Application to Food Standards Australia and New Zealand for the Inclusion of Potatoes with Low Acrylamide Potential and Reduced Black Spot in Standard 1.5.2 Food Produced Using Gene Technology

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VOLUME 2 of 10

Supporting Studies:

– Study 15-29-SPS-MOL-01 - E12 Structure Characterization
– Study 15-52-SPS-MOL-01 Evidence for the Absence of Backbone in Russet Burbank E12
– Study 15-60-SPS-COMP-01 Compositional Assessment of E12 Compared to Russet Burbank
– Study 15-64-SPS-MOL-01 Stability of DNA Insert in Russet Burbank E12
– Study 15-71-SPS-MOL RNA Expression of Down-Regulated Genes in Russet Burbank E12
– Study 15-75-SPS-MOL-01 Construction of pSIM1278
– Study 15-79-SPS-MOL E12 start to stop ORF
– Study 15-84-SPS-MOL- PPO Efficacy of E12

This document does not contain confidential information

STUDY TITLE

Molecular Characterization of the DNA Insert in Russet Burbank E12

AUTHORS

[redacted]

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PERFORMING LABORATORIES

SPS Regulatory Lab

STUDY NUMBER

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CERTIFICATION PAGE

This report is an accurate and complete representation of the study activities.

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Date

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12/9/15
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ABSTRACT

Event E12 (E12) was created by transformation of the Russet Burbank potato variety with the plasmid, pSIM1278. The T-DNA region of pSIM1278 consists of two cassettes designed to down-regulate potato genes, *Asn1*, *Ppo5*, *R1*, and *PhL*, in tubers. Southern blot analysis was conducted to determine the insert structure and copy number in E12. A series of overlapping probes spanning the T-DNA were used to demonstrate that transformation with pSIM1278 led to introduction of a single insert within the Russet Burbank genome. Using probes that hybridize to genetic elements within the insert, Southern blot analysis revealed the insert consists of a single, nearly full-length T-DNA from pSIM1278.

INTRODUCTION

Event E12 was developed by transforming the Russet Burbank potato variety with the plasmid, pSIM1278 resulting in reduced acrylamide potential and reduced black spot in comparison to non-transformed Russet Burbank (WT). The T-DNA region of pSIM1278 consists of two cassettes designed to down-regulate up to four potato genes, *Asn1*, *Ppo5*, *R1*, and *PhL*, in tubers. The Asn1/Ppo5 cassette is comprised of fragments of the potato asparagine synthetase-1 (*Asn*1) and polyphenol oxidase-5 genes (*Ppo5*), arranged as inverted repeats separated by a non-coding potato spacer element (spacer 1). The PhL/R1 cassette is comprised of fragments from the potato phosphorylase-L (*PhL*) and water dikinase genes (*R1*). The fragments of *PhL* and *R1* are arranged as inverted repeats separated by a non-coding potato spacer element (spacer 2). Both cassettes are driven by two convergent native potato promoters; one for the ADP glucose pyrophosphorylase gene (*Agp*) and the other for the granule-bound starch synthase gene (*Gbss*). These promoters drive expression of the inverted repeats to generate double-stranded RNA to target *Asn1, Ppo5, R1, and PhL* for down-regulation in tubers.

The molecular characterization of E12 presented here, shows that transformation with pSIM1278 led to introduction of a single insert within the Russet Burbank genome. The E12 insert consists of a single nearly full-length T-DNA from pSIM1278 with small deletions in the Left Border (LB) and Right Border (RB) sequences.

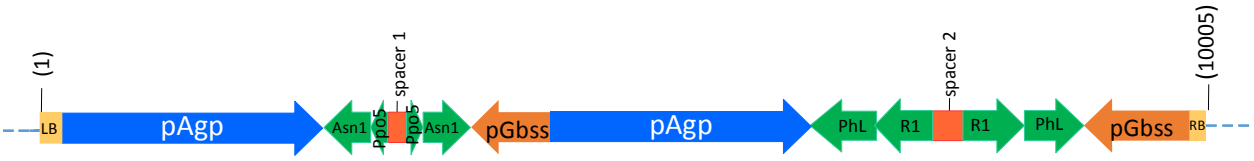


Figure 1. Structure of the pSIM1278 T-DNA Insert in E12
The E12 insert consists of a single nearly full-length T-DNA from pSIM1278 with small deletions in the Left Border (LB) and Right Border (RB) sequences. The Asn1/Ppo5 down-regulation cassette contains an inverted repeat flanked by converging Agp and Gbss promoters. Similarly, the PhL/R1 down-regulation cassette contains an inverted repeat flanked by the same set of promoters. The left border (LB) and right border (RB) regions are not functional components of the insert.

STUDY OBJECTIVES

The objectives of this study were to:

1. Determine the number of pSIM1278 insertion sites.
2. Determine the molecular structure of the pSIM1278 insert

STUDY DATES

01/2008 - 07/2015

KEY STUDY PERSONNEL

[redacted]

MATERIALS AND METHODS

Plant Material

Russet Burbank and E12 plants (G0) were grown in Sunshine mix-1 (www.sungro.com) in two-gallon pots in a greenhouse controlled for temperature (18 °C minimum/27 °C maximum) and light (16-h photoperiod with an intensity of about 1500 µmol/m^2/s). After one to two months of growth, leaf tissue was collected and used for genomic DNA isolation.

DNA Isolation

Isolation of DNA was performed by one of two methods. Control and event DNA were extracted with the same method for any given comparison. For the first method, a 1.0 g sample of young potato leaves was ground into a fine powder using a mortar and pestle under liquid nitrogen. The ground tissue was transferred to a pre-cooled 15 mL conical tube with a pre-cooled spatula and stored at -80 °C. Powdered tissue was thoroughly mixed with 10 mL extraction buffer (0.35 M Sorbitol, 0.1 M Tris-HCl, pH 8.0, 0.05 M EDTA) and the suspension was centrifuged at 3,000 rpm for 15 min at room temperature. The pellet was resuspended in 2 mL extraction buffer containing 200 µg RNase A. After incubating the suspended DNA at 65 °C for 20 min with 2 mL nuclear lysis buffer (0.2 M Tris-HCl pH 7.5, 0.005 M EDTA pH 8.0, 20 mg/mL CTAB, 800 µL 5% Sarcosyl) it was mixed with an equal volume of chloroform: isoamyl alcohol (24:1), vortexed for 1 min, and centrifuged at 3,000 rpm for 5 min at room temperature. The DNA was precipitated with an equal volume of isopropyl alcohol, washed with 70% ethanol, air dried, and dissolved in 400-700 µL 1X Tris/EDTA buffer (TE). DNA concentration was measured using Qubit Fluorometric Quantitation (Life Technologies). DNA quality was confirmed by running the DNA on a 0.8% agarose gel in 1X Tris/Acetate/EDTA (TAE) for 30-40 min at 80 volts.

For the second method, 0.7 g of young leaves was ground and mixed with 7 mL CTAB buffer (2% CTAB, 1.4 M NaCl, 0.1 M Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 100 µg/mL RNase) and incubated at 55°- 65 °C for 30 min followed by centrifugation at 3,000 rpm for 15 min. DNA was extracted twice with equal volumes of chloroform: isoamyl alcohol (24:1) by shaking for 10 min and centrifuged at 3,000 rpm for 5 min at room temperature. The DNA was precipitated with equal volumes of ethanol and rinsed with 70% ethanol. The resulting pellet was air dried and resuspended in TE buffer. DNA concentration was measured using Qubit Fluorometric Quantitation (Life Technologies). DNA quality was confirmed by running the DNA on a 0.8% agarose gel in 1X Tris/Acetate/EDTA (TAE) for 30-40 min at 80 volts.

DNA Restriction Digestion

4 µg of plant DNA was digested overnight in 400 µL final volume with at least 5 µL (10 units/µL) restriction enzyme (Invitrogen) at 37 °C. Digested DNA was concentrated by ethanol precipitation (40 µL of 3M NaOAc pH 5.3 and 1 mL ethanol) at -80 °C for 10 min followed by a wash with 70% ethanol. The DNA pellet was dissolved in 20 µL 1X TE followed by addition of 2 µL DNA gel loading buffer, which consisted of 40% sucrose and 0.35% Orange G (Sigma) in water.

Membrane Preparation and Transfer

Digested plant DNA was loaded on a 0.7% agarose gel containing 1X Tris/Acetate/EDTA (TAE) buffer with 3-5 µL ethidium bromide (10 mg/mL) and run at 35 volts for 18 h. The gel was photographed using a gel documentation system from Alpha Innotech (Santa Clara, California), and depurinated by submerging it in 0.25 N HCl for 2 x 10 min. The gel was subsequently placed in denaturation solution (0.5 M NaOH /1.5 M NaCl) for 2 x 15 min then neutralization solution (1.5 M NaCl and 0.5 M Tris-HCl pH7.5) for 2 x 15 min on a shaker at room temperature. The gel was then equilibrated with 10X SSC for 10 min. The transfer of DNA to the nylon membrane was carried out using 10X SSC according to a standard capillary transfer method.

DIG-Labeled Probe Preparation

The labeling of the PCR-derived probe was achieved using Hotmaster *Taq* enzyme and buffer (Fisher BioReagents) according to Roche's DIG labeling instructions. A standard 50 µL reaction consisted of 5 µL of 10x Hotmaster *Taq* Buffer, 2-5 µL of 10 µM forward primer, 2-5 µL of 10 µM reverse primer, 5 µL DIG-labeled dNTP (Roche), 10 ng plasmid template, and 0.75 µL Hotmaster *Taq* polymerase. The PCR amplification conditions were dependent on each DIG-labeled probe. PCR with regular dNTP instead of DIG labeled dNTP was used as control. Quality of the DIG-labeled probe was assessed by running a small amount of the probe on 1% agarose DNA gel (due to the presence of the DIG moieties in the DNA, probes migrate slower than the control PCR product). The probe was denatured by incubating the probe at 95 °C for 5 min and cooled on ice for 2 min before use.

Probe Hybridization

The cross-linked nylon membrane was prehybridized in 40 mL pre-warmed DIG Easy Hybridization solution (Roche) at 42 °C for 1-4 h in a bottle using a standard hybridization oven (Amerex Instruments Inc.) at 20-25 rpm. Hybridization was carried out by replacing the prehybridization buffer with fresh preheated prehybridization solution, containing 25-50 µL denatured DIG labeled probe. The membrane was incubated at 42 °C, 20-25 rpm for 16 h. The hybridization solution was stored at -20 °C and reused up to 3 times. The reused hybridization solution was heated at 68 °C for 10 min before use.

Detection

The hybridization solution was removed and replaced with 100 mL washing solution I (2X SSC/0.1% SDS). The membrane was washed twice in washing solution I for 10 min at room temperature. This low stringency buffer was poured off and preheated high stringency washing solution II (0.5X SSC/0.1% SDS,

60 °C) was added immediately. The membrane was washed twice in washing solution II at 68 °C for 20 min each at 25-30 rpm, followed by a brief rinse with 2X SSC to remove SDS. The membrane was rinsed with 150 mL 1X DIG Washing Solution (Roche) in a tray for 2 min and incubated in 1X Blocking solution (Roche) for 0.5-3 h on a low-speed shaker. The blocked membrane was incubated with DIG antibody solution (1:10,000 dilution of Anti-DIG-alkaline phosphate conjugate with 1X Blocking solution) for 30 min on a shaker at room temperature. The membrane was washed twice (15 min each) with 1X DIG Washing Solution (Roche) and equilibrated with 1X detection buffer. The detection reaction was carried out with 2 mL CDP-Star solution (1:100 diluted stock of CDP-Star with 1X detection buffer) for 5 min. The membrane was wrapped in a plastic film and either exposed to film or developed on an Amersham Imager 600 (GE Healthcare Life Sciences). Films were developed with a Konica SRX-101A Z-ray film developer.

Southern Blot Presentation Notes

Southern blots were presented along with a table that indicates the approximate size of the bands associated with the T-DNA insert and any digested pSIM1278 plasmid bands used as controls. The bands associated with inserts are distinguished as either junction bands (JB), plasmid bands (PB), or internal bands (IB) depending on their location. Plasmid bands refer to digestion products of predictable sizes and are associated with digested pSIM1278 while internal bands refer to digestion products of predictable sizes that are contained entirely within the boundaries of the T-DNA insert. Junction bands are those bands that extend from the T-DNA insert into the adjacent, native potato DNA. The exact size of these bands can only be predicted when restriction sites exist within the identified flanking sequences. However, the minimum size of these bands can be calculated for a simple T-DNA insert based upon the distance from a given restriction site to the end of the identified flanking sequences (separate study). Junction bands are useful for confirming the number of integration sites in the genome as each integration event will possess two junction bands. Since the T-DNA sequence is largely derived from potatoes, Southern blot probes targeting the insert will also detect a few to many endogenous bands, depending upon the frequency of that sequence in the genome. These bands are not labeled in the Southern blots for simplicity, but consist of all bands common in size and intensity between control and event samples.

Occasionally, bands greater than 3 kb migrate faster than expected due to the presence of residual polysaccharides in the isolated DNA. The size of these bands were verified where possible, by including control samples spiked with pSIM1278 plasmid DNA that produces the same sized bands for comparison.

RESULTS

Characterization of Insert Copy Number Following pSIM1278 Transformation

The pSIM1278 T-DNA consists of an Asn1/Ppo5 down-regulation cassette flanked by converging *Agp* and *Gbss* promoters and a second PhL/R1 down-regulation cassette flanked by the same set of converging promoters (Figure 2). The number of inserts in E12 was evaluated by Southern blot analysis of genomic DNA digested with the NdeI restriction enzyme and hybridized using a series of seven probes that span the length of the T-DNA (Figure 2). NdeI was chosen as it cuts frequently within the potato genome, but does not cut within the boundaries of the T-DNA itself. Thus, a single contiguous band is expected for each insert. Moreover, an intact insert will have the same size when using any of the probes. The presence of additional bands would indicate the presence of a second insert. Finally, since NdeI cuts frequently in the potato genome, it is unlikely that two inserts would have the same size.

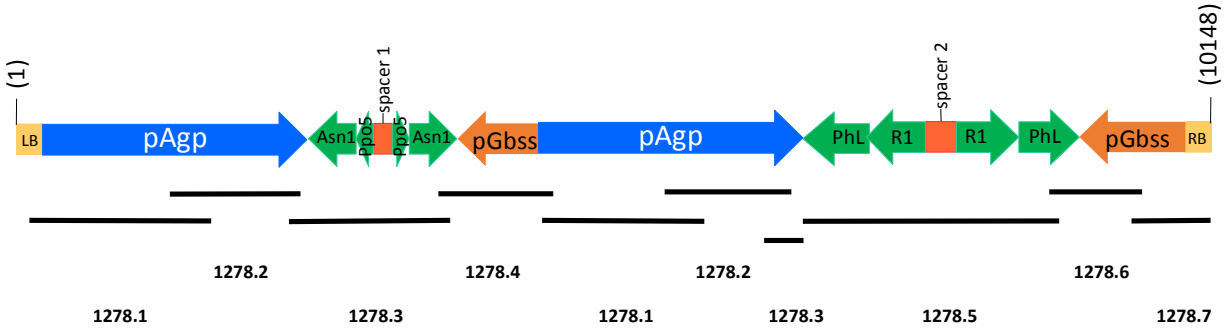


Figure 2. pSIM1278 T-DNA Spanning Probes used to Characterize Insert Number
A schematic of the full structure of the pSIM1278 T-DNA is shown along with the binding sites for each of the seven probes (1278.1 – 1278.7). The probes were designed to overlap and cover the T-DNA sequence. Due to redundancy of sequence in the insert, some probes hybridized to more than one location within the T-DNA. Probes 1278.4 and 1278.6 are only shown once in the figure, but overlap in their coverage of the insert.

Following digestion with NdeI, DNA was separated on agarose gels using extended electrophoresis to ensure adequate separation and resolution of high-molecular weight bands. Southern blots were hybridized with each of the probes described in Figure 2. In each case, a single, high molecular weight band (about 12 kb) was detected using each of the seven probes (Figure 3). The band unique to E12 is indicated in each gel with a black arrow. All other bands exist in both the WT and E12 samples indicating they are endogenous bands not related to the transformation. Since the insert consists of a single copy of the T-DNA in a tetraploid potato (shown below), the detection of this band establishes that the sensitivity of the assay is sufficient to detect a single copy in the genome. Collectively, these data show the pSIM1278 insert was integrated at a single locus within the Russet Burbank genome.

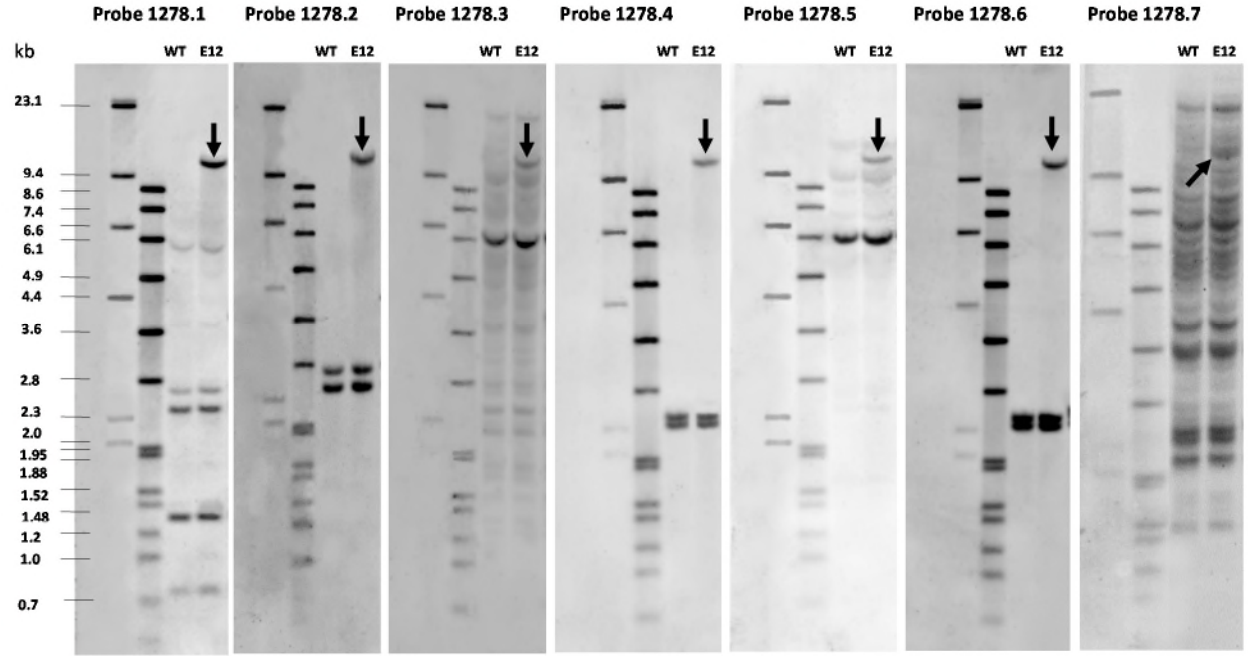


Figure 3. Southern Blots Showing the Presence of a Single Insert in E12
Southern blots of NdeI-digested genomic DNA isolated from Russet Burbank and E12 plants. Both samples were digested with NdeI, which does not cut within the T-DNA insert, but does cut frequently in the potato genome. The single T-DNA insert is indicated in each gel (Probes 1278.1 – 1278.7) by a black arrow. The molecular weight markers, DIGII and DIGVII, were included in each gel and are labeled in kilobases (kb) at the left of the first gel.

Characterization of the Structure of the pSIM1278 Insert

A series of Southern blots were performed to elucidate the structure of the single insert. Southern blots were performed using a different set of probes. The probes described in Figure 2 would result in very complex banding patterns due to the number of hybridization sites in the genome and their juxtaposition to the restriction sites. Instead, a combination of probes and restriction digests were chosen that would result in an informative mixture of digestion products with predicable sizes based upon the structure of the T-DNA in pSIM1278 (Figure 4). Probes hybridizing to the Agp, Asn1, pGbss, and R1 elements were used following digestion with: (1) EcoRV, (2) HindIII, or (3) a double digest with EcoRI and ScaI.

The location of the binding site for each probe and a schematic of the restriction patterns of the final structure (grey box) are provided in Figure 4 for ease of comparison with the Southern blot results. The expected bands, their sizes, and a colored marker indicating probes expected to hybridize with each band are provided. Genomic DNA of non-transformed Russet Burbank was used as a wild-type comparison. A second wild-type sample was spiked with pSIM1278 plasmid prior to digestion to serve as a copy number control and size marker when appropriate. The plasmid was spiked at a target concentration of roughly one copy per genome equivalent. As genomic DNA isolated from potato plants does not always migrate true to size when compared to a molecular weight marker, presumably due to the presence of polysaccharides, the plasmid control provides a valuable size comparison. All Southern blots (Figures 5 – 8) are presented adjacent to a table indicating the observed bands and their molecular

weights to simplify comparison with Figure 4. The table denotes all bands, internal bands (IB) and junction bands (JB), in red; whereas bands (PB) associated with the pSIM1278 plasmid DNA are indicated associated in black in the adjacent tables.

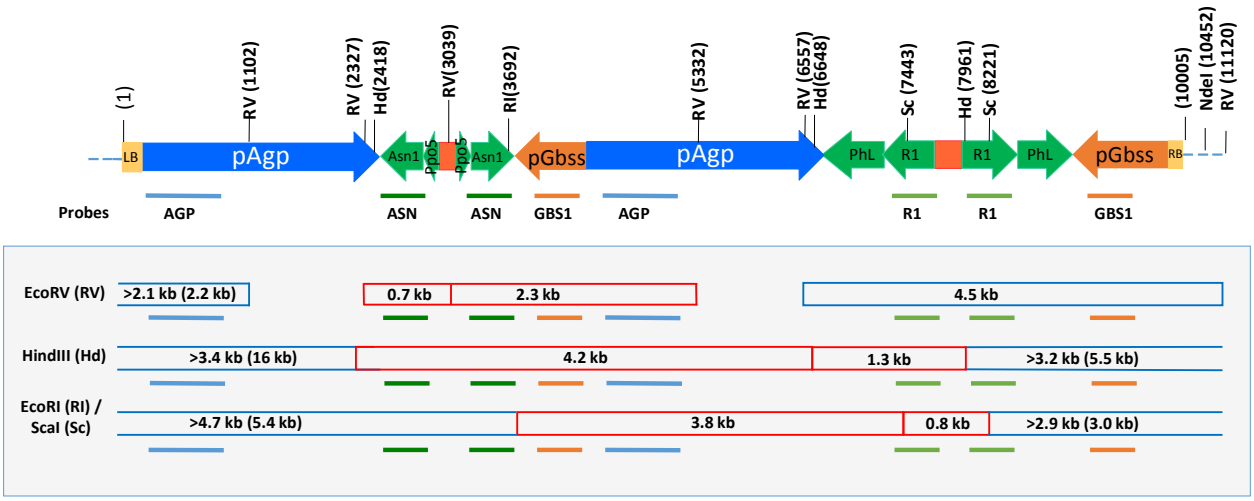


Figure 4. Structure of the pSIM1278 T-DNA Insert in E12 with Digestion Patterns and Probe Binding Sites

A schematic of the full structure of the pSIM1278 T-DNA insert is shown along with the binding sites for each of the four probes (AGP, ASN, GBS1, and R1). The probes all bind to two different locations within the insert. The restriction products that are recognized by the probes are shown within the grey box where the expected sizes are indicated. The actual size of junction bands is shown in parenthesis (sizes of junction bands are estimates, except the 4.5 kb EcoRV band).

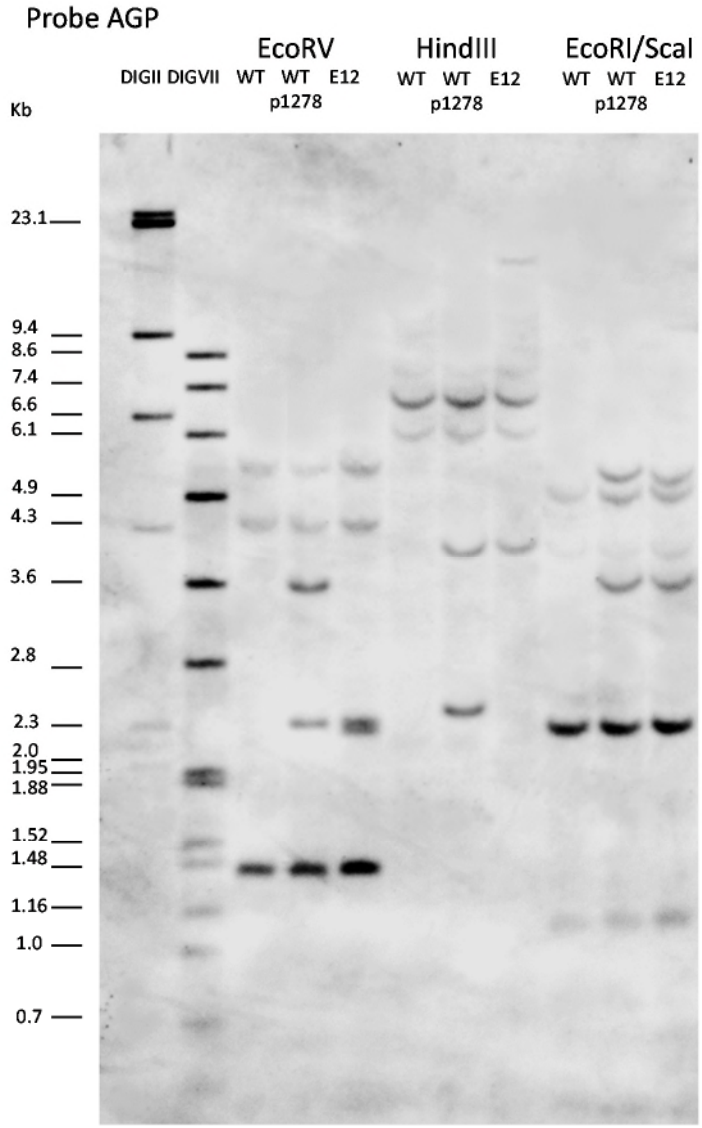
As shown by the red boxes in Figure 4, the digests are expected to produce six bands that are completely contained within the boundaries of the T-DNA (internal bands, IB). These same bands are expected from digestion of the pSIM1278 plasmid DNA and thus provide an ideal molecular weight comparison. Each digest resulted in two internal bands; 0.7 kb and 2.3 kb for EcoRV, 4.2 kb and 1.3 kb for HindIII, and 3.8 and 0.8 kb for EcoRI/ScaI. Southern blots hybridized separately with the AGP, ASN, GBS1 or R1 probes confirmed the presence and size of each of the bands (Figures 5 - 8). In the EcoRV digests, the 0.7 kb band was uniquely detected by the ASN probe (Figure 6), whereas the 2.3 kb fragment was detected by the ASN, GBS1, and AGP probes (Figures 5, 6, and 7), as anticipated. In the HindIII digests, the 4.2 kb fragment was detected by the ASN, GBS1, and AGP probes (Figures 5, 6, and 7), whereas the 1.3 kb fragment was detected by the R1 probe (Figure 8). As expected, the 4.2 kb band had a higher intensity when hybridized with the ASN probe (Figure 6) due to multiple binding sites associated with the inverted repeat (Figure 4). In the EcoRI/ScaI digests, the 3.8 kb fragment was detected in the GBS1, AGP, and R1 blots (Figures 5, 7, and 8), whereas the 0.8 kb fragment was only detected by the R1 probe (Figure 8). All of the internal bands migrated equivalently with similar intensities to the plasmid DNA controls spiked into the wild-type samples. These data indicate the presence of a single intact copy of the internal region of the T-DNA insert in E12.

The remaining structure of the insert was assessed through analysis of the junction bands associated with these same restriction digests. There were six junction bands (blue boxes) associated with the digests as shown in Figure 4. The open-ended blue boxes denote junction bands where only one of the

two restriction site locations is known and thus the exact size cannot be predicted. Each of these junction bands is labeled with the expected minimum size and the observed size (shown in parentheses) from the Southern blots (Figures 5 – 8). Characterization of the junction sites and flanking sequences identified the presence of an EcoRV site in the right flanking region as shown in Figure 4 (separate study). Identification of this site predicted the EcoRV right junction band should be 4.5 kb and was thus represented by a closed box in Figure 4.

As with the internal bands, each restriction digest resulted in two junction bands: >2.1 kb and 4.5 kb for EcoRV, >3.4 kb and >3.2 kb for HindIII, and >4.7 kb and >2.9 kb for EcoRI/ScaI. As summarized in Figure 4 and shown in the Southern blots in Figures 5-8, all of the expected junction bands were observed with the expected sizes. As expected for the structure presented in Figure 4, the AGP probe detected a junction fragment with each of the digests: 2.2 kb EcoRV, 16 kb HindIII, and 5.4 kb EcoRI/ScaI bands (Figure 5). The 5.4 kb band was also detected by the ASN probe with higher intensity as predicted by the multiple binding sites (Figure 6). Similarly, the GBS1 and R1 probes detected the three expected junction fragments associated with the right side of the structure: 4.5 kb EcoRV, 5.5 kb HindIII, and 3.0 kb EcoRI/ScaI bands (Figures 7 and 8). The EcoRV junction band had higher intensity confirming the presence of an entire inverted repeat on the right side of the insert (Figure 8).

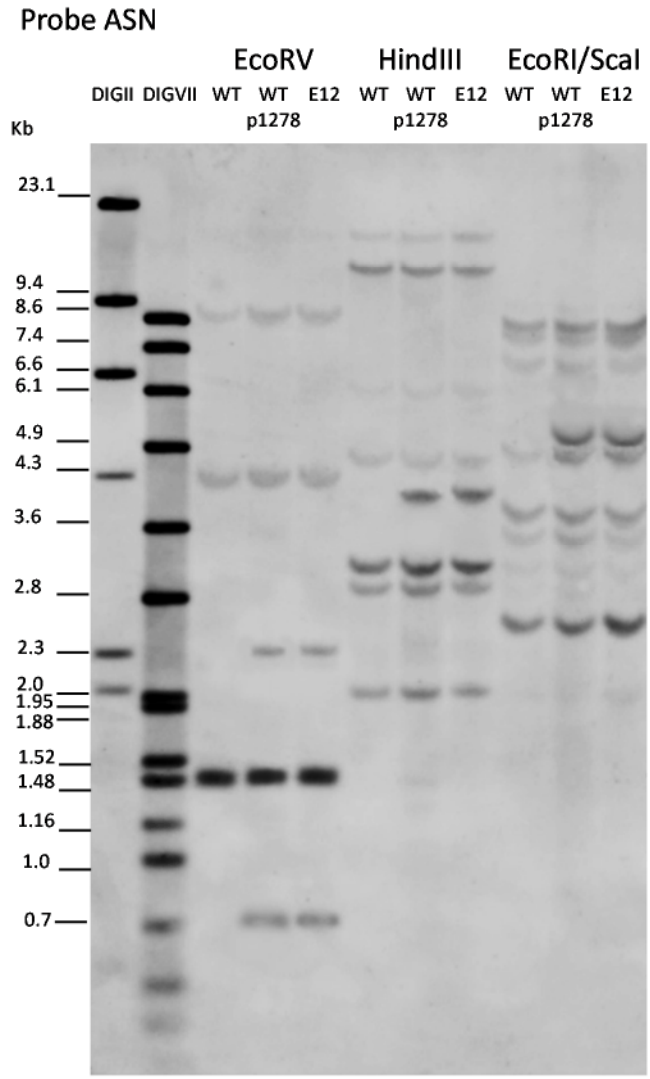
In summary, the overlapping restriction fragments cover the entire insert and therefore confirms the model that the E12 insert consists of a single nearly full-length T-DNA from pSIM1278. The left and right borders were shown to consist of small deletions of non-functional DNA during the characterization of the flanking regions (Layne et al. 2015).



EcoRV	HindIII	EcoRI/ScaI
	JB 16	
		PB 5.6 JB 5.4
	PB 4.2 IB 4.2	
PB 3.8		PB 3.8 IB 3.8
	PB 2.4	
PB 2.3 IB 2.3 JB 2.2		

Figure 5. Southern blots with AGP Probe

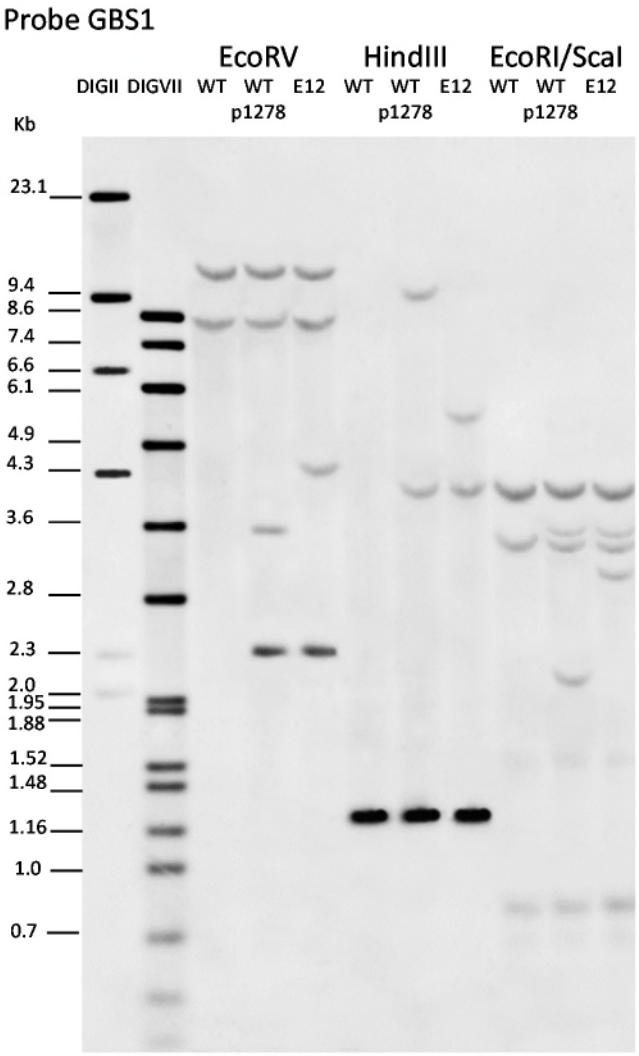
Genomic DNA of Burbank control (WT) and event E12 were digested with EcoRV, HindIII, and EcoRI/ScaI and hybridized with the AGP probe. The estimated sizes of bands are summarized in the table and classified into three groups based on the structure of the DNA insert: T-DNA insert fragments for both IBs and JBs are in red. Plasmid bands (PB) associated with digested pSIM1278 (p1278) are in black. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image. All molecular weights are presented in kilobases (kb).



EcoRV	HindIII	EcoRI/ScaI
		PB 5.6 JB 5.4
	PB 4.2 IB 4.2	
PB 2.3 IB 2.3		
PB 0.7 IB 0.7		

Figure 6. Southern blots with ASN Probe

Genomic DNA of Burbank control (WT) and event E12 were digested with EcoRV, HindIII, and EcoRI/ScaI and hybridized with the ASN probe. The estimated sizes of bands are summarized in the table and classified into three groups based on the structure of the DNA insert: T-DNA insert fragments for both IBs and JBs are in red. Plasmid bands (PB) associated with digested pSIM1278 (p1278) are in black. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image. All molecular weights are presented in kilobases (kb).



EcoRV	HindIII	EcoRI/ScaI
	PB 10.6	
	JB 5.5	
JB 4.5	PB 4.2 IB 4.2	
PB 3.8		PB 3.8 IB 3.8
		JB 3.0
PB 2.3 IB 2.3		PB 2.1

Figure 7. Southern blots with GBS1 Probe

Genomic DNA of Burbank control (WT) and event E12 were digested with EcoRV, HindIII, and EcoRI/ScaI and hybridized with the GBS1 probe. The estimated sizes of bands are summarized in the table and classified into three groups based on the structure of the DNA insert: T-DNA insert fragments for both IBs and JBs are in red. Plasmid bands (PB) associated with digested pSIM1278 (p1278) are in black. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image. All molecular weights are presented in kilobases (kb).

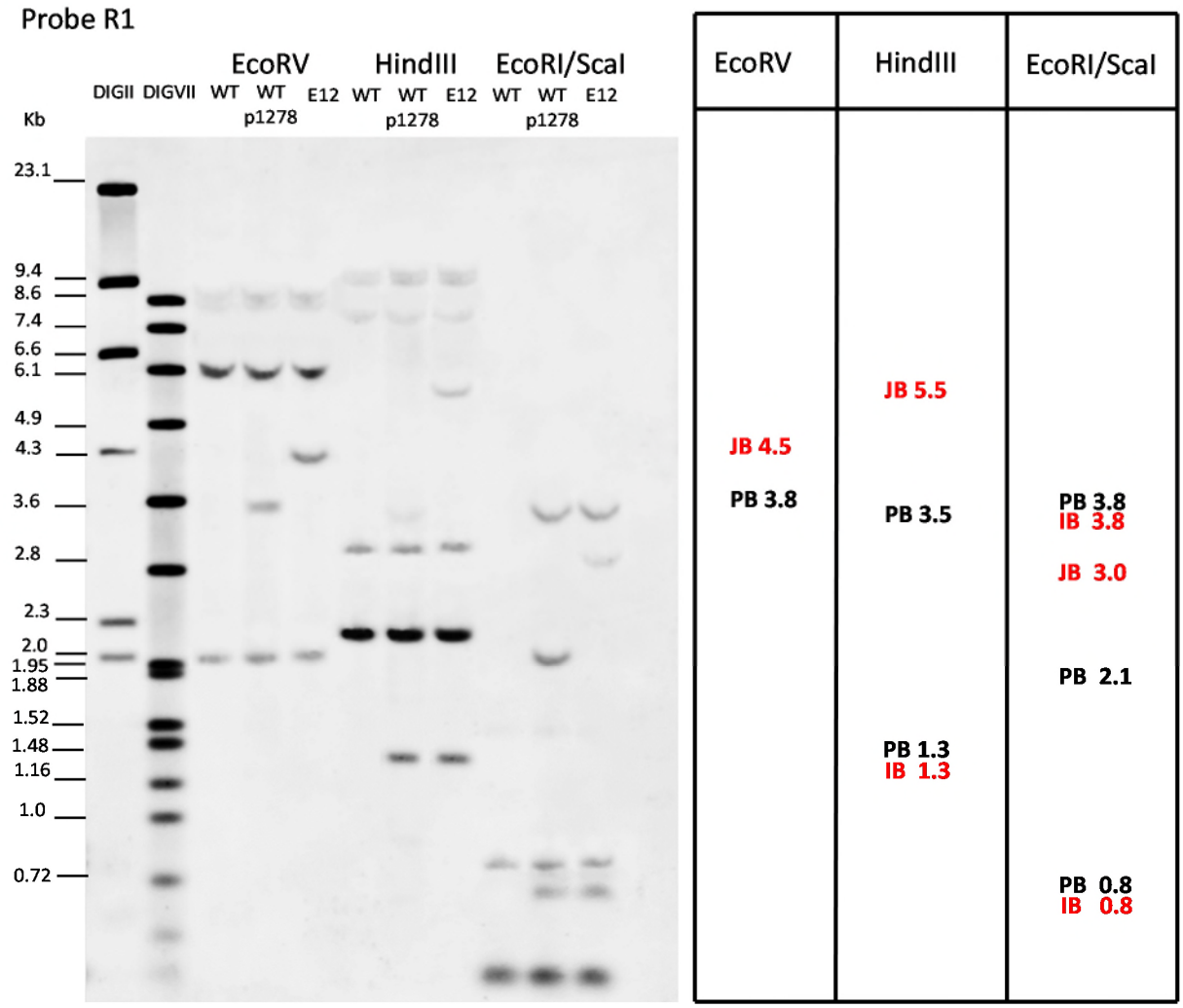


Figure 8. Southern blots with R1 Probe

Genomic DNA of Burbank control (WT) and event E12 were digested with EcoRV, HindIII, and EcoRI/ScaI and hybridized with the R1 probe. The estimated sizes of bands are summarized in the table and classified into three groups based on the structure of the DNA insert: T-DNA insert fragments for both IBs and JBs are in red. Plasmid bands (PB) associated with digested pSIM1278 (p1278) are in black. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image. All molecular weights are presented in kilobases (kb).

CONCLUSION

The molecular characterization of E12 presented here, demonstrates that transformation with pSIM1278 led to introduction of a single insert within the Russet Burbank genome. The E12 insert consists of a single, nearly full-length T-DNA from pSIM1278 in the Left Border (LB) and Right Border (RB) regions that are not necessary for any of the desired traits and are consequences of Agrobacterium-mediated transformation.

REFERENCES

[redacted] (2015). Characterization of the Insertion Site in Russet Burbank E12. Study Report 15-57-SPS-MOL.

STUDY TITLE

Evidence for the Absence of Plasmid Backbone DNA in Russet Burbank E12

AUTHORS

[redacted]

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12/10/2015

PERFORMING LABORATORIES

SPS Regulatory Lab

STUDY NUMBERS

15-52-SPS-MOL

CERTIFICATION PAGE

This report is an accurate and complete representation of the study activities.

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Date

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Senior Regulatory Science Manager

12/9/15
Date

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ABSTRACT

Event E12 (E12) was generated by transformation of Russet Burbank with pSIM1278. *Agrobacterium*-mediated transformation can lead to the integration of plasmid backbone sequences into a host genome along with the desired T-DNA. Following *Agrobacterium*-mediated transformation, E12 was evaluated for the presence of backbone DNA by Southern Blot analysis. No hybridization signal was detected in Southern blots using a series of eight probes that span the backbone of the plasmid, pSIM1278. Thus, there is no evidence of backbone DNA in the E12 genome.

INTRODUCTION

E12 was generated by the transformation of Russet Burbank with pSIM1278. *Agrobacterium*-mediated transformation can lead to the integration of plasmid backbone sequences into a host genome along with the desired T-DNA. The plasmid backbone is important for replication and selection of the plasmid in bacteria but is not intended to transfer into the genome during transformation. The absence of backbone in E12 was assessed by Southern blot analysis using a series of eight probes that span the backbone of the plasmid, pSIM1278. There was no evidence of backbone DNA in the E12 genome.

STUDY OBJECTIVES

The objective of this study is to verify the absence of backbone from the transformation plasmid, pSIM1278, in the E12 genome.

STUDY DATES

01/2008 - 07/2015

KEY STUDY PERSONNEL

[redacted]

MATERIALS AND METHODS

Plant Material

Russet Burbank and E12 (G0) plants were grown in Sunshine mix-1 (www.sungro.com) in two-gallon pots in a greenhouse controlled for temperature (18 °C minimum/27 °C maximum) and light (16 h photoperiod with an intensity of about 1,500 µmol/m2/s). After one to two months of growth, leaf tissue was collected and used for genomic DNA isolation.

DNA Isolation

A 1.0 g sample of young potato leaves was ground into a fine powder using a mortar and pestle under liquid nitrogen. The ground tissue was transferred to a pre-cooled 15 mL conical tube with a pre-cooled spatula and stored at -80 °C. Powdered tissue was thoroughly mixed with 10 mL extraction buffer (0.35 M sorbitol, 0.1 M Tris-HCl, pH 8.0, 0.05 M EDTA) and the suspension was centrifuged at 3,000 rpm for 15 min at room temperature. The pellet was resuspended in 2 mL extraction buffer containing 200 µg RNase A. After incubating the suspended DNA at 65 °C for 20 min with 2 mL nuclear lysis buffer (0.2 M Tris-HCl pH 7.5, 0.005 M EDTA pH 8.0, 20 mg/mL CTAB, 800 µL 5 % sarcosyl) it was mixed with an equal volume of chloroform: isoamyl alcohol (24:1), vortexed for 1 min, and centrifuged at 3,000 rpm for 5 min at room temperature. The DNA was precipitated with an equal volume of isopropyl alcohol, washed with 70 % ethanol, air dried, and dissolved in 400-700 µL 1X Tris/EDTA buffer (TE). DNA concentration was measured using Qubit Fluorometric Quantitation (Life Technologies). DNA quality was confirmed by running the DNA on a 0.8 % agarose gel in 1X Tris/Acetate/EDTA (TAE) for 30-40 min at 80 volts.

DNA Restriction Digestion

Plasmid pSIM1278 containing a full backbone, served as a positive control. A 4.0 µg sample of plant DNA was digested overnight in 400 µL final volume with at least 5 µL (10 units/µL) restriction enzyme (Invitrogen) at 37 °C. Digested DNA was concentrated by ethanol precipitation (40 µL of 3M NaOAc, pH 5.3 and 1 mL ethanol) at -80 °C for 10 min followed by a wash with 70 % ethanol. The DNA pellet was dissolved in 20 µL 1X TE followed by addition of 2 µL DNA gel loading buffer, which consists of 40 % sucrose and 0.35 % Orange G (Sigma) in water.

Gel Preparation

Digested DNA was separated by electrophoresis on a large 0.7 % agarose gel containing 0.5X Tris-borate-EDTA (TBE) buffer for 18 h using 35 volts. The gel was stained with ethidium bromide (10 mg/mL), photographed using a gel documentation system from Alpha Innotech (Santa Clara, California), and depurinated by submersion in 0.25N HCl for 20 min. After subsequent denaturation in 0.5 M NaOH / 1.5 M NaCl for 2 x 15 min and neutralization in 1.5 M NaCl / 0.5 M Tris-HCl (pH 7.5), for 2 x 15 min on a shaker at room temperature, the gel was equilibrated with 10X SSC for 10 min. The transfer of DNA to the nylon membrane was carried out with 10X SSC using capillary transfer.

DIG-Labeled Probe Preparation

The labeling of PCR-derived probes were achieved using Hotmaster Taq enzyme and buffer (Fisher BioReagents) according to Roche's DIG labeling protocol. A standard 50 µL reaction consisted of 5 µL of 10X Hotmaster Taq Buffer, 2-5 µL of 10 µM forward primer, 2-5 µL of 10 µM reverse primer, 5 µL of DIG labeled dNTPs (Roche), 10 ng plasmid template, 0.75 µL Hotmaster Taq polymerase, and water. The PCR amplification conditions were optimized for each DIG-labeled probe. PCR with dNTPs instead of DIG labeled dNTPs were used as a control. Quality of the DIG labeled probe was assessed by analyzing a fraction of the product on a 1 % agarose gel alongside control (unlabeled) PCR product. The probe was denatured before use by incubating the probe at 95 °C for 5 min, and then quenched on ice for 2 min.

Hybridization

The nylon membrane carrying transferred DNA was prehybridized in 40 mL pre-warmed DIG Easy Hybridization solution (Roche) at 42 °C for 1-4 h in a hybridization oven (Amerex Instruments Inc.) rotating at 20-25 rpm. Nylon filters cross-linked with DNA digested with EcoRI were hybridized independently with six different probes and DNA digested with EcoRI/ScaI with two different probes, spanning the plasmid backbone (see Figure 1 for the linear arrangements of the probes described). Hybridization was carried out by replacing the prehybridization buffer with a fresh amount of the same preheated solution containing 25-50 µL denatured DIG labeled probe, and continuing the incubation with rotation (20-25 rpm) at 42 °C for about 16 h. The probe-containing hybridization solution was stored (-20 °C) and reused up to three times. The reused hybridization solution was heated at 68 °C for 10 minutes before use.

Detection

The hybridization solution was removed and replaced by 100 mL washing solution I (2X SSC/0.1 % SDS). The membrane was washed twice in washing solution I for 10 min at room temperature. This low stringency buffer was poured off and preheated high stringency washing solution II (0.5X SSC/0.1 % SDS, 60 °C) was added immediately. The membrane was washed twice in washing solution II at 60-63 °C for 20 min each at 25-30 rpm. This was followed by a rinse with 2X SSC to remove SDS. The membrane was rinsed with 150 mL of 1X DIG Washing Solution (Roche) in a tray for 2 min and incubated in 1X Blocking solution (Roche) for 0.5-3 hours on a low-speed shaker. The blocked membrane was incubated with DIG antibody solution (1:10,000 dilution of Anti-DIG-alkaline phosphate conjugate with 1X Blocking solution) for 30 min on a shaker. The membrane was washed twice (15 min each) with 1X DIG Washing Solution (Roche) and equilibrated with 1X detection buffer. The detection reaction was carried out with 2 mL CDP-Star solution (1:100 diluted stock of CDP-Star with 1X detection buffer) for 5 min. The membrane was wrapped in plastic film. Depending on the experiment multiple exposures were taken from 30 sec to 30 min. Images were developed with an Amersham Imager 600 (GE). Following detection, membranes were washed twice (15 min each) at 37 °C with stripping buffer (0.2M NaOH and 0.1 % SDS) and rinsed with 2x SSC for 5 min. Membranes were checked with Amersham Imager 600 to confirm the absence of signal prior to incubation with additional backbone probes. Each membrane was stripped once and probed twice.

RESULTS

To detect any backbone DNA that may be present in E12, genomic DNA was isolated from Russet Burbank (WT) and E12 plants and analyzed by Southern blotting following digestion with EcoRI or EcoRI/ScaI. The pSIM1278 plasmid served as a positive control (1278) and was spiked into WT genomic DNA at a targeted concentration of one copy / genome equivalent (23.4 pg) prior to digestion. Detection of backbone at this concentration showed the probes are sensitive enough to detect a single copy of backbone DNA in the genome. A set of eight probes were used that span the backbone sequence. A linear representation of the plasmid backbone, including probe binding sites is shown in Figure 1A along with the expected fragment sizes.

As indicated in Figure 1A, digestion of pSIM1278 with EcoRI produces three bands (12.5 kb, 1.57 kb, and 5.6 kb) detectable by the probe set. Samples were digested with EcoRI for the Southern blots hybridized with probes BB1-BB6. A double digest (EcoRI/ScaI) was used for Southern blots hybridized with probes BB7 and BB8 due to cross reactivity of probe BB8 with the pSIM1278 T-DNA. The sequence of the spacer 2 element within the PhL/R1 inverted repeat is derived from the Ubi7 promoter and is therefore detected by the probe BB8 in the pSIM1278 plasmid control and the pSIM1278 insert in E12 (Figure 1B). The EcoRI/ScaI digest of the plasmid control and E12 produces a 0.8 kb band. The structure of the pSIM1278 insert in E12 is shown in Figure 1B along with the expected ScaI digestion product.

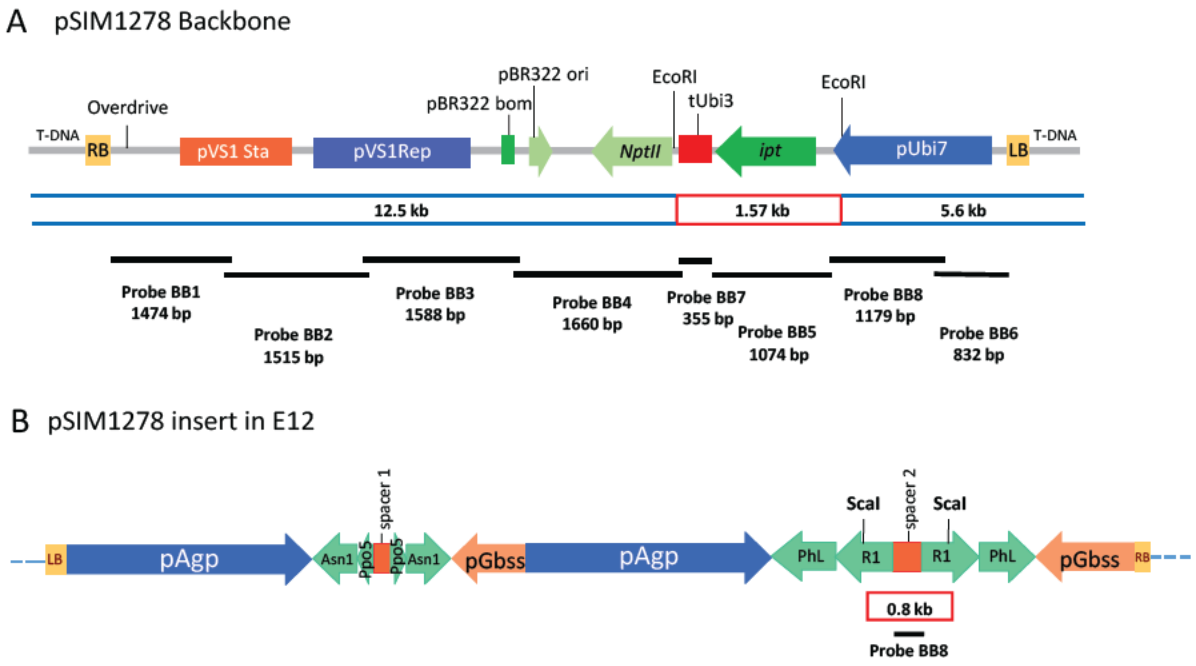

Figure 1. Linear Structure of the pSIM1278 Backbone and Insert in E12
(A) Linear map of the backbone for pSIM1278. The eight probes used to detect the backbone are indicated below (BB1-8) with the size of the probe indicated in base pairs (bp). The EcoRI sites and expected band sizes for the pSIM1278 plasmid control are indicated as colored boxes. (B) Structure of the pSIM1278 insert in E12 is shown. The BB8 probe detects the spacer 2 region of the insert and produces a 0.8 kb band when digested by ScaI.

The concentration of DNA samples were normalized following digestion, and equal loading was verified by staining the agarose gels used in Southern analysis with ethidium bromide (Figure 2).

No hybridization signal was detected in Southern blots hybridized with probes BB1 - BB4 corresponding to backbone DNA in the E12 genome (Figure 3). The expected 12.5 kb fragment was readily detected for the positive control samples in each of these blots. Endogenous bands were detected in all samples of the blot hybridized with probe BB3, which were not related to the transformation.

There were no backbone fragments detected in the genome of E12 when hybridized with probes BB5-BB8 (Figure 4). The expected 1.57 kb plasmid fragment described in Figure 1A was detected in the positive control samples for blots hybridized with probes BB5, BB7, and BB8. Similarly, the 5.6 kb plasmid fragment (Figure 1A) was detected specifically in positive control samples by probes BB6 and BB8 (Figure 4). Lastly, the expected 0.8 kb band was detected in the E12 and pSIM1278 control sample in the blot probed with BB8. This band corresponds to the spacer 2 element of the pSIM1278 insert flanked by ScaI restriction sites as shown in Figure 1B. Endogenous bands not related to the transformations were observed in all samples in the blots hybridized with probes BB6 – BB8.

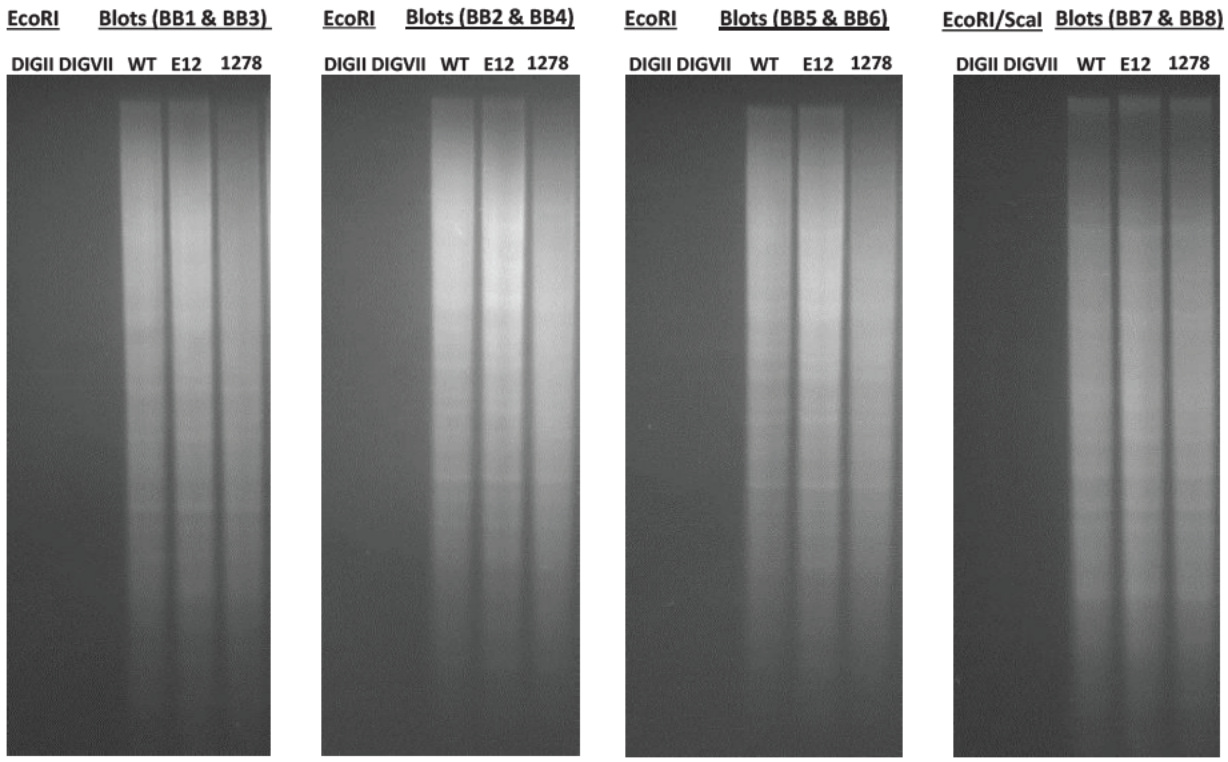

Figure 2. Evidence of Equal Loading of Genomic DNA during Southern Blotting
Four separate agarose gels were loaded with an equivalent amount (4 µg) of EcoRI or EcoRI/ScaI digested DNA of Russet Burbank control (WT), E12, and WT spiked with plasmid pSIM1278 (1278) as evidenced by similar signal intensities in each lane after staining with ethidium bromide to detect DNA. Lanes 1 and 2 include molecular weight markers, DIGII and DIGVII, which were loaded at concentrations too low to be detected by the ethidium stains.

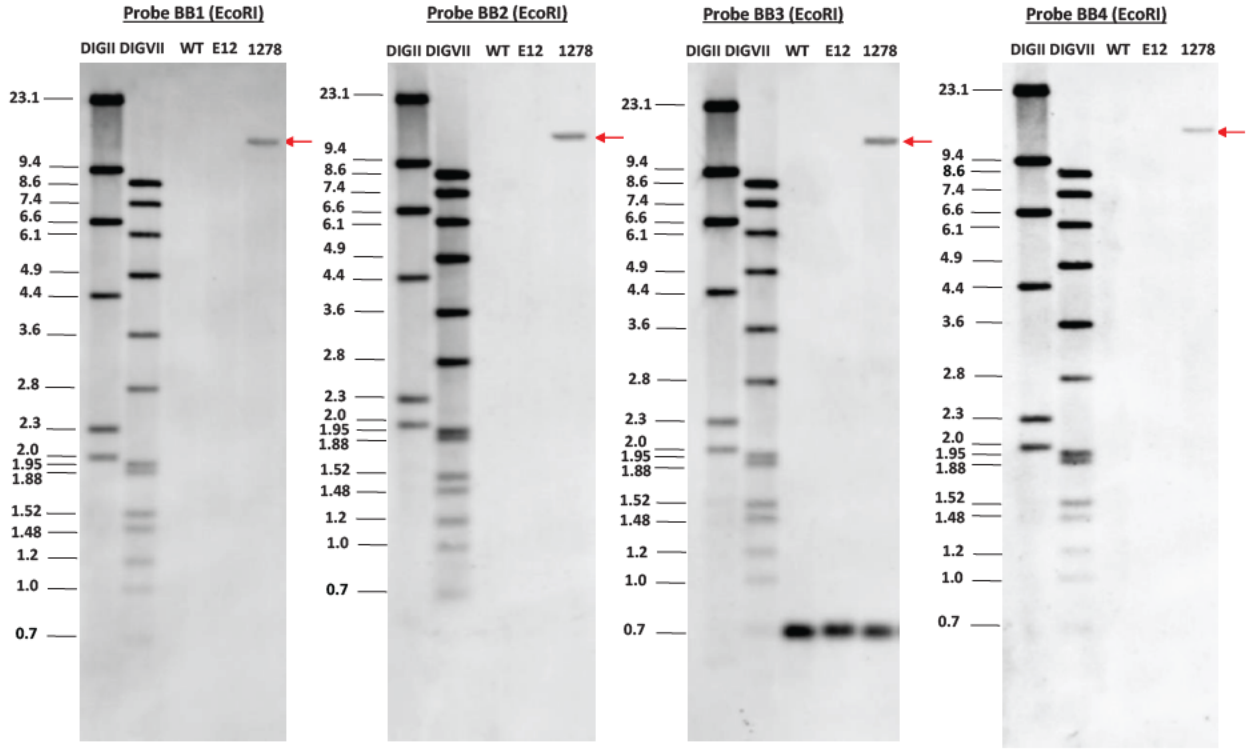

Figure 3. Southern Blots Probed with BB1-4 Show No Evidence of Backbone DNA in E12
Southern blots of genomic DNA isolated from Russet Burbank (WT), E12, and WT spiked with pSIM1278 (1278). Molecular weight markers, DIGII and DIGVII, are labeled in kilobases (kb). Red arrows indicate expected 12.5 kb bands unique to the plasmid control (1278).

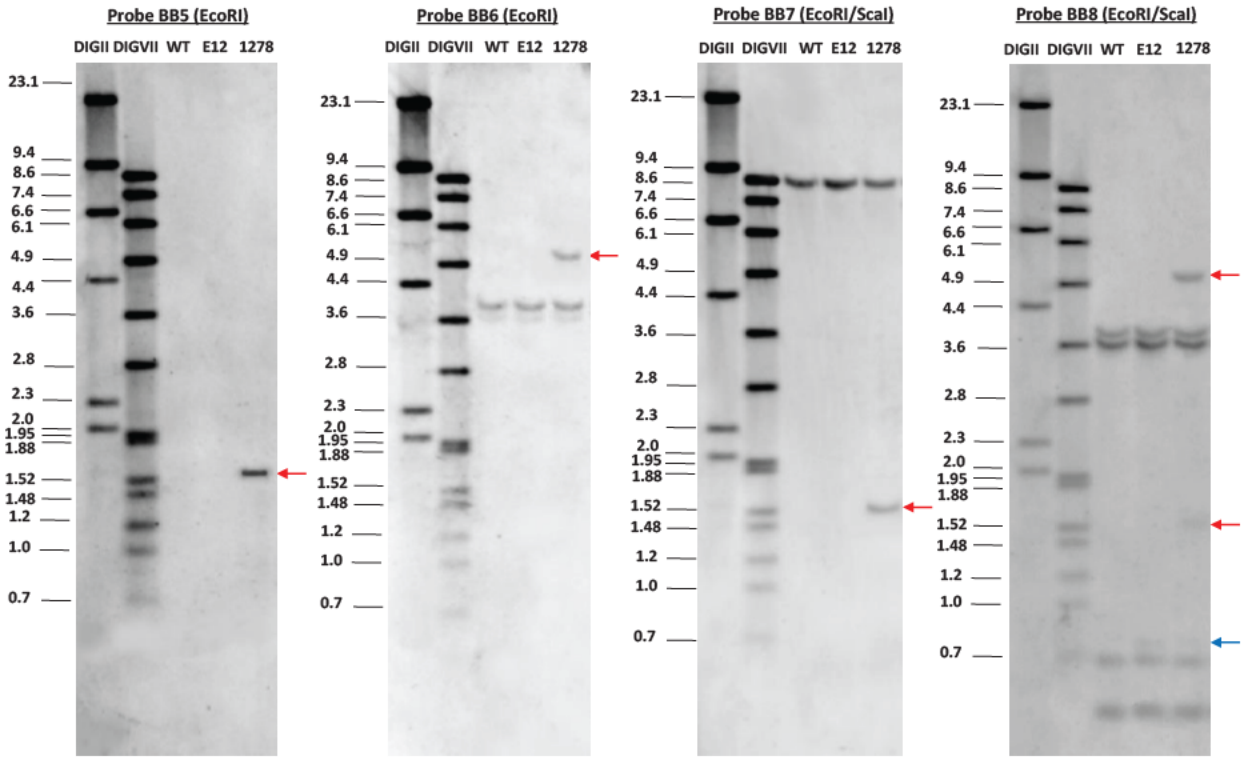

Figure 4. No Evidence of Backbone DNA Corresponding to Probes BB5-BB8 in E12
Southern blots of genomic DNA isolated from Russet Burbank (WT), E12, and WT spiked with pSIM1278 (1278). Molecular weight markers, DIGII and DIGVII, are labeled in kilobases (kb). Red arrows indicate expected bands (1.57 kb and 5.6 kb) unique to the plasmid control (1278). Blue arrow indicates 0.8 kb band associated with the spacer 2 element of the pSIM1278 insert in E12.

CONCLUSION

In this study, Southern blotting was used to probe for the presence of backbone DNA in the E12 genome. No hybridization signal was detected corresponding to backbone DNA in the E12 genome in Southern blots hybridized with probes that spanned the length of the backbone. Thus, this report confirms the absence of backbone DNA in E12.

STUDY TITLE

Compositional Assessment of E12 Compared to Russet Burbank

AUTHOR(S)

[redacted]

REPORT DATE

October 14, 2015

PERFORMING LABORATORIES

Simplot Plant Sciences, Boise, ID
Covance Laboratories, Inc., Madison, WI

STUDY NUMBER

15-60-SPS-COMP-01

QUALITY CONTROL STATEMENT

This report was reviewed to assure that it accurately reflects the raw data of this study. The raw data were audited for compliance with the protocol, study notebook, and Standard Operating Procedures where applicable.

[redacted]

Quality Control Reviewer

10-14-15

Date

CERTIFICATION PAGE

This report is an accurate and complete representation of the study activities.

[redacted] 10-14-15

Study Coordinator and Author — Date

[redacted] 10/14/15

Author — Date

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ABSTRACT

The purpose of this study was to evaluate the nutritional composition and trait efficacy of potato event E12 compared with its parental control, Russet Burbank. Commercially available reference varieties with a history of safe use for food and feed were also grown as comparators. Field trials were conducted at a total of eleven sites during the 2009, 2010, and 2011 growing seasons. Plots of the test, control, and reference varieties were harvested, and tubers were assessed for those analytes important to potato nutrition as well as those related specifically to gene down-regulation and trait efficacy. The nutritional assessment, evaluating proximates, vitamins, minerals, amino acids, and glycoalkaloids demonstrated that E12 is compositionally equivalent to Russet Burbank. As expected, the efficacy assessment evaluating free amino acids and reducing sugars as well as acrylamide concentrations in fries demonstrated that E12 has lower levels of free asparagine, lower levels of reducing sugars, and lower acrylamide potential in fries than Russet Burbank.

INTRODUCTION

Potato event E12 was generated by transforming the Russet Burbank variety with pSIM1278 using *Agrobacterium* transformation. Traits conferred by the genetic elements of the T-DNA include lower free asparagine and reducing sugars, which together contribute to lower acrylamide potential in cooked potatoes, as well as reduced black spot.

STUDY OBJECTIVES

1. Compare the nutrient composition of E12 to its parental control and conventional potato varieties.
2. Determine trait efficacy of E12 with respect to free asparagine, reducing sugars, and acrylamide.

STUDY DATES

Field trials for tuber generation for compositional analysis were conducted during the 2009, 2010 and 2011 field seasons.

PERFORMING LABORATORIES

Simplot Plant Sciences, Boise, ID
Covance Laboratories, Inc., Madison, WI

In 2009 and 2010, samples for E12 and its parental control Russet Burbank, were analyzed in combination by the J.R. Simplot Company and Covance Laboratories, Inc.

In 2011, analyses were completed by Covance Laboratories, Inc.

METHODS

Selection of Control Varieties

To ensure accurate evaluation of E12, proper selection of control varieties was important. For E12, the most relevant comparator is Russet Burbank, its parental variety. The only difference between E12 and Russet Burbank is that E12 underwent transformation and contains a pSIM1278 insert.

Conventional non-transformed potato varieties with a history of safe use for food and feed were used as reference varieties. These varieties are commonly used in the chip, fry, dehydrated and fresh markets. The following reference varieties were grown to provide a range of values common to conventional potatoes: Atlantic, Chieftain, IdaRose, Red Norland, Ranger Russet, Snowden, and two proprietary varieties.

Field Trials

During the growing seasons of 2009, 2010 and 2011, E12 and its parental control were grown at eleven locations in potato growing regions of the United States. At most sites, additional varieties were grown as references to provide a range of values common to conventional potatoes. All reference varieties summarized in this study can be found inTable 1.

The agronomic practices and pest control measures used were location-specific and were typical for all aspects of potato cultivation including soil preparation, fertilizer application, irrigation and pesticide-based control methods.

Location, seed type, trial design and size are provided in Table 1. A combination of field-grown G1 and G2 seed as well as mini-tubers were used as planting material. These mini-tubers were produced by growing tissue culture plants hydroponically, enabling multiple seed harvests from each plant. The generation of seed used was not expected to impact composition or trait efficacy.

The experiments were established in a randomized complete block design (RCB). The RCB design is typical for the evaluation of new potato varieties and events.

In an RCB field trial, the experimental unit is a plot. A plot contains only one treatment (potato variety) and is planted with a specified number of seed tubers. Plots were typically 1-3 rows wide and 20 feet long. Typical seed spacing for potatoes is one tuber per foot of row. Therefore, 60 seed tubers would be planted to create a plot 3 rows wide and 20 feet long.

The treatments included the test, control, and reference varieties. In some cases, additional varieties being evaluated were included in the experimental design. A plot of each treatment is included once in each block (replicate). All plots within each block are independently randomized so that the treatments are in random order. There were typically three blocks in each experiment at each site.

Table 1 details the experimental design specifics for each site such as the generation of the seed tubers, the number of rows per plot, the number of seed pieces per row, and the number of blocks.

Table 1. Field Trial Locations for E12 and Russet Burbank

Year	State	County	Trial Design[1]	Rows x Planted Tubers/Row	Seed Type
2009	Idaho	Canyon	RCB, 3 reps/entry	1x20	Mini-tubers (GO)
2009	Idaho	Bingham	RCB, 3 reps/entry	1x20	Mini-tubers (GO)
2009	Michigan	Montcalm	RCB, 3 reps/entry	1x20	Mini-tubers (GO)
2010	Idaho	Bingham	RCB, 3 reps/entry	3x20	Field seed (G1)
2010	Idaho	Canyon	RCB, 3 reps/entry	3x20	Field seed (G1)
2010	North Dakota	Grand Forks	RCB, 3 reps/entry	3x20	Field seed (G1)
2010	Washington	Adams	RCB, 3 reps/entry	3x20	Field seed (G1)
2011	Idaho	Canyon	RCB, 3 reps/entry	3x20	Field seed (G2)
2011	Idaho	Bingham	RCB, 3 reps/entry	3x20	Field seed (G2)
2011	North Dakota	Grand Forks	RCB, 3 reps/entry	3x20	Field seed (G2)
2011	Washington	Adams	RCB, 3 reps/entry	3x20	Field seed (G2)

[1]Randomized Complete Block Designs contained the same number of blocks as the number of reps in the table.

Table 2. Field Trial List for Reference Varieties

Year	State	County	Reference Varieties[1]
2009	Idaho	Bingham	Ranger Russet, Russet Burbank
2009	Idaho	Canyon	Atlantic, Ranger Russet, Russet Burbank
2009	Michigan	Montcalm	Ranger Russet, Russet Burbank
2010	Florida	St. John's	2 Proprietary Varieties
2010	Idaho	Bingham	Atlantic, IdaRose, Ranger Russet, Russet Burbank, Snowden, 2 Proprietary Varieties
2010	Idaho	Canyon	Atlantic, Ranger Russet, Red Norland, Russet Burbank, Snowden
2010	Michigan	Missaukee	Atlantic, Snowden, 2 Proprietary Varieties
2010	Michigan	Montcalm	Atlantic, Snowden, 2 Proprietary Varieties
2010	Michigan	Montcalm	Red Norland, Snowden
2010	Nebraska	Cherry	Atlantic
2010	North Dakota	Grand Forks	Atlantic, Ranger Russet, Red Norland, Russet Burbank, Snowden
2010	Washington	Adams	Atlantic, Chieftain, Ranger Russet, Red Norland, Russet Burbank, Snowden
2010	Wisconsin	Oneida	Atlantic, Snowden, 2 Proprietary Varieties
2010	Wisconsin	Adams	Atlantic, Red Norland, Snowden
2011	Florida	St John's	Atlantic, Snowden
2011	Idaho	Bingham	Atlantic, Ranger Russet, Russet Burbank, Snowden
2011	Idaho	Canyon	Ranger Russet, Russet Burbank, Snowden
2011	Indiana	Pulaski	Atlantic, Snowden
2011	North Dakota	Grand Forks	Atlantic, Ranger Russet, Russet Burbank, Snowden
2011	Michigan	Montcalm	Atlantic
2011	Michigan	Montcalm	Snowden
2011	Washington	Adams	Atlantic, Ranger Russet, Russet Burbank, Snowden
2011	Wisconsin	Adams	Atlantic , Snowden

[1]Because Ranger Russet is both the control and a conventional potato variety with history of safe use as food and feed, it was used as both the control and in calculating tolerance intervals. The inclusion of Ranger Russet in the tolerance interval did not impact the statistical analysis, since it was calculated separately from the statistical comparisons.

Trials in the same county over multiple years were not in the same location. Plots were in different fields, or in different places on the farm due to crop rotation requirements. Field conditions such as environment, field history, soil type, pest presence, and drainage, for example, can differ from year to year. Each county and year combination was considered a unique site.

MATERIALS

Sample Collection. Test, control and reference tubers for the compositional assessment were collected from the 2009, 2010 and 2011 field trial locations listed in Table 1 and 2. Each sample consisted of five randomly selected tubers, from each site and replicate. Selected tubers were about 6 inches in length.

Testing Facility. Analytical testing for Russet Burbank samples was completed partially by the J.R. Simplot Co., Boise, ID in 2009 and 2010, with the remainder done by Covance Laboratories, Inc., Madison, WI. In 2011, all analyses were completed at Covance. Clarification of testing location by analysis is provided in Table 3. Although data were generated in two laboratories, both used published procedures for the analyses. Therefore, the data from both labs were combined in the statistical analysis.

Table 3. Laboratory Facilities Used for Compositional Analysis

Analysis	2009	2010	2011
	Ranger Russet, Russet Burbank	Ranger Russet, Russet Burbank, Atlantic, Snowden, Chieftain, Red Norland, IdaRose	Ranger Russet, Russet Burbank, Atlantic, Snowden
Key Proximates, Vitamins and Minerals	Covance	Covance	Covance
Total Amino Acids	Simplot	Simplot	Covance
Glycoalkaloids	Simplot	Simplot	Covance
Vitamin C	Simplot	Simplot	Covance
Free Amino Acids	Simplot	Simplot	Covance
Sugars	Simplot	Simplot	Covance
Acrylamide	Covance	Covance	Covance

Sample preparation. Three to nine longitudinal slices with skin from each tuber were taken from the top, bottom, and center of the tuber. Samples were immediately frozen in liquid N_2. These samples were freeze-dried, ground and stored at -80 °C until ready for analysis.

For acrylamide testing, samples of E12 and Russet Burbank potatoes were processed into fries, using standard practice for the foodservice fry industry. Reference varieties were not made into fries.

Moisture content: Simplot Lab. The percentage moisture content was determined by measuring the mass of a tuber before and after the water was removed by freeze-drying. The equation used for calculation was:

$$\% \text{ Moisture} = [(M_{Initial} - M_{Dried})/M_{Initial}] \times 100$$

Moisture content: Covance Labs. Moisture levels were determined by Covance Laboratories using Covance protocol M100T100_S:4 (Method 925.09 and Method 926.08. AOAC, 2005).

Ash: Covance Labs. Ash levels were determined by Covance Laboratories using Covance protocol ASHM_S:5 (Method 923.03. AOAC, 2005).

Carbohydrates: Covance Labs. Carbohydrate levels were determined by Covance Laboratories using Covance protocol CHO:6 (USDA, 1973).

Calories: Covance Labs. Total calories were determined by Covance Laboratories using Covance protocol CALC:4 (USDA, 1975).

Crude Fiber: Covance Labs. Crude fiber was determined by Covance Laboratories using Covance protocol CFIB_S:2 (Method 962.09. AOAC, 2005).

Elements by ICP Emission Spectrometry: Covance Labs. The minerals Copper (Cu), Magnesium (Mg), and Potassium (K) were determined by Covance Laboratories using Covance protocol (ICP_S:13) (Method 984.27 and Method 985.01. AOAC, 2005).

Fat: Covance Labs. Fat was determined by Acid Hydrolysis using Covance protocol FAAH_S:7(Method 922.06 and Method 954.02. AOAC, 2005).

Niacin: Covance Labs. Niacin was determined by Covance Laboratories using Covance protocol NIAP_S:11 (Method 944.13 and Method 960.46. AOAC, 2005).

Protein: Covance Labs. Protein was determined using the Kjeldahl method, approximating protein by multiplying Nitrogen by 6.25, as per Covance protocol PGEN_S:4 (Method 955.04 and Method 979.09. AOAC, 2005).

Pyridoxine: Pyridoxine was determined by Covance Laboratories using Covance protocol B6A_S:11 (Method 961.15. AOAC, 2005; Atkin, 1943).

Vitamin C and glycoalkaloids: Simplot Lab. Vitamin C and total glycoalkaloids (sum of α-chaconine and α-solanine) were extracted by following a fast extraction method as previously described by Shakya and Navarre, 2006, with slight modification. Freeze-dried tuber powder (about 200 mg) was placed into a 2 ml screw cap tube with 0.9 ml of extraction buffer (50% methanol, 3.0% metaphosphoric acid, 1 mM EDTA) and 500 mg of 1.0 mm glass beads. Tubes were shaken in a BeadBeater (Biospec, Bartelsville, OK) for 10 min at maximum speed, centrifuged for 5 min at 4 °C and the supernatant was transferred to a clean tube. The remaining pellet was re-extracted with 0.6 ml of extraction buffer and centrifuged. The supernatants were combined, centrifuged, and concentrated in a SpeedVac (Thermo Savant, Waltham, MA) prior to HPLC analysis. Gallic acid was added as an internal standard for quantification. Samples were kept chilled and protected from bright light at all times.

An Agilent 1200 HPLC system (Agilent Technologies) equipped with an on-line solvent degasser, quaternary pump, refrigerated autosampler, column heater, and variable wavelength diode-array (DAD) and MS detectors was used for sample analysis. Monolithic HPLC column Onyx C18, 100 x 4.6

(Phenomenex) was used with optimized parameters. Column temperature was 35 °C, flow of 1 ml/min with a gradient elution of 0−1 min 100% buffer A (10 mM formic acid, pH 3.5, with NH_4OH), 1−9 min 0−30% buffer B (100% methanol with 5 mM ammonium formate), 9−10.5 min 30% buffer B, 10.5−14 min 35−65% buffer B, 14-16 min 65%-100% buffer B, and 16-16.5 min 100% buffer B. Data acquisition and instrument parameters were controlled using the Agilent ChemStation. UV Diode-Array Detector (DAD) detection and quantification were at wavelength 244 nm for vitamin C and 210 nm for glycoalkaloids. The external standard method of calibration was used, with each curve prepared from 6 to 8 different concentrations of standard solutions.

Vitamin C and glycoalkaloid levels were further confirmed by LC-MS analysis with an Agilent 1200 HPLC coupled to 6320 ion trap LC/MS. Experiments were carried out with an ESI source in negative ion mode for vitamin C and positive ion mode for glycoalkaloids. The source was operated using 350 °C drying gas (N2) at 12 L/min, 55 psi nebulizer gas (N_2),and the source voltage with a scan range of *m*/*z* 100-1300. Automated MS (*2*) analysis was conducted using SmartFrag and Agilent ChemStation software (Agilent) with a ramp range from 1500-4500 V.

Vitamin C: Covance Labs. Vitamin C levels were determined by Covance Laboratories using protocol VCF_S:5 (Method 967.22. AOAC, 2005).

Glycoalkaloids: Covance Labs. Glycoalkaloid levels were determined by Covance Laboratories using protocol COID_S:2 (AOAC, 2006).

Free amino acids: Simplot Lab. Free amino acids were extracted by homogenizing 250 mg ground freeze-dried tubers with 5 µmol sarcosine as an internal standard in 3.0 ml 0.03 M triethylamine HCl buffer and adding (a) 150 µ 85 mM K hexacyanoferrate trihydrate ($K_4Fe(CN)_63H_2O$), (b) 150 µL 100 mM zinc sulfate ($ZnSO_4.7H_2O$), and (c) 250 µL 0.1 N NaOH with 3.0 ml 0.03 M TEA buffer pH 7.0, vortexing the mixture after each addition. The extract was centrifuged for 15 min at 4 °C at 4,000 rpm, and the supernatant was transferred to a new tube. The pellet was re-suspended in 5 ml nanopure water and centrifuged. The first and second supernatants were pooled and the final volume was adjusted to 12.5 ml with water. The extracted free amino acids were derivatized using the EZ:faast method according to the user's manual from the manufacturer (Phenomenex, Torrance, CA). Derivatized samples were analyzed by liquid chromatography-mass spectrometry (LC-MS) using an Agilent 1200 series HPLC system that was coupled to an Agilent 6300 series ion trap. Bruker's quant analysis software was used for quantification. For HPLC, we used a 25.0 x 0.3 cm EZ:faast AAA-MS column, and the mobile phase was 10 mM ammonium formate in water (A) and 10 mM ammonium formate in methanol 1:2, v/v (B), flow rate 0.25 min/ml with a gradient of 68-83% B in 13 min and 13-18 min 68% buffer B. MS was run in the positive mode with ESI and auto MS^n. The limit of quantitation for most matrices was 10mg/100 g dry weight (or 100 ppm in the fresh weight).

Free amino acids: Covance Labs. Free amino acid levels were determined by Covance Laboratories using the Covance protocol FAALC_S:6 (Barkholt and Jensen, 1989; Henderson, 2000; Schuster, 1988).

Tryptophan: Covance Labs. Tryptophan levels were determined by Covance Laboratories using the Covance protocol -TRPLC_S:3 (Method 988.15. AOAC, 2005).

Total amino acids: Simplot Lab. Amino acid hydrolysis for total amino acid analysis was performed as previously described (Miedel et al., 1989; Purcell et al., 1972) with optimization. About 50 mg freeze dried tissue was weighed and transferred into the bottom of a vacuum hydrolysis tube (5 ml, 10mm x 100 mm (Wilmad LabGlass, NJ). A 1 ml aliquot of constant boiling, 6N hydrochloric acid (Thermo Scientific/Pierce, IL) in the presence of 4 % thioglycholic acid was added directly to the sample. Vacuum was applied until no air bubbles were seen and the tube was sealed slowly by screwing the PTFE plug down. The hydrolysis was carried at 110 °C for 24 hr on a dry block heating system (Reacti-therm heating module, Thermo Scientific/Pierce, IL). Following hydrolysis and cooling, pressure in the tube was released slowly. The sample was removed by Pasteur pipette and a 20 µL aliquot of the sample was vacuum dried using an acid resistant speedVac (Savant SC250DDA, Thermo Scientific, IL). Samples were re-suspended in water to make a 10 fold dilution and centrifuged. TRP, cystine and CYS are unstable during acid hydrolysis and so were not estimated quantitatively. GLN and ASN are deaminated to form GLU and ASP. An aliquot of the supernatant was subjected to solid phase extraction (SPE) and derivatization steps using the EZ-faast: easy-fast amino acid sample testing kit (Phenomenex, Torrance, CA) as per manual. The derivatized amino acids were analyzed using a LC/MS instrument (Agilent Technologies) as described in free amino acid analysis.

Total amino acids: Covance Labs. Total amino acid levels were determined by Covance Laboratories using the Covance protocol TAALC_S:6 (Barkholt and Jensen, 1989; Henderson, 2000; Schuster, 1988).

Sugars: Simplot Lab. Sugars were extracted by shaking approximately 150 mg freeze-dried tissues in 1 ml 60% ethanol at 80 °C for 1 h. The supernatant was transferred into a fresh tube, and the pellet was re-extracted with 1 ml 60% ethanol for 30 min at 80 °C. The supernatant volume was reduced in a Speedvac to 60 to 70 µL. A known amount of ribose was added as an internal standard. Sugar analyses were performed on an Agilent 1200 series HPLC system, which consisted of an auto sampler, Zorbax carbohydrate column (4.6 × 150 mm, Agilent Technologies, USA), a solvent system of acetonitrile-water (75 : 25), and a refractive index detector, at a flow rate of 1 ml/min. Sugars were quantified using Agilent ChemStation software with external calibration.

Sugars: Covance Labs. Sugar levels were determined by Covance Laboratories using High Performance Anion Exchange Chromatograph (HPAEC) equipped with a Pulsed Amperometric Detector (PAD) and following Covance protocol SWET_S:9 (Lilla et al., 2005).

Acrylamide: The acrylamide levels were determined by Covance Laboratories in Greenfield, IN using the Covance protocol ACMS_GRN_S:4 (FDA, 2003).

Statistical Analysis. All attributes were analyzed using JMP (SAS Institute, Cary, NC) by combining data from multiple test years and locations using the following linear mixed model:

- $Y_{ijklm} = \alpha_{i} + \beta_{j} + \gamma_{k(j)} + \delta_{l(j,k)} + (\alpha\gamma)_{ik} + \varepsilon_{ijklm}$
- α = mean of treatment (fixed)
- β = year (random)
- γ = effect of location [year] (random)
- δ = rep[year, location] (random)
- ε = residual random error

Where α_i denotes the mean of the i^{th} treatment (fixed effect), β_j denotes the effect of the j^{th} year (random effect), $\gamma_{k(j)}\gamma_{k(i)}$ are the random location (within year effect), $\delta_{l(j,k)}$ are the rep within year and location effect, $(\alpha\,\gamma)_{ik}$ denotes the interaction between the i^{th} treatment and random k^{th} location within year effect, and ε_{ijklm} denotes the residual random error.

All data was tested for normality before conducting the analysis mentioned here.

In cases where all data were from one trial year, the statistical model did not include the year term. Data from multiple locations were analyzed together using the following linear mixed model:

- $Y_{ijk} = \alpha_i + \beta_j + \gamma_{k(j)} + (\alpha\,\beta)_{ij} + \varepsilon_{ijk}$
- α = mean of treatment (fixed effect)
- β = effect of location (random)
- γ = rep within location effect (random)
- ε = residual random error

Where α_i denotes the mean of the i^{th} treatment (fixed effect), β_j denotes the effect of the j^{th} location (random effect), $\gamma_{k(j)}$ are the random replicate (within location) effects, $(\alpha\,\beta)_{ij}$ denotes the interaction between i^{th} treatment and j^{th} location (random effect), and ε_{ijk} denotes the residual random error.

For the by site analyses, data from each location were analyzed using the following linear mixed model:

- $Y_{ij} = \alpha_i + \beta_j + \varepsilon_{ij}$
- α = mean of treatment (fixed effect)
- β = effect of rep (random)
- ε = residual random error

Where α_i denotes the mean of the i^{th} treatment (fixed effect), β_j denotes the effect of the j^{th} rep (random effect), and ε_{ij} denotes the residual random error.

A significant difference was established with a p-value ≤ 0.05.

The following varieties, all grown in the field trial sites, were used to calculate tolerance intervals (Vardeman, 1992): Atlantic, Chieftain, IdaRose, Ranger Russet, Red Norland, Russet Burbank, Snowden and two proprietary varieties. The tolerance interval represents measurements from conventional varieties by using data from all controls and reference varieties from all sites in this study.

The parental control, Russet Burbank was used as a reference variety because of its widespread popularity and its history of safe use as food and feed. The inclusion of the control in the tolerance interval did not impact the statistical analysis because the tolerance interval was a separate calculation.

The tolerance intervals were calculated using JMP to contain, with 95% confidence, 99% of the values contained in the population.

This tolerance interval and the combined range of values for each analyte available from the published literature were used to interpret the composition results. In interpreting the data, emphasis was placed on the analyte means; means that fell within the tolerance interval and/or combined literature range for the analyte were considered to be within the normal variability of commercial potato varieties.

RESULTS

A compositional analysis of E12 was conducted to evaluate the levels of key nutrients (proximates, vitamins, amino acids, and minerals) and glycoalkaloids compared to the parental control, Russet Burbank. In addition, concentrations of free amino acids, sugars, and acrylamide were evaluated in E12 and its parental control to measure efficacy of the low acrylamide potential and lowered reducing sugars traits. A summary of analytes tested can be found in

Table 4.

These analytes were selected by considering the important nutritional components of potatoes (OECD, 2002), the analytes expected to be altered based on the inserted DNA, and those analytes considered important in the potato industry. Tables 5 through 10 summarize data from across all sites and years. By-site data tables can be found in Appendix A.

Table 4. Tuber Composition Analytes Measured

Proximates and Fiber (7)		
Protein	Fat	Ash
Crude Fiber	Carbohydrates	Calories
Moisture		
Vitamins (3)		
Vitamin B3	Vitamin B6	Vitamin C
Minerals (3)		
Copper	Magnesium	Potassium
Total Amino Acids (18)		
Alanine	Histidine	Proline
Arginine	Isoleucine	Serine
Aspartic Acid + Asparagine	Leucine	Threonine
Cystine (including cysteine)	Lysine	Tryptophan
Glutamic Acid + Glutamine	Methionine	Tyrosine
Glycine	Phenylalanine	Valine
Free Amino Acids (4)		
Asparagine	Aspartic Acid	Glutamine
Glutamic Acid		
Sugars (2)		
Fructose + Glucose[1]	Sucrose[1]	
Anti-Nutrients (2)		
Glycoalkaloids	Acrylamide[2]	

[1]analyzed in both fresh tissue and at various monthly intervals.
[2]analyzed in processed materials from both fresh tissue and from tubers at various monthly intervals.

NUTRITIONAL ANALYSIS RESULTS FOR RUSSET BURBANK AND E12

These analyses were conducted to confirm that composition of E12 remained within the range for commercial potato varieties and has equivalent food quality, feed quality, and safety when compared to its parental control, Russet Burbank, and conventional potatoes. The compositional assessments determined the concentrations of:

1) Proximates, vitamins, and minerals (Table 5)
2) Total amino acids (Table 6)
3) Glycoalkaloids (Table 7)

Proximates, Vitamins, and Minerals

Table 5. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank

Variable	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval		Combined Literature Range[2]	
						Min	Max	Min	Max	Min	Max
Protein (%)	E12	2.30	0.8796	0.443	33	1.47	3.02	1.26	3.59	0.7	4.6
Protein (%)	Control	2.32	.	0.431	33	1.64	3.10				
Fat (%)	E12	0.100	0.9772	0.0323	33	0.0500	0.200	0	0.341	0.02	0.2
Fat (%)	Control	0.100	.	0.0365	32	0.0300	0.200				
Ash (%)	E12	1.05	0.5871	0.294	32	0	1.46	0.391	1.89	0.44	1.9
Ash (%)	Control	1.08	.	0.307	33	0.150	2.00				
CrudeFiber (%)	E12	0.470	0.8625	0.107	33	0.330	0.700	0.142	0.690	0.17	3.5
CrudeFiber (%)	Control	0.470	.	0.0979	33	0.320	0.640				
Carbohydrates (%)	E12	18.5	0.9866	1.78	33	14.2	22.2	12.3	25.9	13.3	30.53
Carbohydrates (%)	Control	18.5	.	1.55	33	16.1	21.1				
Calories (kcal/100 g)	E12	83.9	0.9537	6.49	33	64.9	96.3	59.1	115	70	110.2
Calories (kcal/100 g)	Control	84.0	.	5.54	33	73.1	93.3				
Moisture (%)	E12	78.1	0.8815	1.52	33	75.5	82.5	70.3	83.9	63.2	86.9
Moisture (%)	Control	78.0	.	1.36	33	75.5	80.4				
Vitamin B3 (mg/100 g)	E12	1.90	0.7264	0.277	33	1.43	2.53	0.922	3.12	0.09	3.1
Vitamin B3 (mg/100 g)	Control	1.87	.	0.268	33	1.44	2.38				
Vitamin B6 (mg/100 g)	E12	0.140	0.9380	0.0317	33	0.110	0.230	0.0590	0.192	0.13	0.41
Vitamin B6 (mg/100 g)	Control	0.14	.	0.0241	33	0.110	0.200				
Vitamin C (mg/110 g)	E12	18.3	0.3510	5.50	33	11.8	32.9	0	129	1	54
Vitamin C (mg/110 g)	Control	16.8	.	8.20	33	8.88	42.9				
Copper (ppm)	E12	1	0.6422	0.227	33	0.540	1.52	0.111	2.24	0.15	7
Copper (ppm)	Control	0.960	.	0.242	33	0.500	1.34				
Magnesium (ppm)	E12	246	0.7238	49.4	33	188	405	102	372	112.5	550
Magnesium (ppm)	Control	242	.	45.9	33	165	350				
Potassium (ppm)	E12	4,777	0.6940	1,042	33	3,550	6,537	2,711	6,882	3,500	6,250
Potassium (ppm)	Control	4,681	.	951	33	3,040	6,339				

[1]P-values indicating significant differences with controls are underlined.
[2]Combined literature ranges are from, Horton and Anderson, 1992; Lisinska and Leszczynski, 1989; Rogan et al., 2000; Talburt and Smith, 1987.

No statistical differences were found between E12 and Burbank for the proximates, vitamins and minerals measured (Table 5). All mean values for E12 were within the tolerance interval and/or the combined literature range.

Total Amino Acids

A total of eighteen amino acids were measured as seen in Table 6.

Although expected, a significant difference between E12 and the control, Russet Burbank, was noted for ASP+ASN and GLU+GLN. ASP+ASN was expected to be lower and GLU+GLN was expected to be higher in E12 than in Russet Burbank because of the down-regulation of the *Asn1* gene. In each case, the mean for E12 was within the tolerance interval and/or the combined literature range, so E12 was considered equivalent to conventional potatoes.

A significant difference between E12 and the control was also noted for three additional total amino acids (Table 6): arginine, cystine (including cysteine) and methionine. In all cases, the mean values for E12 were within the tolerance interval and/or the combined literature range.

Table 6. Total Amino Acids in E12 and Control Russet Burbank

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval		Combined Literature Range[2]	
						Min	Max	Min	Max	Min	Max
Alanine	E12	718	0.8299	145	33	430	1,053	100	1,335	392	952
Alanine	Control	704	.	245	33	323	1,258				
Arginine	E12	1,080	**0.0314**	454	33	490	2,159	368	1,922	700	1,383
Arginine	Control	945	.	296	33	508	1,731				
ASP+ASN	E12	2,846	**<.0001**	947	33	1,874	5,114	100	13,340	3,385	7,380
ASP+ASN	Control	5,391	.	1,492	33	2,090	8,228				
Cystine[3]	E12	219	**0.0455**	39.8	12	162	282	0	471	286	12,500
Cystine[3]	Control	186		38.8	12	118	251				
GLU+GLN	E12	4,843	**<.0001**	1,010	33	3,517	7,376	568	7,011	2,915	6,035
GLU+GLN	Control	3,051	.	549	33	1,840	3,982				
Glycine	E12	1,329	0.4886	760	33	489	2,885	100	2,865	500	1,990
Glycine	Control	1,459	.	937	33	307	3,548				
Histidine	E12	426	0.2571	216	33	170	991	100	761	133	469
Histidine	Control	373	.	157	33	100	762				
Isoleucine	E12	705	0.7803	197	33	406	1,266	145	1,346	525	953
Isoleucine	Control	687	.	128	33	372	912				
Leucine	E12	1,266	0.6831	329	33	804	2,024	477	2,174	685	1,383
Leucine	Control	1,228	.	295	33	530	1,783				
Lysine	E12	1,111	0.2049	248	33	763	1,795	100	3,363	687	1,368
Lysine	Control	894	.	176	33	560	1,304				
Methionine	E12	418	**0.0410**	152	33	228	826	100	593	300	500
Methionine	Control	368	.	104	33	186	617				
Phenylalanine	E12	799	0.7459	201	33	542	1,247	397	1,395	552	1087
Phenylalanine	Control	787	.	178	33	417	1,150				
Proline	E12	768	0.4801	239	33	487	1,425	100	1,492	355	1,464
Proline	Control	718	.	229	33	321	1,415				
Serine	E12	575	0.5840	209	33	220	985	100	1,362	500	1,022
Serine	Control	548	.	169	33	214	817				
Threonine	E12	659	0.7744	286	33	299	1,347	226	1,315	500	1,022
Threonine	Control	646	.	243	33	220	1,331				
Tryptophan[3]	E12	204	0.5724	24.9	12	163	237	115	435	436	855
Tryptophan[3]	Control	200	.	14.9	12	182	224				
Tyrosine	E12	620	0.3040	203	33	266	989	100	1,523	457	942
Tyrosine	Control	562	.	127	33	328	792				
Valine	E12	1,076	0.2361	196	33	612	1,519	100	1,860	752	1,450
Valine	Control	1,176	.	324	33	564	2,012				

[1]P-values indicating significant differences with controls are underlined.
[2]Combined literature ranges are from Lisinska and Leszczynski, 1989; OECD, 2002; Rogan et al., 2000; Talley et al., 1984.
[3]Cystine and tryptophan measured in 2011 only.

Glycoalkaloids

Glycoalkaloids are toxins commonly found in Solanaceous crops, including potato, and 95% of the total glycoalkaloids in potato tubers consists of α-solanine and α-chaconine (OECD, 2002). The widely accepted safety limit for total glycoalkaloids in tubers is 20 mg/100 gm fresh weight (Smith et al., 1996).

The mean concentration of glycoalkaloids in E12 was not statistically different from the control, was lower than the generally accepted safety limit, and fell within the tolerance interval and/or the combined literature range (Table 7).

Table 7. Glycoalkaloids in E12 and Control Russet Burbank

Variable	Variety	Mean (mg/100g)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval		Combined Literature Range[2]	
						Min	Max	Min	Max	Min	Max
Glycoalkaloids	E12	5.68	0.0524	2.06	33	1.69	10.5	0	33.1	3.20	210.4
	Control	7.40	.	3.49	33	2.74	18.6				

[1]P-values indicating significant differences with controls are underlined.
[2]Combined literature ranges from Kozukue et al., 2008.

EFFICACY ANALYSIS RESULTS FOR RUSSET BURBANK AND E12

An assessment of trait efficacy of E12 for low acrylamide potential and lowered reducing sugars consisted of the following analyses:

1) Free amino acids (Table 8) in tubers
2) Reducing Sugars (Table 9) in tubers
3) Acrylamide (Table 10) in tubers

Free Amino Acids

Free amino acid analysis demonstrated that, as expected, down-regulation of *Asn1* was effective in reducing free asparagine in tubers. The results show that E12 tubers contained statistically less free asparagine and statistically more free glutamine than Russet Burbank tubers (Table 8). However, the mean concentrations of free asparagine and free glutamine for E12 were still within the tolerance intervals and/or the combined literature range and therefore considered within the normal range for potatoes.

Table 8. Free Amino Acids in Tubers of E12 and Control Russet Burbank[1]

Variable	Variety	Mean (ppm)	P-Value[2]	Standard Deviation	N	Range		Tolerance Interval		Combined Literature Range[3]	
						Min	Max	Min	Max	Min	Max
Asparagine	E12	618	**<.0001**	173	33	425	1,250	500	4,952	312	6,890
Asparagine	Control	2,421	.	901	33	822	4,030				
Aspartic Acid	E12	448	0.9073	130	33	293	824	100	1,411	64	752
Aspartic Acid	Control	439	.	137	33	280	760				
Glutamine	E12	1997	**<.0001**	663	33	865	3,490	100	3,449	440	5,396[4]
Glutamine	Control	1111	.	292	33	605	1,700				
Glutamic Acid	E12	460	0.9008	133	33	227	741	100	1,192	450	742
Glutamic Acid	Control	466	.	133	33	221	689				

[1]Free amino acids were included to show efficacy of reducing free ASN and thus reducing acrylamide. If values fell below detection limits, they were adjusted to the limit of detection (100 ppm) for statistical analysis.
[2]P-values indicating significant differences with controls are underlined.
[3]Combined literature ranges are from Davies, 1977; Lisinska and Leszczynski, 1989; Shepherd et al., 2010.
[4]For glutamine, the maximum value from the CLR high level is reported as 5,396 ppm from the mean of four sites (Davies, 1977). A value of 18,244 ppm from a single site was not included because it appeared to be an outlier.

Reducing Sugars

The E12 event contains expression cassettes designed to lower levels of reducing sugars fructose and glucose in tubers. A down-regulation cassette for the promoters of the starch-associated gene (*R1*) and the phosphorylase-L gene (*PhL*) was introduced in E12. These traits are intended to function by slowing the conversion of starch to the reducing sugars glucose and fructose.

Mean results for fructose plus glucose and sucrose levels were within range of the tolerance interval values for fresh, one, three and five months storage for event E12. Partial down-regulation of *R1* and *PhL* resulted in lower levels of reducing sugars at harvest and after one month storage with statistical differences found only at 1 month of storage (Table 9). Values for three and five months were similar for both E12 and the control.

Table 9. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage

Variable	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval		Combined Literature Range[2]	
						Min	Max	Min	Max	Min	Max
Fructose and Glucose (%)											
Fresh[3]	E12	0.160	0.1101	0.0760	12	0.0690	0.339	0	0.307	0.018	0.803
	Control	0.195	.	0.0943	12	0.0530	0.357				
Month 1[4]	E12	0.107	**0.0051**	0.0612	21	0.0340	0.251	0	0.307	0.018	0.803
	Control	0.162	.	0.0946	21	0.0530	0.393				
Month 3[5]	E12	0.249	0.2001	0.151	25	0.0810	0.580	0	0.307	0.018	0.803
	Control	0.290	.	0.155	27	0.117	0.660				
Month 5[6]	E12	0.145	0.2885	0.0909	18	0.0850	0.489	0	0.307	0.018	0.803
	Control	0.190	.	0.151	20	0.0920	0.630				
Sucrose (%)											
Fresh[3]	E12	0.213	0.2115	0.0653	12	0.134	0.299	0	0.315	0.0397	1.39
	Control	0.179	.	0.0608	11	0.113	0.288				
Month 1[4]	E12	0.132	**0.0123**	0.0583	21	0.0600	0.258	0	0.315	0.0397	1.39
	Control	0.168	.	0.0601	21	0.103	0.252				
Month 3[5]	E12	0.0734	0.5109	0.0246	25	0.0340	0.126	0	0.315	0.0397	1.39
	Control	0.0793	.	0.0203	27	0.0470	0.121				
Month 5[6]	E12	0.0882	0.5554	0.0259	18	0.0510	0.141	0	0.315	0.0397	1.39
	Control	0.0792	.	0.0288	21	0.0350	0.127				

[1] P-values indicating significant differences with controls are underlined.
[2] Literature Ranges from Amrein et al., 2003; Vivanti et al., 2006.
[3] Tubers from the fresh time point were analyzed from all sites in 2011.
[4] Tubers from the 1 month time point were analyzed from all sites in 2009 and 2010.
[5] Tubers from the 3 month time point were analyzed from all sites in 2009 and 2010 and from Bingham and Adams counties in 2011.
[6] Tubers from the 5 month time point were analyzed from all sites in 2009 and 2010.

Acrylamide

Lowered asparagine, fructose and glucose levels led to an overall reduction of acrylamide in processed potato as they are reactants in the formation of acrylamide. To demonstrate the lower potential acrylamide trait, field-grown tubers of E12 and Russet Burbank at harvest and after 2, 3, 5, 6, and 7 months of storage were processed into cooked fries, and the acrylamide concentration was measured (Table 10).

At the time of harvest, fries made with E12 tubers contained 67.2% less acrylamide than fries made with Russet Burbank (Table 10). When potatoes were stored for 2 months, acrylamide concentrations in E12 were 69.6% lower than the control. Acrylamide concentrations in E12 fries were statistically lower than Russet Burbank fries after tuber storage for 3, 5, and 6 months. The significantly lower acrylamide levels after storage were expected from down-regulation of the *Asn1, R1 and PhL* genes, thus reducing the

reactants free asparagine and reducing sugars. Similar reductions in reducing sugars and acrylamide were reported by Zhu et al., 2014.

A combined literature range is not provided due to the fact different cooking temperatures and times vary and affect acrylamide levels in processed potatoes. Because of this, acrylamide levels from literature may not be comparable to event E12 or Russet Burbank. Mean results for acrylamide levels were within range of the tolerance interval.

Table 10. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage

Variable	Variety	Mean (ppb)	P-Value[1]	Standard Deviation	N	Percent Reduction	Range		Tolerance Interval	
							Min	Max	Min	Max
Fresh[2]	E12	162	**0.0340**	56	24	67.2	95.0	298	0	3,198
	Control	494	.	130	24		286	770		
Month 2[3]	E12	237	0.1700	13	9	69.6	209	251	0	3,198
	Control	779	.	125	9		619	950		
Month 3[4]	E12	315	**<.0001**	97	24	61.3	169	254	0	3,198
	Control	814	.	230	24		502	1,240		
Month 5[5]	E12	82.0	**<.0001**	10	9	64.3	67.0	97.0	0	3,198
	Control	230	.	20	9		207	260		
Month 6[6]	E12	192	**0.0019**	66	12	52.1	105	272	0	3,198
	Control	401	.	175	12		234	776		
Month 7[7]	E12	192	0.8835	26	3	71.0	165	217	0	3,198
	Control	661	.	55	3		598	693		

[1]P-values indicating significant differences with controls are underlined.
[2]Tubers from the fresh time point were analyzed from all sites in 2010 and 2011.
[3]Tubers from the 2 month time point were analyzed from all sites in 2009.
[4]Tubers from the 3 month time point were analyzed from all sites in 2010 and 2011.
[5]Tubers from the 5 month time point were analyzed from all sites in 2009.
[6]Tubers from the 6 month time point were analyzed from all sites in 2010.
[7]Tubers from the 7 month time point were analyzed only from the Canyon County site in 2009.

CONCLUSION

A compositional assessment was conducted on E12 and its parental control, Russet Burbank. Two types of analyses were conducted:

- Compositional nutritional assessment, for those analytes important to potato nutrition; and
- Traits affecting composition, for those analytes related specifically to gene down-regulation and trait efficacy.

The nutritional assessment, evaluating proximates, vitamins, minerals, amino acids, and glycoalkaloids demonstrated that E12 is compositionally equivalent to Russet Burbank and is as safe and nutritious for food and feed as conventional potatoes that have a long history of safe consumption.

As expected, the efficacy assessment evaluating free amino acids and reducing sugars as well as acrylamide concentrations in fries demonstrated that E12 has lower levels of free asparagine, lower levels of reducing sugars, and lower acrylamide potential in fries than Russet Burbank.

APPENDIX A

The following analytes are summarized for each individual field trial site throughout the growing seasons of 2009, 2010, and 2011 in tables A1 through A66:

1) Proximates, vitamins, and minerals
2) Total amino acids
3) Glycoalkaloids
4) Free amino acids
5) Reducing sugars
6) Acrylamide

As mentioned in the footnotes in tables 6, 9, and 10, exceptions in data collection are summarized below:

Total Amino Acids

- Cystine and tryptophan were measured only in 2011

Reducing Sugars

- Tubers from the fresh time point were analyzed from all sites in 2011.
- Tubers from the 1 month time point were analyzed from all sites in 2009 and 2010.
- Tubers from the 3 month time point were analyzed from all sites in 2009 and 2010 and from Bingham and Adams counties in 2011.
- Tubers from the 5 month time point were analyzed from all sites in 2009 and 2010.

Acrylamide

- Tubers from the fresh time point were analyzed from all sites in 2010 and 2011.
- Tubers from the 2 month time point were analyzed from all sites in 2009.
- Tubers from the 3 month time point were analyzed from all sites in 2010 and 2011.
- Tubers from the 5 month time point were analyzed from all sites in 2009.
- Tubers from the 6 month time point were analyzed from all sites in 2010.
- Tubers from the 7 month time point were analyzed from the Canyon County site in 2009.

Table A-1. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Adams County, Washington in 2010

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.66	0.2286	0.182	3	2.50	2.86
Protein (%)	Control	2.86	.	0.223	3	2.65	3.10
Fat (%)	E12	0.0900	0.8842	0.0441	3	0.0600	0.140
Fat (%)	Control	0.0900	.	0.0149	3	0.0800	0.110
Ash (%)	E12	1.27	0.1283	0.0595	3	1.21	1.33
Ash (%)	Control	1.34	.	0.0872	3	1.24	1.41
Crude Fiber (%)	E12	0.540	0.5502	0.0042	3	0.530	0.540
Crude Fiber (%)	Control	0.520	.	0.0494	3	0.480	0.570
Carbohydrates (%)	E12	17.9	0.2275	0.422	3	17.4	18.2
Carbohydrates (%)	Control	17.3	.	0.922	3	16.3	18.2
Calories (kcal/100g)	E12	83.1	0.4071	2.36	3	80.4	84.7
Calories (kcal/100g)	Control	81.3	.	3.23	3	78.5	84.8
Moist (%)	E12	78.1	0.4823	0.580	3	77.7	78.7
Moist (%)	Control	78.5	.	0.808	3	77.5	79.1
Vitamin B3 (mg/100 g)	E12	1.98	0.8789	0.0380	3	1.94	2.02
Vitamin B3 (mg/100 g)	Control	2.00	.	0.105	3	1.88	2.07
Vitamin B6 (mg/100 g)	E12	0.120	0.5137	0.0082	3	0.120	0.130
Vitamin B6 (mg/100 g)	Control	0.130	.	0.0130	3	0.120	0.140
Vitamin C (mg/110 g)	E12	13.3	**0.0064**	1.49	3	11.8	14.8
Vitamin C (mg/110 g)	Control	9.59	.	0.500	3	9.24	10.2
Copper (ppm)	E12	1.20	0.7262	0.0649	3	1.15	1.27
Copper (ppm)	Control	1.24	.	0.0809	3	1.19	1.34
Magnesium (ppm)	E12	286	0.4169	7.23	3	281	294
Magnesium (ppm)	Control	270	.	9.88	3	261	281
Potassium (ppm)	E12	6,426	0.1984	127	3	6,288	6,537
Potassium (ppm)	Control	6,073	.	150	3	5,918	6,217

Table A-2. Total Amino Acids in E12 and Control Russet Burbank at Adams County, Washington in 2010

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	583	0.5838	42.3	3	538	623
Alanine	Control	641	.	58.6	3	585	702
Arginine	E12	840	0.4064	98.9	3	738	935
Arginine	Control	737	.	59.2	3	696	805
ASP+ASN	E12	2,625	**0.0012**	540	3	2,206	3,235
ASP+ASN	Control	6,915	.	539	3	6,466	7,513
Cystine	E12	.	.	.	0	.	.
Cystine	Control	.	.	.	0	.	.
GLU+GLN	E12	4,848	**0.0035**	795	3	4,240	5,747
GLU+GLN	Control	3,099	.	306	3	2,796	3,408
Glycine	E12	1,467	0.7257	54.8	3	1,404	1,507
Glycine	Control	1,414	.	41.7	3	1,389	1,462
Histidine	E12	212	0.3345	38.6	3	190	256
Histidine	Control	312	.	62.2	3	262	382
Isoleucine	E12	552	0.1286	104	3	446	653
Isoleucine	Control	729	.	33.3	3	691	753
Leucine	E12	1,223	0.7600	235	3	968	1,431
Leucine	Control	1,273	.	37.4	3	1,243	1,315
Lysine	E12	907	0.7239	146	3	763	1,056
Lysine	Control	791	.	28.8	3	758	809
Methionine	E12	328	0.3990	34.4	3	301	367
Methionine	Control	369	.	32.3	3	337	402
Phenylalanine	E12	683	0.2205	109	3	577	794
Phenylalanine	Control	785	.	71.0	3	711	852
Proline	E12	587	0.7228	106	3	497	704
Proline	Control	651	.	34.0	3	614	681
Serine	E12	529	0.7015	9.30	3	521	539
Serine	Control	554	.	44.3	3	513	601
Threonine	E12	544	0.7933	137	3	403	675
Threonine	Control	507	.	61.3	3	436	546
Tryptophan	E12	.	.	NA	0	.	.
Tryptophan	Control	.	.	NA	0	.	.
Tyrosine	E12	495	0.4356	108	3	384	600
Tyrosine	Control	611	.	25.9	3	585	637
Valine	E12	809	**0.0188**	144	3	643	896
Valine	Control	1,193	.	77.7	3	1,142	1,282

Table A-3. Glycoalkaloids in E12 and Control Russet Burbank in Adams County, Washington in 2010

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	2.16	0.1815	0.758	3	1.69	3.04
	Control	3.91	.	1.39	3	2.74	5.45

Table A-4. Free Amino Acids in Tubers of E12 and Control Russet Burbank in Adams County, Washington in 2010

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	541	**<.0001**	59.3	3	490	606
Asparagine	Control	3,052	.	164	3	2,956	3,242
Aspartic Acid	E12	671	0.7587	134	3	577	824
Aspartic Acid	Control	720	.	40.1	3	680	760
Glutamine	E12	2,164	**0.0239**	432	3	1,899	2,662
Glutamine	Control	1,296	.	47.6	3	1,243	1,333
Glutamic Acid	E12	470	0.3217	35.5	3	435	506
Glutamic Acid	Control	571	.	80.7	3	515	664

Table A-5. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Adams County, Washington in 2010

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.0433	0.1267	0.0082	3	0.0340	0.0494
	Control	0.0621	.	0.0106	3	0.0532	0.0738
Month 3	E12	0.146	**0.0055**	0.0058	3	0.139	0.149
	Control	0.227	.	0.0419	3	0.186	0.270
Month 5	E12	0.171	0.6205	0.0338	3	0.135	0.202
	Control	0.145	.	0.0415	3	0.111	0.192
Sucrose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.103	0.8188	0.0151	3	0.0898	0.120
	Control	0.107	.	0.0040	3	0.103	0.111
Month 3	E12	0.0427	**0.0018**	0.0078	3	0.0339	0.0485
	Control	0.0795	.	0.0249	3	0.0555	0.105
Month 5	E12	0.0616	**0.0116**	0.0084	3	0.0528	0.0696
	Control	0.0420	.	0.0092	3	0.0366	0.0527

Table A-6. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Adams County, Washington in 2010

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	111	**0.0035**	13.7	3	102	127
	Control	422	.	64.5	3	348	468
Month 3	E12	238	**<.0001**	27.9	3	206	257
	Control	759	.	96.8	3	654	845
Month 6	E12	109	**<.0001**	3.51	3	105	112
	Control	350	.	47.6	3	322	405

Table A-7. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Adams County, Washington in 2011

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.50	0.4151	0.117	3	2.37	2.59
Protein (%)	Control	2.37	.	0.0404	3	2.33	2.41
Fat (%)	E12	0.100	1.0000	0	3	0.100	0.100
Fat (%)	Control	0.100	.	0	3	0.100	0.100
Ash (%)	E12	1.08	0.7980	0.110	3	0.980	1.20
Ash (%)	Control	1.06	.	0.100	3	0.990	1.17
Crude Fiber (%)	E12	0.350	0.5286	0.0199	3	0.330	0.370
Crude Fiber (%)	Control	0.330	.	0.0023	3	0.330	0.340
Carbohydrates (%)	E12	19.0	0.3160	0.351	3	18.6	19.3
Carbohydrates (%)	Control	19.8	.	0.513	3	19.2	20.2
Calories (kcal/100 g)	E12	86.0	0.4235	1.50	3	84.8	87.7
Calories (kcal/100 g)	Control	88.9	.	2.45	3	86.4	91.3
Moisture (%)	E12	77.4	0.4545	0.265	3	77.1	77.6
Moisture (%)	Control	76.8	.	0.451	3	76.3	77.2
Vitamin B3 (mg/100 g)	E12	1.76	0.7151	0.0153	3	1.75	1.78
Vitamin B3 (mg/100 g)	Control	1.72	.	0.104	3	1.60	1.80
Vitamin B6 (mg/100 g)	E12	0.140	0.7863	0.0036	3	0.140	0.140
Vitamin B6 (mg/100 g)	Control	0.140	.	0.0071	3	0.140	0.150
Vitamin C (mg/110 g)	E12	22.7	0.8505	1.63	3	20.9	24.1
Vitamin C (mg/110 g)	Control	23.5	.	0.551	3	22.9	24.0
Copper (ppm)	E12	0.870	0.8022	0.0800	3	0.780	0.940
Copper (ppm)	Control	0.850	.	0.114	3	0.770	0.980
Magnesium (ppm)	E12	205	0.6556	8.50	3	195	211
Magnesium (ppm)	Control	211	.	4.16	3	206	214
Potassium (ppm)	E12	3,720	0.9362	70.0	3	3,640	3,770
Potassium (ppm)	Control	3,700	.	87.2	3	3,640	3,800

Table A-8. Total Amino Acids in E12 and Control Russet Burbank at Adams County, Washington in 2011

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	806	**0.0012**	16.2	3	797	825
Alanine	Control	689	.	27.9	3	657	708
Arginine	E12	1,347	**0.0049**	50.3	3	1,300	1,400
Arginine	Control	1,153	.	11.5	3	1,140	1,160
ASP+ASN	E12	2,923	**<.0001**	297	3	2,670	3,250
ASP+ASN	Control	5,610	.	139	3	5,530	5,770
Cystine	E12	268	**0.0007**	12.9	3	257	282
Cystine	Control	199		6.66	3	192	205
GLU+GLN	E12	6,063	**<.0001**	817	3	5,120	6,560
GLU+GLN	Control	3,500	.	276	3	3,210	3,760
Glycine	E12	648	**0.0216**	19.0	3	627	664
Glycine	Control	571	.	16.7	3	558	590
Histidine	E12	356	0.0708	11.2	3	344	366
Histidine	Control	327	.	6.66	3	323	335
Isoleucine	E12	879	**0.0288**	1.73	3	878	881
Isoleucine	Control	803	.	20.7	3	784	825
Leucine	E12	1,233	**0.0375**	20.8	3	1,210	1,250
Leucine	Control	1,097	.	46.2	3	1,070	1,150
Lysine	E12	1,183	**0.0451**	40.4	3	1,140	1,220
Lysine	Control	1,093	.	15.3	3	1,080	1,110
Methionine	E12	459	**0.0018**	3.79	3	456	463
Methionine	Control	400	.	11.0	3	389	411
Phenylalanine	E12	1,027	**0.0497**	15.3	3	1,010	1,040
Phenylalanine	Control	935	.	6.35	3	928	939
Proline	E12	781	0.1345	38.9	3	746	823
Proline	Control	671	.	38.7	3	635	712
Serine	E12	899	**0.0091**	17.2	3	880	914
Serine	Control	792	.	13.6	3	779	806
Threonine	E12	812	0.0155	32.0	3	775	831
Threonine	Control	708	.	20.8	3	691	731
Tryptophan	E12	223	0.4390	11.5	3	210	232
Tryptophan	Control	213	.	4.36	3	208	216
Tyrosine	E12	919	**<.0001**	18.0	3	902	938
Tyrosine	Control	690	.	22.6	3	671	715
Valine	E12	1,303	**0.0029**	23.1	3	1,290	1,330
Valine	Control	1,170	.	10	3	1,160	1,180

Table A-9. Glycoalkaloids in E12 and Control Russet Burbank in Adams County, Washington in 2011

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	5.00	0.7496	0	3	5.00	5.00
	Control	5.29	.	0.502	3	5.00	5.87

Table A-10. Free Amino Acids in Tubers of E12 and Control Russet Burbank in Adams County, Washington in 2011

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	995	**<.0001**	221	3	859	1,250
Asparagine	Control	3,703	.	181	3	3,570	3,910
Aspartic Acid	E12	499	0.0814	5.86	3	492	503
Aspartic Acid	Control	458	.	9.29	3	448	466
Glutamine	E12	3,263	**<.0001**	375	3	2,830	3,490
Glutamine	Control	1,427	.	246	3	1,170	1,660
Glutamic Acid	E12	676	**0.0418**	56.1	3	640	741
Glutamic Acid	Control	588	.	25.0	3	571	617

Table A-11. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Adams County, Washington in 2011

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	0.130	0.1650	0.0235	3	0.103	0.144
	Control	0.187	.	0.0185	3	0.169	0.206
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	0.383	0.4482	0.0521	3	0.349	0.443
	Control	0.346	.	0.0080	3	0.338	0.354
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Sucrose (%)							
Fresh	E12	0.144	0.7689	0.0068	3	0.136	0.149
	Control	0.154	.	0.0182	3	0.139	0.174
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	0.0974	0.1818	0.0039	3	0.0932	0.101
	Control	0.105	.	0.0044	3	0.102	0.110
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.

Table A-12. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Adams County, Washington in 2011

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	170	**<.0001**	4.04	3	165	172
	Control	400	.	29.7	3	366	422
Month 3	E12	308	**<.0001**	35.9	3	281	349
	Control	699	.	50.3	3	662	756

Table A-13. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Bingham County, Idaho in 2009

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.18	**<.0001**	0.0564	3	2.12	2.23
Protein (%)	Control	2.40	.	0.0034	3	2.40	2.40
Fat (%)	E12	0.0900	0.0904	0.0205	3	0.0700	0.110
Fat (%)	Control	0.0700	.	0.0061	3	0.0600	0.0700
Ash (%)	E12	0.960	0.7838	0.0681	3	0.890	1.01
Ash (%)	Control	0.970	.	0.0198	3	0.960	0.990
Crude Fiber (%)	E12	0.410	0.4644	0.0126	3	0.400	0.430
Crude Fiber (%)	Control	0.420	.	0.0043	3	0.420	0.430
Carbohydrates (%)	E12	17.3	**<.0001**	0.471	3	17.0	17.9
Carbohydrates (%)	Control	16.3	.	0.0847	3	16.2	16.4
Calories (kcal/100 g)	E12	78.9	**0.0008**	2.18	3	77.5	81.4
Calories (kcal/100 g)	Control	75.4	.	0.317	3	75.1	75.7
Moisture (%)	E12	79.4	**0.0014**	0.570	3	78.8	79.8
Moisture (%)	Control	80.3	.	0.0666	3	80.2	80.3
Vitamin B3 (mg/100 g)	E12	1.70	0.1607	0.0625	3	1.63	1.74
Vitamin B3 (mg/100 g)	Control	1.58	.	0.101	3	1.47	1.66
Vitamin B6 (mg/100 g)	E12	0.180	0.1707	0.0505	3	0.130	0.230
Vitamin B6 (mg/100 g)	Control	0.150	.	0.0078	3	0.140	0.160
Vitamin C (mg/110 g)	E12	13.7	**<.0001**	0.294	3	13.4	13.9
Vitamin C (mg/110 g)	Control	11.3	.	0.266	3	11.1	11.6
Copper (ppm)	E12	0.850	0.4325	0.119	3	0.770	0.990
Copper (ppm)	Control	0.890	.	0.0216	3	0.860	0.910
Magnesium (ppm)	E12	198	0.8474	6.07	3	191	203
Magnesium (ppm)	Control	197	.	1.93	3	195	199
Potassium (ppm)	E12	4,369	**0.0431**	117	3	4,278	4,501
Potassium (ppm)	Control	4,195	.	74.1	3	4,133	4,277

Table A-14. Total Amino Acids in E12 and Control Russet Burbank at Bingham County, Idaho in 2009

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	680	0.0698	66.0	3	620	751
Alanine	Control	565	.	26.1	3	536	587
Arginine	E12	2,076	**0.0172**	109	3	1,952	2,159
Arginine	Control	1,468	.	312	3	1,123	1,731
ASP+ASN	E12	4,668	**<.0001**	519	3	4,098	5,114
ASP+ASN	Control	7,318	.	393	3	6,886	7,654
Cystine	E12	.	.	.	0	.	.
Cystine	Control	.	.	.	0	.	.
GLU+GLN	E12	6,784	**<.0001**	519	3	6,405	7,376
GLU+GLN	Control	3,768	.	125	3	3,676	3,910
Glycine	E12	1,738	0.4181	162	3	1,574	1,898
Glycine	Control	1,476	.	248	3	1,215	1,710
Histidine	E12	677	**0.0009**	13.1	3	663	689
Histidine	Control	430	.	62.8	3	390	502
Isoleucine	E12	941	**0.0135**	55.6	3	880	990
Isoleucine	Control	743	.	125	3	604	846
Leucine	E12	1,837	0.1572	39.3	3	1,799	1,878
Leucine	Control	1,536	.	191	3	1,386	1,752
Lysine	E12	1,574	**0.0002**	207	3	1,383	1,795
Lysine	Control	942	.	118	3	871	1,078
Methionine	E12	568	0.4766	50.5	3	535	626
Methionine	Control	513	.	100	3	417	617
Phenylalanine	E12	1,120	0.5163	129	3	977	1,229
Phenylalanine	Control	1,034	.	136	3	884	1,150
Proline	E12	915	0.4361	52.2	3	860	963
Proline	Control	805	.	64.6	3	737	865
Serine	E12	846	**0.0167**	127	3	734	985
Serine	Control	492	.	55.9	3	435	546
Threonine	E12	1,040	**0.0264**	128	3	898	1,146
Threonine	Control	809	.	139	3	675	952
Tryptophan	E12	.	.	.	0	.	.
Tryptophan	Control	.	.	.	0	.	.
Tyrosine	E12	893	**0.0002**	86.3	3	823	989
Tyrosine	Control	662	.	73.8	3	594	741
Valine	E12	1,125	0.7229	49.3	3	1,073	1,171
Valine	Control	1,090	.	194	3	933	1,307

Table A-15. Glycoalkaloids in E12 and Control Russet Burbank at Bingham County, Idaho in 2009

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	4.58	**<.0001**	0.111	3	4.50	4.71
	Control	10.1	.	0.094	3	10.0	10.2

Table A-16. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Bingham County, Idaho in 2009

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	589	**<.0001**	20.8	3	565	604
Asparagine	Control	1,703	.	130	3	1,562	1,819
Aspartic Acid	E12	527	0.6836	29.2	3	494	544
Aspartic Acid	Control	512	.	39.9	3	469	548
Glutamine	E12	1,783	**<.0001**	40.6	3	1,738	1,815
Glutamine	Control	1,193	.	121	3	1,062	1,301
Glutamic Acid	E12	596	0.6734	72.9	3	545	679
Glutamic Acid	Control	571	.	129	3	433	689

Table A-17. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Bingham County, Idaho in 2009

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.109	**<.0001**	0.0114	3	0.101	0.122
	Control	0.157	.	0.0124	3	0.146	0.170
Month 3	E12	0.120	0.6565	0.0172	3	0.102	0.136
	Control	0.126	.	0.0076	3	0.117	0.130
Month 5	E12	0.102	0.7172	0.0030	3	0.0988	0.104
	Control	0.104	.	0.0106	3	0.0920	0.112
Sucrose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.0655	**<.0001**	0.0045	3	0.0603	0.0688
	Control	0.138	.	0.0062	3	0.132	0.145
Month 3	E12	0.0636	0.5711	0.0095	3	0.0528	0.0707
	Control	0.0678	.	0.0081	3	0.0587	0.0743
Month 5	E12	0.0988	0.7169	0.0071	3	0.0945	0.107
	Control	0.0964	.	0.0087	3	0.0865	0.103

Table A-18. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Bingham County, Idaho in 2009

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 2	E12	243	**<.0001**	3	3	240	246
	Control	795	.	21.9	3	770	811
Month 5	E12	85.3	**<.0001**	4.51	3	81.0	90.0
	Control	235	.	23.7	3	208	250
Month 7	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.

Table A-19. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Bingham County, Idaho in 2010

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.95	0.2034	0.0872	3	2.85	3.02
Protein (%)	Control	2.68	.	0.234	3	2.52	2.95
Fat (%)	E12	0.160	**0.0095**	0.0067	3	0.160	0.170
Fat (%)	Control	0.110	.	0.0057	3	0.100	0.110
Ash (%)	E12	1.30	0.5195	0.0454	3	1.25	1.33
Ash (%)	Control	1.25	.	0.0565	3	1.21	1.31
Crude Fiber (%)	E12	0.620	0.9139	0.0447	3	0.570	0.660
Crude Fiber (%)	Control	0.610	.	0.0278	3	0.580	0.640
Carbohydrates (%)	E12	17.1	0.6951	0.562	3	16.5	17.6
Carbohydrates (%)	Control	17.3	.	0.769	3	16.5	18.0
Calories (kcal/100 g)	E12	81.6	0.7959	2.60	3	78.7	83.8
Calories (kcal/100 g)	Control	81.0	.	3.47	3	77.0	83.5
Moisture (%)	E12	78.5	0.8195	0.686	3	77.9	79.3
Moisture (%)	Control	78.7	.	0.883	3	78.1	79.7
Vitamin B3 (mg/100 g)	E12	2.44	0.4115	0.108	3	2.32	2.53
Vitamin B3 (mg/100 g)	Control	2.28	.	0.123	3	2.14	2.38
Vitamin B6 (mg/100 g)	E12	0.120	0.4008	0.0082	3	0.110	0.120
Vitamin B6 (mg/100 g)	Control	0.120	.	0.0053	3	0.120	0.130
Vitamin C (mg/110 g)	E12	16.8	**0.0043**	0.929	3	15.8	17.6
Vitamin C (mg/110 g)	Control	12.7	.	1.83	3	11.0	14.6
Copper (ppm)	E12	1.35	0.1920	0.182	3	1.16	1.52
Copper (ppm)	Control	1.19	.	0.0598	3	1.14	1.26
Magnesium (ppm)	E12	287	0.8551	15.1	3	269	297
Magnesium (ppm)	Control	282	.	14.7	3	266	292
Potassium (ppm)	E12	6,172	0.4669	255	3	5,886	6,374
Potassium (ppm)	Control	5,840	.	152	3	5,735	6,014

Table A-20. Total Amino Acids in E12 and Control Russet Burbank at Bingham County, Idaho in 2010

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	955	0.4089	64.2	3	899	1,025
Alanine	Control	1,178	.	61.7	3	1,112	1,234
Arginine	E12	846	0.0953	65.8	3	779	911
Arginine	Control	644	.	19.7	3	624	664
ASP+ASN	E12	1,975	**0.0276**	94.2	3	1,874	2,061
ASP+ASN	Control	4,337	.	196	3	4,170	4,553
Cystine	E12	.	.	.	0	.	.
Cystine	Control	.	.	.	0	.	.
GLU+GLN	E12	4,292	**<.0001**	226	3	4,045	4,488
GLU+GLN	Control	2,435	.	388	3	2,163	2,879
Glycine	E12	2,635	0.1651	216	3	2,508	2,885
Glycine	Control	3,468	.	102	3	3,353	3,548
Histidine	E12	410	**0.0457**	134	3	263	525
Histidine	Control	247	.	30.0	3	214	272
Isoleucine	E12	765	0.5948	13.7	3	749	774
Isoleucine	Control	841	.	91.9	3	735	900
Leucine	E12	1,574	0.8112	59.7	3	1,511	1,630
Leucine	Control	1,516	.	92.2	3	1,410	1,577
Lysine	E12	1,194	0.1185	51.9	3	1,140	1,243
Lysine	Control	684	.	84.4	3	592	757
Methionine	E12	232	0.9937	4.89	3	228	237
Methionine	Control	232	.	47.1	3	186	280
Phenylalanine	E12	702	0.8972	14.3	3	685	711
Phenylalanine	Control	709	.	32.4	3	672	730
Proline	E12	1,363	0.7051	85.4	3	1,266	1,425
Proline	Control	1,291	.	108	3	1,218	1,415
Serine	E12	389	0.1698	19.2	3	371	409
Serine	Control	305	.	25.8	3	275	321
Threonine	E12	404	0.1125	11.8	3	393	416
Threonine	Control	258	.	41.7	3	220	303
Tryptophan	E12	.	.	.	0	.	.
Tryptophan	Control	.	.	.	0	.	.
Tyrosine	E12	372	0.9774	71.6	3	292	430
Tyrosine	Control	374	.	45.9	3	328	420
Valine	E12	1,302	0.2096	55.4	3	1,252	1,361
Valine	Control	1,785	.	136	3	1,634	1,897

Table A-21. Glycoalkaloids in E12 and Control Russet Burbank at Bingham County, Idaho in 2010

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	5.14	0.4275	1.02	3	4.24	6.26
	Control	6.03	.	0.818	3	5.11	6.68

Table A-22. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Bingham County, Idaho in 2010

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	567	**<.0001**	34.7	3	531	600
Asparagine	Control	2,735	.	158	3	2,557	2,861
Aspartic Acid	E12	448	0.8411	15.6	3	430	459
Aspartic Acid	Control	402	.	18.7	3	386	422
Glutamine	E12	2,182	**0.0109**	130	3	2,049	2,308
Glutamine	Control	1,216	.	114	3	1,095	1,322
Glutamic Acid	E12	340	0.7765	5.18	3	335	345
Glutamic Acid	Control	288	.	23.1	3	262	305

Table A-23. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Bingham County, Idaho in 2010

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.0918	**0.0187**	0.0331	3	0.0552	0.120
	Control	0.160	.	0.0081	3	0.153	0.169
Month 3	E12	0.339	0.9634	0.0902	3	0.236	0.406
	Control	0.337	.	0.0219	3	0.314	0.357
Month 5	E12	0.136	0.5632	0.0249	2	0.119	0.154
	Control	0.163	.	0.0357	3	0.137	0.204
Sucrose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.197	0.0796	0.0640	3	0.131	0.258
	Control	0.248	.	0.0052	3	0.242	0.252
Month 3	E12	0.0754	0.9891	0.0149	3	0.0632	0.0919
	Control	0.0752	.	0.0033	3	0.0718	0.0784
Month 5	E12	0.0624	0.2018	0.0105	3	0.0526	0.0734
	Control	0.0719	.	0.0157	3	0.0584	0.0891

Table A-24. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Bingham County, Idaho in 2010

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	112	0.6184	3.79	3	108	115
	Control	451	.	14.6	3	436	465
Month 3	E12	240	**<.0001**	55.4	3	176	277
	Control	900	.	105	3	828	1,020
Month 6	E12	238	**<.0001**	12	3	226	250
	Control	674	.	88.9	3	615	776

Table A-25. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Bingham County, Idaho in 2011

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.06	**0.0297**	0.0520	3	2.00	2.09
Protein (%)	Control	2.28	.	0.0854	3	2.19	2.36
Fat (%)	E12	0.100	0.1613	0	3	0.100	0.100
Fat (%)	Control	0.130	.	0.0577	3	0.100	0.200
Ash (%)	E12	1.16	0.2985	0.148	3	0.990	1.28
Ash (%)	Control	1.27	.	0.163	3	1.08	1.38
Crude Fiber (%)	E12	0.350	0.6248	0.0214	3	0.340	0.370
Crude Fiber (%)	Control	0.360	.	0.0626	3	0.320	0.430
Carbohydrates (%)	E12	15.6	0.0588	1.21	3	14.2	16.3
Carbohydrates (%)	Control	17.3	.	1.12	3	16.1	18.3
Calories (kcal/100 g)	E12	71.0	**0.0320**	5.27	3	64.9	74.4
Calories (kcal/100 g)	Control	79.3	**.**	5.71	3	73.1	84.3
Moisture (%)	E12	81.1	**0.0221**	1.19	3	80.3	82.5
Moisture (%)	Control	79.0	**.**	1.15	3	78.1	80.3
Vitamin B3 (mg/100 g)	E12	1.61	**0.0041**	0.0361	3	1.57	1.64
Vitamin B3 (mg/100 g)	Control	2.05	.	0.166	3	1.87	2.20
Vitamin B6 (mg/100 g)	E12	0.110	0.3404	0.0036	3	0.110	0.120
Vitamin B6 (mg/100 g)	Control	0.120	.	0.0031	3	0.120	0.120
Vitamin C (mg/110 g)	E12	18.6	0.0807	0.907	3	17.8	19.6
Vitamin C (mg/110 g)	Control	26.6	.	14.4	3	15.7	42.9
Copper (ppm)	E12	0.920	0.3738	0.0751	3	0.840	0.980
Copper (ppm)	Control	1.02	.	0.228	3	0.880	1.28
Magnesium (ppm)	E12	222	0.0550	1.53	3	220	223
Magnesium (ppm)	Control	253	.	19.0	3	234	272
Potassium (ppm)	E12	4,217	0.7909	491	3	3,660	4,590
Potassium (ppm)	Control	4,390	.	610	3	3,970	5,090

Table A-26. Total Amino Acids in E12 and Control Russet Burbank at Bingham County, Idaho in 2011

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	690	0.1816	9.02	3	681	699
Alanine	Control	655	.	32.9	3	635	693
Arginine	E12	929	0.1036	62.6	3	862	986
Arginine	Control	1,077	.	155	3	952	1,250
ASP+ASN	E12	2,213	**<.0001**	116	3	2,090	2,320
ASP+ASN	Control	5,123	.	622	3	4,730	5,840
Cystine	E12	185	0.5488	29.3	3	162	218
Cystine	Control	198		58.6	3	135	251
GLU+GLN	E12	4,757	**<.0001**	233	3	4,490	4,920
GLU+GLN	Control	3,100	.	207	3	2,910	3,320
Glycine	E12	510	**0.0133**	18.1	3	489	523
Glycine	Control	585	.	86.7	3	513	681
Histidine	E12	311	**0.0154**	14.7	3	302	328
Histidine	Control	352	.	9.07	3	342	359
Isoleucine	E12	716	**0.0238**	14.2	3	707	732
Isoleucine	Control	781	.	42.4	3	746	828
Leucine	E12	998	0.1142	47.0	3	944	1,030
Leucine	Control	1,081	.	159	3	922	1,240
Lysine	E12	1,000	0.6499	43.0	3	951	1,030
Lysine	Control	1,019	.	64.1	3	947	1,070
Methionine	E12	398	0.6528	5.86	3	391	402
Methionine	Control	389	.	2.65	3	387	392
Phenylalanine	E12	790	**0.0051**	23.2	3	763	805
Phenylalanine	Control	881	.	68.4	3	806	940
Proline	E12	743	0.8566	76.2	3	670	822
Proline	Control	718	.	119	3	580	787
Serine	E12	657	**0.0046**	19.4	3	635	670
Serine	Control	745	.	68.4	3	681	817
Threonine	E12	658	**0.0469**	32.1	3	621	679
Threonine	Control	717	.	76.5	3	640	793
Tryptophan	E12	223	0.3522	17.2	3	204	237
Tryptophan	Control	210	.	17.2	3	191	224
Tyrosine	E12	692	**0.0149**	44.2	3	642	725
Tyrosine	Control	601	.	93.7	3	511	698
Valine	E12	1,030	0.0793	0	3	1,030	1,030
Valine	Control	1,100	.	20.0	3	1,080	1,120

Table A-27. Glycoalkaloids in E12 and Control Russet Burbank at Bingham County, Idaho in 2011

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	7.98	0.8067	2.08	3	5.67	9.70
	Control	7.62	.	1.70	3	6.45	9.57

Table A-28. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Bingham County, Idaho in 2011

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	699	**<.0001**	20.6	3	678	719
Asparagine	Control	3,393	.	576	3	2,910	4,030
Aspartic Acid	E12	331	0.1929	26.7	3	310	361
Aspartic Acid	Control	365	.	74.2	3	294	442
Glutamine	E12	2,883	**<.0001**	148	3	2,720	3,010
Glutamine	Control	1,360	.	311	3	1,090	1,700
Glutamic Acid	E12	409	**0.0208**	21.8	3	385	428
Glutamic Acid	Control	502	.	63.6	3	441	568

Table A-29. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Bingham County, Idaho in 2011

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	0.242	0.7891	0.0841	3	0.186	0.339
	Control	0.259	.	0.0856	3	0.202	0.357
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	0.511	0.9149	0.0621	3	0.460	0.580
	Control	0.516	.	0.131	3	0.403	0.660
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Sucrose (%)							
Fresh	E12	0.209	0.4182	0.0722	3	0.134	0.278
	Control	0.175	.	0.0978	3	0.113	0.288
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	0.0527	0.1946	0.0140	3	0.0370	0.0639
	Control	0.0661	.	0.0170	3	0.0465	0.0763
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
		.	.	.	0	.	.

Table A-30. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Bingham County, Idaho in 2011

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	99	**<.0001**	4.54	3	95.1	104
	Control	315	.	28.5	3	286	343
Month 3	E12	414	**<.0001**	58.7	3	365	479
	Control	1157	.	80.2	3	1,080	1,240

Table A-31. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Canyon County, Idaho in 2009

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	1.53	0.4095	0.0554	3	1.47	1.56
Protein (%)	Control	1.67	.	0.0267	3	1.64	1.68
Fat (%)	E12	0.0900	0.2921	0.0062	3	0.0800	0.0900
Fat (%)	Control	0.0700	.	0.0056	3	0.0700	0.0800
Ash (%)	E12	0.980	0.7799	0.0352	3	0.940	1.01
Ash (%)	Control	0.950	.	0.0317	3	0.910	0.970
Crude Fiber (%)	E12	0.440	0.3484	0.0085	3	0.430	0.450
Crude Fiber (%)	Control	0.420	.	0.0043	3	0.420	0.430
Carbohydrates (%)	E12	20.7	**0.0001**	0.126	3	20.6	20.8
Carbohydrates (%)	Control	19.4	.	0.0304	3	19.3	19.4
Calories (kcal/100 g)	E12	89.8	**0.0001**	0.386	3	89.4	90.2
Calories (kcal/100 g)	Control	84.8	.	0.184	3	84.6	85.0
Moisture (%)	E12	76.7	**0.0005**	0.105	3	76.6	76.8
Moisture (%)	Control	78.0	.	0.0650	3	77.9	78.0
Vitamin B3 (mg/100 g)	E12	1.93	**0.0091**	0.0695	3	1.85	1.99
Vitamin B3 (mg/100 g)	Control	1.64	.	0.118	3	1.53	1.77
Vitamin B6 (mg/100 g)	E12	0.170	**0.0071**	0.0041	3	0.160	0.170
Vitamin B6 (mg/100 g)	Control	0.150	.	0.0076	3	0.140	0.160
Vitamin C (mg/110 g)	E12	13.2	**<.0001**	0.209	3	13.0	13.4
Vitamin C (mg/110 g)	Control	9.84	.	0.157	3	9.66	9.95
Copper (ppm)	E12	1.24	0.4238	0.0292	3	1.22	1.27
Copper (ppm)	Control	1.29	.	0.0304	3	1.26	1.32
Magnesium (ppm)	E12	231	**0.0056**	5.23	3	226	237
Magnesium (ppm)	Control	203	.	5.43	3	197	207
Potassium (ppm)	E12	4,021	0.7400	71.5	3	3,966	4,102
Potassium (ppm)	Control	3,895	.	62.9	3	3,825	3,946

Table A-32. Total Amino Acids in E12 and Control Russet Burbank at Canyon County, Idaho in 2009

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	511	0.1065	71.4	3	429	563
Alanine	Control	450	.	23.7	3	423	468
Arginine	E12	828	0.7288	68.7	3	780	907
Arginine	Control	877	.	153	3	723	1,029
ASP+ASN	E12	3,039	**<.0001**	156	3	2,869	3,177
ASP+ASN	Control	5,134	.	75.8	3	5,071	5,218
Cystine	E12	.	.	.	0	.	.
Cystine	Control	.	.	.	0	.	.
GLU+GLN	E12	3,538	**0.0385**	35.6	3	3,517	3,579
GLU+GLN	Control	2,688	.	172	3	2,489	2,788
Glycine	E12	675	**0.0040**	137	3	568	830
Glycine	Control	1,559	.	178	3	1,422	1,760
Histidine	E12	605	0.4265	46.6	3	557	650
Histidine	Control	577	.	56.1	3	542	642
Isoleucine	E12	422	0.3582	18.9	3	406	443
Isoleucine	Control	538	.	79.8	3	467	625
Leucine	E12	988	0.9798	162	3	804	1,110
Leucine	Control	984	.	171	3	810	1,152
Lysine	E12	1,117	**0.0465**	106	3	1,031	1,235
Lysine	Control	788	.	47.9	3	743	838
Methionine	E12	570	**0.0061**	97.0	3	464	654
Methionine	Control	410	.	60.8	3	355	475
Phenylalanine	E12	704	0.2838	29.7	3	676	735
Phenylalanine	Control	614	.	133	3	466	720
Proline	E12	829	0.2133	112	3	704	921
Proline	Control	725	.	52.3	3	672	776
Serine	E12	283	0.6652	23.4	3	265	310
Serine	Control	319	.	48.5	3	275	371
Threonine	E12	504	0.1013	55.4	3	454	563
Threonine	Control	718	.	70.0	3	672	799
Tryptophan	E12	.	.	.	0	.	.
Tryptophan	Control	.	.	.	0	.	.
Tyrosine	E12	296	0.1252	26.8	3	266	318
Tyrosine	Control	423	.	123	3	350	565
Valine	E12	894	0.0768	245	3	612	1,055
Valine	Control	1,065	.	154	3	905	1,212

Table A-33. Glycoalkaloids in E12 and Control Russet Burbank at Canyon County, Idaho in 2009

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	4.15	0.2611	0.319	3	3.85	4.48
	Control	3.56	.	0.203	3	3.33	3.71

Table A-34. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Canyon County, Idaho in 2009

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	447	**<.0001**	7.08	3	440	454
Asparagine	Control	842	.	25.9	3	822	871
Aspartic Acid	E12	302	0.8807	10.4	3	293	313
Aspartic Acid	Control	294	.	17.4	3	280	313
Glutamine	E12	891	0.0552	22.2	3	865	907
Glutamine	Control	612	.	10.0	3	605	623
Glutamic Acid	E12	458	0.3735	25.3	3	428	474
Glutamic Acid	Control	496	.	55.4	3	448	556

Table A-35. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Canyon County, Idaho in 2009

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.140	**<.0001**	0.0056	3	0.134	0.144
	Control	0.209	.	0.0035	3	0.205	0.212
Month 3	E12	0.155	**0.0014**	0.0075	3	0.149	0.163
	Control	0.215	.	0.0288	3	0.189	0.246
Month 5	E12	0.120	**0.0067**	0.0028	3	0.117	0.123
	Control	0.140	.	0.0072	3	0.132	0.146
Sucrose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.107	0.2931	0.0038	3	0.103	0.111
	Control	0.117	.	0.0010	3	0.116	0.118
Month 3	E12	0.0900	0.0804	0.0054	3	0.0845	0.0953
	Control	0.103	.	0.0137	3	0.0878	0.114
Month 5	E12	0.101	0.6054	0.0024	3	0.0992	0.103
	Control	0.0965	.	0.0057	3	0.0905	0.102

Table A-36. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Canyon County, Idaho in 2009

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 2	E12	222	**<.0001**	11.7	3	209	232
	Control	629	.	10.0	3	619	639
Month 5	E12	90.7	**<.0001**	6.03	3	85.0	97.0
	Control	241	.	16.7	3	231	260
Month 7	E12	192	**<.0001**	26.1	3	165	217
	Control	661	.	54.6	3	598	693

Table A-37. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Canyon County, Idaho in 2010

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.62	0.7238	0.163	3	2.45	2.78
Protein (%)	Control	2.73	.	0.185	3	2.60	2.94
Fat (%)	E12	0.0700	0.8288	0.0176	3	0.0500	0.0900
Fat (%)	Control	0.0700	.	0.0204	2	0.0500	0.0800
Ash (%)	E12	1.25	0.6349	0.0882	3	1.19	1.35
Ash (%)	Control	1.19	.	0.126	3	1.11	1.34
Crude Fiber (%)	E12	0.510	0.1139	0.0255	3	0.480	0.520
Crude Fiber (%)	Control	0.560	.	0.0407	3	0.520	0.600
Carbohydrates (%)	E12	17.4	0.7661	0.607	3	16.9	18.1
Carbohydrates (%)	Control	17.2	.	0.273	3	17.0	17.5
Calories (kcal/100 g)	E12	80.7	0.8314	3.16	3	78.0	84.2
Calories (kcal/100 g)	Control	80.2	.	0.908	3	79.2	80.9
Moisture (%)	E12	78.7	0.7674	0.714	3	77.9	79.2
Moisture (%)	Control	78.8	.	0.283	3	78.6	79.1
Vitamin B3 (mg/100 g)	E12	2.02	0.6061	0.0794	3	1.94	2.09
Vitamin B3 (mg/100 g)	Control	2.10	.	0.128	3	2.01	2.24
Vitamin B6 (mg/100 g)	E12	0.130	0.6779	0.0053	3	0.120	0.130
Vitamin B6 (mg/100 g)	Control	0.130	.	0.0098	3	0.120	0.140
Vitamin C (mg/110 g)	E12	17.8	0.5567	0.475	3	17.5	18.3
Vitamin C (mg/110 g)	Control	15.1	.	1.45	3	13.9	16.7
Copper (ppm)	E12	1.07	0.7776	0.137	3	0.970	1.23
Copper (ppm)	Control	1.03	.	0.206	3	0.820	1.23
Magnesium (ppm)	E12	271	0.7252	31.8	3	246	307
Magnesium (ppm)	Control	264	.	21.1	3	240	280
Potassium (ppm)	E12	5,872	0.6672	212	3	5,707	6,112
Potassium (ppm)	Control	5,681	.	584	3	5,223	6,339

Table A-38. Total Amino Acids in E12 and Control Russet Burbank at Canyon County, Idaho in 2010

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	930	**0.0270**	117	3	820	1,053
Alanine	Control	1,154	.	140	3	995	1,258
Arginine	E12	968	0.5039	74.6	3	882	1,016
Arginine	Control	1,039	.	60.0	3	970	1,077
ASP+ASN	E12	2,673	**<.0001**	479	3	2,335	3,221
ASP+ASN	Control	5,122	.	511	3	4,574	5,585
Cystine	E12	.	.	.	0	.	.
Cystine	Control	.	.	.	0	.	.
GLU+GLN	E12	5,332	**<.0001**	567	3	4,683	5,733
GLU+GLN	Control	3,444	.	169	3	3,281	3,619
Glycine	E12	2,055	0.2365	127	3	1,913	2,157
Glycine	Control	2,653	.	318	3	2,443	3,020
Histidine	E12	221	**0.0074**	47.0	3	170	263
Histidine	Control	308	.	62.0	3	237	349
Isoleucine	E12	523	**0.0220**	62.2	3	455	577
Isoleucine	Control	640	.	58.6	3	586	702
Leucine	E12	1,094	**<.0001**	132	3	942	1,177
Leucine	Control	1,681	.	90.9	3	1,609	1,783
Lysine	E12	969	0.6632	58.4	3	904	1,017
Lysine	Control	1,000	.	53.8	3	943	1,050
Methionine	E12	304	0.1534	1.25	3	302	305
Methionine	Control	348	.	20.7	3	330	371
Phenylalanine	E12	655	**0.0004**	45.1	3	605	694
Phenylalanine	Control	889	.	65.2	3	814	927
Proline	E12	572	**0.0499**	60.2	3	502	607
Proline	Control	685	.	38.7	3	643	720
Serine	E12	509	0.0561	73.1	3	428	568
Serine	Control	592	.	90.1	3	509	688
Threonine	E12	349	**0.0004**	29.7	3	323	381
Threonine	Control	670	.	147	3	549	834
Tryptophan	E12	.	.	.	0	.	.
Tryptophan	Control	.	.	.	0	.	.
Tyrosine	E12	626	0.7891	31.5	3	608	663
Tyrosine	Control	648	.	127	3	505	751
Valine	E12	1,202	**0.0008**	152	3	1,029	1,317
Valine	Control	1,728	.	282	3	1,447	2,012

Table A-39. Glycoalkaloids in E12 and Control Russet Burbank at Canyon County, Idaho in 2010

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	4.31	0.4422	0.255	3	4.11	4.60
	Control	5.18	.	1.82	3	3.90	7.26

Table A-40. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Canyon County, Idaho in 2010

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	740	**<.0001**	196	3	591	962
Asparagine	Control	3,155	.	88.2	3	3,060	3,234
Aspartic Acid	E12	612	0.6313	86.9	3	523	697
Aspartic Acid	Control	649	.	40.1	3	605	683
Glutamine	E12	2,122	**0.0019**	165	3	1,946	2,274
Glutamine	Control	1,433	.	111	3	1,321	1,543
Glutamic Acid	E12	462	0.1608	97.7	3	384	572
Glutamic Acid	Control	549	.	23.9	3	527	574

Table A-41. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Canyon County, Idaho in 2010

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.0624	**0.0069**	0.0064	3	0.0567	0.0692
	Control	0.0935	.	0.0125	3	0.0797	0.104
Month 3	E12	0.188	0.8687	0.0778	3	0.134	0.277
	Control	0.198	.	0.0144	3	0.183	0.212
Month 5	E12	0.134	0.2471	0.0048	3	0.131	0.140
	Control	0.168	.	0.0156	3	0.155	0.185
Sucrose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.177	**<.0001**	0.0269	3	0.146	0.194
	Control	0.244	.	0.0055	3	0.237	0.248
Month 3	E12	0.114	**<.0001**	0.0106	3	0.106	0.126
	Control	0.0643	.	0.0066	3	0.0602	0.0718
Month 5	E12	0.0962	0.0994	0.0054	2	0.0924	0.100
	Control	0.0816	.	0.0041	3	0.0783	0.0862

Table A-42. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Canyon County, Idaho in 2010

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	185	**0.0421**	16.3	3	172	203
	Control	575	.	56.2	3	530	638
Month 3	E12	342	**<.0001**	55.1	3	285	395
	Control	559	.	20.9	3	545	583
Month 6	E12	156	**0.0002**	10	3	146	166
	Control	254	.	17.3	3	234	265

Table A-43. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Canyon County, Idaho in 2011

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	1.86	0.2612	0.0907	3	1.76	1.94
Protein (%)	Control	1.79	.	0.0300	3	1.76	1.82
Fat (%)	E12	0.100	0.1139	0	3	0.100	0.100
Fat (%)	Control	0.130	.	0.0577	3	0.100	0.200
Ash (%)	E12	0.740	0.8895	0.395	2	0.460	1.02
Ash (%)	Control	0.760	.	0.0847	3	0.670	0.840
Crude Fiber (%)	E12	0.420	0.1143	0.0452	3	0.390	0.470
Crude Fiber (%)	Control	0.470	.	0.0509	3	0.440	0.530
Carbohydrates (%)	E12	21.9	0.0866	0.493	3	21.3	22.2
Carbohydrates (%)	Control	21.0	.	0.115	3	20.9	21.1
Calories (kcal/100 g)	E12	95.1	0.1117	1.88	3	92.9	96.3
Calories (kcal/100 g)	Control	92.1	.	0.569	3	91.6	92.7
Moisture (%)	E12	75.7	0.1919	0.153	3	75.5	75.8
Moisture (%)	Control	76.3	.	0.100	3	76.2	76.4
Vitamin B3 (mg/100 g)	E12	1.89	0.7778	0.0700	3	1.84	1.97
Vitamin B3 (mg/100 g)	Control	1.87	.	0.0153	3	1.86	1.89
Vitamin B6 (mg/100 g)	E12	0.130	0.0950	0.0015	3	0.130	0.130
Vitamin B6 (mg/100 g)	Control	0.150	.	0.0257	3	0.130	0.180
Vitamin C (mg/110 g)	E12	30.6	0.8816	1.97	3	29.4	32.9
Vitamin C (mg/110 g)	Control	30.9	.	1.82	3	29.3	32.9
Copper (ppm)	E12	0.770	0.4507	0.104	3	0.710	0.890
Copper (ppm)	Control	0.710	.	0.0415	3	0.670	0.750
Magnesium (ppm)	E12	267	0.5748	10.0	3	257	277
Magnesium (ppm)	Control	275	.	18.5	3	263	296
Potassium (ppm)	E12	4,243	0.1682	178	3	4,050	4,400
Potassium (ppm)	Control	4,487	.	218	3	4,310	4,730

Table A-44. Total Amino Acids in E12 and Control Russet Burbank at Canyon County, Idaho in 2011

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	631	0.0798	19.9	3	615	653
Alanine	Control	571	.	48.4	3	517	610
Arginine	E12	918	0.3033	30.8	3	883	941
Arginine	Control	855	.	27.6	3	836	887
ASP+ASN	E12	2,223	**<.0001**	49.3	3	2,190	2,280
ASP+ASN	Control	4,067	.	163	3	3,940	4,250
Cystine	E12	185	0.2197	5.69	3	180	191
Cystine	Control	148		27.1	3	118	171
GLU+GLN	E12	3,960	**<.0001**	72.1	3	3,900	4,040
GLU+GLN	Control	2,707	.	40.4	3	2,670	2,750
Glycine	E12	553	0.3706	14.2	3	544	569
Glycine	Control	533	.	42.1	3	489	573
Histidine	E12	296	0.8242	10	3	286	306
Histidine	Control	293	.	18.0	3	281	314
Isoleucine	E12	644	0.6001	24.2	3	622	670
Isoleucine	Control	629	.	47.6	3	583	678
Leucine	E12	1,006	0.3524	22.1	3	987	1,030
Leucine	Control	965	.	63.2	3	896	1,020
Lysine	E12	918	0.8001	34.8	3	893	958
Lysine	Control	910	.	37.2	3	875	949
Methionine	E12	364	0.1964	5.77	3	357	367
Methionine	Control	348	.	18.1	3	331	367
Phenylalanine	E12	681	0.9860	56.9	3	618	728
Phenylalanine	Control	681	.	54.7	3	637	742
Proline	E12	575	0.0692	11.7	3	566	588
Proline	Control	517	.	33.5	3	484	551
Serine	E12	671	0.2296	20.2	3	649	689
Serine	Control	647	.	27.8	3	617	672
Threonine	E12	707	0.1409	11.8	3	700	721
Threonine	Control	667	.	44.7	3	618	705
Tryptophan	E12	176	0.2812	20.8	3	163	200
Tryptophan	Control	186	.	6.08	3	182	193
Tyrosine	E12	640	**0.0126**	25.7	3	625	670
Tyrosine	Control	564	.	44.5	3	520	609
Valine	E12	972	0.5251	18.6	3	959	993
Valine	Control	953	.	59.0	3	909	1,020

Table A-45. Glycoalkaloids in E12 and Control Russet Burbank at Canyon County, Idaho in 2011

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	6.41	**0.0005**	0.473	3	6.07	6.95
	Control	9.50	.	1.51	3	8.58	11.2

Table A-46. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Canyon County, Idaho in 2011

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	706	**<.0001**	27.4	3	690	738
Asparagine	Control	2,640	.	108	3	2,550	2,760
Aspartic Acid	E12	396	**0.0168**	7.09	3	388	402
Aspartic Acid	Control	359	.	21.0	3	338	380
Glutamine	E12	1,973	**<.0001**	15.3	3	1,960	1,990
Glutamine	Control	969	.	79.4	3	916	1,060
Glutamic Acid	E12	633	0.1149	24.2	3	607	655
Glutamic Acid	Control	591	.	41.7	3	543	618

Table A-47. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Canyon County, Idaho in 2011

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	0.085	0.3413	0.027	3	0.0685	0.116
	Control	0.072	.	0.021	3	0.0534	0.0944
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Sucrose (%)							
Fresh	E12	0.276	0.0530	0.020	3	0.264	0.299
	Control	0.222	.	0.065	3	0.149	0.275
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.

Table A-48. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Canyon County, Idaho in 2011

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	217	**<.0001**	18.0	3	198	234
	Control	529	.	23.1	3	515	556
Month 3	E12	193	**<.0001**	26.2	3	169	221
	Control	563	.	60.0	3	502	622

Table A-49. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Larimore County, North Dakota in 2010

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.80	0.7488	0.148	3	2.71	2.97
Protein (%)	Control	2.72	.	0.251	3	2.57	3.01
Fat (%)	E12	0.0900	0.9492	0.0049	3	0.0900	0.0900
Fat (%)	Control	0.0900	.	0.0317	3	0.0600	0.120
Ash (%)	E12	1.32	0.1069	0.121	3	1.23	1.46
Ash (%)	Control	1.17	.	0.130	3	1.03	1.29
Crude Fiber (%)	E12	0.680	0.1986	0.0164	3	0.670	0.700
Crude Fiber (%)	Control	0.620	.	0.0199	3	0.590	0.630
Carbohydrates (%)	E12	18.9	0.5466	0.799	3	18.0	19.5
Carbohydrates (%)	Control	19.4	.	1.16	3	18.2	20.5
Calories (kcal/100 g)	E12	87.8	0.5791	3.62	3	83.8	90.8
Calories (kcal/100 g)	Control	89.3	.	3.94	3	85.4	93.3
Moisture (%)	E12	76.8	0.7396	0.975	3	76.0	77.9
Moisture (%)	Control	76.6	.	1.03	3	75.5	77.5
Vitamin Vitamin B3 (mg/100 g)	E12	2.28	0.5665	0.0440	3	2.23	2.32
Vitamin Vitamin B3 (mg/100 g)	Control	2.13	.	0.0681	3	2.08	2.20
Vitamin B6 (mg/100 g)	E12	0.210	0.1958	0.0055	3	0.200	0.210
Vitamin B6 (mg/100 g)	Control	0.190	.	0.0133	3	0.180	0.200
Vitamin C (mg/110 g)	E12	16.6	**0.0003**	2.91	3	14.2	19.8
Vitamin C (mg/110 g)	Control	10.3	.	1.33	3	8.88	11.5
Copper (ppm)	E12	0.630	0.6195	0.0835	3	0.540	0.710
Copper (ppm)	Control	0.680	.	0.0963	3	0.620	0.790
Magnesium (ppm)	E12	344	0.4377	53.5	3	307	405
Magnesium (ppm)	Control	323	.	31.6	3	288	350
Potassium (ppm)	E12	5,802	0.6508	428	3	5,421	6,265
Potassium (ppm)	Control	5,586	.	659	3	4,825	5,966

Table A-50. Total Amino Acids in E12 and Control Russet Burbank at Larimore County, North Dakota in 2010

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	815	0.7236	12.2	3	801	824
Alanine	Control	732	.	95.2	3	672	842
Arginine	E12	494	0.6650	4.02	3	490	498
Arginine	Control	583	.	43.2	3	534	613
ASP+ASN	E12	2,338	0.3339	61.3	3	2,291	2,408
ASP+ASN	Control	4,852	.	507	3	4,378	5,387
Cystine	E12	.	.	.	0	.	.
Cystine	Control	.	.	.	0	.	.
GLU+GLN	E12	4,132	0.1854	192	3	3,918	4,288
GLU+GLN	Control	2,633	.	400	3	2,354	3,092
Glycine	E12	1,620	0.8841	181	3	1,475	1,823
Glycine	Control	1,697	.	293	3	1,396	1,981
Histidine	E12	404	0.2688	19.0	3	388	425
Histidine	Control	289	.	167	3	100	415
Isoleucine	E12	598	0.8121	27.2	3	575	628
Isoleucine	Control	642	.	86.2	3	589	741
Leucine	E12	1,234	0.9799	52.6	3	1,192	1,293
Leucine	Control	1,239	.	78.6	3	1,152	1,304
Lysine	E12	956	0.7601	35.5	3	921	992
Lysine	Control	694	.	70.5	3	646	775
Methionine	E12	240	0.4906	9.36	3	232	250
Methionine	Control	217	.	22.3	3	197	241
Phenylalanine	E12	544	0.7345	4.06	3	541	549
Phenylalanine	Control	577	.	55.2	3	536	639
Proline	E12	657	0.5218	18.3	3	638	675
Proline	Control	582	.	77.0	3	521	669
Serine	E12	258	0.2432	33.4	3	220	281
Serine	Control	398	.	160	3	214	499
Threonine	E12	314	0.5381	20.1	3	299	337
Threonine	Control	378	.	81.9	3	313	470
Tryptophan	E12	.	.	.	0	.	.
Tryptophan	Control	.	.	.	0	.	.
Tyrosine	E12	432	0.9137	4.13	3	428	436
Tyrosine	Control	469	.	74.4	3	386	529
Valine	E12	952	0.5070	90.1	3	865	1,045
Valine	Control	1,124	.	161	3	962	1,285

Table A-51. Glycoalkaloids in E12 and Control Russet Burbank at Larimore County, North Dakota in 2010

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	7.66	**0.0188**	2.54	3	5.69	10.5
	Control	15.2	.	3.49	3	11.6	18.6

Table A-52. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Larimore County, North Dakota in 2010

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	463	**<.0001**	34.9	3	425	493
Asparagine	Control	2,223	.	68.8	3	2,164	2,298
Aspartic Acid	E12	320	0.9989	9.90	3	314	332
Aspartic Acid	Control	321	.	23.0	3	303	347
Glutamine	E12	1,656	0.1094	94.9	3	1,595	1,765
Glutamine	Control	983	.	160	3	809	1,124
Glutamic Acid	E12	232	0.8952	4.50	3	227	236
Glutamic Acid	Control	251	.	28.1	3	221	277

Table A-53. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Larimore County, North Dakota in 2010

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.229	**<.0001**	0.0262	3	0.200	0.251
	Control	0.355	.	0.0431	3	0.308	0.393
Month 3	E12	0.418	0.4079	.	1	0.418	0.418
	Control	0.519	.	0.134	3	0.366	0.617
Month 5	E12	0.489	0.4385	.	1	0.489	0.489
	Control	0.624	.	0.0092	2	0.617	0.630
Sucrose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.191	0.5311	0.0377	3	0.150	0.223
	Control	0.206	.	0.0137	3	0.192	0.219
Month 3	E12	0.0568	0.5921	.	1	0.0568	0.0568
	Control	0.0777	.	0.0375	3	0.0542	0.121
Month 5	E12	0.0507	0.6828	.	1	0.0507	0.0507
	Control	0.0447	.	0.0151	3	0.0350	0.0621

Table A-54. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Larimore County, North Dakota in 2010

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	145	0.0591	15	3	128	157
	Control	539	.	135	3	459	694
Month 3	E12	315	**<.0001**	50.7	3	257	351
	Control	750	.	57	3	684	787
Month 6	E12	263	**0.0067**	15.6	3	245	272
	Control	327	.	46.7	3	286	378

Table A-55. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Larimore County, North Dakota in 2011

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	1.86	0.1030	0.0764	3	1.78	1.93
Protein (%)	Control	1.72	.	0.0862	3	1.64	1.81
Fat (%)	E12	0.130	1.0000	0.0577	3	0.100	0.200
Fat (%)	Control	0.130	.	0.0577	3	0.100	0.200
Ash (%)	E12	0.420	0.1440	0.364	3	0	0.640
Ash (%)	Control	0.900	.	0.973	3	0.150	2.00
Crude Fiber (%)	E12	0.380	0.9127	0.0303	3	0.350	0.410
Crude Fiber (%)	Control	0.380	.	0.0291	3	0.350	0.410
Carbohydrates (%)	E12	19.5	0.7363	0.321	3	19.3	19.9
Carbohydrates (%)	Control	19.2	.	1.37	3	17.7	20.4
Calories (kcal/100 g)	E12	86.8	0.6102	1.11	3	85.6	87.8
Calories (kcal/100 g)	Control	84.8	.	5.56	3	78.5	89.0
Moisture (%)	E12	78.0	0.9732	0.611	3	77.5	78.7
Moisture (%)	Control	78.1	.	2.06	3	76.5	80.4
Vitamin B3 (mg/100 g)	E12	1.47	0.9499	0.0404	3	1.43	1.51
Vitamin B3 (mg/100 g)	Control	1.47	.	0.0306	3	1.44	1.50
Vitamin B6 (mg/100 g)	E12	0.120	0.1884	0.0069	3	0.110	0.130
Vitamin B6 (mg/100 g)	Control	0.110	.	0.0050	3	0.110	0.120
Vitamin C (mg/110 g)	E12	24.2	0.2853	1.91	3	22.0	25.6
Vitamin C (mg/110 g)	Control	21.6	.	1.83	3	19.6	23.2
Copper (ppm)	E12	1.11	**0.0210**	0.0950	3	1.01	1.20
Copper (ppm)	Control	0.660	.	0.214	3	0.500	0.900
Magnesium (ppm)	E12	210	0.1061	9.50	3	201	220
Magnesium (ppm)	Control	192	.	23.6	3	165	210
Potassium (ppm)	E12	3,690	0.5968	201	3	3,550	3,920
Potassium (ppm)	Control	3,533	.	437	3	3,040	3,870

Table A-56. Total Amino Acids in E12 and Control Russet Burbank at Larimore County, North Dakota in 2011

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	620	**0.0001**	18.0	3	602	638
Alanine	Control	452	.	112	3	323	526
Arginine	E12	889	**0.0183**	21.7	3	866	909
Arginine	Control	687	.	156	3	508	794
ASP+ASN	E12	2,040	0.0562	78.1	3	1,950	2,090
ASP+ASN	Control	2,867	.	698	3	2,090	3,440
Cystine	E12	238	0.0926	6.24	3	231	243
Cystine	Control	199		33.8	3	161	225
GLU+GLN	E12	4,203	**<.0001**	263	3	3,900	4,360
GLU+GLN	Control	2,413	.	509	3	1,840	2,810
Glycine	E12	537	**0.0024**	9.61	3	527	546
Glycine	Control	421	.	99.2	3	307	484
Histidine	E12	281	**0.0146**	2.00	3	279	283
Histidine	Control	233	.	41.1	3	186	262
Isoleucine	E12	646	**0.0038**	29.4	3	612	666
Isoleucine	Control	509	.	119	3	372	590
Leucine	E12	955	**0.0024**	18.5	3	937	974
Leucine	Control	739	.	181	3	530	845
Lysine	E12	902	**0.0164**	44.5	3	851	930
Lysine	Control	737	.	154	3	560	845
Methionine	E12	432	**0.0006**	21.2	3	408	447
Methionine	Control	290	.	92.1	3	200	384
Phenylalanine	E12	751	**0.0008**	18.7	3	730	765
Phenylalanine	Control	563	.	127	3	417	653
Proline	E12	528	**0.0059**	69.9	3	487	609
Proline	Control	400	.	69.0	3	321	446
Serine	E12	712	**0.0016**	9.87	3	701	719
Serine	Control	542	.	132	3	390	625
Threonine	E12	672	**0.0023**	20.2	3	659	695
Threonine	Control	524	.	127	3	381	623
Tryptophan	E12	195	0.5960	12.5	3	181	204
Tryptophan	Control	190	.	2.31	3	187	191
Tyrosine	E12	668	**0.0003**	27.2	3	639	693
Tyrosine	Control	495	.	121	3	358	589
Valine	E12	1,034	**0.0003**	39.3	3	992	1,070
Valine	Control	775	.	183	3	564	894

Table A-57. Glycoalkaloids in E12 and Control Russet Burbank at Larimore County, North Dakota in 2011

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	6.94	0.8085	1.08	3	5.72	7.76
	Control	6.58	.	1.31	3	5.25	7.86

Table A-58. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Larimore County, North Dakota in 2011

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	560	**<.0001**	63.5	3	496	623
Asparagine	Control	1,933	.	194	3	1,710	2,060
Aspartic Acid	E12	310	0.6954	4.36	3	307	315
Aspartic Acid	Control	319	.	22.7	3	300	344
Glutamine	E12	1,747	**<.0001**	163	3	1,570	1,890
Glutamine	Control	955	.	166	3	789	1,120
Glutamic Acid	E12	385	0.2389	30.4	3	351	409
Glutamic Acid	Control	331	.	38.0	3	302	374

Table A-59. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Larimore County, North Dakota in 2011

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	0.183	**0.0188**	0.0517	3	0.130	0.233
	Control	0.261	.	0.0733	3	0.195	0.340
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Sucrose (%)							
Fresh	E12	0.222	**0.0200**	0.0676	3	0.153	0.288
	Control	0.156	.	0.0078	2	0.150	0.161
Month 1	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 3	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 5	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.

Table A-60. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Larimore County, North Dakota in 2011

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 0	E12	261	**<.0001**	35.6	3	227	298
	Control	720	.	44.9	3	683	770
Month 3	E12	466	**<.0001**	57.5	3	409	524
	Control	1,128	.	121	3	995	1,230

Table A-61. Proximates, Vitamins, and Minerals in E12 and Control Russet Burbank at Montcalm County, Michigan in 2009

Variable	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Protein (%)	E12	2.25	0.0849	0.0191	3	2.23	2.26
Protein (%)	Control	2.28	.	0.0294	3	2.25	2.31
Fat (%)	E12	0.0800	0.0578	0.0217	3	0.0600	0.110
Fat (%)	Control	0.110	.	0.0110	3	0.100	0.120
Ash (%)	E12	1.02	0.8206	0.0395	3	0.980	1.06
Ash (%)	Control	1.01	.	0.0175	3	1	1.03
Crude Fiber (%)	E12	0.430	0.0670	0.0114	3	0.420	0.440
Crude Fiber (%)	Control	0.470	.	0.0077	3	0.470	0.480
Carbohydrates (%)	E12	18.1	**<.0001**	0.0295	3	18.1	18.1
Carbohydrates (%)	Control	19.2	.	0.0108	3	19.2	19.3
Calories (kcal/100 g)	E12	82.2	**<.0001**	0.345	3	81.9	82.6
Calories (kcal/100 g)	Control	87.1	.	0.138	3	86.9	87.2
Moisture (%)	E12	78.5	**<.0001**	0.0987	3	78.4	78.6
Moisture (%)	Control	77.4	.	0.0379	3	77.3	77.4
Vitamin B3 (mg/100 g)	E12	1.84	**0.0204**	0.0373	3	1.80	1.88
Vitamin B3 (mg/100 g)	Control	1.69	.	0.117	3	1.59	1.82
Vitamin B6 (mg/100 g)	E12	0.150	**0.0002**	0.0065	3	0.140	0.150
Vitamin B6 (mg/100 g)	Control	0.170	.	0.0028	3	0.170	0.170
Vitamin C (mg/110 g)	E12	13.3	0.2581	0.181	3	13.0	13.4
Vitamin C (mg/110 g)	Control	13.0	.	0.378	3	12.6	13.4
Copper (ppm)	E12	1	0.1400	0.0172	3	0.990	1.02
Copper (ppm)	Control	1.06	.	0.0432	3	1.01	1.09
Magnesium (ppm)	E12	190	0.6785	4.14	3	188	195
Magnesium (ppm)	Control	189	.	2.38	3	187	191
Potassium (ppm)	E12	4,013	0.1014	125	3	3,875	4,120
Potassium (ppm)	Control	4,114	.	52.7	3	4,068	4,171

Table A-62. Total Amino Acids in E12 and Control Russet Burbank at Montcalm County, Michigan in 2009

Variable	Variety	Mean (ppm)	P-Value[1]	Standard Deviation	N	Min	Max
Alanine	E12	681	0.7209	70.7	3	618	758
Alanine	Control	654	.	48.0	3	609	705
Arginine	E12	1,744	**0.0221**	271	3	1,559	2,055
Arginine	Control	1,276	.	157	3	1,131	1,443
ASP+ASN	E12	4,590	**<.0001**	102	3	4,512	4,705
ASP+ASN	Control	7,956	.	275	3	7,678	8,228
Cystine	E12	.	.	.	0	.	.
Cystine	Control	.	.	.	0	.	.
GLU+GLN	E12	5,366	**<.0001**	121	3	5,228	5,452
GLU+GLN	Control	3,777	.	190	3	3,605	3,982
Glycine	E12	2,187	**0.0498**	204	3	2,048	2,421
Glycine	Control	1,673	.	352	3	1,467	2,079
Histidine	E12	911	**0.0052**	107	3	790	991
Histidine	Control	732	.	28.2	3	705	762
Isoleucine	E12	1,072	**0.0155**	177	3	919	1,266
Isoleucine	Control	705	.	181	3	573	912
Leucine	E12	1,786	**0.0189**	243	3	1,537	2,024
Leucine	Control	1,394	.	107	3	1,283	1,498
Lysine	E12	1,501	**0.0437**	241	3	1,235	1,705
Lysine	Control	1,176	.	111	3	1,099	1,304
Methionine	E12	710	0.0727	139	3	556	826
Methionine	Control	527	.	25.7	3	506	555
Phenylalanine	E12	1,129	0.0815	116	3	1,014	1,247
Phenylalanine	Control	991	.	69.0	3	916	1,051
Proline	E12	895	0.5202	26.9	3	872	925
Proline	Control	857	.	73.0	3	784	930
Serine	E12	573	0.6295	130	3	425	671
Serine	Control	638	.	108	3	514	707
Threonine	E12	1,240	0.5177	167	3	1,048	1,347
Threonine	Control	1,152	.	180	3	970	1,331
Tryptophan	E12	.	.	.	0	.	.
Tryptophan	Control	.	.	.	0	.	.
Tyrosine	E12	787	0.0613	66.1	3	748	863
Tyrosine	Control	649	.	126	3	555	792
Valine	E12	1,212	0.1247	285	3	955	1,519
Valine	Control	959	.	92.3	3	857	1,037

Table A-63. Glycoalkaloids in E12 and Control Russet Burbank at Montcalm County, Michigan in 2009

Variable	Variety	Mean mg/100 g	P-Value	Standard Deviation	N	Min	Max
Glycoalkaloids	E12	8.11	0.3624	0.283	3	7.93	8.44
	Control	8.42	.	0.249	3	8.13	8.56

Table A-64. Free Amino Acids in Tubers of E12 and Control Russet Burbank at Montcalm County, Michigan in 2009

Variable	Line	Mean (ppm)	P-Value	Standard Deviation	N	Min	Max
Asparagine	E12	492	**<.0001**	12.5	3	484	506
Asparagine	Control	1,252	.	10.4	3	1,240	1,261
Aspartic Acid	E12	506	0.0997	11.4	3	495	518
Aspartic Acid	Control	430	.	11.3	3	417	437
Glutamine	E12	1,298	**<.0001**	29.4	3	1,270	1,329
Glutamine	Control	777	.	18.9	3	759	797
Glutamic Acid	E12	399	0.6549	29.2	3	373	430
Glutamic Acid	Control	385	.	7.07	3	377	391

Table A-65. Russet Burbank and E12 Sugars in Tubers at Harvest and After Storage at Montcalm County, Michigan in 2009

Timing	Variety	Mean	P-Value	Standard Deviation	N	Min	Max
Fructose and Glucose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.0736	**0.0197**	0.0134	3	0.0595	0.0863
	Control	0.100	.	0.0183	3	0.0892	0.122
Month 3	E12	0.0960	**0.0014**	0.0133	3	0.0810	0.106
	Control	0.123	.	0.0055	3	0.117	0.127
Month 5	E12	0.0893	**<.0001**	0.0038	3	0.0851	0.0924
	Control	0.131	.	0.0067	3	0.125	0.138
Sucrose (%)							
Fresh	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.
Month 1	E12	0.0809	**0.0094**	0.0072	3	0.0754	0.0891
	Control	0.114	.	0.0162	3	0.104	0.132
Month 3	E12	0.0573	**0.0016**	0.0099	3	0.0458	0.0636
	Control	0.0752	.	0.0033	3	0.0723	0.0788
Month 5	E12	0.125	0.4345	0.0143	3	0.113	0.141
	Control	0.122	.	0.0078	3	0.113	0.127

Table A-66. Acrylamide in Fries from Russet Burbank and E12 at Harvest and After Storage at Montcalm County, Michigan in 2009

Timing	Variety	Mean (ppb)	P-Value	Standard Deviation	N	Min	Max
Month 2	E12	247	**<.0001**	4.58	3	242	251
	Control	912	.	34.9	3	881	950
Month 5	E12	70	**<.0001**	3.61	3	67.0	74.0
	Control	215	.	14.4	3	207	232
Month 7	E12	.	.	.	0	.	.
	Control	.	.	.	0	.	.

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STUDY TITLE

Stability of the DNA Insert in Russet Burbank E12

AUTHORS

[redacted]

REPORT DATE

12/10/2015

PERFORMING LABORATORIES

SPS Regulatory Lab

STUDY NUMBER

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This report is an accurate and complete representation of the study activities.

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ABSTRACT

The Russet Burbank potato variety was transformed with the plasmid pSIM1278 to produce event E12, which contains a single, intact insert. Southern blot analysis was conducted to verify the stability of the DNA insert in E12. Using probes that hybridize to genetic elements within the T-DNA, Southern blot analysis revealed consistent banding patterns demonstrating that the insert in E12 has been maintained across clonal cycles. These findings confirm the stability of the insert in event E12.

INTRODUCTION

Commercial potatoes are clonally propagated to obtain genetically identical clonal progeny. Since the clonal progeny do not undergo meiotic recombination, this would make them genetically and phenotypically stable. Nonetheless, stability of the insert in E12 was assessed using Southern blot analyses of genomic DNA isolated from potato plants across clonal cycles.

The Russet Burbank potato variety was transformed with the plasmid pSIM1278 to produce event E12. Event E12 contains a single insert from the plasmid pSIM1278. In this study, the genetic stability of the pSIM1278 insert was assessed by Southern blot analysis.

STUDY OBJECTIVES

The objective of this study is to confirm that E12 retains a stable genetic insert across clonal cycles.

STUDY DATES

01/2008 – 05/2015

KEY STUDY PERSONNEL

[redacted]

MATERIALS AND METHODS

Plant Materials

E12 and non-transformed Russet Burbank (WT) G0 plants were grown in Sunshine mix-1 (www.sungro.com) in two-gallon pots in a greenhouse controlled for temperature (18 °C minimum/27 °C maximum) and light (16-h photoperiod with an intensity of about 1,500 µmol/m2/s). G0 leaf tissue was sampled after one to two months of growth and stored at -80 °C. Sprouted mini-tubers (G0) were planted to produce G1 plants. G1 plants were grown until maturity to produce G1 tubers that were planted to produce G2 plants. This process was repeated to obtain G3 plants. Tubers were collected from G3 plants.

DNA Isolation

DNA was isolated from G0 leaf tissue and G3 tuber tissue. DNA from leaf and tuber tissue was extracted using the same protocol. A 1.0 g sample collected was ground into a fine powder using a mortar and pestle under liquid nitrogen. The ground tissue was transferred to a pre-cooled 15 mL conical tube with a pre-cooled spatula and stored at -80 °C. Tissue was thoroughly mixed with 10 mL extraction buffer (0.35 M sorbitol, 0.1 M Tris-HCl, pH 8.0, 0.05 M EDTA) and the suspension was centrifuged at 3,000 rpm for 15 min at room temperature. The pellet was resuspended in 2 mL extraction buffer containing 200 µg RNase A. After incubating the suspended DNA at 65 °C for 20 min with 2 mL nuclear lysis buffer (0.2 M Tris-HCl pH 7.5, 0.005 M EDTA pH 8.0 and 20 mg/mL CTAB Hexadecyl Trimethyl Ammonium Bromide) and 800 µL 5 % sarcosyl, it was mixed with an equal volume of chloroform: isoamyl alcohol (24:1), vortexed for 1 min, and centrifuged at 3,000 rpm for 5 min at room temperature. The DNA was precipitated with an equal volume of isopropyl alcohol, washed with 70 % ethanol, air dried, and dissolved in 400-700 µL 1X Tris/EDTA buffer (TE). DNA concentration was measured using Qubit Fluorometric Quantitation (Life Technologies). DNA quality was confirmed by running the DNA on a 0.8 % agarose gel in 1X Tris/Acetate/EDTA (TAE) for 30-40 min at 80 volts.

DNA Restriction Digestion

A 4 µg sample of plant DNA was digested overnight in 400 µL final volume with at least 5 µL (10 units/µL) restriction enzyme (Invitrogen) at 37 °C. Digested DNA was concentrated by ethanol precipitation (40 µL of 3M NaOAc, pH 5.3 and 1 mL ethanol) at -80 °C for 10 min followed by a wash with 70 % ethanol. The DNA pellet was dissolved in 20 µL 1X TE followed by addition of 2 µL DNA gel loading buffer, which consists of 40 % sucrose and 0.35 % Orange G (Sigma) in water.

Membrane Preparation and Transfer

Digested plant DNA was loaded on a 0.7 % agarose gel containing 1X Tris/Acetate/EDTA (TAE) buffer with 3-5 µL ethidium bromide (10 mg/mL) and run at 35 volts for 18 h. The gel was photographed using a gel documentation system from Alpha Innotech (Santa Clara, California), then depurinated by submerging it in 0.25 N HCl for 2 x 10 min. The gel was subsequently placed in denaturation solution (0.5 M NaOH /1.5 M NaCl) for 2 x 15 min then neutralization solution (1.5 M NaCl and 0.5 M Tris-HCl pH 7.5) for 2 x 15 min on a shaker at room temperature. The gel was then equilibrated with 10X SSC for 10 min. The transfer of DNA to the nylon membrane was carried out using 10X SSC according to a standard capillary transfer method.

DIG-Labeled Probe Preparation

The labeling of the PCR-derived probe was achieved using Hotmaster *Taq* enzyme and buffer (Fisher BioReagents) according to Roche's DIG labeling instructions. A standard 50 µL reaction consisted of 5 µL of 10x Hotmaster *Taq* Buffer, 2-5 µL of 10 µM forward primer, 2-5 µL of 10 µM reverse primer, 5 µL DIG-labeled dNTP (Roche), 10 ng plasmid template, and 0.75 µL Hotmaster *Taq* polymerase. The PCR amplification conditions were dependent on each DIG-labeled probe. PCR with regular dNTP instead of DIG labeled dNTP was used as control. Quality of the DIG-labeled probe was assessed by running a small amount of the probe on 1 % agarose DNA gel (due to the presence of the DIG moieties on the DNA, probes migrate slower than the control PCR product). The probe was denatured before use by incubating the probe at 95 °C for 5 min followed by placing on ice for 2 min.

Probe Hybridization

The cross-linked nylon membrane was prehybridized in 40 mL pre-warmed DIG Easy Hybridization solution (Roche) at 42 °C for 1-4 h in a bottle using a standard hybridization oven (Amerex Instruments Inc.) at 20-25 rpm. Hybridization was carried out by replacing the prehybridization buffer with fresh preheated prehybridization solution, containing 25-50 µL denatured DIG labeled probe. The membrane was incubated at 42 °C, 20-25 rpm for 16 h. The hybridization solution was stored at -20 °C and reused up to 3 times. The reused hybridization solution was heated at 68 °C for 10 min before use.

Detection

The hybridization solution was removed and replaced with 100 mL washing solution I (2X SSC/0.1 % SDS). The membrane was washed twice in washing solution I for 10 min at room temperature. This low stringency buffer was poured off and preheated high stringency washing solution II (0.5X SSC/0.1 % SDS, 60 °C) was added immediately. The membrane was washed twice in washing solution II at 68 °C for 20 min each at 25-30 rpm, followed by a brief rinse with 2X SSC to remove SDS. The membrane was rinsed with 150 mL 1X DIG Washing Solution (Roche) in a tray for 2 min and incubated in 1X Blocking solution (Roche) for 0.5-3 h on a low-speed shaker. The blocked membrane was incubated with DIG antibody solution (1:10,000 dilution of Anti-DIG-alkaline phosphate conjugate with 1X Blocking solution) for 30 min on a shaker at room temperature. The membrane was washed twice (15 min each) with 1X DIG Washing Solution (Roche) and equilibrated with 1X detection buffer. The detection reaction was carried out with 2 mL CDP-Star solution (1:100 diluted stock of CDP-Star with 1X detection buffer) for 5 min. The membrane was wrapped in a plastic film and either exposed to film or developed on an Amersham

Imager 600 (GE Healthcare Life Sciences). Films were developed with a Konica SRX-101A Z-ray film developer.

RESULTS

The E12 insert consists of a single nearly full-length T-DNA from plasmid, pSIM1278. The stability of the insert in E12 was assessed at the G0 and G3 clonal cycles using Southern blot analysis, demonstrating stability across clonal cycles. The GBS and AGP probes were used as they hybridize to multiple regions spanning the insert (Figure 1).

Evidence of genetic stability would entail consistent banding patterns on Southern blots across clonal cycles. Russet Burbank (WT) and E12 DNA was digested with the restriction enzyme, EcoRV. The EcoRV restriction enzyme digests the pSIM1278 insert into multiple bands that can be distinguished by size and probe specificity (Figure 1).

The probes used in this study bind to potato genes, so the Russet Burbank (WT) bands result from detection of endogenous genes. Only bands unique to E12 are associated with the insert.

Insert Stability in E12

The red boxes in Figure 1 show that the EcoRV digest is expected to produce three bands (2.2 kb, 2.3 kb, and 4.5 kb) associated with the pSIM1278 DNA. Southern blots hybridized separately with the GBS or AGP probes confirm the presence and size these bands (Figure 2 and Figure 3).

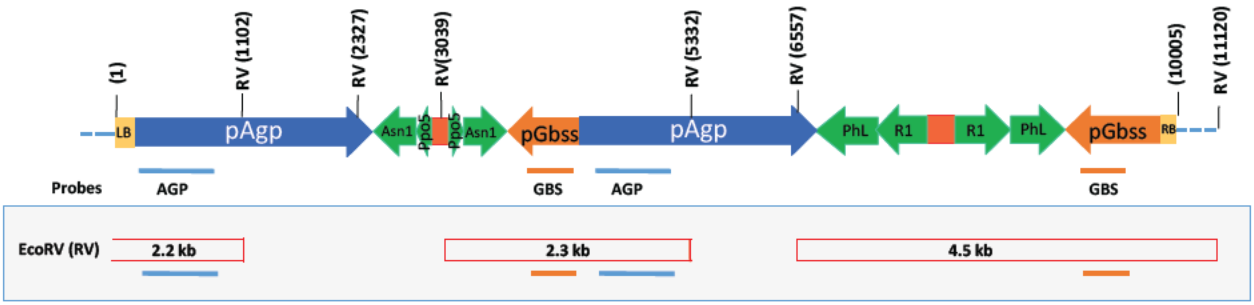

Figure 1. Structure of the pSIM1278 Insert in E12
The pSIM1278 insert in E12 is shown with the EcoRV restriction sites. The bands expected following EcoRV digestion are indicated below the insert map as red boxes with their observed size. A colored indicator notes the binding site for each probe.

The AGP probe hybridized to two bands associated with the pSIM1278 insert (2.2 kb and 2.3 kb; Figure 1). These bands were detected consistently in the E12 plants over clonal cycles (Figure 2). The GBS probe was expected to hybridize to two bands associated with the pSIM1278 insert (2.3 kb and 4.5 kb). These bands were observed in each of the E12 samples over clonal cycles (Figure 3), indicating the stability of

the insert. The banding pattern of the WT remained constant over the clonal cycles (Figure 2 and Figure 3). These endogenous bands are observed in E12 and are not related to the insert.

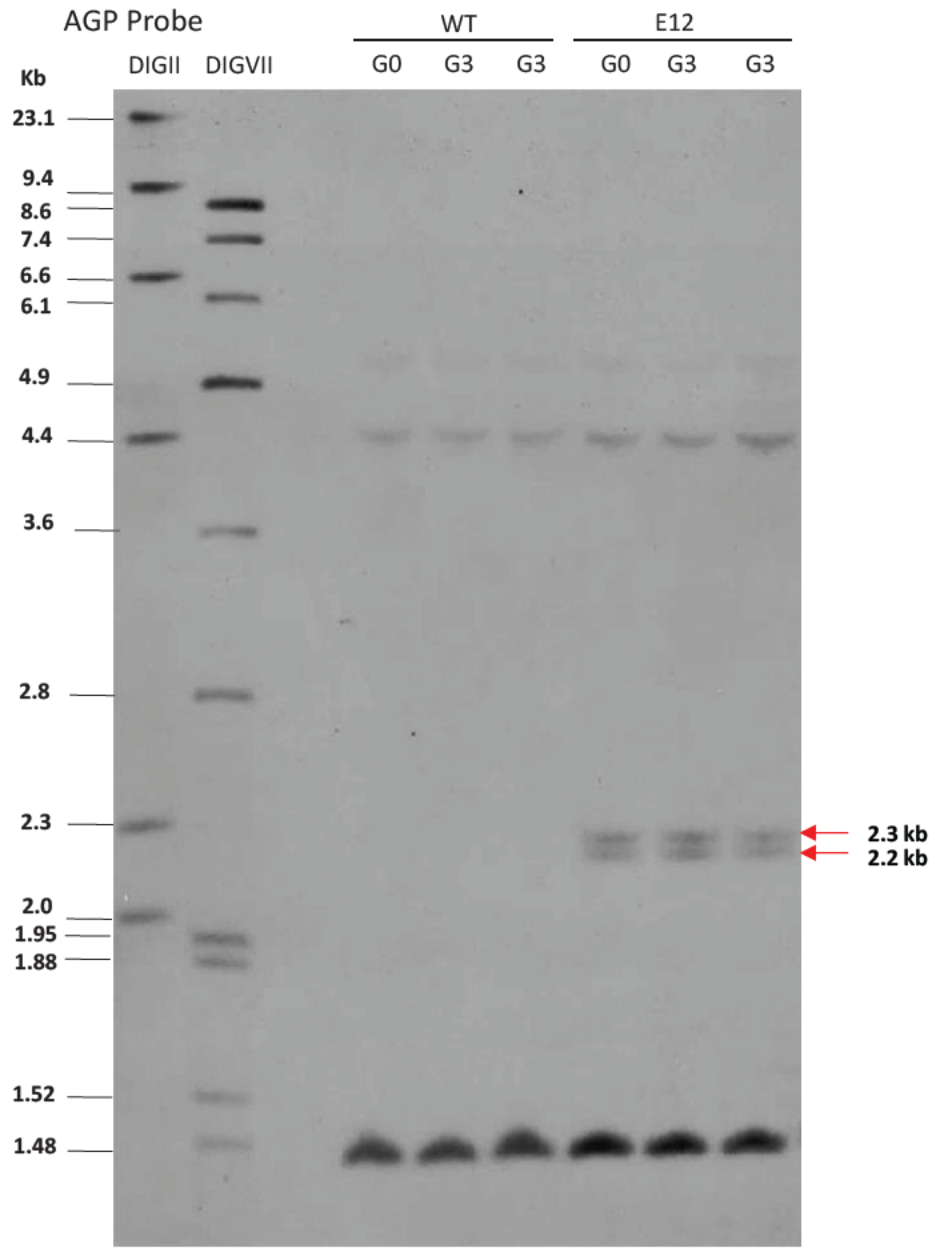

Figure 2. E12 Insert Stability in G0 and G3 Clonal Cycles Visualized with the AGP Probe
Southern blots of genomic DNA isolated from G0 leaves and G3 tubers of Russet Burbank control (WT) and E12 hybridized with the AGP probe. Four µg of each sample were digested with EcoRV and separated by electrophoresis prior to transfer and hybridization with the indicated probe. Red arrows indicate pSIM1278 insert bands with the indicated sizes. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

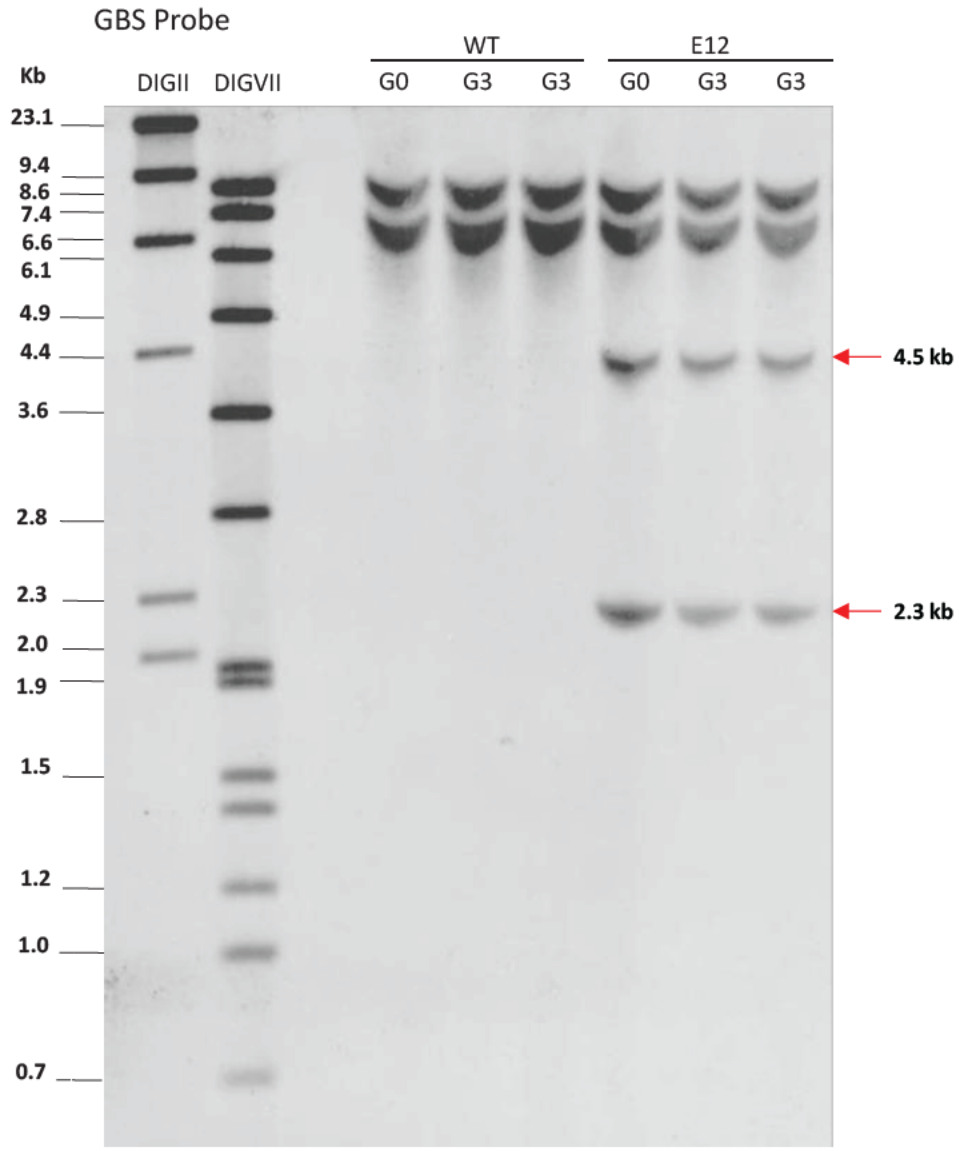

Figure 3. E12 Insert Stability in G0 and G3 Clonal Cycles Visualized with the GBS Probe
Southern blots of genomic DNA isolated from G0 leaves and G3 tubers of Russet Burbank control (WT) and E12 hybridized with the GBS probe. Four µg of each sample were digested with EcoRV and separated by electrophoresis prior to transfer and hybridization with the indicated probe. Red arrows indicate pSIM1278 insert bands with the indicated sizes. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

CONCLUSION

The Southern blot results showed consistent banding patterns between G0 and G3 clonal cycles of E12 when analyzed by Southern blot. As potatoes are clonally propagated, all progeny produced from an individual plant are considered genetically identical. Consequently, evaluating insert stability by examining inheritance across clonal cycles using Mendelian segregation analysis is not applicable for potatoes. This study demonstrates that the insert in E12 is stable and was maintained across clonal cycles.

STUDY TITLE

RNA Expression of Down-Regulated Genes in Russet Burbank E12

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12/10/15

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ABSTRACT

Event E12 was developed by transforming Russet Burbank with pSIM1278. The inserted cassettes were designed to down-regulate expression of four genes: *Asn1*, *Ppo5*, *PhL*, and *R1*. The results demonstrate reduced expression of the *Asn1* and *Ppo5* genes in tubers. Gene expression in other tissues was unaffected, except for a small reduction of *Asn1* RNA levels in flowers.

INTRODUCTION

Event E12 was developed by transforming Russet Burbank (RB) with pSIM1278. The pSIM1278 construct introduced two cassettes intended to down-regulate expression of the *Asn1*, *Ppo5, PhL*, and *R1* genes.

Each cassette is driven by promoters primarily active in tubers to facilitate tissue-specific down-regulation. The effectiveness of target gene down-regulation and tissue-specificity was evaluated by northern blot analysis in potato tuber, leaf, stem, root, and flower tissues.

STUDY OBJECTIVES

The objectives of this study are:

1. Demonstrate down-regulation of target genes in E12 tubers.
2. Determine the tissue-specificity of target gene down-regulation in E12 plants.

STUDY DATES

01/2008 - 12/2015

KEY STUDY PERSONNEL

[redacted]

MATERIALS AND METHODS

Plant Material

Leaf, stem, root, and flower tissues of first generation field grown E12 and RB plants (G1) were collected from Jerome, ID and Parma, ID. Tubers were collected at harvest. All samples were flash frozen in liquid nitrogen and stored at -80 °C.

RNA Isolation

RNA was extracted from tuber and root tissues using Plant RNA reagent (Invitrogen™, Thermo Fisher Scientific, Waltham, MA). Trizol reagent (Invitrogen™) was used to extract RNA from leaf, flower, and stem tissues. The concentration of isolated RNA was measured using a Qubit 2.0 fluorometer (Invitrogen™) and RNA quality was confirmed by electrophoresis on 1 % agarose gels in 200 mM MOPS buffer containing 50 mM NaOAc and 20 mM EDTA (pH 7.0) for 30-60 min at 90 volts.

RNA Transfer

RNA was denatured by heating at 65 °C for 10 min followed by 5 min incubation on ice. RNA samples were electrophoresed on 1 % agarose gels containing 0.1-0.25 µg/mL ethidium bromide and 2 % formaldehyde. Gels were run at 80-85 volts for 2-3 h and imaged using an AlphaImager HP instrument (ProteinSimple, San Jose, CA). The gels were washed twice in 10X Saline Sodium Citrate (SSC) for 15 min to remove formaldehyde. RNA was transferred to a nylon membrane (Roche, Indianapolis, IN) by capillary transfer in 10X SSC buffer for 16-18 h and stabilized by UV cross-linking (UVP, Upland, CA). Transferred membranes were stored at 4 °C until probed.

Probe Labelling

Probes were DIG labeled using PCR. Probes were made complementary to the four target genes and to the 18S rRNA as a control. Primers used for probe amplification and probe lengths are shown in Table 1. A typical reaction (50 µL) contained 5 µL HotMaster™ Taq Buffer (Thermo Fisher), 0.4-1 µM forward primer, 0.4-1 µM reverse primer, 5 µL DIG-labeled dNTP (Roche), 5-30 ng plasmid template, and 0.5-0.75 µL HotMaster™ Taq polymerase. PCR conditions were specific to each probe. The size and purity of the DIG-labeled probe was confirmed by agarose gel electrophoresis. As expected, the labeled probe migrated slower than the unlabeled control. The probe was denatured before use (5 min at 95 °C) and quenched on ice.

Table 1. Primers and Probes Used for Northern Analysis of Potato Genes

Probe	Target	Primer Sequences (5′ to 3′)	Size (bp)
ASN1	*Asn1* transcript	GGTTGATGACTGATGTCCCCTTTG	1115
		TAGTTAGCTCCTTATTGTGAGCTC	
PPO5[1]	*Ppo5* transcript	ATCTTCCACTCCTAAGCCCTCTCAAC	864
		CCGCCAAAGAACATCCGAGG	
PhL	*PhL* transcript	CAATTCCAGATTCATCCATTTCAC	968
		CATCCCCAGGGTAGAGTATGTAAC	
R1	*R1* transcript	ACCAGGGATTCCTAACCTCAAC	852
		CAGGTATATCACTCTTTGTTAC	
18S	18S rRNA	GCATTTGCCAAGGATGTTTT	694
		GTACAAAGGGCAGGGACGTA	

[1]The PPO probe was derived from the expressed *Ppo5* gene from *S. tuberosum*. The probe hybridizes with *Ppo* genes expressed in other tissues. The *Ppo5* sequence in pSIM1278 comes from the untranslated trailer of the *S. verrucosum Ppo5* gene.

Hybridization

Prior to use, hybridization solution was heated to 68 °C for 10 min. Membranes were incubated in 40 mL warm DIG Easy Hybridization solution (Roche) at 42 °C for 1-4 h with rotation at 20-25 rpm. The hybridization solution was replaced with 40 mL warm hybridization solution containing 25-50 µL of the DIG-labeled probe and incubated for 3-16 h at 42 °C. For each tissue, blots were stripped and probed again using the indicated probe.

Detection

Blots were washed twice with solution I (2X SSC/0.1 % SDS) for 10 min at room temperature (RT) with rotation at 25-30 rpm. Blots were washed twice with solution II (0.5X SSC/ 0.1 % SDS) for 20 min at 60 °C with rotation at 25-30 rpm. A final wash step was carried out in solution III (0.1X SSC/ 0.1 % SDS) for 20 min at 65 °C. Blots were rinsed with 2X SSC to remove SDS. The membrane was rinsed with 100 mL 1X DIG Washing Solution (Roche) for 2 min at RT and then blocked with 1X Blocking solution (Roche) for 30 min to 3 h at RT with constant shaking. A 1:10,000 dilution of anti-DIG-alkaline phosphate conjugate was added and blots incubated for 30 min at RT with constant shaking. Membranes were washed twice (15 min each) with 1X DIG Washing Solution and equilibrated in 1X detection buffer. A 1:100 dilution of CDP-Star was added. After 5 min the membrane was wrapped in plastic and developed at exposure times ranging from 1 to 25 min using an Amersham™ Imager 600 instrument (GE Healthcare Life Sciences, Pittsburgh, PA).

RESULTS

RNA was isolated from field grown E12 and RB control plants (WT) and analyzed by northern blot to determine the extent of down-regulation of the target genes. Tissue specificity was determined by comparing gene expression levels between tuber, leaf, stem, root, and flower tissues.

The results showed that *Asn1* and *Ppo5* were down-regulated in tubers (Figure 1). No differences were observed in other tissues, except for a partial down-regulation of *Asn1* in flower tissue (Figure 2, Figure 3, Figure 4, and Figure 5). The 18S RNA and total RNA levels were consistent across samples allowing for direct comparisons of *Asn1*, *Ppo5*, *PhL*, and *R1* transcripts between samples in a given tissue. Results of this study are summarized in Table 2.

Table 2. Summary of the Expression of Genes Targeted for Down-Regulation in E12 Tissues

Gene	Tuber	Leaf	Stem	Root	Flower
Asn1	✓	-	-	-	✓
Ppo5	✓	-	-	-	-
PhL	-	-	-	-	-
R1	-	-	-	-	-

✓ = down-regulated, - = not down-regulated.

Tuber

The absence of a band in the E12 samples probed for ASN1 and PPO5 indicated down-regulation of *Asn1* and *Ppo5* gene expression in E12 tubers compared to the RB controls (WT) (Figure 1). The down-regulation was particularly strong as nearly complete absence of the target RNA signal was observed. *PhL* and *R1* expression levels were unchanged between E12 and WT tuber samples (Figure 1).

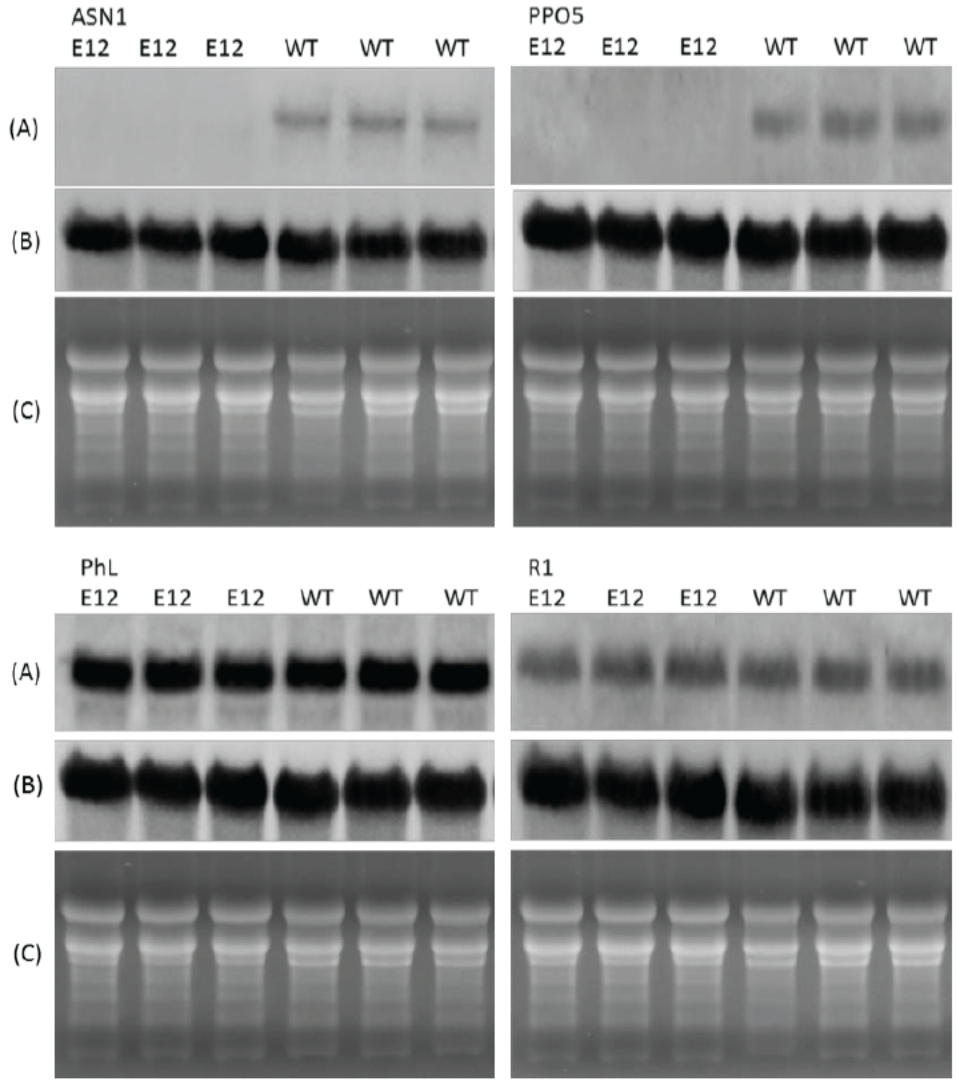

Figure 1. Changes in *Asn1* and *Ppo5* Gene Expression in E12 Tubers
Northern blots were probed to detect each of the RNAi targets: *Asn1*, *Ppo5*, *PhL*, and *R1*. (A) Samples were analyzed with the indicated probe. (B) 18S RNA provides a gel loading control. (C) Total RNA is shown after staining with ethidium bromide.

Leaf

Down-regulation was not observed for any of the four target genes in E12 leaf samples compared to the RB control (WT) consistent with tuber-specific gene silencing (Figure 2).

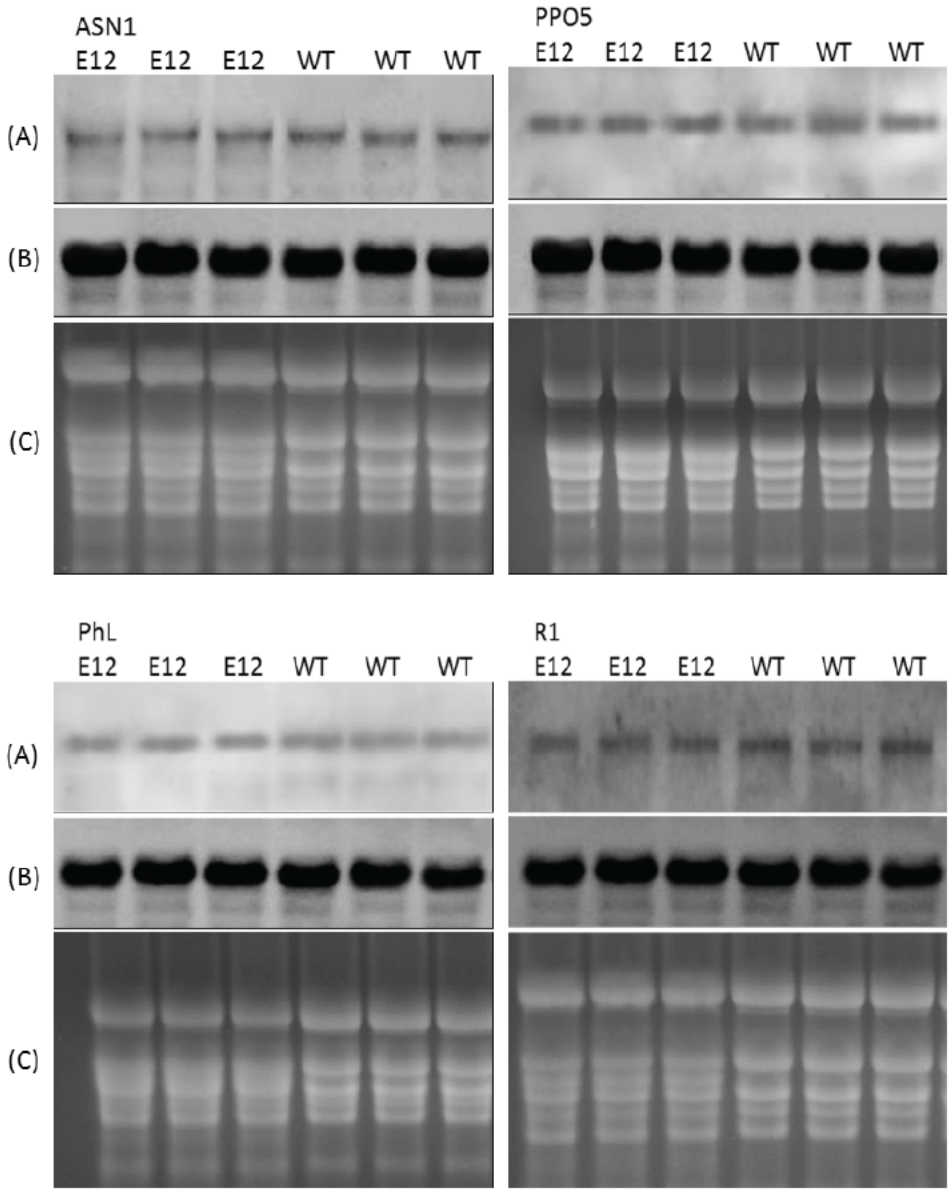

Figure 2. No Changes in RNAi Target Gene Expression in E12 Leaves
Northern blots were probed to detect each of the RNAi targets: *Asn1*, *Ppo5*, *PhL*, and *R1*. (A) Samples were analyzed with the indicated probe. (B) 18S RNA provides a gel loading control. (C) Total RNA is shown after staining with ethidium bromide.

Stem

Down-regulation was not observed for any of the four target genes in E12 stem samples compared to the RB control (WT) consistent with tuber-specific gene silencing (Figure 3).

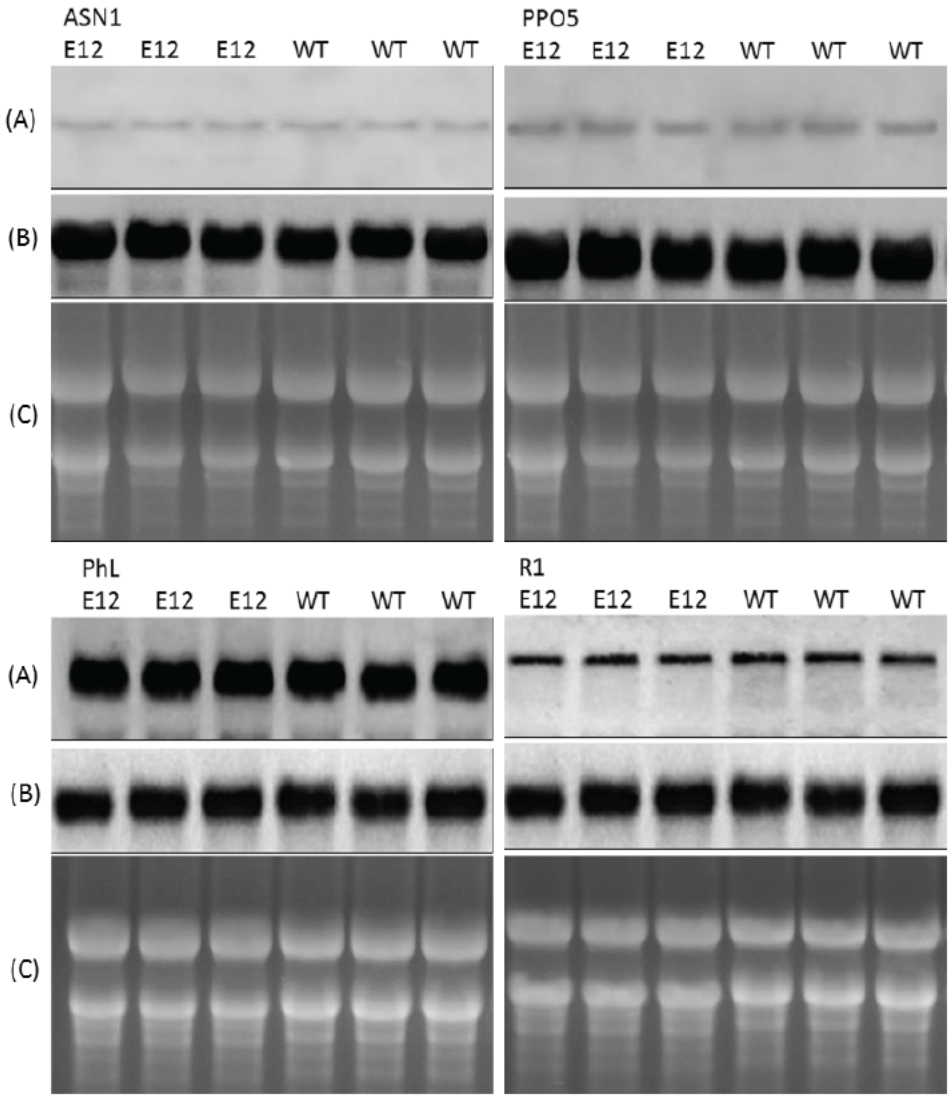

Figure 3. No Changes in RNAi Target Gene Expression in E12 Stems
Northern blots were probed to detect each of the RNAi targets: *Asn1*, *Ppo5*, *PhL*, and *R1*. (A) Samples were analyzed with the indicated probe. (B) 18S RNA provides a gel loading control. (C) Total RNA is shown after staining with ethidium bromide.

Root

Down-regulation was not observed for any of the four target genes in E12 root samples compared to the RB control (WT) consistent with tuber-specific gene silencing (Figure 4).

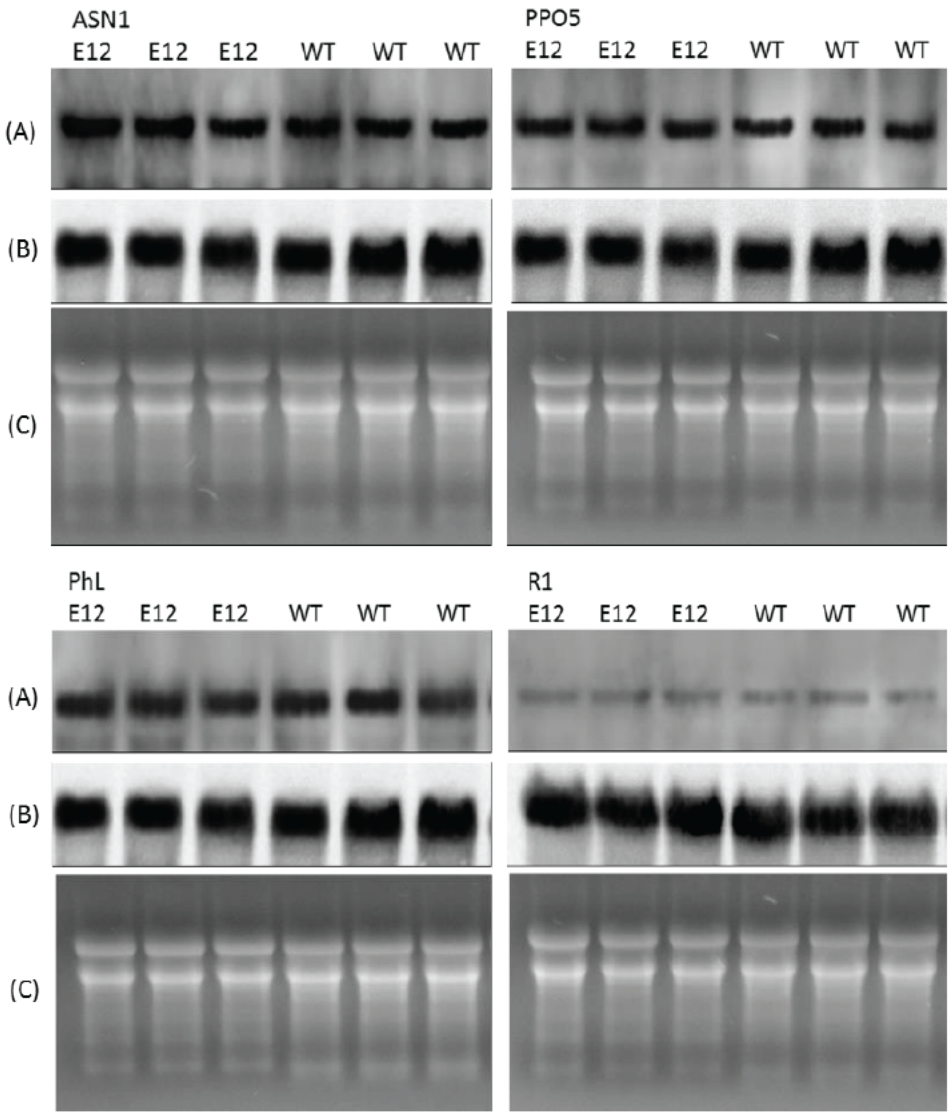

Figure 4. No Changes in RNAi Target Gene Expression in E12 Roots
Northern blots were probed to detect each of the RNAi targets: *Asn1*, *Ppo5*, *PhL*, and *R1*. (A) Samples were analyzed with the indicated probe. (B) 18S RNA provides a gel loading control. (C) Total RNA is shown after staining with ethidium bromide.

Flower

A minor decrease in *Asn1* gene expression was observed in E12 flower samples (Figure 5). There was no observable down-regulation of other genes in E12 flower samples consistent with tuber-specific gene silencing (Figure 5).

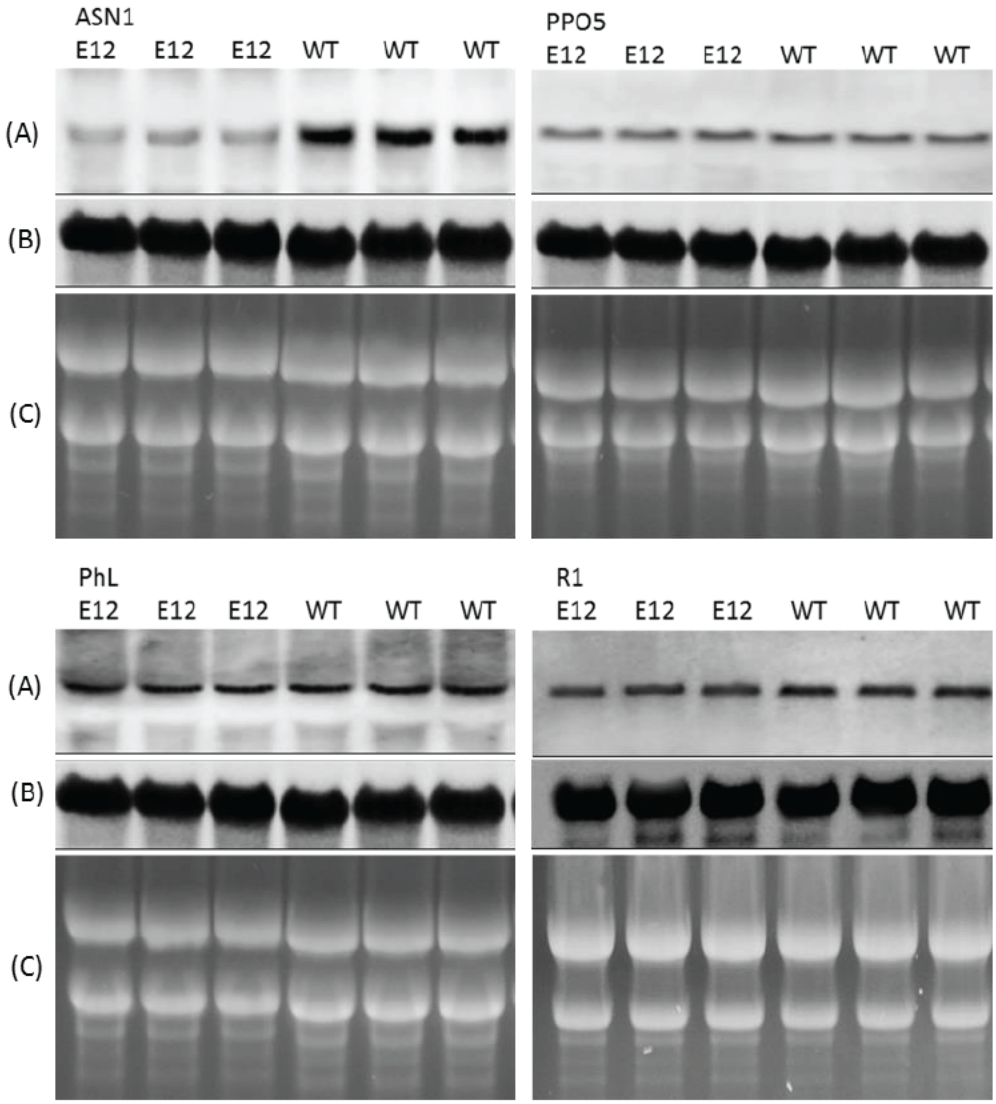

Figure 5. Minor Changes in *Asn1* Gene Expression in E12 Flowers
Northern blots were probed to detect each of the RNAi targets: *Asn1*, *Ppo5*, *PhL*, and *R1*. (A) Samples were analyzed with the indicated probe. (B) 18S RNA provides a gel loading control. (C) Total RNA is shown after staining with ethidium bromide.

CONCLUSION

The pSIM1278 construct introduced two cassettes intended to down-regulate expression of the *Asn1*, *Ppo5, PhL*, and *R1* genes. The cassettes are driven by promoters primarily active in tubers to facilitate tissue-specific down-regulation. The effectiveness of target gene down-regulation and tissue-specificity was evaluated by northern blot analysis in potato tuber, leaf, stem, root, and flower tissues. *Asn1* and *Ppo5* gene levels were down-regulated in E12 tubers as intended. The expression level of the target genes was unaffected in other tissues, except for a partial down-regulation of *Asn1* in flower, consistent with tuber-specific gene silencing.

STUDY TITLE

Construction of pSIM1278

AUTHORS

[redacted]

REPORT DATE

12/28/2015

PERFORMING LABORATORIES

SPS Regulatory Lab

STUDY NUMBER

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This report is an accurate and complete representation of the study activities.

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12/28/15

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TABLE OF CONTENTS

ABSTRACT

The plasmid pSIM1278 is a 19.7 kb binary vector used to transform potatoes. Details are included for both backbone and T-DNA genetic elements and the cloning steps used to create the plasmid. The T-DNA region of pSIM1278 consists of two cassettes designed to down-regulate potato genes, *Asn1*, *Ppo5*, *R1*, and *PhL*, in tubers. The plasmid backbone contains sequence for maintaining the plasmid in *Agrobacterium* and *Escherichia coli*, an overdrive sequence to enhance cleavage at the right border, a kanamycin selectable marker, and *ipt* for screening to select against plants containing vector backbone DNA.

INTRODUCTION

This report provides detailed information on the assembly of plasmid pSIM1278, a 19.7 kb binary transformation vector used to transform potatoes. This report shows the source of the genetic elements, the cloning steps for the backbone and T-DNA sequences, and the order of the elements in the plasmid.

STUDY OBJECTIVES

The objective of this study was to characterize the pSIM1278 plasmid and its assembly.

STUDY DATES

2/2007 - 9/2007

KEY STUDY PERSONNEL

[redacted]

DESCRIPTION of pSIM1278

pSIM1278 Backbone

The plasmid backbone (Figure 1; Table 1) contains two well-characterized bacterial origins of replication. pVS1 (pVS1 Sta and Rep) enables maintenance of the plasmid in *Agrobacterium*, and pBR322 (pBR322 bom and ori) enables maintenance of the plasmid in *Escherichia coli*. The *Agrobacterium* DNA overdrive sequence enhances cleavage at the RB, and the *E. coli. nptII* gene is a bacterial kanamycin selectable marker. The backbone contains an expression cassette comprising the *Agrobacterium* isopentenyl transferase (*ipt*) gene flanked by the Ranger Russet potato polyubiquitin (*Ubi7*) promoter and the Ranger Russet potato polyubiquitin (*Ubi3*) terminator (Garbarino and Belknap, 1994). The *ipt* cassette introduces a phenotype used to select against plasmid backbone DNA integration in the host plant. When present in transformed plant tissue, overexpression of *ipt* results in the overproduction of the plant hormone cytokinin resulting in plants with stunted phenotypes, abnormal leaves and the inability to root.

pSIM1278 T-DNA

Plasmid pSIM1278 T-DNA contains two expression cassettes (Figure 1):

- The first cassette (elements 4 to 12, Table 2) results in down-regulation of *Asn1* and *Ppo5* in the transformed potato variety. It is comprised of two identical 405 bp fragments of *Asn*1 and two identical 144 bp fragments of *Ppo5*. The fragments of *Asn*1 and *Ppo5* are arranged as inverted repeats separated by a non-coding 157 bp Ranger Russet potato nucleotide spacer element. The *Asn1* and *Ppo5* fragments are arranged between the two convergent potato promoters; the *Agp* promoter of the ADP glucose pyrophosphorylase gene (*Agp*) and the Gbss promoter of the granule-bound starch synthase gene (*Gbss*) that are primarily active in tubers. These promoters drive expression of the inverted repeats to generate double-stranded RNA and down-regulate *Asn1* and *Ppo5*.

- The second cassette (elements 14 to 21, Table 2) results in down-regulation of *PhL* and *R1* in the transformed potato variety. It is comprised of two identical 509 bp fragments of the *PhL* promoter region and two identical 532 bp fragments of *R1* promoter region. The PhL and R1 fragments are arranged as inverted repeats separated by a non-coding 258 bp fragment of the Ranger Russet potato polyubiquitin gene. Like the first cassette, the PhL and R1 fragments are arranged between and transcribed by the potato *Agp* and *Gbss* promoters.

DESCRIPTION of pSIM1278 GENETIC ELEMENTS

The genetic elements in the pSIM1278 backbone region are detailed in Table 1 and the genetic elements in the T-DNA region are detailed in Table 2.

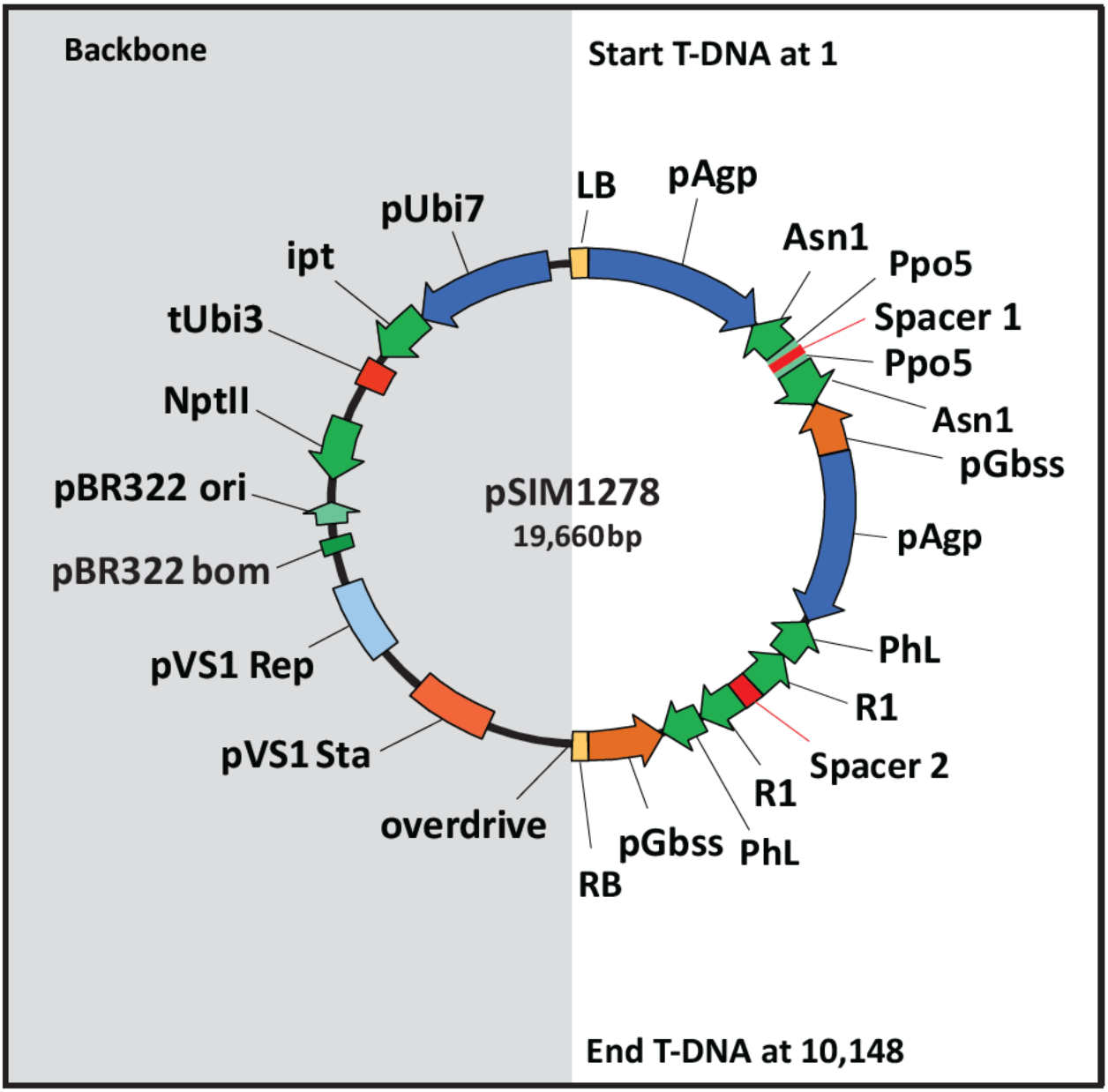

Figure 1. Schematic Diagram of pSIM1278

Table 1. Genetic Elements of the pSIM1278 Backbone

Genetic Element	Origin	Accession Number[1]	Position	Size (bp)	Function
1. Intervening sequence	Synthetic DNA		10,149-10,154	6	Sequence used for cloning
2. Overdrive	*Agrobacterium tumefaciens* Ti-plasmid	NC_002377	10,155-10,184	30	Enhances cleavage of *A. tumefaciens* Right Border site [1]
3. Intervening sequence	*Pseudomonas fluorescens* pVS1	AJ537514	10,185-11,266	1,082	pVS1 backbone[1]
4. pVS1 partitioning protein StaA (PVS1 Sta)	*P. fluorescens* pVS1	AJ537514	11,267-12,267	1,001	pVS1 stability [1]
5. Intervening sequence	*P. fluorescens* pVS1	AJ537514	12,268-12,860	593	pVS1 backbone[1]
6. pVS1 replicon (pVS1Rep)	*P. fluorescens* pVS1	AJ537514	12,861-13,861	1,001	pVS1 replication region in *Agrobacterium* [1]
7. Intervening sequence	*P. fluorescens* pVS1	AJ537514	13,862-14,099	238	pVS1 backbone[1]
8. Intervening sequence	pBR322	AF234297	14,100-14,270	171	pCambia1301 backbone[1]
9. pBR322 bom	pBR322	AF234297	14,271-14,531	261	pBR322 region for replication in *E. coli* [1]
10. Intervening sequence	pBR322	AF234297	14,532-14,670	139	pBR322 backbone[1]
11. Origin of replication for pBR322 (pBR322 ori)	pBR322	AF234297	14,671-14,951	281	Bacterial origin of replication [1]
12. Intervening sequence	pBR322	AF234297	14,952-15,241	290	pCambia1301 backbone[1]
13. Neomycin phosphotransferase II (*nptII*) gene	Tn*5* transposon	FJ362602	15,242-16,036	795	Aminoglycoside phosphotransferase[1] (Simpson et al., 1985)
14. Intervening sequence	Vector DNA	FJ362602	16,037-16,231	195	pCAMBIA vector backbone[1]
15. Terminator of the ubiquitin-3 gene (tUbi3)	*S. tuberosum*	GP755544	16,232-16,586	355	Terminator for *ipt* gene transcription (Garbarino and Belknap, 1994)
16. Intervening sequence	*A. tumefaciens* Ti-plasmid	NC_002377	16,587-16,937	351	Sequence used for DNA cloning
17. Isopentenyl transferase (*ipt*) gene	*A. tumefaciens* Ti-plasmid	NC_002377	16,938-17,660	723	Condensation of AMP and isopentenyl-pyrophosphate to form isopentenyl-AMP, a cytokinin in the plant. Results in abnormal growth phenotypes in plant (Smigocki and Owens, 1988)
18. Intervening sequence	Synthetic DNA		17,661-17,672	12	Sequence used for DNA cloning
19. Polyubiquitin promoter (Ubi7)	*S. tuberosum* var. Ranger Russet	U26831	17,673-19,410	1,738	Promoter to drive expression of the *ipt* backbone marker gene (Garbarino et al., 1995)
20. Intervening sequence	Vector DNA	U10460	19,411-19,660	250	pZP200 vector backbone[1]

[1] http://www.ncbi.nlm.nih.gov/nuccore/AF234297.1

Table 2. Genetic Elements of pSIM1278 T-DNA, from Left Border Site to Right Border

Genetic Element	*Origin*	Accession Number	Position (pSIM1278)	Size (bp)	Intended Function
1. Left Border (LB) site[1]	Synthetic	AY566555[2] (bases 1-25)	1 – 25	25	Site for secondary cleavage to release single-stranded DNA insert from pSIM1278 (van Haaren et al., 1989)
2. Left Border region sequence	*S. tuberosum* var. Ranger Russet.	AY566555[2] (bases 26-187)	26 – 187	162	Supports secondary cleavage at LB
3. Intervening Sequence	*S. tuberosum*	AF393847	188 –193	6	Sequence used for DNA cloning
4. Promoter for the ADP glucose pyrophosphorylase gene (pAgp), 1st copy	*S. tuberosum* var. Ranger Russet	HM363752	194-2,453	2260	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers
5. Fragment of the asparagine synthetase-1 (Asn1) gene (1st copy antisense orientation)	*S. tuberosum* var. Ranger Russet	HM363759	2,454-2,858	405	Generates with (11) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation (Chawla et al., 2012[2])
6. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (1st copy, in antisense orientation)	*S. verrucosum*	HM363754	2,859-3,002	144	Generates with (9) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot development
7. Intervening Sequence	*S. tuberosum*	DQ478950	3,003-3,008	6	Sequence used for DNA cloning
8. Spacer-1	*S. tuberosum* var. Ranger Russet	HM363753	3,009-3,165	157	Sequence between the 1st inverted repeats
9. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (2nd copy, in sense orientation)	*S. verrucosum*	HM363754	3,166-3,309	144	Generates with (6) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot development
10. Fragment of the asparagine synthetase-1 (Asn1) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	HM363759	3,310-3,715	406	Generates with (5) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation (Chawla et al., 2012[2])
11. Intervening Sequence	*S. tuberosum*	X73477	3,716-3,721	6	Sequence used for DNA cloning
12. Promoter for the granule-bound starch synthase (pGbss) gene (1st copy, convergent orientation relative to the 1st copy of pAgp)	*S. tuberosum* var. Ranger Russet	HM363755	3,722-4,407	686	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers
13. Intervening Sequence	*S. tuberosum*	X95996 / AF393847	4,408-4,423	16	Sequence used for DNA cloning
14. pAgp, 2nd copy	*S. tuberosum* var. Ranger Russet	HM363752	4,424-6,683	2260	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of the promoters of PhL and R1, especially in tubers
15. Fragment of promoter for the potato phosphorylase-L (PhL) gene (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	HM363758	6,684-7,192	509	Generates with (21) double stranded RNA that triggers the degradation of PhL transcripts to limit the formation of reducing sugars through starch degradation

16. Fragment of promoter for the potato R1 gene (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	HM363757	7,193-7,724	532	Generates with (20) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation
17. Intervening Sequence	*S. tuberosum*	DQ478950	7,725-7,730	6	Sequence used for DNA cloning
18. Spacer-2	*S. tuberosum* var. Ranger Russet	U26831[3]	7,731-7,988	258	Sequence between the 2nd inverted repeat
19. Fragment of promoter for the potato R1 gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	HM363757	7,989-8,520	532	Generates with (20) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation
20. Fragment of promoter for the potato phosphorylase-L (PhL) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	HM363758	8,521-9,029	509	Generates with (16) double stranded RNA that triggers the degradation of PhL transcript to limit the formation of reducing sugars through starch degradation
21. pGbss (2nd copy, convergent orientation relative to the 2nd copy of pAgp)	*S. tuberosum* var. Ranger Russet	X83220[4]	9,030-9,953	924	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of the promoters of PhL and R1, especially in tubers
22. Intervening Sequence	*S. tuberosum*	AF143202	9,954 – 9,962	9	Sequence used for DNA cloning
23. Right Border region sequence	*S. tuberosum* var. Ranger Russet	AY566555[5] (bases 231-391)	9,963 – 10,123	161	Supports primary cleavage at RB site
24. Right Border (RB) sequence[1]	Synthetic	AY566555[5] (bases 392-416)	10,124 – 10,148	25	Site for primary cleavage to release single stranded DNA insert from pSIM1278 (van Haaren et al., 1989)

[1]The LB and RB sequences (25-bp each) were synthetically designed to be similar to and function like T-DNA borders from *Agrobacterium tumefaciens*.

[2]ASN1 described as genetic elements 5 and 11 is referred to as StAst1 in Chawla et al., 2012.

[3] GenBank Accession HM363756 is replaced with a citation to GenBank Accession U26831 to properly include four 3' end nucleotides present in the pGbss DNA element of the pSIM1278 construct.

[4] GenBank Accession HM363755 is replaced with a citation to GenBank Accession X83220 to properly include the full pGbss (2nd copy) DNA insert sequence present in the pSIM1278 construct.

[5]GenBank Accession AY566555 was revised to clarify the sources of DNA for the Border regions.

ASSEMBLY OF PLASMID pSIM1278

The plasmid, pSIM1278, was constructed as outlined in Figure 2 using DNA sequences as described in Table 1 and Table 2. The starting vector, pCAMBIA1301, contains the origins of replications in the final pSIM1278 backbone.

1. Deleted T-DNA region between the SacII and SphI restriction sites of pCAMBIA1301.

2. Inserted new T-DNA region between the SacII and SphI restriction sites adding KpnI and SacI restriction sites.

3. Inserted ipt cassette (pUbi7, *ipt*, tUbi3) into SacII restriction site.

4. Deleted DNA between KpnI and SacI. Inserted T-DNA region assembled from potato DNA sequence (Figure 3) into Kpn1 and SacI.

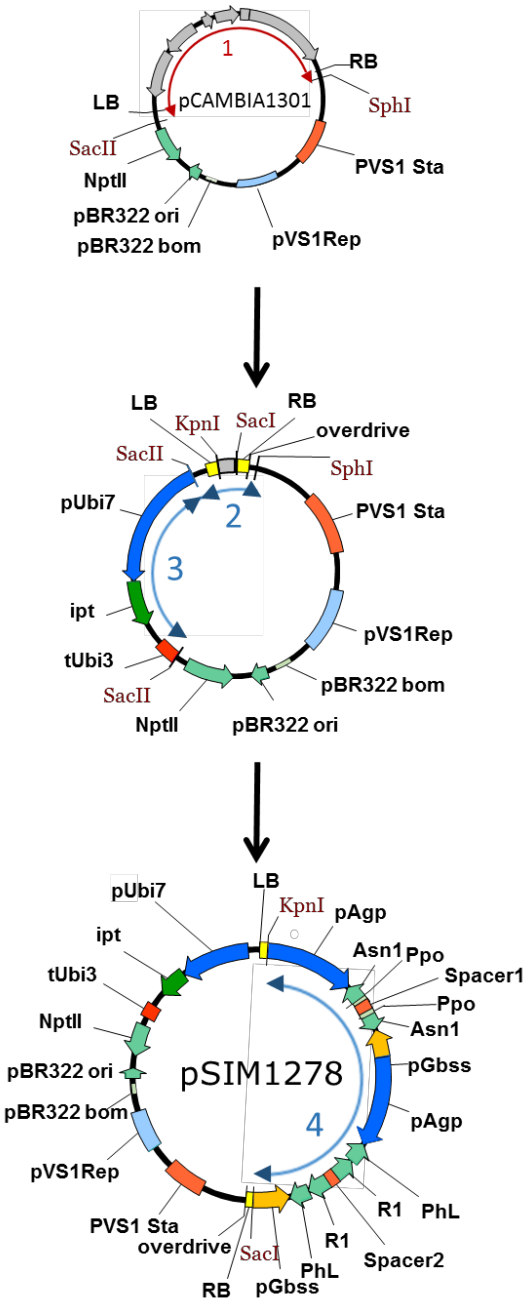

Figure 2. Construction of pSIM1278

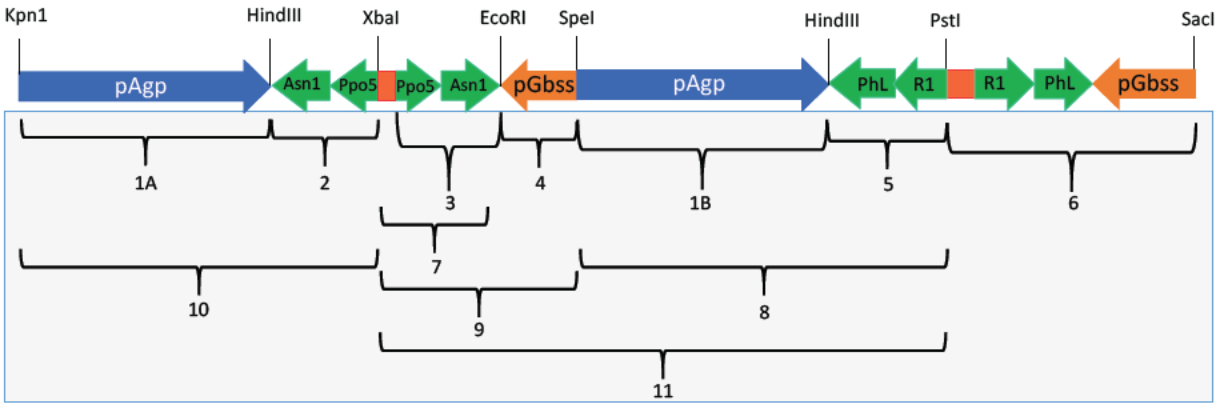

Figure 3. Construction of T-DNA Expression Cassettes
Fusion PCR was used to amplify elements 1A (pAgp – 1st copy), 1B (pAgp-2nd copy), 2 (Asn1, Ppo5), 3 (Ppo5, Asn1), 4 (pGbss -1st copy) and 7 (Spacer1, Ppo5, Asn1). Elements 5 (PhL, R1) and 6 (Spacer2, R1, PhL, pGbss) were synthesized by the Blue Heron Biotechnology, Inc. (Bothell, WA) based on the sequence from the potato genome. Elements 8, 9, and 10 were generated by ligating building blocks shown in the figure. In the end, three fragments, 10, 11 and 6 were created to span the desired expression cassette. These three fragments were ligated and inserted into the KpnI – SacI restriction sites shown in Figure 2 to generate pSIM1278.

CONCLUSIONS

The pSIM1278 plasmid is a binary vector designed for potato plant transformation. The vector backbone contains sequences for replication in both *E. coli* and *Agrobacterium* along with an *ipt* marker for screening to eliminate plants with vector backbone DNA. The T-DNA region consists of two expression cassettes flanked by LB and RB sequences. Upon inoculation of host plant tissue with *Agrobacterium* containing the pSIM1278 vector, the T-DNA region of pSIM1278 is transferred into the host genome.

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STUDY TITLE

Evaluation of Allergen and Toxin Homology of
Start-to-Stop Open Reading Frames in Event E12

AUTHOR

[redacted]

REPORT DATE

09/23/15

PERFORMING LABORATORIES

SPS Regulatory Lab

STUDY NUMBER(S)

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CERTIFICATION PAGE

This report is an accurate and complete representation of the study activities.

[redacted]

Senior Regulatory Science Manager

9/23/15
Date

TABLE OF CONTENTS

ABSTRACT

An analysis was completed to assess open reading frames (ORFs) in event E12, which was generated by transformation of Russet Burbank potatoes with pSIM1278 T-DNA. Novel ORFs were evaluated using bioinformatics (i.e., amino acid sequence comparison techniques) to assess their similarity to known toxins or allergens. Bioinformatic analyses of 27 start-to-stop ORFs within the DNA insert and genomic junction regions demonstrated no putative polypeptides with relevant homology to proteins that are known to be toxic, allergenic, or to have other biologically adverse properties.

INTRODUCTION

An analysis was completed to determine the toxin or allergen homology, if any, of open reading frames (ORFs) introduced into event E12 through transformation with pSIM1278 T-DNA. This report assesses the allergen and toxin homology of the start-to-stop open reading frames in the E12 insert and junction regions using the standard bioinformatic techniques summarized in Table 1.

Expression of toxins or allergens is unlikely, particularly in potatoes transformed with potato DNA. Most of the ORFs contained in the E12 T-DNA insert exist naturally elsewhere in the potato genome.

Well-established techniques in bioinformatics were used to evaluate the insert in E12 (Ladics, 2007; Goodman, 2008; Terrat, 2013).

Table 1: Overview of Analyses using Bioinformatics

Analysis	Purpose	Approach
Start-to-stop ORF Analysis	Identify all open reading frames associated with the T-DNA insert, including junction regions	Internally developed Python script systematically identifies all ORFs (≥30 amino acids) located between a start codon and a stop codon where all six reading frames are considered
Allergenicity Analysis	Ensure that known allergenic sequences have not been introduced through transformation	AllergenOnline (FASTA Search): identify any small regions of similarity or larger regions of homology between ORFs and known allergens
Toxicity Analysis	Ensure that sequences similar to known toxins have not been introduced through transformation	FASTA Search (fasta36): Identify any annotated toxins with regions of similarity to ORFs associated with the insert

STUDY OBJECTIVES

The objectives of this study were:

1. Perform a comprehensive start-to-stop ORF analysis covering the DNA insert and its genomic junctions with the flanking regions;
2. Identify and evaluate any potential allergens based upon similarity between the ORFs and known allergens; and
3. Identify and evaluate any potential toxins based upon similarity between the ORFs and known toxins.

STUDY DATES

06/2015 - 09/2015

KEY STUDY PERSONNEL

[redacted]

MATERIALS AND METHODS

ORF detection

All open reading frames (ORFs) created as a result of the T-DNA insertion were identified using a Simplot-developed Python script (BioPython). The search parameters were defined to identify all ORFs with at least 30 amino acids located between the first ATG start codon in an ORF and the subsequent stop codon. Since a nucleotide sequence can be translated in three reading frames from two directions, all six reading frames of the E12 insert and flanking regions were analyzed for potential ORFs. The results were converted into FASTA-formatted files containing all unique protein sequences for further analysis. In addition, a FASTA-formatted file was generated where 20 ORFs were concatenated together and separated by the string "xxxxxx" to expedite the 80mer allergen scanning algorithm by allowing it to search multiple ORFs at a time.

The input and output file names for all analyses are described in Table 2.

Table 2: Data Files for Bioinformatics Analyses

Analysis	Input File	Output File
ORF Finder	E12.fasta	E12_start_to_stop.fasta E12_start_to_stop_concat.fasta
Toxin Search	E12_start_to_stop.fasta	E12_start_toxin15.txt
Allergen – Full	E12_start_to_stop.fasta	E12_start_allergen_full.txt
Allergen – 80mer	E12_start_to_stop_concat.fasta	E12_start_allergen_80mer.txt
Allergen – 8mer	E12_start_to_stop.fasta	E12_start_allergen_8mer.txt

Allergenicity database searches

Allergenicity potential was evaluated using the public, allergen-specific search engine (http://www.allergenonline.org/databasefasta.shtml) available through the Food Allergy Research and Resource Program (FARRP) at the University of Nebraska. All searches were performed using the most current database (version 15; January 12, 2015). Version 15 contains 1897 protein sequence entries that are categorized into 744 taxonomic-protein groups of unique proven or putative allergens (food, airway, venom/salivary and contact). Some of the allergenic wheat gliadins or glutenins may also cause celiac disease, however they are listed on the allergen site if there is evidence of IgE binding. The ORFs were analyzed using full-length, 80-mer local, and 8-mer exact match alignments.

Toxin database searches

The most recent UniProt database (49,293,307 entries) was used to extract proteins containing the keyword, "toxin", in the annotation for protein function. The toxin-related proteins were downloaded from the UniProt website (www.uniprot.org/downloads) on June 19, 2015 into a local, FASTA-formatted file (referred to as *toxin15*), which contained 10,555 protein sequences. In addition to actual toxins, this database includes proteins that have roles related to toxin production, exposure, or protection.

The FASTA36 software package (http://faculty.virginia.edu/wrpearson/fasta/fasta36/) was installed locally and used to perform protein homology searches (Pearson 1988, 2000, and 2003). All ORFs were queried against the toxin15 database with an E-value cutoff of $1x10^{-4}$:

>fasta36 -p -E 0.0001 *E12_start_to_stop.fasta* toxin15.fasta > *E12_start_toxin15*.txt

RESULTS

Start-to-stop ORF identification

A start-to-stop open reading frame is a contiguous sequence located between a canonical start codon and the next in-frame downstream stop codon. A Python script was used to identify all start-to-stop ORFs contained in the T-DNA insert and the adjacent potato flanking sequence, as shown schematically in Figure 1.

For E12 there were 27 unique ORFs identified, which were used in the subsequent allergen and toxin analyses.

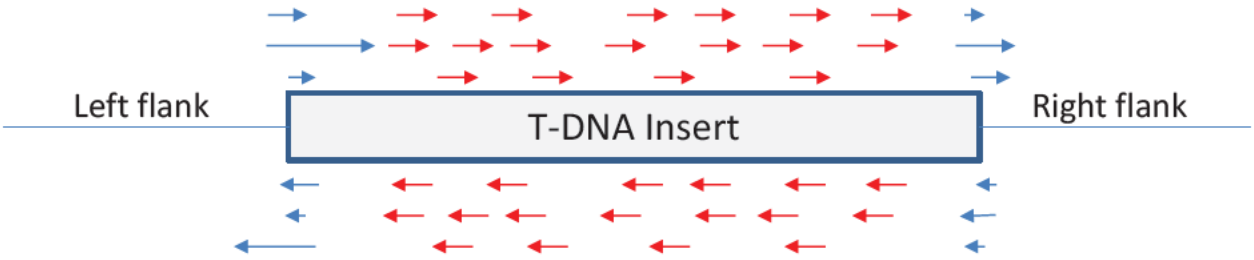

Figure 1 - Complete ORF Analysis Scheme. A schematic diagram illustrating any T-DNA insertion site in the plant genome. All ORFs (≥30 amino acids, colored lines) contained within the DNA insert (red lines) or overlapping the genomic flanking sites on either end (blue lines) are identified for each insert and used in subsequent analyses.

Allergenicity Assessment

Evaluation of the 27 ORFs for similarity or identity to known allergens did not identify any significant matches. There are no allergen-based safety concerns associated with the E12 insert, including ORFs extending into flanking sequences.

The allergenic potential of the ORFs was assessed using the web-based tool (http://www.allergenonline.org/databasefasta.shtml) provided by the Food Allergy Research and Resource Program (FARRP). This tool allowed comparison of ORFs associated with the E12 insert to known allergens using multiple sequence identity searches:

- full-length alignments;
- 80 amino acid (80-mer) high local-identity alignments; and
- 8 amino acid (8-mer) exact matches.

Full-length matches consisting of greater than 50% identity are considered the most predictive of potential allergens (Aalberse, 2000), whereas the 80-mer search is a precautionary tool to identify smaller regions of high identity between any ORFs and those of known allergens. The allergen database contains the sequence of known allergens, but the specific sequence responsible for allergenicity is not necessarily known, nor is it known whether an 8-mer is capable of inducing an allergic response (Goodman, 2008).

Full-length homology search

A full-length homology search was performed to identify homology between the individual ORFs and known allergens. This analysis compared each of the 27 ORFs with the sequence of known allergens and reported any matches with greater than 35 % homology (E-value cutoff = 10^{-4}). The algorithm did not identify any potential allergens (Table 3) associated with the E12 insert.

80-mer homology search

A second analysis identified localized regions of similarity between the ORFs and known allergens. This analysis compared all contiguous 80 amino acid sequences within an ORF and identified any matches with greater than 35% homology to known allergens (E-value cutoff = 10). The algorithm did not identify any potential allergens (Table 3) associated with the E12 insert.

8-mer identity search

Lastly, a short identity analysis was performed to identify short (8-mer) regions of exact identity between ORFs and known allergens. The algorithm did not identify any potential allergens (Table 3) associated with the E12 insert.

Toxicity Assessment

Evaluation of the ORFs for homology to known toxins did not identify any significant matches, and indicated that there are no toxicity-based safety concerns associated with the E12 insert.

There are no well-curated toxin-specific databases. However, the UniProt/Swissprot database is an expansive and well-annotated protein database that can be queried for protein homology. A query against the entire database, much like a query against the NCBI database, will produce many non-specific hits that must be manually and somewhat subjectively inspected to determine whether the identified proteins present toxicity safety concerns.

In an effort to develop an approach that more closely parallels the allergenicity analysis performed using FARPP, a more selective database was generated by Simplot through guidance from bioinformatics experts at UniProt. The database was limited to proteins annotated to be toxins or associated with toxin production or exposure. A toxin-related database (toxin15.fasta) was generated by extracting all protein sequences from the UniProt database containing the keyword, "toxin".

The ORFs associated with the E12 insert were queried against this database using search parameters similar to the full-length allergen search at FARPP (FASTA36; E-value cutoff, 10^{-4}). A summary of the results is included in Table 3.

Table 3. Summary of Allergenicity and Toxicity Matches for ORFs Associated with E12 Insertion Site

Allergenicity Search Alignments			Toxicity Search Alignments
Full-length alignment	**Scanning 80-mer**	**8-mer identity**	**Full-length**
0	0	0	0

CONCLUSION

Using well-established bioinformatic tools, a comprehensive analysis of the open reading frames in the insert and flanking regions of E12 was conducted. This bioinformatic analysis did not identify any potential toxin or allergen safety concerns with start-to-stop open reading frames associated with E12.

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Efficacy of Polyphenol Oxidase Downregulation in E12 Tubers

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This report is an accurate and complete representation of the study activities.

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Date

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ABSTRACT

The purpose of this study was to evaluate the trait efficacy of polyphenol oxidase (PPO) downregulation in potato event E12 compared with its parental control Russet Burbank. A field trial was conducted in Canyon County, Idaho during the 2015 growing season. Plots of the test and control varieties were harvested, and tubers were assessed for the darkening associated with the PPO enzyme using a colorimetric assay. As expected, the assessment evaluating PPO activity demonstrated that E12 tubers have consistently lower levels of PPO activity than its parental control.

INTRODUCTION

Potato event E12 was generated by transforming Russet Burbank with pSIM1278 using *Agrobacterium* transformation. One of the traits conferred by the T-DNA of pSIM1278 is reduced black spot.

Black spot is a post-harvest physiological phenomenon primarily resulting from the handling of potato tubers during harvest, transport, and processing, and refers to the black or grayish color that may form in the interior of damaged potatoes. The enzymatic darkening and discoloration, associated with the enzyme polyphenol oxidase (PPO), occurs when PPO leaks out from the plastids of damaged potatoes. Potatoes that show black spot are typically trimmed, or oftentimes the entire potato is rejected before processing. This results in quality control challenges, economic loss, or both.

The PPO cassette in pSIM1278 targets the native *Ppo5* gene to downregulate gene expression via RNAi. Reducing the PPO concentrations in E12 tubers reduces the occurrence of black spot and therefore potato waste.

STUDY OBJECTIVES

Compare the PPO activity of E12 to its control, Russet Burbank, using a colorimetric assay that measures the conversion of a PPO substrate, catechol, into melanin.

STUDY DATES

Field trials for tuber generation for PPO analysis were conducted during the 2015 field season. Tubers were harvested and analyzed in September, 2015.

KEY PERSONNEL

[redacted].

MATERIALS AND METHODS

Field Trials

During the growing season of 2015, the event and its parental control were grown in Canyon County, Idaho. The field trial study report number is 15-03-SPS-EXP. The agronomic practices and pest control measures used were location-specific and were typical for all aspects of potato cultivation including soil preparation, fertilizer application, irrigation and pesticide-based control methods.

All seed was field-grown, with generation one (G1) seed for Russet Burbank control and generation two (G2) seed for E12. The generation of seed used is unlikely to impact trait efficacy since potatoes are clonally propagated. Plots were organized with events located adjacent to the appropriate parental controls. Each plot consisted of two rows, approximately 20 feet in length, planted with 20 seed pieces per row and approximately 8 and 12 inches between pieces.

PPO Activity Assay

Buffer Solution Preparation and Treatment. The assay involves a 15 minute incubation with one of two buffers: Buffer A (no substrate control) and Buffer B (containing catechol substrate). Buffer A is comprised of 0.1 M citric acid and 0.2 M Na_2HPO_4. Buffer B is comprised of 0.1 M citric acid, 0.2 M Na_2HPO_4, and 0.2% catechol.

Tuber Sample Preparation. A total of 10 tubers (1 tuber from each plant) was collected for the event and its respective control. Using disposable plastic scoops, two scoops from each tuber were transferred to a 1.7 mL Eppendorf tube. Six independent tubes were prepared: three treated with 1 mL of Buffer A and three with 1 mL of Buffer B. Photographs were taken at two time points: immediately following addition of buffers (T_0) and after 15 minutes of incubation (T_{15}).

Sample Scoring Protocol and Data Analysis. Ten tubers from both field grown E12 and Russet Burbank tubers were analyzed in triplicate. The color of the solution for each sample (T_0 and T_{15}) was compared to the gradient shown in the scoring scale depicted in Figure 1. Each tuber was given a score based upon the mean of the three technical replicates that were each scored by two independent personnel.

A mean score and standard deviation was calculated for each treatment (Buffer A or Buffer B) and sample (WT or E12) at each time point. A Student's t-test was used to assess statistical differences between WT and E12 after the 15 minute incubation (T_{15}).

Figure 1. PPO Activity Colorimetric Scoring Scale
A color scale was established that visually depicts the polyphenol oxidase (PPO) mediated conversion of a catechol substrate to melanin (colored). The extent of conversion can be visually assayed from little to no melanin (1-3), intermediate melanin (4-7), and extensive melanin formation (8-10).

RESULTS

Oxidation of diphenolic compounds catalyzed by polyphenol oxidase (PPO) results in formation of o-quinones, leading to the formation of melanin (Albarran et al., 2010; Behbahani et al., 1993; Muneta, 1977). In this study, PPO activity was measured by monitoring the conversion of an exogenous diphenolic substrate, catechol, into melanin which is associated with a visible color change. An exogenous substrate was used to increase the rate and extent of color change in the samples.

Ten tubers from both field grown E12 and Russet Burbank tubers were analyzed using the PPO activity assay in triplicate. As shown in Figure 2A, there was no color change in either WT or E12 samples when treated with buffer lacking the PPO substrate, catechol. Addition of catechol resulted in a visible color change in the conventional control, Russet Burbank (WT), but not in E12 (Figure 2B).

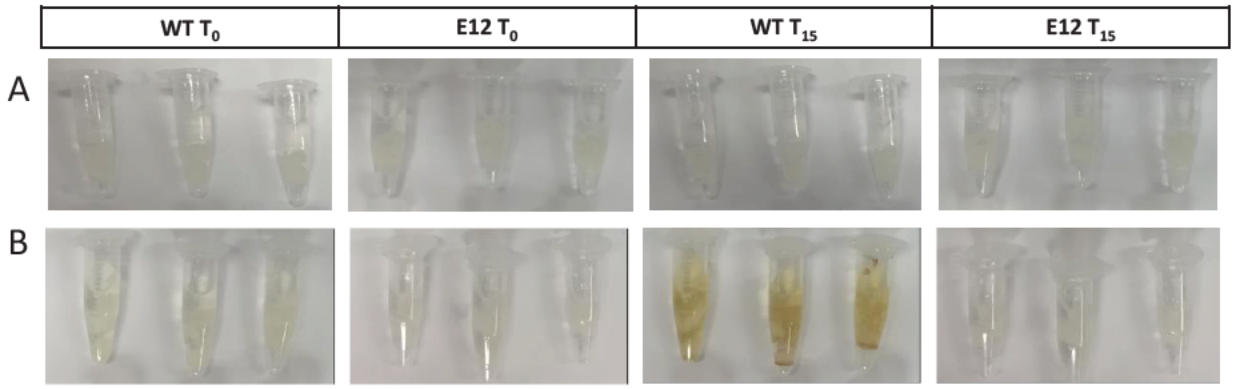
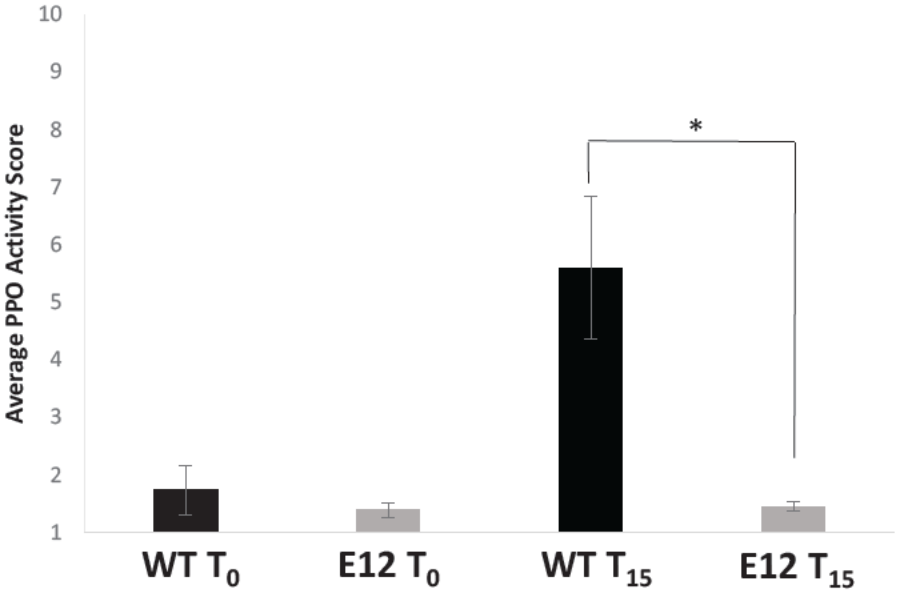


Figure 2. PPO Activity is Not Observed in E12 Tubers
Representative results from one conventional WT and E12 tuber exposed to either Buffer A or Buffer B. (A) No color change observed in WT and E12 after incubation in Buffer A (no catechol) for 15 minutes. (B) Dark color observed in WT, but not E12, after incubation in Buffer B (+ catechol) for 15 minutes. Color change indicates PPO activity.

The color change associated with each sample was scored using the PPO Activity Colorimetric Scoring Scale (Figure 1) with a summary of the results depicted in Figure 3. After 15 minutes, the color of E12 samples was similar to the T_0 time point, whereas significant color change occurred in the WT samples

after 15 minutes (Figure 3). The difference between the WT and E12 T_{15} samples was statistically significant ($p<0.01$). These results confirm a reduction of PPO activity in E12 tubers.



	WT T_0	E12 T_0	WT T_{15}	E12 T_{15}
Mean	1.733	1.383	5.600	1.450
Std Dev	0.417	0.137	1.240	0.081

Figure 3. Reduced PPO Activity in E12 Compared with Control
PPO activity was scored using the PPO Activity Colorimetric Scoring Scale for each of the 10 biological samples. The mean and standard deviation (Std Dev) values are listed above (n=10). The data compares activity scores between E12 and WT Control at T0 and T15 in the presence of catechol. The average PPO activity score at 15 minutes catechol exposure is significantly lower in E12 when compared with the conventional control (* denotes $p<0.01$).

CONCLUSION

Trait efficacy associated with down regulation of *Ppo5* in E12 tubers was assessed in this study. Compared to the Russet Burbank conventional variety, PPO activity was significantly reduced in E12 tubers, consistent with effective downregulation of *Ppo5* in E12 potatoes.

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