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Identification of Genetically Modified Potato (*Solanum tuberosum*) Cultivars Using Event Specific Polymerase Chain Reaction

MARIE-JOSÉ CÔTÉ,*,† ALLISON J. MELDRUM,† PHILIPPE RAYMOND,‡ AND CHERYL DOLLARD†

Center for Plant Quarantine Pests, Ottawa Laboratory (Fallowfield), Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, Ontario K2H 8P9, Canada, and St. Hyacinthe Laboratory, Canadian Food Inspection Agency, 3400 Casavant Boulevard West, St. Hyacinthe, Québec J2S 8E3, Canada

Several genetically modified (GM) cultivars are registered in Canada although they are not currently in commercial production. The GM cultivars can be distinguished from the non-GM and other GM cultivars by analyzing the DNA nucleotide sequence at the insertion site of the transgene corresponding to a single transformation event in the plant genome. Techniques based on modified polymerase chain reaction (PCR) strategies were used to generate sequence information from the plant genome flanking the insertion site of transgenic DNA for specific GM potato events. The plant genome sequence adjacent to the transgenic insertion was used to design PCR primers, which could be used in combination with a primer annealing to one of the nearby inserted genetic elements to amplify an event specific DNA fragment. The event specific PCR fragments generated were sequenced to confirm the specificity of the method.

KEYWORDS: ***Solanum tuberosum*; GMO; NewLeaf; NewLeaf Y; NewLeaf Plus; genetically modified potato; polymerase chain reaction; plant border region; event specific identification**

INTRODUCTION

Many genetically modified (GM) plant cultivars have been registered worldwide. For example, in 2000, there were seven cultivars of GM potatoes registered in Canada (*1*). It is not possible to distinguish the GM cultivars from the non-GM and other GM cultivars on the basis of morphological plant characteristics, but they can be distinguished by characterizing the engineered modification of the plant genome. Polymerase chain reaction (PCR)-based methods can be used to amplify genetic elements inserted in plants to detect the presence of GM events. While particular GM potato events can be detected by these amplification methods, the methods are limited in being able to discriminate among GM cultivars as they target only the genetic modification. Genetic modifications could be similar from one GM cultivar to another (*2*–*5*).

The registered GM potato cultivars in Canada are Monsanto's trademark NewLeaf lines (*1*). For some of these lines, there are distinct GM potato cultivars sharing the same genetic construct or a potato cultivar transformed with a genetic construct for which two or more events are registered under the same GM cultivar name (*6*). In some cases, only a subset of the events approved for unconfined release into the environment are registered cultivar in Canada (*1*). The sequence of the insertion site in the plant genome corresponding to a single transformation event can be characterized, and this information can be used to design a PCR method to identify a specific GM potato events. Because of differences in regulatory status of present and future GM potato cultivars worldwide, it is important to develop methodologies that can specifically identify each event to allow proper regulatory control of GM cultivars.

The characterization of the Roundup Ready soybean genetic insert (*7*) provided information on the sequences flanking the insertion site, which was then used to develop a GM event specific identification method. One of the techniques the authors used for the determination of Roundup Ready soybean plant border regions is based on adaptor-mediated PCR (*8*). This technique, the supported PCR (*9*) technique, and the universal fast walking (UFW) (*10*) technique were adapted and established in our laboratory for potato to accomplish the first objective of this work, which is to determine the DNA nucleotide sequence of the genome–insertion border region. The sequence of the plant region bordering the transgenic insertion site was determined for five GM potato events. The second objective is to develop a PCR method, based on the nucleotide sequence of the border area, to identify GM potato events. Border specific primers were each paired with a primer designed from a nearby transgenic element to direct the amplification of an event specific DNA fragment across the plant–insertion junction. All event

* To whom correspondence should be addressed. Tel: 613-228-6698. Fax: 613-228-6676. E-mail: cotemj@inspection.gc.ca.
† Ottawa Laboratory (Fallowfield).
‡ St. Hyacinthe Laboratory.

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Table 1. List and Description of Primers and Their Sequences

primer name	target element	sequence
AP1	original adaptor (1st PCR)	5′-GGATCCTAATACGACTCACTATAGGGC-3′
AP2	original adaptor (2nd PCR)	5′-TATAGGGCTCGAGCGGC-3′
NewAP1	modified adaptor (1st PCR)	5′-GGATCCTATTAGGACACAGTATTGGGC-3′
NewAP2	modified adaptor (2nd PCR)	5′-TATTGGGCTCCAGCGGC-3′
FL	linker and primer	5′-ACCCGGAATTCCGTACCGCGACTTG-3′
FLS	linker used with FL	5′-CAAGTCGCGGT-3′
LBDR-1	ti-plasmid left border region	5′-GCCTATAAATACGACGGATC-3′
LBDR-2	ti-plasmid left border region	5′-ATTGCTGATCCATGTAGATTTCC-3′
LBDR-3	ti-plasmid left border region	5′-ATAACGCTGCGGACATCTAC-3′
NOSP-1R	nopaline synthase promoter	5′-CGTTGCGGTTCTGTCAGTTCC-3′
RTBDR-3R	ti-plasmid right border region	5′-GATTGTCGTTTCCCGCCTTC-3′
UFW#1	pea rubisco E9 terminator	5′-CTAGGATCACAGAGTCATGACGAGTATTATGGCATTGGGAAAACTG-3′
UFW#2	pea rubisco E9 terminator	5′-GGATGGAGAAGAGTTAATGAATGATATGGTCCNNNNNNNNNN-3′
UFW#3	pea rubisco E9 terminator	5′-TACCATTTGTTGTGCTTGTAATTTA-3′
UFW#4	pea rubisco E9 terminator	5′-TGTTGTGTGTTGAATTTGAAA-3′
UFW#5	pea rubisco E9 terminator	5′-AACTGTGAAATGGAAATG-3′
UFW#6	pea rubisco E9 terminator	5′-TAAGAGATATGCAAACAT-3′
UFW#7	pea rubisco E9 terminator	5′-AATCGTGGCCTCTAATGACC-3′

specific primer pairs were tested on several GM potato events and PCR with each primer pair specifically amplifying a DNA fragment of the event for which it was designed.

MATERIALS AND METHODS

Potato Cultivars. The following potato cultivars used for this work were obtained as tubers from the Canadian Food Inspection Agency, Centre for Animal and Plant Health, Charlottetown, PEI, Canada: non-GM Atlantic, Shepody, Superior, Russet Burbank, and GM NL10-ATL (two events), NL10-SUP, NL20-SHE, NL20-RBK, NL30-RBK-082, and NL30-RBK-350. Plantlets were grown from tubers in Murashige and Skoog basal medium with sucrose and agar (Sigma, St. Louis, MO) until the plantlet could provide a minimum of 0.3 g of tissue for DNA extraction.

DNA Extraction. Using a mortar and pestle, 0.3−0.5 g of frozen plant tissue was ground to a powder in liquid nitrogen. Five milliliters of CTAB lysis buffer [1% (w/v) CTAB, 5% poly(vinylpyrrolidone) (PVP), 1.4 M NaCl, 20 mM EDTA, 10 mM Tris-HCl, pH 8.0, and 350 mM 2-mercaptoethanol] was added to the powdered plant tissue in a 50 mL polypropylene tube. After gentle swirling, the mixture was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). The aqueous phase was transferred to a clean polypropylene tube and extracted again with an equal volume of chloroform/isoamyl alcohol (24:1). After extraction, the aqueous phase was transferred to a clean tube and DNA was precipitated with 2.5 volumes of 95% ethanol. After centrifugation at 4000*g* for 5 min at room temperature, the supernatant was poured off and the pellet was resuspended in 2 × 100 μL of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0) and transferred to a 1.5 mL tube. The DNA was precipitated again using 1 volume of 5 M ammonium acetate and 2 volumes of 95% ethanol. After centrifugation at maximum speed for 30 min at 4 °C, the ethanol was discarded and the pellet was washed with 70% ethanol. Once dried, the DNA pellet was resuspended in 50 μL of 1 × TE and treated with 1.0 μg of RNase (DNase-free; Roche, Laval, QC, Canada). DNA was then visualized on a 1% agarose gel and quantified by comparison of the intensity relative to the High DNA Mass Ladder (Invitrogen Life Technologies, Burlington, ON, Canada) band of the most comparable size.

Supported PCR. Supported PCR was carried out according to the protocol of Rudenko et al. (*9*) using the GeneAmp PCR system 9600 thermal cycler (Applied Biosystems, Foster City, CA). Essentially, the technique involves the amplification of digested plant DNA in the presence of biotin targeting the desired area. After selective isolation of the fragment on streptavidin-agarose, linkers are ligated to the fragment and it is amplified using primers specific to the linkers. The restriction enzyme used was *Bfa*I (New England Biolabs, Beverly, MA). The primers (**Table 1**) were synthesized by Invitrogen Life Technologies. The primer used for the target enrichment step was designed from the ti-plasmid right border region (*11*). The plant border region of the GM potato event obtained using this technique is shown in **Table 2**.

Table 2. List of GM Potato Events, Methods, and Primers Used for the Cloning of Plant DNA Bordering the Transgenic Insertion

GM potato cultivar name	method	primers used for the amplification of plant border sequences
NL10-ATL	adaptor-ligation with *Mse* I restriction digest	1st PCR: AP1 + LBDR-1 2nd PCR: AP2 + LBDR-2
NL10-SUP	UFW	UFW#1, UFW#2 1st PCR: UFW#3 +UFW#4 2nd PCR: UFW#5 +UFW#6 UFW#7 for sequencing
NL20-RBK	supported PCR	ligated linkers: FL + FLS PCR: RTBDR-3R + FL
NL20-SHE	adaptor-ligation with *Mse* I restriction digest	1st PCR: AP1 + NOSP-1R 2nd PCR: AP2 + LBDR-3
NL30-RBK-350	adaptor-ligation with *Mse* I restriction digest	1st PCR: NewAP1 + LBDR-1 2nd PCR: NewAP2 + LBDR-3

Adaptor-Ligation PCR. Adaptor-ligation PCR was carried out following a modification of the protocol of Spertini et al. (*8*). Briefly, digested plant DNA is ligated to linkers and amplified using primers specific to the linker sequence and to the desired area. The restriction enzymes used were *Mse*I, *Taq*I, and *Bfa*I. A description and sequences of the primers used are listed in **Table 1**. Primers used for the specific portion of the technique were designed from the ti-plasmid left border region (*12*) and the nopaline synthase promoter region (*11*). The list of primers used for each GM potato event is shown in **Table 2**. The amplifications were carried out on a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems) using FastStart Taq Polymerase (Roche). Cycling conditions were modified for the PCR reactions by changing the first heating step to 95 °C for 4 min and performing 37 cycles in the primary PCR and 30 cycles in the nested PCR. Primers were synthesized by Invitrogen Life Technologies.

UFW. An adaptation of the UFW technique (*10*) was used with primers listed in **Table 1**. Briefly, UFW involves five primers targeting known sequences in the desired area. One of the primers has a string of random nucleotides allowing for nonspecific annealing to the neighboring unknown region forming a lariat. After digestion in the known sequence to release the lariat, the targeted unknown region can be amplified using primers specific to the known sequence now on each side the desired area. Primers were synthesized by Invitrogen Life Technologies and based on the terminator region of the pea rubisco small subunit gene (*11*). All steps were carried out on a GeneAmp

Table 3. List of the Event Specific PCR Established and PCR Product Generated

registered cultivar	commercial name	event name	traits conferring resistance to	availability of plant material	event specific plant PCR primer	transgenic element PCR primer	PCR product (bp)
NL10-ATL	NewLeaf Atlantic	ATBT04-06 ATBT04-31 ATBT04-36	Colorado potato beetle (CPB)	2 of the 3 events were available	NL10ATL01PLT5[a]: 5′-AGT GAA GCA TCA CGC CAT C-3′	NPTII-3R: 5′-AGT CAT AGC CGA ATA GCC TC-3′	829
NL10-RBK	NewLeaf Russet Burbank	BT06, BT10, BT12, BT17	Colorado potato beetle (CPB)	not available	not applicable	not applicable	NA
NL10-SUP	NewLeaf Superior	SPBT02-05	Colorado potato beetle (CPB)	yes	NL10SUPPLT3: 5′-GCT TAT GGA GAA CAC AGT GG-3′	NOST-3R: 5′-ACA TGC TTA ACG TAA TTC AAC AG-3′	265
NL20-RBK	NewLeaf Y Russet Burbank	RBMT15-101	CPB and potato virus Y (PVY)	yes	NL20RBKPLT1: 5′-AAC TGA AAG TGG TAT AAT TG-3′	FMVP-1: 5′-GTG ATA TGG CTC GTA CCT TG-3′	192
NL20-SHE	NewLeaf Y Shepody	SEMT15-02	CPB and potato virus Y (PVY)	not available	not applicable	not applicable	388
		SEMT15-15	CPB and potato virus Y (PVY)	yes[b]	NL20SHEPLT3[a]: 5′-TCG TAT ATA CAC TTT ATG CG-3′	NOSP-1R: 5′-CGT TGC GGT TCT GTC AGT TCC-3′	
NL30-RBK-082	NewLeaf Plus Russet Burbank	RBMT22-082	CPB, PVY, and potato leaf roll	yes	not available	not applicable	NA
NL30-RBK-350	NewLeaf Plus Russet Burbank	RBMT21-350	CPB, PVY, and potato leaf roll	yes	NL30RBK350PLT2: 5′-ACA ACA TAG CAA ACA CAA TC-3′	NOSP-1R	419

[a] Available for one event. [b] Event name confirmed using the method described by Watanabe et al. (*5*).

PCR system 9700 thermal cycler (Applied Biosystems). Primer-directed specific first strand synthesis was performed using 7.5 pmol of primer UFW#1 with the following modifications. Deep Vent (exo-) Polymerase (New England Biolabs) was used in 25 μL of UFW buffer (2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 M KCl, and 0.2 M Tris-HCl, pH 8.4). The annealing temperature was modified to 62 °C for 1 min, and the extension was done at 72 °C. Exonuclease I digestion was performed as described and then inactivated at 80 °C for 20 min. Tagged random priming was done using 25 pmol of primer UFW#2. After denaturation, the primer was annealed at a temperature decreasing from 70 to 37 °C (1 °C per 30 s). Digestion of free primer and first strand trimming was performed with 5 units of Exonuclease 1. The sequence conversion at the 3′-end of the first strand was performed at 72 °C instead of 68 °C. The first strand lariat was formed by first heating the mixture at 94 °C for 5 min followed by a slow cooling from 70 to 40 °C (1 °C per 30 s) allowing intrastrand annealing. The strand was then extended at 72 °C for 5 min. An unrolling amplification step was added using 2 units of N.BstNB1 (New England Biolabs) diluted in 5 μL of UFW buffer with incubation steps at 55 °C for 30 min and 72 °C for 15 min followed by a 20 min enzyme inactivation at 80 °C. The primary PCR was performed using 0.5 units of Deep Vent (exo-) Polymerase and 10 pmol of each of the UFW#3 and UFW#4 primers. Cycling parameters were 40 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 2 min, and with a final extension step at 72 °C for 5 min. A secondary PCR was performed as well using 1 μL of the primary PCR amplification, 4 pmol each of the UFW#5 and UFW#6 primers, 1 unit of platinum Taq DNA polymerase (Invitrogen Life Technologies) all in a final volume of 20 μL of UFW buffer. After initial denaturation at 94 °C for 2 min, the primary PCR product was incubated at 94 °C for 30s, then 60 °C for 30 s, and at 72 °C for 1 min for 35 cycles before a final extension at 72 °C for 7 min.

Cloning and Sequencing. DNA fragments generated from supported PCR, adaptor-ligation PCR, or the UFW technique were eluted from 1.5% (w/v) agarose gels run in 1 × TBE (0.089 M Tris-borate, pH 8.3, and 0.002 M EDTA) using either the MinElute Gel Extraction kit, the QIAquick gel extraction kit (Qiagen, Mississauga, ON, Canada), or the Geneclean II kit (Q-Biogene, Carlsbad, CA). Eluted DNA was treated with T4 DNA polymerase (Invitrogen Life Technologies) to generate blunt ends and phosphorylated using T4 polynucleotide kinase (Invitrogen Life Technologies) following the manufacturer's instructions. These fragments were then cloned into pBluescript II SK+ (Stratagene, La Jolla, CA), and plasmid DNA was transformed into chemically induced competent DH5α *Escherichia coli* cells (Invitrogen Life Technologies). Plasmid DNA was extracted using the GenElute Plasmid Miniprep Kit (Sigma). Sequencing was carried out by Mobix Lab (McMaster University, Hamilton, ON, Canada) or using the SequiTherm EXCEL II DNA Sequencing Kit-LC (Epicenter Technologies, Madison, WI) on the LI-COR DNA analyzer (LI-COR Biosciences, Lincoln, NE).

Event Specific PCR. PCR reactions using event specific primers were performed using Invitrogen Life Technologies reagents unless otherwise indicated. Approximately 3−75 ng of GM and non-GM potato DNA was amplified in 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 200 μM each dNTP, 2.5 mM $MgCl_2$, 0.4 μM of each of the specific primers, and 0.5 units of HotStarTaq DNA polymerase (Qiagen) in a total volume of 20 μL. The reactions were carried out in a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems), with an initial denaturation at 95 °C for 10 min followed by 40 cycles of 95 °C for 30 s, 58−60 °C for 30 s, an extension at 72 °C for 30 s and, at the end of the last cycle, a final extension at 72 °C for 7 min. The event specific primers used are described in **Table 3**. Primers for neighboring transgenic regions were designed from the nopaline synthase promoter and terminator regions (*11*, *14*), the neomycin phosphotransferase II gene (*15*), and the promoter region of the figwort mosaic virus (*16*). Potato sucrose synthase primers (Pss01n-5′, 5′-TGA CCT GGA CAC CAC AGT TAT-3′; Pss01n-3′, 5′-GTG GAT TTC AGG AGT TCT

TCG A-3′) were used to verify the quality of the GM and non-GM DNAs (*2*). Amplified products were electrophoresed through 1.5% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light.

RESULTS AND DISCUSSION

Availability of GM Potato Cultivars. Because GM potato cultivars are currently not grown commercially in Canada, it was only possible to obtain six of the seven GM cultivars registered in Canada. The cultivar registered under the New Leaf Russet Burbank name was not available, and only two of the three events registered under the New Leaf Atlantic name were obtained (**Table 3**). For some GM potato cultivars, different events are registered under the same cultivar name and each event is the same GM construct but inserted at different locations in the plant genome. Obtaining plants with specific events registered under the same cultivar name was challenging. However, the acquisition of the two events registered under the cultivar name New Leaf Atlantic allowed us to validate the event specific method described herein.

Determination of the GM Plant Genome Sequence Next to the Transgenic Insertion. Three techniques were used for generating DNA fragments containing sequences of the transgenic insertion from either the 5′- or the 3′-end (**Table 2**). The first technique, supported PCR (*9*), is based on enrichment of the desired DNA fragment using biotin labeling and selective isolation on streptavidin-agarose and was first applied to retrieve T-DNA flanking sequences of transgenic tomato plants (*9*). The selected DNA fragment is ligated to linkers and amplified. Using the primers listed in **Table 2**, it was possible to obtain plant sequences ∼75 bp long for the GM cultivar NL20-RBK (NewLeaf Y Russet Burbank, event RBMT15-101) adjacent to the inserted T-DNA right border region and the FMV promoter sequence designed to drive the transcription of the potato virus Y coat protein gene (*6*). This method was also used for other transgenic events but was unsuccessful possibly due to poor biotinylation of the targeted fragment resulting from an inefficient PCR. The adaptor-ligation PCR (*8*) was used as an alternative method. Following restriction digestion of the total GM plant DNA, adaptors were ligated to the plant DNA to allow two series of amplifications using primers specific to the ligated adaptor and a specific sequence in the target region. Using the nonmodified adaptor listed in **Table 1**, we obtained plant sequences ∼80 bp long followed by the T-DNA integrated left border region for one of the events of the GM cultivar NL10-ATL (NewLeaf Atlantic, one of the ATBT04 events). A GenBank search revealed that some nonspecific sequences recovered at a high rate after performing the adaptor-ligation PCR technique corresponded to promoter consensus sequences. We therefore redesigned primers AP1 and AP2 (**Table 1**) so that they would no longer resemble the promoter sequences. Following this modification, it was possible to obtain ∼60 and ∼35 bp of plant sequences adjacent to the inserted T-DNA border region for NL20-SHE (NewLeaf Y Shepody, event SEMT15-15) and NL30-RBK-350 (NewLeaf Plus Russet Burbank, event RBMT21-350), respectively. Both supported PCR and adaptor-ligation PCR techniques failed to amplify border regions for cultivar NL10-SUP (NewLeaf Superior). This failure is attributed to the uncertainty surrounding the sequences of the T-DNA left border region inserted into the plant genome (*6*). The need to recover DNA fragments of a large size from the site of a transgenic element confirmed to be present in the plant genome led us to use the UFW technique (*10*). This technique relies on a series of five primers derived from the sequence of the transgenic element, which, for NL10-SUP (NewLeaf Superior, event SPBT02-05), is the pea E9 terminator (*6*). The key step of this technique is the use of primers (**Table 1**; UFW#2) with sequences found in the E9 terminator and adjacent to a string of random nucleotides allowing nonspecific annealing into the unknown region. After nonspecific annealing, the known portion of these primers, which are E9 terminator sequences, causes a lariat formation leading to the extension of the unknown sequence with E9 terminator sequences. The end result is the formation of a DNA fragment of the unknown sequence bordered on each side with E9 terminator sequences, which is amplified using primers that anneal to each side of the E9 terminator sequences. Using this technique, the sequence of the DNA fragment generated for NL10-SUP revealed the presence of the NOS terminator sequence followed by sequences aligned to a portion of the NPTII gene and close to 90 bp of the plant genome. The presence of a partial sequence of NPTII is probably due to the insertion of a truncated version of the T-DNA and therefore would explain the problems encountered using the two previous techniques (*6*).

The plant genomic sequences that we obtained did not align significantly to any of the sequences in GenBank. It was therefore assumed that our sequences correspond to plant sequences bridging the T-DNA insertion site. Several sequences were obtained for each of the other transgenic events, NL10-ATL-02 (NewLeaf Atlantic, another line of the ATBT04 events) and NL30-RBK-082 (NewLeaf Plus Russet Burbank, event RBMT22-082). However, the identity of the PCR DNA fragments generated could not be confirmed because none of the T-DNA transgenic elements expected to be present in these events were recognized. Lack of publically available specific sequence information for the border regions might be partly the cause as we may have been designing primers for T-DNA transgenic areas that were never inserted in these particular potato events. It has been shown that T-DNA insertions are not precise, resulting in loss of large segments of either the right or the left border regions (*17*). Another possibility is that the targeted transgenic insertions (NL10-ATL-02 and NL30-RBK-082) are situated in a very highly repetitive area of the plant genome. Therefore, generating amplification problems as the plant primer would anneal to multiple sites leading to the characterization of artifactual PCR DNA fragments.

Identification of GM Potato Lines Using Event Specific PCR. Primers corresponding to plant sequences bordering the transgenic insertion site were designed within 35−90 bases away from the last recognizable left or right border of the T-DNA inserted into the plant genome. DNA isolated from single GM potato events was amplified using the plant genome primer and a primer annealing to a transgenic element within a distance that would produce a DNA fragment between 190 and 830 base pairs. For some of the events, there were several attempts to design a plant primer that would specifically amplify the event as a single band, but instead, several PCR bands or smears were obtained from the primers. These nonspecific results would be likely due to the probable repetitive nature of the plant genome disrupted by the T-DNA. Plant primers used in combination with transgenic element primers and leading to the amplification of an event specific PCR product were retained and are listed in **Table 3**. **Figure 1B−E** shows the specificity of all event specific primer combinations developed. Each event specific primer pair only amplified a DNA fragment from potato tissue containing the transgenic event for which it was designed. This specificity is clearly demonstrated by the amplification of a PCR product for only one of the two NL10-ATL lines (**Figure 1C**).

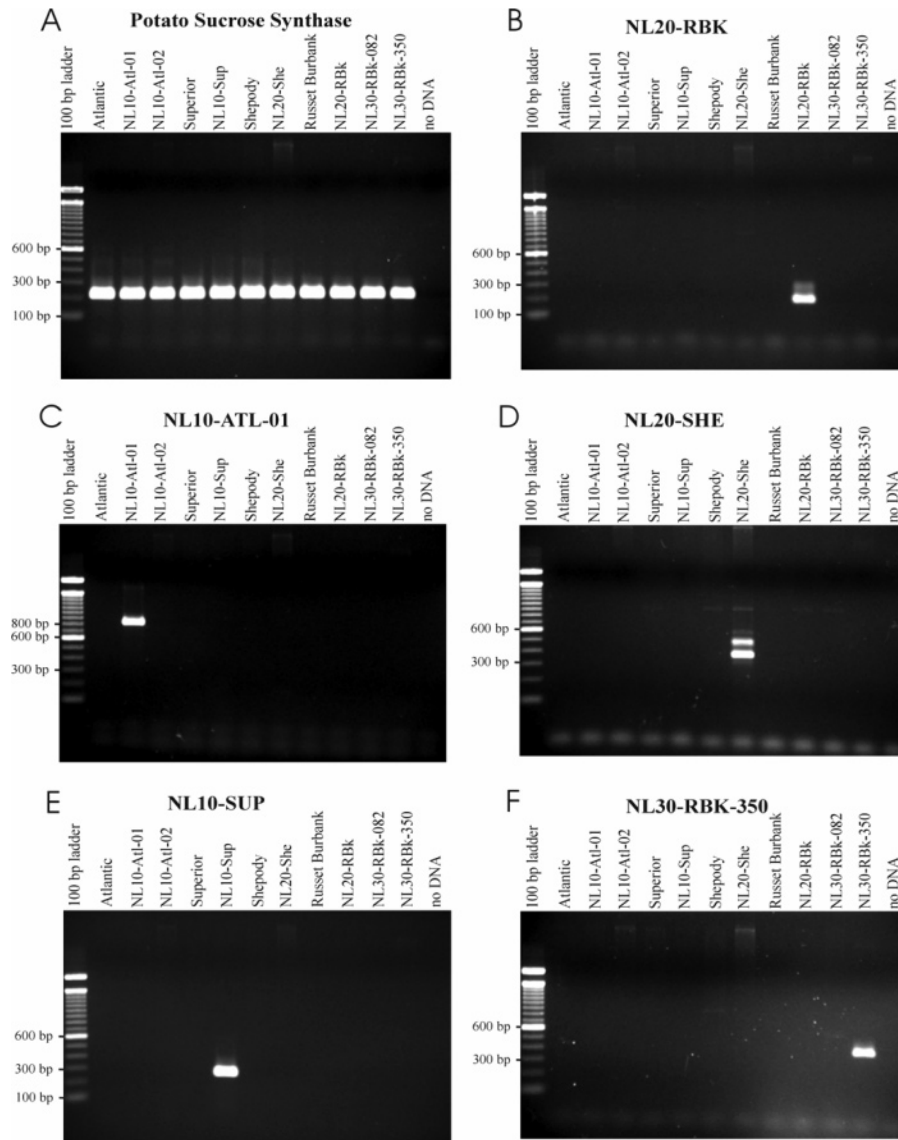

Figure 1. Event specific PCR assay using DNA extracted from GM and non-GM equivalent potato cultivars. The primers used are as follows: Pss01n-5′ with Pss01n-3′ (**A**), NL20RBKPLT1 with FMVP-1 (**B**), NL10ATLPLT5 with NPTII-3R (**C**), NL20SHEPLT3 with NOSP-1R (**D**), NL10SUPPLT3 with NOST-3R (**E**), and NL30RBKPLT2 with NOSP-1R (**F**). The sizes (base pairs) of selected fragments of the 100 bp ladder are indicated on the left.

All event specific PCR products were sequenced to confirm the identity of the fragments amplified (data not shown). **Figure 1A** shows the amplified DNA fragment representing the potato sucrose synthase gene when primers (*2*) were used in PCR of all DNA extracts, demonstrating that lack of amplification using event specific primers was not caused by inhibitors or poor DNA quality.

Applicability of GM Potato Event Specific PCR Method for Cultivar Identification. The design of PCR primers to amplify a DNA fragment corresponding to the junction between the plant genome and the transgenic insertion led to the development of a PCR method specific to the event. Event specific methods have been developed for GM crops such as corn, potato, and soybean (*5*, *7*, *18*−*22*). For each of these methods, the amplification of a DNA fragment corresponding to the junction between the plant genome and the transgenic insert has resulted in a highly specific method of identification, which would likely not become obsolete with the increasing development of GM cultivars. Recently, plant border region sequences were used to design event specific primers (NL20 series) for the last step of a GM potato detection scheme (*5*). For some of the GM potato cultivars registered in Canada (*1*), a single event is registered under one cultivar name; therefore, the event specific method developed herein would allow the differentiation of the registered cultivar in case of dispute. For cultivars NL10-ATL, NL10-RBK, and NL20-SHE (**Table 3**), three, four, and two events, respectively, were registered under the same cultivar name, therefore requiring the design of more than one set of event specific primers. This is well-demonstrated for NL10-ATL where event specific primers used for PCR in **Figure 1C** amplified a DNA fragment from one of the two lines tested. Similar results were obtained by Watanabe et al. (*5*) for NL20-SHE. For regulatory purposes, the event specific methods would effectively identify cultivars if event specific PCR methods are available for all multiple events approved under a single cultivar designation. An obvious challenge in this approach is the difficulty in the acquisition of representative material for all events registered under the same cultivar name. This is directly related to the difficulty in identifying these commodities at the event level in any way other than by using an event specific identification method such as the one described herein. Detection of GM cultivars is further complicated by the lack of reference material. The future registration of only one

event for each GM potato cultivar would definitely simplify cultivar identification.

ABBREVIATIONS USED

GM, genetically modified; PCR, polymerase chain reaction; UFW, universal fast walking.

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LITERATURE CITED

(1) Canadian Food Inspection Agency Internet site: http://www.inspection.gc.ca/english/plaveg/variet/pntvcne.shtml. http://www.inspection.gc.ca/english/plaveg/bio/pntvcne.shtml.

(2) Akiyama, H.; Sugimoto, K.; Matsumoto, M.; Isuzugawa, K.; Shibuya, M.; Goda, Y.; Toyoda, M. A detection method of recombinant DNA from genetically modified potato (NewLeaf Plus potato) and detection of NewLeaf Plus potato in snack. *Shokuhin Eiseigaku Zasshi* **2002**, *43*, 24−29.

(3) Jaccaud, E.; Hohne, M.; Meyer, R. Assessment of screening methods for identification of genetically modified potatoes in raw materials and finished products. *J. Agric. Food Chem.* **2003**, *51*, 550−557.

(4) Rho, J. K.; Lee, T.; Jung, S. I.; Kim, T. S.; Park, Y. H.; Kim, Y. M. Qualitative and quantitative PCR methods for detection of three lines of genetically modified potatoes. *J. Agric. Food Chem.* **2004**, *52*, 3269−3274.

(5) Watanabe, T.; Kuribara, H.; Mishima, T.; Kikuchi, H.; Kodama, T.; Futo, S.; Kasama, K.; Toyota, A.; Nouno, M.; Saita, A.; Takahashi, K.; Hino, A.; Akiyama, H.; Maitani, T. New qualitative detection methods of genetically modified potatoes. *Biol. Pharm. Bull.* **2004**, *27*, 1333−1339.

(6) BATS. Centre for Biosafety and Sustainability Internet site: http://www.gmo-watch.org/GVO-report140703.pdf.

(7) Windels, P.; Taverniers, I.; Depicker, A.; Van Bockstaele, E.; De Loose, M. Characterisation of the Roundup Ready soybean insert. *Eur. Food Res. Technol.* **2001**, *213*, 107−112.

(8) Spertini, D.; Béliveau, C.; Bellemare, G. Screening of transgenic plants by amplification of unknown genomic DNA flanking T-DNA. *BioTechniques* **1999**, *27*, 308−312.

(9) Rudenko, G. N.; Rommens, C. M. T.; Nijkamp, H. J. J.; Hille, J. Supported PCR: An efficient procedure to amplify sequences flanking a known DNA segment. *Plant Mol. Biol.* **1993**, *21*, 723−728.

(10) Myrick, K. V.; Gelbart, W. M. Universal Fast Walking for direct and versatile determination of flanking sequence. *Gene* **2002**, *284*, 125−131.

(11) Depicker, A.; Stachel, S.; Dhaese, P.; Zambryski, P.; Goodman, H. M. Nopaline synthase: Transcript mapping and DNA sequence. *J. Mol. Appl. Genet.* **1982**, *1*, 561−573.

(12) Barker, R. F.; Idler, K. B,; Thompson, D. V.; Kemp, J. D. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. *Plant Mol. Biol.* **1983**, *2*, 335−350.

(13) Coruzzi, G.; Broglie, R.; Edwards, C.; Chua, N.-H. Tissue-specific and light-regulated expression of pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. *EMBO J.* **1984**, *3*, 1671−1679.

(14) Bevan, M.; Barnes, W. M.; Chilton, M.-D. Structure and transcription of the nopaline synthase gene region of T-DNA. *Nucleic Acids Res.* **1983**, *11*, 369−385.

(15) Beck, E.; Ludwig, G.; Auerswald, E. A.; Reiss, B.; Schaller, H. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene* **1982**, *19*, 327−336.

(16) Richins, R.; Scholthof, H. B.; Shepherd, R. J. Sequence of figwort mosaic virus DNA (caulimovirus group). *Nucleic Acids Res.* **1987**, *15*, 8451−8466.

(17) Binns, A. N.; Thomashow, M. F. Cell biology of *Agrobacterium* infection and transformation of plants. *Annu. Rev. Microbiol.* **1988**, *42*, 575−606.

(18) Hernández, M.; Pla, M.; Esteve, T.; Prat, S.; Puigdomènech, P.; Ferrando, A. A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard based on the 3′-transgene integration sequence. *Transgenic Res.* **2003**, *12*, 179−189.

(19) Windels, P.; Bertrand, S.; Depicker, A.; Moens, W.; Bockstaele, E.; Loose, M. Qualitative and event-specific PCR real-time detection methods for StarLink maize. *Eur. Food Res. Technol.*, **2003**, *216*, 259−263.

(20) Rønning, S. B.; Vaïtilingom, M.; Berdal, K. G.; Holst-Jensen, A. Event specific real-time quantitative PCR for genetically modified Bt11 maize (*Zea mays*). *Eur. Food Res. Technol.* **2003**, *216*, 347−354.

(21) Huang, H.-Y.; Pan, T.-M. Detection of genetically modified maize MON810 and NK603 by multiplex and real-time polymerase chain reaction methods. *J. Agric. Food Chem.* **2004**, *52*, 3264−3268.

(22) Nielsen, C. R.; Berdal, K. G.; Holst-Jensen, A. Characterisation of the 5′ integration site and development of an event-specific real-time PCR assay for NK603 maize form a low starting copy number. *Eur. Food Res. Technol.* **2004**, *219*, 421−427.

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