



Assessment of DNA extraction methods for PCR testing of discontinued or unapproved biotech events in single seeds of canola, flax and soybean[☆]

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ABSTRACT

The purpose of the study was to assess the suitability of three DNA extraction methods (Fast ID, SDS-based and relatively high-throughput methods) for single seeds (kernels) of canola, flax and broken pieces of soybean seeds. The extracted DNA was used for conventional and real-time qualitative PCR detection of the biotech events OXY235 canola, FP967 flax and DP305423 soybean. The mean weight of single canola and flaxseeds ranged from 2.3 to 3.2 mg and 5.5 to 5.8 mg, respectively. For canola, mean total DNA yields of 216, 796 and 377 ng per seed were obtained for Fast ID, SDS and relatively high-throughput DNA extraction methods, respectively. For flax, mean total DNA yields of 329, 826 and 957 ng per seed were obtained for Fast ID, SDS and relatively high-throughput DNA extraction methods, respectively. For soybean, mean DNA yields of 58, 102 and 175 ng per mg of broken seed were obtained for Fast ID, SDS and relatively high-throughput DNA extraction methods, respectively. Comparison of mean DNA yields indicated statistically significant differences among the three DNA extraction methods for all the three grain types. There was either weak or no correlation between seed weight and DNA yield for all the three DNA extraction methods. Abs260/280 ratio of ≥ 1.8 was obtained for DNA extracted with Fast ID and SDS-based methods. DNA isolated with all three extraction methods had low Abs260/230 ratios indicating the presence of contaminants that absorb at 230 nm. Consistent and repeatable PCR amplification was obtained for DNA extracted with the Fast ID and SDS-based extraction methods. Inhibition of PCR was observed for flax DNA extracted using relatively high-throughput method; however, reducing the amount of DNA to 10 ng in the PCR resulted in consistent amplification. Overall, all three DNA extraction methods can be used for fast DNA-based detection of unapproved or discontinued biotech events in single seeds of canola and flax as well as broken pieces of soybean seeds.

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1. Introduction

Global expansion in the development and cultivation of biotech crops has raised concern about the low level (adventitious) presence of biotech materials in non-biotech commodity grains and oilseeds. Many countries have developed domestic legislation with regards to the development, production, traceability, threshold level, etc. of biotech grains and their derived products. One of the challenges with low level presence is detection of discontinued and unapproved biotech events (e.g., FP967 flax event in 2009 in Canada). Asynchronous approval of biotech events (e.g., approved in the exporting country, but not approved in the importing country)

and the presence of unapproved events in any country (e.g., Bt10 corn in 2005 and liberty link rice 601 in 2006) can adversely disrupt the grain trade, and elimination of the low level presence from the affected grain handling system is costly and time-consuming.

The OXY235 biotech canola event (Oxynil herbicide tolerance) was approved in Australia, Canada, China, Japan and USA between the late 1990s and early 2000 (GM Crop Database, OXY235). However, the OXY235 canola event did not go through the regulatory approval process in the European Union (EU). Commercial cultivar production containing the OXY235 canola event was discontinued in Canada in 2001 (Demeke, Perry, & Scowcroft, 2006). The FP967 flax biotech event (CDC Trifid variety) was approved for food, feed and environmental use in Canada (1996–1998) and the USA (1998–1999) (GM Crop Database, FP967). However, CDC Trifid was deregistered in 2001 because of concerns about market access into the EU (major customer for Canadian flax). Detection of trace

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Table 1

Primer and probe DNA sequences and concentrations used for qualitative and real-time PCR assays.

Primer Name	Crop & event	Sequence (5'-3')	Primer/probe concentration	Amplicon size (bp)	Source
NOST-Spec-F	Flax (FP967)	AGC GCG CAA ACT AGG ATA AA	800 nM	105	CRL-JRC-EC, 2009a
NOST-Spec-R		ACC TTC CGG CTC GAT GTC TA	800 nM		
NOST-Spec probe		FAM-CGC GCG CGG TGT CAT CTA TG-BHQ1	100 nM		
OXYRG	Canola (OXY235)	GAT AGA TGG TGG TGT GAG TCT TGT	300 nM	124	Wu, Wu, Xiao, & Lu, 2008
OXYRV		CCT AAC TTT TGG TGT GAT GAT GCT	300 nM		
OXYRP- probe		6-FAM-TGC CAT CAG CTG ACA CGC CGT GC-TAMRA	150 nM		
DP-305-F1	Soybean (DP305423)	CGT GTT CTC TTT TTG GCT AGC	800 nM	93	CRL-JRC-EC, 2009b
DP-305-R5		GTG ACC AAT GAA TAC ATA ACA CAA ACT A	500 nM		
DP-305 probe		6-FAM-TGA CAC AAA TGA TTT TCA TAC AAA AGT CGA GA-TAMRA	220 nM		

OXY235 and DP305 primers are event-specific while the NOST-Spec primer set is construct-specific.

levels of the FP967 event in flaxseed shipments to EU in 2009 negatively affected exports of Canadian flaxseed to the EU (<http://www.grainscanada.gc.ca/gmflax-lingm/stpf-peeavl-eng.htm>). The DP-305423 soybean event (high oleic acid) has received regulatory approval in Australia, Canada, Mexico and the USA (GM Crop Database, DP-305423). Regulatory approval process for the DP-305423 soybean event is currently underway in Europe, Japan and other soybean importing countries.

For grains and oilseeds, most transgenic event detection is based on ground materials or flour samples. When low level presence of biotech seed or grain (e.g., canola and soybean) is detected in non-biotech grain shipments such as wheat, there could be a need to test the single or broken pieces of biotech seeds for the presence of unapproved or discontinued biotech event (s). Some importing countries request analysis of unapproved or discontinued biotech events in single or broken pieces of canola, soybean and maize seeds commingled with non-biotech grain shipments such as wheat. In such a case, individual seeds are tested for the presence of unapproved or discontinued biotech events. Single seed analysis also enables detection of stacked biotech events. Overall, there is a lack of information on the most suitable DNA extraction methods for single or broken pieces of seeds. In addition, DNA-based analysis of single seeds for detection of unapproved or discontinued biotech events is not well documented.

The objectives of this study were to: 1. establish simple DNA extraction method (s) for analysis of single seeds of canola, flax and broken pieces of soybean seeds for presence of unapproved or discontinued biotech events; 2. determine the yield of DNA obtained from single seeds of canola, flax and broken pieces of soybean seeds, 3. determine if conventional and real-time qualitative PCR can be successfully carried out on DNA extracted from single seeds of canola, flax and broken pieces of soybean seeds, and 4. carry out statistical analysis to evaluate the results for the methods and grains tested.

2. Materials and methods

2.1. Seed sources

Seeds of Armor BX canola variety (event OXY235) and Legend (non-biotech canola variety) were received from Oilseed Monitoring unit of the Canadian Grain Commission. Breeder seeds of CDC Trifid flax (FP967 event) and Norlin (non-biotech flax variety) were obtained from Canadian Food Inspection Agency. Partially ground seed material of soybean variety 92Y61 (event DP-305423) was received from Pioneer HiBred. Broken pieces of soybean seeds were washed with mild bleach (1.2% sodium hypochlorite), thoroughly rinsed with de-ionized distilled water and 70% ethanol and dried in an incubator (55 °C for 1 h). Colby, a non-biotech soybean variety, was obtained from Thompsons Ltd. (Chatham, Ontario). The mass of each canola and flaxseed as well as broken piece of soybean seed was measured before DNA extraction. For canola, flax and soybean, 100% reference DNA was prepared from Armor BX, CDC Trifid and 92Y61 varieties, respectively.

2.2. DNA extraction and quantification

Fast ID Genomic DNA Extraction Kit (Genetic ID, Fairfield, Iowa) was used following the manufacturer's recommendations. For each extraction method and grain type, DNA was extracted from at least 30 seeds (Table 2). For Fast ID DNA extraction method, each single or broken piece of seed was crushed with a hammer and placed in a 2.0 mL centrifuge tube. A single 0.25 inch ceramic grinding ball (Qbiogen, Carlsbad, California) was added to each 2.0 mL centrifuge tube. The centrifuge tubes were placed in a rack with appropriate adapters and the seeds were pulverized at 30 Hz in a mixer mill (Qiagen TissueLyser II, Toronto, Ontario) for 1 min. The samples were then spun in a centrifuge at

Table 2

Seed weight and DNA yield of single canola, flax and broken soybean seeds.

Crop	DNA extraction	No. of seeds	Mean seed weight (mg)	Mean DNA yield (ng/mg) ^a	Mean total DNA yield/seed (ng)
Canola	Fast ID	35	2.83 ± 0.08	75.41 ± 4.07 ^C	215.84 ± 14.19
	SDS-based	30	3.18 ± 0.07	251.16 ± 6.88 ^A	795.58 ± 26.30
	High-throughput	32	2.28 ± 0.06	170.48 ± 15.59 ^B	377.26 ± 33.03
Flaxseed	Fast ID	34	5.7 ± 0.1	57.78 ± 3.63 ^C	329.08 ± 21.70
	SDS-based	33	5.8 ± 0.1	144.88 ± 12.22 ^B	826.03 ± 65.59
	High-throughput	30	5.5 ± 0.1	175.35 ± 5.15 ^A	957.10 ± 23.77
Soybean	Fast ID	35	21.67 ± 1.18	57.89 ± 4.59 ^C	1258.93 ± 118.89
	SDS-based	35	14.37 ± 0.47	102.19 ± 6.54 ^B	1509.04 ± 114.54
	High-throughput	32	12.16 ± 0.65	174.97 ± 11.11 ^A	2019.61 ± 100.95

^a Means followed by different capital letters denote statistical difference ($p = 0.05$).

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