the public, however, has largely greeted foods derived from biotechnology with varying degrees of skepticism, cynicism, and mistrust (*2*−*4*).

Although the strategies for regulating the production of novel commodities vary with every jurisdiction, public pressure has generated a growing demand for the monitoring and testing of crops and foods for genetic modifications for several purposes (*5*). First, testing for novel traits will likely be implemented in identity preservation (IP) systems (*6*) in which traceable seed or grain lots are tested for the adventitious presence of genetic modifications within accepted tolerances (*7*). This approach to verification of identity is likely to become more common in global commodities trading because of differences in the way novel crops are regulated in different parts of the world (*6*, *7*). Second, testing is required in many jurisdictions to support regulatory requirements for labeling. Many countries support either mandatory or voluntary labeling for novel foods to provide information and allow consumer choice (*2*−*5*). Finally, surveillance of the incidence of volunteer plants with novel traits or the pollen-mediated gene flow between transgenic crops and their nontransgenic counterparts has generated an additional requirement for novel trait-testing in some parts of the world. The various analytical approaches to the detection of novel traits in crops and foods have been recently reviewed (*8*).

Between 1996 and 2001, seven cultivars of transgenic potato *Solanum tuberosum* L. received full regulatory approval in Canada and the United States. These cultivars were designated NL 10-RBK, NL 10-SUP, NL 10-ATL, NL 20-RBK, NL 20-SHE, NL 30-RBK-350, and NL 30-RBK-082. NL 10-RBK and NL 10-SUP may also be cultivated in Europe, as they have received full regulatory approval in Romania and biosafety approval in Russia.

Developed by the Monsanto Corp. and sold under the brand name NewLeaf, these cultivars are resistant to the Colorado potato beetle, an economically important pest of potato, by virtue of a synthetic transgene, *cry3A*. This gene, originally isolated from the bacterium *Bacillus thuringiensis tenebrionis* (*Btt*), encoded Cry3A, a delta endotoxin that was highly toxic to the Colorado potato beetle. Although these cultivars had received regulatory approval for food use, public pressure had forced many potato-processing companies to stop accepting transgenic tubers (*10*), and all of the cultivars of NewLeaf seed potatoes were officially withdrawn from commercial production in North America in 2001.

Because all of the approved NewLeaf cultivars contained *cry3A* (*11*−*14*), this gene was considered to be a suitable target for a detection method for these transgenic potatoes. Although the antibiotic resistance gene *nptII* derived from *Escherichia coli*, and regulatory elements such as the *nos* terminator from

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Table 1. Potato Cultivars Used in This Study

transgenic cv.	line	*cry3A* copy[a] no.	brand name	parental cv. (nontransgenic)
NL 10-SUP	SPBT02–5	1 (*20*)	NewLeaf Superior	Superior
NL 10-ATL	ATBT04-6, ATBT04-30[b]	3[c] (*21*) 1 (*21*)	NewLeaf Atlantic	Atlantic
NL 20-RBK	RBMT15-101	3 (*21*)	NewLeaf Y Russet Burbank	Russet Burbank
NL 20-SHE	SEMT15-02, SEMT15-15[b]	≥5 (*21*)	NewLeaf Y Shepody	Shepody
NL 30-RBK-350	RBMT21-350	2 (*21*)	NewLeaf Plus Russet Burbank	Russet Burbank
NL 30-RBK-082	RBMT22-82	3 (*21*)	NewLeaf Plus Russet Burbank	Russet Burbank

[a] Refers to the number of intact copies of *cry3A*. [b] Possible lines: The NL 10-ATL and NL 20-SHE lines used in this study could not be absolutely identified. [c] Copy number was dependent on the line. ATBT04-6 had three copies, whereas ATBT04-30 had one copy (*21*).

Agrobacterium tumefaciens, and the 35S promoter from the cauliflower mosaic virus (p35S CaMV) have been used for general screening for genetically modified crops (*8*), these were not considered to be the best targets for the detection of NewLeaf potatoes. According to the USDA/APHIS decision documents, p35S CaMV was not used in all of the transgenic lines that were developed (*11*−*14*). The *nos* terminator was used in all of the transgenic NewLeaf lines (*11*−*14*), but this sequence, as well as *nptII*, is a naturally occurring sequence present in common soil bacteria and may introduce an increased risk of false positive results in tuber testing. Although *cry3A* also occurs naturally in the soil bacterium *Btt*, the *cry3A* sequence used in NewLeaf potatoes was synthetic, as bacterial codons were replaced with plant-preferred codons in order to enhance expression (*9*). Thus, the synthetic *cry3A* should be distinguishable from the natural *cry3A*.

Potential applications of a method for the detection of NewLeaf potatoes include the screening of seed tuber lots for identity preservation purposes, verification of varietal purity, screening tuber lots bound for processing plants, and detection of transgenic potato material in processed foods. This study describes an assessment of a Polymerase Chain Reaction (PCR) method for the detection of synthetic *cry3A* in transgenic potatoes.

MATERIALS AND METHODS

Potato Material. Nontransgenic potatoes, cultivars Russet Burbank, Shepody, Atlantic, and Superior, were obtained as certified seed tubers from six different growers over two seasons. The transgenic cultivars NL 10-ATL, NL 20-SHE, and NL-30-RBK-350 were obtained as field tubers grown in different fields over two seasons. Tubers from the remaining transgenic cultivars used in this study were produced from tissue culture plantlets in the laboratory greenhouse over two growing seasons. Samples of NL 10-RBK tubers were not available for this study. The transgenic cultivars and their respective negative control parental cultivars are listed in **Table 1**. In this study, NewLeaf, NewLeaf Y, and NewLeaf Plus will all be referred to collectively as NewLeaf potatoes.

***Bacillus thuringiensis tenebrionis*.** *Btt* was acquired as a 3.0% suspension of endospores in an organic pesticide preparation purchased from Bionide Products Inc. (Oriskany, NY).

Processed Foods. Samples of potato chips, shoestring potatoes, a snack made from potato flour, and frozen French fries were acquired in the latter part of 2001. Samples of potato flour and instant mashed potatoes were purchased in the first half of 2003. These products were purchased from two local grocery retailers.

DNA Extraction of Tuber Tissue (Wizard Method). The DNA extraction method was slightly modified from the Wizard method (*15*). Fresh tuber materials were homogenized 1:3 (w/v) in an extraction buffer consisting of 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% lauryl sulfate, and 500 mM guanidine−HCl (Sigma Chemical Co., Oakville ON, Canada). Proteinase K (Roche Applied Science, Laval, PQ, Canada) was added to a final concentration of 0.8 mg/mL, and the homogenates were incubated for 3 h at 58 ± 2 °C. After cooling at room temperature for 10 min, the lysates were centrifuged at 14000*g* for 10 min. Meanwhile, sterile, 3 mL syringe barrels were attached to minicolumns and placed onto a VacMan vacuum manifold. Wizard resin (1 mL) (Promega Corp., Madison, WI) was added to the barrel of the syringe.

Cleared supernatant (300 μL) was loaded into the syringe barrel and allowed to mix with the Wizard resin. The mixture was pulled into the minicolumn using the laboratory house vacuum. The contents of the minicolumn were washed once with 2 mL of 80% 2-propanol. The minicolumns were removed from the vacuum manifold and spun inside a clean microfuge tube at 10000*g* for 2 min to remove excess 2-propanol. DNA was eluted in 50 μL of DNase-free water preheated to ∼70 °C using a final centrifugation of 10000*g* for 1 min.

DNA Extraction from Processed Foods. The frozen French fries were homogenized, and DNA was extracted using the Wizard method as described for the tuber material. The potato chips, potato shoestrings, and the snack made from potato flour were each pounded and ground to fine crumbs inside resealable plastic bags; 100−150 mg of each of the six sample potato products was mixed with 10× (v/w) extraction buffer and Proteinase K (0.8 mg/mL final concentration) as described above for the potato tubers. Samples were incubated 3 h at 58 °C and centrifuged 14000*g* for 10 min. DNA was extracted according to the Wizard method as described above for the tubers, except that 600 μL of the postlysis supernatant from each sample was loaded onto each minicolumn. The columns were washed and the DNA was eluted as described above.

DNA Extraction from *Btt* Endospores. DNA was extracted from 1.0 mL aliquots of the organic pesticide, consisting of a suspension of *Btt* endospores, using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Solana Beach, CA) according to kit instructions.

DNA Quantification. DNA was quantified using the Picogreen dsDNA quantitation kit (Molecular Probes, Eugene OR). Lambda DNA provided with the kit was used as a standard between 10 and 1000 pg/mL, and all sample extracts were diluted 1/100 or more. The assay was performed in a microplate format according to kit instructions. Fluorescence was measured (λ_{ex} 485; λ_{em} 528) using microplate fluorescence reader model FLx800 (Biotek Instruments, Winooski, VT).

Oligonucleotide Primers. The primers for synthetic *cry3A* were designed using Primer3 http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi (*16*) against Genbank accession no. X70979 (*9*). The forward (C3A1) and reverse (C3A2) primer sequences for synthetic *cry3A* detection were (respectively) 5′ AAG CCC TCG ACA GTT CTA CC 3′ [positions 23−42 (X70979, *9*)] and 5′ TCG TAT CCC CAC TCT TCT CC 3′ [positions 521−502 (X70979, *9*)]. These primers were expected to produce an amplicon of 499 base pairs (bp) in length.

Forward (PSS1) and reverse (PSS2) primer sequences used for the detection of potato sucrose synthase were 5′ TGC TCA CCG CAA TGA GAT AC 3′ [positions 1874−1893 (U21129, *17*)] and 5′ GGC AGA ACA ATC GCT TCC TA 3′ [positions 2409−2390 (U21129, *17*)]. These primers were designed using Clone Manager Professional Suite v. 6.00 (Science and Educational Software, Durham, NC) and were expected to produce an amplicon of 536 bp.

Forward (NC3A1) and reverse (NC3A2) primer sequences used for the detection of natural *cry3A* in *Btt* were 5′ TGA GGT GCC AAC TAA CCA 3′ [positions 294−311 on J02978 (*18*)] and 5′ GGC AGC

TTG TGC ATA TGT 3′ [positions 843−826 on J02978 (*18*)]. These primers were designed using Clone Manager Professional Suite v. 6.00 and were expected to produce an amplicon of 550 bp.

Primer sequences used for the detection of the *nos* terminator were NOS1 5′ GAA TCC TGT TGC CGG TCT TG 3′ and NOS3 5′ TTA TCC TAG TTT GCG CGC TA 3′, producing a 180 bp amplicon in PCR (*19*). The primers used for synthetic *cry3A*, natural *cry3A*, and potato sucrose synthase detection were synthesized to order by Invitrogen Canada Inc. (Burlington, ON, Canada), whereas NOS1 and NOS3 were purchased from Sigma Genosys (The Woodlands, TX).

PCR. All amplification reactions were performed in a 50 μL volume using a Perkin-Elmer GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA). Final concentrations of the master mix components for the detection of the synthetic *cry3A*, natural *cry3A*, potato sucrose synthase, and *nos* terminator were as follows: 1× PCR buffer (Roche Applied Science), 1.5 mM $MgCl_2$ (Roche Applied Science), 0.2 mM dNTPs (Applied Biosystems), 1:M primers, 100 μg/mL bovine serum albumin (New England Biolabs, Beverly, MA), and 0.04 U/μL FastStart *Taq* DNA polymerase (Roche Applied Science). One or two microliters of DNA extracted from tuber material was used as a template in the reaction. This represented between 0.1 pg and 40 ng of DNA, depending on the experiment. For the processed foods, 2.5:1 of template DNA was used.

Following an initial denaturation step at 95 °C for 4 min, the reaction for the detection of both synthetic *cry3A* and natural *cry3A* and sucrose synthase proceeded for 35 cycles as follows: 30 s at 95 °C, 30 s at 57 °C, and 40 s at 72 °C. A final extension step of 3 min at 72 °C was also performed. Cycling conditions for the detection of the *nos* terminator were as follows (*19*): 95 °C for 4 min followed by 40 cycles of 30 s at 95 °C, 40 s at 64 °C, and 60 s at 72 °C, with a final extension of 3 min at 72 °C.

Agarose Gel Electrophoresis. PCR products were analyzed on 1.5% (synthetic *cry3A*, natural *cry3A*, and potato sucrose synthase) or 2% (*nos* terminator) agarose (Invitrogen) gels prepared and run in 0.5× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA; Sigma). A lane containing either a 50 bp ladder (Invitrogen) or a 100 bp ladder (New England Biolabs) was included on every gel as a molecular weight standard. All gels were run at 140 V (constant voltage) and stained with 0.5 μg/mL ethidium bromide (Invitrogen). Digital images of the gels were viewed and captured using the GeneGenius BioImaging system (Syngene, Cambridge, U.K.).

***Pvu*II Restriction Confirmation.** A 15 μL aliquot of the *cry3A* post-PCR reaction mix was directly digested with 10 units of *Pvu*II (New England Biolabs) for 2 h at 37 °C. Digestion products were analyzed on a 2% agarose gel as described above.

Sequencing. PCR product generated using DNA extracted from NL 30-RBK-350 leaf material and PCR product generated using DNA extracted from the *Btt* suspension were purified using the Qiaquick PCR purification kit (Qiagen Inc., Mississauga, ON, Canada). Sequencing of the purified products was performed by the Core Molecular Biology Facility at York University (Toronto, ON, Canada). C3A1 and C3A2 were used as primers to sequence both forward and reverse strands of the synthetic *cry3A* amplicon, whereas NC3A1 and NC3A2 were used to prime the forward and reverse reactions of the natural *cry3A* sequence. Sequence alignments were performed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and Clone Manager Professional Suite v. 6.00.

Practical Limit of Detection. Tuber tissue (∼200 g) was collected from 8 to 12 tubers from each of the four parental cultivars listed in **Table 1** and divided into two parts. Excess soil was first washed from the tubers, and tissue was collected to a depth of ∼0.5 cm from the surface and included the skin. To determine the practical limit of detection, 0.10 g of transgenic tuber material was homogenized with 100 g of tuber material from the appropriate parental cultivar (**Table 1**) in 3× v/w extraction buffer (described above) using a Waring blender. The remainder of the parental material was homogenized in the same manner without any transgenic material added. The homogenate containing the 0.1% transgenic material was serially diluted into a homogenate of the nontransgenic parental cultivar and the DNA extracted using the Wizard method as described above. Detection of synthetic *cry3A* in each extract was then attempted using PCR.

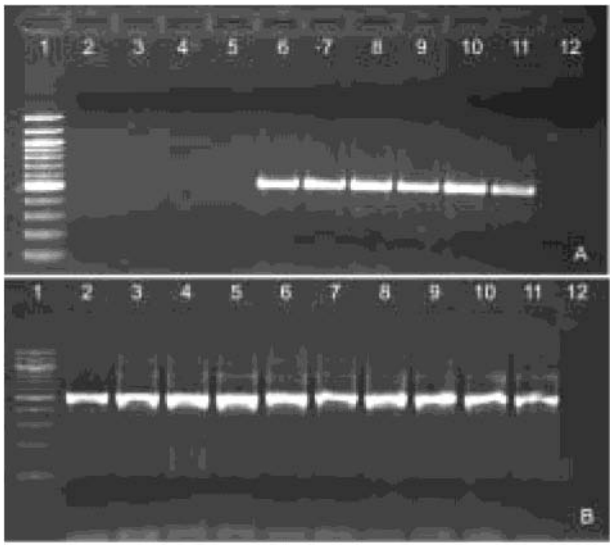

Figure 1. PCR of potato tuber extracts from different cultivars for synthetic *cry3A* (**A**) and sucrose synthase (**B**): (lane 1) 100 bp ladder; (lanes 2–5) nontransgenic cultivars Russet Burbank, Atlantic, Superior, and Shepody, respectively; (lanes 6–11) transgenic cultivars NL 30-RBK-350, NL 10-ATL, NL 20-SHE, NL 10-SUP, NL 20-RBK, NL 30-RBK-082, respectively; (lane 12) reagent control.

RESULTS AND DISCUSSION

Specificity. The primer sets C3A1 (forward primer) and C3A2 (reverse primer) were tested in PCR using template DNA extracted from several transgenic and nontransgenic tubers representing several different cultivars (**Table 1**). A product of 499 bp was successfully amplified from all of the transgenic cultivars, whereas no amplification products were obtained when template DNA extracted from the parental cultivars was used in PCR (**Figure 1A**). No inhibition of PCR was observed, as potato sucrose synthase was successfully amplified in all the cultivars (**Figure 1B**).

The identity of the amplicon generated in PCR using primers C3A1 and C3A2 was confirmed, as the sequence of the amplicon was identical to the sequence between bases 23 and 521 (inclusive) of the synthetic *cry3A* sequence reported by Perlak et al. (*9*) (**Table 2**). Homology of the C3A1/C3A2 amplicon to several naturally occurring *cry3A* sequences (*18*, *22*−*27*) was considerably less, at 76% (**Table 2**). The sequences of the seven naturally occurring sequences (**Table 2**) were all identical within this region.

Further sequence analysis indicated only the synthetic sequence had a *Pvu*II restriction site within the region homologous to the C3A1 + 2 amplicon. When digested with *Pvu*II, the PCR product from all of the transgenic cultivars yielded identical results, as the same two bands (at 216 and 276 bp) were generated (**Figure 2**). This strongly suggested that the same synthetic *cry3A* sequence was used in all of the registered NewLeaf cultivars. Because all of the documented naturally occurring *cry3A* sequences lack this site within the region homologous to the amplicon (*18*, *22*−*27*), this method was unlikely to detect naturally occurring *Btt*. This may be significant, because *Btt* is a naturally occurring soil bacterium that is sometimes used on potato fields as an organic pesticide.

The possibility that this method might detect naturally occurring *cry3A* was tested using an organic pesticide product containing a suspension of *Btt* endospores. **Figure 3** (lanes 1 and 2) shows that a primer set designed to amplify a 550 bp fragment of natural *cry3A* (NC3A1 + NC3A2) successfully

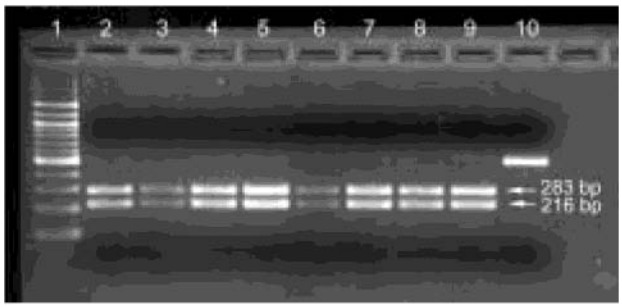

Figure 2. Confirmatory *Pvu*II restriction digest of the C3A1, C3A2 amplicon generated in PCR using DNA extracted from different transgenic cultivars: (lane 1) 100 bp ladder; (lanes 2–8) transgenic cultivars NL 30-RBK-350, NL 10-ATL (field-grown), NL 20-SHE, NL 10-ATL (greenhouse-grown), NL 10-SUP, NL 20-RBK, and NL 30-RBK-350, respectively; (lane 9) sequenced amplicon (digested); (lane 10) undigested amplicon.

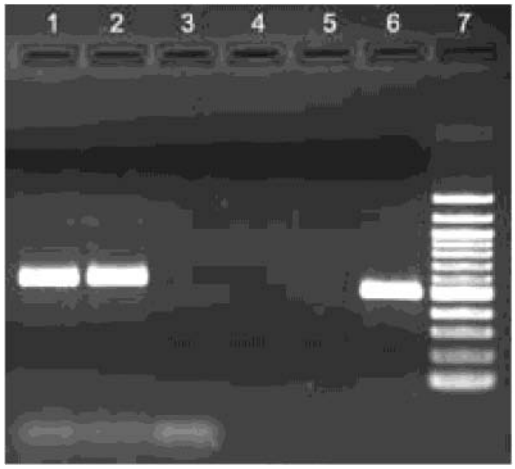

Figure 3. Demonstration of the specificity of primer pair C3A1 and C3A2 for synthetic *cry3A*: (lanes 1 and 2) *Btt* extract amplified using natural *cry3A*-specific primers NC3A1 and NC3A2; (lane 3) no template control of NC3A1/2 reaction; (lanes 4 and 5) PCR of *Btt* extracts using synthetic *cry3A*-specific primers C3A1 and C3A2; (lane 6) PCR of NL 10-ATL extract containing synthetic *cry3A* using primers C3A1 and C3A2 (postive control); (lane 7) 100 bp ladder.

Table 2. Comparison of the Sequence of the Amplicons Generated with Synthetic *cry3A*—Specific Primers C3A1 and C3A2 (Bases 1–499) and Natural *cry3A*—Specific Primers NC3A1 + NC3A2 (Bases 1–550) with Known cry3A Sequences[a]

	C3A1 + C3A2 (499 bp)		NC3A1 + NC3A2 (550 bp)	
GenBank accession no. (and ref)	homologous region (base no.)	homology (%)	homologous region (base no.)	homology (%)
X70979 (*9*)	23–521	100	1–462	63
J02978 (*18*)	404–903	76	294–843	>99
M22472 (*22*)	188–686	76	78–627	>99
Y00420 (*23*)	729–1227	76	619–1168	>99
M37207 (*24*)	732–1230	76	622–1171	>99
M30503 (*25*)	364–863	76	254–803	>99
U10985 (*26*)	732–1230	76	622–1171	>99
AJ237900 (*27*)	732–1230	76	622–1171	>99

[a] All sequences except X70979 (*9*) were identical within the regions of homology indicated (data not shown).

detected the presence of this gene in the extract. Sequence analysis of this amplicon indicated that it shared 99.8% homology with all reported naturally occurring *cry3A* sequences

Table 3. Specificity of PCR for the Detection of Synthetic *cry3A* in NewLeaf Transgenic Potato Tubers

cv.	no. of extracts tested	no. of positives detected
	Negative Controls	
Russet Burbank	6	0
Shepody	7	0
Atlantic	5	0
Superior	6	0
	Positive Controls	
NL 10-SUP	4	4
NL 10-ATL	5	5
NL 20-RBK	4	4
NL 20-SHE	5	5
NL 30-RBK-350	6	6
NL 30-RBK-082	4	4

(*18*, *22*−*27*) but shared only 63% homology with synthetic *cry3A* (*9*; **Table 2**).

The *Btt* extract containing the natural *cry3A* gene was then analyzed in PCR with the primer pair C3A1 and C3A2. There were no amplification products detected using the *Btt* extracts (**Figure 3**, lanes 4 and 5), whereas the positive control for the reaction, an extract of transgenic tuber cv. NL 10-ATL, was strongly positive (**Figure 3**, lane 6). This confirmed that primers C3A1 and C3A2 were specific for synthetic *cry3A* and that natural *cry3A* in *Btt*, which could be present in the soil associated with potato tubers, would not generate false positive results in seed potato testing.

The specificity of this PCR method for the detection of synthetic *cry3A* in potatoes was further evaluated. A total of 52 extracts of field-grown and greenhouse-grown tubers were tested for the presence of *cry3A* using this method. The samples represented all of the available transgenic NewLeaf cultivars (28 extracts) as well as the four parental cultivars (24 extracts). The PCR yielded no false negative or false positive results with any of the cultivars tested (**Table 3**). This strongly suggested that this PCR was specific for the detection of synthetic *cry3A* in NewLeaf potatoes.

Sensitivity. The sensitivity of the PCR method was evaluated in two ways: first, by examining the absolute limit of detection (LoD) by diluting pure DNA extracted from a transgenic tuber until *cry3A* could no longer be detected; and, second, by determining the lowest mass fraction of transgenic tuber material (as a proportion of nontransgenic tuber material) that could be detected following DNA extraction. Often referred to as the practical LoD, the latter approach takes into account the effects of the matrix on the DNA extraction (*28*), whereas the absolute LoD can be a useful comparative estimate of how well an amplification reaction is optimized (*28*).

To determine the absolute LoD, DNA from a transgenic NL 30-RBK-350 tuber was serially diluted 10-fold in nuclease-free water. PCR was performed in an attempt to detect *cry*3A in each dilution using 1000, 100, 10, 1, and 0.1 pg of template DNA in each reaction. **Figure 4** shows that *cry3A* could be detected in as little as 1 pg of total tuber DNA. This indicated that between one and two haploid copies of the genome could be detected using this method, as the size of a haploid copy of the potato genome has been estimated to be 0.9 pg (*29*).

The line of NL 30-RBK-350 used in this study contained two copies of the *cry3A* transgene (**Table 1**); therefore, the absolute limit of detection for the other lines that contained only one *cry3A* copy (i.e., SPBT02-5 of NL 10-SUP; **Table 1**) would likely be higher and would probably require four haploid copies of genomic DNA for detection. This range in absolute LoD of *cry3A* (two to four hapliod copies) is comparable to reported

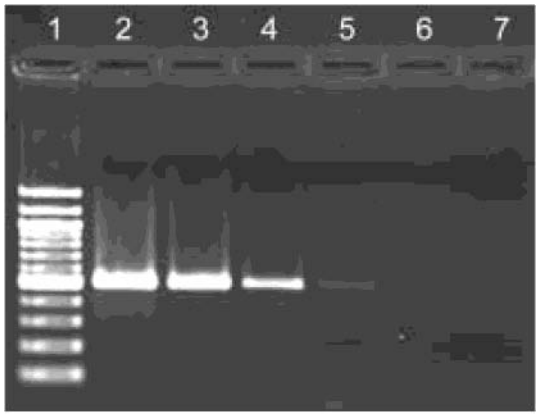

Figure 4. Absolute limit of detection in PCR of synthetic *cry3A* in a 10-fold dilution series of transgenic potato tuber DNA extract: (lane 1) 100 bp ladder; (lanes 2–7) PCR products generated with 1000, 100, 10, 1, 0.1, and 0 pg of DNA, respectively.

absolute LoDs of PCR methods for other novel *cry* genes. Event Bt 176 (*cry1Ab*) could be detected in two haploid copies of Maximizer corn DNA (*28*), Bt 11 (*cry1Ab*) could be detected in five haploid copies of corn DNA (*30*), and *cry9C* could be detected in five haploid copies of Starlink corn DNA (*31*). These results indicated, therefore, that the PCR for *cry3A* was performing with optimal efficiency compared with other methods for similar novel traits.

While determination of the absolute LoD can be useful in comparing how well a PCR method performs or how well it is optimized, it is not necessarily a useful measure of sensitivity in diagnostic samples containing small amounts of transgenic target in an excess of nontransgenic material (*28*, *30*). Under these circumstances, matrix composition, DNA extraction efficiency, and the quality of the extracted DNA can profoundly affect the LoD of any PCR method (*8*, *28*). The practical LoD, therefore, is a more realistic estimate of the LoD, which includes the effects of these influences on the PCR (*28*).

Sampling strategies used for seed potato certification often require a sample size of >400 tubers (or tuber pieces) for various analyses. It is critical, therefore, to determine the practical LoD of any method used for seed potato testing so that an appropriate sample composite size for each analysis can be chosen. The lower the practical LoD, the larger the composite size can be, and, consequently, fewer extractions and analyses need to be performed.

The practical LoD of this PCR method was determined by preparing homogenates of nontransgenic potato tuber tissue containing transgenic tuber tissue (NL 10-ATL, NL 20-SHE, NL 20-RBK, NL 30-RBK-350, and NL 30-RBK-082) at relative concentrations of 0.1, 0.01, 0.001, and 0% (w/w). Tuber tissue from the transgenic cultivar NL 10-SUP was blended at 0.1, 0.02, 0.01, and 0% (w/w) with nontransgenic tuber tissue (cv. Superior). DNA was subsequently extracted from these homogenates, and PCR for the detection of *cry*3A was performed using 1.0 μL (25−40 ng) of each of the DNA extracts as a template.

The practical LoD for *cry3A* in NL 10-ATL, NL 20-SHE, NL 20-RBK, NL 30-RBK-350, and NL 30-RBK-082 was 0.01%, whereas the LoD in NL 10-SUP was 0.02% (**Figure 5**). The LoD for *cry3A* may have been lower in this cultivar than for the other cultivars because the transgenic line used (SPBT02-5) contained only one copy of *cry3A* (**Table 1**). This is comparable, however, to the practical LoDs of the transgenic events Bt11, Bt 176, and MON810 in corn, which ranged

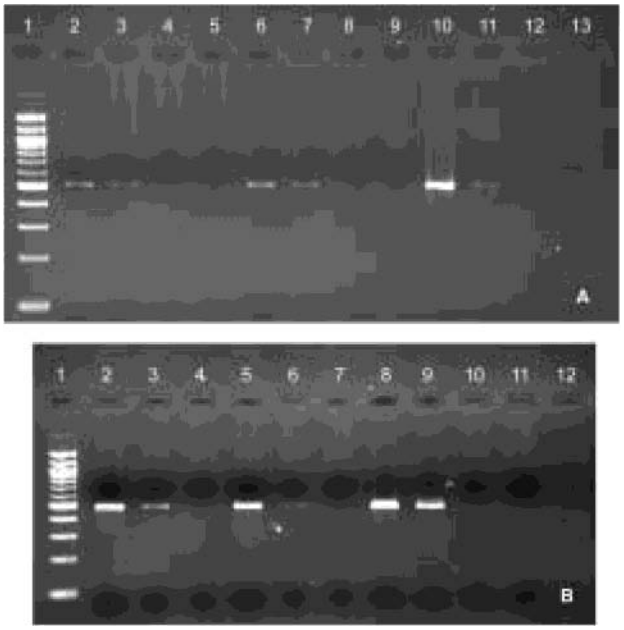

Figure 5. Practical limits of detection in PCR of synthetic *cry3A* in the different transgenic cultivars. Percentages refer to mass fraction of sample prior to extraction. (**A**) (lane 1) 100 bp ladder; (lanes 2–5) 0.1, 0.01, 0.001, and 0% NL 10-ATL, respectively, in Atlantic; (lanes 6–9) 0.1, 0.02, 0.01, and 0% NL 10-SUP, respectively, in Superior; (lanes 10–13) 0.1, 0.01, 0.001, and 0% NL 20-SHE, respectively, in Shepody. (**B**) (lane 1) 100 bp ladder; (lanes 2–4) 0.1, 0.01, and 0.001% NL 20-RBK, respectively, in Russet Burbank; (lanes 5–7) 0.1, 0.01, and 0.001% NL 30-RBK-350, respectively, in Russet Burbank; (lanes 8–10) 0.1, 0.01, and 0.001% NL 30-RBK-082, respectively, in Russet Burbank; (lane 11) Russet Burbank (0% transgenic); (lane 12) PCR reagent control.

between 0.01 and 0.05% using PCR (*32*). This is also comparable to the practical LoD of 0.05% in a test developed for the detection of NewLeaf Plus potatoes (*33*).

The respective intensities of the amplicon bands produced using template DNA extracted from NL 10-SUP and NL 10-ATL tubers at 0.1% (w/w nontransgenic cultivar) were less than those generated from the NL 20 and NL 30 cultivars (**Figure 5**). The reason for this is not clear at this time, but the fact that the NL 20 and NL 30 series of cultivars could have contained more copies of transgenic *cry3A* than the NL 10 series (**Table 1**) may have been a contributing factor. Alternatively, the Superior and Atlantic cultivars, used as the diluent homogenate in this experiment for the NL 10 series, may have had a greater inhibitory effect on PCR efficiency than the Shepody or Russet Burbank homogenates, which were used for the NL 20 and NL 30 series. Differences in the levels of substances that inhibit PCR, such as chlorogenic acid, have been previously observed in various potato cultivars (*34*).

With a practical LoD of ≤0.02%, this PCR for *cry3A* should be sufficiently sensitive for the screening of seed potato lots for the presence of transgenic NewLeaf tubers. Sample composites of several hundred tuber pieces could be used reliably. Because of the low band intensities observed with the NL 10 cultivars, sample composite size should perhaps be limited to 500 to reduce the risk of a false negative result.

Detection of *cry3A* in Processed Foods. Potatoes are used in a wide variety of processed food products. Treatments such as heating, blending, and bleaching, however, can all have a deleterious effect on the quality of the DNA that can be extracted from some processed foods, and many highly refined foods contain no detectable DNA (*8*). In an effort to determine whether

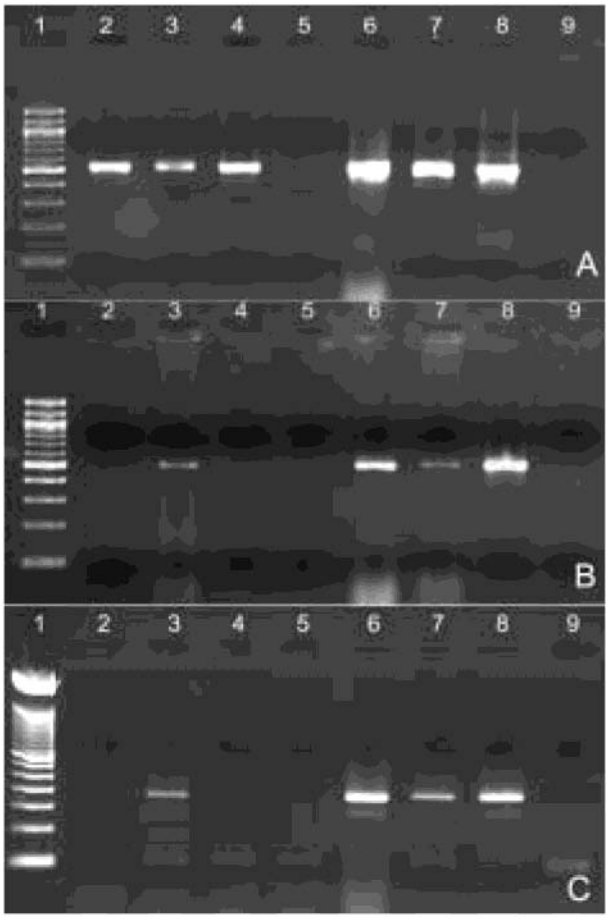

Figure 6. Detection of sucrose synthase (**A**), synthetic *cry3A* (**B**), and *nos* terminator (**C**) in processed foods: (lane 1) 100 bp marker (**A** and **B**), 50 bp marker (**C**); (lane 2) instant mashed potatoes; (lane 3) potato chips; (lane 4) potato flour; (lane 5) snack made from potato flour; (lane 6) potato shoestrings; (lane 7) frozen French fries; (lane 8) cv. NL 10-ATL tuber extract (positive control); (lane 9) no template control.

transgenic potatoes could be detected in processed foods using PCR, six products representing a variety of potato-derived foods were chosen for *cry3*A analysis. These products were instant mashed potatoes, potato chips, potato shoestrings, potato flour, a snack made from potato flour, and frozen French fries. These samples were tested for the presence of NewLeaf potatoes using this method for the detection of synthetic *cry3A*. PCR for the *nos* terminator was also performed as a confirmation test. As a check for the presence of amplifiable DNA of a suitable size, PCR for the detection of potato sucrose synthase was also carried out.

Potato sucrose synthase could be detected in all of the extracts except for the potato snack (**Figure 6**). Synthetic *cry*3A was detected in three of the six potato products tested (**Figure 6**). The potato chips, shoestring snacks, and frozen French fries all tested positive for synthetic *cry3A*. All three positive results were confirmed positive with *Pvu*II restriction digestion (**Table 4**). The presence of transgenic material in these products was also confirmed by the presence of the *nos* terminator (**Table 4**), which has been used to regulate the expression of transgenes in several novel crops. This indicated that this method for the detection of synthetic *cry3A* could be used in the qualitative screening for the presence of NewLeaf potatoes in at least some processed foods. However, because sucrose synthase could not be detected in the snack made from potato flour (**Figure 6**; **Table 4**), a conclusive result for *cry3A* (**Table 4**) in this product could not be obtained (**Table 4**). Although amplifiable DNA >536 bp was successfully recovered from all of the products including potato flour (**Figure 6**; **Table 4**), extraction of sufficient amplifiable DNA of a suitable size (i.e., >536 bp) may not be possible with more highly processed products such as the snack that was made from potato flour.

Table 4. Analyses of Processed Potato Food Products for Total DNA Yield and PCR Detection of Potato Sucrose Synthase, *cry*3A, and *nos* Terminator (T-*nos*)

		cry3A		
food product	sucrose synthase PCR	PCR	*Pvu*II digest[a]	T-*nos* PCR
instant mashed potatoes	+	–	nd[b]	–
potato chips	+	+	conf[c]	+
potato flour	+	–	nd	–
snack (made from potato flour)	–	inc[d]	nd	inc
potato shoestrings	+	+	conf	+
frozen French fries	+	+	conf	+

[a] *Pvu*II restriction digest of amplicon generated in PCR. The generation of two bands at 283 and 216 bp indicated confirmation of a positive result. [b] Not done, as there was no product to digest. [c] Confirmed. [d] Inconclusvie.

Although NewLeaf seed potatoes were withdrawn from the North American market prior to the 2001 planting season, some table acreage was planted that year; therefore, it was not surprising that transgenic potato material was detected in the processed foods. It should be noted, however, that this procedure was intended as a screening method and could not provide a measure of the proportion of transgenic material in the products.

Conclusions. The primers C3A1 and C3A2 specifically amplified the synthetic *cry3A* gene in all of the transgenic cultivars tested; synthetic *cry3A* could be detected in as few as one or two haploid copies of the transgenic potato genome. It should be noted that new transgenic cultivars may not contain the synthetic *cry3A* gene and therefore will not be detected with this method. The practical limit of detection was determined to be 0.02% for the NL 10-SUP and 0.01% in the remaining cultivars on a fresh weight basis. This would be sufficiently sensitive for the screening of seed tuber lots or processing lots for the presence of volunteer or adventitious transgenic tubers. It was also demonstrated that this method could detect transgenic potato material in processed foods such as potato chips, shoestring potatoes, and French fries but should probably not be used for more highly processed products.

SAFETY

Both ethidium bromide and Picogreen dye intercalate or bind DNA and should be treated as mutagenic and potentially carcinogenic. Picogreen reagent is supplied as a solution in dimethyl sulfoxide, which could facilitate the absorption of the dye through the skin. Appropriate personal protective equipment, particularly gloves, should be worn when handling these reagents. Refer to the Material Safety Data Sheets supplied with the reagents for further information.

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